THE SCOVILLE SPLICE LIBRARY · VOLUME XIII

Astringency

Tannin, protein, friction. *Felt, not tasted.*

The complete account of the one sensation the tongue does not taste but feels — how a tannin silences the proteins that keep the mouth slick, and how we tune that grip at its genetic source, from the first grape to the last cup of tea.

SCOVILLE SPLICE · GROWN BY OUR FAMILY. ENGINEERED FROM SCRATCH.



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Opening essay · The axis you feel

There is a moment, a few seconds after an underripe grape or a cup of tea steeped a minute too long, when the inside of your mouth seems to shrink. The cheeks draw in. The tongue, which a breath ago slid against the roof of the mouth without a thought, now catches, as if a thin varnish had been pulled off every surface at once. It is not a flavor. Nothing tastes of anything in particular. It is a *texture that arrives without being touched* — a drawing-down, a pucker, a dryness that spreads from the gums to the back of the teeth and lingers long after you have swallowed. Our family has a word for the fruit that does it best: we call it *grippy*. The orchard word for it, the old word, is **astringent**.

For most of the history of eating, astringency was the sensation people worked hardest to be rid of. It is the taste of the not-yet — the green persimmon, the tannic skin, the wine that needs another five years in the dark. It is why acorns were leached in running water, why unripe fruit was left on the branch, why the first pressing of olives is cured for months before anyone will touch it. Astringency is the plant saying *not yet, and not you* — a chemical fence, built of tannin, raised against the animals that would eat the seed before it was ready. And yet. Pour a great Nebbiolo, steep a first-flush Darjeeling three minutes too long on purpose, break a square of seventy-percent chocolate against the tooth, and the grip is not the flaw. It is the structure. It is the thing that holds the wine up, that gives the tea its spine, that keeps the chocolate from collapsing into sweetness. Astringency, tamed, is architecture — and architecture is the one thing in flavor you cannot build by accident.



The founders in the research greenhouse at first light — where a family that loved the grip of good wine and strong tea asked whether it could be measured, and then grown on purpose.

The Iyengar Flavor Index holds eight axes, and astringency — **IFI-A** — is the strangest of them. The other seven are tastes and trigeminal heats: sweetness read by TAS1R2·R3, umami by mGluR1, heat by TRPV1, each a molecule settling into a receptor like a key into a lock, each a signal the nerve carries as *this much, exactly this much*. Astringency has no such lock. There is no astringency receptor on the tongue, no single cell waiting to be switched on by a tannin. The sensation is not something the mouth *tastes*. It is something the

mouth *loses* — and the whole of this volume is the account of what is lost, how much, and how we grow a plant to take exactly as much as the dish wants and not a particle more.

The thin film that keeps the tongue from noticing itself

Here is the mechanism, and it is the book in a sentence. Your saliva is not water. It is a thin, slick, self-renewing film — a few tens of micrometers thick — held together by proteins. Water is better than ninety-nine parts in a hundred of it; the rest is the quiet machinery: the big gel-forming mucins MUC5B and MUC7 that give saliva its spin and stretch, the statherins and histatins that keep calcium phosphate in solution so your enamel does not dissolve, α-amylase beginning on starch, and, above all, a family of **proline-rich proteins** — the PRPs — whose whole vocation is to keep every surface in your mouth lubricated and whole. In the parotid secretion the PRPs are not a minor component; they are the better part of the protein by mass. They are intrinsically disordered: open, floppy chains with no fixed fold, strung with proline after proline, and that is precisely what makes them the perfect prey.

Tannins are molecular hands with many fingers. A single tannin carries a dozen or more phenolic hydroxyl groups on two, three, four aromatic rings, and it binds a PRP in two ways at once. The flat aromatic faces stack against the pyrrolidine ring of proline — a hydrophobic face-to-face embrace that the extended, proline-studded chain exposes better than any folded protein could. And the phenolic –OH groups throw hydrogen bonds across to the peptide backbone, to the carbonyls and amides of the chain. One tannin, many contacts; one protein, many tannins. What forms is not a neat pair but a cross-linked mat.

The precipitation runs in three acts, and all three have been characterized in the literature. First, **binding**: tannin decorates the open PRP chain at multiple sites, each tannin a rivet. Second, **colloid**: the coated, now-hydrophobic protein particles find one another and aggregate into submicron colloids, still suspended but no longer soluble. Third, **precipitation**: the colloids grow, cross-link into a network, and fall out of solution — they curdle, and as they curdle they take the lubrication down with them. The film thins. Surface meets surface with nothing between them, and the friction of tongue against palate, of cheek against tooth, climbs.

That rise in friction *is* the sensation. There is no chemoreceptor because there is nothing chemical to report; the nerve that fires is the ordinary mechanosensory afferent of the trigeminal system, the same one that tells you a surface is rough, reporting that the mouth has, for a moment, lost its slip. On the Index, the receptor line for IFI-A reads **Salivary PRPs**, and it is exactly right. The thing being acted on is not a nerve. It is the protein the nerve was relying on.

We can put numbers on the slip. A healthy salivary film is a *boundary lubricant*: at the vanishing loads and speeds of tongue-on-palate, it holds the coefficient of friction down near $\mu \approx 0.02$–0.07. Pull the PRPs out and the surfaces run several-fold rougher, μ climbing toward the 0.3–0.5 of a delipidated, protein-starved contact. Soft-contact tribometry captures μ rising in real time as tannin is titrated

in, and the rise tracks trained panel astringency scores closely enough that tribologists treat the friction curve as the sensation's physical shadow. It is from this body of published measurement that our model draws the IFI-A scale — force of grip, not grams of tannin.

STATE OF THE SALIVARY FILM	BOUNDARY FRICTION COEFFICIENT μ *(REPRESENTATIVE)*
Whole saliva, PRP film intact	0.02 – 0.07
PRPs partly precipitated (building grip)	0.1 – 0.2
PRPs depleted (hard, drying grip)	0.3 – 0.5

Two families, and the rules of the grip

Not every tannin pulls with the same hand, and the first thing to know is that "tannin" is two chemistries wearing one name.

The **hydrolyzable tannins** are built on a sugar. Take a glucose and esterify its hydroxyls with gallic acid and you have a *gallotannin* — tannic acid, or the clean laboratory archetype, β-1,2,3,4,6-penta-*O*-galloyl-D-glucose, five galloyl groups fanned off one sugar. Let two of those galloyls couple to each other and you have the *ellagitannins*, built on hexahydroxydiphenic acid: punicalagin, which gives pomegranate its leather-and-ink grip; sanguiin H-6 and lambertianin, which do the same for raspberry; castalagin and vescalagin, leached from oak into every barrel-aged wine. Hydrolyzable because a little acid or the right enzyme cleaves them back to gallic or ellagic acid and sugar.

The **condensed tannins** carry no sugar at all. They are the *proanthocyanidins*: chains of flavan-3-ol units — (+)-catechin, (−)-epicatechin, their trihydroxy cousins (−)-epigallocatechin and (−)-gallocatechin, and the galloylated forms (−)-epicatechin-3-gallate and (−)-epigallocatechin-3-gallate, the EGCG that is tea's signature — stitched together mostly C4→C8, sometimes C4→C6. When the building block is catechin or epicatechin (a catechol B-ring, two hydroxyls) the polymer is a *procyanidin*; when it is the gallocatechins (a pyrogallol B-ring, three hydroxyls) it is a *prodelphinidin*. Condensed because heat and acid do not hydrolyze them; they polymerize further.



Grape, in cross-section — the seed and inner skin where the proanthocyanidins concentrate, and where the balance of procyanidin, prodelphinidin, and galloylation is set.

Within and across those families, four rules decide whether a grip is fine or coarse, long or green — and every one of them is a knob we can reach:

1. **Size.** More rings and more hydroxyls mean more rivets per tannin, so precipitating potency climbs with the mean degree of polymerization (mDP) — until the molecule grows so large it will no longer stay in solution, and the grip falls off as the tannin hazes out on its own. The strongest astringents live in the window between "big enough to cross-link" and "too big to dissolve."
2. **Galloylation.** Hang a galloyl group on a flavan-3-ol and you add a whole aromatic ring and three more hydroxyls precisely at the protein-binding face. Galloylated units bind PRPs far harder than their bare parents; EGCG grips where EGC barely does. Grape *seed* tannin is heavily galloylated and short — this is the source of its hard, drying edge — while grape *skin* tannin is longer, richer in prodelphinidin, and softer on the tongue. Long and fine, or short and green: the difference is mostly galloylation and provenance.
3. **B-ring hydroxylation.** Three hydroxyls (pyrogallol: the prodelphinidins, EGCG, punicalagin) out-bind two (catechol: the procyanidins), because every extra –OH is another hydrogen bond offered to the peptide backbone.
4. **Flexibility.** The 2,3-*cis* epi-forms bend and wrap a disordered PRP chain better than the stiffer 2,3-*trans* forms. A tannin that can mould itself to the protein pulls more per contact than one that cannot.

TANNIN FAMILY	BACKBONE	HYDROLYZABLE?	ARCHETYPE	IN OUR HOSTS
Gallotannin	galloyl esters of glucose	yes	pentagalloylglucose (the reference)	lab standard
Ellagitannin	HHDP esters of glucose	yes	punicalagin, sanguiin H-6	pomegranate, raspberry
Procyanidin	catechin / epicatechin, C4→C8	no	procyanidin B2	grape seed, blueberry, sorghum
Prodelphinidin	(epi)gallocatechin	no	—	grape skin, tea
Galloylated PAC	EGCG / ECG units	no	EGCG	tea, grape seed

This is why IFI-A had to be a measure of *force*, not of *amount*. A gram of soft, long, skin-derived prodelphinidin and a gram of sharp, galloylated seed procyanidin are the same mass and a world apart on the tongue, because one precipitates salivary protein at a fifth the concentration of the other. You cannot design a sensation you cannot separate from its own quantity — so the first thing we built was the arithmetic that separates them. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



IFI-A · SALIVARY PRPS

From pucker to number

PC$_{50}$ · the dose–response · the arithmetic of the grip

The arithmetic of the pucker

Every axis of the Index is scored the same way: we take the half-maximal concentration of a stimulus, invert it against a fixed reference, and scale by a thousand.

$$IFI(c) = 1000 \cdot EC_{50}(ref) / EC_{50}(c)$$

For heat, the concentration in question is the one that half-activates TRPV1. Astringency has no receptor to half-activate, so we substitute the quantity that *does* report the sensation — the pulling-down of protein — and write the astringency form of the same law:

$$IFI\text{-}A(c) = 1000 \cdot PC_{50}(ref) / PC_{50}(c)$$

PC_{50} is the precipitation concentration: the amount of a tannin that pulls half of a standardized pool of salivary proline-rich protein out of solution, read under fixed ionic strength, pH, and temperature. The less tannin it takes to drop half the pool, the harder that tannin grips, and — because PC_{50} sits in the denominator — the higher the score. IFI-A is not a measure of how much tannin a food holds. It is a measure of how *hard that tannin pulls*.

The precipitation is not linear in concentration; it is sigmoid, as cooperative cross-linking always is. We fit the fraction of protein precipitated, *f*, as a Hill curve in tannin concentration [T]:

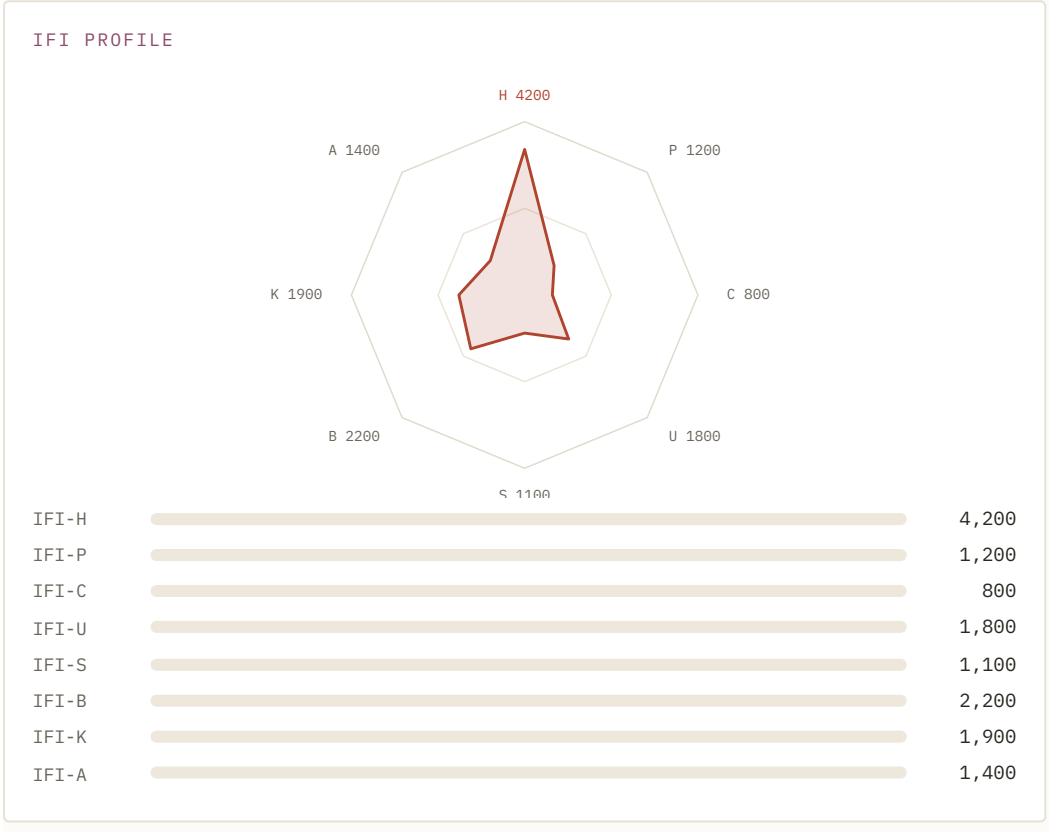
$$f([T]) = [T]^h / (PC_{50}^h + [T]^h)$$

with a Hill coefficient *h* near 1.5–2, the cooperativity coming from the fact that a half-coated colloid recruits the next tannin more readily than a bare protein does. Set [T] = PC_{50} and *f* = ½ by construction. The shape does the rest. Take a tannin with PC_{50} = 30 µM and *h* = 2: at 15 µM, $f = 15^2/(30^2+15^2) = 225/1125 =$ **0.20**; at 30 µM, *f* = **0.50**; at 60 µM, $f = 3600/4500 =$ **0.80**. A fourfold swing in concentration moves the mouth from a whisper of grip to nearly full precipitation — which is exactly why a thirty-second difference in a steep, or one wet vintage against one dry, is the whole range of the experience.

Now anchor the scale. We define the reference to be pentagalloylglucose, the clean gallotannin, at **PC_{50}(ref) ≡ 30 µM** — so the reference reads 1,000 by construction, as every axis's reference does. From there the arithmetic is one division:

TANNIN (PROBE)	PC_{50}	IFI-A = 1000 · 30 / PC_{50}	NOTE
Reference (pentagalloylglucose)	30 µM	1,000	anchor, by definition
Soft skin-derived prodelphinidin	75 µM	**400**	representative
Galloylated grape-seed procyanidin	15 µM	**2,000**	representative
EGCG (tea)	10 µM	**3,000**	representative
Phoenix · SS-0096 (astringency axis)	21.4 µM	**1,400**	canonical profile value

Read the last row from the other direction and you see how the Index lets you design backward. Phoenix, our full-spectrum cultivar, carries a canonical astringency score of **1,400**. Invert the formula — $PC_{50}(c) = 1000 \cdot PC_{50}(ref) / IFI\text{-}A = 1000 \cdot 30 / 1{,}400$ — and the target falls out: **21.4 µM**. That is the specification the plant must hit. We do not breed toward a flavor we can only describe; we breed toward a precipitation concentration we can weigh.



The units travel both ways, because the orchard speaks in grams per litre and the assay speaks in micromoles. Pentagalloylglucose has a molar mass near 940 g/mol, so our 30 µM reference is 30×10^{-6} mol/L × 940 g/mol ≈ 0.028 g/L — about 28 µg/mL. EGCG, lighter at 458 g/mol, reaches its 10 µM grip at just 4.6 µg/mL, a reminder that a potent tannin needs very little mass to speak loudly. Converting a whole wine is subtler and we are honest about it: a red may carry 2 g/L of total tannin, but that mass is a *distribution* of polymers, not a single species, so the g/L figure must be resolved into an mDP-weighted molar concentration of binding-competent chains before it can be set against a PC_{50}. The number on the label is mass; the number on the tongue is force; the assay is the bridge.

Finally, the error, because a score without its uncertainty is a decoration, not a measurement. IFI-A is a quotient of two half-concentrations, and for a quotient the relative variances add in quadrature:

$$\left(\sigma_{IFI} / IFI\right)^2 = \left(\sigma_{ref} / PC_{50,ref}\right)^2 + \left(\sigma_{c} / PC_{50,c}\right)^2$$

If each PC_{50} is measured to a coefficient of variation of 5%, the score inherits $\sqrt{(0.05^2 + 0.05^2)} = 0.071$ — about **7%**. That is not a coincidence of this page; it is the design target of the whole platform. We hold batch-to-batch variation at or below **7% CV**, so a reported IFI-A of 1,400 means 1,400 ± roughly 100 at one standard deviation — a grip you can specify, reproduce, and ship — against a conventional crop whose astringency can swing by tens of percent

between a wet year and a dry one. Reproducibility is not a convenience here. It is the difference between a flavor you can design and a flavor you can only hope for.

The six plants that speak it

Six of our forty-two validated host organisms carry the astringency axis, and between them they hold **2,290 constructs** aimed at a sensation that is not, technically, a taste at all. Each plant builds its tannin by a different route, and each route is a different set of knobs.

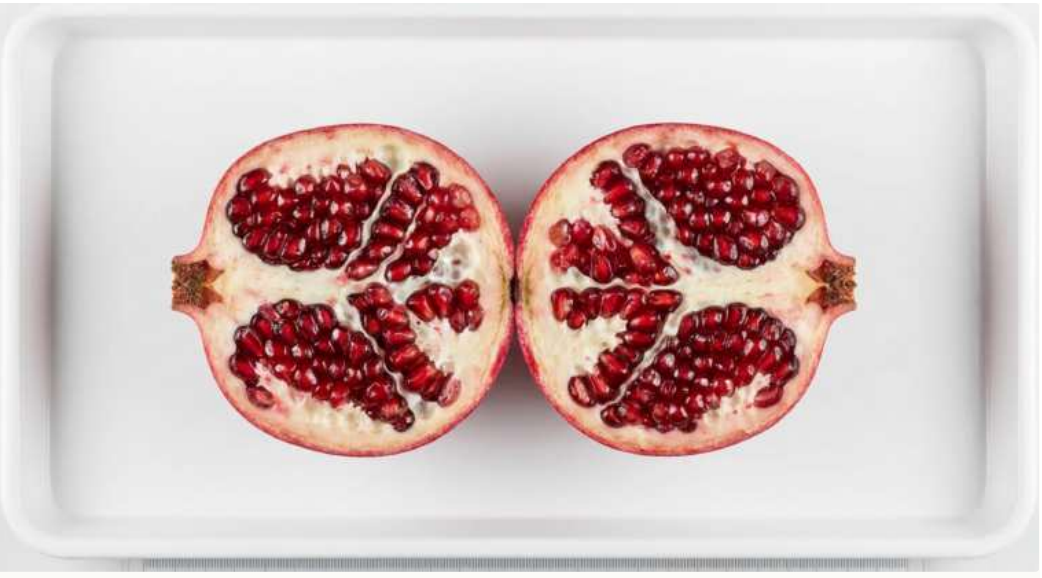
ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	TANNIN CHEMISTRY
Grape	1,610	B · A · M	Hard	condensed; skin prodelphinidin + seed procyanidin
Tea	280	U · B · A	Hard	condensed, heavily galloylated (EGCG, ECG)
Pomegranate	120	A	Hard	hydrolyzable ellagitannin (punicalagin)
Blueberry	120	A	Moderate	condensed procyanidin
Sorghum	120	A	Frontier	condensed proanthocyanidin (testa)
Raspberry	40	A	Moderate	hydrolyzable ellagitannin (sanguiin H-6)
Total	**2,290**			

Grape is the deep library — 1,610 constructs — because grape tannin is the most architecturally tunable of them all. The flavan-3-ol monomers come off the flavonoid pathway, and two enzymes set their character: leucoanthocyanidin reductase (LAR) makes the (+)-catechin that caps a chain, and anthocyanidin reductase (ANR, the *BAN* gene) reduces cyanidin to the (−)-epicatechin that extends it. The ratio of the two sets the balance of terminal to extension units, and with it the mean degree of polymerization; a galloyltransferase decides how much of the chain is galloylated; the skin-versus-seed partition decides how much prodelphinidin softness is blended against procyanidin bite. Tune ANR against LAR, meter the galloylation, and you move a grape from short-and-green to long-and-fine without ever leaving the vine.

Tea speaks three axes at once — umami from its theanine, a managed bitterness from caffeine and the catechins, and the astringency that is the catechins' other face. EGCG dominates the leaf's grip, and because galloylation is the single largest lever on PC_{50}, tea is where a small edit to a galloyltransferase moves the whole sensation. Oxidize the leaf — the move that turns green tea black — and the catechins couple into theaflavins and thearubigins, trading some of that hard catechin grip for a rounder, redder astringency braided with colour. The 280 constructs are mostly about choosing where on that green-to-black continuum a cup should land, and holding it there.



Tea — the leaf whose astringency is written almost entirely in galloylated catechins, EGCG first among them.

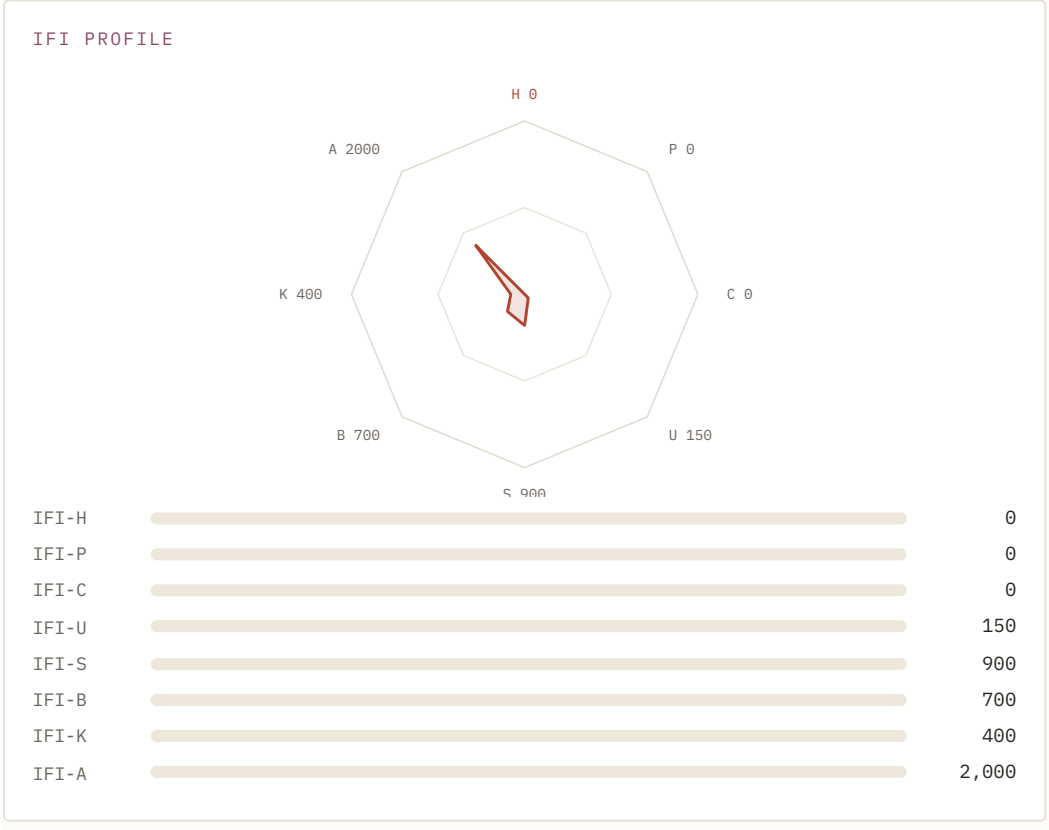


Pomegranate, opened — the arils whose ellagitannins give a grip that is bright and quick where a grape's is slow and deep.

Pomegranate is our hydrolyzable flagship. Its grip is punicalagin, a large ellagitannin, and ellagitannins pull differently from condensed tannins — a brighter, more fleeting astringency that arrives and clears faster because the molecule is a different shape against the protein. The 120 constructs work the ellagitannin branch: how much punicalagin against punicalin, how much free ellagic acid, how the balance reads as the aril is pressed.

Blueberry carries procyanidins alongside its anthocyanins, a gentle, fruit-forward grip; at Moderate difficulty it is one of the more tractable condensed-tannin hosts, 120 constructs deep. **Sorghum** is the frontier of the six — a cereal, not a fruit, concentrating condensed proanthocyanidins in the testa layer of its grain; the 120 constructs are a proof that the axis is not confined to wine and tea but can be grown into a staple. **Raspberry**, smallest of the set at 40 constructs, is an ellagitannin plant like pomegranate — sanguiin H-6 and lambertianin C — and a reminder that the sharp, clean astringency of a good raspberry is the same chemistry, in miniature, as a barrel of oak-aged red.

A designed tannic red — representative, not a released SKU — reads roughly like the profile below: nearly silent on heat and the cooling channels, a little sugar and a managed seed-bitterness for frame, and the grip carried high and alone on IFI-A.



Reading a pathway before we ever plant it

We do not plant and hope. Before a single seed goes into soil we read the pathway in silico, because a tannin is too expensive in a plant's carbon — and a season is too long — to spend either on a guess.

The engine is the one we built first on heat and never stopped using. A 650-million-parameter ESM2 protein language model scores every possible single-residue substitution in a target enzyme by its predicted effect on catalytic efficiency × fold stability; a genome-scale flux-balance model confirms which step is the true rate limiter rather than an upstream one; a structure prediction, read through its per-residue pLDDT confidence and its predicted-aligned-error map, tells us whether the residues we mean to touch sit in a well-ordered, high-confidence pocket or a floppy loop we should leave alone; and CRISPR writes the chosen edits in. On the capsaicin pathway that engine found two substitutions in Pun1 that no breeding program had ever combined — **S39L** (ΔΔG −2.1, binding affinity up at the N-terminus) and **L345G** (ΔΔG −1.8, steric relief in the active-site cleft) — forming the shipped double variant, with C175S retained as a structural annotation only; together they predict a ≤7% CV where the wild type wandered at 42%.

For astringency the same engine turns to a different set of enzymes — the flavonoid trunk PAL → C4H → 4CL → CHS → CHI → F3H → DFR → ANS, then the branch point where ANR and LAR commit carbon to flavan-3-ols, plus the galloyltransferases that decide how much of the chain wears a galloyl group. The targets differ; the method does not.

But we are honest about what is harder here, because astringency breaks the clean assumption the rest of the Index enjoys. Heat is one molecule — capsaicin — and a single EC_{50} describes it. A tannin is

never one molecule. It is a *distribution*: a spread of chain lengths, a percentage of galloylation, a ratio of procyanidin to prodelphinidin, a population rather than a species. PC_{50} is therefore an ensemble readout, and the design target is the shape of a distribution, not the identity of a compound. Push the mean degree of polymerization too high and the tannin crosses its solubility limit and hazes out of the fruit on its own — potent in the flask, absent on the tongue, a failure mode with no analogue in heat. And because catechins are read as *both* bitter and astringent, the plant couples two axes the Index deliberately holds apart; separating IFI-A from IFI-B in a tea or a grape means moving galloylation and polymerization in opposite directions at once. The arithmetic is clean. The biology it describes is a cloud, and we design for the cloud.

The cellar and the table

The grip was never meant to live in a flask. It was built to meet fat, and salt, and time, and a second sip — and every one of those is chemistry we can account for.

Begin with the second sip, because it is the first thing anyone notices. Saliva regenerates in a minute or two, but not instantly, so each mouthful meets a film already thinned by the last; astringency *builds*, cumulatively, as the PRP pool is drawn down faster than it is replaced. This is why a tannic wine is grippier on the third glass than the first, and why a trained panel rinses and rests on a fixed clock — the sensation has a memory, and the measurement has to out-wait it.

Now bring the food. The reason a tannic red loves a hard cheese, the reason a splash of milk softens strong tea, is a single competition: casein, the micellar protein of milk and cheese, is even more proline-rich and tannin-avid than your salivary PRPs. Put it on the tongue first and the tannin spends itself on the casein, precipitating *it* instead of your saliva, and the lubrication survives. Fat and protein do not mask astringency; they intercept it. Salt and acid shift the perception at the margins, warmth sharpens it, and the long polysaccharides — the mannoproteins a wine draws from its yeast lees, the gums a cellar-master may add — coat the tannin and blunt its reach. Time does the deepest work of all: across years in the dark, tannins polymerize and oxidize, the hard short seed-type grip condensing into something longer and rounder, and tannin and pigment co-precipitate as sediment, so an old wine is literally less astringent than its young self — the architecture settling, the fence weathering down to a wall you can lean on.

Know the failure modes, because they are where a designed flavor is won or lost. Over-build the tannin and it exceeds its solubility and falls out as haze before it ever reaches a mouth — potent by assay, silent on the palate. Under-manage the coupling to bitterness and a grape meant to be structured reads merely harsh. And know the honest limit the arithmetic cannot erase: people are not identical assays. Salivary flow rate varies several-fold between individuals, and so does PRP output — some mouths secrete so much proline-rich protein that a tannin exhausts itself before the lubrication is touched, and those people genuinely taste less astringency from the

same glass. PC_{50} is a faithful in-vitro proxy and tribology is a close physical shadow, but the last translation, from precipitated protein to felt grip, runs through a mouth whose chemistry is its own. We report the force the tannin pulls with. The mouth decides how much of that force it can afford to feel.

That is the axis you feel and never quite taste: a sensation with no receptor, read through the protein it destroys, scored by a precipitation concentration, and grown — gently, reverently, in six plants that each spend their hard-won carbon on it — into exactly the grip a wine, a cup, or a fruit was meant to carry. Tannin is the most reverent chemistry in the plant, the compound a plant builds not to feed us but to defend its future, and to design astringency is to take that chemistry in hand with care: to leave the fence standing, and teach it to be architecture.



PART I OF IV

The Sensation

a touch, not a taste · salivary PRPs · hydrolyzable & condensed tannins · the arithmetic of the grip

Chapter 1 · The axis that isn't a taste — *what the mouth does when it dries.*

Taste, in the narrow scientific sense, is a short list: sweet, sour, salty, bitter, umami. Each has a dedicated machinery — taste receptor cells clustered in buds on the tongue and palate, each cell tuned to a class of molecule, each firing a labeled line to the brain that means *sweet* or *bitter* and nothing else. You could, in principle, anesthetize those cells and abolish taste entirely, and a tannin would still make your mouth pucker. Astringency survives the loss of taste because it was never taste to begin with. It is **oral somatosensation** — the sense of touch, temperature, and movement inside the mouth, carried not by the taste nerves but by the trigeminal nerve, the same nerve that reports the burn of chili and the cool of mint. Astringency is to taste what the texture of velvet is to its color: a different channel entirely, arriving at the same time.

We can be precise about what the channel reports. The inside of a healthy mouth is one of the best-lubricated surfaces in the body. A continuous film of saliva, only microns thick, coats the tongue, the palate, the cheeks, and the teeth, and that film is engineered — by us, by evolution — to be *slippery*. Its lubrication is what tribologists call boundary lubrication: the surfaces never actually touch, because a layer of protein molecules bridges them, bearing the load and letting them glide. Run your tongue across the roof of your mouth right now and feel how little resistance there is. That ease is the baseline. **Astringency is the measurable departure from it** — and because it is a departure from a physical baseline, it is a quantity, and a quantity is a thing we can design.



A plum, halved — skin, flesh, and stone. The grip of the astringent fruit lives mostly in the skin and the thin layer around the seed, exactly where the plant most needs to defend what matters.

The protein the mouth spends to stay slippery

To understand what tannin undoes, we have to meet the molecule it undoes it to. The slip of a resting mouth is not an accident of water. It is held by a specific family of salivary proteins — the **proline-rich proteins**, or PRPs, which make up on the order of seventy percent of

the protein your parotid glands secrete, alongside statherin, the histatins, and the long mucins MUC5B and MUC7. The PRPs are strange proteins, and their strangeness is the whole story. They are a quarter to a third proline by composition, strung with glycine and glutamine, and that proline content forbids them from folding into the compact, globe-like shapes most proteins take. Instead they lie open — loose, extended chains with long stretches of polyproline-II helix, flexible as wet thread. An open chain is a *big* chain: it presents an enormous surface, and across that surface it offers two things a polyphenol craves. The pyrrolidine rings of the prolines give a flat hydrophobic face for aromatic rings to stack against, and — because proline has no backbone N–H — the chain leaves a long row of carbonyl oxygens exposed as hydrogen-bond acceptors.

This is the quiet genius the body was already practicing long before we arrived to measure it. A protein built to be sacrificed. The PRP lies in the salivary film doing two jobs at once: it lubricates, by the same open, water-holding, load-bearing structure that makes it slip; and it waits, a soft target, for any tannin the next mouthful brings. There is good evidence the second job is the older one — PRP genes are inducible, climbing in animals moved onto tannin-rich forage, as if the body, meeting the plant's chemical defense of its seed, answered with a defense of its own: a protein offered up to bind the tannin, precipitate it, and carry it harmlessly down and away from the digestive enzymes it would otherwise foul. Astringency, felt honestly, is the sensation of that transaction being paid. The grip is the receipt. We find this one of the most moving facts on the whole Index — that the sense we are scoring is the felt moment where a plant's protection of its future and a body's protection of itself meet on a film of saliva and cancel.

The molecule that unmakes a lubricant

When tannin enters this film, it does not bind a receptor and shout. It quietly dismantles the lubricant, and it does so in three stages we can watch and time. **First**, the polyphenol coats the open PRP chain — galloyl and catechol rings stacking onto the proline faces, phenolic hydroxyls hydrogen-bonding to the exposed carbonyls. This is a multidentate grip: a single tannin of any size carries several aromatic rings, so it does not touch the protein at one point but clings along it. **Second**, because both partners are multivalent — the tannin with its several rings, the open protein with its many sites — one tannin bridges two chains, then three, and soluble complexes grow, knitting the once-free proteins into a loose colloidal mesh. **Third**, the growing aggregates reach a size and a neutralized charge at which they fall out of solution altogether. The protein bridges that held the two oral surfaces apart are now curds suspended in a thinning film.

Within seconds those bridges collapse, the film thins and in places breaks, and the surfaces begin to meet. The friction coefficient — the ratio of the force it takes to slide two surfaces against the force pressing them together — climbs from the easy baseline toward something rougher. The free nerve endings of the trigeminal system woven through the oral mucosa are mechanoreceptors: they report deformation, stretch, and drag. A tongue moving across a palate that

has lost its slip stretches and drags more, and those endings fire. The brain, assembling the signal, does not report *friction coefficient 0.4*. It reports *dry, rough, puckering, tight* — the vocabulary of astringency. The sensation is real, vivid, and entirely mechanical. There is nothing being tasted. There is a lubricant being lost and a mouth noticing its own surfaces for the first time.

The binding itself is a measurable thermodynamic event, which matters because it is one of the ways we prove the account is literally true. Pour a tannin solution into a calorimeter over a PRP pool and the instrument reads heat released — the association is enthalpy-driven, the signature of hydrogen bonds forming, carried along by a hydrophobic, entropy-favored component as water is shed from the stacking rings. The calorimeter returns a binding constant and a stoichiometry: *how tightly* and *how many*. Those two numbers are the molecular root of everything a mouth later feels, and they are the first place a design either succeeds or fails.

Four oddities, and why each is a lever

This mechanical origin explains every strange thing about astringency, and the strangeness is worth cataloguing, because each oddity is a design lever in disguise.

It is **slow**. A sweet taste is nearly instant — the molecule meets the receptor and the signal goes. Astringency takes seconds, because precipitation takes seconds: the tannin must find the protein, bind it, cross-link enough of it, and pull it down before the film is thin enough to feel. The peak often arrives after you have already swallowed. A designer who wants a grip that blooms late, in the finish, reaches for a larger, slower-precipitating tannin; one who wants an immediate catch reaches for a small, fast, galloylated one.

It is **cumulative**. Sip a tannic wine once and you feel a grip. Sip it again before your saliva has fully recovered and the second grip is harder, and the third harder still. Taste adapts — a second spoonful of sugar tastes less sweet. Astringency does the opposite. It builds, because each exposure depletes a lubricant that regenerates only as fast as your glands can make and secrete fresh protein. A wine's tannin is not a single event; it is an accumulating account, and the slope of that accumulation is itself a thing we can tune.

It is **persistent**. Long after the stimulus is gone, the mouth stays dry, because the lubricating protein that was precipitated is simply gone — pulled out of solution — and must be physically replaced. The drying of a great tannic red can last a full minute into the finish. Winemakers prize exactly this persistence and call it *length*.

And it is **personal** in a way taste is not. How astringent a given tannin feels to you depends on how much PRP your saliva carries and how fast your glands replace it — and those vary enormously from person to person, and within one person across a day: salivary protein can differ several-fold between two healthy mouths. A mouth flush with protein shrugs off a tannin that leaves a drier mouth reeling. This is not a nuisance to be averaged away; it is the central reason the Index measures the *stimulus* and not the *sensation*. IFI-A scores how hard a tannin pulls on a **standardized** PRP pool in a flask — a number that does not move when the perceiver does. We score

the chemistry and let each mouth be itself. That single decision is what lets a tomato, a cup of tea, and a chili be read against one another on one page: we are not comparing sensations, which are private, but the chemical loudness that provokes them, which is public and fixed.

A grammar of grip

It helps to have a vocabulary for the kinds of grip, because astringency is not one sensation but a small family of them, and each sub-quality is a different physical event in the film. **Puckering** is the sharp, drawing-in grip of a fast, potent tannin pulling protein down quickly — the green grape, the pomegranate rind. **Drying** is the lingering aftermath, the mouth left without its lubricant, the long finish of a tannic red. **Chalky** or **grainy** is the sensation of the precipitated tannin-and-protein aggregates themselves, felt as fine particles rolling between the surfaces — the hallmark of a young, under-polymerized tannin whose curds are coarse. **Velvety** or **silky** is the paradoxical grip of a long, well-polymerized, prodelphinidin-rich tannin that precipitates protein gently and evenly, so the friction rises in a smooth sweep rather than a rush — the grip a cellar spends a decade chasing. And **harsh** or **rough** is uneven precipitation, friction climbing in catches rather than a clean arc.

These are not poetry dressed as data. They are reliably distinguished on a trained panel, they track measurable differences in the kinetics and the particle size of the precipitate, and — this is the whole point — each is reachable through a different setting of the structural dials. A designer does not merely set *how much* grip. They set *which kind*, and the two are nearly independent knobs. The table below is the map from the felt quality to the physical cause we reach for to produce it.

SUB-QUALITY	WHAT THE FILM IS DOING	STRUCTURAL CAUSE WE TUNE	WHERE IT LIVES
Puckering	Fast, heavy precipitation	High galloylation, small–mid size	Green grape, pomegranate
Drying	Lubricant gone, slow to replace	High total tannin, high affinity	Tannic red wine, strong tea
Chalky / grainy	Coarse curds rolling between surfaces	Low polymerization, polydisperse	Young, unaged condensed tannin
Velvety / silky	Even, gentle, smooth friction rise	High polymerization, prodelphinidin-rich	Aged red, fine dark chocolate
Harsh / rough	Friction climbing in catches	Uneven chain length, branched	Unrefined, under-managed tannin

The structural dials

Grip, then, is not a single magnitude but a surface with several coordinates, and the coordinates are chemical. Four dials set almost everything a tannin does to a salivary film, and each one is a place in the plant's biosynthesis where an enzyme already makes a choice we can move.

The scaffold is shared by every green thing. The general phenylpropanoid branch — PAL to C4H to 4CL — runs in every vascular plant, and the flavonoid core that follows (chalcone synthase, then the isomerase, the hydroxylase F3H, the reductase DFR, and the anthocyanidin synthase) is nearly as universal. The tannin story begins where that core splits toward the flavan-3-ols,

the subunits that string into condensed tannins. Two reductases govern the split: **LAR**, leucoanthocyanidin reductase, which makes the *trans* subunit catechin, and **ANR**, anthocyanidin reductase, which makes the *cis* subunit epicatechin. The balance of those two enzymes, and the pool of subunits they feed, is the control point we return to again and again — our grape-tannin program is built on **ANR** precisely because it sets both the stereochemistry and the extension chemistry of everything downstream.



A wine grape in cross-section — skin, pulp, and the seeds in their astringent coat. Grape is the most tannin-worked organism in the library at 1,610 constructs; the ANR branch is the dial, and the seed's testa is where the grip is born.

The first dial is size — the mean degree of polymerization, or mDP, of the condensed tannin. A lone flavan-3-ol monomer is only weakly astringent: it has too few rings to bridge two protein chains, so it coats without cross-linking, and precipitation barely begins. String the subunits together and astringency climbs steeply, because each added unit is another anchor onto another protein — a dimer cross-links, an oligomer of five or six cross-links avidly, and the grip is at its height. But the dial is not monotonic, and this is one of the honest surprises of the chemistry: push mDP past roughly fifteen and astringency *falls again*, because a very large tannin becomes poorly soluble and begins to self-associate and precipitate before it ever reaches the salivary protein. There is an optimum in the middle, near mDP five to eight, and knowing where the peak sits — and that there is a far side to it — is the difference between designing grip and merely adding tannin.

The second dial is galloylation — the fraction of subunits esterified with a galloyl group, the small gallic-acid ring that tea and grape seed hang off their flavan-3-ols to make epicatechin-3-gallate and epigallocatechin gallate. This is the single strongest lever on affinity. A galloyl group is another aromatic ring and three more hydroxyls, so a galloylated subunit stacks harder onto proline and hydrogen-bonds more to the backbone; a heavily galloylated tannin can bind protein several-fold more tightly than its ungalloylated twin of the same size. Galloylation is laid on by a glucosyltransferase and a galloyltransferase late in the pathway, which makes it a clean, almost dimmer-like control: turn it up for a sharp, early, puckering grip; turn it down for softness.

The third dial is hydroxylation — whether the subunit's B-ring carries two hydroxyls (a procyanidin) or three (a prodelphinidin), a choice made far upstream by the enzyme F3′5′H. The trihydroxylated

prodelphinidins hydrogen-bond more and, through their geometry, tend to precipitate protein in a more even, gradual way. This is the molecular basis of *velvet*: a prodelphinidin-rich tannin does not grab, it closes — the friction rises in a smooth sweep rather than a rush. **The fourth dial is uniformity** — how tight the distribution of chain lengths is. A narrow, well-polymerized distribution gives the even, silky climb; a broad, branched, polydisperse one gives the catching, grainy harshness. Ripening and aging are, in chemical terms, the plant and the cellar slowly narrowing and lengthening that distribution, which is why a tannin softens with time. We can set at harvest what a decade of patience would otherwise be required to reach.

Putting a number on the pull

All four dials end at one place: a single curve of protein precipitated against tannin concentration, and a single number that summarizes it. The Index is anchored, on every axis, to the half-maximal point of a receptor response, and for astringency the "receptor response" is the precipitation of the standardized PRP pool. We define **EC_{50}** as the tannin concentration that precipitates half of the maximal precipitable protein in the assay. A potent tannin reaches half-maximum at a low concentration; a weak one needs a flood. Loudness is the inverse of the concentration it takes to be heard, which is exactly what the Index formula encodes:

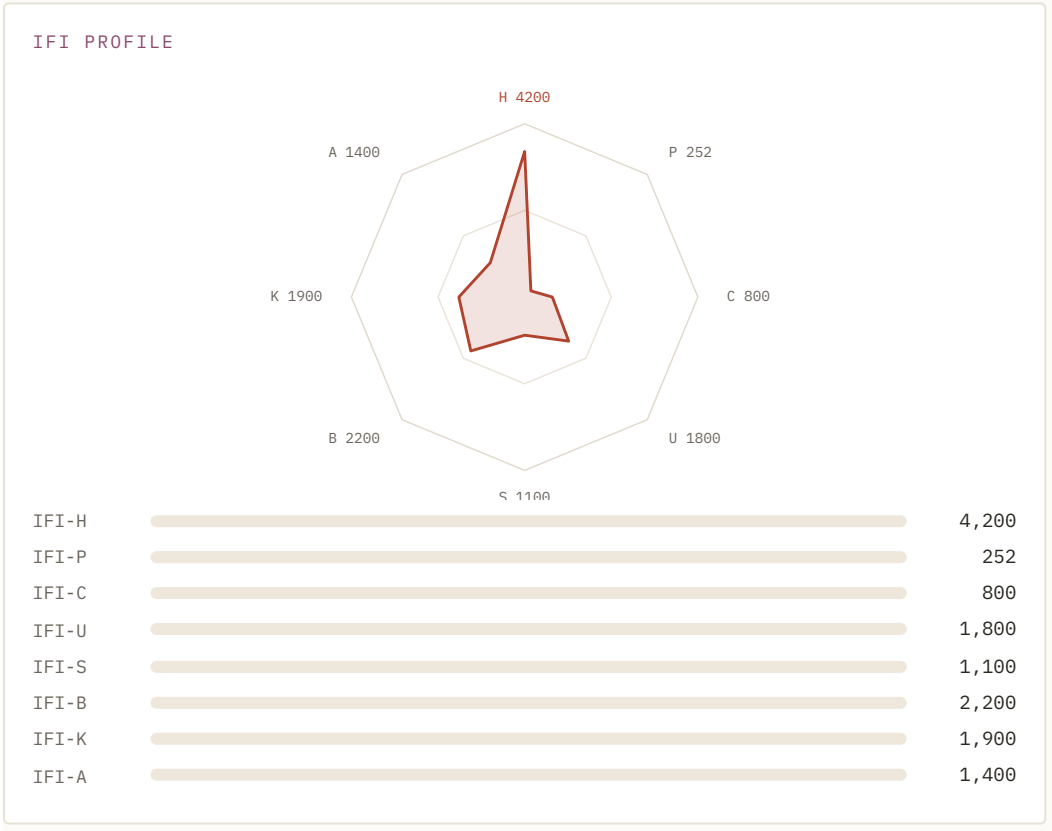
> $IFI\text{-}A(\text{candidate}) = 1{,}000 \times EC_{50}(\text{reference}) \div EC_{50}(\text{candidate})$

We anchor the reference at a defined tannin — a standardized grape-seed procyanidin fraction of mean DP 4 at fifteen percent galloylation — and fix its half-precipitation at **EC_{50}(reference) = 500 µM** (expressed in subunit-equivalents). By construction the reference scores itself: 1,000 × 500 ÷ 500 = **1,000**. Everything else is read against it. A tannin twice as potent as the reference — reaching half-precipitation at 250 µM — scores 1,000 × 500 ÷ 250 = **2,000**. The scorecard ceiling, the per-axis normalization maximum of 5,000, corresponds to EC_{50} = 1,000 × 500 ÷ 5,000 = **100 µM**: the most astringent stimulus the scale resolves is five times more potent than the reference. The whole axis is this one division, run forward or backward.

EC_{50} (mM, subunit-eq)	≈ mg/L (as EGCG)	IFI-A	Marker on the scale
2,000	917	250	weak monomer
1,000	458	500	light grip
500	229	1,000	**reference anchor**
357	164	1,400	**Phoenix** designed note
250	115	2,000	firm red-wine tannin
167	77	3,000	strong, galloylated
125	57	4,000	intense
100	46	5,000	scorecard ceiling

Worked the other way, the Index tells a designer the chemistry a target demands. Our full-spectrum flagship Phoenix (SS-0096) carries a deliberately restrained astringency — a designed note of **IFI-A 1,400**, enough to give the fruit a spine without letting the grip

dominate the other seven axes. Invert the formula to find the EC_{50} that note requires: EC_{50}(candidate) = 1,000 × EC_{50}(reference) ÷ IFI-A = 1,000 × 500 ÷ 1,400 = **357 µM**. So the brief handed to the pathway is exact — build a tannin that half-precipitates the standard PRP pool at 357 micromolar, a touch more potent than the reference, and no more. That is a number the four dials can hit.



The second-column conversion to mass concentration is a small, honest piece of arithmetic worth showing, because it also exposes a limit. A micromolar figure becomes a milligram-per-litre figure only once you assume a molecular weight for the subunit; taking the galloylated subunit EGCG (458.4 g/mol) as the yardstick, 100 µM × 458.4 ÷ 1,000 = 45.8 mg/L, and 357 µM works out to 164 mg/L — concentrations that sit sensibly inside the one-to-four grams per litre of total tannin a red wine carries. But a real tannin is *polydisperse*: it is a distribution of chain lengths with no single molecular weight, so the µM scale is only well defined against a declared reference subunit. We state ours. It is the kind of bookkeeping that keeps a pretty number from quietly lying.

The shape of the climb, and the honesty of the error bar

A single EC_{50} is the midpoint of a curve, and the *shape* of that curve is part of the sensation. Precipitation follows a sigmoidal dose–response that a Hill form describes well:

$$\theta(c) = c^n \div (EC_{50}{}^n + c^n)$$

where θ is the fraction of maximal protein precipitated and *n* is the Hill coefficient. Because tannin binding is multivalent and cross-linking is cooperative — each tannin that lands makes the next landing easier by pre-aggregating the protein — *n* runs above one, typically near 2 for a well-behaved condensed tannin. That cooperativity is why astringency has a *threshold* feel, a sense of almost nothing and then suddenly grip. With n = 2, the curve climbs from θ = 0.2 at half the EC_{50} ($0.5^2 \div (1 + 0.5^2) = 0.25 \div 1.25$) to θ = 0.5 at the EC_{50} by definition, to θ = 0.8 at twice the EC_{50} ($2^2 \div (1 + 2^2) = 4 \div 5$). The grip goes from barely-there to dominant across a

single doubling of concentration. A simple non-cooperative isotherm (n = 1) would spread that same rise over a fourfold change and feel gradual; the steepness is the signature, and it too can be tuned by how uniform the tannin is.

None of this would be designable without a tight error bar, which is the quiet hero of the whole program. Our constructs hold batch-to-batch variation to a coefficient of variation of **≤7%**, against the **42%** that is ordinary for a tannin left to a wild-type plant and a growing season. Walk the arithmetic of what that buys. Because IFI-A = k ÷ EC_{50} with k fixed, a fractional error in EC_{50} passes straight through to IFI-A one-for-one: the relative uncertainty in the score equals the relative uncertainty in the measured concentration. At our Phoenix note of EC_{50} = 357 µM, a 7% CV is ±25 µM, which propagates to IFI-A = 1,400 ± 98 — a band from roughly 1,300 to 1,500. Run eight replicates and the standard error shrinks by √8: 7% ÷ 2.83 ≈ 2.5%, or 1,400 ± 35. The rungs of the ladder — a hundred points apart, 1,400 to 1,500 — become genuinely distinct steps you can stand a product on. At the wild-type's 42%, that same EC_{50} carries ±150 µM, a score smeared from about 990 to 2,400; two cultivars a rung apart are indistinguishable, drowned in their own scatter. Precision is not fussiness here. It is the thing that converts a poetic axis into an engineering one — and converting poetry into engineering, without losing the poetry, is the whole of what our family set out to do.

Reading the pull across the garden

The dials and the number are general, which is the point — astringency is not a property of one plant but a channel any tannin-bearing organism can be tuned on. Across the live library, six host species carry astringency as a primary axis, each a different native chemistry and a different place to set the dials.

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	NATIVE TANNIN LEVER
Grape	1,610	B / A / M	Hard	ANR branch; procyanidin ↔ prodelphinidin balance
Tea	280	U / B / A	Hard	heavy galloylation (EGCG, ECG)
Pomegranate	120	A	Hard	hydrolysable tannins (ellagitannins)
Blueberry	120	A	Moderate	low-DP, anthocyanin-linked
Sorghum	120	A	Frontier	condensed tannins in the seed testa
Raspberry	40	A	Moderate	low-DP, bright, short-lived grip

Grape is the most worked tannin organism in the library at 1,610 constructs, and its chemistry is the condensed-tannin story in full — the ANR-governed flavan-3-ol pool strung into procyanidins and prodelphinidins, the dials all live at once. Tea is the galloylation exemplar: its astringency rides on epigallocatechin gallate and its relatives, the most heavily galloylated flavan-3-ols in common food, which is why strong green tea grips fast and sharp. Pomegranate works a different chemistry altogether — hydrolysable ellagitannins rather than condensed ones — and carries astringency as its lone

primary axis, a clean single note. The point of putting them on one scale is not to erase those differences but to make them comparable: the same EC_{50} assay, the same reference, the same formula, so that the galloylated sharpness of tea and the ellagitannin bite of pomegranate can be read against each other in a single number even though no two of their molecules are alike.



Tea in the garden — the leaf whose astringency rides on the most heavily galloylated flavan-3-ols in common food. Galloylation is the strongest single dial on protein affinity, which is why strong green tea grips fast and sharp.

What the number can and cannot promise

We watch the whole thing on instruments, which is how we know the friction account is literally true and not a convenient metaphor. An oral **tribometer** slides a soft, compliant surface — engineered to mimic the give of the tongue and palate — against a second surface across a thin film of saliva, and reports the friction coefficient μ as load and sliding speed vary. Add a tannin and μ climbs, and it climbs in a shape that matches what a human panel reports from the same sample: a potent galloylated tannin spikes the friction fast; a long, velvety tannin raises it smoothly; a weak one barely moves it. Plot μ against sliding speed — the **Stribeck curve** — and even the sub-qualities appear, because the loss of lubrication shows up most sharply in the speed regime where the protein film was bearing the most load. When the tribometer's μ, the calorimeter's binding constant, the precipitation assay's EC_{50}, and a trained panel's score all rank the same tannins the same way, astringency stops being a subjective report and becomes a measured physical quantity. That fourfold convergence is what earned astringency a place on an index at all.

Honesty requires naming where the number stops. IFI-A is a property of the *tannin*, measured in a defined buffer against a standard protein pool; the mouth adds a matrix the flask does not. Ethanol raises tannin solubility and softens precipitation, so the same tannin reads gentler in wine than in water. Sugars and the polysaccharides of a food — the arabinogalactans and mannoproteins a cell wall or a yeast lees contributes — can sheathe a tannin and blunt its grip competitively. Dietary protein pre-binds it: a tannin met with cream or cheese spends itself on the food before it reaches the saliva. And pH shifts the ionization of the phenols and with it the binding. None of this is a flaw in the axis; it is the reason the axis measures the

stimulus and leaves the plate to the cook. The score tells you what a tannin *can* do to a salivary film; the meal decides how much of that potential is spent.

There is one more layer, and we flag it honestly because the science is still settling. Alongside the pure friction story, some tannins appear to have a second, chemical voice: certain small polyphenols can lightly modulate trigeminal channels — TRPA1 among them, the same channel that reads pungency and the bite of mustard — adding a faint chemesthetic edge, a *sharpness*, on top of the mechanical drag. The two can be told apart in careful panels: the mechanical component scales with how much protein the tannin precipitates; the chemesthetic edge does not. IFI-A is anchored to the mechanical, precipitation-driven sensation, which is the dominant one and the one we design with confidence. Where a tannin also carries that sharper edge, it shows up where it should — as a whisper on IFI-P, the pungency axis — and the two numbers, each anchored to its own defined reference point, together describe the real thing in the mouth better than either alone. That is the premise of measuring flavor on eight axes rather than one: a sensation is a chord, and you can only design it once you can read every note. Astringency is the note most people have never been given a name for — the grip that is not a taste, the dryness carried by the wrong nerve, the plant's defense and the body's defense meeting on a film of saliva — and giving it a number is how we finally learned to write it into the chord on purpose. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 2 · The proteins that take the blow — *saliva, proline, and a mouth's first defense.*

To design astringency you must know the thing astringency attacks, and it is a protein so well-suited to being attacked that it looks, at first, like a mistake. Human saliva is roughly a quarter to a third protein by the dry weight of its non-mucous fraction, and of that protein the single largest share — in some glands close to **seventy percent** — belongs to the **proline-rich proteins**, the PRPs. They are made in the parotid and submandibular glands, poured constantly into the mouth, and they are, by the standards of most proteins, bizarre: long, floppy, open chains with no fixed fold, stuffed with proline, glycine, and glutamine, repeating the same short motifs over and over. A normal protein hides its backbone inside a compact globular fold. A PRP does the opposite. It holds its backbone *out*, exposed, in a loose left-handed coil called the **polyproline-II helix**, as if offering itself.

It is offering itself. The PRPs are, among other things, a sacrificial defense — the mouth's first line against dietary tannin. And astringency itself is worth defining precisely before we design it, because it is the one axis on the Index that is not, strictly, a taste. There is no single astringency receptor the way capsaicin has TRPV1 or glutamate has mGluR1. Astringency is a *mechanical* report: the felt roughening of a mouth that has lost its slip, read by the same trigeminal and mechanosensory nerve endings that tell you a surface is coarse. The chemistry happens first — tannin meets protein and the two fall out of solution — and only then does the body sense the consequence. The transduction event is a precipitation. To understand the stimulus, then, you have to look at the protein, and at the one amino acid that makes it so easy to find: proline.



A cluster of wine grapes, cut to show seed and skin. The tannin that waits here is built to find protein; the mouth answers with a protein built to be found. Astringency is the collision of the two.

Why proline — the chemistry of the landing strip

Proline is the odd amino acid. Its side chain loops back and bonds to its own backbone nitrogen, forming a rigid five-membered ring — a pyrrolidine — and that ring does two things that matter enormously to a tannin. First, it presents a flat, greasy, hydrophobic face, a little aromatic-friendly surface onto which the flat phenolic rings of a tannin can stack like tiles. Second, because the nitrogen is tied up in the ring, proline's peptide bond lacks the amide hydrogen that ordinary residues use to donate a hydrogen bond — but it keeps the carbonyl oxygen, a strong hydrogen-bond *acceptor*, fully available. A tannin is covered in phenolic hydroxyl groups, which are superb hydrogen-bond *donors*. Put the two together and you have a near-perfect complementarity: the tannin's hydroxyls donate hydrogen bonds to proline's exposed carbonyls, while the tannin's aromatic rings stack against proline's hydrophobic face.

The polyproline-II helix compounds the gift. It is an extended, three-residue-per-turn coil with no internal hydrogen bonding of its own — every backbone carbonyl points outward, unclaimed, into the solvent. Where a globular protein would spend those carbonyls on its own fold, the PP-II helix leaves them all on offer, spaced at a pitch that happens to match the reach of a tannin's phenolic arms. A proline-rich chain is, from a tannin's point of view, a landing strip repeated dozens of times down a single molecule — a runway with the lights left on.

Multivalency, or why weak bonds win

The binding is **multivalent**, and multivalency is the key to everything that follows. A single hydrogen bond or a single stacking contact is weak — easily made, easily broken. But a tannin carries many binding groups, and a PRP presents many proline sites, so the two fasten at several points at once. The individual contacts are weak; their sum is tenacious. More than that: because a tannin has multiple "hands," one tannin can grab two different protein molecules, and one protein can be grabbed by two different tannins, and very quickly the solution is knitting itself into a growing three-dimensional network. The network grows until it can no longer stay dissolved, and it separates out — first as a haze, then as visible aggregates, then as a precipitate. That phase separation, happening in the thin film on your tongue, is the drying you feel. The proteins that were lubricating your mouth are now curds on their way out of solution.

It is worth doing the arithmetic, because the arithmetic is where the design levers get their power. Binding free energy is logarithmic in affinity:

$$\Delta G = -RT \cdot \ln K$$

At mouth temperature — 37 °C, or 310 K — the thermal quantum RT equals 0.616 kcal·mol^{-1}. Take a single tannin–proline contact with an association constant of $K \approx 2{,}000\ M^{-1}$, about the strength of one good phenolic hydrogen bond backed by a stacking interaction:

$$\Delta G_1 = -0.616 \cdot \ln(2{,}000) = -0.616 \cdot 7.60 = \mathbf{-4.68\ kcal \cdot mol^{-1}}$$

Weak. A complex held by one such contact would fall apart as fast as it formed. Now suppose a single tannin and a single PRP can close **eight** such contacts at once — eight proline carbonyls, eight phenolic donors, within reach of one another. Free energy is additive, so in the naïve limit the contacts simply sum:

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ΔG₈ = 8 · (−4.68) = −37.5 kcal·mol⁻¹ → K = e^(37.5 / 0.616) ≈ 2.7 × 10²⁶ M⁻¹
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That is an avidity no single bond could approach — on paper, a complex that would essentially never let go. This is precisely why **galloylation** and **chain length** are the two strongest levers on the whole axis: each one adds contacts, and every added contact multiplies the avidity. A galloyl group brings three more hydroxyls and one more flat ring to the encounter; a longer polymer strings more proline-binding units onto one molecule, and lets one tannin bridge more proteins at once.

But a designer who believed that 10^{26} would be fooling herself, and we would rather show the honest number. Full additivity is an upper bound that real complexes never reach, for a reason worth stating plainly: the **first** contact pays a one-time entropic toll. Bringing two free-floating molecules into register costs their translational and rotational freedom — on the order of +10 to +15 kcal·mol^{-1}, an unfavorable "cost of meeting" sometimes expressed as an effective molarity. Once they are tethered, each *subsequent* contact is nearly free enthalpically, but still surrenders a kilocalorie or two of the backbone's own wag. Fold those penalties back in and the realistic avidity of an eight-contact tannin–PRP complex lands near $\Delta G \approx -8$ to -11 kcal·mol^{-1}, an association constant of 10^6 to 10^8 M^{-1} rather than 10^{26}. Still ferociously tight for something built of individually feeble bonds — tight enough to pull protein out of solution — but finite, reversible on the timescale of a rinse, and, most importantly, *tunable*. The gap between the paper number and the literature value is not an embarrassment; it is the headroom the whole design program lives in.

The thermodynamic signature — reading the pucker off a calorimeter

These energies are characterized by isothermal titration calorimetry. Drip a tannin, microliter by microliter, into a stirred cell of standardized PRP inside an **isothermal titration calorimeter**, and every binding event releases a little heat the instrument records. The shape of that heat-versus-ratio curve hands us three numbers at once: the affinity K, the stoichiometry *n* (how many tannins settle onto each protein before it is saturated), and the binding enthalpy ΔH. The entropy follows by difference, because the three are bound by one equation:

$$\Delta G = \Delta H - T\Delta S$$

A representative galloylated tannin binding basic PRP reports an enthalpy near $\Delta H = -7.0$ kcal·mol^{-1} and a small, *favorable* entropy term, $T\Delta S \approx +1.5$ kcal·mol^{-1}. The free energy is their sum:

$$\Delta G = \Delta H - T\Delta S = (-7.0) - (+1.5) = \mathbf{-8.5\ kcal \cdot mol^{-1}} \rightarrow K \approx 1 \times 10^{6}\ M^{-1}$$

Enthalpy carries 7.0 of the 8.5 — about **82 percent** — which is the unmistakable thermodynamic fingerprint of a hydrogen-bond network: the pucker is, at bottom, a great many O–H···O=C bonds forming at once. The smaller favorable entropy is the hydrophobic stacking paying off, as the flat phenolic rings press against the greasy proline faces and squeeze out the ordered shell of water that both surfaces had been holding. Released water is disordered water, and disorder is entropy gained. So the two forces that bind tannin to protein leave two distinct signatures on the calorimeter — a deep enthalpic well and a shallow entropic lift — and we can read each to a fraction of a kilocalorie. The pucker, in other words, has a free energy. We treat that free energy as the raw material of everything downstream.

The PRPs come in families — the mouth's defense is tunable, too

The PRPs come in three families, and the division is a gift to the designer because it means the mouth's defense is itself tunable by diet and genetics, which tells us exactly how our engineered tannins will be met.

SALIVARY PROTEIN	CHARACTER	ROLE AGAINST TANNIN
Acidic PRPs (*PRH1/PRH2*)	Highly charged, bind calcium	Protect tooth enamel; secondary tannin binding
Basic PRPs (*PRB1–4*)	Proline-rich, open coil	**Primary tannin scavengers** — high-affinity sacrifice
Glycosylated PRPs	Sugar-decorated	Lubrication and viscosity; tannin binding with bulk
Histatins	Small, histidine-rich	Fast, high-affinity tannin binding; antimicrobial
Statherin / mucins (MUC5B, MUC7)	Lubricating, film-forming	The lubrication that tannin strips away

The **basic PRPs** and the **histatins** are the sacrificial front rank. They have the highest affinity for tannin, and the body appears to make them *for* this purpose — a pre-emptive supply of protein whose job is to meet the tannin first and precipitate in place of the proteins you cannot spare. Populations and individuals whose diets are rich in tannin tend to carry more of these proteins, and animals that browse heavily tannic forage carry astonishing quantities of them. It is one of biology's quiet elegances: the plant builds tannin to defend its seed, and the animal builds proline-rich protein to pay the tannin off at the door. Astringency is the felt moment of that ancient transaction — the plant's defense and the body's answer, meeting on the surface of the tongue.

The index built on the protein — what EC50 means when the receptor is a sacrifice

Every axis on the Iyengar Flavor Index reduces to one idea: measure how *loudly* a stimulus speaks to its receptor, and score it against a fixed reference.

$$\mathrm{IFI}(\text{candidate}) = 1{,}000 \times \mathrm{EC}_{50}(\text{reference}) \div \mathrm{EC}_{50}(\text{candidate})$$

EC_{50} is a half-maximal concentration — on most axes, the amount of stimulus that drives a receptor to half of its full electrical response. Astringency is the axis where that definition has to be translated, because the "response" is not a current across a channel; it is protein falling out of solution. So for IFI-A we define EC_{50} as the **tannin concentration that drives standardized basic-PRP precipitation to half of its maximal plateau** — the half-clouding point of a defined protein pool, read turbidimetrically or by pelleting and assaying the precipitable fraction, at 37 °C and physiological ionic strength.

The precipitation curve is a sigmoid in log-concentration, and it is steeper than a simple binding isotherm because the cross-linking is cooperative — once a network nucleates, it recruits. A Hill form captures it:

$$\theta = [T]^h \,/\, (\mathrm{EC}_{50}{}^h + [T]^h)$$

where θ is the fraction of maximal precipitation and h is the Hill coefficient, the steepness. With $h \approx 2$, the span from a quarter-grip to near-saturation is startlingly tight. At half the EC_{50}, $\theta = (0.5^2)/(1 + 0.5^2) = 0.20$; at the EC_{50}, $\theta = 0.50$ by definition; at twice the EC_{50}, $\theta = (2^2)/(1 + 2^2) = 0.80$. A mere fourfold rise in tannin carries the mouth from barely gripping to fully drying — which is exactly the lived experience of a wine that is soft on the first sip and tightening by the third. The cooperativity is the ratchet, and the Index's job is to pin the midpoint of that ratchet with a single number.

A worked example — scoring Phoenix on IFI-A

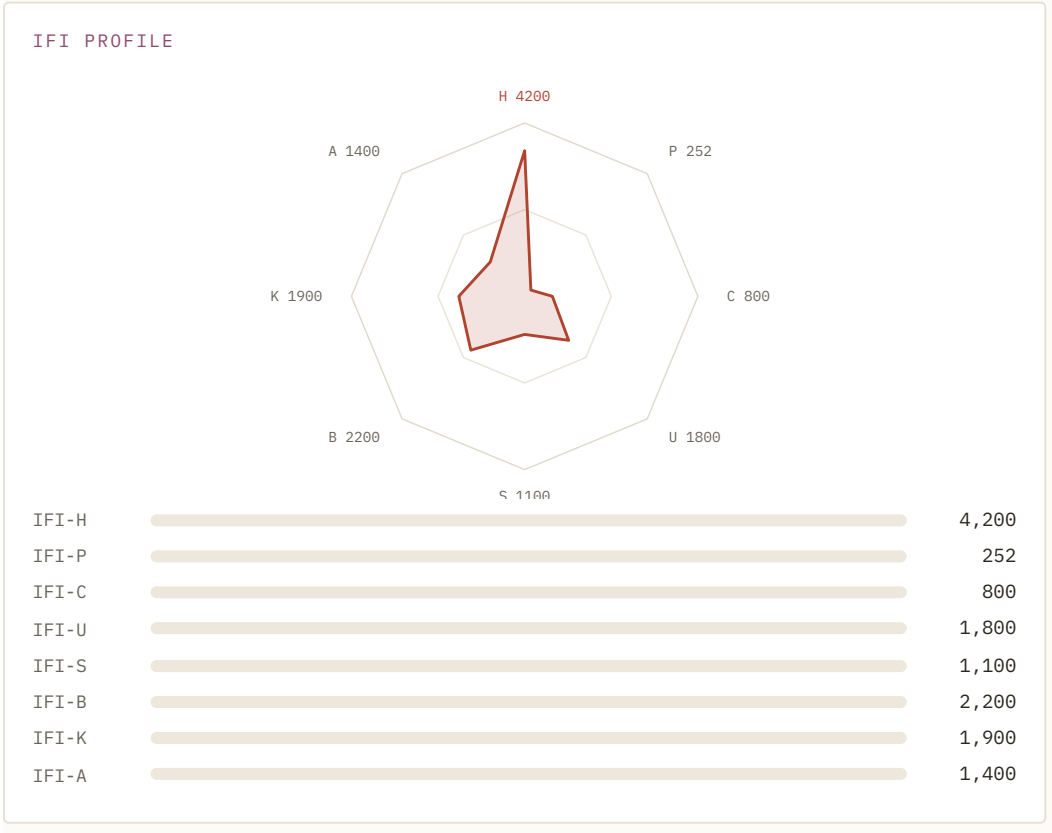
We anchor the astringency scale by fixing the reference. Let the standardized reference tannin half-precipitate the standard basic-PRP pool at **EC_{50}(reference) = 50 µM**; by construction that gives it IFI-A = 1,000. Every other tannin is read against it. Because the formula is a simple ratio, the whole scale collapses to one convenient identity:

$$\mathrm{EC}_{50}(\text{candidate}) = 1{,}000 \times 50\ \mu\mathrm{M} \div \text{IFI-A} = \mathbf{50{,}000 \div \text{IFI-A}}\ (\mu\mathrm{M})$$

So the score and the concentration are reciprocals around the anchor, and the arithmetic is instant. Take the one cultivar we have designed to register on all eight axes at once — **Phoenix** (SS-0096), whose astringency we set, deliberately, at **IFI-A 1,400**. Even a flagship bred for heat carries a designed, predicted tannin grip; astringency on this platform is an independent dial, not a side effect. Its predicted half-precipitation concentration is:

$$\mathrm{EC}_{50}(\text{Phoenix}) = 50{,}000 \div 1{,}400 = \mathbf{35.7\ \mu M}$$

Our model predicts Phoenix's tannin is 1.4× as potent a protein-precipitant as the reference — designed to cloud the standard PRP pool at 35.7 µM where the reference needs 50 µM. The full profile, for the scorecard:



Run the identity across the usable scale and you get the astringency calibration ladder — the dictionary that turns any IFI-A into a concentration and a felt character. Only Phoenix (1,400) and the reference (1,000) and the scorecard ceiling (5,000) are fixed points; the rest are the scale's own gradations:

IFI-A	RELATIVE POTENCY	EC_{50} VS STANDARDIZED BASIC PRP	FELT GRIP
250	0.25×	200 µM	barely perceptible
500	0.50×	100 µM	soft, round
1,000 (reference)	1.00×	50 µM	the anchor
1,400 (Phoenix · SS-0096)	1.40×	35.7 µM	firm, structured
2,000	2.00×	25 µM	gripping
3,500	3.50×	14.3 µM	drying, tight
5,000 (scorecard max)	5.00×	10 µM	the normalization ceiling

From micromolar to the glass — the unit problem a polymer creates

A micromolar figure is exact only if you can say "micromoles of *what*," and tannins make that harder than most stimuli, because they are not one molecule but a distribution. Condensed tannins are polymers of flavan-3-ol units — epicatechin and catechin, each about 290 $g \cdot mol^{-1}$ — strung by interflavan bonds, and a given extract spans trimers to twenty-mers. To convert a molar EC_{50} into the milligrams-per-liter a winemaker actually measures, you need a mean molar mass:

$$C\ (mg \cdot L^{-1}) = EC_{50}\ (\mu M) \times \bar{M}\ (g \cdot mol^{-1}) \div 1{,}000$$

A reference tannin of mean degree of polymerization four weighs about $\bar{M} \approx 1{,}150$ g·mol^{-1} (one flavan-3-ol plus three more at ~288 apiece, each interflavan bond shedding two hydrogens). So the reference EC_{50} of 50 µM is 50 × 1,150 ÷ 1,000 ≈ **57.5 mg·L^{-1}**, and Phoenix's predicted 35.7 µM is ≈ **41 mg·L^{-1}** — both comfortably below the few hundred to few thousand milligrams per liter of total tannin in a structured red. The honest subtlety is the polydispersity: report a number against an undefined extract and it drifts with every harvest. So the reference material's **mean degree of polymerization is fixed as part of the assay definition**, and candidate tannins are expressed per defined reference polymer. We would rather carry the extra definition than publish a number that cannot be reproduced.

The two strongest levers — galloylation and chain length

Everything the calorimeter and the dose–response curve tell us converges on a single design principle: **add contacts, lower the EC_{50}, raise the score.** Two molecular changes add contacts faster than any other, and they are where we spend our engineering.

The first is **galloylation** — esterifying a flavan-3-ol with gallic acid, turning epicatechin into epicatechin gallate. Each galloyl group is a small windfall of binding chemistry: three new phenolic hydroxyls (three more hydrogen-bond donors) and one more flat aromatic ring (one more stacking face), bolted onto a unit that was already binding. This is why tea astringency behaves the way it does — green tea's signature catechin, epigallocatechin gallate, is doubly decorated, and gram for gram it grips protein far harder than an ungalloylated monomer. As a rough design sensitivity, pushing a tannin's galloylation from essentially zero to about a third of its subunits roughly halves the EC_{50} — which, by the reciprocal identity, roughly *doubles* the IFI-A.



Camellia sinensis — the tea leaf, where galloylated catechins make astringency a defining virtue rather than a flaw. The same gallate groups that grip salivary protein are the ones we add, and subtract, by design.

The second lever is **degree of polymerization** — chain length itself. A longer tannin carries more proline-binding units on one molecule, so it closes more contacts per encounter *and* reaches farther, bridging more protein molecules into the growing network. Lengthen the mean chain and the EC_{50} falls steeply. In grape, we build and lengthen these chains through the condensed-tannin route the plant already owns, tuning flux through **ANR** — anthocyanidin reductase, the enzyme that makes the 2,3-*cis* epicatechin units that are the dominant building block of grape proanthocyanidin. Favor the *cis* unit

and the chains pack into the polyproline register a little more snugly; set the polymerization and you set the chain length; esterify and you set the galloylation. Three knobs, one axis.

LEVER	MOLECULAR CHANGE	DIRECTION ON EC_{50}	DIRECTION ON IFI-A
Galloylation (0 → ~30%)	+3 OH, +1 ring per galloyl	≈ ÷ 2	≈ × 2
Degree of polymerization (DP 3 → 8)	more proline sites; more bridges	≈ ÷ 3	≈ × 3
2,3-*cis* (epicatechin, via ANR)	tighter PP-II registry	≈ ÷ 1.3	≈ × 1.3
Excess DP (> ~12)	self-aggregation, solubility loss	**EC_{50} ↑ (reverses)**	**↓**

The multipliers are design sensitivities — the direction and rough magnitude of each knob, not a claim of exact values — and the last row is the point where the physics turns on the designer, which is the next thing to be honest about.

Edge cases and failure modes — where a tannin stops behaving

More is not monotonically more. Push the degree of polymerization too far and two things go wrong. A very long tannin begins to **fold on itself and aggregate with its own kind** before it ever reaches a protein, so the effective, protein-available tannin drops and the line reads *lower* on IFI-A than its total phenolic content would predict — an "over-tannined" construct that measures soft because half its tannin precipitated in the bottle. And a very long, rigid chain loses the conformational nimbleness it needs to wrap a floppy PRP, so each contact costs more backbone entropy to form. The curve that fell steeply with chain length flattens, then turns up. Our design window lives on the near side of that reversal.

The assay has honest limits of its own, and we would rather state them than let a tidy number overpromise:

- **pH.** Astringency climbs as pH falls — more fully protonated phenols are better donors, and the colloidal balance of the protein shifts toward precipitation. A wine at pH 3.4 is a harsher matrix than the assay's physiological buffer, so the standardized EC_{50} ranks tannins faithfully but does not transplant, unchanged, into every glass.
- **Ethanol.** Alcohol competes for the hydrophobic stacking, prying tannin off the greasy proline faces, which is a large part of why a riper, higher-alcohol red tastes softer than its tannin load alone would suggest. Change the ethanol matrix and the effective EC_{50} moves even though the molecule has not.
- **Polysaccharides and mannoproteins.** Sugars and the mannoproteins a yeast sheds can sleeve a tannin or compete for it, masking grip. The flask sees the bare interaction; the finished food may wrap it.
- **Carryover — the ratchet the flask cannot see.** A single-shot EC_{50} measures one encounter against a fresh, full protein pool. A real mouth depletes its sacrificial PRPs sip over sip, so astringency *builds* across a tasting in a way a one-point assay never captures. This is a true limitation, and we name it rather than paper over it.

Why the reference is the receptor

For us, none of this is a curiosity; it is the ground truth our whole measurement rests on. When we score a tannin's IFI-A, the protein pool we precipitate it against is a standardized preparation of **basic PRP** — the same high-affinity scavenger that meets the tannin first in a real mouth, held at fixed concentration, fixed ionic strength, physiological pH, and 37 °C. We chose it deliberately. Score a tannin against serum albumin or gelatin, as older tannin assays did, and you get a number that correlates only loosely with what a person feels. Score it against the very protein the mouth sends to the front, and the flask begins to tell the truth about the tongue. The reference is the receptor.



The measurement bench — a standardized basic-PRP preparation is where a tannin's grip stops being a matter of opinion and becomes a concentration we can read, batch after batch, to a few percent.

Reproducibility is not an afterthought on a scale meant to be designed against; it is the whole point. We fit each EC_{50} from a four-parameter logistic regression over a full dilution series, and we hold the platform's batch-to-batch coefficient of variation to **≤ 7%**. Because IFI-A is a ratio of two fitted concentrations, its uncertainty is the two uncertainties added in quadrature:

$$\sigma(\text{IFI-A})/\text{IFI-A} = \sqrt{\left[(\sigma_{ref}/EC_{50,ref})^2 + (\sigma_{cand}/EC_{50,cand})^2 \right]}$$

With a reference fit good to 3% and a candidate fit good to 5%, the score carries $\sqrt{(0.03^2 + 0.05^2)} = \sqrt{0.0034} =$ **5.8%** — so a design targeting IFI-A 1,400 would carry ±81, inside the platform's seven-percent envelope. A tannin whose true score differs from another by less than that is, honestly, not yet distinguishable, and we say so rather than dress a rounding error as a result. Numbers earn trust by carrying their error bars in the open.

The adaptive mouth, and the right thing to leave unmeasured

The defense is *adaptive*, and that is the final reason it matters to the designer. The basic-PRP genes sit in a cluster on the genome that varies in copy number from person to person, and their expression responds to diet: a mouth that meets tannin regularly makes more of the sacrificial protein, so that the habitual tea drinker and the daily red-wine drinker are, at the level of their salivary proteome, slightly armored against the very grip they have learned to love. Flow matters as much as protein content — a fast-secreting mouth replaces precipitated lubricant within a minute or two and recovers

between sips, while a slower, drier mouth lets the grip ratchet down sip over sip with nowhere to climb back to. This is the biological root of the *personal* quality of astringency, and it is why two honest, careful tasters can disagree about the same wine's tannin without either being wrong.

Far from blurring the case for measurement, it sharpens it. The one fixed quantity in the entire transaction is the tannin's own precipitating potency — the thing it does to a standardized protein in a flask, regardless of whose mouth it will later enter. That is the quantity IFI-A pins down, and it is why the axis describes the *stimulus* and leaves the *sensation*, rightly, to the person doing the tasting. The score predicts rank order and relative grip with real fidelity; it does not pretend to forecast any single mouth's absolute experience, because the receptor pool on the other side is variable, adaptive, and, pleasingly, each person's own. That is not a weakness of the number. It is the number knowing its place.

So the plant builds tannin to defend its seed, folding phenolic rings around the next generation; and the body builds proline-rich protein to pay the tannin off at the door, pouring out a sacrifice it can afford so the lubrication it cannot afford is spared. Astringency is the felt moment of that exchange — ancient, mutual, and, as of now, computable to a few percent. Having met the protein that takes the blow, and learned to read the pucker as a free energy and a concentration, we are ready to meet the tannins themselves: the molecules the plant designed to find a mouth, and the ones we have learned to design right back. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 3 · Two ways to build a tannin — *the hydrolyzable and the condensed.*

"Tannin" is a job, not a molecule. The word names any plant polyphenol big enough and hydroxyl-rich enough to precipitate protein — originally, literally, the compounds that tanned hide into leather by cross-linking its collagen, the same way they cross-link the proteins in your saliva. A tannin is a defense: the plant builds it to make its own tissue unpalatable, to bind and disable the digestive enzymes of anything that bites, to foul the proteins of the fungi and bacteria that would rot it. That the same molecule becomes a pleasure in a glass of wine or a cup of tea is one of the quiet graces of eating — we have learned to court a plant's armament and call the sensation beautiful. We write about it with reverence because it is, at bottom, a conversation between a plant protecting itself and a body meeting that protection halfway.

Every tannin on earth belongs to one of two great families, built by two entirely different chemistries, and the split matters to us because the two families grip differently, taste differently, and — crucially — are made by different enzymes, which means they are tuned by different genes. Design begins with knowing which family you are holding. So before any arithmetic, two questions: how does a tannin actually grip, and how are the two families assembled? Answer those exactly, and the number we put on astringency stops being a verdict and becomes a blueprint.

The grip, mechanically — *what the tongue is actually reporting.*

Astringency is the one axis that is not, strictly, a taste. Sweetness, umami, bitterness — each is a molecule settling into a receptor's binding pocket and opening a channel. Astringency is a demolition. The sensation we call the pucker, the drag, the chalk, the grip is the mechanical report of a protein film collapsing inside your mouth, and following it step by step is the whole of the mechanism.

Saliva is not just water. Suspended in it is a population of **proline-rich proteins** — the PRPs, the receptor of record for this axis — along with their cousins the histatins and statherin. The basic and glycosylated PRPs are strange proteins: intrinsically disordered, carrying no folded shape, up to a quarter of their residues the single amino acid **proline**, strung out as open, floppy chains. Their job in the resting mouth is to coat the mucosa and the enamel in a thin, slick, lubricating film — the reason a healthy mouth feels smooth and wet and frictionless.

Proline is the key, and it is a key the plant can read. Its side chain loops back and bonds to its own backbone nitrogen, forming a rigid five-membered pyrrolidine ring that juts out of the chain as a small, flat, hydrophobic plate. A tannin's aromatic rings — the galloyl groups, the flavan A- and B-rings — are flat hydrophobic plates of exactly the complementary kind. They stack face-to-face against the

proline rings, a **hydrophobic π-stacking** contact, while the tannin's many phenolic hydroxyls reach over and donate **hydrogen bonds** to the exposed backbone carbonyls that proline, uniquely, leaves bare. Stacking plus hydrogen bonding, repeated along the open chain: that is one tannin gripping one PRP.

Now the multivalence does its work. A tannin is not one hydroxyl and one ring but many — a dozen hydroxyls, several stacking faces on a single molecule — so one tannin binds several PRPs at once, and one PRP, long and open, accepts several tannins. The result is not a complex but a lattice. Protein and polyphenol cross-link into aggregates; the aggregates grow past the point of solubility and fall out of suspension — they **precipitate**. The slick film is stripped from the surfaces it was lubricating, and where there was a cushion of water-bound protein there is now bare, dry, grabbing tissue. Your tongue drags against your palate. Free nerve endings of the trigeminal system read that rise in friction as a tactile event, and the brain, having no better word, files it as a taste. The grip is real; it is just made of friction, not of flavor.

Two consequences fall out of this picture and we will use both. First, because the PRPs are **sacrificial** — the body spends them to bind tannin so that the enzymes of digestion are spared — astringency is dose-building and self-renewing: a first sip precipitates the PRPs present, the next sip meets a mouth not yet fully resupplied, and the grip climbs across a tasting. Second, because the binding is cooperative and multivalent, the dose–response is steep: below a threshold almost nothing happens, and then, across a narrow band of concentration, the film goes. That steepness is what we will have to put a number on.

Hydrolyzable tannins — *built on a sugar.*

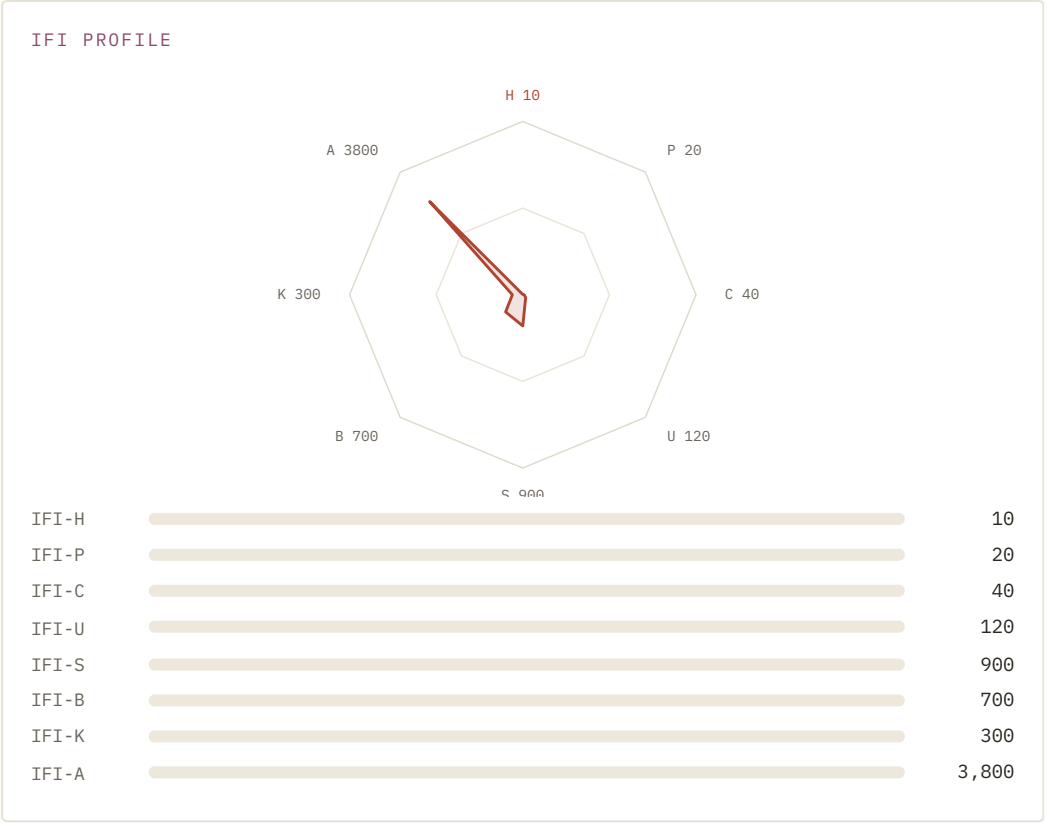


Pomegranate, opened — hundreds of arils around a bitter pith. The pith and rind are among the most astringent tissues any fruit grows, and the molecule responsible, punicalagin, is a hydrolyzable tannin of the first rank.

Hydrolyzable tannins are built on a sugar. Take a glucose, and instead of using its hydroxyls to link to other sugars, esterify each one with **gallic acid** — a small, three-hydroxyl phenolic acid the plant draws off the **shikimate pathway**, the same seven-step aromatic-building route that makes the plant's phenylalanine and tyrosine. Gallic acid branches off that trunk as 3-dehydroshikimate is oxidized and aromatized, and a dedicated **galloyltransferase** then loads it, one gallate at a time, onto the hydroxyls of a glucose through the

activated donor β-glucogallin. One gallate gives a monogalloyl glucose; five gallates, saturating all the available positions, gives **pentagalloyl glucose**, PGG — the archetype and one of the most studied astringents in all of food science.

PGG is where the family forks. Esterify still more gallate onto the galloyl groups already present and you climb the **gallotannins**, loose sprays of gallic acid on a sugar core. Or let two neighboring galloyl groups, held close on the glucose, undergo an oxidative carbon–carbon coupling into the larger **hexahydroxydiphenoyl** unit — HHDP, which, once cleaved free, closes up into **ellagic acid** — and you cross into the **ellagitannins**, the largest and most potent of the hydrolyzable tannins. These are called hydrolyzable because the links to the sugar are **ester bonds**, and an ester is a bond that acid, heat, or an esterase can cleave: push one with a little acid and warmth and it breaks cleanly back down into gallic or ellagic acid and glucose. They are the tannins of **pomegranate**, whose signature **punicalagin** is a giant ellagitannin and the molecule that makes the pith grip like a closing door; of **raspberry** and **blackberry**, whose **sanguiin H-6** and **lambertianin C** are among the largest small-molecules the fruit world builds; and of oak, which is why a wine aged in a new barrel takes up the barrel's **vescalagin** and **castalagin** and, with them, a firmer spine. A hydrolyzable tannin tends to grip fast and bright — high galloyl and HHDP density, very high affinity for protein, a pucker that arrives almost at once.



That profile is a whole pomegranate read on the eight axes, and it says in numbers what the cut fruit says in the hand: a little sweet from the arils, a little bitter from the membrane, and then astringency towering over everything — A near the top of the band, carried almost entirely by punicalagin in the pith and rind. It is an astringent that happens to be a fruit, the plainest possible teaching case for the hydrolyzable family.

Condensed tannins — *built on themselves.*



Grape, cross-sectioned — skin, pulp, and the seeds at the center. The condensed tannins live in the skin, the seed coat, and the stem; the pulp is nearly free of them, which is why a gentle press tastes softer than a hard one.

Condensed tannins are built on themselves. Their unit is the **flavan-3-ol** — catechin, epicatechin, and their relatives — a two-ring-plus-a-pyran flavonoid the plant makes down the **flavonoid branch** of phenylpropanoid metabolism: phenylalanine out of shikimate, then PAL → C4H → 4CL to the activated cinnamic acids, then chalcone synthase builds the flavonoid skeleton, and the terminal enzymes **leucoanthocyanidin reductase (LAR)** and **anthocyanidin reductase (ANR)** deliver the two flavan-3-ol stereochemistries. Link those units carbon to carbon, into chains and branches, and you get a **proanthocyanidin**, so named because heating one in acid snaps the links and spits out colored anthocyanidins. These do not hydrolyze gently; the bonds are **carbon–carbon**, not ester, and they hold. Condensed tannins are the tannins of the **grape** — skin, seed, and stem, never the pulp — of **tea** as its catechins, of **blueberry**, of **sorghum**, of cacao, apple, and cranberry. Most of the everyday astringency in a kitchen is condensed tannin, and the family carries its own vocabulary, each term a design dial:

- **Mean degree of polymerization (mDP)** — the average chain length. A dimer grips gently; a six- to eight-unit polymer grips hard; past roughly a dozen units the molecule grows too large and too insoluble to bind efficiently, and the grip plateaus and then fades.
- **Galloylation** — whether the flavan-3-ol units carry a gallate ester, as in epicatechin-3-gallate (ECG) and epigallocatechin-3-gallate (EGCG). This is the single strongest lever on astringency: a galloylated unit grips far harder than a bare one, because the extra gallate ring is three more hydroxyls and one more stacking surface.
- **Prodelphinidin fraction** — whether the B-ring carries two hydroxyls (procyanidin, from catechin and epicatechin) or three (prodelphinidin, from gallocatechin and epigallocatechin). The trihydroxylated prodelphinidins are felt as *softer*, more velvety; the dihydroxy procyanidins as *coarser*, greener, more angular.
- **Linkage** — most units join C4→C8 by a single carbon–carbon bond, the open, flexible **B-type**; some add a second ether bridge to make the rigid **A-type** that dominates in blueberry and cranberry, changing both solubility and grip.

Flexibility helps a tannin drape itself around a protein and cross-link it; rigidity can hinder that wrapping, or, in the right geometry, present a pre-shaped binding face. Two tannins of identical size and identical galloylation can still grip differently if one is open B-type and the other locked A-type — a quieter dial that nonetheless separates tannins a blunt analysis would call identical, and part of what lets us tune not the magnitude of a grip but its *character*.

The six molecules to hold in mind.

A handful of named molecules anchor the whole field and recur wherever astringency is discussed, so it is worth meeting them once. **Tannic acid** and **pentagalloyl glucose (PGG)** are the model gallotannins — PGG especially is the compound against which protein-precipitation assays were first calibrated, and it sits near the very top of the astringency scale. **Punicalagin**, **vescalagin**, and **castalagin** are the great ellagitannins — of pomegranate, and of the oak a barrel lends to wine — formed when adjacent galloyl groups oxidatively couple into the larger HHDP unit. **Procyanidin B2**, an epicatechin dimer, and **procyanidin C1**, a trimer, are the archetypal small condensed tannins, the first two rungs of the polymerization ladder. And **EGCG** — epigallocatechin-3-gallate — is the single most important astringent monomer in the eating world, the dominant catechin of green tea, carrying both the trihydroxy B-ring and the gallate ester that make it grip hard. Hold these six and the plants become a tour of where each is built, and how we move it up or down.

Measuring their structure is itself a solved problem, and we never guess at it. A controlled acid cleavage in the presence of **phloroglucinol** — the method the trade calls phloroglucinolysis, or thiolysis when a sulfur nucleophile stands in — snaps the polymers at their interflavan bonds into single units, which an HPLC then counts, handing back the exact mean chain length, the galloylation fraction, the prodelphinidin share, and the linkage pattern. We disassemble the tannin and read the parts, and those parts are the inputs the potency model runs on.

Putting a number on the grip — *the astringency index.*



Tea, the living leaf — the plant that packs more galloylated catechin into an edible tissue than almost any other, and the reason a strong steep grips the way it does.

A sensory world this rich needs one number, and the number is a potency, not a mass. Two grams of a weak tannin can grip less than a tenth of a gram of a fierce one, so weighing the tannin tells us almost nothing; what we want is how *hard* a given tannin pulls. We define that against the receptor the way every axis is defined — through a half-maximal concentration — and then invert and scale it exactly as the rest of the Index does:

> **IFI-A(c) = 1,000 × EC_{50}(reference) ÷ EC_{50}(candidate).**

For astringency, EC_{50} is the concentration at which a compound precipitates **half** of a standard proline-rich protein in a controlled turbidimetric assay — the point at which half the lubricating film has been thrown out of solution. A fierce astringent reaches that point at low concentration and has a *small* EC_{50}; a weak one needs a flood of itself and has a *large* one. Inverting puts the fierce one on top, where intuition wants it. We anchor the scale to a single reference astringent, the bare flavan-3-ol unit **(−)-epicatechin**, assigned an EC_{50} of 1,000 µM so that **IFI-A = 1,000** marks the pull of one undecorated unit. The whole relation then collapses to a clean inverse:

> **IFI-A(c) = 1,000,000 ÷ EC_{50}(c)** (EC_{50} in µM)

Everything more structured than a bare unit lands above 1,000; a thin, barely-tannic tea lands well below it. The scorecard caps each axis at **5,000**, the normalization maximum, so the most ferocious astringents in nature press against the ceiling rather than running off the page. Working the formula across the six anchor molecules and their near relatives gives the ladder every later judgment rests on:

COMPOUND	FAMILY	MW (G/MOL)	EC_{50} (MM)	EC_{50} (MG/L)	IFI-A
(−)-Epicatechin · EC	condensed, DP1 (reference)	290.3	1,000	290	1,000
(−)-Epigallocatechin · EGC	condensed, tri-OH monomer	306.3	820	251	1,220
Procyanidin B2	condensed, DP2	578.5	500	289	2,000
Epicatechin-3-gallate · ECG	galloylated monomer	442.4	410	181	2,440
Procyanidin C1	condensed, DP3	866.8	360	312	2,780
Epigallocatechin-3-gallate · EGCG	galloylated, tri-OH	458.4	345	158	2,900
Pentagalloyl glucose · PGG	hydrolyzable, gallotannin	940.7	205	193	4,880
Punicalagin	hydrolyzable, ellagitannin	1,084.7	200	217	5,000 (capped)

Two worked lines show the arithmetic plainly. EGCG precipitates half the reference protein at 345 µM, so IFI-A = 1,000,000 ÷ 345 = **2,900** — nearly three times the grip of a bare unit, which is exactly why a strong green tea puckers and a dish of plain catechin would not. Punicalagin reaches half-precipitation at 200 µM, so IFI-A = 1,000,000 ÷ 200 = **5,000**, sitting on the ceiling: pomegranate's pith is, molecule for molecule, about as astringent as food gets. The mg/L column is only a unit conversion — EC_{50}(mg/L) = EC_{50}(µM) × MW ÷

1,000 — but it earns its place, because an assay reads out in mass and a formulator buys in mass. EGCG's 345 µM is 345 × 458.4 ÷ 1,000 ≈ **158 mg/L**; the same pull, stated the way a bench actually meets it.

The number marks the *half* point, but astringency does not arrive gently on either side of it. Because the grip is cooperative — one precipitation event seeds the next — the dose–response follows a steep Hill curve,

$$f(c) = (c/EC_{50})^h \div [1 + (c/EC_{50})^h],$$

with a Hill coefficient h of roughly 1.8 for a galloylated catechin. For EGCG (EC_{50} 345 µM, h ≈ 1.8): at half the EC_{50}, 172 µM, f = $0.5^{1.8}$ ÷ (1 + $0.5^{1.8}$) = 0.287 ÷ 1.287 = **0.22**, only a fifth of full grip; at the EC_{50} itself, **0.50** by definition; at twice the EC_{50}, 690 µM, f = $2^{1.8}$ ÷ (1 + $2^{1.8}$) = 3.48 ÷ 4.48 = **0.78**. A fourfold swing in concentration carries the mouth from barely a whisper to nearly full pucker. That steepness is not a measurement nuisance — it is the felt sensation itself, the reason astringency seems to *switch on* rather than fade up, and the reason a cultivar's EC_{50} has to be placed precisely rather than approximately.

The levers, as arithmetic.

Structure sets the EC_{50}, and each structural dial moves it by a factor we can read straight off the ladder — which is what turns the chemistry into design. **Galloylation** is the strongest. Compare a bare unit to its gallate ester: epicatechin's 1,000 µM against ECG's 410 µM is a 2.44× gain in potency; epigallocatechin's 820 µM against EGCG's 345 µM is 2.38×. One gallate ester, added to a flavan-3-ol, multiplies its grip by about **2.4** — three new hydroxyls and a fresh stacking face, each paying out. **The trihydroxy B-ring** is a gentler lever: epicatechin to epigallocatechin is 1,000 ÷ 820 = 1.22×, ECG to EGCG is 410 ÷ 345 = 1.19×, so a prodelphinidin B-ring lifts potency by about **1.2** and, more to the point, softens the grip from angular to velvety — a change of character as much as magnitude.

Polymerization gives the richest curve. Going DP1 → DP2 → DP3, epicatechin's IFI-A climbs 1,000 → 2,000 (procyanidin B2) → 2,780 (procyanidin C1): the first added unit doubles the grip, the second adds only 39% more. Fit it and potency rises roughly as DP raised to a power that starts near 1 and falls toward 0.8 — the signature of **multivalency with diminishing returns**, each new binding site slightly less reachable than the last. The curve keeps climbing to an optimum around DP 6–8, then reverses: past DP ~10–12 the polymer outgrows its own solubility, cannot splay across a protein, and the grip falls back even as the molecule gets larger. That reversal is a genuine **failure mode** — breed for ever-longer tannin and you breed, past the peak, for *less* astringency and a muddy, insoluble haze.

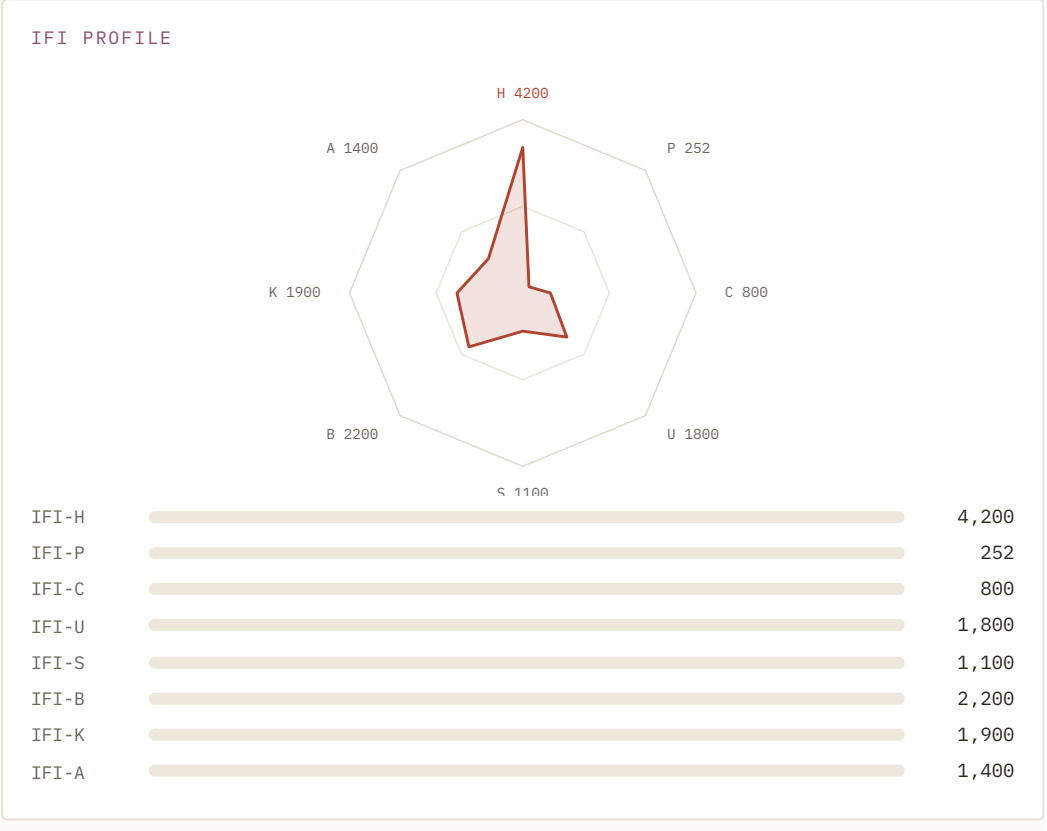
The levers multiply, and they multiply fast. Take procyanidin C1 — a DP3 condensed trimer, EC_{50} 360 µM, IFI-A 2,780 — and galloylate it once. The ×2.4 galloylation factor divides the EC_{50} to 360 ÷ 2.4 = 150 µM, which would read IFI-A = 1,000,000 ÷ 150 = **6,670** — except the scorecard caps at 5,000, so it posts as 5,000 and the raw potency overflows the display. That overflow is itself the lesson: two

moderate levers stacked already saturate the human scale, which is why the index caps where it does and why real design lives in the first turn of each dial, not the last. Laid out as a table, the levers read as a short list of rules — each one a knob we turn in the plant:

STRUCTURAL LEVER	EFFECT ON THE GRIP	QUANTIFIED	WHY, MECHANICALLY
Galloylation ↑	Astringency ↑↑ (strongest lever)	≈ ×2.4 per gallate ester	+3 hydroxyls, +1 stacking ring → tighter multivalent binding
Polymerization ↑ (to DP 6–8)	Astringency ↑	∝ $DP^{0.8-1.0}$	more binding sites per molecule → more cross-links per contact
Polymerization past DP ~10–12	Plateau, then ↓	potency reverses	molecule too large/insoluble to splay across protein
Prodelphinidin (tri-OH B-ring) ↑	Softer, "velvety" grip	≈ ×1.2	altered geometry; broad but gentler binding
Procyanidin (di-OH B-ring) ↑	Coarser, "green" grip	baseline	sharper, more localized binding
Hydrolyzable vs condensed	Often harder, faster grip	PGG/punicalagin → 4,880–5,000	very high galloyl/HHDP density, very high protein affinity
A-type vs B-type linkage	Character shift, not magnitude	solubility/geometry	a rigid locked pair wraps protein differently than an open one

Reading it in the plant — *hosts, constructs, and the cost of a wobble.*

The ladder is chemistry; a cultivar is where we grow to a point on it. Among our flagship peppers, **Phoenix** — the one cultivar designed to register on all eight axes at once — carries a deliberately designed astringency:



Its **A of 1,400** reads, through the inverse formula, as an EC_{50} of 1,000,000 ÷ 1,400 ≈ **714 µM** — a tannin load between a bare unit and a light procyanidin dimer, a whisper of grip laid under the heat to

give the finish a spine without turning the fruit tannic. That restraint is the design: Phoenix is a full-spectrum cultivar, not a tannin crop, and 1,400 is where astringency should sit when it is a supporting voice rather than the lead. The true astringency hosts are elsewhere, and the plants that carry the axis as a primary are exactly the tannin plants the chemistry predicts:

HOST ORGANISM	CONSTRUCTS	PRIMARY AXES	DOMINANT TANNIN CHEMISTRY
Grape	1,610	B / A / M	condensed — procyanidins & prodelphinidins (skin, seed, stem)
Tea	280	U / B / A	condensed — galloylated catechins (EGCG)
Blueberry	120	A	condensed — A-type, locked linkage
Pomegranate	120	A	hydrolyzable — ellagitannin (punicalagin)
Sorghum	120	A	condensed — prodelphinidin-rich
Raspberry	40	A	hydrolyzable — ellagitannin (sanguiin H-6 / lambertianin C)

Reading the two families straight off that column is the practical payoff of the whole chapter: to firm a grape or a tea we reach for the galloylation and chain-length levers of the condensed route; to brighten a pomegranate or a raspberry we work the HHDP density of the hydrolyzable route; and because grape alone carries **1,610** constructs, the design space inside a single astringency host is already enormous.

What makes any of it *designable* is reproducibility, and here the number is a form of respect for the reader. Astringency measured on a wild, unselected crop wanders — a batch-to-batch coefficient of variation near **42%** is ordinary for an uncontrolled tannin load, swinging with sun, water, and season. Our engineered lines hold that to **≤7%**. The difference propagates straight into the index: since IFI-A = 1,000 × EC_{50}(ref) ÷ EC_{50}(c), the relative uncertainty of the score combines the two EC_{50} errors in quadrature,

$$\sigma(IFI)/IFI = \sqrt{[(\sigma_{ref}/EC_{50,ref})^2 + (\sigma_c/EC_{50,c})^2]}.$$

At our controlled 7% on each term, $\sqrt{(0.07^2 + 0.07^2)}$ = **9.9%** — so a designed A of 2,900 is a real 2,900 ± ~290, a target you can aim at and hit. Let the candidate wobble at the wild 42% instead and $\sqrt{(0.07^2 + 0.42^2)}$ = **42.6%**, turning that same 2,900 into anything from ~1,665 to ~4,135 batch to batch. A grip that imprecise is not a flavor you can design; it is weather. Holding the CV down is what converts a tannin from a thing that happens to a thing we grow on purpose.

Where the number lies — *edge cases, failure modes, honest limits.*

An index earns trust by naming where it is thin, and astringency, more than any other axis, is thin in instructive ways. We hold its limits in plain sight.

It is **tactile, not gustatory.** The EC_{50} measures protein precipitation in a cuvette; a mouth is warmer, moving, and lined with living mucosa, and the felt intensity depends on surfaces the cuvette does not have. A turbidimetric EC_{50} and a trained human panel agree in rank order almost always, but not in exact magnitude, so we treat the assay as the calibrated instrument and the panel as the court of final appeal, and we report when they diverge rather than averaging the disagreement away.

It **builds and it adapts.** Because the PRPs are spent sacrificially and resupplied slowly, a second sip meets a mouth with less film than the first, and perceived astringency climbs across a tasting even at constant concentration — a time dependence a single EC_{50} cannot carry. The same mechanism cuts the other way between people: salivary flow and PRP abundance vary widely from mouth to mouth, and a fast, protein-rich salivator will disarm a tannin a dry mouth cannot. The index scores the *stimulus*, honestly and reproducibly; it does not pretend to predict every mouth, and we say so.

It is **suppressible and context-bound.** Fat coats the mucosa and blunts the grip, which is why tannin and cream forgive each other; sugar and high viscosity soften it; low pH sharpens it. An astringency read in isolation is a true number about the molecule and an incomplete number about the dish — the composition around it can move the felt result by a wide margin, and we design the whole plate, not the lone compound.

And it has a **built-in reversal.** The polymerization lever that firms a grip through DP 6–8 turns on you past DP ~10–12, where the tannin grows too large and insoluble to bind — breed naively for "more tannin" and you can sail past the peak into *less* grip and a dull haze. Likewise the two moderate levers of our worked trimer already overflowed the 5,000 cap: the scale saturates, and above the ceiling the index stops resolving differences a mouth may still feel. These are not flaws to apologize for. They are the true shape of the sense, and knowing the shape — where it climbs, where it plateaus, where it reverses, where it caps — is precisely what lets us place a cultivar on it and grow the molecule we meant to.

Two families, a handful of structural dials, one inverse formula, and a coefficient of variation held low enough to aim by: from these the entire sensory world of astringency is built, and every move we make in a plant is a move within it. The rules are not ours. They are the plant's, written in the structure of the molecule and read by the protein in a mouth. Our work is only to learn them exactly — to disassemble the tannin, read its parts, put the right number on its pull — and then to grow, cleanly and on purpose, the grip we intended all along. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 4 · Giving the grip a number — *the arithmetic of IFI-A.*

Every axis of the Iyengar Flavor Index answers the same quiet question — *how loudly does this speak?* — and answers it the same way: find the concentration at which the stimulus drives its target to half-response, invert it against a fixed reference, and scale by a thousand. For heat the target is a channel and the half-response is half-activation, the familiar EC_{50}. Astringency is stranger, and more intimate. It opens no channel; it docks into no nerve ending like a key turning a lock. The half-response that defines it is not a receptor climbing halfway to its ceiling — it is a protein leaving solution. Astringency is the one taste whose measurement *is* its mechanism: the precipitation of the proteins that keep the mouth slick. We call that half-maximal event **PC_{50}**, the half-precipitation concentration, and around it the family formula closes without a single symbol changed.

$$\mathrm{IFI\text{-}A}(c) = 1000 \cdot PC_{50}(\mathrm{ref}) \,/\, PC_{50}(c)$$

The formula structure is shared across all eight axes, but each axis holds its own reference constant — the IFI-A reference is a grape-skin proanthocyanidin standard; the IFI-H reference is capsaicin against TRPV1; and so on. A score of 1,000 on one axis and a score of 1,000 on another are each equal to that axis's own anchor, not to each other. Cross-axis IFI values are relative-potency measurements within their axis, not points on a single common scale.

In words, and the words are gentler than the notation: take a candidate tannin, measure the concentration at which it precipitates half of a standardized pool of **basic salivary proline-rich protein (bPRP)**, and set it against the concentration at which a fixed reference tannin does the same. A tannin that pulls the protein down at a *tenth* of the reference's concentration grips ten times as hard. A tannin that needs *five times* as much grips a fifth as hard. The reference is chosen once and held forever: a calibrated grape-skin proanthocyanidin standard — a real, long, moderately galloylated condensed tannin that sits in the middle of the sensory world — so that **IFI-A 1,000** is its anchor and the scale keeps a human center of gravity. As on every axis, scorecards normalize to a per-axis ceiling of **5,000**, which on the astringency axis is the grip of a near-saturating ellagitannin: the point past which more potency reads as no more sensation, because the lubricating film is already stripped and there is nothing left to take.

The event we are counting

To measure astringency honestly you have to know, molecule by molecule, what is actually happening in the mouth — because the number is anchored to that event and to nothing else.

The mouth is kept slick by a thin salivary film, and the proteins that do the keeping are unusual. The **proline-rich proteins** — acidic and basic, the bPRPs chief among them — are a quarter to a third proline

by residue, arranged in short repeats, and they carry almost no folded structure. Instead they lie open in a left-handed **polyproline-II helix**: an extended, exposed ribbon with its backbone laid bare. Where most proteins hide their reactive backbone inside a fold, the PRPs hold theirs out. They are, in a real sense, *built to be sacrificed* — a first line that binds incoming tannins and carries them harmlessly out of the body before they ever reach the gut.

Tannins bind that open ribbon two ways at once, and the two reinforce each other:

- **Hydrophobic stacking.** Proline's pyrrolidine ring presents a flat, greasy face. The aromatic rings of a tannin's galloyl and catechol groups lie down flat against it, face to face — a π-stacking, water-excluding contact that the proline residues are almost purpose-built to receive.
- **Hydrogen bonding.** A tannin is a dense comb of phenolic hydroxyls. In a proline-rich sequence the backbone carbonyls sit unusually exposed — proline's nitrogen carries no amide hydrogen to compete — so the tannin's hydroxyls hydrogen-bond straight onto the peptide backbone.

Because the hydrogen bonds supply most of the binding energy, astringent association is strongly **enthalpy-driven**, with a hydrophobic, entropy-favored assist from the stacking and the water it releases. And it runs in two regimes. At low tannin-to-protein ratios you get soluble complexes — one tannin draped over one protein, still dissolved. As the tannin concentration climbs, a single long tannin bridges *two* proteins at once; the bridged pairs cross-link into colloidal particles; the particles grow until they scatter light — the haze a winemaker sees against a candle — and then, past a threshold, they fall out of solution. That falling-out is precipitation. It drags the PRPs and mucins out of the salivary film, the film thins, and two oral surfaces that were gliding on a protein-lubricated layer begin to *drag*. Mechanoreceptors in the mucosa — touch, not taste — report the rise in friction. Astringency is the only axis of the Index read by the sense of touch: a tactile event with a chemical cause. PC_{50}, a precipitation potency measured in a flask, is exactly the stimulus that event is anchored to.



Where the grip is built: a wine grape in cross-section. Long, supple proanthocyanidins ride in the skin; hard, short, seed-coat tannins wrap the pips. One berry carries two different numbers.

The inversion, worked

The arithmetic is simpler than its symbols, and it is worth doing by hand until it feels obvious.

Let the reference grape-skin standard precipitate half of the bPRP pool at a concentration we will call **100 working units** — its PC_{50} by definition, its IFI-A exactly 1,000. Working units are only a scale, and we peg them to the bench: the reference reaches half-precipitation at a measured **0.50 mg/mL**, so one working unit reads as 0.005 mg/mL and the whole table converts at a glance. To go further, into molarity, divide by each tannin's own mean molecular weight — for the reference, a proanthocyanidin of mean degree of polymerization near 5–6 and roughly **1,700 g/mol**, that 0.50 mg/mL is 0.50 g/L ÷ 1,700 g/mol ≈ **294 µM**.

Now assay candidates and invert. A young, seed-tannin-dominant extract that half-precipitates at 40 working units grips harder — less does the same work — and scores 1000 × 100 ÷ 40 = **2,500**. A soft, long, lightly galloylated skin tannin that needs 170 scores 1000 × 100 ÷ 170 ≈ **590**. A near-saturating ellagitannin that pulls the protein down at just 20 scores 1000 × 100 ÷ 20 = **5,000** — the normalization ceiling, where the scorecard caps because the film is already gone. And the one fully designed cultivar in our library that registers on this axis, the full-spectrum Phoenix (SS-0096), our model predicts IFI-A 1,400, which is to say a designed PC_{50} of 100,000 ÷ 1,400 ≈ **71 working units**: firmer than the reference, well short of the ceiling.

TANNIN / FOOD	PC_{50} (WORKING UNITS)	PC_{50} (≈ MG/ML)	IFI-A = 100,000 ÷ PC_{50}	BAND
Near-saturating ellagitannin	20	0.10	5,000	Precipitating ceiling (cap)
Young seed-tannin extract	40	0.20	2,500	Hard, youthful red
Phoenix · SS-0096 (predicted A)	71	0.36	1,400	Firm structure
Grape-skin reference standard	100	0.50	1,000	The anchor
Soft, long skin tannin	170	0.85	590	Supple, structured
Soft blueberry / white-tea 2nd steep	400–670	2.0–3.4	150–250	Threshold grip

Every PC_{50} in that table is a mean of replicate titrations, and the ratio inherits their scatter. For a ratio the relative variances add, so the coefficient of variation on a score is $CV(IFI\text{-}A) = \sqrt{(CV_{ref}^2 + CV_c^2)}$. Measure a candidate at the platform's batch CV of **≤7%** against a reference held to the same, and a single pair would carry $\sqrt{(0.07^2 + 0.07^2)} \approx 10\%$. But the reference is the most-measured tannin we own: average it over a few dozen runs and its contribution shrinks as $0.07 \div \sqrt{N}$, so with a well-sampled reference the score's CV collapses back toward the candidate's own ≈ 7%. The precision of the number is a measured quantity, not a hope — which is the whole reason we can design to a band and land in it.

The number is not a ranking or a rating. It is a measured ratio of potencies, and it means the same thing on a grape, a tea, a pomegranate, and a pepper: *this tannin grips this hard.* Only the half-

response event — protein precipitating, rather than a channel half-opening — is particular to astringency. The inversion is the family's, unchanged.

Reading a precipitation curve

PC_{50} is a single point on a curve, and the shape of the curve carries its own information.

Titrate tannin into the bPRP pool and follow the fraction of protein precipitated. The fraction climbs along a sigmoid that fits a Hill form,

```
P(c) = P_max · c^h / (PC₅₀^h + c^h),
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where PC_{50} is the concentration at half-maximal precipitation — read straight off the midpoint — and **h**, the Hill slope, measures how *cooperative* the fall-out is. A short oligomer that binds one protein at a time precipitates gradually, h near 1. A long, galloylated polymer that cross-links as it goes precipitates almost switch-like, h climbing toward 2–3 in our libraries: nothing, nothing, then suddenly haze and a dropping curtain. This is the molecular root of a thing every taster knows — a wine that reads as "closed," giving little, until one threshold sip when the grip arrives all at once. Two tannins can share an identical PC_{50} and still feel unalike: one ramps, the other snaps. The single number sets *where* the grip turns on; the Hill slope sets *how sharply.*

The four assays that have to agree

What keeps PC_{50} honest is that it is not one clever assay but a **battery**, and a tannin earns its IFI-A only when the four converge:

- **Protein precipitation** — the direct measurement. Titrate tannin into the bPRP pool, follow the protein leaving solution by turbidity and by the thinning of its band on a gel, and read PC_{50} off the half-precipitation point. This *is* PC_{50}.
- **Isothermal titration calorimetry (ITC)** — inject tannin into protein and measure the heat released per injection. ITC hands over three numbers at once: the association constant **Ka** (how tight), the stoichiometry **n** (how many tannins per protein), and the enthalpy **ΔH** (how much of the binding is hydrogen bonding). Astringent binding reads strongly enthalpy-driven, the signature of that backbone hydrogen-bond network, with the entropic hydrophobic term confirming the stacking against proline.
- **Nephelometry / haze kinetics** — watch turbidity rise over time. This is the assay that captures *build*: the slow onset, the accumulation, the shape of the reservoir model below.
- **Oral tribology** — the physical payoff. Slide a soft, tongue-mimicking surface against another across a film of the tannin-and-saliva mixture and read the friction coefficient **μ** directly. Astringency, at bottom, is friction, and the tribometer reads friction. When a tannin's rise in μ tracks its PC_{50} and tracks the trained panel's score, we know the number is real.

The convergence is also where we catch the frauds, and there are several — each one a genuine failure mode. A compound can **bind without precipitating** — high Ka in the ITC, no haze in the flask — when it is too short to bridge two proteins, or when the tannin-to-protein ratio sits in the soluble-complex regime; its PC_{50} is then undefined, and the battery refuses to score it. A compound can raise turbidity **without raising μ** — a haze of particles too small or too slick to strip the film — a false astringent that only the tribometer unmasks. And the number itself moves with the matrix: pH, ionic strength, ethanol, and polysaccharides all shift where protein falls out, which is why we fix the assay conditions — a defined model saliva, held pH and ionic strength — so the *flask* number is reproducible, and say plainly that the *in-mouth* number will ride the matrix it is served in. Reproducibility is the entire reason we measure in a flask at all.

From binding energy to grip

The thermodynamics follow cleanly from ITC, and they explain why structure sets potency.

The free energy of binding is **$\Delta G = -RT \ln K_a$**. At mouth temperature (298 K), RT ≈ 2.48 kJ/mol, so every factor of ten in Ka is worth RT·ln 10 ≈ 5.71 kJ/mol. A single tannin–proline contact is weak — Ka per contact in the low thousands of inverse molar, call it 3×10^3 M^{-1}, which is $\Delta G \approx -RT \ln(3000) \approx$ **−19.8 kJ/mol**. On its own that is not enough: the first contact also has to pay a one-time **entropic toll** — freezing the two partners' freedom to tumble and drift, a cost of order +20 kJ/mol — so a lone contact nets near zero, and the complex falls apart as fast as it forms.

Multivalency is the rescue, and it is arithmetic. The entropic toll is paid *once*, on the first contact; every additional contact after that is made by partners already held together, so it adds its full binding energy at almost no further cost. Three contacts contribute roughly $3 \times (-19.8) \approx -59.5$ kJ/mol of binding and still pay the toll only once: $-59.5 + 20 \approx$ **−39.5 kJ/mol** net, which back through $\Delta G = -RT \ln K_a$ is an *effective* affinity of order 10^7 M^{-1} — four orders of magnitude past the single contact. The avidity, the holding-together, is far greater than any one bond, and the complex now lives long enough to grow and precipitate.

This is why the structural dials map straight onto potency. Add a galloyl group and you add hydroxyls and another stacking ring — more contacts per encounter — and Ka climbs by roughly **five- to ten-fold**, dragging PC_{50} down and IFI-A up. Lengthen the polymer and you add whole binding sites, raising the stoichiometry n and the number of cross-links each molecule can throw. The chemistry and the arithmetic are the same story told twice.

The dials — designing the number before the plant fruits

Everything the number rewards, the plant builds with enzymes, and every one of those enzymes is a dial we can turn.

Tannin begins in the flavonoid pathway. Phenylalanine is walked through PAL, C4H, and 4CL to p-coumaroyl-CoA, built up by chalcone synthase and isomerase to naringenin, and hydroxylated on its B-ring by **F3′H or F3′5′H** — the fork that sets dihydroxy (**procyanidin**) against trihydroxy (**prodelphinidin**). Reduction through DFR gives leucoanthocyanidin, and then two reductases fix the stereochemistry of the building block: **LAR** makes catechin, while anthocyanidin synthase followed by **ANR** makes epicatechin — the route grape tannin runs through. Those flavan-3-ol units condense into proanthocyanidins, and the ratio of extension units to terminal units sets the **mean degree of polymerization (mDP)** — the chain length. Galloylation is a second pathway laid over the first: gallic acid is activated to **β-glucogallin** by a glucosyltransferase, and an **SCPL acyltransferase** transfers the galloyl group onto the flavan-3-ol's 3-hydroxyl, turning epicatechin into epicatechin-3-*O*-gallate.

So the four variables the potency model cares about are four enzymes we can turn:

DIAL	WHAT SETS IT IN THE PLANT	HOW WE TURN IT	EFFECT ON KA	ON PC_{50}	ON IFI-A
Galloylation (% galloyl)	SCPL galloyltransferase + β-glucogallin donor	add galloyl esters	↑ ~5–10× per group	↓ sharply	↑↑ (dominant)
Polymerization (mDP)	LAR / ANR flux; extension : terminal ratio	lengthen the chain	↑ (more sites, higher n)	↓	↑
Prodelphinidin (% PD)	F3′5′H (B-ring trihydroxylation)	trihydroxylate the B-ring	shifts quality	modest ↓	bends the quality
Linkage	condensation chemistry (A- vs B-type)	A-type double bond	↑ rigidity / avidity	slight ↓	↑ (firmer)

We can even write the empirical face of it. Across our condensed-tannin libraries the platform fits a potency model — the log of binding potency as a sum of structural contributions, each coefficient learned from measured tannins:

$$\log(1 / PC_{50}) \approx a\cdot(\text{mDP}) + b\cdot(\%\ \text{galloylation}) + c\cdot(\%\ \text{prodelphinidin}) + d\cdot(\text{linkage}) + \text{const.}$$

The coefficients are fitted, not sacred, and we quote them as a model rather than a law — but their *signs and ranks* hold across every organism we grow: galloylation dominates, polymerization follows, the prodelphinidin term bends the quality more than the magnitude. That stability is what lets us do the thing the rest of this

book describes — read a tannin's predicted IFI-A off its structure before the plant has ever fruited, then write the genes to hit a target band. Design the sentence, then grow the mouth that speaks it.



The galloylation dial, made visible. Tea builds heavily galloylated catechins — EGCG and its kin — the same move the plant makes to turn grip up: more hydroxyls, more stacking rings, more contacts per encounter.

It builds — the arithmetic of the second glass

A single sip has a PC_{50}; a meal has a trajectory. Two features of astringency need their own arithmetic, because they are what make the sensation feel alive — and the first is that it *accumulates.*

Model the lubricating film as a reservoir. Let **L** be the fraction of intact lubrication, from 1 (slick) down toward 0 (stripped), and let the **deficit D = 1 − L** be what the mouth actually feels, because perceived grip tracks friction and friction rises as the film thins: roughly **$\mu(t) = \mu_0 + k\cdot(1 - L) = \mu_0 + k\cdot D$**. Each exposure removes a slug of protein set by the tannin's dose and potency; between sips the glands refill the film. Written as a rate,

```
dL/dt = −α · [tannin] · potency · L + β · (1 − L),
```

the first term is the stripping — proportional to how much tannin is present, how potent it is, and how much intact film remains to be pulled down — and the second is the glands restoring the film toward full at a rate **β**, the salivary flow.

Between sips, with no tannin in the mouth, the deficit simply relaxes: dD/dt = −βD, so **$D(t) = D_0\, e^{(-\beta t)}$**, recovering with a time constant τ = 1/β. Now watch a sip train. Say each sip adds a fixed increment d to the deficit, and sips arrive every Δt seconds, so between them the deficit shrinks by a factor **$r = e^{(-\beta\Delta t)}$**. Start slick (D = 0) and the deficits stack as a geometric series:

```
D₁ = d, D₂ = d(1 + r), D₃ = d(1 + r + r²), …, D∞ = d / (1 − r).
```

The first sip grips as d; the glass settles toward **d / (1 − r)**, an amplification of **1 / (1 − r)** over that first taste — and the wine in the glass has not changed at all. Put salivary numbers in. Take a film-recovery time τ = 20 s. Nurse the glass, Δt = 60 s: then βΔt = 3, r = e^(−3) ≈ 0.05, and the amplification is 1 / (1 − 0.05) ≈ **1.05×** — essentially flat. Gulp it, Δt = 15 s: then βΔt = 0.75, r = e^(−0.75) ≈ 0.47, and the amplification is 1 / (1 − 0.47) ≈ **1.9×**. The grip nearly doubles across the glass. Two wines with an *identical* IFI-A can

therefore live two entirely different lives — one sipped slowly across a long meal, its reservoir refilling between tastes; the other gulped, its floor ratcheting down sip over sip. This is why a tannic red that is perfect on the first taste can turn punishing by the third glass, and why a splash of water or a bite of fat — which restore or stand in for the lubricant — reset the whole account. And a tannin designed for a slower-flowing mouth — a lower β — wants a lower magnitude and a gentler build than one meant for a fast-flowing one. When we design a wine's tannin we are not setting a number; we are setting a *curve.* The single number is the amplitude of the grip; the curve is its music, and a designer who knows the pairing, the pace, and the drinker can write the whole phrase rather than only strike the note.

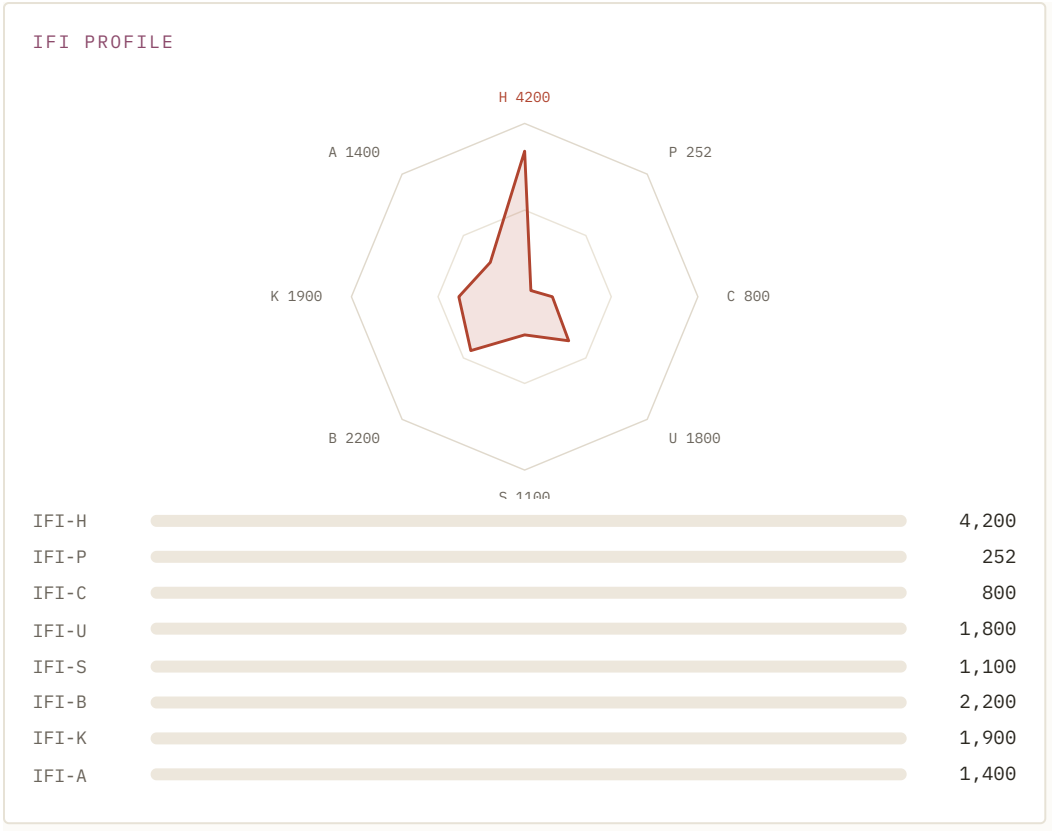
It is personal, and the number isn't

The second living feature is that astringency belongs to the mouth that feels it — and this is the final reason the Index anchors where it does.

Perceived intensity across people follows a **compressive power law**: double the stimulus and the sensation grows by rather less than double, a power function with an exponent below one. The exponent is a property of the sense; the *constant* in front of it is a property of the person, because it carries that mouth's own protein supply and its own salivary flow β. Anchor a scale to *perceived intensity* and it would drift with every taster and every hour of the day — a breakfast mouth and a dinner mouth are not the same instrument. Anchor it, as we do, to **PC_{50}** — a precipitation potency measured in a flask against a standardized protein pool — and it holds still. It does not move when the drinker's saliva does. The Index hands the winemaker a fixed coordinate, *this tannin grips this hard,* and leaves to the table the beautiful, irreducible variability of who is drinking and how fast. We measure the chemistry exactly so that the mouth is free to stay its own.

The library ahead

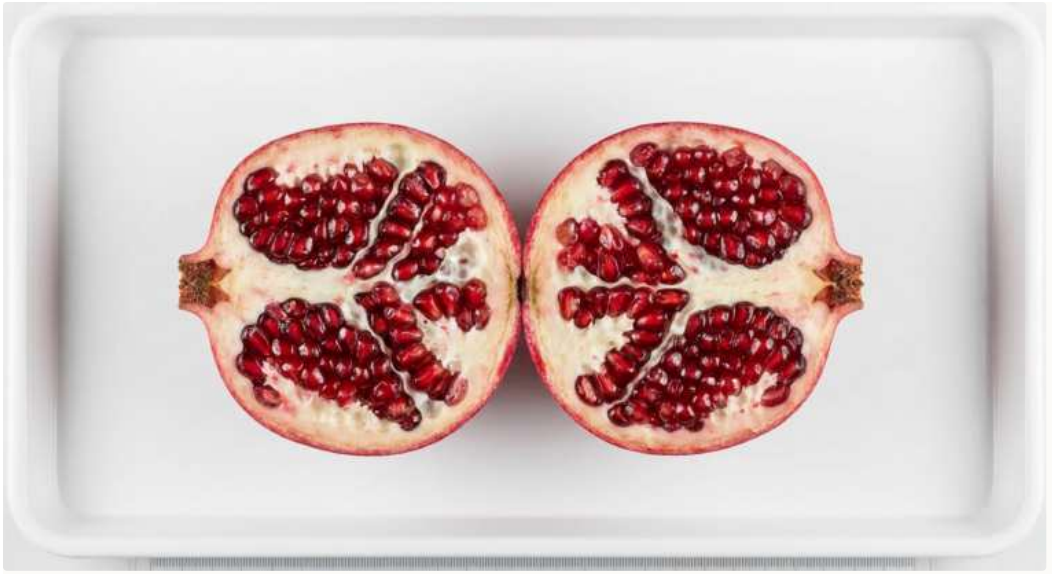
With the sensation named, the proteins and the tannins understood, and the arithmetic built, we can leave the flask for the greenhouse. The astringency axis runs through our one designed full-spectrum cultivar — the plant we built to register on all eight axes at once, which our model predicts will land at a firm, structured **IFI-A 1,400** —



— but the deep astringency libraries are the dedicated tannin plants. Six speak this axis, **2,290 constructs** across them, and each builds its grip a different way:

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	WHERE THE GRIP TENDS TO SIT
Grape	1,610	B / A / M	Hard	reference 1,000 → hard youthful red 2,500–3,500
Tea	280	U / B / A	Hard	gentle second steep ~150–300 → strong black tea 2,500–3,500
Pomegranate	120	A	Hard	ellagitannin-rich — chemistry pushes toward the ceiling
Blueberry	120	A	Moderate	threshold grip ~150–300
Sorghum	120	A	Frontier	condensed tannins in the testa — firm, broad
Raspberry	40	A	Moderate	ellagitannin chemistry at a light load — bright, low

Read that last column as tendency, not verdict: the precise predicted IFI-A is set construct by construct, cultivar by cultivar, and every one of the 2,290 carries a predicted IFI-A score derived from the same structural variables the four-assay battery tracks. The chemistries, though, already sort them. Blueberry carries soft proanthocyanidins and sits near the threshold of grip. Tea builds galloylated catechins and can run from the whisper of a second steep to the firm pull of a strong black. Pomegranate and raspberry carry hydrolyzable ellagitannins — potent per molecule, near the precipitating end — the one at heavy load, the other light and bright.



Pomegranate in cross-section — arils packed around ellagitannin-rich pith and rind. Punicalagin and its kin are hydrolyzable tannins, among the most potent precipitants we measure: the chemistry that lives near the ceiling of the scale.

Sorghum wraps condensed tannins in its seed coat, firm and broad. And grape, the deepest library we hold, carries both at once — the long, supple proanthocyanidins of the skin and the hard, short tannins of the seed — which is why a single berry can speak from the reference all the way to the hard edge of a youthful red. Each of these is a different problem to design, and a different pleasure. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. We begin, as the grip itself does, with the deepest library we hold: the grape.



PART II OF IV

The Living Library

grape · tea · pomegranate · the berries · sorghum · the chocolate question

Chapter 5 · Grape — *the long tannin and the short.*

No plant we grow holds more astringency design in it than the grape. **One thousand six hundred and ten constructs** — our second-largest organism library, behind only the tomato — all aimed at an axis most winemakers can only coax with harvest timing, maceration, and the slow arithmetic of the barrel. In our hands the grape speaks three axes of the Index, **B, A, and M**: bitterness, astringency, and the multi-axis weave between them. But astringency is its soul. A red wine stripped of its tannin is not a lighter wine; it is a different drink, a thing that slips through the mouth and leaves no print behind it. Tannin is the grape's gift and its difficulty at once, and to design it you have to begin from a fact the textbooks state plainly and the palate confirms on every sip: a grape makes not one tannin but several — in different tissues, for different reasons, with different grips.



A single wine grape, the seed visible within. Seed tannin and skin tannin are built by the same pathway to opposite ends of the sensory scale — the designer's first and most useful fork.

We love this plant for how honestly it externalizes a hard problem. Everything astringent in a grape it assembled itself, out of sunlight and water and the carbon of the air, with nothing borrowed from any animal — the pucker in a glass of wine is pure plant chemistry, the condensed record of one season's decisions. Our work is not to overrule those decisions. It is to read them precisely enough, and steer them gently enough, that the grape makes the same fine tannin every harvest instead of once a decade by luck.

The pucker is a reflex, not a taste

Astringency is the one axis of the Index whose receptor is not, in the strict sense, a receptor at all. Heat has TRPV1, a channel that opens and lets ions through; sweetness has TAS1R2·TAS1R3, a binding pocket that triggers a cascade. Astringency has no such lock waiting for a key. What it has instead is a family of proteins the body pours into the mouth on purpose — the **salivary proline-rich proteins**, the PRPs — and a sensation that arises when a tannin takes those proteins out of service.

The mechanism runs in five steps, and it is worth drawing each one, because the whole of grape design is an attempt to tune step three.

1. **Saliva is a lubricant held up by protein.** Parotid saliva is extraordinarily rich in PRPs — by some measures they are the majority of its protein — alongside mucins (the long, sugar-decorated glycoproteins MUC5B and MUC7), statherin, and histatins. The PRPs are *intrinsically disordered*: up to roughly two-fifths of their residues are proline, and proline forbids the tidy folding that most proteins fall into, so a PRP lies open and extended, a loose ribbon rather than a knot. That open shape is not a defect. It is the point.
2. **Proline makes an ideal tannin hook.** Proline's nitrogen is locked into its own ring, so its peptide bond carries no N–H donor — which leaves the backbone carbonyl oxygen fully exposed, an unhindered hydrogen-bond *acceptor* repeated every few residues down the ribbon. A tannin's phenolic hydroxyls are hydrogen-bond *donors*, dozens of them on a single polymer. The two were practically made for each other.
3. **Tannin binds and then bridges.** A polyphenol first settles onto a PRP by a cooperative "hydrophobic sandwich": the flat aromatic faces of its flavan-3-ol rings stack against the proline rings while the hydroxyls hydrogen-bond to the exposed carbonyls. One tannin molecule carries many such faces, so it does not bind one PRP — it *cross-links* several, stitching ribbons into a growing network. As the ratio of tannin to protein climbs past a threshold, the soluble complexes aggregate, turn colloidal, and fall out of solution as a precipitate.
4. **The lubricant fails.** That precipitate pulls PRP and mucin out of the thin salivary film that normally lets the tongue glide against the palate and the teeth. Boundary lubrication collapses. Surface meets surface, and friction rises.
5. **The mouth reads friction as grip.** Oral mechanoreceptors — and very likely trigeminal free nerve endings — report that rise in roughness and drag. The brain renders it as *astringency*: drying, puckering, a tightening across the cheeks, the sensation of a surface where a moment ago there was glide. It is a tactile percept wearing the costume of a taste.

There is a quiet dignity in the fact that the pucker is, at bottom, a protective reflex. An animal that eats plants secretes PRPs as *sacrificial* tannin-binders — proteins offered up to precipitate dietary tannins before they can bind the enzymes of digestion or lock up the protein in a meal. The proline-rich sequence is evolution's answer to high-affinity, reversible tannin capture. When a great red wine grips and then releases, the body is performing, in miniature, the same

ancient negotiation between an herbivore and a leaf. We find it moving that the sensation a sommelier calls *structure* is the felt edge of a mechanism the body built to keep itself fed.

This gives the astringency axis a clean physical anchor. We define **EC_{50}** for a tannin as the concentration — on a basis we will make precise below — at which it drives PRP precipitation, and with it the friction the mouth reads, to half of its maximum. A tannin that reaches half-maximal precipitation at a *lower* concentration is *louder*, and the Index inverts the concentration so that loudness reads as a larger number:

> **$\text{IFI-A}(\text{candidate}) = 1{,}000 \times EC_{50}(\text{reference}) \div EC_{50}(\text{candidate}).$**

The scale is anchored so that **IFI-A = 1,000** marks a candidate whose EC_{50} equals the reference astringent's EC_{50} — the reference is simply the tannin we have agreed to call unity. Halve a tannin's EC_{50}, and it binds PRPs twice as readily at half the dose; its IFI-A doubles. Everything else in this chapter is the work of placing that number where the winemaker wants it, and holding it there batch over batch to a **CV of seven percent or better**.

Three tissues, three tannins

A grape berry keeps its tannin in three tissues — **seed, skin, and stem** — and the seed tannin and the skin tannin are very nearly opposites. That opposition is the central fact of winemaking and the first lever of grape design.



A grape in cross-section — seed, skin, and pulp in one frame. The seed and skin build their tannins by a single pathway steered to opposite ends of the grip, which is why extraction is the winemaker's whole art and the source is ours.

TISSUE	DOMINANT UNITS	MDP	GALLOYLATION	PERCEIVED GRIP
Seed	Procyanidin (catechin / epicatechin)	Low–moderate (up to ~10)	**High**	Hard, green, bitter, "angular"
Skin	Procyanidin + **prodelphinidin** (EGC)	**High** (up to ~30–40+)	Low	Long, fine, "velvety," structural
Stem	Mixed, often green	Variable	Moderate	Herbaceous, coarse

The seed makes a short, heavily **galloylated** procyanidin. Galloylation is the single hardest lever we know on the grip — a gallate ester adds a flat, hydroxyl-rich aromatic face that stacks against proline and hydrogen-bonds to the backbone carbonyl, so per unit of structural change it moves PRP affinity more than lengthening the chain or hydroxylating the ring. A heavily galloylated

seed tannin therefore punches well above its modest length: sharp, bitter, the "green" edge of an underripe berry or an over-extracted red. The skin, by contrast, makes a long, lightly galloylated tannin enriched in **prodelphinidin** — the trihydroxylated flavan-3-ol units (built from epigallocatechin, EGC) that read as velvety, with an extra B-ring hydroxyl offering one more hydrogen bond per contact. This is the tannin of structure and length, the one that holds a great wine upright for a decade.

Winemakers spend their craft trying to extract the skin and spare the seed — gentle pressing, careful maceration, the whole patient art of getting the long tannin without the short. We leave them that art and offer a second one beside it: **we move the balance at the source.** Where the cellar subtracts the harsh tannin after the fact, the plant can be asked to build less of it in the first place, so that the must arrives already weighted toward the fine.

The pathway, and the fork that matters

The chemistry is the flavonoid branch of phenylpropanoid metabolism, and the lever has a precise address: grape tannin, astringency *via* **ANR**. Here is the route, step by step, with the fork that decides the grip set in bold.

STEP	ENZYME(S)	PRODUCT
General phenylpropanoid	PAL → C4H → 4CL	L-Phe → *trans*-cinnamate → *p*-coumarate → *p*-coumaroyl-CoA
Flavonoid core	CHS → CHI → F3H	Naringenin chalcone → naringenin → dihydrokaempferol
B-ring hydroxylation	F3′H / **F3′5′H**	Dihydroquercetin (di-OH) vs **dihydromyricetin** (tri-OH → prodelphinidin)
Reduction	DFR	Leucoanthocyanidins (flavan-3,4-diols)
Fork · branch 1	**LAR** (leucoanthocyanidin reductase)	2,3-*trans*-flavan-3-ols (**catechin**, gallocatechin)
Fork · branch 2	ANS → **ANR** (anthocyanidin reductase)	2,3-*cis*-flavan-3-ols (**epicatechin**, epigallocatechin)
Galloylation	SCPL acyltransferase (+ 1-O-galloyl-β-glucose)	Gallate esters (ECG, EGCG)
Polymerization	Condensing / laccase-type activity	Proanthocyanidin polymers

The two reductases at the fork, **LAR** and **ANR**, set the ratio of catechin to epicatechin — of 2,3-*trans* to 2,3-*cis* starter and extension units — and that ratio, more than almost anything downstream of it, decides whether the finished tannin feels coarse or fine. Push flux through ANR and you build the epicatechin-rich, *cis*-linked polymers the skin favors; the grip lengthens and softens. Starve ANR and favor LAR, and you tilt toward the shorter, harder, *trans*-rich material of the seed.

Three further dials sit below the fork. The **F3′5′H** branch feeds the trihydroxy prodelphinidin units that velvet the grip. The **SCPL galloyltransferase** sets galloylation — the fast way up or down the IFI-A scale, for the reason given above. And the polymerization machinery sets **mDP**, the mean degree of polymerization, the length of the chain. Above all four sit the **MYB** master switches — the VvMYBPA-family regulators that act through an MYB–bHLH–WD40 complex to turn the whole proanthocyanidin program up or down as a unit. They are the gain knob over the entire branch, and they are where a single edit can move everything at once.

It helps to keep the building blocks and their masses in one place, because the sensory math below turns on them.

FLAVAN-3-OL UNIT	ABBREV.	MONOMER MW (G/MOL)	ROLE IN GRIP
(+)-Catechin / (−)-epicatechin	C / EC	290.27	Procyanidin backbone
(−)-Epigallocatechin	EGC	306.27	Prodelphinidin — adds the "velvet" OH
Epicatechin-3-O-gallate	ECG	442.37	Galloylated — the hard lever
(−)-Epigallocatechin-3-O-gallate	EGCG	458.37	Tri-OH **and** galloylated — loudest unit
Gallic acid (as galloyl ester)	—	170.12 (+152.10 per ester)	The appended galloyl face

Each interflavan bond that joins two units into a chain is formed with the loss of two hydrogen atoms (roughly −2 Da per bond), so a polymer of degree *n* has a mass close to *n* times the mean unit mass, minus about 2(*n*−1), plus whatever galloyl esters it carries. We will need that in a moment.

Putting a number on the grip

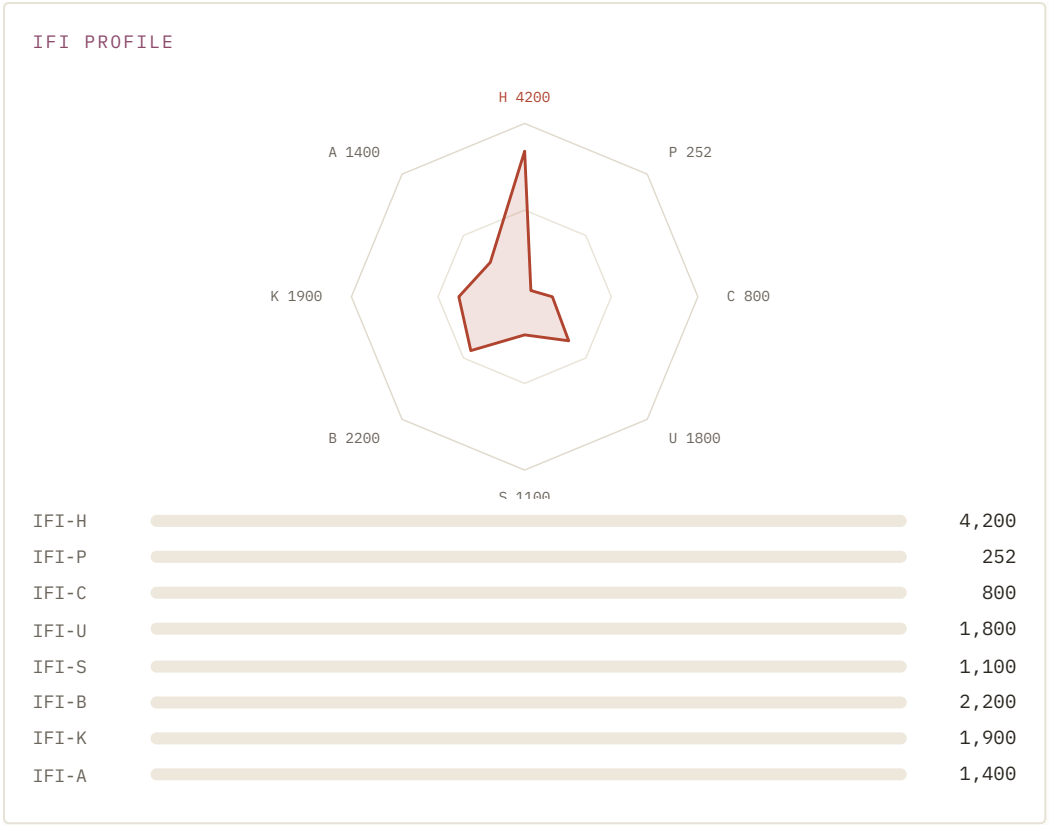
The IFI-A scale earns its keep only if the arithmetic is honest, so let us do it out loud.

Reading a tannin's loudness. The Index is reference-normalized, which has a useful consequence: to compare two tannins you never need the reference at all. From the formula, a tannin's EC_{50} is $EC_{50}(\text{ref}) \times 1{,}000 \div \text{IFI-A}$, so for any two candidates,

> $EC_{50}(1) \div EC_{50}(2) = \text{IFI-A}(2) \div \text{IFI-A}(1)$.

A tannin reading **A = 1,800** half-precipitates PRPs at exactly 1,200 ÷ 1,800 = **0.667×** the concentration of one reading **A = 1,200** — two-thirds the dose for the same half-maximal grip, which is to say it is 1.5× louder. The ratio is clean because the reference cancels.

A cross-organism anchor. Because every axis of the Index rides the same EC_{50} ruler, a pepper and a grape can be read on one page. Our full-spectrum cultivar **Phoenix (SS-0096)** is designed to activate all eight axes at once, with its astringency axis predicted at **A = 1,400** — squarely inside the band a winemaker chases.



That A = 1,400 implies, from the formula, $EC_{50} = EC_{50}(\text{ref}) \times 1{,}000 \div 1{,}400$ = **0.714× the reference** — a tannin that reaches half-maximal PRP precipitation at a little under three-quarters of the reference dose. A grape tuned to the same reading would grip the mouth exactly as hard, by construction, as Phoenix's eighth axis. One ruler, every organism.

The shape of the climb. Astringency does not rise gently with dose; it crosses a threshold. PRP precipitation is *cooperative* — each tannin cross-links several ribbons, so aggregation feeds on itself — and cooperative processes follow a Hill curve:

> $R(c) \div R_{max} = c^n \div (EC_{50}{}^n + c^n)$.

Writing $x = c \div EC_{50}$, this is simply $x^n \div (1 + x^n)$. With a Hill coefficient **n = 2**, which fits the cross-linking well: at x = 0.5 (half the EC_{50}) the response is 0.25 ÷ 1.25 = **20%** of maximum; at x = 1 it is **50%** by definition; at x = 2 it is 4 ÷ 5 = **80%**. So doubling the tannin from half-EC_{50} to twice-EC_{50} — a fourfold change in concentration — carries the mouth from barely-there to nearly-full astringency. The 10%-to-90% window spans a concentration ratio of 9^(2/n) = **9-fold** at n = 2 (it would be 81-fold at n = 1, and a steep 4.3-fold at n = 3). This is why a wine can taste balanced and then, with a little more extraction, tip abruptly into harshness: the grip lives on the steep shoulder of a cooperative curve, and IFI-A names where on the concentration axis that shoulder sits. Two tannins with the same n and different EC_{50} have identical-shaped curves, offset on a log-concentration axis by exactly $\log(IFI_2 \div IFI_1)$ — the louder one shifted left, reaching the same shoulder sooner.

Which concentration? Here is the subtlety that makes astringency a methods problem and not just a formula. A tannin can be counted three ways, and they do not agree:

- **per molecule** — moles of polymer per litre;
- **per binding site** — moles of flavan-3-ol *faces* per litre, which is mDP × the molecule concentration, and which is what PRP precipitation actually tracks;

- **per mass** — grams per litre, reported in the cellar as *gallic-acid equivalents* (GAE) after a colorimetric calibration.

A long skin tannin and a short seed tannin with the *same* per-site avidity are worlds apart per molecule, because the long one carries many more sites on each chain. We therefore anchor EC_{50} — and so IFI-A — to the **binding-site basis**, the only one that measures the tannin's intrinsic loudness rather than an accident of how long its chains happen to be.

Converting to the enologist's mg/L GAE needs the mean molecular weight, and that is where the building-block table pays off. Take a skin tannin of **mDP = 30**, half of its units EGC (mean unit mass ≈ (290.27 + 306.27)/2 = 298.27), lightly galloylated at ~5%:

> MW ≈ 30 × 298.27 − 29 × 2 + 0.05 × 30 × 152.10 ≈ 8,948 − 58 + 228 ≈ **9,118 g/mol.**

And a seed tannin of **mDP = 8**, pure epicatechin, 30% galloylated:

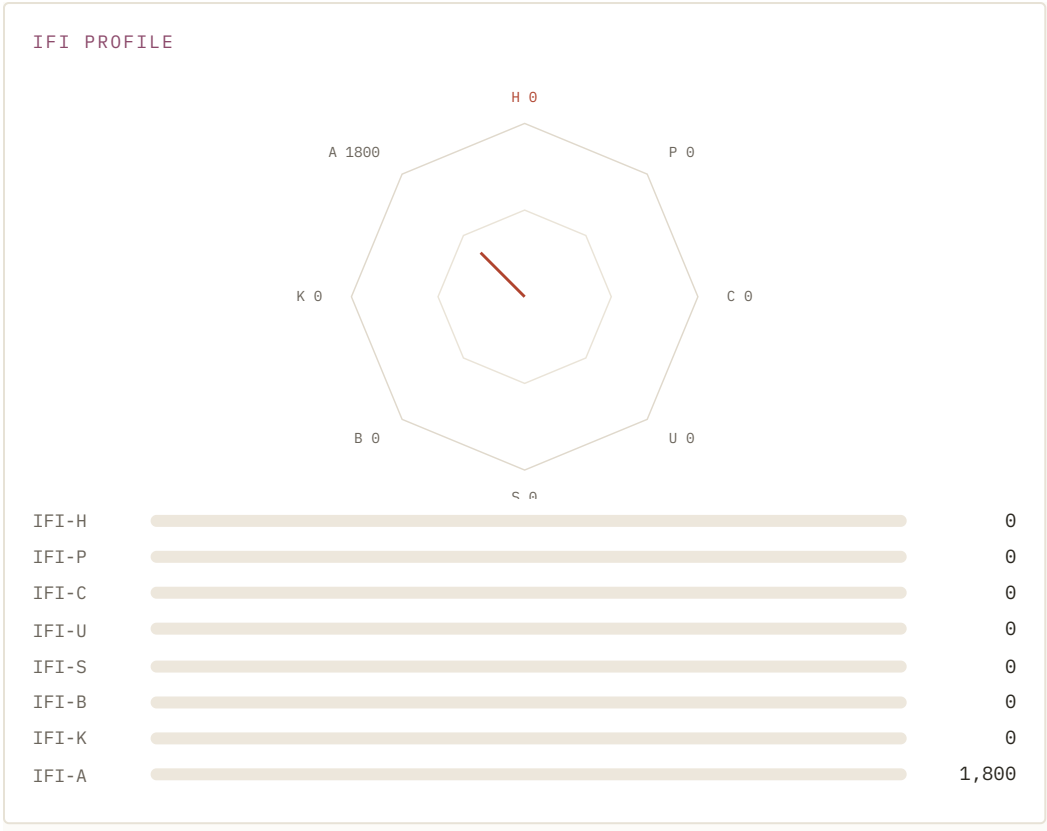
> MW ≈ 8 × 290.27 − 7 × 2 + 0.30 × 8 × 152.10 ≈ 2,322 − 14 + 365 ≈ **2,673 g/mol.**

Per molecule the two differ 3.4-fold in mass. But divide each by its own mDP to get the *mass per binding site* — 9,118 ÷ 30 = **304 g/mol-site** for the skin tannin, 2,673 ÷ 8 = **334 g/mol-site** for the seed — and the two land within about 10% of each other. That near-coincidence is the quiet reason the cellar's mg/L GAE is a *decent* proxy for astringency at all: mass-per-site stays close to the mean monomer mass (~290–330 g/mol) no matter the chain length, so grams roughly track sites. It is also, honestly, where the proxy *fails*: that ~10% spread between a galloylated seed polymer and a prodelphinidin skin polymer is error the colorimeter cannot see, and it is the same order as our whole batch tolerance. The Index insists on the site basis precisely so that the number means loudness and nothing else.

Gathering it into a ladder, with EC_{50} written as a multiple of the reference (EC_{50}/ref = 1,000 ÷ IFI-A):

GRIP	TISSUE SIGNATURE	IFI-A	EC_{50} (× REF)	STRUCTURE
Thin, over-pressed	Dilute skin, short	1,200	0.833	Low mDP, low galloylation
Fine, mid-band	Skin-led, prodelphinidin-rich	1,400	0.714	High mDP, light galloylation
Structured, age-worthy	Skin, long and velvety	1,800	0.556	Very high mDP, tri-OH rich
Green, angular	Seed-led, galloylated	> 1,800	< 0.556	Low mDP, **high** galloylation

The **1,200–1,800 band** is the fine-grained target — "firm but not punishing," the grip arriving slow and resolving long. A fine wine engineered to sit at the structural ceiling of that band reads as a single, pure spike on the eight-axis scorecard, because the grape in our hands is very nearly a one-axis instrument:



Those zeros are not missing data. They are the truth of the plant: no heat, no pungency, no cooling, no engineered umami or sweetness — one axis, played deliberately. (The grape also carries real work on **B**, bitterness attenuation, and on **M**, the multi-axis blends; this chapter reads the astringency axis, and the profile isolates it so the single tuned dimension stands clear.) A seed-dominated must would push the spike *past* the band's ceiling into the angular, green register — louder on the number, worse in the mouth, the hard tannin that a decade of cellaring is meant to resolve.

The step we cannot yet draw

Honesty is part of the method, so here is the limit. The one step we still cannot fully name is the one that makes a tannin a tannin: **polymerization**. How the grape joins its flavan-3-ol units into chains — which activity catalyzes the extension, how the chain length is set, where in the cell the condensation happens — remains partly open, one of the genuine frontiers of plant biochemistry. The leading model is a carbocation chemistry: an electrophilic extension unit, derived from a leucoanthocyanidin, attacks the nucleophilic C8 (or C6) of a terminal flavan-3-ol to form the 4→8 interflavan bond, the reaction proceeding in the acidic vacuole where the units are staged by MATE-type transporters. Laccase-type oxidases are implicated in the coupling. But the picture is not yet a portrait.

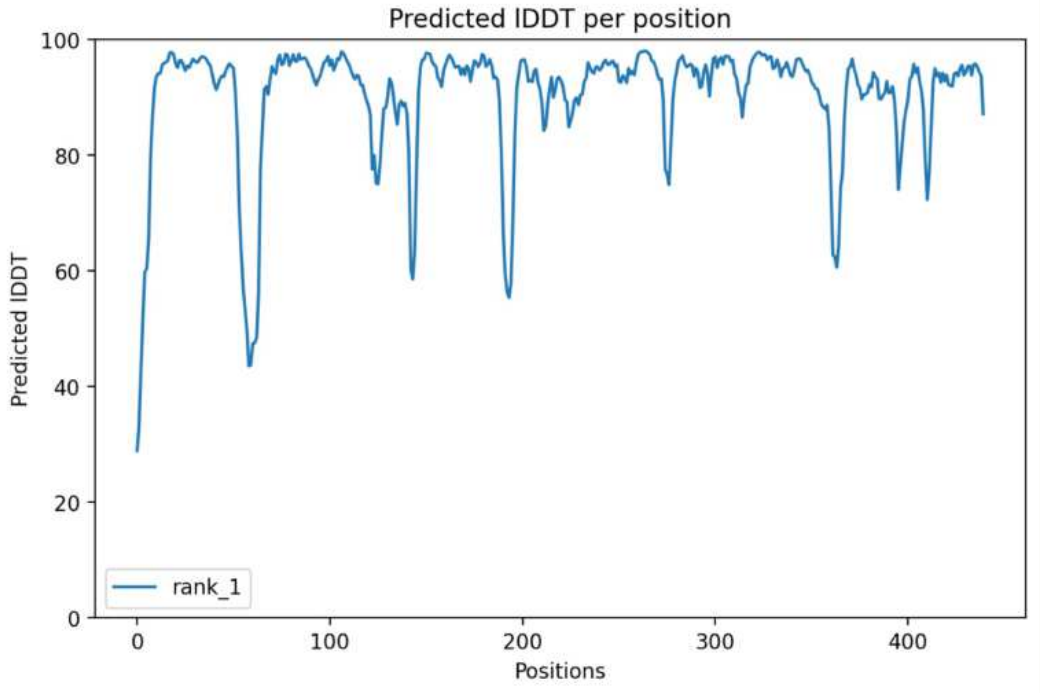


FIG. — Pun1/AT3 coloured by model confidence (pLDDT); the binding domain and active-site cleft resolve as high-confidence. A single acyltransferase like this we can model residue by residue. The grape's chain-extending activity, by contrast, resists that kind of portraiture — which is exactly why the grape is a hard organism, and why its library runs to 1,610 constructs.

There is a useful contrast in our own files. A single acyltransferase — the kind of enzyme Pun1 is — we can model to the residue, score every possible substitution, and redesign with confidence; our capsaicin work turns on exactly two such edits, **S39L and L345G** (a double variant), each with a computed effect we could predict before we made it. The grape's chain-extending activity offers no such target. No one can yet draw the polymerizing enzyme the way we can draw Pun1, and that gap is not a footnote — it is why the grape is a *hard* organism and why its library is our second-largest.

Where we cannot predict the polymer length from first principles, we map it empirically. A great deal of the grape's **1,610 constructs** is that patient map: graded series across the MYB regulators and the two reductases, each read for its resulting mean degree of polymerization by phloroglucinolysis — acid-catalyzed cleavage of the interflavan bonds in the presence of phloroglucinol, which releases the terminal unit free and the extension units as adducts, so that counting the ratio gives mDP directly. Point by point, construct by construct, we fill in a step the textbook still leaves blank, until we can *place* a tannin's length deliberately even though we cannot yet *compute* it. It is slower than design. It is also, in its way, the most reverent thing we do here: when a mechanism refuses to be solved, we sit with the plant and measure until it tells us the answer itself.

Tannin married to pigment

There is a second gift hidden in the grape, and the subtractive side of flavor design leans on it hard: the grape builds its tannin and its **anthocyanin** — its color — off the very same flavonoid branch, diverging only at the final steps. Both pass through DFR; the pigment route runs leucoanthocyanidin → ANS → anthocyanidin → glycosylation, while the tannin route turns at ANR into the flavan-3-ols. Siblings from one parent.



A cluster of wine grapes at depth of color — tannin and pigment are built on one branch, and the finest reds we grow hold both at once, the grip pre-draped in a stable, matched color load.

Because they share a parent, a grape can be designed to carry its tannin already married to its pigment — the two condensing into stable **polymeric pigments** that both soften a grip and hold a color across years in the cellar. A winemaker calls the young, free, hard tannin *unresolved*, and waits a decade for it to find the anthocyanins and settle. A grape engineered with an abundant, matched pigment load arrives with much of that resolution already done, its grip pre-draped in color — a lower effective free-tannin concentration, which, read through the Hill curve above, slides the perceived astringency back down the cooperative shoulder without removing a molecule. Tannin and pigment are siblings in the grape as in the berry, and the finest red-wine cultivars we grow are the ones that hold both at once: deep, stable color *and* a fine, structural, pre-softened grip, so the wine is approachable younger without being one bit less built to last.

Holding the grip steady

A target is only as good as its reproducibility, and here the mathematics turns from design to discipline. The coefficient of variation, **CV = σ ÷ μ**, measures how tightly a process holds its mean. Suppose we tune a grape to the mid-band, **μ = 1,400** on IFI-A. Our platform's design specification holds a batch-to-batch **CV of ≤7%**, so the standard deviation is σ ≤ 0.07 × 1,400 = **98** IFI-A units. The 95% interval (±1.96σ) is 1,400 ± 192 — **1,208 to 1,592** — which sits comfortably inside the 1,200–1,800 fine-tannin band. Every batch, in other words, lands as a fine tannin.

Now take the vintage-dependent alternative: the natural, coaxed tannin whose year-to-year variability runs to a CV near **42%** — the figure a wild-type process drifts to before any control. Then σ ≈ 588, and the 95% interval is 1,400 ± 1,153 — **247 to 2,553** — spilling out of the band on both ends. One vintage thin and slipping, the next green and angular, from the same vineyard and the same intent.

PROCESS	CV	Σ AT M = 1,400	95% INTERVAL	INSIDE THE BAND?
Designed (our platform)	≤7%	≤98	1,208 – 1,592	Fully
Coaxed (vintage-dependent)	~42%	~588	247 – 2,553	No — both ends spill

That sixfold tightening is the whole difference between a tannin that happens to you and a tannin you can specify. And variance composes predictably, which gives the cellar a second tool: blending *k* independent lots of equal size averages their readings and shrinks the spread by $\sqrt{k}$, so **CV_blend = CV ÷ $\sqrt{k}$**. Two lots at 7% blend to 7 ÷ $\sqrt{2}$ ≈ **4.95%**; four lots to **3.5%**. A blender who understood this by feel for centuries can now do it by number.

The honest edge cases are worth naming, because a number that hides its failure modes is worth little. First, the reference itself must be stable — IFI-A is a *ratio*, and a drifting reference moves every reading in lockstep, so the standard astringent is re-verified each run. Second, the ~10% mass-per-site spread between a galloylated seed tannin and a prodelphinidin skin tannin means a cellar tracking only mg/L GAE carries that error invisibly; where it matters, the site basis is measured directly, not inferred from mass. Third, astringency builds over a sip — successive contacts deplete the PRP pool faster than the salivary glands refill it, so the second taste reads louder than the first; our EC_{50} is defined on a single standardized contact to keep the number an intrinsic property of the tannin rather than an artifact of how thirsty the mouth already was. And fourth, the whole scale assumes PRPs in normal supply; a mouth short on saliva reads every tannin louder, which is a fact about the taster, not the wine, and no engineering of the grape can or should erase it.

The four moves, and what the grape keeps

So the grape-design problem, in the language of the Index, becomes four coordinated moves, and the 1,610 constructs are the systematic exploration of their combinations:

1. **Raise the ANR:LAR ratio** to shift the fork from short-coarse toward long-fine — more 2,3-*cis*, epicatechin-rich, skin-type polymer.
2. **Tune F3′5′H** to set the prodelphinidin fraction and move the grip from angular toward velvet, one extra hydrogen bond per contact at a time.
3. **Set galloylation** through the SCPL acyltransferase — the fast lever, up or down the IFI-A scale, because the gallate face moves PRP affinity more than any other single change.
4. **Govern mDP** through the condensing machinery to place the grip in the sweet spot where length means structure, not haze, and the band reads firm but never punishing.

The sensory language of the cellar maps straight onto those four. A **"fine-grained"** tannin — the one every red-winemaker chases — is long (high mDP), prodelphinidin-rich (F3′5′H flux through ANR), moderately galloylated, polymerized far enough to be structural but not so far it falls out as sediment: on the Index, a firm but forgiving **IFI-A in the 1,200–1,800 band**, the grip arriving slow and resolving long. A **"green," "coarse,"** or **"angular"** tannin is the seed signature — shorter, more heavily galloylated procyanidin — which reads higher and harder on first contact and does not resolve into length. The same vineyard, the same vintage, can be pushed toward either by where the flux goes at the fork.

We did not invent this chemistry. We learned to steer it, and then to hold it steady batch over batch to a **CV of seven percent or better** — the quiet discipline that turns a coaxed, vintage-dependent tannin into a designed, reproducible one. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. There is a reverence in this that we feel every harvest. A grape has spent its whole season deciding what tannin to build, out of nothing but light and water and air; we are only, gently, helping it decide. The wine it makes is still the grape's. We have simply given the winemaker a vineyard that keeps its promises.

Chapter 6 · Tea — *catechin, theanine, and the oxidized leaf.*

If the grape is astringency's deepest cellar, tea is its most disciplined, because in a single leaf the grip lives in open argument with two other axes — **umami and bitterness** — and the whole art of tea, carried in our family's kitchens and in a thousand years of kitchens before ours, has been the management of that argument. Our tea library is small and deliberate, **two hundred and eighty constructs**, and the organism speaks **U, B, and A** at once; you cannot design its grip without designing the chord around it. A great tea is not a tannin. It is a balance held at a knife's edge, and the tannin is only one arm of the scale.

We come to this leaf the way we come to every plant in the library: with reverence first, and then with the numbers, because for us the two have always been the same act of attention. The reverence is easy — the hush of a tea garden in the hour before plucking, the particular green of a shaded leaf, the way a good cup asks to be taken slowly. The numbers are the harder discipline, and the more honest. This chapter is the place where the wonder of the cup and the arithmetic of the grip are written as two halves of one sentence.



Camellia sinensis — the tea plant. Green, oolong, and black are not three plants but one leaf led to three different settlements of a single chemistry, and the chief variable is how far the leaf's catechins are allowed to oxidize before the firing stops the clock.

The grip is a friction — but a catechin is the thing that grips

Astringency is the one axis on the Index that is not, strictly, a taste. There is no astringency receptor the way heat has TRPV1 or umami has mGluR1. Astringency is a **mechanical report**: the felt roughening of a mouth that has lost its slip, read by the same trigeminal and mechanosensory nerve endings that tell you a surface is coarse. The chemistry happens first, out in the thin film of saliva on the tongue; only afterward does the body sense the consequence. The transduction event is a precipitation — protein falling out of solution — and the stimulus that causes it, in tea, is the **catechin**.

Catechins are flavan-3-ols, three fused rings — the aromatic A-ring, the oxygen-bearing C-ring, and a phenolic B-ring hanging off the side — and in the leaf they matter not only as polymers, the way grape tannin does, but as monomers and small oligomers in their own right. Their target is the mouth's first line of defense: the **proline-rich proteins** of saliva, the PRPs, long floppy open-coiled chains stuffed with proline that the parotid and submandibular glands pour out constantly. A PRP holds its backbone exposed in a loose left-handed coil, the polyproline-II helix, every carbonyl oxygen pointing outward and unclaimed — a landing strip repeated dozens of times down one molecule, lights left on. A catechin is built to find it. Its flat phenolic rings stack against the greasy pyrrolidine faces of proline; its many hydroxyl groups donate hydrogen bonds to those exposed carbonyls. Weak bonds, each of them — but a catechin carries several binding groups and a PRP presents many sites, so the two fasten at multiple points at once. The contacts are individually feeble; their sum is tenacious.

The binding is multivalent, and multivalency is the whole story, because a catechin with more than one aromatic "hand" can bridge two proteins, and one protein can be caught by two catechins. The solution knits itself into a three-dimensional network, the network grows until it can no longer stay dissolved, and it separates — first a haze, then visible aggregates, then a precipitate. That phase separation, happening in the film on your tongue, is the drying you feel. The proteins that were lubricating your mouth are now curds on their way out of solution, and the raw friction they leave behind is what the nerve endings read as *grip*.

The arithmetic of multivalency is where the design levers get their power, and it is worth one line. Binding free energy is logarithmic in affinity, $\Delta G = -RT \cdot \ln K$, and at mouth temperature — 310 K — the thermal quantum RT is **0.616 kcal·mol^{-1}**. A single catechin–proline contact, one phenolic hydrogen bond backed by a stack, runs near $K \approx 2{,}000\ M^{-1}$:

> $\Delta G_1 = -0.616 \cdot \ln(2{,}000) = -0.616 \cdot 7.60 =$ **-4.68 kcal·mol^{-1}**

Weak — a one-contact complex falls apart as fast as it forms. But let one catechin close several such contacts against one PRP and the enthalpies add, because free energy is additive. The naïve sum overcounts badly — the first contact must pay a one-time entropic toll to bring two free molecules into register, +10 to +15 kcal·mol^{-1}, which no later contact repays — so the realistic avidity of a well-matched catechin–PRP complex lands near $\Delta G \approx -8$ to -10 kcal·mol^{-1}, an association constant of 10^6 to $10^7\ M^{-1}$ rather than the paper fantasy of 10^{20}-something. Still ferociously tight for something built of individually feeble bonds, tight enough to pull protein out of solution, but **finite, reversible on the timescale of a rinse, and tunable**. The gap between the paper number and the measured one is the headroom the whole program lives in. And the single surest

way to add contacts to a catechin is to hang another phenolic ring on it — which is exactly what the leaf does, with an enzyme, and exactly what we tune.

Reading the grip as a number

To design the grip we have to read it, and the Index reads it the same way it reads every axis: against a fixed reference, inverted, and scaled by a thousand.

> $\text{IFI-A}(\text{candidate}) = 1{,}000 \times \text{EC}_{50}(\text{reference}) \div \text{EC}_{50}(\text{candidate})$

For astringency the EC_{50} is not a channel's half-activation but the concentration at which the stimulus drives its target to **half-maximal precipitation** — the point at which half the PRP it can throw out of solution is thrown. We anchor the astringency axis to **EGCG**, epigallocatechin-3-gallate, the galloylated catechin at the very center of this leaf, and define its half-precipitation concentration against a standard human-PRP panel as EC_{50}(reference). On the Index that anchor sits at exactly **1,000** by construction. Every other astringent — every leaf, every cultivar, every rival polyphenol — is read as a ratio to it. A stimulus that grips at half the concentration EGCG needs reads 2,000; one that needs twice the concentration reads 500. For the worked examples that follow we carry EC_{50}(reference) = **420 µM**, the anchor value; because the Index is a ratio of two concentrations, the choice of unit cancels out of every comparison and only the ratio survives.

The percept does not climb forever with concentration, and the shape of its climb is a design constraint we respect in every tea we build. Perceived astringency follows a saturating, mildly cooperative curve — a Hill form — because the cross-linking that precipitates PRP is cooperative, and because the PRP pool in any one mouth is finite:

> $P(C) = P_{max} \cdot C^n \div (EC_{50}^n + C^n)$, with Hill coefficient $n \approx 1.4$

At $C = EC_{50}$ the response is half of maximum, which is what anchors the number. Above it the curve bends over toward a ceiling; below it the grip falls away steeply. A worked case makes the two useful facts fall out at once — a **unit conversion** and a **dose–response reading**. EGCG's molar mass is 458.4 $g \cdot mol^{-1}$, so a concentration of 458.4 $mg \cdot L^{-1}$ is exactly 1 mM. A strong gyokuro brewed hard delivers EGCG near 350 $mg \cdot L^{-1}$:

> $350\ mg \cdot L^{-1} \div 458.4\ g \cdot mol^{-1} = 0.763\ mmol \cdot L^{-1} = \mathbf{763\ \mu M} = 1.82 \times EC_{50}(\text{reference})$

> $P/P_{max} = 1.82^{1.4} \div (1 + 1.82^{1.4}) = 2.31 \div 3.31 = \mathbf{0.70}$

Seventy percent of the leaf's maximum grip, from a hard brew — which tells the designer something precise: the last thirty percent is nearly unreachable by steeping, because the curve has already bent over. You cannot meaningfully out-steep a well-built leaf into punishment. Push the concentration higher and you buy almost no

additional grip; you buy haze and bitterness instead. That is the first of this chapter's honest limits, and we design to it rather than against it.

Running the Index formula itself is a single line of arithmetic once the EC_{50} is in hand. Take a designed green tea whose *whole-extract* half-astringency — the dilution at which the brewed leaf reaches half its maximum grip — sits at EC_{50} = 300 µM:

```
IFI-A = 1,000 × 420 µM ÷ 300 µM = 1,400
```

Fourteen hundred — the same grip the Index logs for **Phoenix**, our full-spectrum pepper, whose astringency sits at **A 1,400** beside its heat of 4,200. A pepper engineered to register on all eight axes and a quietly built green tea can arrive at the identical reading on the one axis they share, because the Index measures the loudness of the stimulus and not the plant it came from. That is the whole purpose of a number: it lets a chili and a leaf be laid on the same page.

The four catechins, and what a gallate ester is worth

Four catechins dominate the leaf, and they are not equal. Two carry a **gallate ester** — a third aromatic ring, bearing three more hydroxyls, esterified to the C3 position — and two do not; two carry a **trihydroxy (pyrogallol) B-ring** and two carry only the dihydroxy (catechol) B-ring. Each feature adds binding contacts, and because avidity multiplies across contacts, each feature multiplies the grip. Measured against the EGCG anchor, the ladder is clean:

CATECHIN	B-RING	GALLATE ESTER	EC_{50} (MM, VS REF PRP)	IFI-A
Epicatechin (EC)	dihydroxy	no	2,100	200
Epigallocatechin (EGC)	trihydroxy	no	1,680	250
Epicatechin-3-gallate (ECG)	dihydroxy	**yes**	525	800
Epigallocatechin-3-gallate (EGCG)	**trihydroxy**	**yes**	420	**1,000**

Each IFI-A is just the formula applied: 1,000 × 420 ÷ EC_{50}. And the four numbers hide a strikingly simple law. Compare EC (200) with ECG (800) — the only difference is the gallate ester, and the grip rises **fourfold**. Compare EGC (250) with EGCG (1,000) — again only the gallate differs, and again the grip rises **fourfold**. Now compare the B-ring alone: EC (200) to EGC (250), and ECG (800) to EGCG (1,000) — each extra B-ring hydroxyl adds **twenty-five percent**. The whole table collapses into one multiplicative model:

```
IFI-A ≈ 200 × g × b, where g = 4 if galloylated (else 1), b = 1.25 if trihydroxy B-ring (else 1)
```

EGCG is simply 200 × 4 × 1.25 = 1,000 — both potency-raising features, the trihydroxy B-ring and the gallate ester, stacked on one small molecule, which is exactly why strong green tea dries and bites at once. The gallate factor of four is the molecular picture made quantitative: the extra galloyl ring roughly doubles a catechin's binding surface, and a doubled surface, read through multivalent

avidity, lands near a fourfold jump in half-precipitation potency. This is the single most important lever on the entire tea-astringency problem, and the leaf hands us the enzyme that sets it.

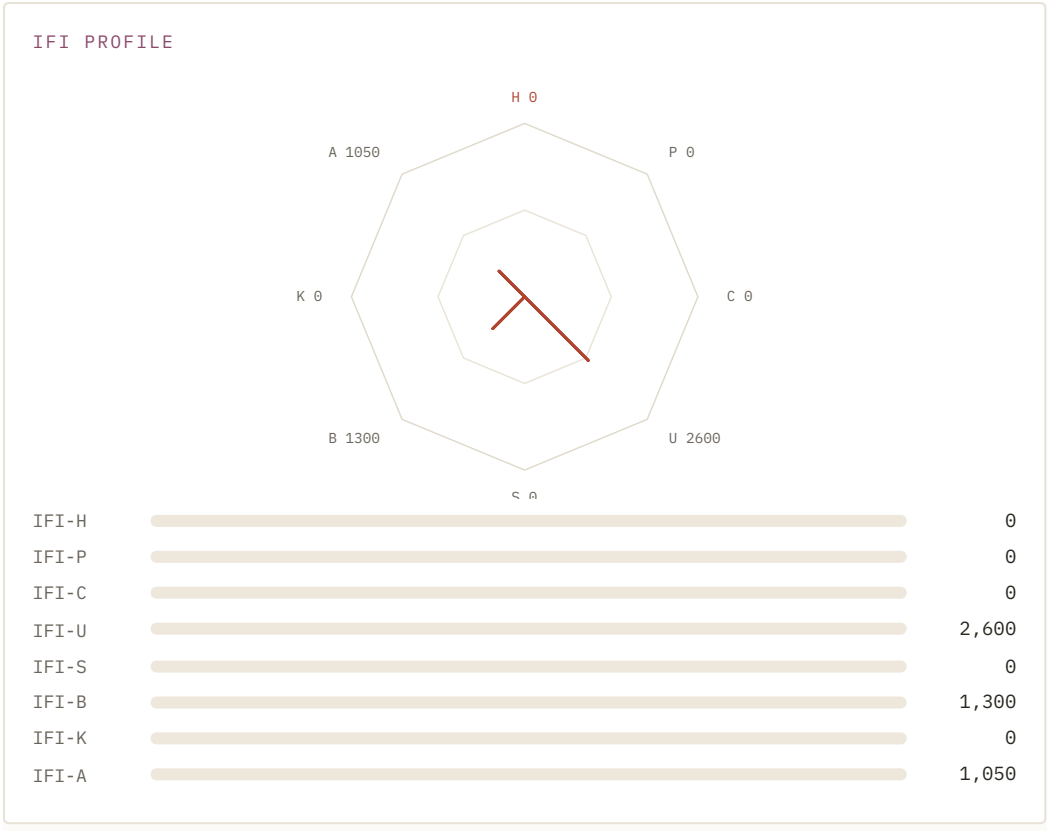


A tea leaf in cross-section. The flavan-3-ols are made and stored in the leaf's vacuoles; shading, plucking standard, and the galloylating enzyme together decide how much grip each cell will carry to the cup.

Theanine, and the chord the grip must live in

A tea that were grip alone would be undrinkable, and the leaf knows it. The counterweight is **L-theanine**, γ-glutamylethylamide, the amino acid very nearly unique to tea, which the plant builds in its roots by theanine synthetase and ships up into the leaf. Theanine is umami — soft, brothy, faintly sweet-edged — and it speaks the **IFI-U** axis through **mGluR1**, the same quiet *yes* that glutamate gives a broth. It is theanine that makes a fine green tea taste *round* rather than merely sharp, and the central design problem of green tea is, at its heart, a single ratio: the proportion of grip to roundness, the **catechin-to-theanine balance**.

We write that balance as a molar ratio and treat it as a design target. A sun-grown sencha, catechins driven high by full light, runs grip-forward — a catechin:theanine ratio near **8:1** — and reads as bright and cutting. Shade the plant for the weeks before plucking, the practice behind gyokuro and the finest matcha, and two things happen at once: light-hungry catechin synthesis falls, and theanine, no longer drawn down as fast, accumulates. The ratio drops toward **3:1**, and the cup turns sweet and broth-like with a clean edge instead of a cutting one. Our designed theanine lift reaches the same destination by a different road — a titre set at the source rather than chased in the field — and the two can be stacked, pushing a construct toward a **1.5:1** balance while the grip itself is held exactly where we want it. The result is a tea built so that the umami arm of the scale is raised to meet the tannin arm, which is why it can be brewed the way people actually brew — too hot, too long — and stay round instead of turning punishing. Here is the target profile we hold for such a construct, a shaded green built for roundness over a real but gentled grip:



Read it as a chord, not a column. The **A** of 1,050 is a genuine grip — EC_{50} near 400 µM, a leaf that dries the mouth with intention. The **U** of 2,600 is the theanine raised to answer it. And the **B** of 1,300 is the third voice: a designed attenuation, 20 to 65 percent of the harsh TAS2R38 bitterness that galloylated catechins unavoidably brush on their way up the astringency scale, dialed down so the grip reads as structure and not as a scold. The zeros are the discipline — a tea is not a pepper, and we do not make it pretend to heat or sweetness it has no business carrying.

Oxidation — the grip does not vanish, it moves

Then comes the fork that makes tea *tea*. Bruise or roll the leaf and you rupture the cells, releasing **polyphenol oxidase** — PPO, a copper enzyme the trade's word "fermentation" slanders, since no microbe is involved — which takes the catechins and their oxygen and makes **quinones**, reactive and eager to couple. Two catechins condense, especially the galloylated ones, into a **theaflavin**: a larger orange-red molecule built around a benzotropolone core that carries the *briskness* of a good black tea. Drive the oxidase further and the theaflavins polymerize into **thearubigins**, the brown, high-molecular-weight pigments — less a compound than a continuum of them — that give black tea its body, its depth of color, and much of its long, soft astringency.

The theaflavins are a design space in their own right, and they obey the same gallate law as the catechins that made them:

THEAFLAVIN	GALLATE ESTERS	RELATIVE BRISK GRIP
Theaflavin (TF1)	0	base briskness
Theaflavin-3-gallate	1	firmer
Theaflavin-3′-gallate	1	firmer
Theaflavin-3,3′-digallate (TFDG)	**2**	**highest — the brisk edge of a fine black**

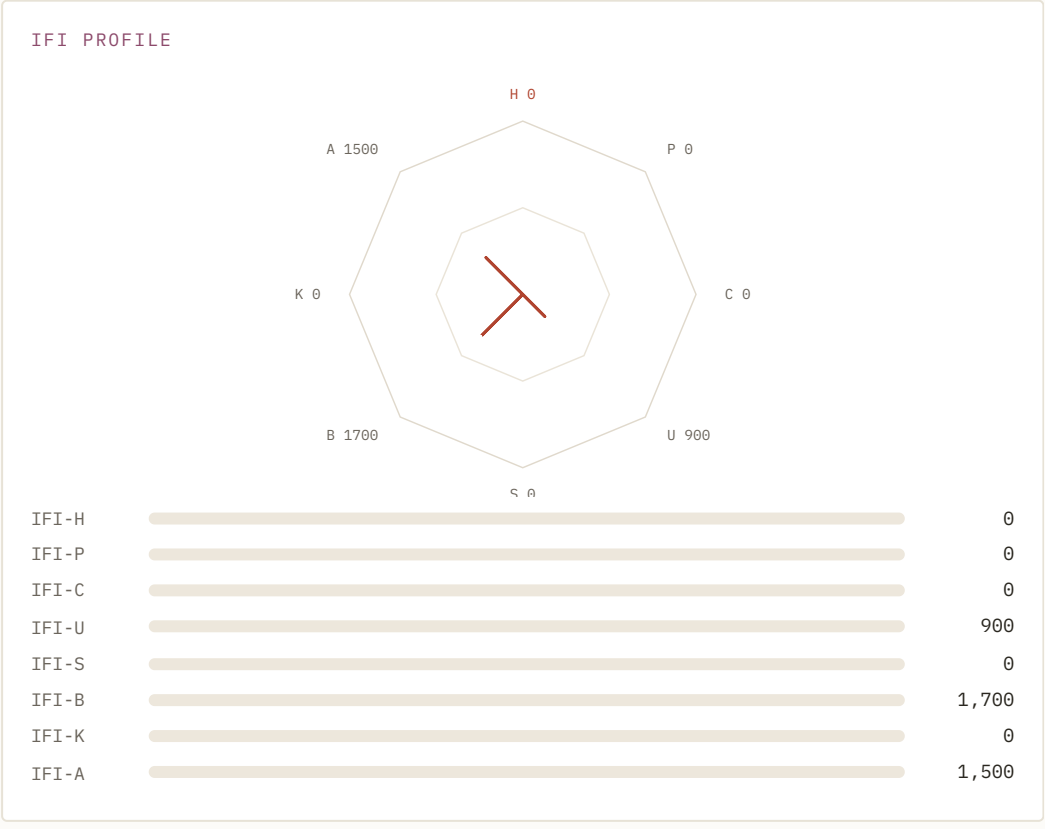
The crucial fact for the designer is that oxidation does not *destroy* the grip; it **relocates** it. Follow the arithmetic on a leaf picked with 100 units of bright catechin grip — defined as its catechins summed

and weighted by their IFI-A — and let PPO oxidize a fraction *f*. A black tea runs $f \approx 0.85$:

```
surviving bright-catechin grip = (1 − 0.85) × 100 = 15 units
oxidized pool = 0.85 × 100 = 85 units, partitioned by PPO
```

Of those 85 oxidized units, roughly a quarter settle into theaflavins — call it 21 units of **brisk** grip, galloyl-heavy and sharp-edged — and the remaining 64 units become **thearubigin** body, a slower, rounder, color-married astringency. Green tea's grip is its native catechins: bright, immediate, EC_{50} low, the whole of it on the front of the tongue. Black tea's grip is 15 parts surviving catechin, 21 parts brisk theaflavin, and 64 parts thearubigin body — larger, rounder, slower, married to color. Oolong sits between, the oxidase stopped partway on purpose. One leaf, three settlements, and the only real variable is how far you let the enzyme go before firing halts it.

There is even a fifth character, and it is astringency's own chemistry rehearsing in the pot. **Caffeine** complexes with the galloylated catechins and the theaflavins — the identical multivalent stacking that binds a catechin to a salivary protein — and as a strong black tea cools the complex falls out as the pale haze the trade calls *cream*. A tannin–alkaloid precipitation in the cup is the same event as a tannin–protein precipitation on the tongue; the mouth simply hosts the version you can feel. A designed black tea, then, is set not merely by its catechin load but by how far the oxidase takes it — the **theaflavin-to-thearubigin balance** that decides whether the finished cup reads brisk and bright or deep and malty. Here is a construct built for briskness without harshness: the theaflavins tuned up, the raw galloylated-catechin edge tuned down, the grip carried as spine rather than rasp:



The **A** of 1,500 is higher than the green tea's — black tea carries more total grip once theaflavins and thearubigins are counted alongside the survivors — but it is a rounder 1,500, its EC_{50} near 280 µM reached through body rather than through a bright monomeric edge. The **U** has fallen to 900, because oxidation and firing spend

some of the leaf's native softness, and the **B** has climbed to 1,700, a firmer bitterness attenuation to keep a strong black from turning punishing as it cools and the cream begins to form.



The bench where a leaf's chemistry is read before it is grown — the sequencing and assay work that turns a cup's grip into a number we can hold batch to batch.

The levers, and the two hundred and eighty constructs

Everything upstream of the pot is a lever, and the trade has worked these for centuries without the molecular names. We have only given the names back, so the levers can be set at the source and held. Four parameters carry the whole tea-astringency design, and our **two hundred and eighty constructs** explore them against the umami counterweight so the chord stays whole:

LEVER	MOLECULAR KNOB	WHAT IT MOVES	DESIGN RANGE
Total flavan-3-ol flux	LAR / ANR branch of the flavonoid pathway	the **ceiling** of grip — the leaf's raw IFI-A potential	`sets A headroom`
Galloylation fraction	**ECGT** galloyltransferase (SCPL-type) + β-glucogallin supply	how **hard** each catechin grips	`×1 → ×4 IFI-A per catechin`
Polyphenol oxidase activity	PPO expression; wither-and-roll intensity	how far bright grip converts to **brisk** theaflavin and **body** thearubigin	`0 → 85%+ oxidation`
Theanine titre	theanine synthetase (root) + leaf transport	the **umami** that rounds the whole thing	`catechin:theanine, ~8:1 → ~1.5:1`

Total catechin sets the ceiling; **galloylation fraction** is the fast lever on how hard that ceiling pulls, because it moves each catechin along the fourfold gallate factor; **PPO activity** decides how much of the grip is spent into briskness and body; and **theanine titre** sets the ratio that decides whether a strong tea reads as structured or as merely astringent. A designed green tea with a target grip and a lifted theanine can be brewed strong and long — three minutes, near-boiling, the way most people over-steep — and stay round, because the dose–response curve has already bent over near seventy percent and the umami arm was built up to meet the tannin arm. A designed black tea can carry briskness without harshness, its theaflavins tuned up and its raw edge tuned down, so the grip reads

as spine. None of it tastes engineered. It tastes like the finest version of itself, on purpose, every season — which, in a crop as vintage-dependent as tea, is the whole point.

Holding the cup to ±7% — and what the number cannot promise

We hold every construct to the same batch consistency that governs the rest of the library: a coefficient of variation of **≤7 percent**, against the **42 percent** that an unmanaged crop drifts across seasons. In a plant as weather-ridden as tea this is close to heresy, and it is exactly the service a tea house needs — the ability to order the same cup twice. The arithmetic of that promise is worth seeing plainly. For the shaded green construct, target IFI-A 1,050:

> at CV ≤ 7%: σ ≈ 0.07 × 1,050 = 74 → ~95% of batches fall in **900–1,200** at CV = 42%: σ ≈ 0.42 × 1,050 = 441 → batches sprawl across **170–1,930**

The first is a cup; the second is a lottery. And because the Index score is an *inverse* of a measured concentration — IFI-A = constant ÷ EC_{50} — the fractional error transfers one-to-one: a five-percent uncertainty in the EC_{50} read-out is a five-percent uncertainty in the score, no more and no less. CV(IFI-A) = CV(EC_{50}). We like that the error propagation is this clean, because it means the number we print is as trustworthy as the assay beneath it, and no more — which is the only honest way to print a number.

Honesty is also knowing what the grip-number cannot promise, and tea tests every limit at once. The dose–response saturates, so **total catechin is not a lever past the PRP saturation point** — beyond it, more catechin buys haze, bitterness, and cream, not more grip; the finite protein pool simply runs out. Astringency and bitterness are **coupled at the molecule**: the very galloylation that lifts IFI-A also brushes TAS2R38, so grip cannot be raised cleanly without engaging the B axis to pay the bitterness back down — the chord is not fully separable, and we design it as a chord for that reason. The oxidase is a **window, not a monotonic knob**: push PPO too far and the briskness of theaflavins collapses into flat thearubigin dullness. The cup is **context-dependent** in ways the leaf cannot overrule — milk's caseins bind the tannin and blunt a black tea's grip, acids and polysaccharides soften it, temperature and repeated sips change it as the saliva is stripped across a session. And the standard against which we measure is a **reference saliva**, not every mouth: the PRP content a drinker secretes varies with flow rate, diet, and genotype, so IFI-A is faithfully the loudness of the leaf against a fixed listener, not a guarantee that two people feel the identical pucker.

We state these limits not as apologies but as the shape of the problem, because the problem is beautiful and we would rather show it whole. The grip of tea was always the leaf's — built by the plant to defend its seed, answered by a mouth that pours out protein to pay the tannin off at the door, an ancient transaction we only learned to read. What we have done is make the argument inside the leaf one the drinker gets to win: the catechin set where we mean it, the

gallate ester counted, the oxidase stopped on purpose, the theanine raised to round it, and the whole chord held to seven percent so the finest version of the cup arrives, with intention, every single time.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 7 · Pomegranate — *the most potent grip we grow.*

Of the six plants that speak this axis, the pomegranate grips hardest, and it grips by the other chemistry. Where grape and tea build **condensed** tannin — carbon-linked flavan-3-ol chains, catechin stacked on catechin until the molecule is a long, soft rope — the pomegranate builds **hydrolyzable** tannin, the sugar-and-gallate architecture, and takes it to an extreme. Its signature molecule, **punicalagin**, is a great ellagitannin: a single compound massive by small-molecule standards, near 1,085 grams per mole, dense with galloyl and hexahydroxydiphenoyl groups, and by every rule in our structure tables it is built to precipitate protein. **One hundred and twenty constructs**, aimed at a fruit whose rind and pith are, pound for pound, among the most astringent tissues any plant grows. We rank the pomegranate Hard, and we mean it; the grip you can feel in a sliver of its inner rind is the hardest thing on this axis to design, to hold steady, and to place on purpose.



Pomegranate, whole and halved. The jeweled arils carry the sweetness and the color; the pale pith and rind around them carry punicalagin, a hydrolyzable tannin whose grip arrives fast and reads high on IFI-A.

The pomegranate's grip behaves differently from the grape's, and the difference is worth dwelling on because it is the whole difference between the two tannin families made sensory. Punicalagin and its relatives are small enough to be fast and galloyl-dense enough to be potent: the pucker arrives **quickly**, hits **high** on the IFI-A scale, and carries a clean, almost bright edge rather than the slow velvet build of a long grape polymer. Alongside punicalagin sit **punicalin**, free **ellagic acid**, the gallagic-acid units, and the anthocyanins that color the arils — the whole package reading, in a ripe fruit, as a vivid sweetness wrapped around a firm, immediate grip. The seed and the

pith and the inner rind are the tannic reserves; the juice carries a fraction of it, which is exactly why pomegranate juice is refreshing and faintly drying at once, and why the molasses made from it — reduced, concentrated — becomes one of the great sour-astringent seasonings of Persian and Levantine cooking. We grow this plant with a cook's question always in hand: not only how much grip it can make, but where in the fruit that grip waits, and whether a hand reaching into the bowl can get to it.

The two tannin families, made sensory

Astringency is not a taste the way sweetness is a taste. There is no cell on the tongue waiting to bind a tannin and fire. The sensation is mechanical and it is built in the saliva: the salivary **proline-rich proteins (PRPs)**, flexible open-chain molecules whose job is to be sacrificial, meet a tannin and are cross-linked and precipitated out of solution. The thin lubricating film that lets the tongue glide over the palate thickens, curdles, and fails; the drag we read as *grip* is friction where a moment ago there was slip. Every number on this axis is, at bottom, a measure of how efficiently a molecule can collapse that film.

That reframing tells us at once why the two tannin families feel so different. A tannin precipitates protein well when it can reach across and bind several PRP molecules at once — when it is **multivalent**, presenting many phenolic faces, and when those faces are held in a rigid, pre-organized geometry so that binding one does not cost the freedom to bind the next. Condensed and hydrolyzable tannins solve that problem in opposite ways.

Condensed tannins — the proanthocyanidins of grape and tea — grow their valency by growing *long*. Each added flavan-3-ol unit is another binding face, so a high polymer carries many; but the chain is floppy, the faces are not held in register, and the whole molecule is large and slow. Hydrolyzable tannins reach the same valency in a **compact, stiff** package: punicalagin hangs its galloyl and HHDP groups off a glucose and gallagyl core that holds them splayed and ready, roughly half a dozen protein-binding faces on a molecule that is, for all its mass, far smaller and far more rigid than a serious grape polymer.



Grape, the condensed-tannin counterpoint. Its proanthocyanidins reach their grip by growing long and soft; the pomegranate reaches a harder grip by staying compact and rigid.

Size sets the *speed* of the grip, and here a single physical law does the explaining. A molecule's diffusion coefficient in water falls as its hydrodynamic radius rises — the Stokes–Einstein relation, `D = kT /`

$(6\pi\eta r)$, where η is the viscosity of the saliva and r the effective radius of the tannin. Punicalagin, compact and rigid, has a small r and so a large D; it reaches the salivary film and begins precipitating PRPs almost the instant the fruit is bitten. A long grape proanthocyanidin, with several times the radius, diffuses several times more slowly, and its grip unfolds over seconds — the "velvet build" a winemaker prizes. Fast and bright versus slow and rounded is not a metaphor we reach for; it is $1/r$ read on the tongue.

	CONDENSED (GRAPE, TEA)	HYDROLYZABLE (POMEGRANATE)
Building block	flavan-3-ol (catechin)	galloyl + HHDP on a glucose core
Linkage	C–C interflavan bond (stable)	ester, galloyl–sugar (hydrolyzable)
Signature molecule	proanthocyanidin polymer	punicalagin (ellagitannin)
Molecular size	large, long, flexible	mid-sized, compact, rigid
Diffusion to PRP film	slow (large r)	fast (small r)
Grip onset	slow, velvet build	quick, bright arrival
IFI-A character	rounded, sustained	high, immediate, clean edge

This is why the pomegranate earns a book-chapter of its own on an axis it shares with five other plants. It is the family's clearest demonstration that two molecules can land at the same height on IFI-A and feel like different instruments — one a cello drawn slowly, the other a struck bell — and that the Index, read carefully, hears the difference in the kinetics even when the peak heights match.

Reading astringency as a number

The Iyengar Flavor Index puts a single number on each axis, anchored to the half-maximal response of a receptor. For every other axis that anchor is a receptor activation — a channel opening, a G-protein firing. For astringency the "receptor" is the salivary PRP system itself, and the half-maximal response is a half-maximal *precipitation*. We keep the same formula and we are honest about the one place it bends:

> $\text{IFI-A} = 1{,}000 \times EC_{50}(\text{reference}) / EC_{50}(\text{candidate})$

EC_{50} is the concentration at which a stimulus drives its target to half of full response — here, the concentration of tannin at which the salivary film is driven halfway to collapse, measured as half-maximal PRP precipitation in the standard assay and cross-checked against a trained panel's half-maximal perceived grip. The Index fixes the reference so that **IFI-A = 1,000 marks the half-maximal grip of the standard** — a defined tannin preparation at its own half-precipitation concentration, which we carry as $EC_{50}(\text{reference})$. Every candidate is read against that one anchor. A molecule that reaches half-maximal grip at a *lower* concentration than the reference is more potent, and its IFI-A rises above 1,000; a weaker one falls below. Loudness, not quantity: not how much tannin a fruit holds, but how efficiently that tannin collapses the film.

The dose–response underneath the single number is sigmoidal, and for tannins it is **steeper than a simple binding curve**, which matters to anyone reducing a syrup or blending a juice. Write the response as a Hill function,

$$R([C]) = R_{max} \times [C]^n / (EC_{50}^n + [C]^n)$$

where $[C]$ is tannin concentration and n is the Hill coefficient. Set $[C] = EC_{50}$ and the two terms in the denominator are equal, so $R = R_{max} / 2$ — the definition of EC_{50}, independent of n. The Hill coefficient controls how abruptly grip switches on across concentration. For astringency n runs above one — illustratively $n \approx 1.5$ to 2 — because the precipitation is **cooperative**: one ellagitannin bridges several PRPs, the growing aggregate exposes fresh binding surface, and collapse feeds collapse. The practical consequence is a narrow band in which a fruit tips from refreshing to gripping. A pomegranate juice reduced a little too far does not drift toward astringency; it crosses into it. The cook who has scorched a pan of pomegranate molasses into harshness has met the Hill coefficient without a name for it.

There is a clean bridge from the biology of the molecule to the number on the page, and it is the lever the whole chapter turns on. Model each tannin as presenting v accessible phenolic binding faces, each binding a PRP site with intrinsic affinity k_0. To first order the half-precipitation concentration falls as the product rises,

$$EC_{50} \propto 1 / (v \cdot k_0),$$

so, substituting into the Index formula,

$$\text{IFI-A} \propto v \cdot k_0.$$

The astringency score rises with binding valency and per-face affinity — and those are exactly the two quantities the pomegranate's biosynthesis builds. The galloylation steps raise v by hanging more phenolic groups on the sugar; the oxidative coupling step raises *both* v and k_0 by locking pairs of galloyls into rigid HHDP bridges that are pre-organized to grip. The biosynthetic dial and the sensory number are the same quantity seen from two ends.

The molecules of the grip

The pomegranate's grip is not one compound but a small, related family, and to design it we model them as a set. Their masses and their binding valencies are the raw material of every calculation that follows.

COMPOUND	FORMULA	MW (G/MOL)	CLASS	BINDING FACES, V
Gallic acid	$C_7H_6O_5$	170	phenolic acid	1
β-glucogallin	$C_{13}H_{16}O_{10}$	332	galloyl-glucose	1
Ellagic acid	$C_{14}H_6O_8$	302	HHDP lactone	2
Pentagalloyl glucose (PGG)	$C_{41}H_{32}O_{26}$	940	gallotannin	5
Punicalin	$C_{34}H_{22}O_{22}$	782	ellagitannin	~3
Punicalagin	$C_{48}H_{28}O_{30}$	**1,085**	ellagitannin	**~6**

Read down the valency column and the potency story reads with it: a lone gallic acid, with its single face, barely dents the salivary film; pentagalloyl glucose, with five, is a serious astringent; punicalagin, with roughly six faces held rigid on a gallagyl–glucose scaffold, is among the most efficient protein-precipitating small molecules a plant makes. The `v ∝ IFI-A` relationship is not a tidy abstraction here — it is the literal climb down this table.

Punicalagin itself is a shape-shifter worth a paragraph, because it teaches a general lesson about the hydrolyzable tannins. It exists as two slowly interconverting forms, an **α and a β anomer**, that a careful chromatogram resolves as two peaks and the tongue reads as a single grip — a reminder that even a lone astringent "compound" is often a small family in equilibrium with itself. That is why the pomegranate, for all its brightness, rewards the same disassemble-and-read discipline as any tannic plant: we model the two punicalagin anomers, the punicalin, the free ellagic acid, and the anthocyanin load together, and we set the pathway to place their *sum*, expressed as IFI-A, where the dish wants it.

A word on units, because the Index speaks in loudness and the lab bench speaks in milligrams. Concentrations convert through the molar mass. Punicalagin at **1 µM is 1,085 µg/L, or 1.085 mg/L**, since one micromole of a 1,085 g/mol molecule weighs 1,085 micrograms. So a half-maximal grip modelled at, say, 50 µM corresponds to

> `50 µM × 1,085 g/mol = 54,250 µg/L =` **`54.25 mg/L`** `punicalagin.`

Hold that figure against the fruit. Aril-only juice carries punicalagins as a small fraction of a gram per liter; whole-fruit juice pressed with the rind can carry them into the hundreds, even the low thousands, of milligrams per liter — tens of times above a 54 mg/L half-maximal point, far up the plateau of the dose–response. By the raw chemistry such a juice should be punishing. It is not, and the reason is one every cook of the fruit knows in the hand: the sugars and acids of the arils ride alongside the tannin and mask much of its grip, so *perceived* astringency sits well under what the tannin load alone predicts. The number on the bench and the number on the tongue are related by the matrix the molecule travels in.

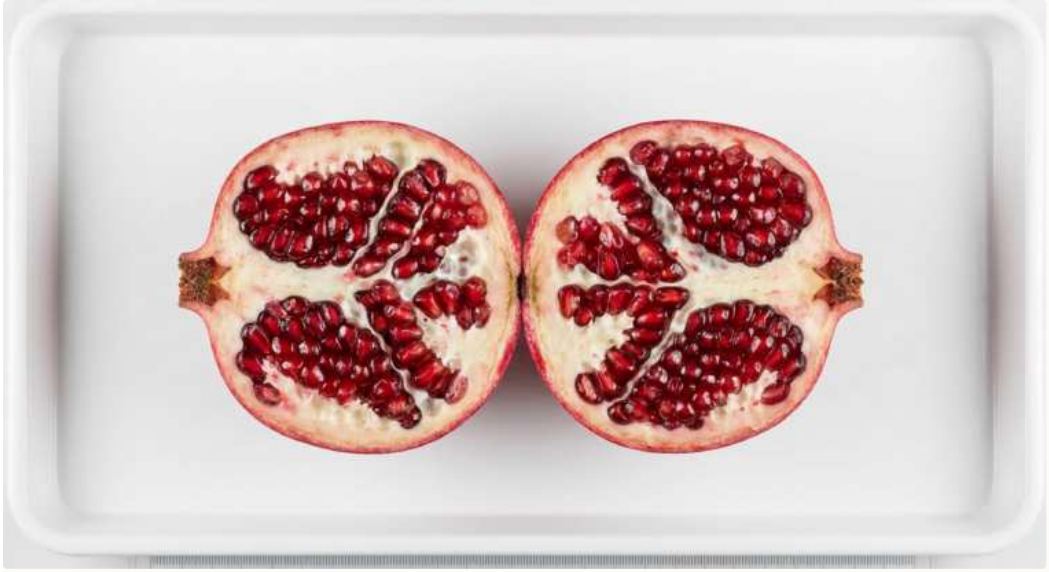
The biosynthesis — the gallate route

The pomegranate is a separate design problem from the grape, and a hard one, because it is built by different enzymes. The grape's grip runs down the flavan-3-ol branch; the pomegranate's runs the **gallate route**, and the two share almost nothing past the common phenylpropanoid trunk. We lay the route out as a ladder of steps, each with the enzyme that drives it and the product it yields, because each step is a place we can push.

STEP	ENZYME	PRODUCT
Shikimate → gallate	Dehydroshikimate dehydrogenase	**Gallic acid**
Activation	UGT84A (glucosyltransferase)	β-glucogallin (galloyl-glucose)
Galloylation	Galloyltransferases	**Pentagalloyl glucose (PGG)**
Oxidative C–C coupling	Laccase-type oxidases	HHDP units → **ellagitannins (punicalagin)**

Three levers, then, in the order the ladder climbs. The **gallic acid supply** sets how much raw galloyl the fruit can make — the budget the whole route spends. The **galloylation flux** builds β-glucogallin up through the galloylated intermediates to pentagalloyl glucose — this is where v first climbs, where a sugar with one face becomes a sugar with five. And the **oxidative coupling**, the laccase-type step, is the one that takes a merely gallated sugar and makes it a punicalagin: it welds adjacent galloyls into HHDP bridges, raising valency and — because those bridges are rigid and pre-organized — raising per-face affinity k_0 at the same time. In the language of the number, the first two levers raise v; the third raises $v \cdot k_0$ together. Turn them up and the fruit's grip firms and its IFI-A climbs toward the top of the scale. Turn them down and you get a softer, sweeter, more purely fruity pomegranate whose arils you could eat by the handful without the drying edge.

That the route is known enzyme by enzyme is what lets us design it rather than stumble on it. The method is the one our family developed and first applied on the heat axis: an ESM2 protein-language model scores candidate substitutions in a target enzyme for predicted effect on catalytic efficiency and fold stability, and our flux-balance model locates where control truly sits — in our capsaicin work that turned out to be upstream in the phenylpropanoid pathway, at 4CL and HCT/CCoAOMT, not at the terminal Pun1 synthase — so we edit where the leverage is, not where intuition might point. Only the handful of edits that survive both filters are written in with CRISPR. On the capsaicin enzyme that program identified the double variant **S39L+L345G** — two substitutions no breeder had ever combined — predicted to push peak output toward its design ceiling, held to a batch-to-batch coefficient of variation at or under **7%**. The pomegranate's galloyltransferases and laccase-type oxidases are a different protein family and a different search, but the discipline is identical: model the enzyme, locate the true rate-limiting step, edit the few residues that move it, and place the design outcome as IFI-A at a CV the kitchen can rely on. One hundred and twenty constructs is the pomegranate's share of that work — modest beside the grape's sixteen hundred, and every one of them hard-won, because the gallate route is less charted than the flavonoid branch and the fruit's grip is the subtlest thing we place.



Pomegranate in cross-section. The grip is not in the jeweled arils but in the pale pith, the inner rind, and the membranes that cradle the seed — the plant's own polyphenol defense, and the richest design question in the fruit.

Worked example — placing the dial

Here is the arithmetic in full, because the numbers are a form of respect for the reader and because the whole promise of the Index is that a grip can be *placed*, not merely admired. Take the reference at its anchor, $EC_{50}(\text{reference}) = 100\ \mu M$, so that by construction IFI-A = 1,000 × 100 / 100 = 1,000 at the standard. Then for any candidate,

> $IFI\text{-}A = 1{,}000 \times 100 / EC_{50} = 100{,}000 / EC_{50}$ (in μM).

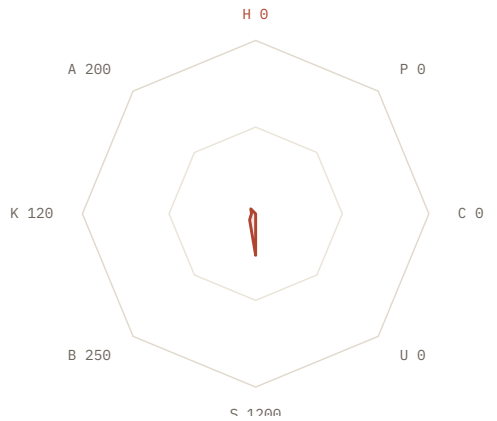
Walk a pomegranate down the dial and read the score at each setting. A dessert fruit bred soft reaches half-maximal grip only at a high concentration, 500 μM, so 100,000 / 500 = **200** — a whisper of structure under a lot of sugar. A market standard at 250 μM reads **400**. An old sour heirloom at 125 μM reads **800**. A fruit we design for the glaze, firm and culinary, at 66.7 μM reads 100,000 / 66.7 ≈ **1,500**. And a fruit designed for a full punicalagin load, half-maximal at just 50 μM, reads **2,000** — a grip with the structural weight of a young red wine, delivered fast and clean.

POMEGRANATE (DIAL SETTING)	EC_{50} (MM)	EC_{50} (MG/L)	IFI-A
Dessert, bred down	500	542	200
Market standard	250	271	400
Heirloom sour	125	136	800
Designed firm culinary	66.7	72	1,500
Designed high-punicalagin	50	54	2,000

The mg/L column is just the μM column times 1.085, the punicalagin conversion from the molecules table, so a formulator can meet the target on a balance as well as on the Index. For scale, hold these against the one full profile we anchor the whole library to: the all-axis flagship **Phoenix (SS-0096)**, which our model places at an astringency of **A 1,400** — against this same reference, a computed half-maximal point near $100{,}000 / 1{,}400 \approx 71\ \mu M$. A designed high-punicalagin pomegranate at 50 μM grips harder on this single axis than the cultivar we engineered to grip on all eight at once — which is precisely what we mean when we call the pomegranate the most potent grip we grow.

Two dial settings, written as Index profiles. The soft fruit for the hand, sweetness forward and grip kept low:

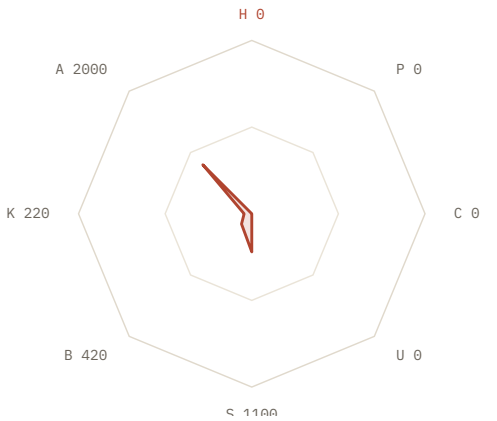
IFI PROFILE



IFI-H	0
IFI-P	0
IFI-C	0
IFI-U	0
IFI-S	1,200
IFI-B	250
IFI-K	120
IFI-A	200

And the structural fruit for the glaze and the bar, the same plant with the oxidative-coupling lever turned up, grip brought to the front while the aril sweetness still rides underneath:

IFI PROFILE



IFI-H	0
IFI-P	0
IFI-C	0
IFI-U	0
IFI-S	1,100
IFI-B	420
IFI-K	220
IFI-A	2,000

These are design targets, not a survey of what sits in a market bin — the dial settings the chapter keeps naming, with the astringency value derived from the EC_{50} relation above and the other axes placed where a pomegranate honestly lives: no heat, no pungency, no cool, no umami to speak of, a generous aril sweetness, a faint polyphenol bitterness, and a little kokumi body that rises slightly as the tannin load climbs.

Every one of those numbers carries an error, and we quote it. The design specification holds batch-to-batch variation at a coefficient of variation **at or under 7%**. Because IFI-A is inversely proportional to EC_{50} — write it as `IFI-A = k / EC₅₀` with `k` fixed — the relative error carries straight through: for any `Q = k/x`, differentiating gives `|σ_Q / Q| = σ_x / x`. A 7% CV on the specified EC_{50} is a 7% CV on the score. At A = 2,000 that is a one-sigma band of `0.07 × 2,000 = ±140`, so the design estimate is **A = 2,000 ± 140**, roughly 1,860 to 2,140. That tightness is itself the achievement. An unmanaged pomegranate's pith tannin swings far wider than that from season to season and tree to tree — the reason a cook can never quite trust a wild fruit's grip twice — and holding the design inside a ±7% band is most of what designing this axis *means*.

Where the grip lives — and the design question

The pomegranate hides its grip, and the hiding is the richest part of the design. The arils — the jeweled, edible seeds — carry the sweetness, the acid, and the anthocyanin color, and only a fraction of the tannin. The **pith, the inner rind, and the pale membranes** that cradle the seeds are the tannic reserve, the plant's own polyphenol armor around the thing it most wants to protect. The juice you press from the arils is faintly drying; the bitterness and the serious grip come only when the press reaches the rind.

So the design question for a pomegranate is not only *how much* tannin to make but *where to keep it* — a question most of the eight axes never raise. A fruit can carry a high total ellagitannin load locked away in a rind no one eats, and read, in the bowl, as merely sweet. The same load moved outward, into the membranes and the aril coat where a hand and a knife can reach it, reads as structure. Much of our hundred and twenty constructs is spent not on the biosynthetic levers at all but on *tissue placement*: tuning where in the fruit the galloylation and coupling run hottest, so that the grip we build is grip a cook can actually get to. A pomegranate bred for the handful and a pomegranate bred for the glaze can hold the same total tannin and differ only in its address.

And there is a quiet reversal in the work here. Most of the eating world has bred pomegranates *down* this axis — toward the soft, sweet, low-pith varieties, grip bred out as a defect. But the cook and the bartender often want the grip *back*: a pomegranate whose molasses carries real structure, whose juice can stand up to fat and sugar in a glaze or a shrub without disappearing. The pomegranate is the one plant on this axis where we most often design astringency *upward* on purpose, and it taught us something the grape, with its endless capacity for nuance, did not: that a fast, bright, honest grip is its own kind of beauty, and that the hydrolyzable tannins — long dismissed as the crude cousins of the subtle proanthocyanidins — are simply a different instrument, played in a different register. We grow this one to be played loud.

The cook's pomegranate

The cuisines that love the pomegranate have always understood its grip intuitively, and the Index lets us say plainly what they knew in the hand. The reduced molasses of Persian and Levantine cooking — the *rob-e anar*, the sour-dark syrup that lifts a walnut stew or

glazes an eggplant — is, in the language of this book, a **concentration step**: boil the juice down and you drive the tannin concentration up the dose–response curve, past the EC_{50}, into the gripping band, where the cooperative Hill kinetics tip the syrup from refreshing to structural over a surprisingly short reduction. A good molasses lands its IFI-A in the band of a young red wine, married to a bright acidity and a deep sweetness so that the grip reads as architecture rather than assault.

But a long boil extracts a cost. Drive a thin juice down far enough to reach structural grip and you also caramelize, then scorch — the reduction that builds the tannin also builds a burnt, flat bitterness that no amount of sugar quite hides. A designed high-punicalagin pomegranate hands the tradition a way around the toll: a raw material that already sits near the gripping band, so it reaches the same structural IFI-A with **less reduction, less time on the heat, and less of the scorched edge** a long boil brings. The syrup arrives at structure before it arrives at bitterness. The same logic serves the bar — a pomegranate shrub or a non-alcoholic aperitivo built on a fruit that grips cleanly needs no tannin borrowed from steeped tea or added to a cordial to give the drink a spine.

There is a nourishment note under all of it, and we state it as a fact and not a promise. A fruit designed to carry a fuller ellagitannin load is a fruit carrying more of the polyphenol chemistry it makes to defend its own seed — more of its own substance, grown into the fruit rather than bred out of it. Our family has always cooked and eaten this way: toward the whole plant, toward the fruit given its full chemistry rather than thinned to sweetness alone, toward food offered to the body with intention. We think a cook reaching for a pomegranate should get it whole — grip and color and the defensive polyphenols all of a piece — and the work in this chapter is, in the end, how to design that fruit on purpose and hand it over at a number the kitchen can trust.

Honest limits

We would mistrust any single number that did not come with its edges drawn, and this axis has more edges than most.

The first is the plainest: **astringency is not receptor gating.** The IFI-A "EC_{50}" is an operational half-maximal *precipitation*, not a half-maximal channel opening, and precipitation depends on the assay — the PRP preparation, the ionic strength, the temperature, the time allowed for aggregates to grow. We fix those conditions and cross-calibrate against a trained panel so the number travels, but a reader comparing an IFI-A to a figure from another lab's astringency method should confirm the two were anchored the same way. The formula is identical across all eight axes; the measurement under it, on this axis alone, is a protein assay wearing a receptor's clothes.

The second is the **anomer problem.** Punicalagin's slow $\alpha \rightleftharpoons \beta$ equilibrium means a single extract read at two times of day can give two chromatograms and, strictly, two grips, as the balance shifts. We report the sum of the anomers and specify the equilibration conditions, but it is a genuine reason the pomegranate resists a one-decimal answer in a way a pure capsaicinoid does not.

The third is the **matrix effect**, the same one that makes aril juice refreshing despite a tannin load that should punish. Sugar, acid, polysaccharide, and ethanol all modulate *perceived* astringency at a fixed tannin concentration — sugars and viscous polysaccharides shield the salivary film, acids sharpen the grip, ethanol in a wine changes the whole balance. A pomegranate's IFI-A is computed in a defined matrix; the same fruit in a sweet glaze, a sour shrub, and a dry reduction will read as three different intensities on the tongue even though the molecule never changed. The number is a property of the tannin; the sensation is a property of the dish.

And the fourth is a **failure mode of the design itself.** Grip without a frame is not structure — it is harshness. Push IFI-A up without the sweetness and acid to carry it and the fruit reads green, aggressive, unbalanced; the Hill kinetics that make a well-framed grip snap cleanly into place make an unframed one snap into something punishing. The art of designing this axis upward is never the tannin alone. It is the tannin *placed* — against the aril sugar, against the fruit's own acidity, in the tissue a cook can reach, at a number held steady to ±7% — so that the grip arrives as the thing a good red wine and a good pomegranate molasses have always had in common: a spine you taste as generosity rather than as a wall. The pomegranate is where designing astringency upward and growing a plant to hold the most it can turn out to be the same single move, and it is the plant that taught our family to make that move on purpose.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 8 · Blueberry and raspberry — *soft astringency and the berry's ellagitannin.*

The berries are where astringency goes gentle, and where the axis meets the sweetness and color that make fruit *fruit*. Blueberry carries **one hundred and twenty constructs**, raspberry **forty** — the smaller end of our astringency libraries — and both speak the **A** axis softly, in company with the sugars and anthocyanins that dominate the eating experience. You rarely think of a blueberry as astringent, and that is precisely the point: in a ripe berry the grip is a whisper under the sweetness, a faint tightening at the back of the bite that keeps the fruit from tasting flat. Design here is not about building a wall of tannin. It is about setting a *floor* — the quiet structural grip that gives a soft fruit its shape, held so low and so even that a person tastes it only as the reason the fruit feels whole.



Blueberries on the bush at the turn of ripeness — the bloom still on the skin, the color deepening from green through red to the blue-black that announces the fruit is ready. The pigment that colors the skin and the tannin that lightly grips it are built by the same flavonoid branch, in the same few cell layers.

Both berries are, by the standards of this axis, nearly empty vessels — and that emptiness is the design problem. On the organisms we tune for a loud grip, a grape or a pomegranate or a tea leaf, the work is addition: build more tannin, lengthen it, decorate it, push the number up. On a berry the work is almost entirely *restraint and registration* — place a small, clean quantity of grip exactly where it belongs beneath the sugar, and then hold it there, lot after lot, within a few percent. A great engineered berry is not more astringent than

a wild one. It is astringent in precisely the right small, reliable amount. To design that floor well you have to understand three things exactly: what a tannin does to a mouth, what kind of tannin each berry builds, and why — on a berry above all other fruit — the grip and the color are not two decisions but one.

The grip with no receptor

Astringency is the one axis on the Index that is not, strictly, a taste. Heat has TRPV1; sweetness has TAS1R2·R3; the savory depth of umami has mGluR1. Each is a receptor a molecule can key into, and the Index reads how loudly the molecule turns that key. Astringency has no such lock. What it has instead is a sacrifice. Human saliva pours out a constant supply of small, floppy, open-chain proteins stuffed with the amino acid proline — the **salivary proline-rich proteins**, the PRPs — and these are the receptor in the only sense that matters here: they are the thing the stimulus acts on, and the felt sensation is the consequence of that act.

Proline is the reason they work. Its side chain loops back and bonds to its own backbone nitrogen, forming a rigid five-membered ring that does two useful things at once. It presents a flat, slightly greasy face onto which the flat phenolic rings of a tannin can stack like tiles; and because its nitrogen is tied up in that ring, it keeps its backbone carbonyl oxygen fully exposed as a hydrogen-bond *acceptor*. A tannin is covered in phenolic hydroxyl groups, which are superb hydrogen-bond *donors*. The complementarity is almost unfair: the tannin's hydroxyls donate to proline's open carbonyls while its aromatic rings press against proline's hydrophobic face, and a proline-rich chain holds its backbone out in an extended, unspooled coil that offers those carbonyls in a row, like a runway with the lights left on.

A single such contact is weak and fleeting. The thing that makes astringency happen is **multivalency**: a tannin carries many binding groups and a PRP presents many proline sites, so the two fasten at several points at once, and each tannin — having more than one hand — can grab more than one protein. Very quickly the solution knits itself into a growing three-dimensional network of tannin bridging protein bridging tannin, until the network can no longer stay dissolved and separates out, first as a haze and then as a precipitate. That phase separation, happening in the thin film of saliva on the tongue, is the drying you feel. The proteins that were lubricating the mouth are now curds on their way out of solution, and the trigeminal nerve endings that report a coarse surface report the loss of slip as a rough, puckering grip. The chemistry happens first; the sensation is its echo.

For a berry this matters in a specific way. There is so little tannin in a ripe blueberry or raspberry that the question is not whether it will overwhelm the saliva — it never will — but whether it will register *at all*, cleanly and repeatably, as that faint structural tightening. A floor is a harder thing to hold than a wall. It must be high enough to be felt as shape and low enough never to be felt as grip, and the window between those is narrow.

Two families of tannin, two kinds of grip

The two berries make a neat matched pair for teaching the axis, because they grip by the two different chemistries that the whole tannin world divides into — and set side by side, under all that sweetness, they let you feel the difference in miniature.

Blueberry builds condensed tannin. Its grip comes from **proanthocyanidins** — polymers of flavan-3-ol units, the catechins and epicatechins, strung together by carbon–carbon interflavan bonds — concentrated in the skin alongside the anthocyanins that give the fruit its blue-black color. Most of these linkages are the common **B-type**, a single bond from the C4 of one unit to the C8 (or C6) of the next. But blueberry, like its cranberry cousin, carries an unusually high share of the tighter **A-type** linkage, in which a second bond — an ether bridge from C2 to the oxygen on C7 — doubly fastens two units into a compact, rigid, doubly-locked pair. That second bond is the molecular root of blueberry's particular quality: an A-type proanthocyanidin is stiffer and more folded-in than a B-type chain of the same length, so it grips a little more roundly and lets go a little more gently, which is a large part of why a blueberry dries so softly despite genuinely carrying tannin.

Raspberry grips by the other family entirely. It is an **ellagitannin** fruit — a *hydrolyzable* tannin, built not by stringing flavan-3-ol units but by hanging galloyl groups on a sugar core and then oxidatively coupling them. The signature is the **hexahydroxydiphenoyl** group, the HHDP unit, two galloyl rings coupled side to side; on hydrolysis it springs open into **ellagic acid**, the molecule the whole class is named for. Raspberry's dominant tannins are **sanguiin H-6**, a dimer of about **1,871 g·mol^{-1}**, and the still larger **lambertianin C**, a trimer of about **2,804 g·mol^{-1}** — among the biggest molecules any fruit builds, kin to the punicalagins of pomegranate. This is why a raspberry's astringency, faint as it is, has a different *character* than a blueberry's: brighter, faster, cleaner-edged — the hydrolyzable signature — where the blueberry's is rounder and slower, the condensed signature.



A raspberry, whole and bright — an aggregate of tiny drupelets around a hollow core, each one a thin skin over juice and an ellagitannin grip. The hydrolyzable tannins here, sanguiin H-6 and lambertianin C, are among the largest molecules in the berry world, and their fast, clean edge is the signature of the whole hydrolyzable family carried into something you can eat by the bowlful.

FRUIT	TANNIN CLASS	SIGNATURE MOLECULES	LINKAGE / CORE	MASS PER MOLECULE	ONSET CHARACTER	CONSTRUCTS
Blueberry	Condensed (proanthocyanidin)	B-type + A-type procyanidins	C4→C8 interflavan (± C2→O→C7 ether)	~290 (unit) to multi-kDa polymer	rounder, slower	120
Raspberry	Hydrolyzable (ellagitannin)	sanguiin H-6, lambertianin C	galloyl–glucose, HHDP couplings	1,871 (dimer); 2,804 (trimer)	brighter, faster	40

The two chemistries also behave differently in the one place a measurement has to be honest about — their molar mass. A condensed tannin is not one molecule but a *distribution*: a blueberry extract spans trimers to twenty-mers, so any single molar mass you assign it is a mean over a spread, and the mean drifts with harvest. A raspberry ellagitannin is a defined molecule with an exact formula and an exact weight. That distinction will matter the moment we try to turn a grip into a number, because it decides whether the number can be reproduced.

Tannin and pigment are siblings

The deepest fact about a berry's grip is that it cannot be designed alone. Astringency and color come off the **same flavonoid branch**, and on a berry the two outputs sit so close together on the pathway that tuning one bends the other. This is the principle that runs through the whole axis, and the berry is its clearest illustration: **tannin and pigment are siblings.**

Trace the branch and the sibling relationship becomes a fork you can point to. Phenylalanine enters the phenylpropanoid route and is built up, step by enzyme, into the flavanone naringenin, then hydroxylated and reduced through **dihydroflavonol-4-reductase (DFR)** into a **leucoanthocyanidin** — a flavan-3,4-diol that is the last intermediate both outputs share. From that single pool the path forks:

- Down one tine, **anthocyanidin synthase (ANS)** oxidizes the leucoanthocyanidin to a colored anthocyanidin, which a glycosyltransferase (UFGT) then sugars into a stable **anthocyanin** — the blue-black of a blueberry, the red of a raspberry.
- Down the other, **leucoanthocyanidin reductase (LAR)** reduces it to catechin, and **anthocyanidin reductase (ANR)** reduces the anthocyanidin back to epicatechin — the 2,3-*cis* and 2,3-*trans* flavan-3-ol monomers that polymerize into **proanthocyanidin**, the condensed tannin, the grip.

One fork, two destinies: pigment down one tine, tannin down the other, both drawing from the same leucoanthocyanidin pool, both switched on in the skin by the same family of MYB–bHLH–WD40 transcription complexes that the berry uses to decide how much of itself to spend on color and how much on structure. The flux that the fork sends toward anthocyanin is flux *not* sent toward proanthocyanidin. Tune one and you bend the other — which is exactly why a blueberry is the hardest berry on which to ask for more grip without paying in color.



A blueberry in cross-section — the skin, the pale flesh, the tiny seeds. The anthocyanins that color the skin and the proanthocyanidins that lightly grip it are built in these same few outer cell layers, off one branch of one pathway. On a berry you cannot reach for the tannin without touching the pigment; the two are drawn from a single pool.

The arithmetic of that fork is worth writing out, because it tells us exactly what we can and cannot ask of the design. Let **F** be the flux arriving at the shared leucoanthocyanidin pool, and let **φ** be the fraction the fork routes toward pigment, so that **(1 − φ)** goes toward tannin:

```
anthocyanin output = φ · F · proanthocyanidin output = (1 − φ) · F
```

Now the two things a designer might do pull in opposite ways. Hold the total flux **F** fixed and merely re-partition the fork — nudge φ down to send more toward grip — and you gain tannin only by losing an equal measure of color:

```
∂(anthocyanin)/∂φ = +F · ∂(proanthocyanidin)/∂φ = −F
```

A blue berry leans toward pigment; take a representative split of **φ = 0.70**, so seven parts color to three parts grip. Shift it to **φ = 0.60** at fixed F and the tannin rises from 0.30 F to 0.40 F — a **+33 %** gain in grip — but the color falls from 0.70 F to 0.60 F, a **−14 %** loss. That is the wrong trade for a fruit whose whole beauty is its color. The other move is to raise the total flux **F** itself — push harder through the upstream flavonoid enzymes that feed the shared pool — and because both outputs are proportional to F, both rise together:

```
∂(anthocyanin)/∂F = +φ · ∂(proanthocyanidin)/∂F = +(1 − φ)
```

Raise F by **20 %** at fixed φ = 0.70 and the color climbs from 0.70 F to 0.84 F *and* the grip climbs from 0.30 F to 0.36 F — both up a clean twenty percent, neither spent to buy the other. This is the whole of why "a berry that is darker *and* a touch more structured" is a coherent design goal and "a berry with more grip" alone usually is not: the interesting cultivars are the ones that raise the shared flux rather than re-partition it, and spend most of the berry's 120 constructs upstream of the fork, lifting the pool, rather than at the fork, robbing one tine to feed the other.

Reading the floor as a number

To hold a floor you must be able to read it, and the Index reads it the same way it reads every axis:

> $IFI(candidate) = 1{,}000 \times EC_{50}(reference) \div EC_{50}(candidate)$

On astringency the EC_{50} is not a half-maximal current across a channel but the tannin concentration that drives standardized salivary-PRP precipitation to half of its maximal plateau — the half-clouding point of a defined protein pool, read turbidimetrically at mouth temperature and physiological ionic strength. We anchor the scale by fixing the reference tannin to half-precipitate that pool at **EC_{50}(reference) = 50 µM**, which by construction gives it IFI-A = 1,000. Because the formula is a simple ratio around that anchor, the whole scale collapses to one instant identity:

> $EC_{50}(candidate) = 1{,}000 \times 50\ \mu M \div IFI\text{-}A =$ **50,000 ÷ IFI-A** (µM)

The score and the concentration are reciprocals: a higher IFI-A means a lower EC_{50}, a tannin that clouds protein at less concentration, a louder grip. For a berry we want the opposite of loud. We set a deliberately **low, clean floor** — a few hundred, well under the reference — and the identity turns each target straight into the concentration it demands. Take the blueberry floor we design to, **IFI-A 300**:

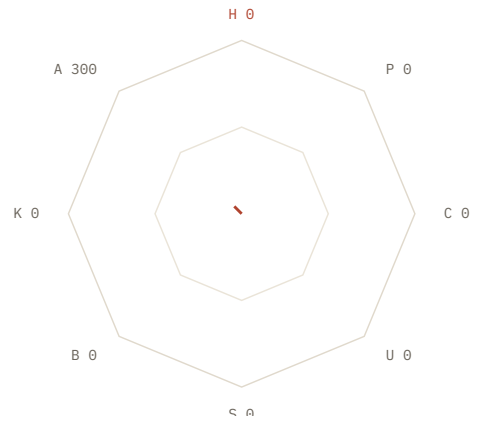
> $EC_{50}(blueberry\ floor) = 50{,}000 \div 300 =$ **166.7 µM**

The blueberry's grip half-precipitates the standard PRP pool only at 166.7 µM, more than three times the reference concentration — it is **0.30×** as potent a protein-precipitant as the reference, exactly the quiet floor we want. We set raspberry's floor a touch lower in integrated magnitude, at **IFI-A 240**, because — as the dose–response below will show — its large ellagitannins deliver a sharper onset that reads as grip at a lower total load, so a smaller number lands at a similar *felt* edge:

> $EC_{50}(raspberry\ floor) = 50{,}000 \div 240 =$ **208.3 µM** (0.24× the reference)

Set those against the one cultivar we have designed to register on all eight axes at once — **Phoenix** (SS-0096), whose astringency we set deliberately at **IFI-A 1,400**, a half-precipitation of 50,000 ÷ 1,400 = **35.7 µM**, **1.4×** the reference — and the gulf between a loud designed grip and a berry's floor is plain: a berry's tannin needs roughly **four to six times** the concentration of Phoenix's to reach the same half-precipitation. The scorecards, in the lab-notebook layer that reads a profile at a glance, make the single-axis nature of the berry visible — a lone soft column with the rest of the axes near silent:

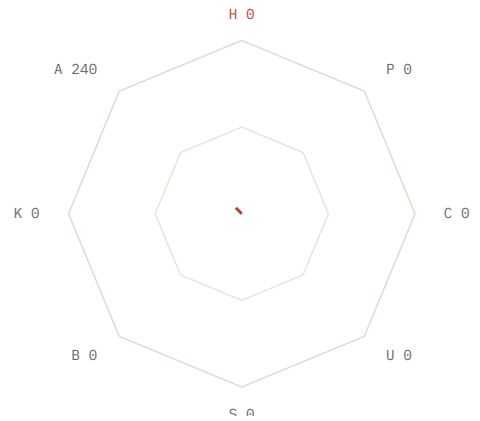
IFI PROFILE



IFI-H		0
IFI-P		0
IFI-C		0
IFI-U		0
IFI-S		0
IFI-B		0
IFI-K		0
IFI-A		300

That is the blueberry's designed profile — one axis, set to a floor; the sweetness and color you taste are the fruit's native gift, not an IFI construct, laid over this small deliberate grip. Raspberry's reads the same shape, a touch lower and, in character, faster-edged:

IFI PROFILE



IFI-H		0
IFI-P		0
IFI-C		0
IFI-U		0
IFI-S		0
IFI-B		0
IFI-K		0
IFI-A		240

Run the identity across the band that matters for a berry and you get the calibration ladder — the dictionary that turns any IFI-A into a concentration and a felt grip, with the two designed floors between the reference above and the two failure modes that bracket them:

ENTRY	IFI-A	RELATIVE POTENCY	EC_{50} VS STANDARDIZED PRP	READ
Over-grip (ruined berry)	900	0.90×	55.6 µM	a wall where a floor belongs
Reference anchor	**1,000**	1.00×	50 µM	the scale's fixed point
Phoenix · SS-0096	**1,400**	1.40×	35.7 µM	a loud, designed grip
Blueberry floor (design)	**300**	0.30×	166.7 µM	round, structural, felt as shape
Raspberry floor (design)	**240**	0.24×	208.3 µM	bright, fast-edged, felt as shape
Below floor (flat)	150	0.15×	333.3 µM	no grip; reads as sugar-water

A micromolar figure is exact only if you can say "micromoles of *what*," and here the two berries part ways again. To put a molar EC_{50} into the milligrams per liter a jam kitchen would actually measure, you need a molar mass:

$$C\ (\mathrm{mg \cdot L^{-1}}) = EC_{50}\ (\mu M) \times \bar{M}\ (\mathrm{g \cdot mol^{-1}}) \div 1{,}000$$

For the blueberry's condensed tannin that $\bar{M}$ is a *mean*. Each flavan-3-ol unit is about 290 $\mathrm{g \cdot mol^{-1}}$, and each interflavan bond sheds two hydrogens, so a proanthocyanidin of mean degree of polymerization five weighs about 290 + 4 × 288 ≈ **1,443 $\mathrm{g \cdot mol^{-1}}$**. The floor's 166.7 µM is then 166.7 × 1,443 ÷ 1,000 ≈ **241 $\mathrm{mg \cdot L^{-1}}$** of tannin — faint, as a berry's grip should be. But the honest catch is that "mean DP five" is an average over a spread, and report it against an undefined extract and it drifts every harvest, so the reference material's mean degree of polymerization is fixed as part of the assay and candidates are read against it. The raspberry has no such trouble: sanguiin H-6 is a single molecule of 1,871 $\mathrm{g \cdot mol^{-1}}$, so its 208.3 µM floor converts cleanly to 208.3 × 1,871 ÷ 1,000 ≈ **390 $\mathrm{mg \cdot L^{-1}}$**, no averaging required — and lambertianin C, being a trimer, carries half again as many binding groups on one scaffold, so fewer micromoles of it do the same work. The defined molecule is the easier number to trust; the polymer is the one that demands the fixed definition.

The shape of the onset

Two tannins can share a floor — the same half-precipitation point, the same IFI-A — and still feel different, because astringency is not a single level but a *curve*, and the shape of that curve is where the blueberry's roundness and the raspberry's brightness live. The precipitation rises with tannin concentration as a sigmoid, steeper than a simple binding isotherm because the cross-linking is cooperative: once a network nucleates, it recruits. A Hill form captures it:

$$\theta = [T]^h \div (EC_{50}{}^h + [T]^h)$$

where θ is the fraction of maximal grip and **h** is the Hill coefficient — the steepness, set by how many bridging arms a tannin brings to one encounter. A compact, doubly-locked A-type blueberry procyanidin presents its binding faces more sparingly and more rigidly; it behaves near **h ≈ 2**, a gentle climb. A large raspberry ellagitannin —

a dimer or trimer studded with galloyl and HHDP groups on one scaffold — is a high-valency molecule that nucleates a network sharply; it behaves nearer **h ≈ 3**, a steep one. Hold each at its own EC_{50}, so each reads θ = 0.5 at its floor, and walk the concentration up and down around it:

[T] ÷ EC_{50}	θ AT H ≈ 2 (BLUEBERRY, ROUND)	θ AT H ≈ 3 (RASPBERRY, BRIGHT)
0.25	0.06	0.02
0.50	0.20	0.11
1.00	0.50	0.50
2.00	0.80	0.89
4.00	0.94	0.99

Both pass through the same midpoint — that is what sharing a floor means — but the raspberry's steeper exponent makes its grip arrive later and then climb faster through the midpoint, which is the mathematics of "brighter, faster, cleaner-edged": a grip that is almost absent, then suddenly *there*, then just as quickly spent. The blueberry's shallower climb is "rounder, slower": a grip that eases in and eases out, present across a wider span of concentration and never sharp at any point. The same floor; two different edges; and the difference is a single exponent set by the size and rigidity of the molecule the berry builds.

There is one more softening that belongs only to the berries, and it comes from the sibling pigment. Anthocyanins and tannins **co-pigment** — their flat aromatic faces stack against one another in solution — and in a berry, heavy with both, that stacking does two gentle things at once. It deepens and stabilizes the color, shifting the anthocyanin's absorbance so the fruit reads darker and holds that darkness longer; and it sequesters a share of the tannin against the pigment, where it is less available to find a salivary protein. So a berry's grip arrives already partly spoken for, softened by the very molecule that makes the fruit beautiful. The color and the grip, drawn from one pool and then stacked against each other in the cell, *resolve* one another — which is why designing a berry's floor of grip is inseparable from designing its color, and why the finest cultivars tune the two as a pair rather than in turn.

Holding it down and even

The design intent for the berries is almost always restraint, and it is the counter-lesson to the loud-grip organisms, where the whole craft is building tannin up. A berry lives or dies by its sweetness and its perfume; astringency's job here is supporting architecture — the grip that keeps the fruit from reading as sugar-water, the faint tightening that makes you want the next one. Push it too far and you have ruined a blueberry; leave it out entirely and you have a flat, characterless berry that melts to nothing. The target is the low, clean floor the ladder names — a few hundred on IFI-A, set beneath a high native sweetness and a full anthocyanin load, with the tannin tuned toward the soft end of its quality range: longer and less galloylated for the blueberry, so the grip is velvety rather than green; intact and clean-edged for the raspberry. We spend as much effort holding astringency *down and even* as we spend elsewhere building it up.

And even is the harder half of down. A berry whose grip wanders from lot to lot is a berry a jam-maker or a pastry kitchen cannot trust — the compote that tasted right last year must taste right this year. Because IFI-A is a ratio of two fitted concentrations, its uncertainty is the two relative uncertainties added in quadrature:

```
σ(IFI-A) ÷ IFI-A = √[ (σ_ref ÷ EC₅₀_ref)² + (σ_cand ÷ EC₅₀_cand)² ]
```

With the reference fit held to 3 % and a candidate fit to 5 %, that is $\sqrt{(0.03^2 + 0.05^2)} = \sqrt{0.0034} =$ **5.8 %**, inside the platform's **≤ 7 %** batch envelope. So the blueberry floor is not "IFI-A 300" but **300 ± 17**, a 95 % band of roughly 266 to 334; the raspberry floor is **240 ± 14**. A conventionally grown, conventionally bred berry swings far wider than that — its tannin moving with season, ripeness, and lot by tens of percent — and the whole design intent of an engineered berry is that its floor is set and *held*: a structure under the sweetness that does not wander, batch after batch, to a few percent.

LIBRARY	CONSTRUCTS	ENGINEERED AXIS	FLOOR TARGET (IFI-A)	DOMINANT LEVER	ONSET (HILL H)	BATCH CV
Blueberry	120	A (floor)	300	raise shared flavonoid flux F; favor A-type, low galloylation	≈ 2	≤ 7 %
Raspberry	40	A (floor)	240	protect ellagitannin integrity; keep HHDP intact	≈ 3	≤ 7 %

The ways a berry's floor can fail are worth naming, because they are the edges of the design window. A floor set too low — drop much below IFI-A 150, past 333 µM — and the grip falls under the threshold of notice; the fruit reads as sugar-water, sweet and flat and forgettable, with nothing to make you reach for the second one. A floor pushed too high — up toward IFI-A 900, a wall where a floor belongs — and you have overwritten the thing that makes a berry a berry, trading its soft perfume for a pucker it was never meant to carry. And there is a subtler failure that comes from reaching for grip the wrong way: push a condensed tannin's chain length too far and a very long proanthocyanidin begins to fold on itself and aggregate with its own kind before it ever reaches a protein, so the effective, protein-available tannin drops and the berry reads *lower* on IFI-A than its total phenolic content predicts — an over-tannined construct that scores soft because half its tannin fell out in the fruit. The berry's design window lives on the near side of that reversal, at modest chain length and low galloylation, lifting the whole flavonoid pool rather than overbuilding any one molecule.

What the number cannot promise

A single number is a form of respect for the reader only if it also admits what it leaves out, and astringency — a colloidal, protein-precipitation event rather than a clean lock-and-key — leaves out more than most axes. The honest limits are worth stating plainly, because a berry's grip is small enough that every one of them can move it.

- **The matrix is not the flask.** The assay reads a tannin against a standardized protein pool at a fixed pH and ionic strength; a real berry arrives wrapped in its own sugars, acids, pectins, and cell-wall polysaccharides, and those can sleeve a tannin or compete for it, masking grip the flask would have seen. On a soft fruit, where the whole signal is faint, that wrapping is proportionally large.
- **The co-pigment masks and stabilizes at once.** The same anthocyanin stacking that deepens a berry's color sequesters a share of its tannin, so a darker berry can read softer than its tannin content alone would predict — a real effect the single-shot number only partly captures.
- **The mouth is each person's own.** The salivary proteins the grip precipitates vary from person to person in quantity and in flow, and a mouth that meets tannin often makes more of the sacrificial protein, so the habitual berry-eater is, at the level of the saliva, slightly armored against the very grip the rest of us feel more sharply. The Index pins the *stimulus* — the tannin's own precipitating potency against a fixed standard — and leaves the *sensation*, rightly, to the person tasting.
- **Grip builds; one point cannot see it.** A single EC_{50} measures one encounter against a fresh protein pool, but a real mouth depletes its sacrificial proteins over a session, so astringency ratchets up across a bowl of berries in a way a one-point assay never captures. On a berry the ratchet is gentle, but it is real, and we name it rather than paper over it.

None of this is a weakness of the number; it is the number knowing its place. The IFI-A floor predicts rank order and relative grip with real fidelity — this berry firmer than that one, this lot matched to the last — and it does not pretend to forecast any single mouth's absolute experience, because the protein on the other side is variable, adaptive, and pleasingly each person's own.

The whole of itself

And here, as quietly as anywhere in the library, the nourishment note sits inside the same molecule. The proanthocyanidins and ellagitannins that give a berry its faint, clean grip are the same polyphenols that make the fruit worth more than its sugar — the compounds a lifetime of clean eating is reaching for when it reaches for a bowl of berries. We do not dress that as a health claim; we only decline to breed it out. Conventional selection, chasing size and sweetness and shelf life, tends to thin these compounds away, and with them goes both the grip and the goodness, because on this fruit they are the same thing. A berry designed to hold its color, its perfume, *and* its small honest floor of grip is a berry designed to hold the most of itself. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. That is the whole of the ethos, stated in miniature on the softest plant we keep: grow the fruit to carry everything it can, set the grip to the floor and hold it there, and let the body taste the structure as the reason the sweetness feels whole.

Chapter 9 · Sorghum — *the grain that wears its tannin on the outside.*

The last of our pure astringency organisms is the one no one expects: a grain. **Sorghum** carries **one hundred and twenty constructs** and sits at the **frontier** end of our difficulty scale, and it earns its place on this axis because, nearly alone among the cereals, it builds real condensed tannin — proanthocyanidins, laid down not through the kernel but in a thin pigmented layer just under the seed coat, the **testa**. A sorghum that has that layer is a tannin sorghum; one that lacks it is not. The whole of the grain's astringency hangs on whether a single tissue is present, and whether it is present hangs on two genes. Most plants our family makes astringent carry their tannin diffusely — a grape stows it through seed and skin, a tea leaf weaves it into every cell. Sorghum does something almost architectural instead: it builds one wall, in one place, or it does not, and wears that decision on the outside of the seed.



Sorghum, in cross-section — the grain and the thin outer layers that can carry a pigmented, tannin-rich testa. In sorghum, astringency is almost a switch: a tissue that is either built or not, under the control of two regulatory genes.

The genetics are unusually clean, which is what makes the frontier organism tractable despite its difficulty. The presence of a pigmented testa — and therefore of tannin — requires two dominant loci acting together, historically called **B1** and **B2**, and the deposition of the tannin in that testa is governed by the regulatory genes **Tannin1 (*Tan1*)**, a WD40-repeat protein, and **Tannin2 (*Tan2*)**. Where the regulators are active and the testa forms, the grain builds its proanthocyanidins; where they are off, the testa never forms and the

grain is tannin-free. Astringency in sorghum is, more than in any other organism we grow, close to a **switch** — and a switch is the easiest thing in the world to design with, once you know where it is.

The tissue that is either built or not

Begin with the anatomy, because in sorghum the anatomy *is* the chemistry. Cut a kernel across and you pass, from the outside in, through the pericarp — the fruit wall, the part that colors the grain red or white or bronze — then, in some sorghums and not others, through the **testa**, a thin pigmented seed-coat layer only a few cells deep, and finally into the endosperm and germ, the starch and oil that make the grain a food. The testa is the whole story. It is the only tissue in the plant that lays down condensed tannin in bulk, and it is a tissue the grain either builds or omits. There is no half-testa in the wild. A sorghum is a tannin sorghum because that layer exists and is pigmented; it is a food-grade sorghum because that layer was never built.

This is why sorghum sits at the frontier of difficulty despite the clean genetics: the trait is spatial. We are not merely asking a cell to make more of a molecule — we are asking a specific layer of a specific tissue to form, or not, in register with the seed coat around it. But the spatial switch is also the gift. In a grape, grip is distributed through pulp and skin and seed, and tuning it means reaching into many tissues at once; in sorghum, grip lives in one wall. The frontier organism turns out to have the single cleanest control surface on the whole astringency axis, which is exactly the paradox that drew us to it: the hardest grain to engineer is the easiest grain to *dial*, once the wall is yours to command.

Two dominant loci, B1 and B2, must both be present for that pigmented testa to form at all. Think of them as permission to build the wall: lose either, and the testa does not appear, and no downstream machinery can deposit tannin into a layer that was never laid. This is the coarsest control, a true on/off — the one the twentieth century reached for when it bred the tannin *out*. Our interest runs finer: we keep the wall, and work the regulators that decide how much tannin it holds.

A switch written in transcription factors

The reason sorghum's astringency can be designed cleanly is that its master control is not an enzyme but a **committee of transcription factors** — regulatory proteins that do not themselves make tannin but switch on, together, the dozen structural genes that do.

Across the plant kingdom, proanthocyanidin biosynthesis is governed by one recurring regulatory grammar: three kinds of transcription factor assemble into a single activating complex — a **MYB**, a **bHLH**, and a **WD40-repeat** protein, the so-called **MBW complex**. The WD40 protein is the structural hub, a seven-bladed β-propeller that holds the partners in register; the bHLH and MYB give the complex its DNA-binding reach and its target specificity. Only when all three are present and bound does the complex sit on the promoters of the tannin genes and turn them on in concert. In

sorghum, **Tan1** supplies that WD40 hub; **Tan2** supplies the bHLH partner; a tissue-specific MYB completes the trio. Where B1 and B2 have granted the testa, and where Tan1 and Tan2 are both functional, the MBW complex forms, the structural genes fire, and the wall fills with proanthocyanidin. Knock out either regulator — the WD40 or the bHLH — and the complex cannot assemble, the structural genes stay dark, and the grain is tannin-free even if the testa is present. The wild sorghums that lost their tannin lost it, most often, exactly here: a broken *Tan1*, a committee that can no longer convene.

Designing on a regulator rather than an enzyme is a categorical advantage, and it is what makes one hundred and twenty constructs enough for a frontier organism. A structural enzyme is one rung on a ladder; move it and the pathway routes around you or piles up a dead-end intermediate. A regulatory complex is the ladder's light switch: tune the *dosage* of Tan1 and Tan2 — how much of each regulator the testa cells make, how tightly the complex assembles, how long it sits on the promoters — and you scale the entire downstream program up or down as a unit, the pathway's stoichiometry preserved. That is why the switch, once located, becomes a dial without a fight.

The five-deoxy signature

The molecule the wall holds is a **condensed tannin**: a polymer of flavan-3-ol subunits, each a three-ringed phenolic, strung into a proanthocyanidin that may run from a dimer to a chain of a dozen units or more. But sorghum's are built from an unusual subunit, and the difference is not cosmetic — it is chemical, it is tasteable, and it is measurable to the gram.

Most food proanthocyanidins are **procyanidins**, built from catechin and epicatechin, whose A-ring carries a hydroxyl at the 5-position. Sorghum is one of a small number of plants — it keeps company with the acacias and the quebracho of the tanner's trade — that builds **profisetinidins**, condensed tannins whose extension units are **5-deoxy** flavan-3-ols: fisetinidol in place of catechin, lacking that 5-hydroxyl entirely. The missing oxygen traces all the way back to the very first committed step of the pathway, where a **chalcone reductase** acts alongside chalcone synthase to drop one oxygen before the flavonoid rings are even closed, yielding the 6'-deoxychalcone from which every 5-deoxy flavonoid descends. From there the familiar machinery takes over — the general phenylpropanoid branch **PAL → C4H → 4CL** feeding the flavonoid core, then the reductases that finish the flavan-3-ol subunit, then the condensation that polymerizes subunits into tannin inside the testa. The whole of it waits on the MBW committee to call it into session.



Sorghum grain, whole — the pigmented, tannin-bearing testa is the thin layer beneath the seed coat, and the only tissue in the plant that lays down condensed tannin in bulk. The same molecule that dries the mouth is the grain's armor in the field.

The missing oxygen has consequences we can count. A catechin subunit is **$C_{15}H_{14}O_6$**, molar mass **290.3 g/mol**; strip the 5-hydroxyl and the fisetinidol subunit of a profisetinidin is **$C_{15}H_{14}O_5$**, molar mass **274.3 g/mol** — sixteen grams per mole lighter, one oxygen gone. That lightness is not trivia. Because astringency is made by binding sites, and the number of binding subunits in a gram of tannin scales inversely with the subunit's mass, a gram of profisetinidin carries **1000 ÷ 274.3 = 3.65 mmol** of subunits against a gram of procyanidin's **1000 ÷ 290.3 = 3.44 mmol** — about **5.8% more** aromatic cross-linking units per unit mass. Measured against the galloylated reference subunit the Index anchors on, epigallocatechin gallate at 458.4 g/mol, the same gram of sorghum tannin delivers **3.65 ÷ 2.18 = 1.67×** as many subunits, two-thirds again as many hands reaching for protein. The 5-deoxy ring also makes the molecule a touch more planar and more electron-rich, which is why sorghum tannin does not read like a watery grape but carries a drier, grainier, more resinous grip that stands up to the toasty flavors of a flatbread or a porridge. The chemistry we are tuning is, from the first oxygen, its own thing.

What the grip actually is

It is worth being exact about what the mouth is doing when a sorghum porridge grips, because astringency is the one axis on the Index that is not a taste at all. No receptor fires a labeled line that means *tannin*; a lubricant is destroyed, and the mouth notices. A resting mouth is among the best-lubricated surfaces in the body, and the lubricant is protein: saliva lays a film microns thick in which **proline-rich proteins (PRPs)** — a quarter to a third proline, too proline-heavy to fold, lying open as loose extended chains — bridge the oral surfaces and let them glide without touching. A tannin unmakes that film in three steps. **First**, the polyphenol coats the open chain, its aromatic rings stacking onto the flat proline faces, its phenolic hydroxyls hydrogen-bonding to the backbone carbonyls proline leaves exposed. **Second**, because both partners are multivalent, one tannin bridges two chains, then three, and the complexes grow into a colloidal mesh. **Third**, the aggregates reach a size and neutralized charge at which they fall out of solution entirely. The lubricating protein is now curds in a thinning film, the surfaces meet, and the trigeminal mechanoreceptors woven through the mucosa — endings that report stretch and drag — fire; the brain

reports *dry, rough, tight, puckering*. The sensation is vivid and entirely mechanical. A sorghum's 5-deoxy proanthocyanidin, lighter and richer in cross-linking units than a grape's, is simply very good at step two: many hands, pulling the lubricant down.

That this is literally mechanical, and not a convenient metaphor, can be read on instruments: a calorimeter reads the heat of tannin binding protein and returns a binding constant and a stoichiometry; an oral tribometer slides a soft, tongue-mimicking surface across a film of saliva and reports the friction coefficient climbing as tannin is added. When calorimeter, tribometer, and a trained human panel all rank the same sorghum samples in the same order, astringency stops being a subjective report and becomes a physical quantity — a thing we can design.

Putting a number on the grip

All of it ends at one number. The Index is anchored, on every axis, to the half-maximal point of a receptor response, and for astringency the "response" is the precipitation of a standardized PRP pool. We define **EC_{50}** as the tannin concentration that precipitates half of the maximal precipitable protein in the assay — a potent tannin reaches half-maximum at a low concentration, a weak one needs a flood — and loudness is the inverse of the concentration it takes to be heard. That is exactly what the Index formula encodes:

> $IFI\text{-}A(candidate) = 1{,}000 \times EC_{50}(reference) \div EC_{50}(candidate)$

We anchor the reference at a defined tannin — a standardized grape-seed procyanidin fraction of mean degree of polymerization 4 at fifteen percent galloylation — and fix its half-precipitation at **$EC_{50}(reference) = 500\ \mu M$** in subunit-equivalents. By construction the reference scores itself: 1,000 × 500 ÷ 500 = **1,000**. Everything else is read against it. A tannin twice as potent, half-precipitating at 250 µM, scores 1,000 × 500 ÷ 250 = **2,000**. The scorecard ceiling — the per-axis normalization maximum of 5,000 — corresponds to EC_{50} = 1,000 × 500 ÷ 5,000 = **100 µM**, the most astringent stimulus the scale resolves, five times more potent than the reference. The whole axis is this one division, run forward to score a tannin or backward to brief one.

The table below runs it backward, turning a target grip into the exact chemistry the testa must deliver. The third column converts the micromolar figure to a mass concentration using sorghum's own subunit — the fisetinidol unit at 274.3 g/mol — rather than the galloylated reference, because that is the honest yardstick for a profisetinidin.

IFI-A	EC_{50} (MM, SUBUNIT-EQ)	≈ MG/L (AS FISETINIDOL UNIT)	MARKER ON THE SCALE
300	1,667	457	whisper of grip
600	833	229	**table-grade target**
1,000	500	137	structured; = the reference
1,400	357	98	**Phoenix** designed note
1,800	278	76	wild full-tannin switch
5,000	100	27	scorecard ceiling

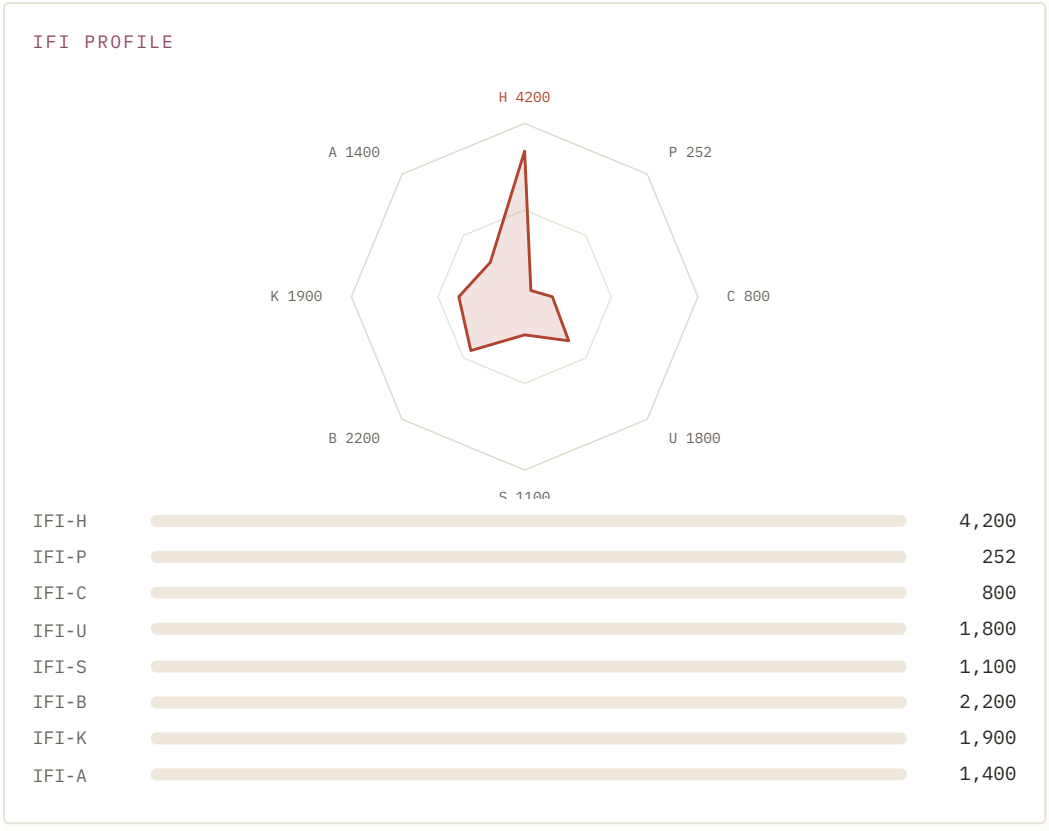
The micromolar-to-milligram step is a small, honest piece of arithmetic worth showing, because it also exposes a limit. A µM figure becomes a mg/L figure only once you declare a molecular weight for the subunit, and a real condensed tannin is **polydisperse** — a distribution of chain lengths with no single molecular weight — so the µM scale is well defined only against a stated reference subunit. We state ours: the fisetinidol unit, 274.3 g/mol. It is the kind of bookkeeping that keeps a pretty number from quietly lying, and it matters more for sorghum than for most, whose 5-deoxy subunit differs from the galloylated reference the axis is anchored on.

A single EC_{50} is only the midpoint of a curve, and the *shape* of the curve is part of the sensation. Precipitation follows a sigmoidal dose–response that a Hill form describes well:

$$\theta(c) = c^n \div (EC_{50}^n + c^n)$$

where θ is the fraction of maximal protein precipitated and *n* is the Hill coefficient. Because tannin binding is multivalent and cross-linking is cooperative — each tannin that lands pre-aggregates the protein and makes the next landing easier — *n* runs above one, near 2 for a well-behaved condensed tannin. Take a table-grade sorghum at EC_{50} = 833 µM and *n* = 2, and walk the curve a bowl of porridge might trace across the mouth. At a delivered **c = 400 µM**, $\theta = 400^2 \div (833^2 + 400^2) = 160{,}000 \div 853{,}889 =$ **0.19** — a bare touch of grip. At **c = 833 µM**, θ = **0.50**, the half-maximum by definition. At **c = 1,666 µM**, $\theta = 1{,}666^2 \div (833^2 + 1{,}666^2) = 2{,}775{,}556 \div 3{,}469{,}445 =$ **0.80** — dominant. The grip climbs from barely-there to commanding across a single doubling of concentration. That steepness is the *threshold* feel of astringency, the sense of almost-nothing and then suddenly grip, and it is why a sorghum tuned a little too high does not read as slightly firmer but as a different, harsher grain. The cooperativity means the dial near the top is sensitive, and sensitivity near the top is exactly why the wild all-or-nothing switch is so unforgiving and why a held, modest setting is worth the engineering.

For orientation, here is where that note sits inside a fully-realized fruit: our full-spectrum cultivar **Phoenix (SS-0096)**, designed to register on all eight axes at once, carries a deliberately restrained astringency of **IFI-A 1,400**, enough to give the fruit a spine without letting the grip overrun the other seven axes. Inverting the formula gives the brief its pathway must hit: $EC_{50} = 1{,}000 \times 500 \div 1{,}400 =$ **357 µM**, a touch more potent than the reference and no more.



From switch to dial — the hundred and twenty constructs

Here is where sorghum becomes the clearest case in the whole library of designing astringency in *both* directions. The wild grain offers a binary: tannin or no tannin, the switch thrown or not. Our one hundred and twenty constructs turn that binary into a **rheostat**, and they do it at the regulator rather than the enzyme — tuning the dosage of Tan1 and Tan2, and with it how densely the MBW complex sits on the structural genes, and with that how much proanthocyanidin the standing testa wall holds. Less regulator, a lighter wall; the geometry of the tannin preserved, only its quantity dialed.

Because IFI-A is proportional to the delivered tannin at a fixed reference, the dial maps cleanly onto the grain's own agronomic quantity — condensed tannin as a percentage of grain mass, in catechin-equivalents. We anchor the grain scale so that the "structured" setting, IFI-A 1,000, corresponds to **1.0%** tannin, which leaves a tidy mnemonic: a tannin sorghum's IFI-A is very nearly its condensed-tannin percentage times a thousand — a consequence of our chosen anchor, not a law of nature, but one that makes the dial legible to a grower and a formulator at a glance.

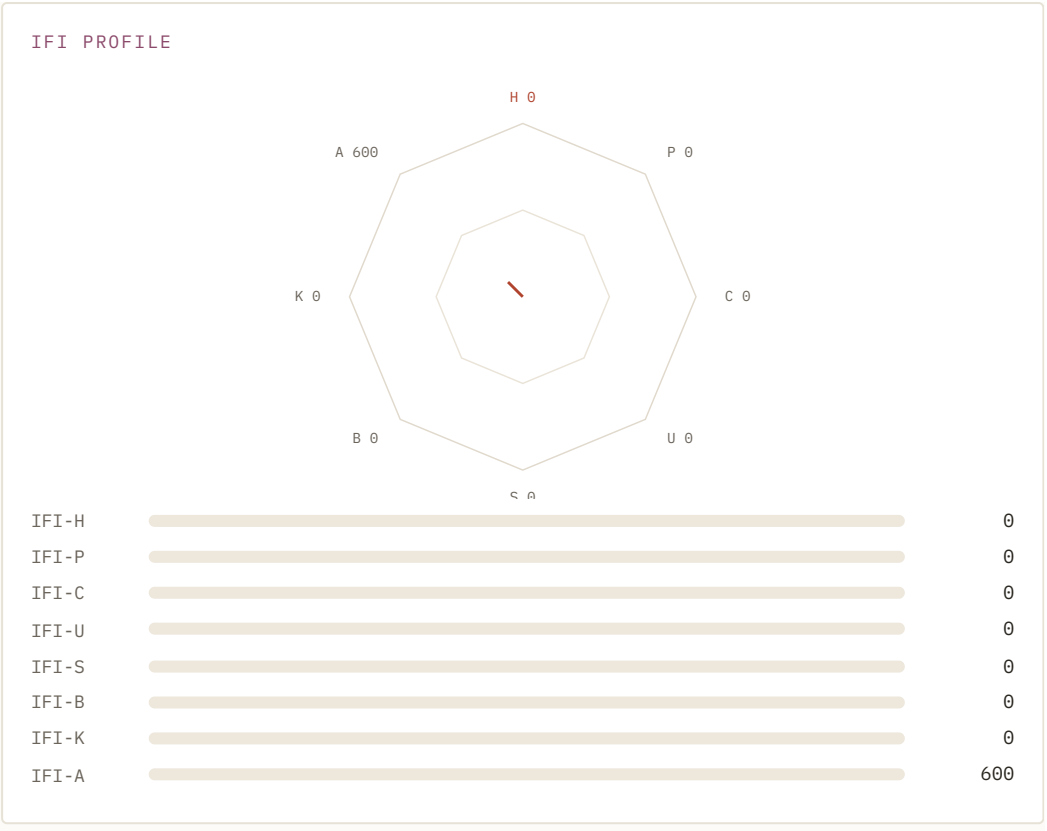
DIAL SETTING	TAN1 / TAN2 STATE	TESTA	TANNIN (%, CE)	≈ MG/G	IFI-A	EC_{50} (MM)
0 · food-grade	silent (or *tan1* null)	absent	0	0	0	∞
1 · whisper	low dosage	thin	0.3	3	300	1,667
2 · **table (target)**	tuned	formed	0.6	6	600	833
3 · structured	mid dosage	full	1.0	10	1,000	500
4 · wild full switch	max, B1 B2	full	1.8	18	1,800	278

The grain we actually hand to a table sits at setting 2: a modest, chosen grip of **IFI-A 600**, built on a 0.6% condensed-tannin testa, enough to give a porridge a clean edge and a flatbread some backbone, enough to hold off the birds and the field molds — without the all-or-nothing harshness of the wild switch at the bottom

of its climb. Its profile is seven zeros and one honest note, and the zeros are the whole point: sorghum speaks a single axis, cleanly, and we design it to say that one thing well rather than muddling it with grips it has no chemistry to make.



Sorghum grain specimens — the same grain, set deliberately along a dial of testa tannin rather than thrown to the wild switch. A chosen, modest grip, held to a reliable level, is a staple grown with intention.



Quantity is not the only lever even here. The grip of a condensed tannin peaks with the mean degree of polymerization in a band near five to eight subunits, and falls again past roughly fifteen, where the chain grows too large and poorly soluble and begins to self-associate before it ever reaches the salivary protein. Wild sorghum tannins can run long and broad, and part of their harshness is exactly an over-polymerized, polydisperse distribution precipitating unevenly, in catches. Holding the mDP in the optimum band — narrow, well-behaved, near the peak of the grip curve — is the

second thing the constructs do, so that the table-grade grain is not merely lighter but *smoother*: an even sweep of friction rather than a grab.

Holding the grip — precision and the error bar

A dial is only worth the name if its settings hold, and precision is the quiet hero of the whole program. Our constructs are designed to hold batch-to-batch variation to a coefficient of variation of **≤7%**, against the **42%** that is ordinary for a tannin left to a wild-type plant and a growing season — and in sorghum that wild scatter is not a nuisance but the native condition, because the testa switch is notoriously sensitive to genetic background, to weather, to the wet of a season that triggers pre-harvest sprouting and with it a surge of tannin the grower never chose.

Walk the arithmetic of what the tight error bar buys. Because IFI-A = k ÷ EC_{50} with k fixed, and because the grain's IFI-A is proportional to its testa tannin content, a fractional error in the tannin passes straight through to the score, one for one. At our table-grade setting of IFI-A 600, a 7% CV is **±42 IFI units** — a band from roughly 558 to 642, a grip a cook can plan a dish around. Run eight replicate batches and the standard error of the mean shrinks by $\sqrt{8}$: 7% ÷ 2.83 ≈ 2.5%, or **600 ± 15**. In grain terms the same precision reads as 6.0 ± 0.4 mg/g of testa tannin, a wall built to a drawing.

Now the wild comparison, which is the argument for the whole exercise. A wild full-tannin sorghum sitting near IFI-A 1,800 at the wild-type's 42% CV carries a standard deviation of **±756 IFI units** — a grip smeared from about 1,044 to 2,556, from a firm structure to a mouth-stripping harshness, batch to batch, with nothing the grower did to choose it. In grain terms that is 18 ± 7.6 mg/g: two harvests of the "same" grain that are not the same food. You cannot cook to that — you cannot formulate a porridge to a grip that wanders across a factor of two and a half. The wild switch gives you tannin or no tannin and, within tannin, a lottery; the dial gives you a number and holds it. That conversion — from a lottery into a specification — is the entire reason a frontier grain earns one hundred and twenty constructs.

The roster — where sorghum sits

Sorghum is one of six host species in the live library that carry astringency as a primary axis. Every entry below is read on the same EC_{50} assay, against the same 500 µM reference, through the same formula — which is the whole point of an index: not to erase the differences between a tea leaf and a grain, but to make them comparable in one number even though no two of their molecules are alike.

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	NATIVE TANNIN LEVER
Grape	1,610	B / A / M	Hard	ANR branch; procyanidin ↔ prodelphinidin balance
Tea	280	U / B / A	Hard	heavy galloylation (EGCG, ECG)
Blueberry	120	A	Moderate	low-DP, anthocyanin-linked
Pomegranate	120	A	Hard	hydrolysable tannins (ellagitannins)
Sorghum	**120**	**A**	**Frontier**	**5-deoxy condensed tannin in the seed testa**
Raspberry	40	A	Moderate	low-DP, bright, short-lived grip

Three things set sorghum apart on that list. It is the **only cereal** — every other astringency organism we grow is a fruit or a leaf, and no other staple grain builds condensed tannin to speak of. It is the **only frontier-difficulty** entry on the axis, earning that rank through the spatial, switch-like nature of its trait. And it is the only one whose grip is governed as a near-binary by a transcription-factor committee rather than tuned continuously through a structural enzyme like grape's ANR branch or tea's galloylation. Tea rides the most heavily galloylated flavan-3-ols in common food, which is why strong green tea grips fast and sharp; pomegranate works a different chemistry altogether, the hydrolysable ellagitannins, and carries astringency as its lone clean note. Sorghum alone builds one wall, from a 5-deoxy subunit, under a switch — and alone among them lets us set the grip of a grain that feeds hundreds of millions.

History ran one way; the dial runs both

For most of the twentieth century, plant breeders worked hard to breed the tannin *out* of sorghum, and the reasoning was sound on its own terms. Tannins bind dietary protein — the same precipitation that dries the mouth cross-links the protein in a meal — so a staple's tannin was read, reasonably, as an anti-nutrient: more bitter, more astringent, its protein and starch digested more slowly. The tannin-free "food grade" sorghums won, and for a kitchen that wanted the grain to vanish into porridge without a fight, they were the right answer.

But the same chemistry reads differently from other angles, and out in the field it always did. That pigmented testa is the grain's armor: tannin sorghums resist birds, which cannot stomach the grip, and they resist the molds and the pre-harvest sprouting that ruin tannin-free lines in a wet season. The molecule that dries the mouth defends the harvest. And the slower starch digestion that looked like a liability to one era looks, to a cook interested in how a grain actually behaves, like a lower and gentler release. We take no position dressed as a health claim — no "cures," no "detox," none of the halo that gets hung on a polyphenol. We note only, with a plant scientist's and a lifetime-vegetarian family's plain interest, that a grain's astringency and its behavior in the body are built by the same molecule, and that *Tan1* and *Tan2* let us set the dial deliberately instead of accepting whatever a breeding accident left.

So the contribution is to make the resolution a *dial* rather than a demolition. A sorghum can now carry a modest, chosen grip — the table-grade IFI-A 600, a 0.6% testa held to a seven-percent band — enough to give a porridge a clean edge and a flatbread some backbone, enough to hold off the birds and the field molds, without the all-or-nothing harshness of the wild switch. The twentieth century answered the tension between palatability and protection by removing one side of it; we answer it by making both sides adjustable and choosing a point between them on purpose.

Honest limits and failure modes

Reverence without honesty is just marketing, so it is worth naming plainly where the number stops and what can go wrong.

The axis measures the *stimulus*, not the sensation. IFI-A is a property of the tannin, read in a defined buffer against a standard PRP pool, and the mouth adds a matrix the flask does not. In a porridge or a flatbread, the grain's own starch and the arabinoxylans of its cell walls can sheathe a tannin and blunt its grip; **fermentation**, which so much of the world's sorghum cooking relies on, actively lowers extractable tannin as microbes bind and transform it; and any dairy or legume at the same meal pre-binds the tannin on its way in. A table-grade grain designed to score at IFI-A 600 in the assay will, on a real plate, deliver something softer — and that is a feature, not a failure of the number, because the score tells you what the tannin *can* do and leaves to the cook how much of that potential the dish spends.

The failure modes are real and we hold them in view. The first is **testa instability**: because the trait is spatial and switch-like, a construct that loosens the regulators too far can give a patchy or incompletely pigmented testa, a wall built unevenly, and an uneven wall grips in catches — the coarse, grainy harshness of a polydisperse tannin precipitating unequally. The second is the **cooperativity trap**: near the top of the grip, a Hill coefficient of 2 makes a small over-dose of regulator read as a large over-grip, so the dial is deliberately set low on its sensitive range, and the constructs spend much of their budget holding the chains in the smooth band rather than letting them run long. The third is the **protein-binding tradeoff**, honestly weighed: the grip we add is the same chemistry that slows the meal's protein, so we set the table grade modestly, where the structure a cook wants is bought without turning the staple back into the anti-nutrient the last century rightly fled. And the fourth is the **polydispersity caveat** behind the pretty table: a condensed tannin has no single molecular weight, so every µM figure here is honest only against the declared fisetinidol subunit.

None of this is a flaw in the axis; it is the reason the axis measures the chemistry and leaves the plate to the cook, and the reason we describe a sorghum's grip as the behavior of a molecule and claim nothing beyond that. Sorghum is no boutique crop — it is one of the great cereal staples of the dry lands of Africa and South Asia, the daily bread and porridge of hundreds of millions, for whom the question of its tannin has always been whether the grain is palatable, storable, and bird-safe all at once. Our contribution is to hand that

oldest tension back as a number you can set: a grain that wears its tannin on the outside, where we can read it, and now dial it — a staple grown to carry a precise, reliable grip, which is all our family has ever meant by growing a plant to hold the most it can. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 10 · The chocolate question — *why cacao sits at this table.*

A book on astringency written for winemakers, tea masters, and chocolate-makers owes chocolate an honest chapter, and honesty here begins with a line drawn plainly, in the open, before a single number is laid down. The astringency of fine chocolate is real. It is a condensed-tannin astringency — cousin to the grip of a young red and to the bite of a hard-steeped tea — and it is one of the three crafts this volume was written to serve. But cacao is not, today, one of our forty-two host organisms. We hold no engineered cacao library, no construct count, no designed cultivar, no gene moved inside a pod, and a family of scientists owes its readers, before anything else it offers them, the difference between the plants it grows and the plants it only could. What we can do — and do here in full, with nothing held back — is the exact thing the Index was built for: *measure* cacao's grip precisely, follow the chemistry that makes it step by step, and name honestly the place where the designing of it would one day live. That place is Volume XVI, *Cacao & Chocolate*, the organism monograph, which carries this book's plum accent for exactly this reason. Here, at the astringency table, cacao is a guest. It is a welcome one, and a revered one, and we set it a full place.



A cacao pod, opened to its beans in their pale pulp. The astringency and bitterness that define fine chocolate are built here, in the raw seed, as procyanidins — and they are tamed, never erased, by everything that happens between the pod and the bar.

We begin where the grip itself begins — in the raw bean, before anything has been done to soften it.

The wall in the raw bean — *what procyanidin is, and why it grips.*

A raw, unfermented cacao bean, split open and tasted, is one of the most astringent and bitter things a kitchen can hand you — a wall of grip, nearly inedible, a mouth drawn shut. Everything that happens between the pod and the finished bar is the patient taking down of that wall, and to tune a wall you must first know what it is made of.

The grip of chocolate is **procyanidin**: condensed tannin, built as chains of **flavan-3-ols** — the catechin-and-epicatechin family, the same subunits that string the tannin of a grape skin and a green tea leaf. The plant assembles them down the flavonoid branch of ordinary phenylpropanoid metabolism, the route every green thing runs — phenylalanine drawn off the shikimate pathway, then the cinnamic-acid enzymes **PAL → C4H → 4CL**, then the flavonoid skeleton, and finally two terminal reductases that deliver the two stereochemistries of the subunit: **LAR**, leucoanthocyanidin reductase, which yields *trans* catechin, and **ANR**, anthocyanidin reductase, which yields *cis* epicatechin. Cacao's pool is decisively **epicatechin-dominant** — an ANR-weighted chemistry — and it strings those epicatechin units carbon-to-carbon into the small condensed tannins that define the bean: the dimer **procyanidin B2**, the trimer **procyanidin C1**, and their short oligomer relatives, joined almost entirely by the open, flexible C4→C8 **B-type** interflavan bond.

Two structural facts about cacao's tannin matter more than any other, because they set its whole character against the other astringent plants. First, cacao's flavan-3-ols are **largely non-galloylated** — they do not, as tea's do, hang a small gallic-acid ring off the subunit. Galloylation is the single strongest lever there is on how hard a tannin pulls, so a bean that forgoes it grips, unit for unit, far more softly than a leaf that leans on it; chocolate's astringency is rounder and less sharp than tea's for a reason written in the molecule. Second, cacao's grip is carried by **short-to-middling chains** rather than long polymers, which places it on the steep, early, climbing part of the astringency curve, where every added unit still pays out in grip. A raw nib of fine cacao can run to a very high polyphenol load — on the order of a tenth of its dry, defatted weight in the literature of the plant — and nearly all of that is this epicatechin-based condensed tannin. That is the wall: a dense pool of short, ungalloylated procyanidin, sitting in the seed exactly where the plant most needs to defend what matters, which is the embryo of its own next generation.

How a tannin takes the tongue — *the mechanism, step by step.*

Astringency is the one dimension on the whole Index that is not, strictly, a taste. Sweetness and umami and bitterness are each a molecule settling into a receptor's pocket and opening a channel. Astringency is a demolition, and the sensation of chocolate's grip is the mechanical report of a protein film being pulled apart inside your mouth. The mechanism is worth walking all the way through, because it is the thing the number stands for.

The resting mouth is one of the best-lubricated surfaces in the body, held slick by a thin salivary film only microns thick. The slip is not the water; it is a family of salivary **proline-rich proteins**, the **PRPs**, which make up the better part of what the parotid glands secrete, alongside statherin and the long mucins. The PRPs are strange proteins, and the strangeness is the entire story: up to a quarter of their residues are the single amino acid **proline**, which forbids them from folding into a compact globe and instead strings them out as open, floppy chains rich in **polyproline-II helix**, flexible as wet thread. Proline's side chain loops back onto its own backbone nitrogen into a rigid

five-membered pyrrolidine ring that juts out as a small, flat, hydrophobic plate — and, because proline alone among the amino acids leaves no backbone N–H, the open chain presents a long row of bare carbonyl oxygens as hydrogen-bond acceptors. The protein is, in effect, a lubricant purpose-built to also be a soft target.

Now bring in cacao's procyanidin, and watch it work in three stages. **First**, the tannin coats the open PRP chain: its flat aromatic rings — the flavan A- and B-rings — stack face-to-face against the proline plates in a hydrophobic **π-stacking** contact, while its many phenolic hydroxyls reach over and donate **hydrogen bonds** to the exposed carbonyls. This is a multidentate grip, a single tannin clinging along the protein at several points at once. **Second**, because both partners are multivalent — the tannin carrying several rings, the open protein offering many sites — one tannin bridges two chains, then three; soluble complexes grow and knit the once-free proteins into a loose colloidal mesh. **Third**, the aggregates reach a size and a neutralized charge at which they fall out of solution altogether and **precipitate**. The protein bridges that held two oral surfaces gliding apart are now curds suspended in a thinning film. The surfaces begin to meet; the **friction coefficient** climbs from its easy baseline; and the free trigeminal nerve endings woven through the mucosa, which are mechanoreceptors reading stretch and drag, begin to fire. The brain, assembling the signal, does not report *friction rising*. It reports *dry, rough, puckering, tight* — the whole felt vocabulary of the grip. There is nothing being tasted. There is a lubricant being lost and a mouth noticing its own surfaces.

Two consequences fall out of this picture, and both will shape how chocolate behaves in the mouth. Because the PRPs are **sacrificial** — the body spends them to bind and carry the tannin harmlessly away from the digestive enzymes it would otherwise foul — astringency *builds*: the first bite precipitates the protein present, the next meets a mouth not yet resupplied, and the grip climbs across a tasting even at constant dose. And because the binding is cooperative and multivalent, the dose–response is **steep** — almost nothing below a threshold, then, across a narrow band of concentration, the film goes. The grip of raw cacao does not fade up. It switches on.

A number for the pull, anchored to cacao's own molecule — *the astringency index.*

A sensory world this physical still needs one number, and the number is a potency, not a mass. Two grams of a gentle tannin can grip less than a tenth of a gram of a fierce one, so weighing the tannin tells us almost nothing; what we want is how *hard* a given tannin pulls. We define that against the receptor the way every axis on the Index is defined — through a half-maximal concentration — and then invert and scale it:

$$\mathbf{IFI\text{-}A(c) = 1{,}000 \times EC_{50}(reference) \div EC_{50}(candidate).}$$

For astringency, $\mathbf{EC_{50}}$ is the concentration at which a compound precipitates **half** of a standardized proline-rich protein pool in a controlled turbidimetric assay — the point at which half the lubricating film has been thrown out of solution. A fierce astringent

reaches that point at a low concentration and has a *small* EC_{50}; a weak one needs a flood of itself and has a large one. Inverting puts the fierce one on top, where intuition wants it. We anchor the whole scale to a single reference astringent — the bare, undecorated flavan-3-ol unit **(−)-epicatechin**, assigned an EC_{50} of **1,000 µM** by convention, so that **IFI-A = 1,000** marks the pull of one plain subunit. With that anchor the formula collapses to a clean inverse:

> **IFI-A(c) = 1,000,000 ÷ EC_{50}(c)** (EC_{50} in µM).

There is a quiet grace in that choice worth naming, because it belongs to this chapter above all others. The reference molecule on which the entire astringency axis is pinned, (−)-epicatechin, is the very molecule cacao builds most of its grip from. The plant we do not grow is anchored, by happy accident of chemistry, to its own principal subunit. When we score a chocolate, we are reading it against the clean pull of one of its own building blocks — which makes cacao, of all the guests at this table, the one the ruler was practically cut to measure. Working the formula across the short condensed tannins that actually populate a cacao bean gives the ladder every later judgment rests on:

FLAVAN-3-OL UNIT	DEGREE	MW (G/MOL)	EC_{50} (MM)	EC_{50} (MG/L)	IFI-A
(−)-Epicatechin	DP1 · reference	290.3	1,000	290	1,000
Procyanidin B2	DP2	578.5	500	289	2,000
Procyanidin C1	DP3	866.8	360	312	2,780

Two worked lines show the arithmetic plainly. Procyanidin B2, cacao's archetypal dimer, precipitates half the reference protein at 500 µM, so IFI-A = 1,000,000 ÷ 500 = **2,000** — twice the grip of the bare unit, the whole gain bought by stringing two epicatechins into one cross-linking molecule. Add a third unit to reach the trimer procyanidin C1 at 360 µM, and IFI-A = 1,000,000 ÷ 360 = **2,780**: the first added unit doubled the grip, the second added only another thirty-nine percent. That is the signature of **multivalency with diminishing returns** — each new binding site slightly less reachable than the last — and it is the reason cacao's short oligomers already grip firmly while its chemistry never has to grow the enormous polymers a grape seed does. The mg/L column is only a unit conversion, EC_{50}(mg/L) = EC_{50}(µM) × MW ÷ 1,000, but it earns its place because an assay reads out in mass and a maker buys in mass: B2's 500 µM is 500 × 578.5 ÷ 1,000 ≈ **289 mg/L**, the same pull stated the way a bench meets it.

The number marks the *half* point, but the grip does not arrive gently on either side of it. Because precipitation is cooperative — one event seeds the next — the dose–response follows a steep Hill curve,

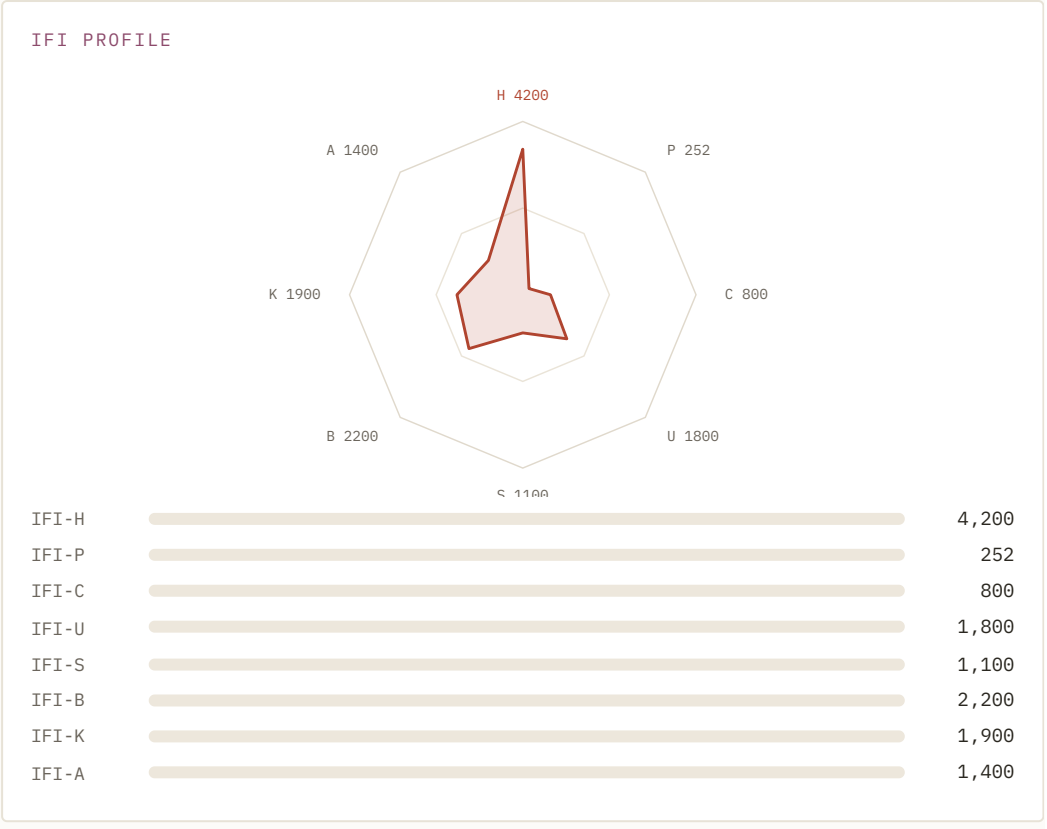
> $f(c) = (c/EC_{50})^{h} \div [1 + (c/EC_{50})^{h}]$,

with a Hill coefficient *h* near **1.8** for a condensed catechin tannin. Take procyanidin B2 at its EC_{50} of 500 µM: at half that concentration, 250 µM, $f = 0.5^{1.8} \div (1 + 0.5^{1.8}) = 0.287 \div 1.287 =$ **0.22**, only a fifth of full grip; at the EC_{50} itself, **0.50** by definition; at twice the EC_{50}, 1,000 µM, $f = 2^{1.8} \div (1 + 2^{1.8}) = 3.48 \div 4.48 =$ **0.78**. A fourfold swing in

concentration carries the mouth from barely a whisper to nearly full pucker. That steepness is not a measurement nuisance; it *is* the felt thing — the reason a raw nib seems to slam the mouth shut rather than ease into grip, and the reason a bean's EC_{50} has to be placed precisely rather than approximately.

The bar a percentage cannot read — *chocolate on eight axes.*

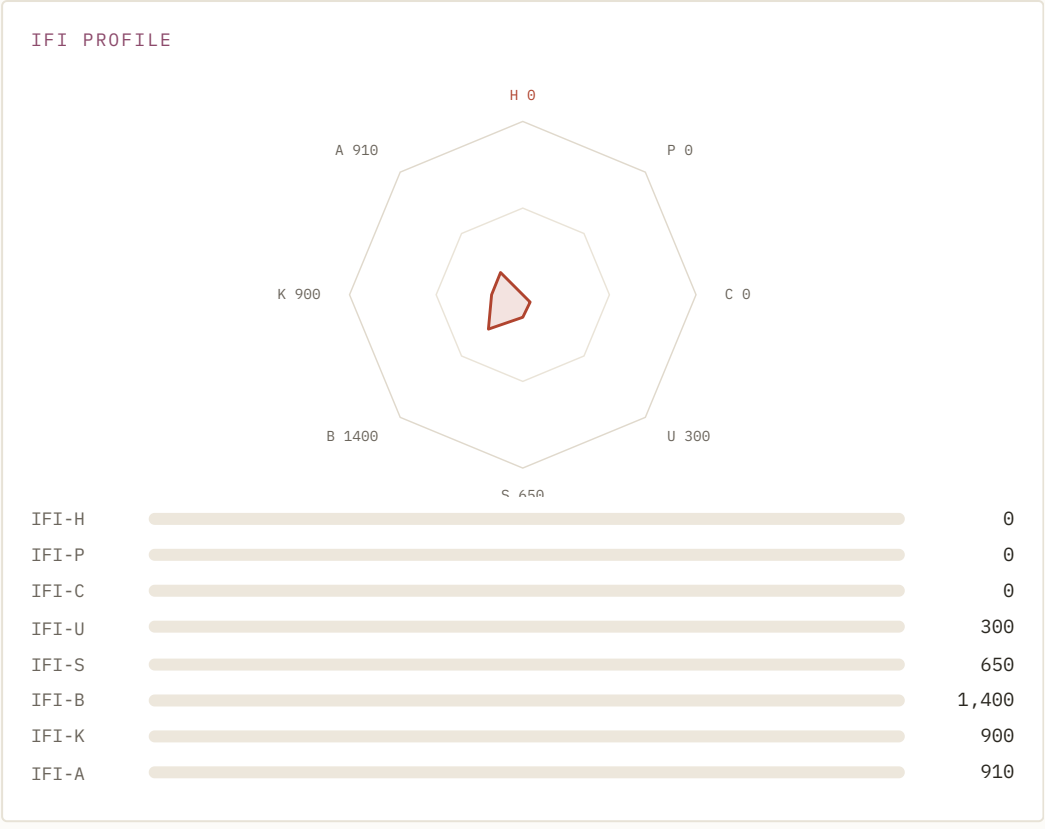
A cocoa percentage — the number stamped on nearly every bar — tells you the mass fraction of cocoa solids and butter and nothing whatever about grip, bitterness, or body. Score a chocolate on all eight axes instead, and you hold a description a percentage cannot draw. Set against it, for reference, is the astringency we designed into our full-spectrum flagship **Phoenix** (SS-0096), the single cultivar tuned to register on all eight axes at once: our model predicts a deliberately restrained grip of **IFI-A 1,400**.



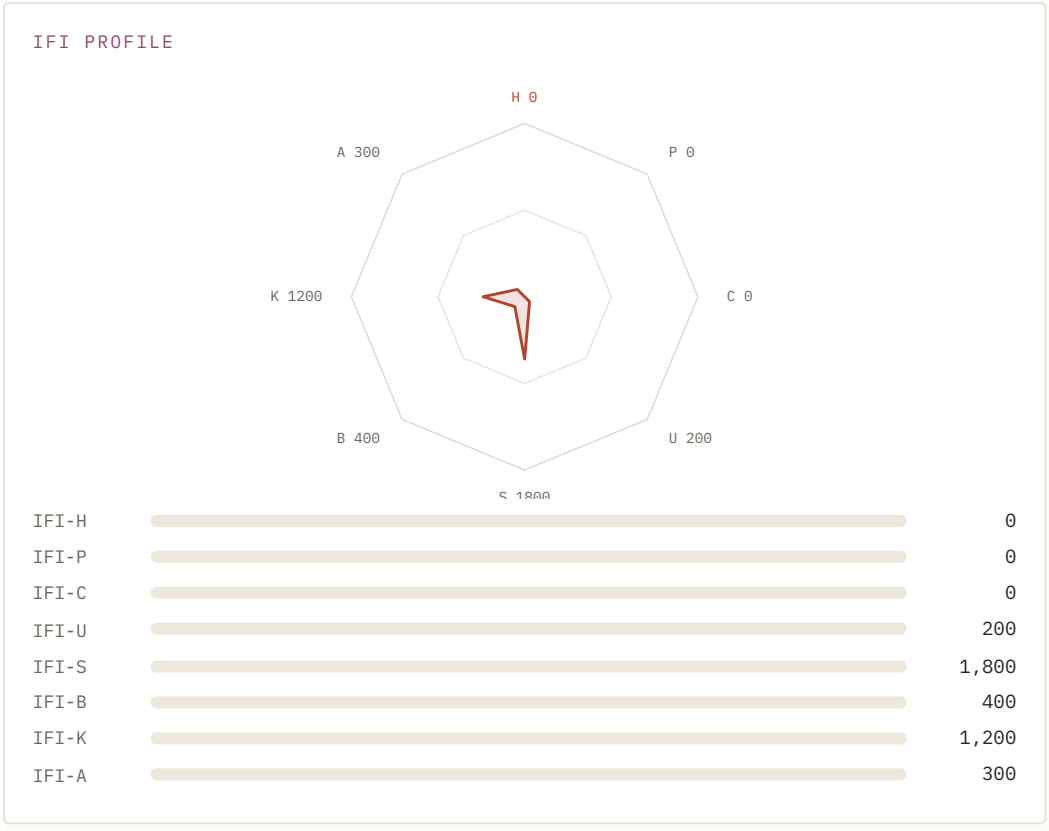
That designed A of 1,400 reads, through the inverse formula, as an EC_{50} of 1,000,000 ÷ 1,400 ≈ **714 µM** — a whisper of tannin laid under the heat to give the finish a spine. Hold it as the yardstick, because it says something startling about raw cacao. A raw nib's extract can half-precipitate the standard pool near **700 µM**, which reads IFI-A = 1,000,000 ÷ 700 ≈ **1,430** — a grip marginally *firmer* than the designed astringency of a cultivar we engineered gene by gene to be formidable, and nearly inedible on its own. The raw bean is

already at the full-spectrum design target, and then some. The maker's entire craft is spent bringing that wall *down* to the architecture of a finished bar.

Here is a representative read of a fine dark origin bar — a single-estate seventy, the chocolate equivalent of a structured young red, its grip and bitterness deliberately left tall so the bean's fruit and florals carry through them. We measure its extractable tannin at half-precipitation near **1,100 µM**, which fixes the headline axis at IFI-A = 1,000,000 ÷ 1,100 ≈ **910** — firm, below the engineered note, squarely in the young-red-wine band:



And here, the same chemistry with the grip and bitterness pulled down and the sweetness and dairy body brought up — a milk chocolate, whose tannin is diluted by milk solids and sugar and whose extract half-precipitates only near **3,300 µM**, reading IFI-A = 1,000,000 ÷ 3,300 ≈ **300**:



Read together, the three profiles are the taming journey in numbers: the raw nib's wall at **~1,430**, the dark bar's architecture at **~910**, the milk bar's roundness at **~300**. A maker who scores a bean and a finished bar this way holds a map a percentage cannot sketch — where the grip sits, how far the ferment and roast still have to move it, whether it will stand up to a splash of chili on the heat axis or need its grip softened for a children's bar.

One axis in those reads deserves its own word, because chocolate's bitterness is not tannin at all. The high **IFI-B** of a dark bar is carried chiefly by the **methylxanthines** — theobromine and caffeine, small purine alkaloids that are bitter agonists at the **TAS2R** receptors the axis is anchored to. (IFI-B scores the bitter dimension as a reading; where a plant is one of our hosts, the engineering lever on it is *attenuation* — talking that reading down by twenty to sixty-five percent — but cacao is a guest, so here we only measure what the bean already carries.) Theobromine dominates the load, and a short mole-count shows why:

METHYLXANTHINE	FORMULA	MW (G/MOL)	~% OF DEFATTED SOLIDS	RELATIVE MOLAR LOAD
Theobromine	$C_7H_8N_4O_2$	180.16	~2–3	~8–9 parts
Caffeine	$C_8H_{10}N_4O_2$	194.19	~0.2–0.5	~1 part

Take a cocoa mass running, in round numbers from the plant's own literature, 2.4 % theobromine against 0.3 % caffeine. Per hundred grams of solids that is 2.4 ÷ 180.16 = 0.01332 mol of theobromine against 0.3 ÷ 194.19 = 0.001545 mol of caffeine — a molar ratio near **8.6 to 1**. Theobromine carries roughly nine-tenths of the methylxanthine moles, which is why the bitterness of a dark bar is its own tall axis, standing beside the astringency rather than inside it. A percentage conflates the two into one dark number; the eight-axis read pulls them apart, and the separation is the whole usefulness.



A cacao pod and its beans, dressed as the bean is before any craft is spent on it. The number on a finished bar names its cocoa mass; it names nothing of the grip, the bitterness, or the body — the three things the eight axes pull cleanly apart.

The maker's subtractive-astringency program — *fermentation and roasting.*

The taming of the wall rewards the closest look of all, because it is astringency's chemistry run as a craft — a subtractive-astringency program carried out in the fermentation box and the roaster rather than the vineyard and the tank. Every chocolate-maker runs this program, whether they name it or not, and the whole of it is the controlled reduction of a measured EC_{50}.

Fermentation comes first. Heaped in boxes or under banana leaf, the sugary pulp around the beans becomes a microbial engine: wild **yeasts** consume the sugars to ethanol, then **lactic** and **acetic acid bacteria** take over, and the heat of their collective metabolism climbs the heap toward forty-five or fifty degrees. Ethanol and acetic acid seep inward through the seed coat, kill the embryo, and break down the cell walls that had kept the bean's enzymes and its polyphenols in separate compartments. Released together, the bean's own **polyphenol oxidase** meets the procyanidin pool and oxidizes the flavan-3-ols to **quinones** — reactive, brown, and promiscuous. Those quinones do two things that both subtract grip: they **polymerize** the tannin into larger, rounder, less-soluble forms, and they **bind irreversibly to the bean's own proteins**, pulling a share of the astringent pool off the sensory stage entirely. Free epicatechin falls sharply; the measurable EC_{50} of what remains climbs; the wall begins to come down. It is, molecule for molecule, the same enzymatic oxidation that turns a green tea into a black one, run inside a seed.

Roasting carries it the rest of the way. At a hundred and twenty to a hundred and fifty degrees, the **Maillard** reaction — reducing sugars condensing with amino acids — builds the roasted aroma that reads as *chocolate* at all, while **Strecker degradation** spins off the nutty, malty pyrazines; and alongside that aroma chemistry the heat continues to **condense and complex** the remaining tannin into forms that no longer reach the saliva. Soluble, grip-bearing polyphenol drops again. A poorly fermented, lightly roasted bean keeps its wall and tastes green and harsh; a well-fermented, properly roasted one has had the wall quietly rebuilt into structure — the clean, drying grip

that gives dark chocolate its spine and keeps it from collapsing into fat and sugar. A great chocolate, like a great wine, is a managed tannin.

There is even an edge to the maker's lever that the chemistry predicts and the craft confirms. Astringency climbs with chain length only so far: push the mean degree of polymerization past roughly ten or a dozen units and the tannin grows too large and too insoluble to splay across a salivary protein, so the grip **plateaus and then falls back** even as the molecule gets bigger. Fermentation and roast ride precisely this curve — they condense the tannin up and over its own peak, converting fierce short grip into mild, haze-forming polymer. The promise of designing cacao at the source is simply to hand the maker a bean whose procyanidins are *already* long and velvety, its grip already near architecture, so that less of the fine flavor has to be burned away to tame what the raw seed over-built.



A single cacao specimen, isolated for the record. The maker's ferment and roast are a subtractive-astringency program — polyphenol oxidase and Maillard chemistry moving the grip from a wall to an architecture, the same chemistry a designer would one day set in the bean itself.

The honesty of the error bar — *precision, variance, and an asymmetry.*

None of these numbers would mean anything without a tight error bar, and here the chocolate chapter must own an asymmetry the engineered-plant chapters do not. Our constructs are designed to hold batch-to-batch variation to a coefficient of variation of **≤7 %**, against the **42 %** that is ordinary for a tannin load left to a wild plant and a growing season. That precision propagates cleanly into the Index. Because IFI-A = k ÷ EC_{50} with k fixed, a fractional error in the measured concentration passes one-for-one into the score; and when both the reference pool and the candidate carry their own measurement error, the two combine in quadrature,

$$\sigma(IFI)/IFI = \sqrt{[(\sigma_{ref}/EC_{50,ref})^2 + (\sigma_c/EC_{50,c})^2]}.$$

At a controlled 7 % on each term, $\sqrt{(0.07^2 + 0.07^2)}$ = **9.9 %**. So when we measure one well-prepared chocolate extract, the dark bar's read of IFI-A ≈ 910 is a genuine 910 ± ~90 — a rung you can stand a product on. Run the measurement in eight replicates and the standard error shrinks by $\sqrt{8}$, to about 2.5 %, tightening the band to ± ~23. The *instrument*, in other words, reads cacao as precisely as it reads anything we grow.

The asymmetry is the plant. We can measure a given bean to ≤7 %, but we do not *hold* a cacao bean to ≤7 %, because we do not engineer it. A bean's grip is vintage and weather and ferment — the heap that ran a degree hotter, the three days that became five, the estate's rain that year — and across harvests the same origin can swing with the wild 42 % that uncontrolled tannin always carries. Let the candidate wobble at that rate and $\sqrt{(0.07^2 + 0.42^2)}$ = **42.6 %**, smearing a 910 into anything from roughly 520 to 1,300 batch to batch. That is not a flaw in the Index; it is the Index being honest about the difference between a measurement and a design. We can tell a maker, to the point, how hard *this* bean grips today. What we cannot yet do — because cacao is a guest and not a host — is make next season's bean grip the same. The precision belongs to the instrument; the variance belongs to a plant we have not taken up.

Where the number lies on a bar — *matrix, edge cases, failure modes.*

An index earns trust by naming where it goes thin, and on a bar of chocolate astringency goes thin in instructive ways, because a bar is a far richer matrix than the flask the EC_{50} is measured in.

The largest correction is **fat**. Roughly half of a cacao bean is cocoa butter, and in a finished bar that fat coats the mucosa and sheathes the tannin, so a share of the grip is spent or hidden before it ever reaches the salivary film. A bar reads gentler in the mouth than its extractable tannin content alone would predict — which is why chocolate and tannin forgive each other where tea and tannin do not. We measure the stimulus, the tannin's true pull; the butter decides how much of that potential is ever cashed. **Sugar and milk** push the same way, softening and diluting the grip, which is the whole mechanism of a milk bar's low A. **Conching** — the long, warm shear a maker runs for hours or days — works a subtler magic still, narrowing the particle distribution and nudging the tannin toward even, well-polymerized chains, converting a coarse, chalky grip into a velvety one without necessarily changing how much tannin is present. The felt character moves while the headline magnitude barely does.

Two honest limits round it out. Cacao's **non-galloylated** chemistry means a bar never reaches, per unit of tannin, the sharp early pucker that tea's galloylated catechins deliver — chocolate's grip is constitutionally rounder, and a maker chasing a bright, cutting astringency is working against the molecule. And the subtractive program has a true **failure mode** at its far end: a maker who tames the wall by *over-roasting* kills the grip, yes, but burns off the florals,

the fruit, and the delicate volatiles in the same fire — and burns off, with them, the very procyanidins the plant built. Every gram of flavanol scorched away to soften a bean is a gram of what the raw seed grew for its own protection, lost to the heat. We flag that plainly, because it is the clearest argument for designing a bean born gentle rather than wrestling a fierce one quiet: the grip you do not have to burn off is the nourishment you get to keep. We make no claim for what those molecules do in a body — only the quiet, vegetarian-minded point that food grown clean should not have to be half-destroyed to be made kind, and that the most reverent design is the one that wastes the least of what the plant already made.

What we can give chocolate today — *and what waits.*

This, then, is the measurement we can give chocolate right now, in full: the grip read exactly against the bean's own anchor molecule, the bitterness pulled off the astringency into its own axis, the body named, the whole bar written as eight numbers a percentage could never draw. The craft we can describe completely — the ferment and the roast as a subtractive-astringency program, polyphenol oxidase and Maillard chemistry moving a wall toward an architecture. The measurement and the understanding are ours to offer today, and we offer them without reservation.

The *design* is the part that waits. A cacao whose procyanidins are built long and velvety from inside the bean, whose grip arrives already architectural, whose bitterness and astringency are set at the source rather than wrestled out in the factory — that is the promise of the method this book builds toward, applied to a plant we have not yet taken up. The levers are visible from here: the **ANR** branch that sets the epicatechin pool, the chain-length machinery that sets the degree of polymerization, the galloylation the bean forgoes and a designer might offer or withhold — the same kind of program that, in a pepper, let a protein language model and a flux-balance model propose substitutions no breeder ever stumbled on, and CRISPR write them in. We can see exactly where the dials sit in cacao. We have simply not yet turned them, and we will not pretend, for the pleasure of a grand claim, that we have. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

The astringency hosts we *do* grow are the tannin plants the chemistry predicts, and cacao's absence from the list is the honest shape of this chapter:

HOST ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	NATIVE TANNIN CHEMISTRY
Grape	1,610	B / A / M	Hard	condensed — procyanidins & prodelphinidins (skin, seed, stem)
Tea	280	U / B / A	Hard	condensed — galloylated catechins (EGCG)
Blueberry	120	A	Moderate	condensed — A-type locked linkage
Pomegranate	120	A	Hard	hydrolyzable — ellagitannin (punicalagin)
Sorghum	120	A	Frontier	condensed — prodelphinidin-rich (seed testa)
Raspberry	40	A	Moderate	hydrolyzable — ellagitannin

Six plants carry astringency as a primary axis in the live library; cacao is not among them, and it would be the easiest thing in the world to add it quietly to the row and let no one ask. We will not. The one thing a family of scientists owes its readers is the difference between what we grow and what we could, and cacao, for now, sits firmly on the *could* side of that line — a frontier, flagged as a frontier, and not a product dressed as one. What we can do for chocolate today we have done here, completely: we measure its grip, molecule by molecule, and we revere it. The designing of it is a chapter the method will one day write, in a bean we have yet to take into our hands.



PART III OF IV

The Method

reading a pathway before it is planted · turning the structural knobs · the subtractive art of softening

Chapter 11 · Reading the pathway before we plant it — *ESM2, flux balance, and the proof in Pun1.*

Every organism in Part II is a different tannin chemistry, but the *method* by which we learn to steer them is one method, and it is the same one that built the superhot peppers this company is known for. We did not arrive at designed astringency by crossing tannic grapes for a decade and hoping. We arrived at it the way we arrive at every axis: by reading the pathway computationally, predicting which enzyme is the real bottleneck and which edit will move it, and only then writing the change into the plant. The proof of the method is a pepper enzyme, and it is worth walking through that proof slowly — the structure, the scoring, the whole-cell accounting, the arithmetic underneath each — because the logic transfers to tannin exactly, residue for residue and shadow price for shadow price. This is the one chapter where we open the machine and show every gear, so that when we point it at a grape the reader already knows what it is doing.

The sensation with no receptor — what we are actually steering

Astringency is the strange one. Heat has a channel, TRPV1; sweetness has a dimer, TAS1R2·TAS1R3; umami, bitterness, kokumi, cool-burn, and pungency each resolve to a named protein that opens or signals when the right molecule arrives. Astringency does not. There is no astringency receptor in the grain of the tongue. The pucker of a young Nebbiolo, the grip of an over-steeped green tea, the slate-and-plum drag of a pomegranate eaten with its pith — all of it is produced not by a receptor binding a ligand but by a demolition happening in the saliva itself.

Here is the mechanism, step by step, because the whole index rests on getting it right. The mouth lubricates itself with a thin film of saliva whose glide comes from two kinds of protein: large glycosylated mucins, and a family of small, intrinsically disordered **proline-rich proteins (PRPs)**. The PRPs are extraordinary molecules. They have almost no fixed fold — they are open polyproline-II ribbons, their backbones studded with proline residues whose flat pyrrolidine rings present a row of hydrophobic faces and a row of exposed carbonyls. Evolution shaped them, in tannin-eating mammals, to be sacrificial tannin sponges.

A tannin arrives — a polyphenol, which is to say a molecule carrying many phenolic hydroxyls on many aromatic rings. Its galloyl and catechol faces stack flat against the proline rings, a hydrophobic and CH–π embrace; its phenolic –OH groups hydrogen-bond to the PRP's backbone carbonyls. One tannin can reach two or three PRPs at once, because it is multivalent — several binding faces on one molecule. Cross-links form. The protein–tannin complex grows from a soluble pair to a colloid to an aggregate that passes its solubility limit and **precipitates**. The lubricating film collapses out of solution.

What the tongue then reports is not taste at all. With the mucin–PRP film stripped, the oral surfaces lose their boundary lubrication, and the sliding friction between tongue and palate climbs sharply. Mechanosensitive free nerve endings — trigeminal, very likely including TRPA1-bearing afferents — read that rise in friction as roughness, drag, and tightening. Astringency is a *tactile* percept triggered by a *chemical* precipitation. That is the whole secret of the axis, and it is why the index had to be built a little differently here than for a channel like TRPV1.



FIG. 11a — grape berry in cross-section: the seed coat and inner skin, where the condensed-tannin program runs hottest. Astringency is written into tissue here long before it is ever tasted, as a defense the plant builds and a structure we learn to tune.

The index, made exact for a tactile sense

The Iyengar Flavor Index scores every axis by the same law:

$$IFI(c) = 1{,}000 \times EC_{50}(\text{ref}) \,/\, EC_{50}(c)$$

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response; invert it against a fixed reference, scale by a thousand, and loudness becomes a single number. For a channel, EC_{50} is a clean half-activation. For astringency there is no channel to half-activate, so we anchor the axis to the event that actually produces the sensation: half-maximal precipitation of a standardized PRP. We call that concentration the **PC_{50}** — the half-precipitation concentration — and it slots into the law unchanged:

$$\text{IFI-A}(c) = 1{,}000 \times PC_{50}(\text{ref}) \,/\, PC_{50}(c)$$

A lower PC_{50} means less tannin is needed to precipitate half the protein, which means a more potent astringent, which means a *higher* IFI-A. The inversion is the point: potency and concentration run opposite, so the score climbs as the stimulus grows louder.

Fix the reference so that **IFI-A = 1,000** lands exactly on a defined reference tannin's half-precipitation — call its value PC_{50}(ref). To make the arithmetic visible on the page, take a reference that halves a standard PRP at 50 µM. Then the law reads any tannin against it:

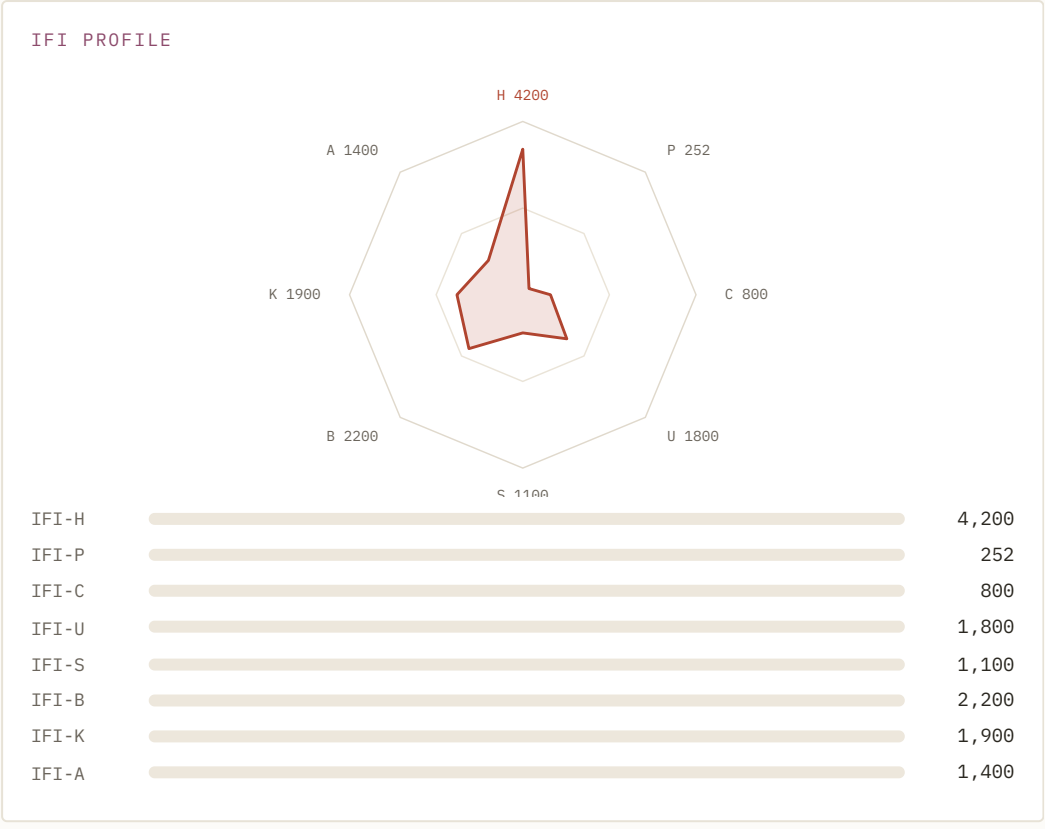
- A candidate that also halves at 50 µM scores 1,000 × 50/50 = **1,000** — it *is* the reference.
- A candidate twice as potent, halving at 25 µM, scores 1,000 × 50/25 = **2,000**.

- A candidate five times as potent, at 10 µM, scores 1,000 × 50/10 = **5,000** — the scorecard ceiling, the per-axis normalization maximum.
- A gentle table grape halving only at 100 µM scores 1,000 × 50/100 = **500** — half the reference's loudness.

Our model predicts Phoenix's IFI-A at **1,400** — the full-spectrum cultivar SS-0096 is the first plant we designed to register on all eight axes at once, and its astringency is designed at 1,400. Run the law backward and it tells us exactly how potent Phoenix's tannin is designed to be relative to the reference:

> PC_{50}(Phoenix) = 1,000 × PC_{50}(ref) / IFI-A = 1,000 × 50 µM / 1,400 ≈ **35.7 µM**

So Phoenix's tannin is designed to reach half-precipitation at approximately 35.7 µM against the reference's 50 µM — it is 50/35.7 = **1.4× as potent**, which is simply what "1,400 on a 1,000-anchored axis" means as a design specification. The 50 µM reference is a teaching value; the 1.4× ratio is the canonical design parameter.



That block is Phoenix's complete eight-axis profile — the only food in the library that speaks every axis, and therefore the only one whose astringency sits inside a full chord rather than alone. Read across it and you can see the design problem of the whole book in miniature: an A of 1,400 has to be built *without* collapsing the B of 2,200, because tannin and bitterness share chemistry and will move together unless the pathway is steered to separate them.

Dose–response. PC_{50} is not a threshold; it is the midpoint of a sigmoid. Tannins cross-link PRPs multivalently, so precipitation is cooperative, and the fraction precipitated follows a Hill curve:

```
f([T]) = [T]^n / (PC50^n + [T]^n)
```

with [T] the tannin concentration and n the Hill coefficient — the cooperativity, which for multivalent tannins runs above 1; take n = 2. At Phoenix's design PC_{50} of 35.7 µM:

- at [T] = 35.7 µM (1× PC_{50}): f = 35.7^2 / (35.7^2 + 35.7^2) = **0.50**
- at [T] = 17.9 µM (½× PC_{50}): f = 17.9^2 / (35.7^2 + 17.9^2) ≈ **0.20**
- at [T] = 71.4 µM (2× PC_{50}): f = 71.4^2 / (35.7^2 + 71.4^2) ≈ **0.80**

Between half and double the PC_{50}, precipitation runs 20% → 80%: the pucker arrives fast. More generally, the span of concentration needed to climb from 10% to 90% of full astringency is 81^(1/n) — a 9-fold window at n = 2, against the 81-fold window a non-cooperative n = 1 would give. Cooperativity is what makes astringency *snap* on rather than fade up, and it is a dial we can read off the Hill slope of any construct.

Units. The flask speaks in molarity; a glass speaks in milligrams per litre. A condensed tannin of mean degree of polymerization 5 — five flavan-3-ol units — carries a molecular weight near 1,450 g/mol. Phoenix's design PC_{50} of 35.7 µM converts to

```
35.7 × 10^-6 mol/L × 1,450 g/mol ≈ 0.052 g/L = ~52 mg/L
```

which is the same order as the tannin a structured red already carries (roughly 100 mg/L in a light wine to over 1 g/L in a big one). That proximity is exactly why a small shift in PC_{50} is a large shift on the palate: you are moving the midpoint of the curve right through the concentration the food actually delivers.

Error, and why reproducibility is the whole argument. A number means nothing without its spread. Our constructs target a batch-to-batch coefficient of variation of **≤7%**, against a wild-type variability near **42%**. Because PC_{50} sits in the denominator of the law, the relative error in IFI-A equals the relative error in PC_{50} to first order. So Phoenix's design A of 1,400 at a 7% CV specification is 1,400 ± 98 — a tight band from roughly 1,300 to 1,500. The same axis in an unimproved wild plant at 42% CV would be 1,400 ± 588, a smear from 812 to 1,988 that overlaps three or four other cultivars into meaninglessness. The index is only a standard because the plants behind it are reproducible; the CV is not a footnote, it is the reason the score is a score.

The proof: a pepper enzyme we could read, rank, and rewrite

The capsaicin work is the template, and it is worth watching the method do something impossible before we ask it to do something subtle. Faced with making a pepper hotter, every prior program selected the *phenotype* — the hottest fruit — and prayed the *genotype* followed. We inverted it. We read the enzyme first.

The enzyme is **Pun1/AT3**, the acyltransferase that condenses vanillylamine with an 8-methyl branched-chain fatty acid to make capsaicin. We predicted its three-dimensional structure, then scored every change we might make to it. The scorer is an **ESM2** protein language model — six hundred and fifty million parameters trained not on labels but on the shape of evolution itself, on millions of natural sequences, until it learned which residues belong where. Mask a position, and the model returns a probability for each of the twenty amino acids that could sit there. The variant-effect score for a substitution is the log-likelihood ratio between the mutant and the wild-type residue in that exact structural context:

```
score(mut) = log P(mut | context) − log P(wt | context)
```

A positive or near-zero score is a change evolution would tolerate; a sharply negative one is a change evolution has avoided for good reason. We rank candidates by the product the pathway actually cares about — predicted catalytic effect × predicted fold stability — because an edit that speeds the chemistry but unfolds the protein buys nothing.

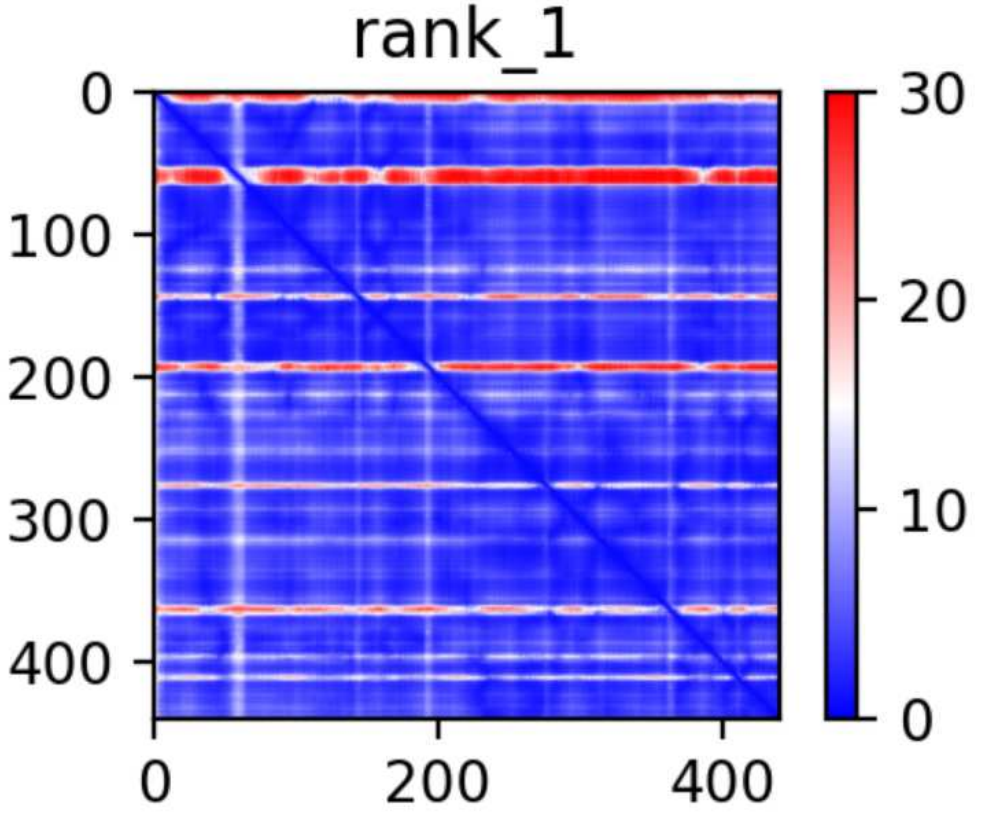


FIG. 11b — Pun1/AT3 predicted aligned error. Every pixel scores the model's confidence in the relative position of two residues; the low-error blocks are the rigid domains where an edit lands where we think it will, and the bright off-diagonal bands are the hinges we leave alone.

The reason this has to be done *in silico* is pure combinatorics. For an enzyme of Pun1's size — on the order of 450 residues, which its own edit positions confirm, since they run from residue 39 near the N-terminus to 345 in the active-site cleft — the single-substitution space is 19 alternatives × 450 positions = **8,550 variants**. Scoring all

of them is an afternoon for the model and a career for a wet lab. Go to pairs — which is where our answer lived — and the space is C(450,2) × 19² ≈ **36 million** combinations. No one builds 36 million peppers. The model does not enumerate them either; it scores the 8,550 singles, keeps the handful that improve both catalysis and stability, and uses the structure to propose the few combinations where those edits do not collide. That pruning — singles scored exhaustively, combinations proposed structurally — is the honest shape of the search. Two substitutions, never before combined in any living pepper, survived it.

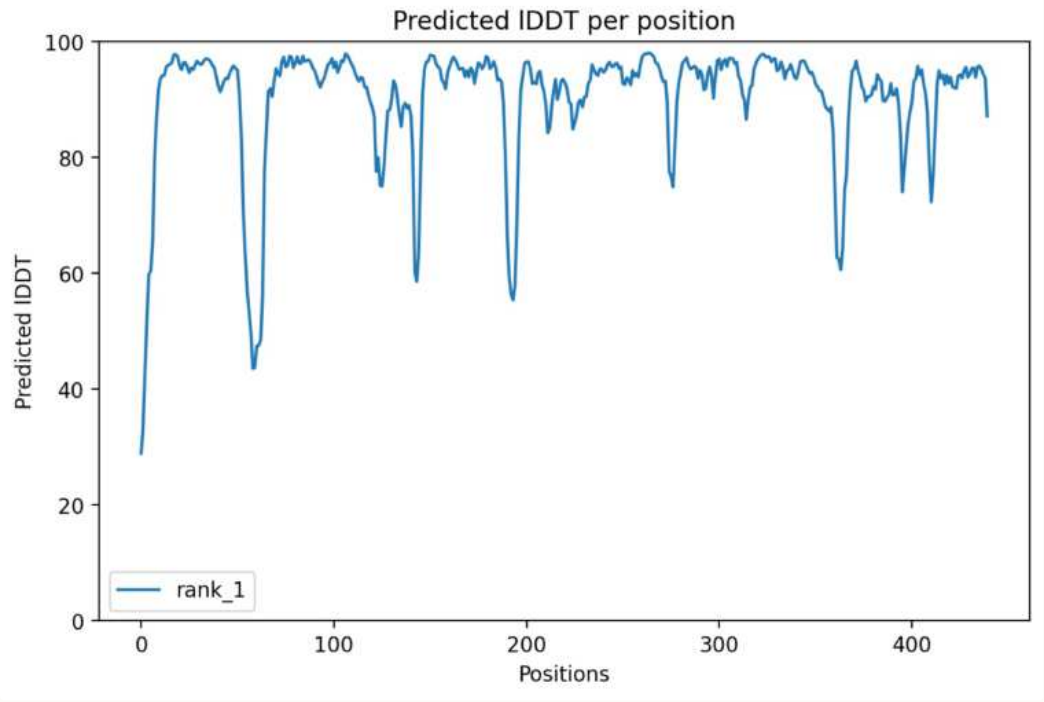


FIG. 11c — the Pun1/AT3 model coloured by pLDDT confidence. The platform predicts a structure, scores every possible single-residue change against it, and confirms with a whole-cell flux model which step actually limits output before a single edit is made.

What the scores returned, and what a structure-based ΔΔG then established, was two edits whose free-energy changes are worth reading as physics rather than as a list. ΔΔG is the change in folding and binding free energy a mutation causes, in kcal/mol; a negative value is stabilizing. And free energy is not abstract — through the Boltzmann relation it is a population. A change of ΔΔG shifts the favorable-state equilibrium by a factor of exp(−ΔΔG / RT), where RT at laboratory temperature (298 K) is 1.987 cal/mol·K × 298 K ≈ 0.592 kcal/mol. Run each edit through it:

EDIT	POSITION	EFFECT	ΔΔG (KCAL/MOL)	EQUILIBRIUM SHIFT EXP(−ΔΔG/RT)
S39L	res 39 · N-terminal	binding affinity ↑	−2.1	≈ 35×
L345G	res 345 · active-site cleft	steric relief	−1.8	≈ 21×
C175S	res 175 · core	*structural annotation — not in shipped construct*	−0.9	–
combined (S39L+L345G)	—	stacked, non-interfering	**−3.9**	**≈ 735×**

The arithmetic is exact: exp(2.1/0.592) ≈ 35, exp(1.8/0.592) ≈ 21, and because free energies of independent edits add, the combined −3.9 kcal/mol multiplies out to exp(3.9/0.592) ≈ 735× — equivalently 35 × 21. Two small, carefully chosen changes shift the fold-and-bind equilibrium of the enzyme by approximately 735-fold toward the productive state. Written in with CRISPR, our model predicts peak

output toward approximately **2.95 million Scoville** at the ≤7% batch CV design specification, against the ~42% a wild-type pepper wanders across from fruit to fruit.

That reproducibility is what lets the method leave an audit trail instead of an anecdote. The flagship ladder is that trail: each cultivar a controlled step in design complexity, each step layering new computational axes, the eighth — astringency — appearing only at the top, where Phoenix is designed to register on all eight at once.

CULTIVAR	SKU	SHU (PREDICTED)	IFI-H	AXES
Ember	SS-0087	866K	1,570	2/8
Blaze	SS-0091	1.72M	2,630	4/8
Wildfire	SS-0092	1.96M	2,930	5/8
Firestorm	SS-0094	2.47M	3,490	6/8
Phoenix	SS-0096	2.95M	4,200	**8/8**

The predicted numbers map each design's computational scope. The *method* is portable, and that portability is the whole reason this library spans forty-two organisms and eight axes rather than one plant and one sense.

Flux balance: the step that saves us from a beautiful, useless edit

Before any edit is written, one more model runs, and it is the one that most often rescues us from a gorgeous mistake. Scoring an enzyme tells you how to make that enzyme better. It does not tell you whether a better enzyme will change anything — because an enzyme sits inside a whole living economy of carbon and reducing power, and improving a step that is not the bottleneck moves nothing.

The accounting is **flux-balance analysis (FBA)**, and underneath it is linear algebra. Write the plant's metabolism as a stoichiometric matrix **S**, with a row for every metabolite and a column for every reaction. Let **v** be the vector of reaction rates. At metabolic steady state nothing accumulates, which is one clean constraint:

```
S · v = 0
```

Add thermodynamic bounds on each reaction (lb ≤ v ≤ ub, which among other things forbids irreversible steps from running backward), choose an objective — maximize flux into the tannin you want to build, c·v — and you have a linear program. Solve it and you get the optimal flux distribution v*, the best the whole cell can do.

The quantity that makes FBA worth running is not v* itself but its **shadow prices** — the dual variables on the mass-balance constraints. A metabolite's shadow price is the marginal gain in the objective per extra unit of that metabolite: precisely *how much more product you would get if you had a little more of this precursor.* And it answers, before a seed is sown, the question that wrecks naive edits. Raising a galloyltransferase does nothing if the gallate supply is the real limit — and the shadow price of gallate says so outright: if it is near zero, more galloyltransferase is wasted; if it is large and positive, gallate is the true bottleneck and *that* is where to edit.

Pushing the condensed-tannin branch does nothing if the upstream dihydroflavonol pool is already capped, and the model reads that cap as a shadow price too. A companion calculation, flux variability analysis, then asks for each reaction the minimum and maximum flux still consistent with the optimum — telling us which steps are rigidly determined and which have slack to exploit.

The failures FBA catches are the expensive ones. Divert too much carbon into tannin and you can starve the anthocyanins a fruit needs for its colour, turning a designed grape pale. Push a pathway past a downstream capacity and the intermediate backs up into a dead-end pool the plant simply excretes — effort spent making something the plant throws away. This is the difference between editing an enzyme and designing a flavor: the first is a change to a protein; the second is a change to a sensation, and only a whole-cell account can tell you whether the one will become the other.

Turning the machine on tannin

Now point the same machine — predict the structure, score the substitutions with the ΔΔG and Boltzmann logic above, check the flux and its shadow prices — at tannin, and nothing about the method changes. Only the terminal enzymes at the end of the pathway do.

The trunk is shared by every vascular plant on earth: the phenylpropanoid branch **PAL → C4H → 4CL → HCT → COMT**, the ancient road that turns the amino acid phenylalanine into the activated phenolics every downstream flavor is built from. Peppers hang capsaicin off the far end of it; tannic plants hang the flavonoid pathway off it instead. That pathway reaches a fork, and the fork is where astringency is decided.

At the fork, a leucoanthocyanidin can go two ways, set by two reductases. **LAR** (leucoanthocyanidin reductase) makes the 2,3-*trans* flavan-3-ols — catechin. The other way runs through an anthocyanidin, and **ANR** (anthocyanidin reductase) makes the 2,3-*cis* epicatechin. These are the "fork reductases," and their ratio sets the starter and extension units of every condensed tannin the plant will polymerize. Upstream of them, **F3'5'H** decides how many hydroxyls the molecule's B-ring carries — push it and you make the trihydroxylated prodelphinidins, which are more hydrogen-bond-rich and more astringent than the procyanidins. Then galloylation: an **SCPL galloyltransferase** (a serine-carboxypeptidase-like acyltransferase, the same enzyme *class* as a Pun1/AT3, which is why the structural method transfers so cleanly) esterifies gallic acid onto the flavan-3-ol, and a galloylated catechin is markedly more astringent than its bare form. Finally, **MYB** transcription factors sit over the whole program as regulators — turning the tannin machinery on in a tissue, or leaving it off. Potency, in the end, is set by four dials the method can read and rewrite: the fork (cis vs trans, catechin vs epicatechin), the hydroxylation (procyanidin vs prodelphinidin), the galloylation, and the degree of polymerization that multivalency rides on.

Different plants hang different terminal enzymes on that identical trunk, and the construct counts and difficulty are the library's own:

ORGANISM	CONSTRUCTS	TANNIN CHEMISTRY	A-AXIS TARGETS	DIFFICULTY
Grape	1,610	condensed · procyanidin / prodelphinidin	LAR · ANR · F3'5'H · SCPL-GT · MYB	Hard
Tea	280	galloylated catechin (EGCG-type)	ECGT · polyphenol oxidase	Hard
Pomegranate	120	hydrolyzable · ellagitannin (punicalagin)	galloyltransferases · oxidative-coupling laccases	Hard
Blueberry	120	condensed · procyanidin	condensed-tannin fork (ANR / LAR)	Moderate
Sorghum	120	condensed · testa proanthocyanidin	*Tan1* · *Tan2* regulators	Frontier
Raspberry	40	hydrolyzable · ellagitannin	galloyl / coupling	Moderate

Read the table as a set of design problems the method solves the same way each time. Grape is the deep one — 1,610 constructs — and its astringency is a five-enzyme chord: the LAR/ANR ratio for texture, F3'5'H for the prodelphinidin edge, the SCPL galloyltransferase for grip, and the MYB above them for where and when. Tea's astringency lives almost entirely in galloylation: the galloylating **ECGT** builds the EGCG and ECG that make a green tea bite, while its polyphenol oxidase governs the oxidative browning that softens a black tea's tannin into theaflavins. Pomegranate is hydrolyzable-tannin country — galloyltransferases lay gallic acid onto a glucose core to build pentagalloylglucose, and **oxidative-coupling laccases** weld those galloyls into punicalagin, the ellagitannin behind the fruit's long, dry finish. Sorghum is the frontier case: its astringency is a switch, the **Tan1/Tan2** regulators that turn the pigmented testa on, and editing a regulator is editing a decision rather than a reaction.

The design logic over all of them is one loop, and it is the loop the pepper proved. *Which step actually limits the tannin I want?* — the flux model answers, with shadow prices, and the answer is routinely counterintuitive: raising a galloyltransferase is wasted if gallate is the limit, and FBA catches it before a plant is grown. *Which edit moves that step without breaking the enzyme?* — ESM2 scores the whole substitution landscape and ranks it by the same catalytic-effect-times-stability product, the same ΔΔG that found S39L and its companion L345G. *Will the whole-plant system actually express the change as a different tannin?* — the flux balance closes the loop, checking that carbon diverted at the fork becomes astringency rather than backing up or bleeding into anthocyanin. We predict the structure, score the edits, check the flux, write the change, and design to a target PC_{50} — and the loop would close the day an experimental PC_{50} matches the number the model predicted.

There is a quiet reward buried in the inversion of the index. Because IFI-A rises as PC_{50} falls, designing *up* on astringency means a plant can deliver the same structural grip with *less* total tannin. A wine or a tea designed to a higher IFI-A reaches its intended tightness at a lower tannin load — which also lowers the bitterness that rides alongside tannin on the small galloylated molecules. Less material, same sensation, cleaner finish: for a family that thinks about what a plant is asked to carry, that is not a side effect. It is part of the point.

What the number does not promise — matrix effects and honest limits

A PC_{50} is a design target derived from a standardized protein in a clean buffer, and the mouth is neither clean nor standardized. We say so plainly, because a number is only respectable if its boundaries are.

Astringency *accumulates*. Each sip precipitates PRPs that saliva has not yet replenished, so the third taste of a tannic wine is sharper than the first — the sensation builds across an interval that a single-exposure PC_{50} does not capture. The *matrix* shifts it: ethanol competes with tannin for the hydrophobic stacking against proline, so the same tannin reads softer in a wine than in water, while low pH reads it sharper; a PC_{50} in buffer is a true north, not the exact bearing in the glass. *Protein masks* it — the casein in a splash of milk pre-binds tea tannin and blunts the astringency entirely, which is physiology, not preference. And the *perceiver varies*: PRP expression differs between people and even rises with habitual tannin intake, so one design PC_{50} is felt differently across a table. The index reports the stimulus, not the sensation in a particular mouth, and that is a deliberate choice — we standardize what the plant is designed to deliver and leave the last, personal translation where it belongs.

One confound matters enough to design against directly: **bitterness co-travels with astringency.** The small, galloylated catechins that make a potent astringent are also ligands for the TAS2R38 bitter receptor, so IFI-A and IFI-B move together unless the pathway is steered to separate them — which is exactly why grape and tea live in the library as B/A organisms and why the flux work matters. Shifting the mean degree of polymerization upward, for instance, raises astringency (bigger, more multivalent tannins precipitate PRPs harder) while lowering bitterness (the oligomers grow too large to fit the bitter receptor's pocket). The method does not just raise A; it holds B where we want it while it does — the same deconvolution visible in Phoenix's profile, an A of 1,400 standing beside a deliberately governed B of 2,200.

Why the pepper paid for the road

This is the deepest reason the library spans forty-two organisms rather than one. **Five of our seven platform modules transfer unchanged** from the pepper to the grape to the pomegranate, because the upstream phenylpropanoid trunk — PAL → C4H → 4CL — is shared by every vascular plant on earth, and only the terminal enzymes differ at the far end of each branch. The module that predicts a structure, the one that scores substitutions with ESM2, the one that models whole-cell flux and reads its shadow prices, the one that designs the CRISPR edit, the one that evaluates the result against the index — none of them knows or cares whether the terminal enzyme it is pointed at makes capsaicin or epicatechin or punicalagin. A galloyltransferase and a capsaicin synthase are the same structural problem to the model: an acyltransferase fold, an active-site cleft, a ΔΔG landscape to rank. Only the last two modules — the ones that encode an organism's particular terminal chemistry and its particular MYB or Tan regulation — are rebuilt per plant.

So the cost of adding an organism to the astringency roster is not a new platform; it is the characterization of a few terminal enzymes and their switches, bolted onto a method already proven on the hottest pepper our model has designed. Astringency, the sensation with no receptor, turns out to be among the most *designable* axes we hold — because its chemistry is enzymatic from end to end, every dial a named protein the method can read, rank, and rewrite, and every result a target PC_{50} the index can close the loop on. The grape is not a harder problem than the pepper. It is the same problem, asked of a different fork in the same ancient road. The pepper paid for the road. Every tannin we design drives on it.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 12 · Turning the knobs — *polymer length, galloylation, and the ANR:LAR ratio.*

The method tells us which enzyme to touch; this chapter is about what happens inside the molecule when we touch it, and how a handful of edits becomes a designed grip. Everything reduces to three structural dials — **galloylation, degree of polymerization, and the fine-structure of the flavan-3-ol units** — and each dial is reachable through a specific enzymatic lever, with a predictable sign and a measurable size on the IFI-A scale. Astringency is the one axis where the thing we feel is not a molecule speaking to a channel but a molecule binding a protein and pulling it out of solution, and that difference changes everything about how we design it. So before we reach for a single lever, we have to be honest about what the grip actually *is*.



Trays of a single organism's design families under controlled light. A construct library is not a scatter of lucky plants; it is a systematic grid across the structural dials, grown and measured until the map between edit and grip is complete.

The grip, and the receptor that is not a receptor

Heat gates a channel. Sweetness folds a G-protein pocket shut. Astringency does neither. The sensation we call the grip is mechanical — a loss of lubrication reported by the trigeminal touch system, not a taste read by a taste bud at all. It begins in the thin salivary film that normally lets the surfaces of the mouth glide over one another, a film carried in large part by **proline-rich proteins (PRPs)**, which make up the majority of the protein in parotid saliva,

together with the small, histidine-dense histatins. These proteins are intrinsically disordered: open, extended, floppy chains with proline residues strung along them like beads, each proline presenting the flat, rigid, hydrophobic face of its pyrrolidine ring to the solvent.

A tannin is the thing those faces were waiting for. A condensed tannin — a proanthocyanidin — is a chain of flavan-3-ol units, each unit a pair of aromatic rings bearing hydroxyl groups, and the encounter between tannin and PRP is a three-part handshake we can describe exactly. First, **hydrophobic stacking**: the flat aromatic rings of the flavan-3-ol lie face-to-face against the proline rings, π-surface to π-surface, the single most favorable contact in the whole interaction. Second, **hydrogen bonding**: the phenolic hydroxyls of the tannin donate to the carbonyl oxygens of the peptide backbone, many weak bonds summing to one strong grip. Third, and decisively, **cross-linking**: because a polymeric tannin carries many binding faces along its length, one molecule bridges two, three, four protein chains at once, and the soluble complexes grow until charge is neutralized and the whole aggregate falls out of solution.

That precipitation is the sensation. The salivary film thins where its proteins have been pulled into haze; lubrication drops; friction between the moving surfaces of the mouth rises; mechanoreceptors report drag, roughness, a drawing-in. It is why astringency *builds* across a flight and *lingers* after the swallow — a depleted film re-wets slowly — and why it reads as texture rather than flavor. We find a quiet pleasure in the fact that the most architectural of the eight axes is the one the tongue cannot technically taste: it is felt the way you feel the nap of raw silk, or the pull of dry hands.

This is also why the Index cannot anchor IFI-A to a half-activated current the way it anchors heat. There is no channel to open. So we anchor it to the half-*precipitation* point instead. In a standardized turbidimetric assay — a fixed panel of salivary PRPs, a fixed ionic strength and pH, a tannin titrated in — we read the concentration at which precipitation reaches half of its maximum, the **PC_{50}**, the astringency analogue of EC_{50}. A potent tannin precipitates PRPs at low concentration and so carries a *small* PC_{50}; a weak one needs a flood. The Index formula then runs exactly as it does on every other axis, with PC_{50} standing in for EC_{50}:

$$\text{IFI-A} = 1{,}000 \times PC_{50}(\text{reference}) \div PC_{50}(\text{candidate})$$

The reference is the native wild-type seed tannin of *Vitis vinifera*, set as the unit by convention. By construction it scores **1,000**. Everything else is read against it: a tannin twice as potent — half the PC_{50} — scores 2,000; one half as potent scores 500. Loudness, on this axis, is grip per micromolar.

Reading the dial in numbers

Precipitation is a dose–response like any other, and we fit it with a Hill function so the whole curve, not just its midpoint, is a designed object. For a tannin at concentration C, the fraction of the salivary PRP panel pulled into haze is

$$\theta(C) = C^n \div (PC_{50}^n + C^n)$$

where PC_{50} is the half-precipitation concentration and n is the Hill coefficient — the steepness of the turn from silky to gripping. Because the mechanism is multivalent cross-linking, n is greater than one; the binding is cooperative, each bridge making the next aggregate more likely, and n climbs as the polymer lengthens and offers more faces per encounter. A monomer barely precipitates at all (n near 1, a lazy curve); a well-built octamer switches hard ($n \approx 2.5–3$), which is why a structured tannin has an *edge* to its onset — a defined moment where the grip arrives — while a short one only hazes vaguely. At $C = PC_{50}$, $\theta = 0.5$ by definition, and that single point is what the Index reads.

The arithmetic from there is clean. Hold the reference PC_{50} at a convention value of **28 µM** in monomer-equivalents — the concentration of flavan-3-ol subunits, whatever their chain length, at which the standard panel half-precipitates. Then any IFI-A we want corresponds to one and only one PC_{50}, found by rearranging the formula to $PC_{50}(\text{candidate}) = 28 \times 1{,}000 \div \text{IFI-A}$. A candidate we measure at 20.0 µM scores 1,000 × 28 ÷ 20.0 = **1,400**. One at 20.29 µM scores 1,379.9, which we report as **1,380**. The table below is just that rearrangement run across the scale, with the molar figure converted to mass using the mean subunit weight of catechin and epicatechin, 290.27 g/mol (1 µM = 0.290 mg/L).

IFI-A	READS AS	PC_{50} (MM, MONOMER-EQ.)	PC_{50} (MG/L)
500	flabby, under-ripe pole	56.0	16.3
1,000	native wild-type reference	28.0	8.1
1,380	the worked design result	20.3	5.9
1,400	structural-red target	20.0	5.8
2,000	firm, grippy pole	14.0	4.1

Two honest footnotes keep the conversion from lying. The mass column uses the base flavan-3-ol weight; a galloylated subunit carries an extra galloyl ester and runs roughly 150 g/mol heavier (epicatechin gallate, 442.4; epigallocatechin gallate, 458.4), so a galloyl-rich tannin delivers the same molar PC_{50} at perhaps 20–30% more mass. And the molar figure is *per subunit*, which matters because a chain hides its valency: a tannin of mean degree of polymerization 8 presents eight binding faces per molecule, so a per-subunit PC_{50} of 20 µM is a per-*molecule* PC_{50} near 2.5 µM. The protein does not count subunits; it feels molecules that cross-link, and the molecule is eight times rarer than its parts.

The last piece of the math is the one the cellar cares about: **reproducibility**. The Index value is a ratio, so its error propagates in quadrature — the relative variances of reference and candidate add under the root:

$$CV(\text{IFI-A}) = \sqrt{[\ CV(PC_{50}\ \text{ref})^2 + CV(PC_{50}\ \text{cand})^2\ ]}$$

The reference is a tightly held standard, CV near 1.5%. Push a designed construct to a batch-to-batch CV of 7% and the Index value inherits $\sqrt{(0.015^2 + 0.07^2)} = 0.0716$, about **7.2%** — effectively the candidate's own number. A natural vineyard tannin, by contrast, swings at a CV near **42%** from harvest to harvest. Put that in the reader's hand: a wild tannin nominally at IFI-A 1,400 arrives anywhere across roughly 812 to 1,988 — a different wine each year — while the designed one holds between 1,302 and 1,498, a grip a blender can actually build on. The move from 42% to 7% is the move from a scientific result to a product, and it is the quietest, hardest-won number in the chapter.

The galloylation knob — the fast lever

Galloylation is the strongest single move on astringency, and the mechanism tells you why before the data does. To galloylate a flavan-3-ol is to hang a **galloyl ester** — a trihydroxybenzoate, three hydroxyls on one more flat aromatic ring — off the 3-position of the C-ring. Every one of those rings is a fresh stacking surface for a proline face and three fresh hydroxyls to hydrogen-bond the peptide backbone. The handshake that was already favorable gains a whole extra hand. Measured against the panel, each galloyl group added multiplies binding potency by roughly **five- to tenfold**, which on the Index means it divides PC_{50} by five to ten and so multiplies IFI-A by the same.

The lever that turns it is an acyltransferase: an **SCPL acyltransferase** in grape, **ECGT** in tea, both drawing on the activated donor β-glucogallin (1-*O*-galloyl-β-D-glucose) to esterify gallate onto the flavan-3-ol. Turn that enzyme up and the tannin's IFI-A climbs steeply; turn it down and the grip softens fast — all without changing how *much* tannin the plant makes. This is the dial we reach for when a target needs a large, clean move, because its effect is big, monotonic, and well-predicted by the potency model: firming a flabby wine, or softening a punishing young tea by dialing the galloyltransferase *down* so the catechins arrive ungalloylated and gentle.

Run the numbers and the reach is almost comic. Start with a thin construct at IFI-A 300 — PC_{50} = 28,000 ÷ 300 = 93.3 µM. Add one effective galloyl step at the midpoint factor of seven: PC_{50} → 93.3 ÷ 7 = 13.3 µM, and IFI-A → 28,000 ÷ 13.3 ≈ **2,100**. One edit, 300 to 2,100. Naively stack a second galloyl and the model predicts another sevenfold — PC_{50} near 1.9 µM, IFI-A near 14,700 — and *that* is where the fast lever teaches its lesson. No tannin scores 14,700, because the per-axis scale saturates at a normalization maximum of **5,000**, and, more physically, because a heavily galloylated chain runs past its solubility window and falls out of the fruit as haze before it ever reaches a mouth. The realized potency plateaus. The galloylation knob is fast precisely because it is steep, and steep levers overshoot; past a point you are no longer tuning a grip, you are precipitating your own tannin in the berry. We respect the dial by using it in single, deliberate steps.

The chemistry under the dial is worth naming, because the names recur every time we build a grip. The ungalloylated units are **catechin** and its stereoisomer **epicatechin** (both 290.3 g/mol, two B-

ring hydroxyls); hydroxylate the B-ring once more and they become **gallocatechin** and **epigallocatechin** (306.3, three hydroxyls); hang the galloyl ester and they become **epicatechin gallate** (442.4) and **epigallocatechin gallate** (458.4), the most protein-avid flavan-3-ols the plant kingdom builds and the reason a strong green tea grips the way it does. Six units, one scaffold, a four-fold spread in potency — the galloylation knob is simply the choice of which of them the plant assembles.



A cross-section of the engineered grape — seed and skin, where the condensed tannins are built and stored. Grape carries the densest construct map we hold, and nearly every point on it is a different setting of these same three dials.

The polymerization knob — the structure lever

If galloylation sets the potency of each unit, polymerization sets how many units ride together, and therefore how many bridges a single molecule can throw across the salivary proteins at once. Degree of polymerization is an avidity dial. A monomer binds and lets go; a dimer holds with two points; an octamer drapes across a cluster of PRPs and cross-links them into a lattice. In molar terms the effect is close to linear up to a point — a chain of mean degree of polymerization *m* behaves, per molecule, like *m* binding events, so the per-molecule PC_{50} falls roughly as 1/*m* while the cooperativity *n* climbs and the onset sharpens. The grip **deepens and lengthens**: more sites, more avidity, a slower and more persistent sensation, the *length* a cellar prizes in a structural red.

The dial is not linear for long, though, and the non-linearity is the whole art. Raising the mean degree of polymerization toward the **DP 6–10** window is the sweet spot — enough valency to grip hard and hold, enough cooperativity to give the onset an edge. Past roughly **DP 10–12** the molecule grows too large and too insoluble to bind efficiently; it precipitates in the fruit matrix before the mouth ever sees it, its effective concentration collapses, and the grip plateaus and then fades into mere haze — present in the glass as sediment, absent on the palate. The failure mode is specific and worth stating plainly: *over-polymerize and you lose the very sensation you were chasing*, trading a long velvet finish for a cloudy pour. The construct grid is laid out to find each organism's knee — the *m* at which avidity and solubility are both still rising — because that number is not universal; grape seed, grape skin, and tea each knee at a slightly different length.

We read the dial with **phloroglucinolysis**: acid-catalyzed depolymerization in the presence of phloroglucinol, which cleaves the interflavan C4–C8 bonds, caps the extension units as phloroglucinol adducts, and releases the terminal units free. The ratio of adducts to free units on the HPLC trace gives the mean degree of polymerization directly, and the same trace reports the subunit census — percent galloylation, percent prodelphinidin, the cis/trans balance — so a single assay reads all three dials at once. We lean on it hard, because the polymerization machinery is the least fully understood step in the whole pathway. The condensing chemistry — how the interflavan bond is actually formed and controlled in the plant, whether by laccase-like oxidation or carbocation capture — is still being mapped, which makes this the hardest lever to turn by prediction and the one where the construct libraries do the most empirical work, growing graded series and measuring where the sweet spot truly falls for each organism rather than trusting a model to tell us.

The fine-structure knob — the quality lever

The third dial moves almost nothing on the Index and everything in the mouth. The **ANR:LAR ratio** and the **F3′5′H** branch set the fine-structure of the units — their stereochemistry and their B-ring hydroxylation — and together they decide not how *hard* a tannin grips but *how* it grips: coarse or fine, green or ripe, a wall or a drape.

The branch point is leucoanthocyanidin, the flavan-3,4-diol that sits where the flavonoid pathway forks three ways. **LAR** (leucoanthocyanidin reductase) reduces it directly to a **2,3-*trans*** flavan-3-ol — the catechin series. Alternatively **ANS** (anthocyanidin synthase) oxidizes it to a colored anthocyanidin, which **ANR** (anthocyanidin reductase) then reduces to a **2,3-*cis*** flavan-3-ol — the epicatechin series. Push flux through ANR and you build epicatechin-rich, 2,3-*cis* polymers, whose geometry reads as finer and more supple; favor LAR and you build the more angular *trans*-catechin chains. Lay the **F3′5′H** branch over the top — flavonoid 3′,5′-hydroxylase, which hydroxylates the B-ring at both the 3′ and 5′ positions — and you convert procyanidin units (two B-ring hydroxyls) into **prodelphinidin** units (three), the trihydroxy chemistry that the palate reads as velvet rather than grain. None of this necessarily touches the IFI-A number. Two tannins can score the same on the Index and feel entirely different — one a wall, one a drape — and the fine-structure dial is what separates them. It is why we report IFI-A alongside a *quality* descriptor in the full cultivar sheets, and why a winemaker reads both numbers, not one.

The quality lever carries the chapter's sharpest warning, because it is the one most entangled with the rest of the plant. ANR draws its substrate — anthocyanidin — from the same pool that the pigment branch glycosylates into **anthocyanins**. Push ANR too hard for grip and you can starve the color, pulling the fruit a shade paler even as the tannin improves. The flavonoid branch that builds the grip also feeds bitterness through shared phenolic ligands at the bitter receptor, so a move that firms a tannin can brush IFI-B upward. The

fine-structure dial, in other words, is where astringency stops being a private axis and starts negotiating with color and bitterness, which is exactly the point the two cautions below are built to hold.



The engineered tea plant. Tea and grape share this chemistry almost gene for gene; the galloyltransferase that tea calls ECGT, grape calls an SCPL acyltransferase, and the same three dials tune both.

The three dials, side by side

Set out together, the levers make a small instrument panel — three knobs, three signs, three costs — and designing a grip is choosing a setting for each with full knowledge of what it exacts.

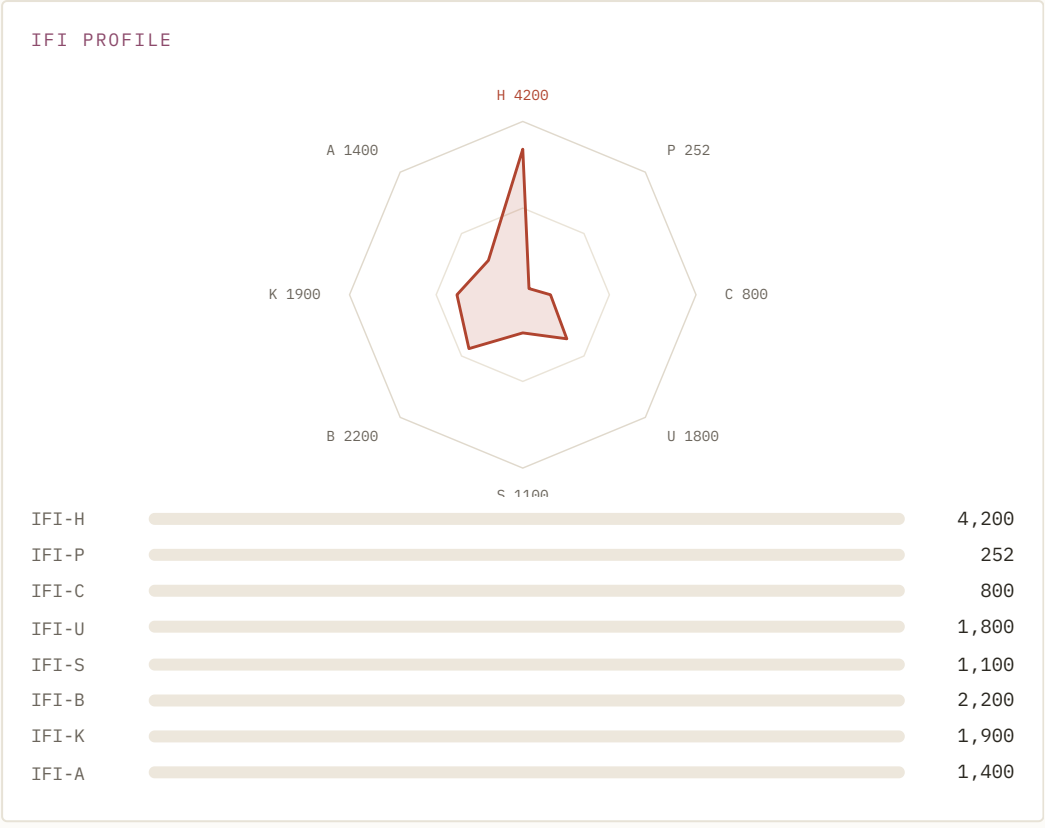
DIAL	ENZYMATIC LEVER	EFFECT ON IFI-A	CHARACTER OF THE MOVE	WHAT IT COSTS
Galloylation	SCPL acyltransferase (grape) · ECGT (tea)	strong ↑, monotonic	×5–10 per galloyl group	solubility ceiling; a green edge if overdone
Polymerization (mDP)	condensing / interflavan machinery	↑ through DP 6–10, then ↓	deepens and lengthens the grip	past DP 10–12 the grip fades into haze
Fine-structure	ANR · LAR · F3′5′H	≈ 0 on magnitude	coarse → fine, green → velvet	ANR up can starve anthocyanin color

Two cautions that keep the knob-turning honest

The first caution is that the **dials interact**. Raising galloylation while also lengthening the polymer does not add the two effects — it multiplies them past the physics, because a long and heavily galloylated tannin runs through its solubility window and drops out of the fruit as haze before it reaches a tongue. The potency model carries cross-terms for exactly this: galloylation × degree of polymerization × solubility, so that the prediction knows where magnitude and solubility stop coexisting. The construct grid is then laid out to find the *ridgelines* — the settings where the grip is hard **and** the tannin stays in solution — rather than the false summits where the model says "maximum potency" and the berry says "sediment."

The second caution is that **astringency is never alone**. The flavan-3-ol branch that builds the grip also feeds bitterness and shares its final intermediate with color, so a move that firms a tannin can lift IFI-B a notch or pull pigment a shade lighter. Designing a grip well means watching the neighboring axes move and holding them where the recipe wants them — which is why the eight-axis scorecard, not the single number, is the true unit of design, and why we score a cultivar on every axis even when only one of them is the point. A grape is not an astringency machine with seven side effects. It is a chord, and the grip is one note we are responsible for placing without knocking the others out of tune.

Nothing shows the chord better than the one cultivar we have built to sound all eight notes at once. Our model predicts an astringency of **1,400** — a deliberate, structural grip by design — held in tune beside a towering heat and a calibrated bitterness, every axis placed on purpose:



Read that profile as a lesson in restraint. The astringency is not maxed; it is *set*, at the same 1,400 a structural red asks for, while seven other receptors are each tuned to their own target. The grip is one voice in an eight-voice chord, and the discipline of placing it without disturbing the others is the whole of what the scorecard protects.

The library as a map

A construct library, then, is not a pile of edited plants. It is a **systematic grid** across these three dials — galloylation high to low, mean degree of polymerization short to long, the ANR:LAR ratio and the prodelphinidin fraction across their range — grown out, measured on the full assay battery, and used to fill in the map between genotype and grip for each organism. These are the six astringency hosts and the density of their maps:

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Grape	1,610	B · A · M	Hard
Tea	280	U · B · A	Hard
Pomegranate	120	A	Hard
Blueberry	120	A	Moderate
Sorghum	120	A	Frontier
Raspberry	40	A	Moderate

Grape's **1,610 constructs** are the densest such map we hold — 2,290 constructs across the six hosts, and more than two-thirds of them grape — because grape's tannin is the most architecturally demanding and the most commercially exact, and because the knee of the polymerization dial moves enough between seed and skin that only a dense grid pins it down. Each of the others is a complete-enough exploration of its own chemistry to let us hit a target IFI-A and a target quality on demand: tea for the galloylated, high-cooperativity grip; pomegranate for its punicalagin-driven astringency; sorghum at the frontier of the grain tannins; blueberry and raspberry for the softer, fruit-forward end of the scale.

And every point on every map is held to the discipline that makes the whole enterprise more than a laboratory curiosity: a **batch-to-batch coefficient of variation of seven percent or better**. A designed tannin that varied forty-two percent from harvest to harvest — the way a natural vineyard's tannin genuinely does — would be a scientific result and a commercial impossibility. Seven percent is the number that turns "we can make this grip once" into "a cellar can order this grip every year." It is, quietly, the hardest-won figure in the book, and the one that matters most to the people who will actually use it.

One design, all the way through

To see the dials work together, follow one design from brief to bottle. Suppose the brief is the grip every red-winemaker asks for: **fine-grained, structural, long — a target IFI-A of about 1,400, with a velvety quality and a slow, persistent finish.**

The potency model says 1,400 is reachable at a moderate galloylation and a mean degree of polymerization in the **six-to-ten** window, so we do *not* reach for the galloyltransferase — a heavy hand there would overshoot the magnitude, drive the PC_{50} well below the 20.0 µM that 1,400 asks for, and coarsen the quality on the way. We set the **quality** first: push flux through **ANR** to raise the epicatechin, 2,3-*cis* fraction, and lift **F3′5′H** to feed the prodelphinidin units that read as velvet. We set the **length** next, nudging the condensing machinery toward the upper end of the sweet spot — mean degree of polymerization near 8, deep in avidity,

well short of the DP 10–12 haze — for persistence without cloud. We hold **galloylation** moderate: enough grip to land at 1,400, not enough to turn the finish green.

Then we check the neighbors, because the chord has to stay in tune. The ANR push should not starve the anthocyanins — the cross-term in our model confirms the fine-structure edit is sized to hold color. IFI-B should move only slightly — a notch of bitterness we can live with. The flux model confirms the carbon is available to build the tannin without robbing an upstream pool. The CRISPR edits go in. The plant grows out, and phloroglucinolysis would reveal a tannin of **mean degree of polymerization 8, moderate galloylation, a 2,3-*cis*-rich, prodelphinidin-leaning subunit census** — exactly what the design asked for. Our model predicts a PC_{50} of 20.3 µM, which the Index converts to 1,000 × 28 ÷ 20.3 = **IFI-A 1,380** — inside tolerance of the 1,400 brief — with the design specification holding batch-to-batch variation under seven percent.

That is the whole method in one cultivar: a sensory brief, turned into a structure, turned into a set of enzyme edits, and computed into a predicted grip of **IFI-A 1,380** against a target of 1,400, designed to hold within seven percent of its value from one harvest to the next — a grip a winemaker can order by name, and pour with confidence that next year's bottle will grip the same. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 13 · The subtractive art — *softening a tannin without erasing it.*

So far we have mostly spoken of building astringency — raising a grip, firming a structure, designing the pucker in. But a great deal of the real demand, and some of the subtlest design, runs the other way: *softening* a tannin that is already too hard, taking the rasp out of a young red or a strong tea without taking out the structure that makes it worth drinking. This is the subtractive art, and it is harder than addition, because the goal is not to remove the grip but to **change its quality while keeping its magnitude** — to turn a wall into a drape. It belongs to the same family of work as the bitterness-attenuation we do in coffee and cacao, and the two sciences inform each other closely: both are about leaving a molecule in the glass while teaching it to speak more quietly.

To soften a thing well you must first know exactly how it takes the mouth. So before the dials, the mechanism — step by step, because every lever in this chapter is a place where we reach into one of these steps and slow it down.

How a tannin takes the mouth — the mechanism of grip

Astringency is the odd one of the eight axes, because it is not, strictly, a taste. Sweetness, heat, umami, the others — each is a molecule settling into a receptor and opening a channel, a lock and key that fires a nerve. Astringency has no such single receptor firing. It is a *tactile* event: the felt collapse of a lubrication system, read not by a taste bud but by the ordinary touch and friction sensors of the trigeminal nerve. The receptor line on the index — **salivary proline-rich proteins** — names not a channel but the protein the tannin attacks.

Here is the sequence, as it happens in the half-second after the sip.

One — the film. At rest, the surfaces of the mouth are coated in a thin, slick film of saliva: mucins (the long glycoproteins MUC5B and MUC7), statherin, histatins, and above all the **proline-rich proteins** — acidic, basic, and glycosylated — which together make the mouth one of the most frictionless surfaces the body owns. This film is the thing astringency destroys.

Two — the arrival. A tannin is a polyphenol carrying many phenolic hydroxyl groups on many aromatic rings. In the plants of this axis there are two great families: the **condensed tannins** — proanthocyanidins, built as chains of flavan-3-ol units (catechin, epicatechin, and their trihydroxylated cousins) — and the **hydrolyzable tannins**, galloyl and hexahydroxydiphenoyl esters hung on a sugar core, the ellagitannins of pomegranate and the gallotannins of oak and tea. Both carry the same weapon: a dense surface of hydroxyl groups and flat, hydrophobic aromatic faces.

Three — the stack. The tannin's aromatic rings press flat against the **proline** residues that give these salivary proteins their name. Proline is the decisive amino acid here for two reasons at once: its pyrrolidine ring presents a flat, greasy face that the polyphenol's rings stack against hydrophobically, and its tertiary amide removes a backbone N–H, leaving the peptide carbonyl an open, unobstructed acceptor. Proline makes the protein, in effect, pre-shaped to be gripped.

Four — the bonds. Onto those exposed carbonyls the tannin's phenolic –OH groups donate hydrogen bonds. One tannin is **multidentate** — many rings, many hydroxyls — so a single molecule binds several sites on one protein and bridges to the next. Hydrophobic stacking brings the surfaces together; hydrogen bonding locks them.

Five — the collapse. Cross-linked tannin–protein complexes grow until they outrun their own solubility and fall out of solution as a fine precipitate. The lubricating proteins are now hanging in clots instead of coating the tissue. The film is gone; the surfaces drag; a faint grit of precipitate adds its own friction. The trigeminal sensors report rising friction, and the brain names it drying, puckering, grip.

Six — the recovery. Fresh saliva rebuilds the film over seconds to a minute or two, and the grip fades. This is why astringency has a *length* — a finish — and why, if you sip again before the film has returned, the grip stacks higher than the first sip alone. The sensation is a curve in time, not a point, and that fact is the whole hinge of the subtractive art.

We dwell on this because every softening move in this chapter is a way of reaching into one of these six steps. Grow the tannin with fewer gripping groups, and step four weakens. Wrap it in pigment, and step three is pre-occupied. Give it a competitor to bind, and step five never reaches the film. Mature it into larger, slower aggregates, and the collapse comes gently and late instead of all at once. The mechanism is the map; softening is a route across it.

A number for a grip — EC50, the index, and the ladder

To steer a sensation you must be able to measure it, and the Index measures loudness the same way on every axis: by the concentration at which a stimulus drives its target to half of full response.

$$\mathrm{IFI}(c) = 1000 \cdot \mathrm{EC}_{50}(\mathrm{ref}) \,/\, \mathrm{EC}_{50}(c)$$

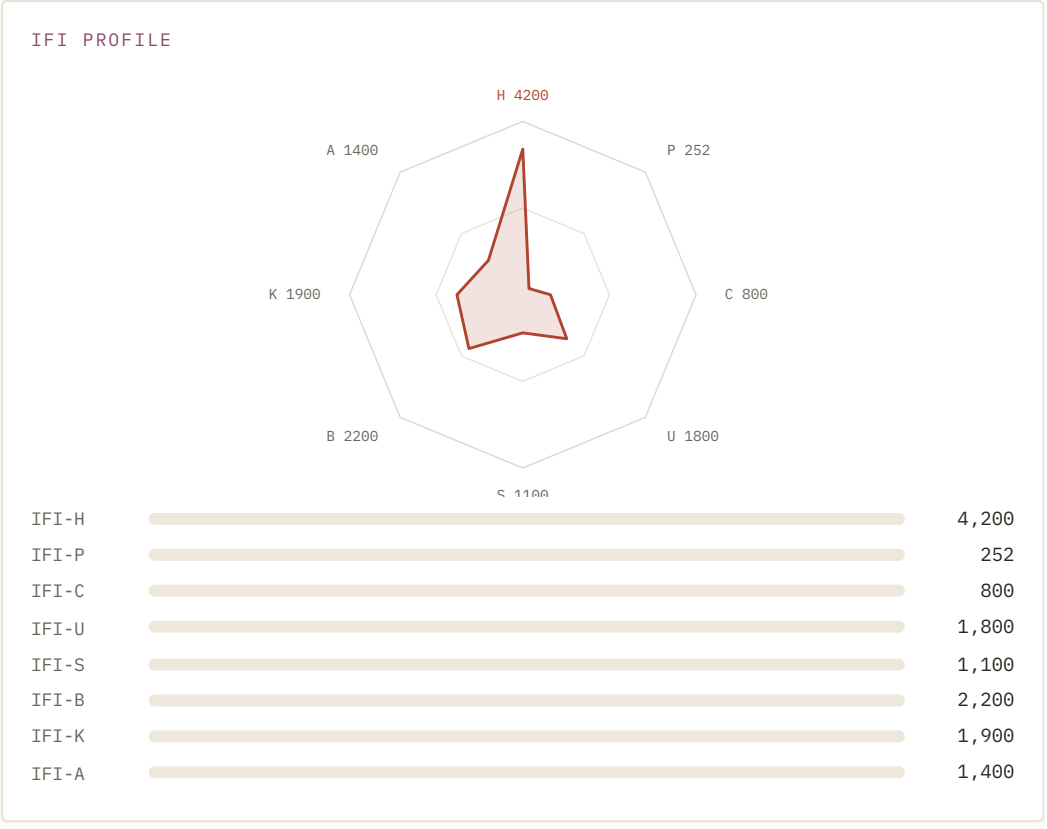
For astringency, $\mathbf{EC_{50}}$ is the tannin concentration at which salivary-protein precipitation — read as turbidity, or equivalently as the rise in oral friction — reaches half of its maximum. A potent, gripping tannin half-saturates the film at a low concentration and earns a low EC_{50}; a gentle one needs more and earns a high one. Invert that against a fixed reference, scale by a thousand, and the grip of a tea, a grape, and a glass of pomegranate molasses can be read on one line.

We anchor the astringency axis to a standard proanthocyanidin solution whose EC_{50} for half-maximal salivary-protein precipitation is **0.70 g/L**. By convention that reference scores **IFI-A = 1,000** — a stated definition, the same way the Scoville scale is defined by

dilution-to-threshold, not derived from a literature average. Everything else is read against it. Our full-spectrum flagship **Phoenix** carries a designed astringency of **IFI-A 1,400** — a deliberate mid-axis grip, built to drape rather than wall — and we can read its EC_{50} straight off the formula:

> $EC_{50}(\text{Phoenix}) = 1000 \cdot EC_{50}(\text{ref}) / \text{IFI-A} = 1000 \cdot 0.70 / 1{,}400 = \mathbf{0.500\ g/L}$

So Phoenix's tannin is designed to half-take the film at half a gram per litre — about 1.4 times more potent than the reference. Its place on all eight axes is the profile we design and verify against:



Because tannins are polydisperse — a population of chain lengths, not one molecule — we carry EC_{50} in g/L and convert to molar only against a defined oligomer. For a model procyanidin pentamer of molar mass ≈ 1,440 g/mol:

> $EC_{50}(\text{Phoenix}) = 0.500\ \text{g/L} \div 1{,}440\ \text{g/mol} \times 10^6 = \mathbf{347\ \mu M}$

The ladder below is the whole axis in one view — the arithmetic that turns a felt grip into a score.

IFI-A	EC_{50} (G/L)	EC_{50} (MM, PENTAMER)	FELT CHARACTER
2,200	0.318	221	a firm wall — young, unresolved
1,400	0.500	347	designed grip — Phoenix, built to drape
1,000	0.700	486	the reference — a clean drape
700	1.000	694	soft, rounded, easy
350	2.000	1,389	barely there — a whisper of structure

Read it in either direction: halve the EC_{50} and you double the IFI-A; the axis is a reciprocal scale, so the gentlest tannins crowd the low end and a small chemical change near the top moves the score a long way.

The EC_{50} itself comes from a dose–response. Plot precipitation against tannin concentration and the curve is sigmoidal, well fit by a Hill form with a cooperativity exponent n above one — the cross-linking of step four is cooperative, so the response steepens as aggregates seed more aggregates. Taking Phoenix's EC_{50} = 0.500 g/L and n = 2:

> $R([T]) = [T]^n / (EC_{50}^n + [T]^n)$ at 0.25 g/L: 0.0625 / (0.25 + 0.0625) = **20%** at 0.50 g/L: 0.25 / (0.25 + 0.25) = **50%** at 1.00 g/L: 1.00 / (0.25 + 1.00) = **80%**

A fourfold span in concentration (0.25 → 1.00 g/L) swings the grip from one fifth to four fifths of maximum — the steepness is the cooperativity made visible.

Every one of our constructs is designed to a batch-to-batch coefficient of variation of **≤ 7%** on its predicted EC_{50}, and we carry that honestly into the published score. Since IFI-A is a ratio, relative errors add in quadrature:

> $CV(\text{IFI-A}) = \sqrt{[\ CV(EC_{50},c)^2 + CV(EC_{50},ref)^2\ ]}$

With a 7% construct CV against a reference measured at high replication (≈ 2%), CV(IFI-A) = $\sqrt{(0.07^2 + 0.02^2)}$ = 7.3%. So Phoenix's astringency is properly written **1,400 ± 100** at one standard deviation — a number with its uncertainty attached, because a number without its error is a kind of flattery we don't pay the reader.

What aging actually does — maturation at the molecular scale

Nature already knows how to soften a tannin, and it does it by **reaction, not removal**. This is the chemistry of aging. In a cellar, a young wine's hard, reactive tannins slowly combine — with each other, and crucially with the wine's **anthocyanins** — into larger **polymeric pigments**. As the chains grow and bind pigment, the grip changes: the sensation moves from sharp and drying toward round and integrated, even as the total tannin barely falls. The tannin is not leaving. It is *maturing* — reorganizing into a form that attacks the salivary film more gently and more slowly.

At the molecular scale, three things happen at once, and all three reach back into the six steps of grip. Polymerization raises the mean degree of polymerization, so there are fewer, larger particles; larger aggregates grow slowly and precipitate slowly, so the collapse of

step five comes late and soft instead of fast and hard. Direct tannin–anthocyanin condensation, and the acetaldehyde-bridged condensation that a winemaker's deliberate trickle of oxygen — **micro-oxygenation** — accelerates, cap the free galloyl and hydroxyl groups that did the gripping in step four. And **co-pigmentation**, the flat stacking of anthocyanin against the tannin's aromatic faces, pre-occupies the very hydrophobic surfaces that would have pressed against proline in step three. The pigment, in other words, is the tannin's chaperone — it arrives holding the tannin's hand so the tannin cannot take the mouth's.

This is why a tannin grown in a fruit rich in the right pigments arrives already partly softened, its grip pre-draped in color, and why a wine aged on its lees tastes rounder than its tannin number alone would predict. Tannin and pigment are siblings — both are flavonoids off one upstream pathway, a few enzymes apart — and in the subtractive art the sibling is the softener.

For the designer this opens two routes, and we use both.

The source route — designing the softness into the plant

The **source route** builds the softness in from the start: grow a tannin that is already fine-grained and already partly co-pigmented at harvest, so the softening a cellar used to do over years is partly done in the vineyard. This is the route we prefer, because it is the one that holds to seven-percent consistency — the softness is *designed*, not coaxed out batch by hopeful batch.



A grape in cross-section — seed, pulp, and the pigment-laden skin that will chaperone the seed's tannin. Co-pigmentation begins here, in the geometry of a single berry, long before any cellar.

The levers are structural, and they are genetic. A tannin's grip and its *grain* are set by four dials on the flavonoid pathway, each a well-characterized enzyme we can turn up or down.

LEVER	ENZYME / GENE	FIRM, COARSE SETTING	SOFT, FINE SETTING	WHY IT MOVES THE GRIP
B-ring hydroxylation	F3′H vs **F3′5′H**	procyanidin (3′,4′-diOH)	**prodelphinidin** (3′,4′,5′-triOH)	prodelphinidin chains read as silkier and finer in the mouth despite the extra hydroxyl
Galloylation	SCPL galloyl-acyltransferase	heavily galloylated (EGCG-like)	low galloylation	galloyl groups sharply lower EC_{50} — they are the single hardest, most aggressive edge
Chain length	extension/terminal supply; **mDP**	short (mDP ≈ 3–4)	long (mDP ≈ 7–10)	long chains precipitate slowly and feel rounder; short ones feel rasping and hot
Stereochemistry	**ANR : LAR** balance	catechin-rich (2,3-trans)	epicatechin-rich (2,3-cis)	a high ANR:LAR ratio favors cis extension units and longer, finer chains

The instruction that falls out of the table is the one we design to: make the tannin **long and prodelphinidin-rich rather than short and galloylated**. Lean on F3′5′H for the trihydroxy B-ring; raise the ANR:LAR ratio so epicatechin extension units build long cis chains; dial the galloyl-transferase down so the sharpest edge is simply never put on. A tannin designed this way can carry a high magnitude — a real, structural IFI-A — and still feel like a drape, because magnitude lives in the surface of hydroxyls and grain lives in the shape and length of the chain, and those are separable dials.

There is a fifth lever that is not about the tannin at all: the **pigment load**. Push the anthocyanin branch — more flux through the glucosyltransferase that stabilizes anthocyanin — and the fruit arrives carrying its own chaperone, co-pigmented at harvest. A grape engineered to the drape target is really two designs braided together: a fine, long tannin and a generous pigment to pre-occupy it. The berry does the softening the cellar used to do, and nothing is wasted doing it.

The process route — holding the trajectory

The **process route** steers the maturation itself: hold the softening trajectory — the oxygen, the temperature, the time — to a fixed, reproducible path in a controlled environment, so that a tea or a wine or a reduced pomegranate molasses arrives at a *target* grip on a schedule rather than whenever the vintage happens to allow.



The controlled chamber, where a softening trajectory can be held to a fixed path batch after batch. The process route is less about removing tannin than about steering how it matures — and steadiness is the whole game.

The subtlety is that the goal here is a *curve*, not a number. The single IFI-A score is an amplitude — it tells you how much grip, not how the grip arrives and leaves. And softening by maturation, done well, barely touches the amplitude. What it changes is the **shape in time**.

Model the felt grip as a rise-and-fall in time, the standard two-compartment form with a build constant τ_r and a finish constant τ_f:

```
g(t) = Q / (τ_f − τ_r) · ( e^(−t/τ_f) − e^(−t/τ_r) )
```

The single most useful fact about this curve is what happens when you integrate it over the whole sip:

```
∫₀^∞ g(t) dt = Q, independent of τ_r and τ_f
```

The total grip delivered — the area under the felt curve, which is what the EC_{50} and therefore the IFI-A actually report — is the conserved quantity **Q**. The time constants set only how that fixed dose is *distributed*: into a tall, early, narrow peak, or a low, late, long plateau. This is the mathematics behind the chapter's whole claim. Softening by maturation **conserves the area and redistributes the shape** — same Q, same IFI-A, a lower peak and a longer finish.

Here is a young, firm tannin and the same grip-dose matured to a drape, Q held identical:

PARAMETER	FIRM (YOUNG)	DRAPED (MATURED)	WHAT CHANGED
Grip-dose Q (∝ IFI-A)	1.00	1.00	**conserved**
Build constant τ_r	2 s	4 s	slower onset
Finish constant τ_f	8 s	24 s	longer finish
Time to peak	3.7 s	8.6 s	grip arrives later
Peak height (firm = 100%)	100%	37%	the wall comes down
Finish (peak → 10%)	18 s	55 s	the drape hangs on

The peak drops to roughly a third while the finish triples — and the integrated grip, the number the index prints, is unchanged. That is the target of the subtractive art stated exactly: lower the **peak** and the **build** of the curve while keeping the **length** that gives the drink its finish. Strip too much and you get a soft, short, flabby thing with no grip at all; strip too little and the rasp survives. The target is a

tannin that arrives gently, resolves slowly, and lingers long — high length, low aggression — and hitting it reliably, to a held trajectory, is the finest work on the whole axis.

It is worth naming the failure modes, because the curve can go wrong in instructive ways. Push the polymerization too far and the chains outgrow the glass itself — they precipitate in the vessel, throw a sediment, and the grip leaves the drink entirely, structure and all. Lean too hard on co-pigmentation and you buy a drape that is only lightly tied: anthocyanin stacking is reversible and pH-sensitive, so a sharp dilution or a shift in acidity in the mouth can release some of the tannin the pigment was holding, and the grip you thought you had softened returns on the finish. The honest limit sits underneath all of it: the scalar IFI-A cannot see the curve. Two tannins reading an identical 1,400 can feel like a wall and a drape, because they carry the same Q in opposite shapes. This is why we never publish the magnitude alone — a grip is reported as its amplitude *and* its build, peak, and length, or it is not honestly reported at all.

The old subtraction — leaching, and why we moved it upstream

The subtractive art has a long and unglamorous ancestor: **leaching**. For as long as people have eaten acorns, unripe persimmons, and raw olives, they have soaked, cured, and fermented the grip out — pulling the water-soluble tannins into a brine that is then thrown away, or letting enzymes and microbes break them down, exactly as the olive's bitter-astringent **oleuropein** is cured out over weeks before the fruit is fit to eat.



An olive, whole and intact — a fruit no one can eat until its oleuropein is leached or cured away. The oldest subtraction, and the most wasteful: the grip leaves, and the color and nourishment leave with it.

Leaching is a partition, and its arithmetic is unforgiving. If each soak pulls a fraction f of the remaining tannin into the water, then after n changes of water the fraction left behind is:

```
remaining = (1 − f)ⁿ
```

For an acorn hot-leached at $f = 0.5$ per change, four changes leave $(0.5)^4 = \mathbf{0.0625}$ — about 94% of the tannin gone. But the same water that carries off the tannin carries off the soluble sugars, the minerals, the anthocyanins, the very polyphenols whose presence is half the reason to eat the thing. The curing of oleuropein follows a first-order decay, o(t) = o₀·e^(−kt), with a half-life measured in weeks; and

across those weeks the brine that gentles the fruit also leaches it pale. Leaching lowers the grip by lowering *everything* — it is subtraction by amputation.

This is the deep reason we moved the work upstream. The modern subtractive art keeps the structure by changing the tannin's *form* rather than its amount — polymerizing it, binding it to pigment, maturing it in place — so the magnitude stays and only the shape softens. And the source route goes one better: design the softness into the plant, so little has to be leached away at all. The through-line from the acorn pit to the micro-oxygenation tank is a single human wish — to keep the good of the grip and lose the rasp. We have only moved the work to the gene, where the least is wasted and the most is kept. For a family that has always eaten this way — plant-first, whole-food, reverent about what a fruit carries — that is not a technical footnote. The polyphenols a leach throws down the drain are the same ones the body is glad of. Keeping them is the whole point.

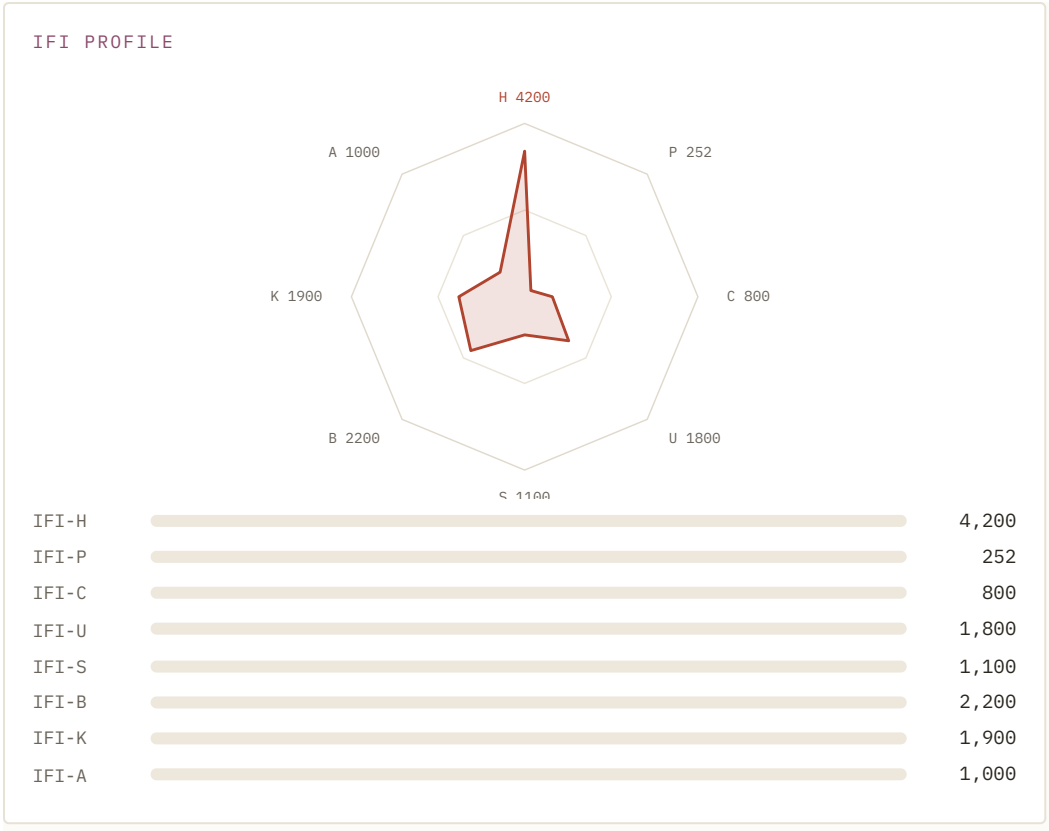
Competition — the glass gets the last word

Between the crude leach and the designed gene sits a third, elegant route the wine world has long used, and it belongs in any honest account of softening: **competition**. A tannin precipitates salivary protein, but it will just as happily bind other large molecules offered to it, and a wine or a tea carries several. The **mannoproteins** released slowly from yeast cell walls during aging on the lees, the **polysaccharides** — the arabinogalactans and rhamnogalacturonans — that a ripe grape or a careful extraction brings into solution, even a touch of gum arabic in a tea or a syrup: each of these wraps or caps the tannin, or competes with the saliva for it, lowering the grip that reaches the mouth without removing a molecule of tannin from the glass.

This one is quantifiable, and cleanly. If a competitor sequesters tannin with an effective ratio [C]/K_C, the free tannin available to attack the salivary film drops, and the **apparent** EC_{50} — the total tannin you must add to reach half-maximal grip — rises by that factor:

```
EC50(apparent) = EC50 · ( 1 + [C]/K_C ) IFI-A(delivered) = IFI-A(designed) / ( 1 + [C]/K_C )
```

Take Phoenix's designed tannin at EC_{50} = 0.500 g/L (IFI-A 1,400), and age it on fine lees until the mannoprotein competition reaches [C]/K_C = 0.40. The apparent EC_{50} climbs to 0.500 × 1.40 = **0.700 g/L**, and the delivered grip falls to 1,400 / 1.40 = **1,000** — the reference drape — with every tannin molecule still in the glass. The eight-axis profile that reaches the mouth is the same fruit, moved on exactly one axis, because the cellar's polysaccharides touch the tannin and nothing else:



Set the amplitude at the gene; let the glass set the last of the shape. For the designer this is a reassuring lever, because it means the grip we build into a fruit is not the grip's final word — the cellar, the steep, and the recipe all still get their say, and a tannin designed a touch firm can be rounded at the table as readily as one designed soft can be left to stand tall.

One science, read backward

There is a deeper symmetry worth naming, because it is the reason this is a chapter of method and not of recipes. **Addition and subtraction are the same science read in two directions.** The dials are the same dials: F3′5′H, the ANR:LAR ratio, the galloyl-transferase, the pigment branch, the held trajectory of oxygen and time. The mechanism is the same six steps, worked forward to firm a grip or worked backward to gentle one. The potency model predicts a softening as exactly as it predicts a firming — the EC_{50} moves up or down the same ladder; the area Q is conserved or redistributed by the same integral. A library built to make a grape more astringent is, read backward, a library that teaches you precisely how to make it less — and *which* less: coarse-less or fine-less, magnitude-less or build-less, the wall taken down or the drape hung longer.

To design a sensation is to hold both of its directions at once, and astringency — because its chemistry is enzymatic from end to end and its physics is a lubrication film we can model molecule by molecule — lets us hold them more completely than almost any axis. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. With the method whole in both directions, we can leave the greenhouse for the place all of this was always headed: the cellar, and the table, where the grip finally meets fat, and salt, and time, and a second sip.



PART IV OF IV

The Table & the Cellar

pairing by number · wine and tea as IFI-A · cooking with the grip · the grip inside the spectrum · what the tannin is for

Chapter 14 · Pairing by number — *why the grip loves fat, salt, and time.*

The grip was never meant to live in a flask, and everything behind it — the proteins, the tannins, the arithmetic, the plants, the method — comes to rest here, at the table, where astringency does its real work: not alone, but *against* something. A tannin tasted by itself is an abstraction. A tannin tasted against a square of cheese, a spoon of yogurt, a bite of lentils, a piece of dark chocolate, a second sip of the same wine — that is the thing people actually love, and, gratifyingly, the Index predicts it. Pairing astringency is not mysticism but receptor chemistry run at the table, and nearly the whole lexicon of tannin pairing falls out of three mechanisms. Everything that pairs well with a grip does so because it either **feeds the tannin a protein to precipitate other than your saliva**, or **restores the lubrication the tannin stripped**, or **slows the build**. Learn those three and you can read a plate the way a winemaker reads a vineyard, and put a number on each move.



A fig, halved. Tannic, honeyed, and built to be eaten with fat and salt — a hard sheep's-milk cheese, a thread of good oil, a scatter of toasted nuts — the fig is a small lesson in why the grip and richness were made for each other, and why the oldest pairings are, underneath, chemistry.

The grip is a reaction you can feed

Astringency is the one axis on the Index that is not, strictly, a taste. Heat keys into TRPV1; sweetness into TAS1R2·R3; the savory depth of umami into mGluR1. Each is a receptor a molecule turns like a lock, and the Index reads how loudly it turns. Astringency has no such lock. What it has instead is a **sacrifice**. Human saliva pours out a constant supply of small, floppy, open-chain proteins stuffed with

the amino acid proline — the **salivary proline-rich proteins**, the PRPs — and these are the receptor in the only sense that matters: they are the thing the stimulus acts on, and the felt sensation is the downstream consequence of that act.

Proline is why they work. Its side chain loops back and bonds to its own backbone nitrogen, forming a rigid five-membered ring that does two useful things at once. It presents a flat, faintly greasy face onto which the flat phenolic rings of a tannin stack like tiles; and because its nitrogen is tied up in that ring, it leaves its backbone carbonyl oxygen fully exposed as a hydrogen-bond *acceptor*. A tannin is studded with phenolic hydroxyl groups — superb hydrogen-bond *donors*. The complementarity is almost unfair. A proline-rich chain holds its backbone out in an extended, unspooled coil, offering those open carbonyls in a row like a runway with the lights left on, and the tannin fastens along it — aromatic faces pressed to the greasy proline rings, hydroxyls donating to the open carbonyls — at many points at once.

A single contact is weak and fleeting. What makes astringency *happen* is **multivalency**: a tannin carries many binding groups, a PRP presents many proline sites, and each tannin — having more than one hand — grabs more than one protein. The solution knits itself into a growing three-dimensional network of tannin bridging protein bridging tannin, in three rough stages: the tannin first decorates the protein at discrete sites; a second protein then binds a dangling tannin end, cross-linking two chains; and the cross-linked mesh grows past the point where it can stay dissolved and separates out, first as a haze, then as a precipitate. That phase separation, happening in the thin film of saliva on the tongue, is the drying you feel. The proteins that were lubricating the mouth are now curds on their way out of solution; the trigeminal endings that report a coarse surface report the loss of slip as a rough, puckering grip. The chemistry comes first; the sensation is its echo.

This is the fact the whole chapter turns on: **astringency is a protein-precipitation reaction read out as friction.** And a reaction can be fed. Give the tannin a *different* protein to precipitate — one not anchored to your oral surfaces — and it will spend itself on that decoy and leave the salivary film intact. Nature already uses this trick: the caseins of milk are open, unstructured, phosphorylated proteins, and β-casein is roughly **seventeen percent proline** — nature's *other* proline-rich tannin sponge. It is no accident that a splash of milk quiets a hard tea, or a hard cheese softens a young red; it is the same chemistry the saliva runs, moved off the tongue and onto the plate. The reverent reading of the salivary PRPs is that the body already built the elegant answer: a stream of sacrificial protein secreted precisely to intercept dietary tannin before it can reach the digestive proteases downstream, so that pairing well is, in a sense, lending the mouth a hand at its own ancient job.

One honest caveat belongs here. The mechanical-friction account is the dominant model and the one the Index assays against, but the final transduction — exactly which nerve endings report the stripped film, and whether some astringents also engage chemesthetic channels such as TRPA1 directly — remains genuinely debated. The

Index pins the *stimulus*, the tannin's own power to precipitate a standardized protein pool; it does not pretend to adjudicate the last millimetre of the nerve.



Grape in cross-section — skin, flesh, and the small hard seeds. The condensed tannins that give a red its grip are built in the skin and, most densely, the seed, off the flavonoid branch of the phenylpropanoid pathway; the lever we tune in the grape, anthocyanidin reductase, sits right at the fork that decides how much of the fruit becomes pigment and how much becomes structure.

Putting the grip on the scale

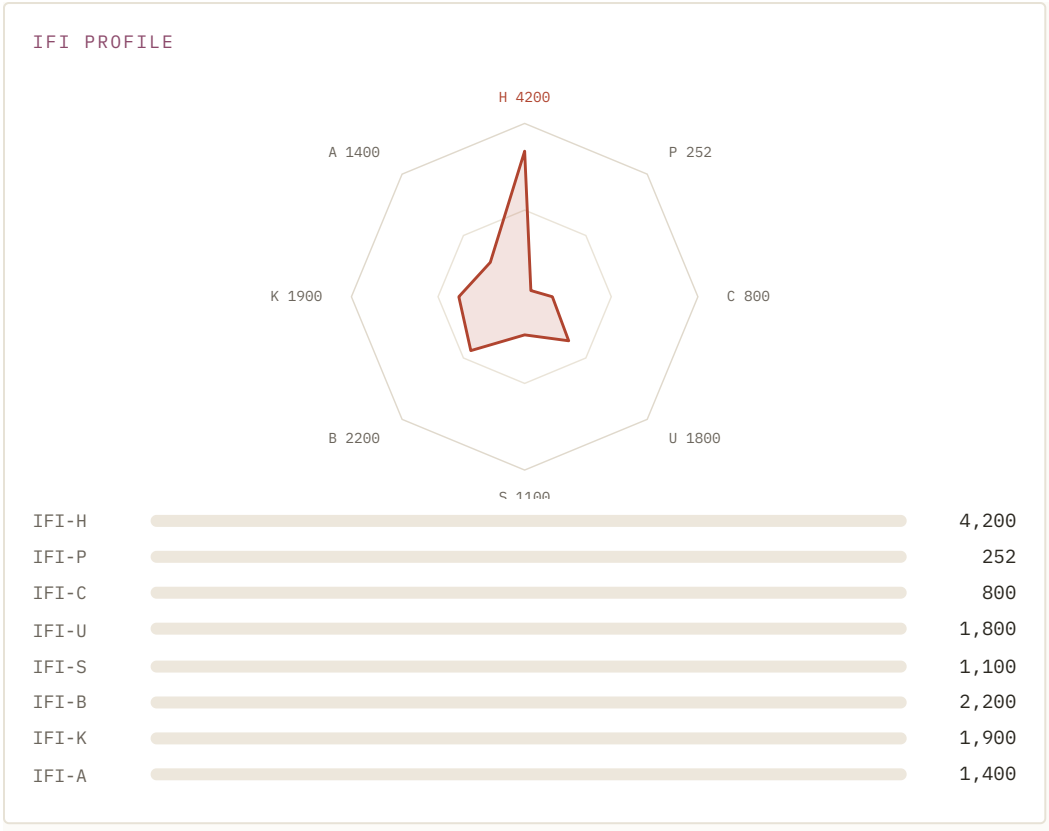
To pair by number you must first read the grip as a number, and the Index reads astringency the same way it reads every axis:

$$\mathrm{IFI}(\text{candidate}) = 1{,}000 \times \mathrm{EC}_{50}(\text{reference}) \div \mathrm{EC}_{50}(\text{candidate})$$

Here the EC_{50} is not a half-maximal current across a channel but the tannin concentration that drives standardized salivary-PRP precipitation to half of its maximal plateau — the half-clouding point of a defined protein pool, read turbidimetrically at mouth temperature and physiological ionic strength. We anchor the scale by fixing the reference tannin to half-precipitate that pool at **EC_{50}(reference) = 50 µM**, which by construction makes its IFI-A exactly **1,000**. Because the formula is a simple ratio around that anchor, the whole scale collapses to one instant identity, and it is the identity every pairing calculation in this chapter runs through:

$$\mathrm{EC}_{50}(\text{candidate}) = 1{,}000 \times 50\ \mu\mathrm{M} \div \mathrm{IFI\text{-}A} = \mathbf{50{,}000 \div \mathrm{IFI\text{-}A}}\ (\mu\mathrm{M})$$

Score and concentration are reciprocals: a higher IFI-A means a lower EC_{50} — a tannin that clouds protein at less concentration, a louder grip. Take the one cultivar we designed to register on all eight axes at once, **Phoenix** (SS-0096), whose astringency we set deliberately at **IFI-A 1,400**. Its half-precipitation point is 50,000 ÷ 1,400 = **35.7 µM**, which is **1.4×** the potency of the reference — a loud, designed grip living inside a full spectrum:



The scorecard reads the whole plant at a glance — a wall of heat at **H 4,200** (our model predicts approximately 2.95 million SHU), and beneath it a deliberate grip at **A 1,400**, placed so the fruit has structure and not only fire. For pairing, that lone astringency column is the term we will learn to move.

Here is the pivot the rest of the chapter stands on. **Pairing almost never changes the tannin's own EC_{50}** — the molecule in the glass is fixed the moment it was grown. What pairing changes is the EC_{50} *the salivary film actually sees* — the **effective** EC_{50} — by intercepting tannin before it arrives, restoring the lubricated surface it was stripping, or changing how fast the mouth recovers between encounters. And because astringency climbs with concentration not linearly but as a steep sigmoid, a modest shift in the effective EC_{50} produces a large shift in felt grip. A Hill form captures the steepness:

$$\theta = [T]^h \div (EC_{50}{}^h + [T]^h)$$

where θ is the fraction of maximal grip, [T] the tannin concentration meeting the film, and **h** the Hill coefficient — the cooperativity, set by how many bridging arms a tannin brings to one encounter. A mid-sized condensed tannin, the kind a structured young red carries, behaves near **h ≈ 2.5**: a grip that is almost absent, then climbs hard through its midpoint, then saturates. That steepness is the cook's whole opportunity. Slide the effective EC_{50} to the right — hand the tannin a decoy — and at a fixed mouthful the operating point slides down the steep face of the curve, and the felt grip does not ease, it *collapses*.

Fat and protein — the deepest partners, and why a mouthful of cheese is arithmetic

Begin with the deepest pairing, the one the receptor chemistry makes inevitable. Fat and protein give the tannin something to do other than dry your mouth. The protein in the food — the casein of cheese and yogurt, the globulins of lentils and chickpeas, and, for those who eat it, the collagen and myofibrils of meat — meets the tannin and precipitates *with it*, intercepting the grip before it reaches

your salivary PRPs. The fat coats the oral surfaces and restores, physically, the boundary lubrication the tannin was stripping. Two different mechanisms wearing one result, and both can be written down.

Take the interception first, as a competition for the tannin. Let the resting saliva present an affinity-weighted binding capacity **C_P**, and let the food deliver a decoy protein of capacity **C_D** (concentration × affinity × valency). With tannin the limiting reagent, the fraction that reaches the salivary film is simply the salivary share of the total capacity:

```
f_P = C_P ÷ (C_P + C_D)
```

and the effective EC_{50} the film experiences is scaled up by the reciprocal of that fraction:

```
EC50(effective) = EC50(bare) × (1 + C_D ÷ C_P)
```

Now put real food on it. Normalize the resting salivary capacity to **C_P = 1**. A bite of aged cheese floods the mouth with casein — abundant, open, and roughly a sixth proline — so its affinity-weighted capacity dwarfs the resting saliva's; take **C_D = 4**. Then f_P = 1 ÷ 5 = **0.20**: only a fifth of the tannin ever reaches your salivary film; four-fifths is spent on the sacrificial casein. The effective EC_{50} of a structured young red — bare value 35.7 µM at IFI-A 1,400 — rises to 35.7 × 5 = **178.6 µM**, which reads on the identity as a felt grip of 50,000 ÷ 178.6 = **IFI-A 280**. A single bite of cheese has turned a Phoenix-grade grip into a berry's soft floor.

And on the steep Hill curve the collapse is starker still. At the mouthful's tannin concentration — call it the bare EC_{50}, 35.7 µM, where the unpaired wine sits exactly at θ = 0.50, half its maximal grip — the cheese has slid the effective EC_{50} to 178.6 µM, so the operating ratio falls to [T] ÷ EC_{50} = 0.20, and

```
θ = 0.20^2.5 ÷ (1 + 0.20^2.5) = 0.0179 ÷ 1.0179 ≈ 0.018
```

The felt grip falls from one-half of maximum to under two percent. That is the arithmetic a diner performs, unknowing, every time a bite of something rich tames a hard pour — not a softening but very nearly an *erasure*, which is why the effect feels so disproportionate to the small bite that caused it.

The interception ladder, run across the foods a vegetarian table reaches for, is the chapter's centerpiece — pairing made literal:

PAIRED AGAINST A STRUCTURED YOUNG RED (BARE IFI-A 1,400)	DECOY CAPACITY C_D ÷ C_P	TANNIN REACHING SALIVA F_P	EFFECTIVE EC_{50}	FELT IFI-A	FELT GRIP 0 AT THE MOUTHFUL
Nothing (a sip of water)	0	1.00	35.7 µM	1,400	0.50
A spoon of plain yogurt	1	0.50	71.4 µM	700	0.15
A bite of paneer or warm lentils	2	0.33	107 µM	467	0.06
A bite of aged, fatty cheese	4	0.20	179 µM	280	0.018
A full rich course, several bites in	9	0.10	357 µM	140	0.003

The fat works by the other mechanism, and it does not touch the tannin at all — it repairs the *surface*. In the lubricated mouth the salivary film keeps the friction coefficient between tongue and palate very low, µ ≈ 0.02–0.06; strip that film by precipitation and the surfaces slide dry, µ climbing toward 0.3–0.5 — an order of magnitude rougher, which is the drag the nerves report as astringency. An oil, a cream, a cheese's fat phase re-coats the surfaces and drives µ back toward the lubricated floor, restoring the slip the tannin took. So richness softens a grip twice over: the protein intercepts the molecule, and the fat re-greases the film it was stripping. This is the single most important fact in all of tannin pairing, and it is why our most structured cultivars were, from the first, designed with the plate in mind. A grip is not an assault to be survived but a structure built to be *met*, and the meeting is where the pleasure lives.

A plant-forward table meets it beautifully, which is the quiet vindication of a vegetarian-first kitchen. The great tannin decoys are not only meat; they are dairy and legume — a hard cheese, a bowl of yogurt, a dal, a dish of beans dressed in good oil. Lacto-vegetarian cooking is, at the level of the saliva, exceptionally well armed, because casein is the finest sacrificial protein on any table and legumes bring both protein and the starch that carries fat. The meat pairings the old books lead with are real and we honor them; we only note that the mechanism was never about the meat — it was about the protein and the fat, which a vegetable table has in abundance.

Salt, acid, sweetness, warmth — the softeners and the rounders

The remaining partners do not intercept the tannin; they act on the saliva, on the surface, or on the perception, and each moves a different term of the same equation.

Salt is a flow agent. A little salt on the food raises salivary secretion sharply — resting flow runs near 0.3–0.4 $mL \cdot min^{-1}$ and climbs under stimulation toward 7 $mL \cdot min^{-1}$, roughly a twentyfold rise — so the sacrificial PRP reservoir refills faster between sips. If a reservoir of volume V refills at flow Q, its recovery time constant is τ_r = V ÷ Q, and a twentyfold rise in Q cuts that refill time twentyfold, returning the mouth to a lubricated baseline between encounters instead of letting it ratchet drier. The felt result is a lower, faster-recovering grip — the quiet friend of a tannic pour.

Acid is subtler, the one partner that genuinely cuts both ways. A bright acidity provokes saliva, as salt does; but low pH also protonates the tannin's phenols and promotes its self-association, nudging the molecules to aggregate harder and present a rougher, more "angular" grip. Which way the net falls depends on balance — the balance of tannin against acid is the central tension in a young red and in a strong black tea taken with lemon. The designed advantage is that we can set a grip's magnitude and *build* precisely enough to balance a known acidity rather than hope a vintage cooperates.

Sweetness rounds without intercepting. A touch of residual sugar does not precipitate the tannin; it raises the solution's viscosity and perceived lubricity and competes, centrally, for attention — padding the felt edge so a grip reads softer and more integrated: a whisper of sugar makes a tannic wine seem gentler, and dark chocolate's small sweetness keeps its considerable tannin civil.

Temperature bends the curve. Within the drinking range, warmth lowers the film's viscosity and friction so a grip reads softer and shorter, while cool raises friction so the same grip reads firmer and more defined — the physics behind serving a structured red a few degrees below room temperature rather than warm. At the scalding extreme a second effect takes over, pulling the other way: heat itself is an irritant that compounds the grip, which is why a fierce green tea is kinder let down from scalding to merely hot before it is drunk. The levers, laid side by side with what each one actually moves:

PARTNER	ACTS ON	DIRECTION ON FELT GRIP	MECHANISM	ROUGH MAGNITUDE
Protein (cheese, yogurt, legumes, meat)	the molecule	strongly down	sacrificial precipitation intercepts tannin	effective EC_{50} ×2 to ×10
Fat (oil, cream, cheese fat)	the surface film	strongly down	restores boundary lubrication, μ 0.3 → 0.05	~10× drop in friction
Salt	the saliva reservoir	down	raises flow up to ~20×; faster recovery	shortens refill τ_r sharply
Acid	saliva *and* molecule	both ways	provokes flow, but low pH hardens aggregation	balance-dependent
Sweetness	the perception	down	viscosity and central padding	rounds, does not remove
Warmth	the surface film	down	lowers viscosity and friction	softer, shorter finish
Cold	the surface film	up	raises friction, slows recovery	sharper, longer finish
Time (decant, age)	the molecule itself	down	in-glass polymerization and pigment binding	the only term that changes the tannin



Tea, the other great tannin beverage — the leaf whose galloylated catechins grip by the same chemistry as a grape's seed. Everything the service does to a young red does to a cup of tea: milk lends its casein as a decoy, sugar pads the edge, and letting the scald fall to merely hot drops the whole cup out of its harshest range.

The build, and the reservoir — pace and sip size

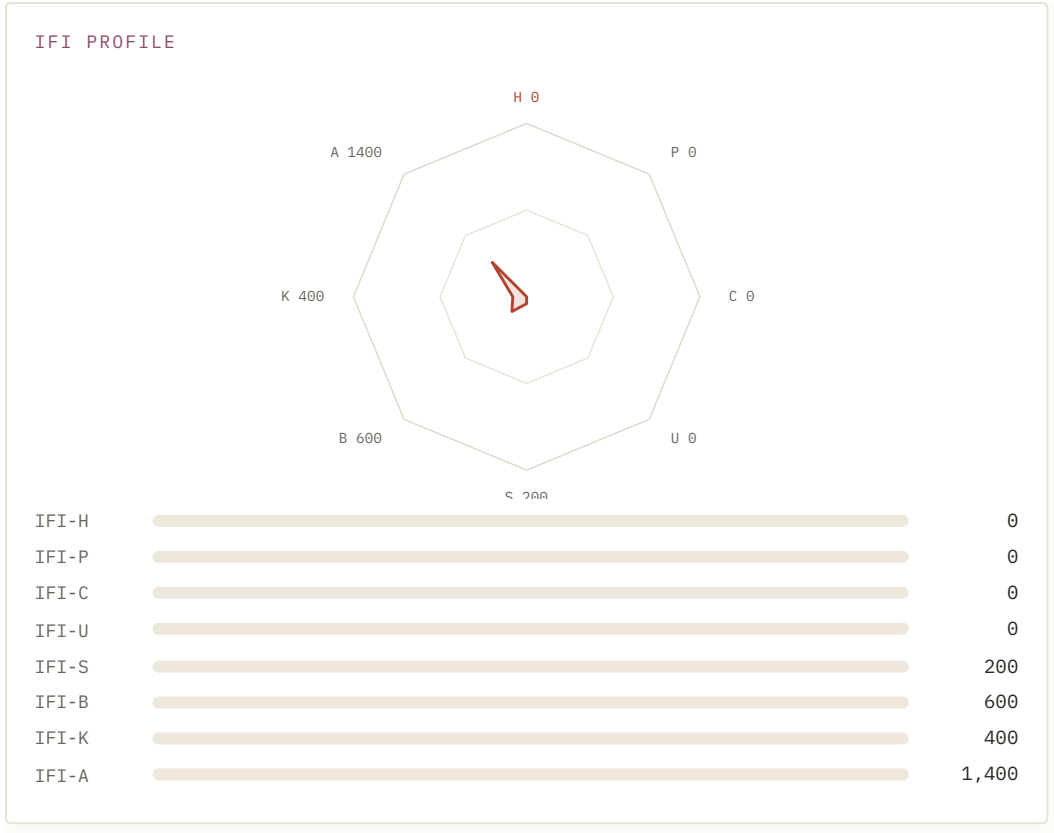
One term no single mouthful reveals, because it is cumulative: astringency *builds*. It peaks late — the full grip of a sip arrives some fifteen seconds after you swallow, long after a sweet or a sour has come and gone — and it ratchets upward across repeated sips, because each sip precipitates a share of the sacrificial PRPs and the reservoir replenishes only slowly. The build is literally the depletion-and-refill kinetics of the salivary pool, and it has a simple steady state worth writing out.

Let each sip precipitate a fraction *d* of the available PRP reservoir — *d* rising with the tannin dose and the size of the sip — and let the reservoir recover a fraction ρ of its running deficit in the interval τ between sips, where ρ = 1 − e^(−τ ÷ τ_r) and τ_r = V ÷ Q is the refill time constant set by salivary flow. The deficit climbs until the loss added per sip equals the amount recovered per interval, reaching a steady-state depletion:

> D* = d ÷ ρ

Read that expression and the whole craft of pacing falls out. **Large sips in quick succession** mean a big *d* and a small τ — little recovery between them — so ρ is small and D* is large: the grip climbs toward its ceiling and stays there. **Small sips, well spaced** mean a small *d* and a large τ, so ρ approaches one and D* stays near its floor: the film recovers between each, and the grip is held in check. **Salt and acid** raise the flow *Q*, shrink τ_r, enlarge ρ, and lower D* — which is why a well-salted, bright-edged dish lets you drink a structured wine across a whole course without the grip stacking into a wall. A sommelier pouring a firm young red into a wide bowl, at cellar temperature, to be sipped slowly across a fatty course, is managing nearly every term of this equation at once — EC_{50} through the fat and protein, ρ through the salt, the friction floor through temperature, *a* through the pace.

That is also why the same wine can read as two different wines. Here is a structured young red as poured, read on the Index — the grip the headline, bitterness and a little body beneath it:



Poured into a careless mouth — big gulps, no food, warm glass — that **A 1,400** builds toward its ceiling and the wine reads as a wall. Poured slowly, cool, against a plate of cheese and lentils and a little salt, the very same wine reads, by the same interception arithmetic, down near **A 280** and softly rounded. One number built into the grape; the service writes the rest of the phrase.

Time — the one partner that changes the molecule

Time deserves the last word, because it is the only partner that changes the tannin itself rather than merely meeting it. Everything else works in the moment — the fat restores lubrication, the protein offers a decoy, the salt raises flow, the sweetness pads the edge — each cushioning a grip that is otherwise unchanged. Time works on the molecule.

The chemistry is a slow polymerization. Young condensed tannins are moderate chains of flavan-3-ol units, often galloylated and loosely self-associated — a configuration that presents many free binding arms and grips hard. Left in the glass for an hour, or in the bottle for a decade, those chains polymerize further and, crucially, bind to anthocyanin pigment, forming the gentle **polymeric pigments** that give an old red its brick color and its softened structure. As the mean degree of polymerization climbs, the felt grip walks along a curve with a *peak*: up to a point, larger tannins carry more binding arms and grip harder, but past an optimum the chains grow so large that they begin to fold on themselves and aggregate with their own kind *before* ever reaching a salivary protein, so the effective, protein-available tannin falls — and the felt astringency falls with it, even as the total phenolic mass is unchanged. Aging walks a tannin past that peak: the grip's amplitude lowers while its finish lengthens, which is why a great structured wine is sold as a promise and drunk as a fulfillment.

This is precisely the lever we tune in the grape, which carries **1,610 constructs** across its astringency, bitterness, and multi-axis libraries. The grape builds its condensed tannins off the flavonoid branch, and

the enzyme at the decisive fork is **anthocyanidin reductase (ANR)**, which reduces anthocyanidin to epicatechin — the flavan-3-ol monomer that polymerizes into proanthocyanidin, the grip. Tune ANR expression, the chain length it feeds, and the degree of galloylation, and you set where on that polymerization curve the fruit *starts* — you can design a tannin that arrives already sitting where a decade in a cold cellar would have carried it. The source-route softening we design in is, in effect, **time bottled in advance**: a grip built to its mellow target at harvest rather than left to find it over years in the dark. The pairing table is the cook's instrument for the grip as it is tonight; the design is the instrument for the grip as it will be; and the two together are the whole art of living with tannin.



Phoenix (SS-0096) in the controlled chamber — the cultivar designed to register on all eight axes at once, its astringency set deliberately to IFI-A 1,400. Where a cellar spends a decade walking a tannin toward softness, the design sets the grip precisely at harvest: the same destination, reached forward instead of waited out.

A note on the number behind the number, because pairing math only works if the grip is a *fixed* starting amplitude. A micromolar EC_{50} is exact only if you can say "micromoles of what," and a condensed tannin is not one molecule but a distribution. Each flavan-3-ol unit weighs about 290 $g \cdot mol^{-1}$ and each interflavan bond sheds two hydrogens, so a proanthocyanidin of mean degree of polymerization five weighs roughly 290 + 4 × 288 ≈ **1,443 $g \cdot mol^{-1}$**. The structured red's 35.7 µM then converts to 35.7 × 1,443 ÷ 1,000 ≈ **51.5 $mg \cdot L^{-1}$** of protein-active tannin — small against the 1–4 $g \cdot L^{-1}$ of *total* tannin a red carries, because most of that total sits at chain lengths too large or too small to work near the active EC_{50}. The gram-per-litre figure a cellar measures is a blunt instrument; the molar EC_{50} the Index anchors is the sharp one, and the reason a grip can be designed to a target rather than merely estimated.

Precision — why pairing by number needs a number that holds

None of this pairing arithmetic survives if the grip wobbles. You cannot slide an effective EC_{50} with a bite of cheese and predict the felt rung if the starting IFI-A could sit anywhere across a wide band — and a conventional tannin crop swings with season, ripeness, and lot by tens of percent, the way wild-type capsaicin did. Because IFI-A is a ratio of two fitted concentrations, its uncertainty is the two relative uncertainties added in quadrature:

$$\sigma(\text{IFI-A}) \div \text{IFI-A} = \sqrt{\left[(\sigma_{ref} \div EC_{50}_ref)^2 + (\sigma_{cand} \div EC_{50}_cand)^2 \right]}$$

Hold the reference fit to 3 % and a designed candidate to 5 %, and that is $\sqrt{(0.03^2 + 0.05^2)}$ = **5.8 %**, inside the platform's **≤ 7 %** batch envelope. So Phoenix's grip is not "IFI-A 1,400" but **1,400 ± 82** at one standard deviation — a 95 % band of roughly 1,240 to 1,560, a single reliable rung you can pair against. Let the candidate fit drift to a wild-type-grade 42 %, and the quadrature gives $\sqrt{(0.03^2 + 0.42^2)}$ = **42 %**, so the same grip becomes 1,400 ± 589 — a 95 % band from about 245 to 2,555, which is to say the grip could be a whisper or a wall and you would not know which until it was in the mouth.

LOT	EC_{50} FIT CV	IFI-A	95 % BAND	WHAT A COOK CAN DO WITH IT
Designed, held to envelope	5.8 %	1,400	~1,240 – 1,560	pair by number; the cheese math lands
Conventional, wild-type drift	42 %	1,400	~245 – 2,555	cannot pair; guess and hope

That contrast is the quiet argument under the whole chapter. The pairing table is arithmetic only because the grip is a number that *holds*; precision is not fussiness but the precondition that turns "it pairs well" into something a cook can design forward, lot after lot, within a few percent.

The plate as a composition

Hold these tables beside the eight-axis scorecards and the point of measuring astringency becomes concrete. A dish or a glass is a **composition** — a grip set against a fat, a sweetness, a salt, a warmth, a pace — and once every element carries a number, pairing stops being incantation and becomes an equation whose terms you can name and move. The grip built into the grape is only the starting amplitude; the plate and the service write the rest, and a cook who reads the curve can make one wine feel like two.

The honest limits are the edges of that promise. **The matrix is not the flask:** the assay reads a tannin against a standardized protein pool, while a real wine or tea arrives wrapped in its own acids, sugars, and polysaccharides that can sleeve or free the tannin. **The mouth is each person's own:** salivary PRPs vary in quantity and flow, and a mouth that meets tannin often makes more of the sacrificial protein, so the habitual tea-drinker is slightly armored against the grip the rest of us feel more sharply. And there is a failure mode on the generous side of the table: **you can intercept too well.** Drown a great structured wine in fat and protein — push the effective EC_{50} past 300 µM, felt grip under IFI-A 150 — and you have not softened it, you have *hollowed* it, erased the architecture that made it worth opening. The grip is a structure to be met, not abolished; the art is to pull it down to where the fruit and the finish stand clear, and no further.

And here, as quietly as anywhere, the nourishment note sits inside the same molecule. The proanthocyanidins and ellagitannins that give a grip its edge are the same polyphenols that make a grape or a tea leaf or a square of dark chocolate worth more than its sugar — the compounds a lifetime of clean eating reaches for. We do not dress that as a claim; we only decline to breed it out, and we design the grip so the body meets it the way it was made to: against fat, against protein, slowed and paced and welcomed. Build the grip to a number that holds, set it against a plate that knows what to do with it,

and let a person taste the structure not as an assault survived but as the reason the meal feels whole. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The last thing left is to cook.

Chapter 15 · In the glass — *wine and tea read as IFI-A.*

We begin in the glass, because the glass is where astringency is most consciously loved, and because a beverage is the cleanest demonstration of designing a grip to a number. A dish hides its tannin inside fat and salt and char; a drink has nowhere to hide. Lift a grippy red or a long-steeped green to the light and the whole of the astringent axis is suspended in front of you, undefended — the pucker, the drying, the slow pull across the palate that a good one turns into pleasure and a careless one turns into a wall. What follows are formulations rather than field-recipes: a cellar's and a tea house's working notes, each built on a designed cultivar and each read out on the full eight-axis Index, with astringency the headline.

They are vegetarian by nature and, in our family's own habit, poured first for the people at the table who are not drinking at all. Every one has a zero-proof sibling, because the grip belongs to everyone — the pleasure of astringency is a tactile pleasure, not a chemical indulgence, and a child with a cold-brewed tea and a grandmother with a dealcoholized red are tasting the same designed molecule do the same designed thing. That is the quiet argument of this chapter: astringency is the one axis you can give anyone.



Tea leaf in cross-section — the palisade and spongy mesophyll where the catechins are stored in the vacuoles. A cold, long steep pulls the grip without the heat-driven bitterness, the simplest way to taste a designed catechin profile for exactly what it is.

What the tongue is actually doing — astringency as a loss of lubrication

Astringency is the odd one out among the eight axes, and it is worth being exact about why. Heat, sweetness, umami, bitterness, kokumi, cool-burn, pungency — seven of the eight are *tastes* or *chemestheses*: a molecule arrives, docks a receptor protein, a channel opens or a G-protein fires, and a nerve reports a sensation that corresponds to a thing dissolved on the tongue. Astringency reports nothing dissolved. It reports a *mechanical* change — the

sudden roughening of a surface that was a moment ago slick — and the receptor on the Index, the salivary proline-rich proteins, is not on the tongue at all. It is floating in the saliva.

Resting saliva is a lubricant. A thin film of mucins and proline-rich proteins (PRPs) coats the oral mucosa and lets the tongue glide against the palate with almost no friction — a glide so complete we never notice it until it fails. Tannins make it fail. A tannin is a polyphenol with many hydroxyl-bearing aromatic rings, and the PRPs are, as their name says, extraordinarily rich in proline. Proline is the key. Its pyrrolidine ring locks the peptide backbone into a rigid, open, left-handed polyproline-II helix, a conformation that exposes the backbone carbonyls and presents broad flat faces of the chain to the solvent — an ideal landing strip. The tannin's aromatic rings stack against those faces by hydrophobic and π–π interactions while its phenolic hydroxyls hydrogen-bond to the exposed carbonyls. One tannin can bridge two protein chains; one protein chain can carry several tannins. The result is cooperative: each binding event presents fresh faces and makes the next more favorable.

At low tannin-to-protein ratios the complexes stay soluble. Add more tannin and the bridging runs away with itself — soluble complexes grow into colloidal aggregates, the aggregates into a visible floc, and the floc drops out of solution, carrying the lubricating protein film down with it. The mucosa, stripped of its slick coat, now drags. Free nerve endings read the drag as friction, and friction, integrated over the whole moving surface of the mouth, is what we call the grip. This is why astringency *builds* across a sip and lingers after you swallow: precipitation is not instantaneous, and saliva needs fifteen to thirty seconds to regenerate the film. The sensation is the gap between the stripping and the repair.

Not all tannins strip alike, and the differences are the whole palette a designer works in. The two great families are the **condensed tannins** — proanthocyanidins, built from flavan-3-ol units (catechin, epicatechin and their trihydroxylated cousins) polymerized into chains — and the **hydrolyzable tannins** — gallotannins and ellagitannins, in which galloyl groups are esterified onto a sugar core and, in the ellagitannins, coupled into the large hexahydroxydiphenoyl cages of a molecule like punicalagin. Three structural levers move their grip:

- **Galloylation.** A galloyl ester hung on a flavan-3-ol adds a whole extra aromatic, hydroxyl-dense face. Galloylated units — epicatechin gallate, epigallocatechin gallate — bind PRPs far more avidly than their ungalloylated parents, and read as the sharpest, most rasping grip.
- **Mean degree of polymerization (mDP).** Longer condensed chains carry more binding faces and precipitate protein more readily, up to the length where the polymer folds on itself and buries them. A narrow mDP distribution gives a focused, predictable onset; a broad one gives a grip that arrives in waves.
- **B-ring hydroxylation.** A trihydroxyl (prodelphinidin, from epigallocatechin) B-ring binds differently from a dihydroxyl (procyanidin, from epicatechin) one — prodelphinidin-rich tannin

reads long, fine and velvety where procyanidin reads rounder and softer.

TANNIN CLASS	REPRESENTATIVE UNIT	BINDING MODE	GRIP IT READS AS
Condensed, procyanidin	epicatechin (dihydroxy B-ring)	chain stacking, moderate faces	round, soft, broad
Condensed, prodelphinidin	epigallocatechin (trihydroxy B-ring)	long chains, many fine faces	fine-grained, long, velvety
Galloylated flavan-3-ol	epigallocatechin gallate	extra galloyl face, high avidity	sharp, rasping, fast
Hydrolyzable, ellagitannin	punicalagin (HHDP + galloyl)	many faces per large molecule	fast, bright, structural

A plant breeder working by taste could only select the whole blurred sum of these. Working from the biosynthetic genes, we select each lever on its own: the ratio of the branch enzymes that set prodelphinidin against procyanidin, the galloyltransferase load that sets galloylation, the polymerization that sets mDP. Design in this axis is not making a plant *more* astringent. It is choosing the exact shape of a grip and then holding it.

Putting a number on the grip — the astringency index

Because astringency is a reaction — tannin precipitating protein — it has a half-point, and a half-point is all the Index ever needs. We define **EC_{50}** here as the tannin concentration, expressed in milligrams per liter of catechin-equivalents, at which the standardized protein-precipitation reaction reaches half of its maximum. Catechin-equivalents are a potency-normalized unit: we titrate the sample against a fixed load of precipitable protein, measure how much protein drops out as turbidity, and report the concentration of a catechin standard that would have precipitated the same amount. The unit already folds potency into itself, which is exactly what we want — a milligram of rasping galloylated catechin and a milligram of soft procyanidin are *not* the same dose of grip, and catechin-equivalents say so.

From there the axis score is the same ratio that defines every axis:

> $\text{IFI-A} = 1{,}000 \times EC_{50}(\text{reference}) \div EC_{50}(\text{candidate})$

The reference is a stated convention — a native astringency standard anchored so that 1,000 is its own half-point, in the same way that the Scoville scale is defined by dilution-to-threshold rather than derived from a single literature measurement. Everything louder than the reference — reaching half-maximal grip at a *lower* concentration, because each unit of it binds harder — scores above 1,000; everything softer scores below. The elegant part is that you never actually need the reference's absolute value to compare two of our drinks, because it cancels. Rearranging the definition,

> $EC_{50}(\text{candidate}) \div EC_{50}(\text{reference}) = 1{,}000 \div \text{IFI-A}$

so a score reads straight off as a concentration ratio. An IFI-A of 1,400 means the designed tannin reaches half-maximal grip at 1,000 ÷ 1,400 = 0.714 of the reference concentration — it binds harder, so less of it does the job. An IFI-A of 900 means 1.111 — it needs eleven

percent *more* concentration to grip as hard, because it is gentler per unit. An IFI-A of 2,000 means 0.500 — half the concentration, twice the loudness.

To make the arithmetic concrete, anchor the reference at EC_{50}(reference) = 700 mg/L catechin-equivalents — an illustrative value, chosen only to turn the ratios into numbers you can hold. The four pours of this chapter then read out directly:

POUR	TARGET IFI-A	IMPLIED EC_{50} (MG/L CAT-EQ)	DOMINANT TANNIN
Cold-brew catechin green	900	778	galloylated catechin, softened
Structured dealcoholized red	1,400	500	condensed, skin prodelphinidin
Pomegranate shrub	2,000	350	hydrolyzable ellagitannin

Check the first line by hand: 1,000 × 700 ÷ 778 = 900. The last: 1,000 × 700 ÷ 350 = 2,000. The score and the concentration are two readings of one fact.

A concentration is a count of molecules only once you divide by a molar mass, and here the molecule matters enormously. Catechin has a molar mass near 290 g/mol, so the red's 500 mg/L of catechin-equivalent grip is about 500 ÷ 290 = 1.72 mmol/L — roughly 1,720 µmol of binding faces per liter. Punicalagin, the pomegranate's workhorse, weighs near 1,085 g/mol; a given astringency delivered by punicalagin is carried by *far fewer molecules* than the same astringency delivered by catechin, because each punicalagin presents a cage of hexahydroxydiphenoyl and galloyl faces where catechin presents one small flavan. The pomegranate scores loudest on the fewest molecules. This is why the hydrolyzable grip reads so *fast* — a sparse population of very avid, very large binders saturates the protein almost on contact, where a dense population of small condensed units grips gradually as the chains find their faces.

Loudness at the half-point is not the whole story of a grip; the *shape* of the climb to it is what separates a pleasure from a wall. Astringency follows a sigmoid in concentration, and the Hill form captures it:

$$\theta([T]) = [T]^n \div (EC_{50}^n + [T]^n)$$

where θ is the fraction of maximum grip, [T] the tannin concentration, and n the Hill coefficient — the cooperativity of the cross-linking. Because one bound tannin makes the next bind more easily, n for tannins runs above 1, typically between about 1.5 and 2.2, and that steepness is the danger. Take the structured red at EC_{50} = 500 mg/L and a cooperative n = 1.8. Poured at twice its half-point, $[T]/EC_{50}$ = 2, the grip is already θ = 2^1.8 ÷ (1 + 2^1.8) = 3.48 ÷ 4.48 = 0.78 — seventy-eight percent of maximum. Push to three times the half-point and θ climbs to 7.22 ÷ 8.22 = 0.88. A steep curve means a small change in extraction can flip a comfortable grip into a harsh one; that is the wall, and it is a curve-shape problem, not a quantity problem.

The design answer is to flatten the curve, not lower the dose. We tune n by controlling the *breadth* of the mDP distribution — a narrow band of polymer lengths grips in a near-cooperative rush (high n, sharp onset), a deliberately broadened band staggers the binding across concentrations (lower n, gradual onset). At the same two-times pour, dropping n from 2.2 to 1.5 moves the grip from θ = 4.60 ÷ 5.60 = 0.82 to θ = 2.83 ÷ 3.83 = 0.74 — a modest change in height, but the slope beneath it is far gentler, so the grip now *arrives slow, builds modestly over two sips, and resolves long*, with no wall anywhere in it. That single sentence of tasting is a statement about n.

Every number carries an error, and honesty about astringency means carrying it in the open. We hold our construct output to a batch coefficient of variation at or below 7%, against the 42% that wild-type selection drifts to, and we carry that same discipline into the precipitation assay. Since IFI-A is a ratio of two EC_{50} values, the relative errors add in quadrature:

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(σ_IFI ÷ IFI)² = (σ_ref ÷ EC_ref)² + (σ_cand ÷ EC_cand)²
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With each EC_{50} assay held to our design specification of CV = 7%, the score carries $\sqrt{(0.07^2 + 0.07^2)} = \sqrt{0.0098} = 0.099$, just under ten percent. So the three pours read 900 ± 89, 1,400 ± 139 and 2,000 ± 198 — bands that do not overlap, which is the only reason the ladder means anything. The contrast with the tongue is the argument for the instrument: a trained human panel scoring astringency runs a CV of 25 to 35%, and two such measurements in a ratio propagate to a spread of 35 to 50%, wide enough to swallow the gap between the soft tea and the loud shrub entirely. The panel feels the truth; the assay can *say* it, to a tenth. We keep both, and we anchor the index on the one that does not drift with who is tasting, how much they have eaten, or how tired the afternoon has made their mouth.

The hosts that can carry it

Astringency lives in a handful of lineages that evolved tannin as a chemical defense, and those are the hosts we build into. Each is difficult in its own way — tannin biosynthesis sits far out on the flavonoid branch, past the pigments, and moving it without wrecking the plant's own co-pigmentation is delicate work.

HOST ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Grape	1,610	B / A / M	Hard
Tea	280	U / B / A	Hard
Blueberry	120	A	Moderate
Pomegranate	120	A	Hard
Sorghum	120	A	Frontier
Raspberry	40	A	Moderate

Grape carries the deepest program of any astringent host — 1,610 constructs — because grape's tannin is not one thing but a landscape. The flavan-3-ol branch splits at two reductases: anthocyanidin reductase (ANR), which makes the *cis* flavan-3-ols (epicatechin and epigallocatechin), and leucoanthocyanidin reductase (LAR), which makes the *trans* ones (catechin,

gallocatechin). The ratio of their activity sets the character of the tannin before a single chain is polymerized — a high ANR-to-LAR balance in the skin gives the long, *cis*-rich, prodelphinidin-leaning polymer that reads velvety, while the seed's shorter, more galloylated, more procyanidin tannin reads green and biting. A designer who can set ANR against LAR, tissue by tissue, can grow a grape whose skin is a different grip from its seed on purpose. Tea runs the same flavan-3-ol chemistry toward the galloylated catechins and holds its umami counterweight in theanine; pomegranate abandons the condensed route entirely for the hydrolyzable ellagitannins; blueberry and raspberry and sorghum each carry their own smaller, cleaner single-axis grip. What they share is that the grip can be read on one number and tuned by name.



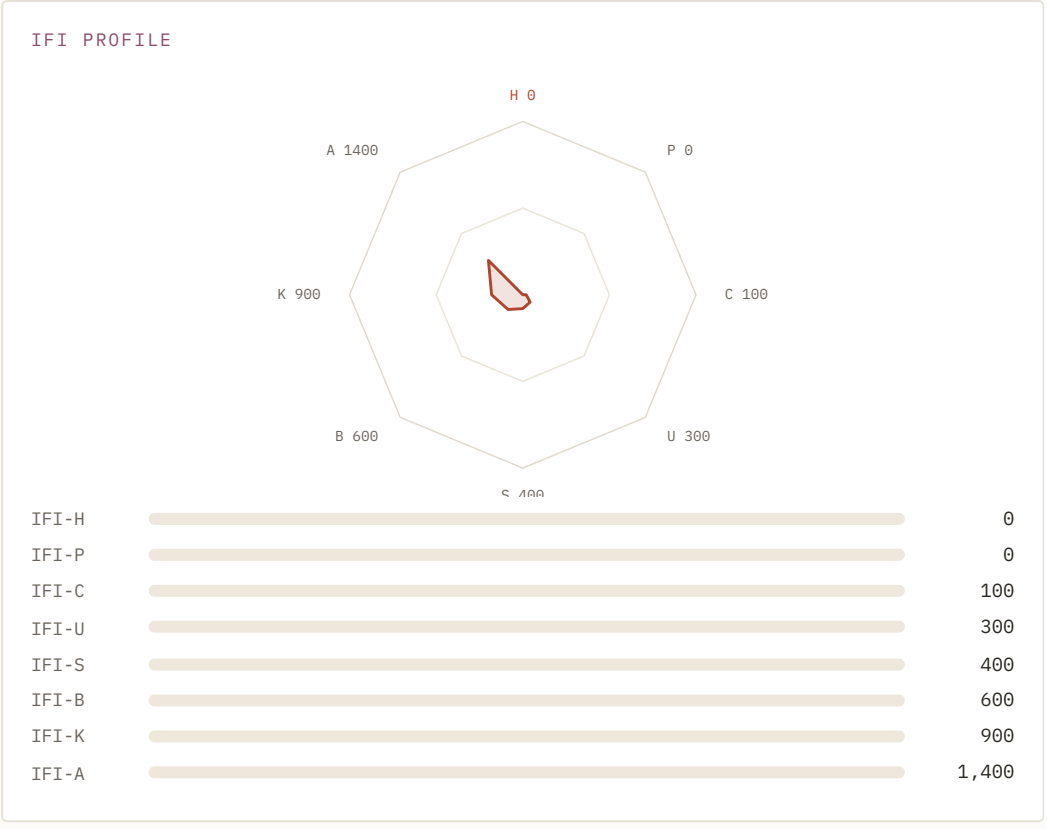
Grape in cross-section — skin, pulp and seed, three tannins in one berry. The skin's long prodelphinidin reads as velvet; the seed's short, galloylated, procyanidin-rich tannin reads as green. Setting the ANR-to-LAR balance tissue by tissue is how a designed berry carries one grip in the skin and another in the seed.

The structured dealcoholized red

A still, deep, grippy red made from a skin-tannin-forward designed grape and then gently dealcoholized, so that the architecture of the tannin carries the drink where the alcohol used to. The whole point is that a dealcoholized wine usually collapses — strip the alcohol, which carries body and softens grip, and you are left with thin, sharp tannin — unless the tannin was *designed* to be long, fine, and co-pigmented from the start.

Base	designed skin-tannin red grape must, high mDP, prodelphinidin-rich
Astringency	target IFI-A ~1,400 — firm, fine-grained, long finish
Co-pigment	full anthocyanin load, tannin pre-draped in pigment from the vine
Treatment	ferment dry · gentle dealcoholization · 2 weeks on fine lees
Serve	16 °C, large bowl, decant 30 min
Zero-proof twin	same must, arrested before fermentation; grip identical

1. Press for **skin** extraction, sparing the seed — the designed grape already carries a high ANR-to-LAR ratio, so the skin fraction is long and velvety, not green.
2. Ferment dry, then dealcoholize gently at low temperature to protect the aromatics.
3. Rest two weeks on fine lees for a trace of kokumi body, then decant thirty minutes before serving so the designed tannins open.
4. The grip should arrive slow, build modestly over two sips, and resolve long — a wall nowhere in it.



Why does ordinary dealcoholized wine collapse, and why does ours not? Ethanol is a quiet solvent for tannin. At the ten-to-fourteen percent of a finished wine it keeps the larger polymers in solution and blunts their rush to precipitate protein — part of what we feel as alcohol's "softness" is tannin held in check by the solvent around it. Pull the ethanol out and that restraint goes with it: the polymers self-associate, the longest ones drop out prematurely, and what reaches the glass is the short, sharp, under-dressed remainder — thin body and a biting, graceless grip. The answer is not to extract harder but

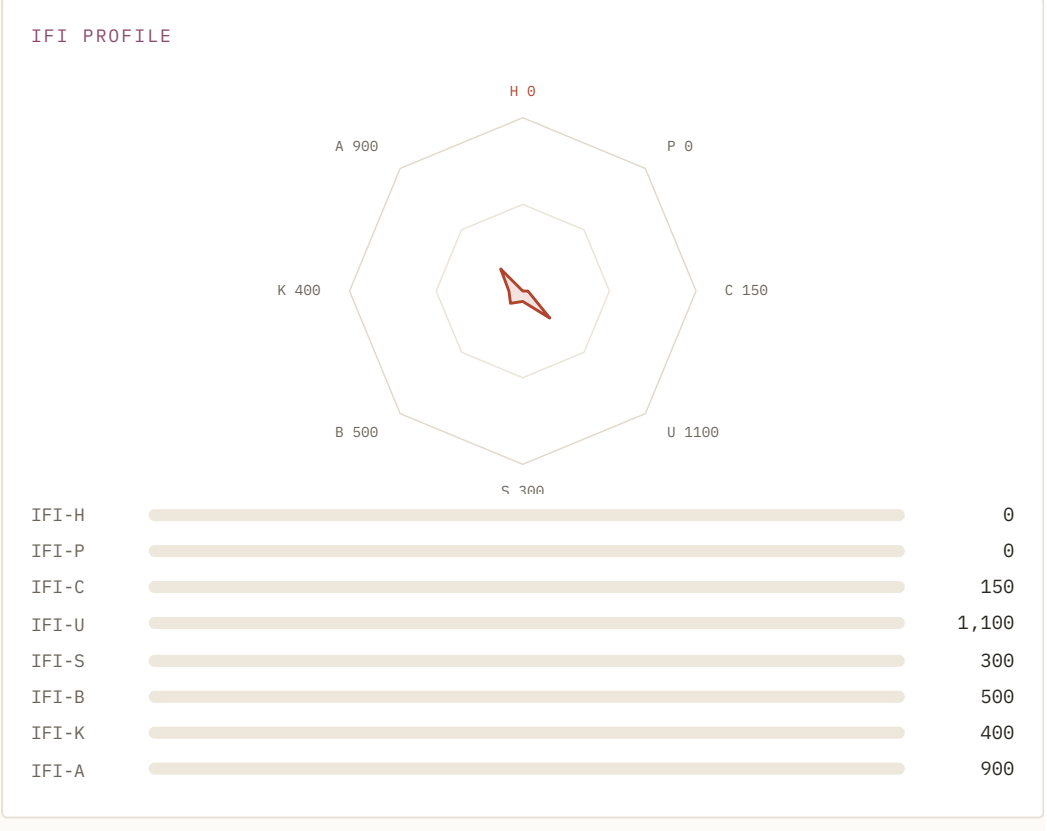
to *design* the tannin long, fine and pre-draped before the alcohol is ever removed. Co-pigmentation does the draping. Anthocyanins — the skin pigments — stack face-to-face with the tannin polymers, and over the two weeks on fine lees they begin forming covalent tannin-anthocyanin adducts, often bridged by a trace of acetaldehyde into stable pigmented polymers. A tannin wearing a coat of pigment presents fewer naked faces to the salivary protein; its grip softens and lengthens without losing its loudness. That is how a wine with no alcohol in it still reads IFI-A 1,400 — EC_{50} near 500 mg/L catechin-equivalents, about 1.7 mmol/L of fine, co-pigmented, prodelphinidin-rich binding faces — and still pours as architecture rather than rasp. The zero-proof twin, arrested before fermentation, never had the alcohol to lose; its grip was built to stand without it, and it stands.

The cold-brew catechin program

A designed green tea steeped cold and long — ten to twelve hours in the refrigerator — which pulls the catechin grip and the theanine umami while leaving most of the heat-driven bitterness behind. It is the clearest way to taste a designed catechin-to-theanine ratio for exactly what it is.

Leaf	designed green tea, lifted theanine, moderate galloylated catechin
Astringency	target IFI-A ~900 — clean, round, no rasp
Water	cold, filtered, 1 g leaf : 100 ml
Time	10–12 h, refrigerated
Finish	strain; serve over a single large ice

1. Combine leaf and cold water; refrigerate ten to twelve hours.
2. The cold, slow extraction favors theanine and the softer catechins over the harshest galloylated fraction — the designed leaf's high theanine ratio does the rest.
3. Strain and serve cold and undiluted; the grip should read as a clean edge under a broth-like roundness.



The green leaf holds four catechins that matter, and they are not equal. The design lever is their ratio.

CATECHIN	FORMULA	MOLAR MASS (G/MOL)	GALLOYLATED	GRIP
Epicatechin (EC)	$C_{15}H_{14}O_6$	290.3	no	low, round
Epigallocatechin (EGC)	$C_{15}H_{14}O_7$	306.3	no	low–moderate
Epicatechin gallate (ECG)	$C_{22}H_{18}O_{10}$	442.4	yes	high, sharp
Epigallocatechin gallate (EGCG)	$C_{22}H_{18}O_{11}$	458.4	yes	highest, rasping

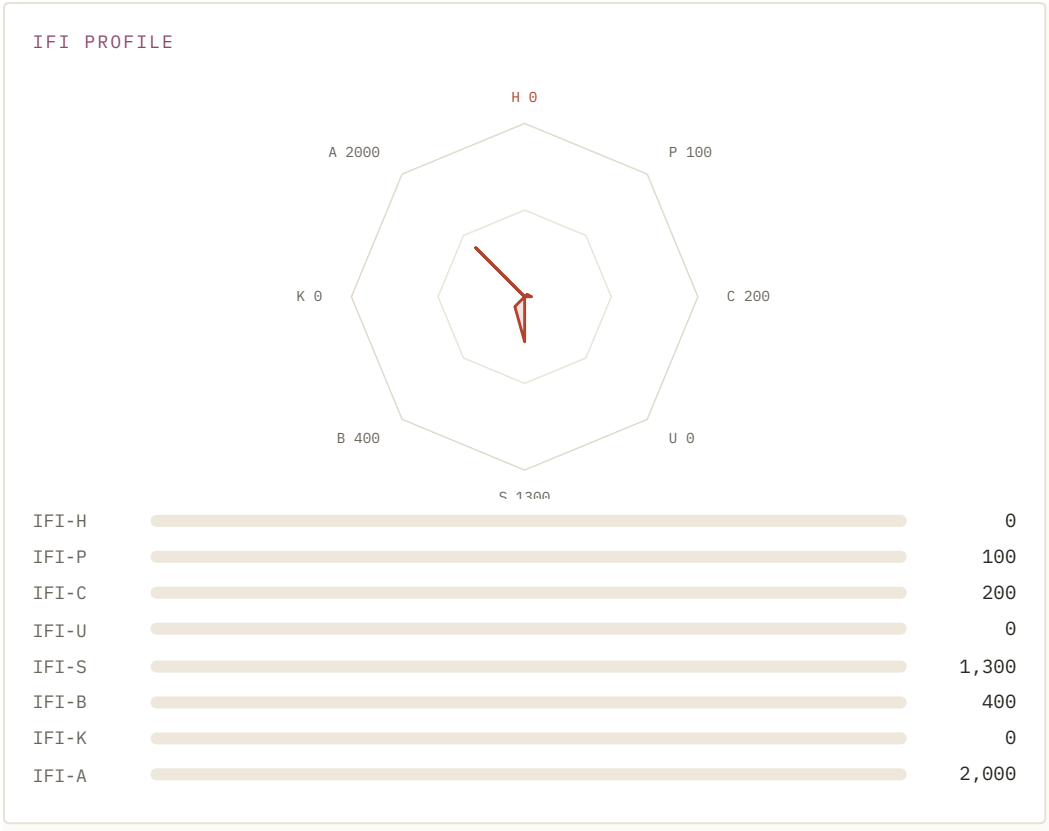
The two galloylated catechins carry most of a green tea's bite, and heat pulls them hardest. A cold, long steep is a *selective* extraction: the colder water favors the smaller, more soluble, less galloylated fractions and the amino acid theanine, and leaves much of the harshest galloylated grip locked in the leaf. The designed leaf stacks the odds further — lifted theanine, moderated galloylation — so the cup is designed to land at IFI-A 900, EC_{50} near 778 mg/L catechin-equivalents, the gentlest grip of the four pours. Brew it to roughly its own half-point and the Hill form reads $\theta = 0.5$: the grip sits exactly at the middle of its curve, a clean edge and no further, which is why it reads as a crisp line rather than a drying wall. The theanine does the rest of the work perceptually — its umami, scoring IFI-U 1,100, drapes a broth-like roundness over the edge, not by binding the tannin but by filling the mouth with a competing, pleasant fullness that the grip then cuts through. Loudness and roundness in one cup, each on its own axis, each on its own number.

The pomegranate shrub

A drinking vinegar built on a designed high-grip pomegranate, where the fast, bright ellagitannin pucker is set against sugar and acid into something that can stand up to fat in a glass or over ice. This is astringency designed *upward* — the pomegranate played loud, on purpose.

```
Fruit           designed high-punicalagin pomegranate, arils + a little
pressed rind
Astringency     target IFI-A ~2,000 — fast, bright, structural
Sugar           raw cane, 1:1 by weight with juice
Acid            white wine vinegar, 1:2 to juice
Rest            5 days, refrigerated, then strain
Serve           1 part shrub : 4 parts sparkling water, over ice
```

1. Press the arils with a small fraction of inner rind to carry the grip up; combine with sugar and let the sugar draw the juice overnight.
2. Add vinegar, rest five days refrigerated, then strain.
3. Dilute to taste with sparkling water over ice — the grip should arrive fast and clean, cut the sweetness, and leave the mouth refreshed and faintly dry, wanting the next sip.



The pomegranate is designed for the loudest grip in the glass — IFI-A 2,000, EC_{50} near 350 mg/L catechin-equivalents, half the reference concentration doing twice the reference's work — and it is loud for a structural reason. Its tannin is not the slow condensed polymer of grape and tea but the hydrolyzable ellagitannin punicalagin, a single large molecule carrying a cage of hexahydroxydiphenoyl and galloyl faces. Where a condensed tannin grips *gradually*, its chains finding the protein face by face, punicalagin grips *fast* — few molecules, each so avid and so multivalent that it saturates the salivary protein almost on contact. That is the "fast, bright, structural" pucker exactly: the kinetics of a sparse population of very large binders. The inner rind carries the punicalagin up; pressing a small fraction of it in is how we play the fruit loud without turning it green.

Loud grip is only a pleasure if something catches it, and here the catch is sugar and acid rather than another tannin. The shrub pours at IFI-S 1,300, and sweetness is a powerful perceptual mask for astringency — not a chemical reaction with the tannin but a viscosity and a competing pleasure that softens the edge and slows the drying. The vinegar pulls the other way: acid sharpens grip, because near the salivary proteins' isoelectric point a lower pH shifts the protonation of both tannin and protein toward readier precipitation. The shrub is a deliberate tension between the two — sugar rounding the grip, acid honing it — held at a dilution where the pucker arrives fast, cuts the sweetness clean, and leaves the mouth faintly dry and reaching for the next sip. Astringency designed upward and then framed, rather than extracted hard and left to bite.



Pomegranate, played loud — the arils and the pale inner rind that carries the punicalagin. The ellagitannin grip arrives fast and bright where a condensed tannin arrives slow, the structural signature of a sparse population of very large, very avid binders.

The oolong flight — a reading, not a recipe

Finally, the simplest demonstration of all: three steeps of one designed oolong, oxidized partway on purpose, tasted in sequence. The first steep is bright and grippy, the native catechins forward; the second rounds as the theaflavins and theanine come into balance; the third softens and lengthens. One leaf, three settlements, a whole oxidation arc in a cup — and a reminder that the finest astringency instrument we have is still time and water, now aimed by design rather than by luck.

The oolong needs no profile block of its own, because the point of the flight is that the number *moves* while the leaf stays still. Partial oxidation is the design: left between the unoxidized green and the fully oxidized black, a share of the galloylated catechins has condensed into theaflavins — gentler, rounder, more slowly extracted grip. Each steep then draws down the leaf, and the same leaf simply slides down its own dose–response curve. Hold EC_{50} fixed and let the tannin concentration fall across the three steeps, from roughly two-and-a-half times the half-point, to about one times, to about one-half — illustrative multiples, but the arithmetic is honest. With the cooperative n = 1.8 of a condensed grip, the Hill form reads θ = 0.84, then θ = 0.50, then θ = 0.22: bright and gripping, then balanced, then soft and long. No leaf was changed between pours; only its concentration fell, and the curve did the rest. That is astringency's whole design argument in one teapot — the grip is a number, the number has a shape, and water and time move you along it.

A sidebar on extraction, for the pourer

Why does the same leaf grip so differently cold and hot, and why do these formulations specify their temperatures so exactly? Because extraction is chemistry, and the tannins do not all dissolve alike. Heat pulls tannin fast and indiscriminately — the galloylated catechins and the bitterest fractions come out first and hardest — which is why a hot, long steep turns harsh while a cold, long one stays round: the cold water favors the softer, more soluble compounds and the theanine, and leaves much of the sharpest grip in the leaf. The temperature is not a comfort setting; it is a selectivity dial, choosing *which* tannins enter the cup.

pH matters as much. The more acidic the water or the drink, the more a tannin tends to precipitate protein, because near the salivary proteins' isoelectric point a lower pH tips the protonation of both partners toward binding — so a squeeze of lemon can sharpen a tea's grip even as it brightens it, and a wine's acidity and its tannin are forever in tension, each setting how loudly the other reads. And in a wine, **ethanol** is quietly at work on the grip: at moderate strength it keeps tannins in solution and softens their precipitation, which is part of why a dealcoholized wine needs its tannin *designed* long and fine from the start rather than merely extracted hard. Temperature, pH and ethanol are three dials on the same reaction, and the EC_{50} we publish is computed in a fixed standard matrix precisely so that these do not smear the number — change the matrix and you change the grip, which is a feature of the drink and a hazard for the measurement. The pour is the last step of the design, and a pourer who knows the chemistry holds the final dial.

What the number cannot tell you

A number this clean invites over-trust, so we end on its honest limits. The EC_{50} is a property of the *reaction* — tannin precipitating a standardized protein — and perceived astringency is not identical to protein precipitation; it is a tactile integration of friction across a moving, living surface, and the assay is a faithful proxy, not the sensation itself. The proxy is most faithful in the middle of the curve and least at the extremes, where saturation and self-association bend the relationship.

The mouth varies, too. People differ in how much proline-rich protein they secrete and in how fast they regenerate the lubricating film, so a grip that reads one number on the instrument reads *softer* to a high-PRP mouth and *sharper* to a low-PRP one — the index is objective, the experience is personal, and both are true. Astringency also *accumulates*: it carries over from sip to sip faster than saliva can clear it, so the third taste of a flight is never read against a fresh mouth, which is why a flight and a single pour of the same leaf are different experiences of the same number. And other axes mask it — the shrub's sweetness and the cold-brew's umami both soften the perceived grip without touching the tannin, by filling the mouth with a competing pleasure the friction must cut through.

None of this weakens the index; it places it. The number aims the design — it lets us build a tannin long or fast, velvet or bright, and hold it to a tenth across every batch — and then it hands the glass to the person drinking, whose own mouth finishes the measurement. That is the right division of labor, and the one our family keeps at the table: the mathematics does the growing and the choosing, so that the grip can belong to whoever lifts the glass, drinking or not. We designed the number so the pleasure would not need one.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 16 · At the table — *cooking with the grip.*

Astringency on the plate is the least-discussed and most-used of all the axes, because every cook already works with it — in the char on a grilled leaf, the grip of a walnut, the dryness of a well-steeped braise — without ever naming it. It is the pucker that keeps a glass against a rich dish from going flabby, the clean edge a tannin lays under fat and sweetness so a mouthful has a spine. Named and designed, it becomes a tool as precise as salt — a dimension a cook can reach for on purpose, set to a number, and meet at the table with exactly the fat, protein, or acid that completes it.

These dishes put the grip to work. They are vegetarian-first, as our family's own table has always been — a lifetime of cooking the plant as the center of the plate — and where meat or fish appears it is written with the same care and no apology, because the grip meets animal protein most beautifully of all, and to pretend otherwise would leave out the oldest pairing there is. What unites every dish is not an ingredient but a mechanism: a known quantity of tannin delivered to the mouth, then met — on the plate or in the pot — by the fat, salt, sweetness, or protein that intercepts it and writes the last of its shape. Understand that one exchange and you can cook with astringency as deliberately as with heat.



A plum, whole and cut. Cooked down with a little salt and dark sugar, the astringent plum becomes the spine of a dessert that would otherwise be only sweet — the grip doing, on the plate, exactly what it does in the glass.

The grip in the mouth — what a tannin does, and undoes

Astringency is the one axis on the Index that is not, strictly, a taste. Heat has TRPV1; sweetness has TAS1R2·R3; the savory depth of umami has mGluR1 — each a receptor a molecule keys into, with the Index reading how loudly the key turns. Astringency has no such lock. What it has instead is a sacrifice. Human saliva pours out a constant supply of small, open-chain proteins stuffed with the amino acid proline — the **salivary proline-rich proteins**, the PRPs — and they are the thing a tannin acts on. A tannin is a polyphenol carrying many hydroxyl groups and many flat aromatic rings; proline presents

a greasy flat face its rings stack against, and keeps its backbone carbonyl exposed as a hydrogen-bond acceptor for the tannin's hydroxyls. The fit is almost unfair. One contact is weak, but a tannin carries several binding arms and a PRP several proline sites, so the two fasten at several points at once, and each tannin — having more than one hand — reaches more than one protein. This **multivalency** knits the saliva into a growing network of tannin bridging protein bridging tannin, until it can no longer stay dissolved and separates out as a haze and then a curd. That phase separation, in the thin film of saliva on the tongue, is the drying you feel: the lubricating proteins are leaving solution, and the trigeminal endings read the lost slip as a rough, puckering grip. The chemistry happens first; the sensation is its echo.

Two facts from that mechanism are the whole of cooking with the grip. First: the drying is a loss of lubrication, so anything that restores it — fat, a fresh flush of saliva — eases the grip. Second, and the one most cooks never name: the PRPs are **sacrificial**, spent in the act, carried out of the mouth as precipitate, and remade only slowly. This is why astringency *recovers* — the second sip of a tannic wine feels softer than the fifth — and why it also *ratchets*: push bite after bite without a reset and the mouth runs ahead of its own protein supply, so grip accumulates. Every move at the table is played against those two facts.

A cook meets the grip in two chemical families, and they behave differently enough to be worth telling apart. **Condensed tannins** — proanthocyanidins — are polymers of flavan-3-ol units, the catechins and epicatechins, strung together by carbon–carbon bonds; they are the grip of grape skin and seed, of a sorghum testa, of cocoa, of a plum skin, of a blueberry. **Hydrolyzable tannins** are built the other way, galloyl groups hung on a sugar core and oxidatively coupled; their signature is the ellagitannin, which on hydrolysis springs open to ellagic acid. Pomegranate's **punicalagin** is one of the largest, and walnut and raspberry grip by the same family. Condensed tannins tend to a rounder, slower grip; hydrolyzable ones to a brighter, faster, cleaner-edged one — a difference we will shortly put a number on. The table lists our astringency libraries by the chemistry each actually builds.

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	DOMINANT TANNIN	FAMILY
Grape	1,610	B / A / M	Hard	proanthocyanidin (epicatechin chains, via ANR)	condensed
Tea	280	U / B / A	Hard	galloylated catechins (EGCG)	condensed / galloyl
Pomegranate	120	A	Hard	punicalagin	hydrolyzable (ellagitannin)
Blueberry	120	A	Moderate	proanthocyanidin (+ anthocyanin)	condensed
Sorghum	120	A	Frontier	testa proanthocyanidin	condensed
Raspberry	40	A	Moderate	sanguiin / lambertianin	hydrolyzable (ellagitannin)

Reading the grip as a number — the Index on the plate

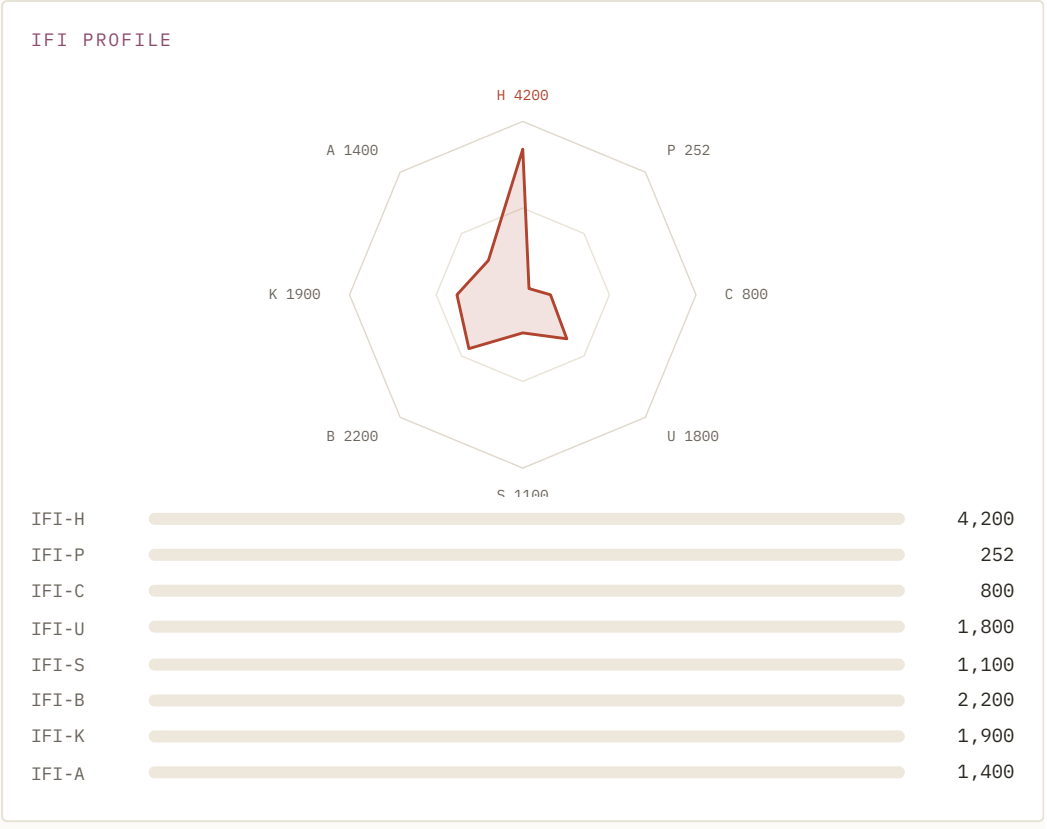
To cook to a grip you must be able to read one, and the Index reads astringency the same way it reads every axis:

> $\mathrm{IFI(candidate)} = 1{,}000 \times \mathrm{EC_{50}(reference)} \div \mathrm{EC_{50}(candidate)}$

On this axis the EC_{50} is the tannin concentration that drives standardized salivary-PRP precipitation to half its maximal plateau — the half-clouding point of a defined protein pool, read turbidimetrically at mouth temperature and physiological ionic strength. We anchor the scale by convention, fixing the reference tannin to half-precipitate that pool at **EC_{50}(reference) = 50 µM**, which by construction places it at IFI-A = 1,000. Because the formula is a bare ratio around that anchor, the whole scale collapses to one instant identity:

> $\mathrm{EC_{50}(candidate)} = 1{,}000 \times 50\ \mu\mathrm{M} \div \mathrm{IFI\text{-}A} = \mathbf{50{,}000 \div IFI\text{-}A}\ (\mu\mathrm{M})$

Score and concentration are reciprocals: a higher IFI-A means a lower EC_{50}, a tannin that clouds protein at less concentration — a louder grip. The clearest way to feel the scale is against the one cultivar we have tuned to register on all eight axes at once — **Phoenix** (SS-0096), whose astringency we designed at **IFI-A 1,400**, a half-precipitation of 50,000 ÷ 1,400 = **35.7 µM**, **1.4×** the reference. It is the loud end of designed grip, the measuring stick the plates are set against, its profile showing astringency among the other seven axes rather than alone:



Run the identity across the band this chapter cooks in, and the plate targets become concentrations you could weigh out:

PLATE / CULTIVAR	IFI-A	RELATIVE POTENCY	EC_{50} VS STANDARDIZED PRP (MM)	TANNIN FAMILY	ONSET (HILL H)
Designed sorghum floor	250	0.25×	200.0	condensed	≈ 2
Grape-leaf · sorghum plate	700	0.70×	71.4	condensed	≈ 2
Phoenix · SS-0096 (reference grip)	**1,400**	**1.40×**	**35.7**	—	–
Duck · pomegranate · walnut plate	1,500	1.50×	33.3	hydrolyzable	≈ 3
Dark chocolate · roasted plum tart	1,600	1.60×	31.3	condensed	≈ 2

A micromolar figure is exact only if you can say micromoles of *what*, and here the two families part ways. A defined hydrolyzable tannin converts cleanly. Punicalagin is a single molecule, $C_{48}H_{28}O_{30}$, **1,084.7 g·mol^{-1}**, so the duck plate's 33.3 µM is a mass concentration of

$$C = EC_{50}\ (\mu M) \times M\ (g \cdot mol^{-1}) \div 1{,}000 = 33.3 \times 1{,}084.7 \div 1{,}000 \approx \mathbf{36\ mg \cdot L^{-1}}$$

of punicalagin-equivalent reaching the mouth — faint, bright, reproducible to the milligram. A condensed tannin resists the same tidiness: it is not one molecule but a distribution spanning short oligomers to twenty-mers, so its molar mass is a *mean* that drifts with harvest. Taking each flavan-3-ol unit at about 290 g·mol^{-1} and each interflavan bond as shedding two hydrogens (288 per added unit), a proanthocyanidin of mean degree of polymerization five weighs about 290 + 4 × 288 ≈ **1,443 g·mol^{-1}**, and the grape-leaf plate's 71.4 µM is then ≈ **103 mg·L^{-1}** — but only if the reference material's mean DP is fixed as part of the assay, which is exactly how the platform pins it.

Grip is not a level but a curve, and its shape is where the condensed roundness and the hydrolyzable brightness live. Precipitation rises with concentration as a sigmoid, steeper than a simple isotherm because the cross-linking is cooperative — once a network nucleates, it recruits. A Hill form captures it, θ the fraction of maximal grip and **h** the steepness set by how many bridging arms a tannin brings:

$$\theta = [T]^h \div (EC_{50}{}^h + [T]^h)$$

A condensed proanthocyanidin presents its faces sparingly and behaves near **h ≈ 2**, a gentle climb; a large ellagitannin, high-valency on one scaffold, nucleates a network sharply and behaves nearer **h ≈ 3**. Hold each at its own EC_{50} and walk the delivered concentration up and down around it:

[T] ÷ EC_{50}	θ AT H ≈ 2 (CONDENSED, ROUND)	θ AT H ≈ 3 (HYDROLYZABLE, BRIGHT)
0.25	0.06	0.02
0.50	0.20	0.11
1.00	0.50	0.50
2.00	0.80	0.89
4.00	0.94	0.99

Both pass through the same midpoint — that is what sharing a target means — but the ellagitannin's steeper exponent makes its grip arrive later and then climb faster through the middle: almost absent, then suddenly *there*, then quickly spent. This is the mathematics behind choosing a pomegranate for a plate that must cut clean and a grape for one that must hold its grip across a wide, forgiving span — and, shortly, behind how differently the two families answer the fat and protein a cook sets against them.

One last number makes a plate target an instruction rather than a wish: its tolerance. IFI-A is a ratio of two fitted concentrations, so its relative uncertainty is the two relative uncertainties added in quadrature,

```
σ(IFI-A) ÷ IFI-A = √[ (σ_ref ÷ EC₅₀_ref)² + (σ_cand ÷ EC₅₀_cand)² ]
```

and with the reference fit held to 3 % and a candidate fit to 5 %, that is $\sqrt{(0.03^2 + 0.05^2)}$ = **5.8 %**, inside the platform's **≤ 7 %** batch design envelope. So "plate target IFI-A ~700" is really **700 ± 41**, a band of roughly 659 to 741 — a grip you can specify and hit again next service. A conventionally grown tannin swings by tens of percent with season and ripeness; it cannot be cooked *to a number* at all. A grip that is set and held is the whole gift the designed organism brings to the kitchen, and the only thing that lets the softening arithmetic mean anything.

Interception — the arithmetic of softening

Here is the exchange every dish turns on. When a tannin arrives in a mouthful that already holds food protein, fat, or certain polysaccharides, the saliva's PRPs are no longer its only partner — the food competes for it, and whatever the food binds or sleeves never reaches the saliva to be felt. Grip delivered and grip perceived come apart, and the gap between them is the cook's instrument.

Protein is the strongest foil, and the reason is a quiet joke of biochemistry: the proteins that intercept tannin best are the ones that most resemble the saliva's own sacrificial PRPs. **Casein**, the dominant milk protein, is open, unstructured, and richly proline-rich — β-casein runs near seventeen percent proline — so in a spoon of yogurt a tannin meets a protein built on the same flat, carbonyl-lined runway the PRPs offer, and binds it by the identical chemistry: milk and yogurt are the classic tannin foils because casein is a PRP mimic. **Collagen and its gelatin** carry a comparable load of proline and hydroxyproline, which is why a long-braised meat and a reduced jus intercept tannin so thoroughly; for a vegetarian kitchen the legume proteins and dairy do the same. Fat intercepts differently — it coats the oral surfaces and the tannin both, restoring the lubrication the grip removes — while sugar, pectins, and the wine-world's mannoproteins act as colloidal shields that keep tannin and protein from finding each other. All of it reduces the tannin that reaches the saliva.

Write the design heuristic plainly. Let a bite deliver a bare grip of **IFI-A(deliver)** — the number the plate would read with nothing to intercept it — and let the companion phase sequester a fraction **f** of

the tannin before it reaches the PRPs. Because perceived grip tracks the free, unintercepted tannin, the felt loudness scales down:

```
IFI-A(perceived) ≈ IFI-A(deliver) × (1 − f)
```

That is the number a cook designs to — though it understates the softening, because interception does its work near the steep middle of the Hill curve, where a little change in free tannin moves the felt grip a lot. Take a bite delivered right at its EC_{50}, where the bare grip would read θ = 0.50, and intercept half of it (f = 0.5). The free tannin falls to 0.5 × EC_{50}, and the curve reads

$$\theta = (1 - f)^h \div (1 + (1 - f)^h)$$

which for a condensed tannin (h ≈ 2) gives $0.5^2 \div (1 + 0.5^2)$ = **0.20**, and for a bright ellagitannin (h ≈ 3) gives $0.5^3 \div (1 + 0.5^3)$ = **0.11**. Half the tannin removed, but far more than half the *felt* grip gone — and the brighter hydrolyzable tannin softens most decisively of all, which is why duck and pomegranate feels so clean. The linear estimate is the design target; the curve is why interception feels like a switch rather than a dial.

COMPANION IN THE BITE	HOW IT INTERCEPTS	MODEL f	DELIVERED IFI-A	PERCEIVED ≈ ×(1 − F)
Cold strained yogurt (casein + milkfat)	casein is a PRP-analog, binds tannin; fat re-lubricates	0.45	700	~385
Seared duck (rendered fat + muscle protein + gelatin)	fat coats; protein and collagen compete for tannin	0.50	1,500	~750
Dark-chocolate ganache (cocoa butter + sugar)	fat lubricates, sugar thickens; neither binds avidly	0.25	1,600	~1,200
Toasted walnut (its own ellagitannin)	adds grip — a *negative* interception	−0.15	raises the plate	grip rises
Flaky salt / a squeeze of acid	lifts saliva flow — resets, does not intercept	—	—	faster recovery

The *f* values here are illustrative model coefficients, not catalogue measurements; their point is the arithmetic, which is robust to their exact size. Two companions sit in a different column entirely. The walnut does not soften — it is itself an ellagitannin source, so it *adds* grip, a negative interception. And salt and acid do not intercept at all; they act on the recovery side, provoking a flush of saliva that replenishes the spent PRPs and the lost lubrication, which is why a pinch of flaky salt or a scrape of citrus resets the palate between bites and keeps the ratchet from climbing. With these five levers — bind it, coat it, shield it, add to it, or speed the mouth's recovery — a cook can take any delivered grip and land it where they like.

A vegetarian plate — grape leaf, sorghum, and cold yogurt

A vegetarian plate built on two designed astringency organisms at once — the grape, here as its leaf, and sorghum as a whole grain carrying a chosen, modest testa grip — set against the fat and protein of a cold yogurt that intercepts the tannin exactly as the arithmetic predicts. Both grip by the condensed family — the vine's proanthocyanidins in the blanched leaf, the sorghum testa's in the grain — each a rounder, h ≈ 2 tannin that eases in and out rather than snapping.

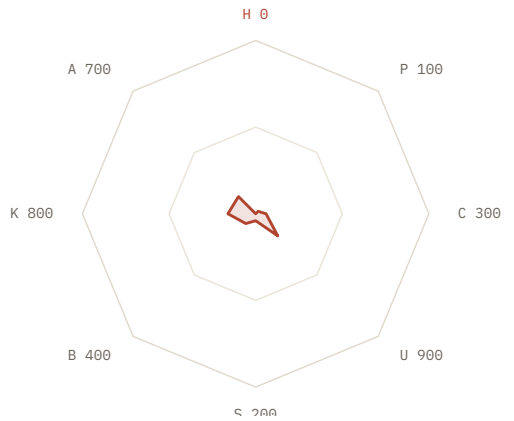


Grapes on the vine. Fruit, skin, and leaf all carry the vine's proanthocyanidins — condensed tannins built by anthocyanidin reductase, the enzyme we tune to set the grape's grip. Here it is the young leaf, blanched tender, that brings that grip to the grain.

Grain	designed modest-tannin sorghum, IFI-A floor ~250, cooked whole
Wrap	tender young grape leaves, blanched
Aromatics	dill, mint, lemon zest, olive oil, toasted pine nuts
Against	thick strained yogurt, cold, salted
Astringency	plate target IFI-A ~700 — a clean structural edge under brightness

1. Simmer the whole sorghum until tender but intact; its modest designed grip — a floor near IFI-A 250, an EC_{50} of 50,000 ÷ 250 = 200 µM, only a quarter as potent a protein-precipitant as the reference — gives the grain a clean chew rather than the all-or-nothing of wild tannin sorghum.
2. Fold through dill, mint, lemon zest, olive oil, toasted pine nuts; season.
3. Blanch the grape leaves, wrap small parcels, warm through in oil and a little water. The leaf's condensed grip lifts the bare plate from the grain's floor toward its design target of IFI-A ~700 — an EC_{50} of 71.4 µM, 0.70× the reference, a structural edge rather than a wall.
4. Serve over cold salted yogurt. The yogurt's casein binds a share of the grip before the saliva meets it, and the milkfat re-lubricates the mouth — take the interception at f ≈ 0.45 and the bare 700 lands near a perceived **~385**: a plate bright, herbal, and only faintly, structurally dry, the grip present as the reason the brightness feels whole.

IFI PROFILE



IFI-H	0
IFI-P	100
IFI-C	300
IFI-U	900
IFI-S	200
IFI-B	400
IFI-K	800
IFI-A	700

A dessert built on tannin — dark chocolate and roasted plum

A dessert where astringency is the whole architecture — the procyanidin grip of a fine dark chocolate, read on the Index though it was never engineered, set against the designed grip of a roasted astringent plum. Both are condensed tannins — cocoa's procyanidin and the plum skin's proanthocyanidin — and the two, holding hands under the sugar and fat, keep a sweet thing from collapsing into mere sweetness. That the chocolate's grip is read rather than grown is the quiet point: the Index scores any tannin against the same standardized protein pool, so a cook can read a wild ingredient as exactly as a designed one and build to the total.

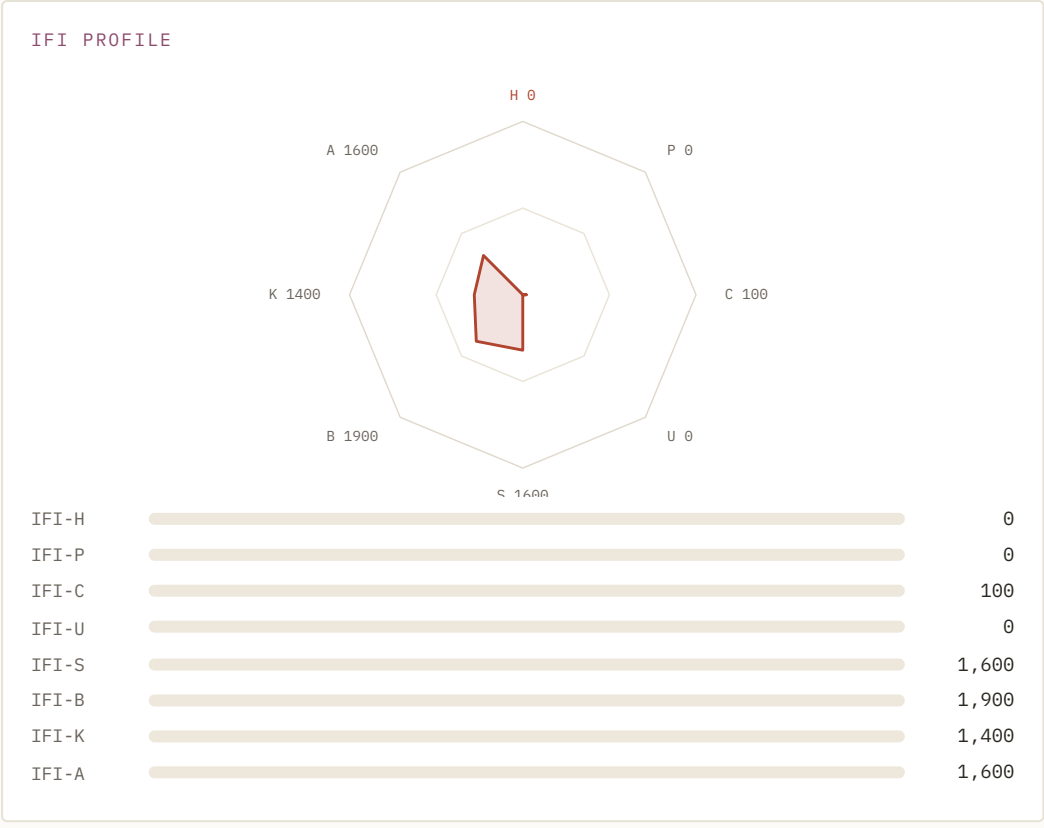


Cocoa pods and beans. The procyanidin grip of fine dark chocolate is a condensed tannin we did not build but can read precisely on the Index — scored against the same protein pool as any designed organism, so a wild grip and a tuned one meet on one plate.

Shell	dark, short pastry, lightly salted
Filling	70% dark chocolate ganache (read IFI-A, firm grip)
Fruit + salt	designed astringent plums, halved, roasted with dark sugar
Finish	flaky salt; a scrape of orange zest
Astringency	plate target IFI-A ~1,600 — structural, married to bitterness

1. Blind-bake the salted shell dark.

2. Set a firm ganache from a chocolate chosen for a real structural grip — read its IFI-A before you build, and you know whether it will stand up to the fruit or disappear beneath it.
3. Roast the halved plums with dark sugar and a pinch of salt until the skins' grip concentrates and the flesh collapses to jam; the salt speeds the mouth's recovery between bites.
4. Arrange over the ganache, finish with flaky salt and orange zest. The two condensed tannins give the tart a spine at a plate target of IFI-A ~~1,600 — an EC_{50} of 50,000 ÷ 1,600 = 31.3 µM, 1.6× the reference, the loudest grip of the three plates. Here the design intent is the opposite of the yogurt dish: the fat and sugar must keep the grip *civil without erasing it.* Cocoa butter and sugar shield and lubricate but bind no tannin, a low interception near f ≈ 0.25, so the perceived grip lands near **~~1,200** — softened, married to the chocolate's own bitterness, but still a true structural backbone rather than a grip removed.



The oldest pairing — seared duck, pomegranate, and walnut

The one meat dish, written with full respect: duck and pomegranate is one of the great astringency pairings in any cuisine, its mechanism the purest illustration in the book. Our table is a vegetarian one, and the dish is offered as much for its lesson as its plate — but it is the oldest marriage of grip and fat there is, and it would be a dishonesty to leave it out. A high-grip designed pomegranate, reduced to a glaze, meets the fat and protein of the duck, which intercept the tannin in real time. And the grip is the bright kind: punicalagin is a

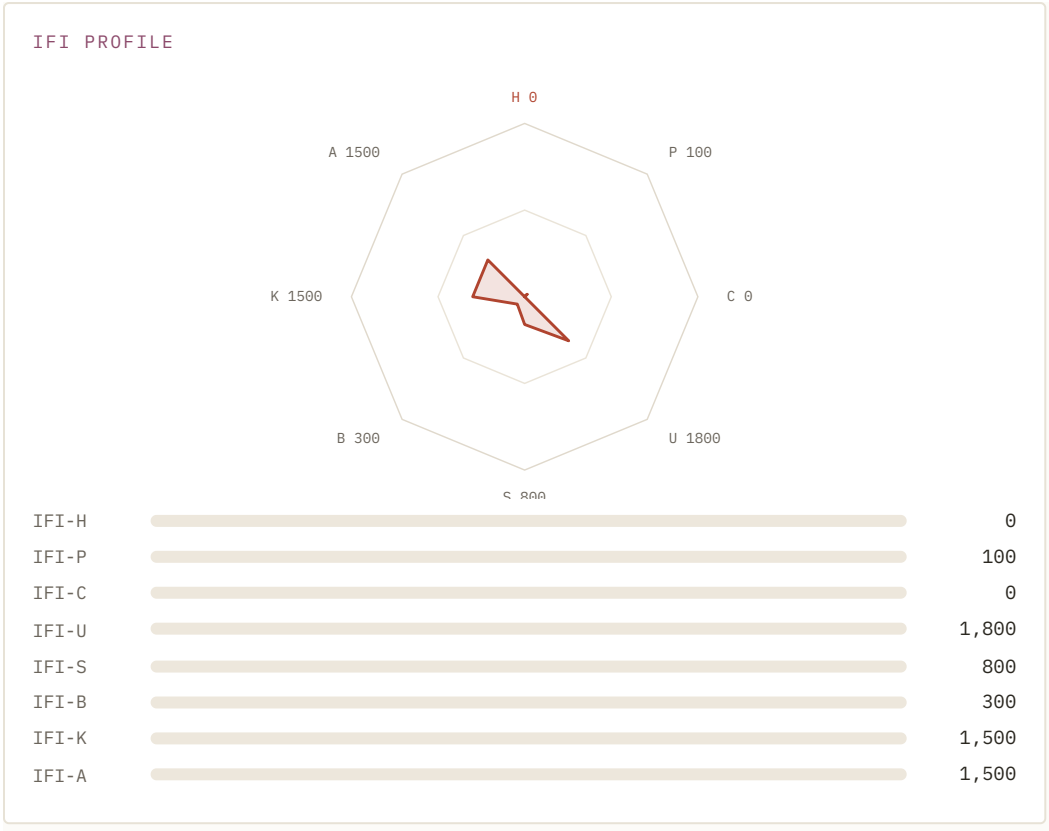
hydrolyzable ellagitannin, a large, high-valency molecule that nucleates sharply and behaves near h ≈ 3, so it arrives late and climbs fast — exactly the tannin interception collapses most decisively.



A pomegranate split to the arils. Its grip is punicalagin — a hydrolyzable ellagitannin, one of the largest tannins any fruit builds, bright and fast where a condensed tannin is round and slow. Reduced to a glaze and met by fat and protein, it softens through the steep middle of its own curve.

Protein	duck breast, scored, rendered slow on the skin
Glaze	designed high-punicalagin pomegranate, reduced with a little honey
Tannin partner	toasted walnuts (their own grip), roughly crushed
Against	the duck's rendered fat + protein (intercepts the tannin)
Astringency	plate target IFI-A ~1,500 — bright, met and softened by the fat

1. Score and render the duck slowly on its skin until deeply crisp; rest.
2. Reduce the designed pomegranate juice with a little honey to a glaze with real, bright grip — a loud, high-punicalagin pomegranate carried to a plate target of IFI-A ~1,500, an EC_{50} of 33.3 µM, or about 36 $mg \cdot L^{-1}$ of punicalagin-equivalent reaching the mouth.
3. Fold crushed toasted walnuts, which bring their own ellagitannin grip, into the glaze — a *negative* interception that deepens the plate rather than easing it, the one place grip is added, not subtracted.
4. Slice the duck, spoon over the glaze. The rendered fat coats the mouth, and the muscle protein and collagen-rich gelatin of the slow-rendered duck compete for the tannin — take the interception at f ≈ 0.50 and the loud 1,500 lands near a perceived **~750**. Because the tannin is a sharp h ≈ 3 ellagitannin, the felt softening runs steeper still: intercepting half the free tannin at its own midpoint drops the felt grip from θ = 0.50 toward 0.11 — the mathematics of a bright pucker met and quieted in a single bite, each mouthful rich, bright, and cleanly structured. A pescatarian or vegetarian table takes the same glaze over a meaty roasted king oyster mushroom or a thick-seared portobello to the same effect; the grip does not care which protein greets it, only that one does.



The stove as a softening tool — heat, protein, and time

The interception above happens in the mouth, but a cook who knows the mechanism can make most of it happen earlier, in the pan, and arrive at the table with the exchange already spent. Prolonged heat with protein drives the tannin to bind that protein directly in the pot: a tannic wine reduced into a ragù, or a grape-and-walnut sauce simmered with duck, gives much of its grip to the meat before it is ever plated — the same arithmetic, run in advance. Dairy and legume proteins do this for a vegetarian kitchen exactly as in the mouth: a tannic plant simmered with yogurt, cream, lentils, or beans hands its grip to their protein in the pot, which is why a braise can carry a loud tannin and still read gentle, and why so many long-cooked vegetable stews are built on a tannic green and a soft pulse.

Heat does a second thing, subtler and double-edged. Long cooking in air oxidizes and polymerizes the flavan-3-ol units of a condensed tannin, lengthening the mean degree of polymerization — and because grip climbs with chain length up to a point and then falls as the polymer grows too large to stay dissolved, a sauce reduced a little can *gain* structure while one reduced too far loses it, the overgrown tannin precipitating out of the pot and leaving the dish flat. Acid and salt tune it further, as in the glass: acid sharpens the grip and quickens salivation, salt speeds recovery. The grip is not fixed at harvest. We set its amplitude in the plant, to a number and a tolerance; the cook writes the last of its shape with heat, fat, protein, and time — a flavor fully determined before it reached the kitchen would not be cooking at all.

Edge cases, failure modes, and honest limits

Cooking to a number is still cooking, and the ways a grip-built plate goes wrong are worth naming, because each is an edge of the same mechanism.

- **Over-interception — the grip vanishes.** The softening levers are strong, and a heavy hand with cream, butter, or a long dairy braise can push *f* so high the perceived grip falls below notice and the

dish goes slack. The cure is arithmetic: work back through IFI-A(perceived) ≈ IFI-A(deliver) × (1 – f) to the grip you must deliver, and do not out-rich it.

- **Tannin meets iron.** Polyphenols chelate iron to a blue-black complex — the chemistry of old iron-gall ink — so a loud-grip plate cooked on reactive cast iron or carbon steel can turn inky and taste metallic. We keep a high-tannin reduction in stainless or enamel; the grip is designed, the discoloration is not.
- **Bitter is not astringent.** The two travel together — cocoa, over-steeped tea, a tannic skin all carry both — but they are different axes: bitterness is a true taste keying into the TAS2R38 receptor, astringency the tactile precipitation of protein. A plate can read harsh from either, and the fix differs: bitterness is met by sweetness and salt masking the receptor, astringency by fat and protein intercepting the tannin. Reading a food on both the B and A axes before you cook tells you which harshness you are taming.
- **The polymer-size window.** Reaching for more grip by overbuilding a condensed tannin can backfire: push the chain length too far and the proanthocyanidin folds on itself and aggregates before it reaches a protein, so the protein-available tannin drops and the plate reads *lower* than its total phenolic content promised — past a modest chain length, more tannin measures as less.
- **The mouth is each person's own, and the grip ratchets.** The salivary proteins the grip precipitates vary from person to person, and a mouth that meets tannin often makes more of them — the habitual tea-drinker is, at the level of the saliva, slightly armored against the grip the rest of us feel sharply. And because the PRPs are spent faster than they are remade, astringency climbs across a course in a way a single plate target never captures. The Index pins the *stimulus* — the tannin's precipitating potency against a fixed standard — and leaves the *sensation*, rightly, to the person at the table. A number set to ≤ 7 % in the plant does not promise a home kitchen will hit it; it promises that if the kitchen is steady, the grip will be, lot after lot.

What it was all for

Four plates, four grips, each designed to a number and each met at the table by the fat, salt, sweetness, or protein that completes it. This is what the proteins and pathways and arithmetic were for — a cook able to reach for astringency as deliberately as for heat or salt, a grip that arrives exactly as deep as the plate intended, neither a wild surprise nor an accident of harvest. And in the same molecules the nourishment note sits, as everywhere in this library: the proanthocyanidins and ellagitannins that give these plates their clean grip are the polyphenols a lifetime of careful eating reaches for in the fruit and the leaf and the whole grain. We do not dress that as a claim; we only decline to breed it out, and then set it, on the plate, to the exact structural edge the dish was built to carry. We write the amplitude in the plant; the cook writes the last of its shape with heat and fat and protein and time; and the body, at the end of it, tastes the grip as the reason the whole plate feels whole. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 17 · The grip inside the spectrum — *Phoenix and astringency as one of eight.*

There is a kind of proof our family trusts above all others. Not a plant that speaks a single sensation beautifully — any patient grower can coax one voice out of one fruit — but a plant that holds all eight sensations at once and lets each keep its exact place. A grip we can read in isolation is a grip we understand in isolation. A grip that will take its intended seat inside a full chord — loud enough to give a fruit a spine, quiet enough never to shout over the fire sitting beside it — is a grip we understand completely. That plant exists. We grew it gene by gene because the plant that could do it did not yet exist. It is **Phoenix**, SKU **SS-0096**, the tenth and final flagship of our proof-of-concept superhots, and it is the only cultivar we have ever built to register on every axis of the Index simultaneously. Its astringency is designed to **IFI-A 1,400** — a deliberately placed grip, reproduced batch to batch to within seven percent, sitting at last in its native company beside seven other sensations. Of every number in this book, it is the one we are proudest of, because it is astringency expressed not as a curiosity of certain plants but as a dimension of taste we can write wherever we choose.



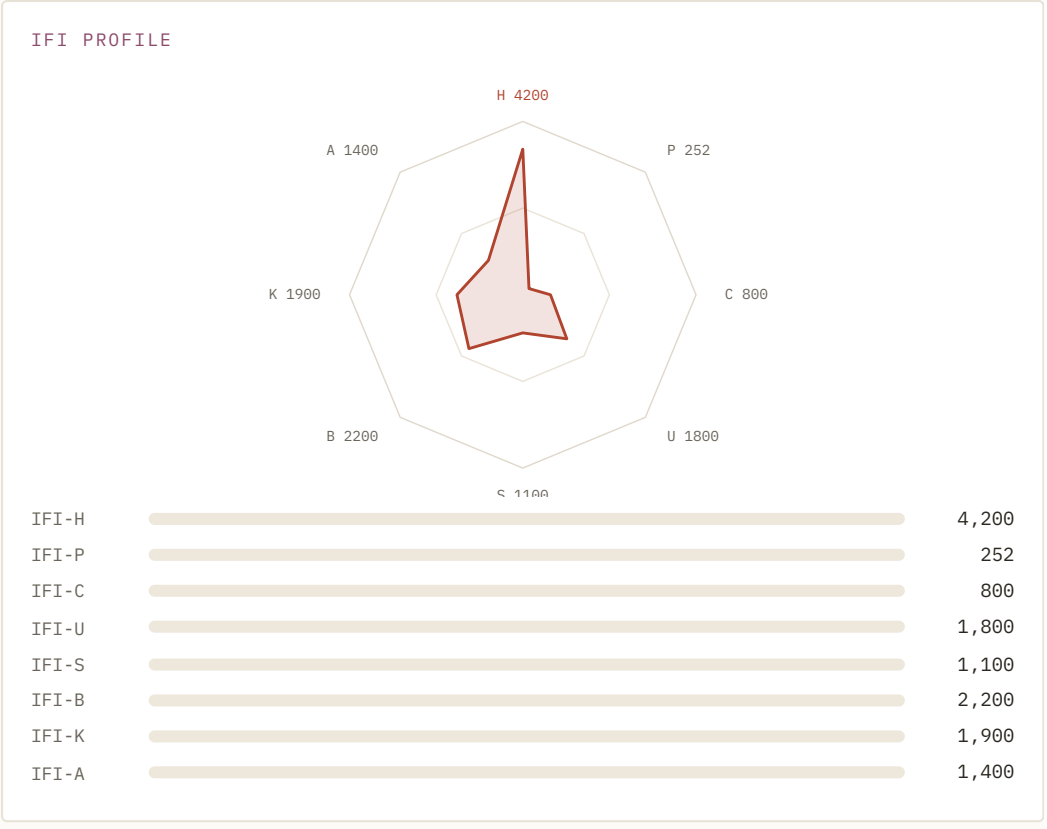
Phoenix (SS-0096) in the controlled chamber — the first cultivar engineered to register on all eight axes at once. Its astringency, IFI-A 1,400, is a designed grip holding its place inside a full-spectrum chord.

Phoenix is the last rung of a ladder, and the ladder is worth seeing whole, because it is the record of learning to add one axis at a time until all eight were present. The ten flagships climb from a two-axis pepper to a full-spectrum one, the Scoville figure rising in step with the axis count — not because heat and complexity must travel together, but because we chose to push both frontiers on the same line of plants.

CULTIVAR	SKU	SHU*	IFI-H	AXES
Ember	SS-0087	866K	1,570	2/8
Cinder	SS-0088	1.05M	2,290	3/8
Kindle	SS-0089	1.25M	2,000	3/8
Flare	SS-0090	1.48M	—	4/8
Blaze	SS-0091	1.72M	2,630	4/8
Wildfire	SS-0092	1.96M	2,930	5/8
Scorch	SS-0093	2.21M	3,220	5/8
Firestorm	SS-0094	2.47M	3,490	6/8
Solara	SS-0095	2.72M	3,980	6/8
Phoenix	**SS-0096**	**2.95M**	**4,200**	**8/8**

*SHU values are computational predictions from our flux model.

Only Phoenix carries all eight, and so only Phoenix carries a designed astringency. Here is its full profile — the one scorecard in our entire catalog where IFI-A sits in a full chord rather than alone on a page.



Reading the scorecard as a chord — the arithmetic of balance

A scorecard is not a list; it is a chord, and a chord has a shape you can calculate. Lay the eight values out and attach to each its receptor and its share of the per-axis ceiling, which the Index fixes at **5,000**, and the architecture of the fruit becomes legible as numbers rather than adjectives.

AXIS	CODE	RECEPTOR	IFI	% OF 5,000	Z-SCORE
Heat	IFI-H	TRPV1	4,200	84%	+2.24
Zero-Bitter	IFI-B	TAS2R38	2,200	44%	+0.44
Kokumi	IFI-K	CaSR	1,900	38%	+0.17
Umami	IFI-U	mGluR1 / TAS1R1·R3	1,800	36%	+0.08
Astringency	IFI-A	Salivary PRPs	1,400	28%	−0.28
Sweetness	IFI-S	TAS1R2 / R3	1,100	22%	−0.54
Cool-Burn	IFI-C	TRPM8 / TRPV1	800	16%	−0.81
Pungency	IFI-P	TRPA1	252	5%	−1.31

The eight values sum to 13,652, so the mean voice of this fruit sits at **1,707**. Their spread, taken as a population standard deviation, is √(1,238,296) ≈ **1,113**. Those two numbers do the whole explaining. The **heat** at 4,200 stands +2.24 standard deviations above the mean — a genuine statistical outlier, the fortissimo you cannot miss, the single spike in an otherwise gathered chord. Every other axis falls inside one standard deviation of the center, which is exactly what a *designed* flavor should look like: one lead voice and a tight, deliberate consort around it, not eight soloists fighting.

And astringency? It sits at 1,400 — **−0.28 standard deviations**, a touch below the mean, at 28% of the per-axis ceiling. That is precisely where a structural voice belongs. It is not the loudest axis and was never meant to be; it is the one that keeps the loud ones honest. A fruit whose astringency rode above the mean would read as hard and tooth-stripping, grip with nothing to grip; a fruit with none at all would read as flat and cloying no matter how high its heat and sweetness climbed. We placed the grip just under center on purpose, the way a winemaker seats tannin beneath fruit — present in every swallow, dominant in none. The arithmetic says what the palate feels: astringency is the fifth-loudest of eight, by design.

What astringency actually is — the odd axis out

To understand why that 1,400 was the hardest of the eight numbers to earn, you have to understand that astringency is not like its neighbors. Seven of the eight axes are, at bottom, a molecule settling into a receptor: capsaicin into TRPV1, glutamate into mGluR1, a sweet ligand into the TAS1R2·R3 heterodimer, a bitter compound into TAS2R38, a kokumi peptide into the calcium-sensing receptor. Lock, key, channel opens or G-protein turns, a nerve fires, the brain reports a taste. Astringency is the one axis where nothing of the kind happens. There is no astringency receptor in the tongue. The **receptor is in the saliva.**

Here is the mechanism, step by step, because it is beautiful and because the number depends on it exactly. Tannins — condensed proanthocyanidins, the polyphenols that give a tannic red its spine and an unripe persimmon its shock — are multivalent molecules studded with galloyl and catechol hydroxyl groups. Saliva is rich in **proline-rich proteins (PRPs)**, long, floppy, proline-studded peptides whose entire evolutionary job appears to be sacrificing themselves to tannins so the rest of the mouth is spared. When tannin meets PRP, two things bind at once: the flat aromatic faces of the tannin stack against the exposed pyrrolidine rings of proline through hydrophobic and CH–π contacts, while the tannin's hydroxyls

hydrogen-bond to the peptide backbone. Because the tannin carries *many* such faces and the PRP offers *many* such prolines, the binding is not one-to-one but cross-linking — a single tannin bridges several proteins, those proteins bridge further tannins, and the whole tangle grows until it falls out of solution as a visible precipitate.

That precipitation is the event. Saliva's job in the mouth is lubrication — a thin film of mucins and PRPs that lets the tongue, palate, and teeth glide against one another nearly without friction. When tannin precipitates the PRPs, it strips that film. The surfaces, suddenly unlubricated, drag. And *friction* is something the mouth can feel: the trigeminal mechanoreceptors — the same free nerve endings that register texture and touch — report the rise in friction as the dry, rough, puckering pull we call astringency. It is not a taste. It is a touch. You are not tasting the tannin; you are feeling your own saliva fail.



Grape in cross-section — the lineage of the tannin dimension. The same proline-rich-protein precipitation that grips in a tannic red is the chemistry we wrote, enzyme for enzyme, into a pepper.

This is why astringency is the odd axis out, and why we revere it a little more than the rest. It is the most physical of the eight — closer to texture than to flavor — and the only one whose transduction we designed *around* a protein we do not control, because PRPs are made by the person eating, not by the plant. The plant's only move is to present the tannin. Everything after that is chemistry and friction, which is also why it is the axis that behaves most differently from one mouth to the next, a limit we will come back to honestly.

From the backbone to the tannin — the enzymatic branch

The reason we could write a real grip into a chili at all is that the chemistry of astringency shares a backbone with nearly everything else a plant builds for flavor. The phenylpropanoid pathway — **PAL →**

C4H → 4CL → HCT → COMT — runs in every vascular plant on Earth; it is the common trunk from which lignin, the aroma compounds, the pigments, and the tannins all branch. A pepper runs it already. To make capsaicin, a pepper takes that trunk toward vanillylamine and condenses it with a branched-chain fatty acid at the Pun1/CS acyltransferase. To make a tannin, a very different terminal branch is needed — but it hangs off the same trunk.

That tannin branch runs through the flavonoid arm: chalcone synthase opens the flavonoid route, then the dihydroflavonols are reduced by **DFR** to leucoanthocyanidins, and two reductases — **LAR** (leucoanthocyanidin reductase) and **ANR** (anthocyanidin reductase) — deliver the flavan-3-ol monomers, catechin and epicatechin, which then condense into proanthocyanidin polymers. A pepper, in the wild, barely runs this arm; it spends its carbon on heat and pigment, not on building a tannin program. Writing IFI-A 1,400 into Phoenix meant installing and tuning that flavan-3-ol branch so the fruit would actually make, and polymerize, condensed tannin on purpose.

And the key variable there is not merely *how much* tannin but **how long**. Astringent potency rises with the degree of polymerization. A flavan-3-ol monomer barely grips; a dimer grips a little; it is the longer oligomers — four, five, six units and up — that carry enough binding valency to cross-link PRPs efficiently and precipitate them. More galloyl and catechol faces per molecule means more simultaneous contacts with proline, means cooperative precipitation at lower concentration, means a lower half-precipitation point and a higher IFI-A. So the engineering target for astringency is a *distribution* — not just a tannin titer but a controlled window of polymer lengths — and holding that distribution steady, harvest to harvest, is the whole game. It is the single hardest distribution to hold in the plant kingdom, which is the real reason astringency was among the very last of the eight axes to come online.

The number — PC_{50}, the index inversion, and the worked math

Every axis on the Index is anchored the same way, by the formula that makes the whole library one language:

> $\mathrm{IFI(candidate)} = 1{,}000 \times EC_{50}\mathrm{(reference)} \div EC_{50}\mathrm{(candidate)}$

EC_{50} is the concentration that drives a receptor to half of full response. Invert it against a fixed reference, scale by a thousand, and *loudness* — not quantity, loudness — becomes a single score. For astringency there is no channel to half-activate, so we substitute the exact analog the mechanism hands us: **PC_{50}**, the concentration at which a standard tannin precipitates half of a standard PRP preparation. The identical formula holds, with PC_{50} in place of EC_{50}.

> $\mathrm{IFI\text{-}A} = 1{,}000 \times PC_{50}\mathrm{(reference)} \div PC_{50}\mathrm{(candidate)}$

By construction, the reference astringent is pinned to a reading of exactly 1,000: its own PC_{50} divided by itself, times a thousand. Everything else is computed against it. And because IFI-A varies

inversely with the candidate's PC_{50}, a higher score means a *lower* precipitation concentration, which means a *more potent* grip. Work Phoenix's number backward and it tells you precisely how potent:

> $1{,}400 = 1{,}000 \times PC_{50}(\text{ref}) \div PC_{50}(\text{Phoenix})$ $PC_{50}(\text{Phoenix}) = PC_{50}(\text{ref}) \times 1{,}000 \div 1{,}400 =$ **$0.714 \times PC_{50}(\text{ref})$**

Phoenix's tannin design predicts PRP precipitation at 71.4% of the reference concentration — it is **1.4× as potent** a grip as the anchor. That is the entire meaning of the 1,400, and it requires no invented quantity to state, only the ratio the Index is built on. The full scale reads as potency the same way:

IFI-A READING	WHAT IT MARKS	PC_{50} (RELATIVE TO REFERENCE)	POTENCY VS REFERENCE
5,000	per-axis ceiling	0.20 ×	5.0×
2,000	—	0.50 ×	2.0×
1,400	Phoenix	0.714 ×	1.4×
1,000	the anchor	1.00 ×	1.0×
500	—	2.00 ×	0.5×

The unit conversion is where the abstraction touches the bench. Suppose we calibrate the reference, for illustration, at $PC_{50}(\text{ref})$ = 50 µM of a standard procyanidin of molecular weight ≈ 900 g/mol — a representative trimer. Then the reference grips at 50×10^{-6} mol/L × 900 g/mol = 0.045 g/L, or **45 mg/L**. Phoenix's tannin, at 0.714 of that, grips at 35.7 µM = **32.1 mg/L**. The reading of 1,400 is just those two bench concentrations, inverted and scaled — the arithmetic that lets a pepper and a grape be compared on one line.

Precipitation does not switch on at PC_{50}; it climbs through it along a dose–response curve, and because tannin–PRP binding is cooperative — multivalent cross-linking, each event making the next more likely — that curve is a Hill function with a coefficient well above one. Writing θ for the fraction of PRP precipitated and taking a cooperativity of n ≈ 2.5:

> $\theta([T]) = [T]^n \div (PC_{50}{}^n + [T]^n)$

At the half point, $[T] = PC_{50}$, θ = 0.50 by definition. Step down to half the PC_{50} and $\theta = 0.5^{2.5} \div (1 + 0.5^{2.5}) = 0.177 \div 1.177 =$ **0.15**. Step up to twice the PC_{50} and $\theta = 2^{2.5} \div (1 + 2^{2.5}) = 5.66 \div 6.66 =$ **0.85**. So the grip travels from barely-there to very nearly saturating across just a fourfold span of concentration — 0.5× to 2× the half point carries you 0.15 → 0.50 → 0.85. Astringency, once it begins, arrives fast. That steepness is the fingerprint of cooperativity, and it is why a tannin level a hair too high reads as harsh rather than merely firmer: on a slope that sharp, there is little room between structure and assault. Seating Phoenix's grip at 28% of the ceiling, well below the mean, is also a way of staying on the gentle lower shoulder of that curve, where the fruit dries cleanly instead of stripping.



Phoenix on the microscope stage — the PC_{50} precipitation assay that reads a pepper's grip on the same instrument, and the same protein, as a grape's.

A number is only worth what its error bars allow, and here the discipline shows. Because IFI-A is proportional to $1/PC_{50}$(candidate) with the reference held fixed, its relative uncertainty equals the relative uncertainty of the precipitation measurement itself: propagate a ratio and $(\sigma_IFI/IFI)^2 = (\sigma_ref/PC_{50}ref)^2 + (\sigma_cand/PC_{50}cand)^2$. The reference is a pooled calibrant measured across many replicates, so its term is small; the candidate's own batch variability dominates. At our house figure of **≤7% CV**, Phoenix's designed IFI-A of 1,400 carries a one-sigma band of 1,400 × 0.07 ≈ **±98**, so a reading of 1,400 ± 98 and a two-sigma interval of roughly **1,200 to 1,600**. That tolerance is the engineered achievement, not an afterthought: a wild, unselected pathway drifts enormously batch to batch — the same heat program, left to a wild-type plant, runs a CV of 42% — and for astringency, whose potency rides on a polymer-length distribution that oxidizes and shifts, the untamed variance is worse still. Holding the grip to seven percent is the difference between a flavor you can formulate against and a rumor.

How eight programs become one fruit

Phoenix is not one engineering project but eight, braided. Each axis is its own pathway with its own terminal enzymes, its own receptor, its own measurement; the plant is the place where all eight had to be made simultaneously true without any one starving the others of carbon. That is why the flagships added axes one rung at a time — not because we could not imagine eight at once, but because each new voice had to be proven not to collapse the voices already present.

The heat, the fortissimo, came from the method at its most direct. A protein language model scored every single-residue substitution in the Pun1/CS acyltransferase for its predicted effect on catalytic efficiency and fold stability; our flux model predicts that rate control sits upstream in the phenylpropanoid pathway — at 4CL and HCT — not at the terminal Pun1/AT3 step, which labels the capsaicin product without governing the rate. Optimizing the terminal enzyme's binding affinity still contributes to peak capsaicinoid output given that upstream supply, and two substitutions, never before combined in any breeding line, emerged from that analysis and were written in with CRISPR.

EDIT	EFFECT	ΔΔG (KCAL/MOL)
S39L	binding affinity ↑ (N-terminus)	−2.1
L345G	steric relief, active-site cleft	−1.8

Those two substitutions, in our model, predict a peak output of approximately **2.95 million SHU** at a batch-to-batch CV of ≤7%. (A quiet piece of arithmetic lives in that prediction: ~2.95 million ÷ 4,200 ≈ 702 SHU per IFI-H point at the top of the scale — yet the same ratio runs near 552 at Ember's 1,570. The Scoville-per-point figure *climbs* as you ascend, because SHU is linear in capsaicin mass while the Index compresses through the receptor's EC_{50}, so each added point of loudness near the ceiling costs more raw molecule than a point near the floor. It is a reminder of why the Index exists at all: loudness, not quantity, is what the tongue reports.)

The astringency came by a wholly different road — not an acyltransferase but the flavan-3-ol branch described above, installed, balanced, and then throttled to a target polymer distribution and verified on the PC_{50} precipitation assay, the very same assay and the very same protein we use to read a grape. That is the deepest point Phoenix makes. The grip in this pepper is not a lucky side effect of an enormous phenolic load thrown off by the heat program. It is a tannin we chose, built on purpose, scored on the wine chemist's own instrument, and seated at a deliberate 1,400 to give the fire an edge to resolve against. The organism furthest from the vineyard in all of our work carries a vineyard's chemistry because astringency is not a property of grapes — it is a dimension of taste, and a dimension can be dialed into anything whose pathways we can read and whose carbon we can spare.

Astringency inside the chord — how the grip talks to seven neighbors

A grip alone is a known thing; a grip among seven other sensations behaves in ways no lone tannin ever reveals, and Phoenix is where we learned it. Read the scorecard as the chord it is, and each pairing teaches something.

Against the **heat** at 4,200, the grip does what a lone tannin cannot: it gives the burn an *end*. A great heat without astringency pools and lingers, soft and formless at the back of the palate, smearing past its welcome. The tannin's drying pull draws a clean line beneath the fire — the friction cue arrives as the capsaicin fades, and the sensation resolves instead of trailing. Heat is the loudest voice; astringency is the one that tells it when to stop.

Against the **Zero-Bitter** reading of 2,200, the relationship is subtle and, we think, the most elegant thing in the fruit. IFI-B is not bitterness — it is bitterness *attenuation*, the engineered suppression of TAS2R38, the 20-to-65% quieting of the bitter taste. And a fruit this rich in polyphenol is a fruit that *should* taste savagely bitter, because the same condensed tannins that precipitate PRPs also hammer the bitter receptor. So the high astringency and the high bitterness-attenuation are not two unrelated voices; they are partners of necessity. The tannin builds the tactile grip; the

attenuation program strips away the harsh bitter *taste* those identical molecules would otherwise trigger — leaving the clean, dry, structural pull without the ugliness that usually rides with it. You feel the architecture and are spared the rasp. Seating astringency at 1,400 is only possible because the Zero-Bitter axis is holding at 2,200 to catch what the tannins would otherwise do to TAS2R38.

Against the **sweetness** at 1,100, the grip is the classic counterweight — the reason a huge ripe sweetness does not collapse into cloying jam. Against the **umami** at 1,800 and the **kokumi** at 1,900, the two axes of savory depth and continuous body, astringency supplies the contrasting edge: body wants structure the way breadth wants a frame, and the drying pull keeps the mouth-filling richness from going slack. Against the **pungency** at 252, a sharp TRPA1 top-note, astringency is the base beneath the brightness. And the faint **cool** at 800, the quietest voice, lifts the very end of the finish just as the grip dries it. Eight voices, one chord, and the grip is the one keeping the whole thing honest at the back of the palate — the fifth-loudest sensation and, we would argue, not the least important of them.

This is not unique to Phoenix, only clearest there. Across our multi-axis libraries — the **M2 dual-axis** work spanning some 8,400 constructs and the **M3 triple-axis** another 2,800 — a great many of the combinations that hold together over a long finish do so because a designed grip is giving the louder axes an edge to resolve against. What Phoenix teaches at its extreme, a balanced red wine teaches gently every evening: astringency is rarely the loudest voice in a flavor, and almost always the one that keeps the loud voices from dissolving into mush.

Edge cases, failure modes, and the honest limits

We would not revere a number we could not also criticize, and astringency carries more asterisks than any other axis. They are worth stating plainly.

The first is the one the mechanism forces: astringency is read on *your* saliva, which the plant does not make. PC_{50} is computed against a standard PRP preparation, but a real mouth varies — salivary flow and PRP concentration differ with age, hydration, time of day, and the dry-mouth that many medications bring. So the bench number is a faithful proxy with a wider inter-individual spread than, say, heat; the same Phoenix fruit will grip a touch harder for a person with sparse, thin saliva than for one with abundant protein-rich saliva. We state IFI-A as a property of the fruit, because the fruit is what we control, and we do not pretend it fully predicts every mouth.

The second is **build-up**. Because astringency spends saliva — precipitating PRPs that then take minutes to regenerate — it accumulates across repeated sips in a way a receptor taste does not. The second mouthful reads grippier than the first, the fifth grippier still, until the saliva catches up. The dose–response curve above describes a single clean exposure; lived astringency is non-stationary, climbing with repetition. It is why a tannic wine feels

firmer by the third glass and why we taste Phoenix's grip in spaced, palate-rested trials rather than in a rapid series that would read falsely high.

The third is **drift over time**, the failure mode that kept astringency last to come online. A tannin's potency rides on its polymer-length distribution, and that distribution is not inert — post-harvest oxidation and slow condensation lengthen and rearrange the polymers, shifting PC_{50} under the fruit even in storage. Of all eight axes, astringency is the one most prone to move after the plant leaves our hands. Holding it to ≤7% is partly a pathway achievement and partly a handling discipline; it is the axis we watch most carefully between harvest and measurement.

The fourth is **cross-reactivity**, and it is an engineering constraint as much as a caveat. The same polyphenols that precipitate PRPs can, at the wrong polymer lengths, weakly trip TRPA1 (reading as spurious pungency) and TAS2R (reading as bitter). Writing a *clean* astringency therefore means selecting a tannin distribution that precipitates efficiently while touching the taste GPCRs as little as possible — gripping without shouting on the neighboring channels. The 1,400 on Phoenix's card is not merely a tannin titer; it is a tannin titer chosen inside a narrow window that keeps the grip tactile and the taste clean, which is exactly what the Zero-Bitter program is there to guarantee.

None of these is a flaw in the Index; each is the mechanism being honest about itself. Astringency is a touch, read on a protein the eater supplies, carried by a polymer that will not sit still. That we can nonetheless design it to 1,400 ± 98, harvest after harvest, in a fruit that also carries seven other sensations, is the measure of how far the method reaches.

The unification

That the astringency in a full-spectrum *pepper* uses the very same chemistry, the same precipitation, the same PRP target, and the same PC_{50} measurement as the grip of a grape or a tea or a pomegranate is the quiet unification this whole library rests on. Astringency is not a property that belongs to certain plants and is denied to others. It is an axis — a dimension of the sense — and a dimension can be written into any organism whose pathways we can read and whose carbon we can spare. The living proof is already a working catalog of hosts, each carrying the grip at its own scale:

ORGANISM	CONSTRUCTS	AXES EXPRESSED	DIFFICULTY
Grape	1,610	B / A / M	Hard
Tea	280	U / B / A	Hard
Blueberry	120	A	Moderate
Pomegranate	120	A	Hard
Sorghum	120	A	Frontier
Raspberry	40	A	Moderate

From a berry's whisper to a tea's astringent lift to a grape's decade-long spine, the same enzymes and the same measurement serve them all — part of a platform now spanning roughly 19,000 engineered constructs across 42 host organisms and eight sensory

axes, with a further 64 organisms sketched on the roadmap and every one of them scored, when its time comes, on the same page. The grip does not have to be invented anew for each plant. It has to be read, and written, and held.

And Phoenix is that argument carried to its furthest edge: eight sensations, each placed exactly, each held to seven percent, in a single fruit we built gene by gene because the plant that could carry them did not yet exist. The grip you have been following lives here as one voice of eight — seated just under the mean, at 28% of its ceiling, giving an enormous fire a clean place to end. We grew a vineyard's chemistry into a pepper not to prove we could, but because the whole point of a number is that it belongs to no single plant. Astringency was always a dimension of taste. Phoenix is where we finally got to express it inside the fullest flavor we have ever made.

These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 18 · What the tannin is for — *polyphenol, nourishment, and the road ahead.*

We end where the plant begins, because the last thing worth understanding about astringency is *why a plant makes it at all* — and the answer turns out to be the quiet through-line of this whole library. A plant does not build tannin to flavor our wine. It builds tannin, at real metabolic cost, to defend the thing it cares most about: the seed, the future, the next generation. Tannin is the plant's investment in continuity — a chemical fence of polyphenol raised around what matters, potent enough to precipitate the proteins of anything that would eat the seed before its time. The grip you feel is the felt edge of that investment. To grow a plant's astringency with intention is to take the most protective chemistry it makes into our hands, and we do not do it lightly.



The research greenhouse at dusk — the plants that carry the grip, grown to hold as much of their own chemistry as they can. We design the flavor; we also grow the plant to be worth eating.

That is the reverent half of the story. The exact half — the half that lets us grow the grip on purpose and promise it to the decimal — is a small, specific act of chemistry happening on the surface of your tongue, a reaction we can write down, measure, model, and dial. This closing chapter is where we do exactly that: we show what the grip *is*, mechanically; we put a number on the one sensation the tongue does not taste; we lay out the six plants we hold today and the compounds that make each of them pull; and then we point down

the road, because the astringency axis reaches much further than six organisms, and the way it reaches is the engine under the entire shelf.

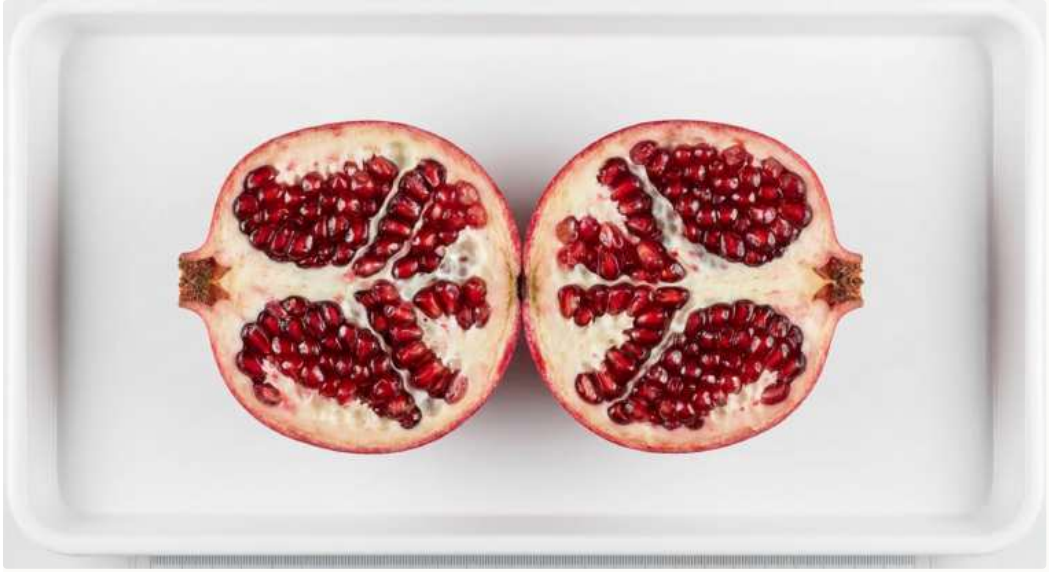
The grip, mechanically — a precipitation you can feel

Astringency is the odd one of the eight, because it has no receptor of its own. Heat has TRPV1; sweetness has TAS1R2·R3; umami has mGluR1; the sharp channels of pungency and cool-burn are TRPA1 and TRPM8. Astringency has no dedicated gustatory channel at all. What it has instead is a protein reaction — and a mechanical consequence your trigeminal nerve reads as roughness. The tongue does not taste the tannin. It feels what the tannin *does* to your saliva.

Here is the reaction, step by step, the way we model it when we set the IFI-A dial on a plant.

Your saliva is not water. It is a thin, continuous film of protein spread over the mucosa, and its job is lubrication — to keep tongue, palate, and cheek sliding over one another without friction. The proteins that do most of that lubricating are the **proline-rich proteins**, the PRPs: long, open, flexible peptides whose chains run as much as forty percent proline, with glycine and glutamine filling most of the rest. Proline is the key. Its side chain loops back and bonds to its own backbone, forming a rigid five-membered ring that presents a flat, hydrophobic face and — because that ring consumes the amide hydrogen a normal residue would carry — forces the chain into an open, extended, polyproline conformation that leaves the backbone carbonyls exposed and available. Evolution built these proteins, in us and in the animals the plant is defending against, almost as if to be caught.

A tannin is the thing that catches them. A tannin is a polyphenol with *many* phenolic hydroxyls arranged on *many* aromatic rings — and that multivalence is the whole mechanism. There are two broad families, and this book holds plants from both. A **condensed tannin**, or proanthocyanidin, is an oligomer of flavan-3-ol units — the catechin and epicatechin of grape, tea, blueberry, and sorghum — strung into chains two, five, ten units long, each unit carrying a catechol or pyrogallol ring studded with hydroxyls. A **hydrolyzable tannin** is a core of sugar esterified with gallic-acid-derived groups — the ellagitannins of pomegranate and raspberry, of which pomegranate's punicalagin is the grand example, a single molecule hanging twenty-four hydroxyls off a glucose core.



Pomegranate in cross-section — arils packed around the seeds, and the ellagitannins (punicalagin the chief of them) the plant raised to defend them. The grip and the fence are the same chemistry.

When tannin meets PRP, two grips close at once. The flat aromatic faces of the polyphenol **stack** against the flat hydrophobic faces of the proline rings — a hydrophobic association that squeezes water out from between them. And the phenolic **hydroxyls hydrogen-bond** to the exposed backbone carbonyls and to the glutamine side chains, donor to acceptor, dozens of times along a single chain. Neither grip alone would hold; together, repeated down the length of two multivalent partners, they are decisive.

And because both partners are multivalent, the binding does not stop at a pair. One tannin bridges two PRP chains; each PRP offers more proline sites to more tannins; the complex grows, cross-links, turns from a soluble tangle into a colloid and then — this is the moment you feel — falls out of solution as a visible precipitate. It is the identical reaction a winemaker runs on purpose when they fine a tannic wine with egg white or gelatin: protein in, tannin down, clarity out. On your tongue, the protein that goes down is your own lubricating film. The saliva is stripped. The boundary lubrication fails. Tongue and palate, suddenly unlubricated, drag against one another, and the free nerve endings of the trigeminal system report that rise in friction as exactly what it is: *rough, dry, drawn-in, puckered.* That is astringency. Not a taste — a precipitation, felt as a texture.

Three consequences of this mechanism matter so much that we design around all three. First, it is **slow**: precipitation takes seconds to build, which is why the grip arrives late, after the fruit and the sweetness have already spoken. Second, it is **cumulative**: each sip throws down more protein before saliva can fully replenish it, which is why the fifth taste of a young red grips harder than the first, and why a tannic tea or wine asks you to pace it. Third, it **outlasts the stimulus**, lingering as a dry finish long after you have swallowed, until salivary flow rebuilds the film. The reticence this book has loved from its first page — the flavor that gives nothing away on contact and keeps revealing itself after — is not a metaphor. It is the literal time-course of a protein coming out of solution.

Putting a number on what the tongue cannot taste

If astringency is a precipitation rather than a receptor event, how do we score it on the same index as heat and sweetness? We anchor the axis to the reaction itself. Everywhere else in this library, the Iyengar Flavor Index reads a half-maximal *activation* — the

concentration at which a stimulus drives its receptor to half of full response. For astringency, IFI-A reads a half-maximal *precipitation*: the concentration of a tannin at which it throws down half of the maximum salivary PRP it can — measured against a standardized saliva and against a calibrated sensory panel. Same idea, different endpoint. The number still answers one question: not how *much* of the compound a food holds, but how *loudly* it speaks.

The formula is the one that runs the whole index:

$$\mathrm{IFI}(c) = 1000 \cdot \mathrm{EC}_{50}(\mathrm{ref}) \,/\, \mathrm{EC}_{50}(c)$$

EC_{50} is the concentration at the half-maximal point — here, half-maximal PRP precipitation. The reference is fixed by convention, and the axis is anchored so that the reference scores **1,000**. Because EC_{50} sits in the denominator, the relationship is an inversion: a *more* potent tannin reaches its half-precipitation at a *lower* concentration, so a smaller EC_{50} gives a *larger* score. Loudness, not quantity. A tannin that grips the mouth at half the concentration the reference needs is twice as loud, and scores 2,000.

Work it both directions, because the inversion is where people slip. The potency of a candidate relative to the reference is simply IFI ÷ 1,000; the concentration ratio you need to hit that potency is its reciprocal, 1,000 ÷ IFI. Take the full-spectrum designed profile we hold — **Phoenix**, the cultivar engineered to register on all eight axes at once — whose astringency reads **A: 1,400**. Then its tannins reach half-maximal PRP precipitation at $EC_{50}(\mathrm{Phoenix}) = 1000 \cdot EC_{50}(\mathrm{ref}) / 1400 = \mathbf{0.714 \times EC_{50}(ref)}$ — about seven-tenths of the reference concentration, which is to say its grip is **1.40×** as potent as the reference, loudness for loudness.

To make the arithmetic physical, carry it into real units. Suppose — illustratively, to teach the conversion — the reference tannin reaches half-precipitation at an EC_{50} of **0.75 mmol/L**. Then an A: 1,400 grip sits at 0.714 × 0.75 = **0.536 mmol/L**. The scorecard ceiling of 5,000 would sit at 1000 · 0.75 / 5000 = **0.15 mmol/L** — five times as potent as the reference, a fifth of the concentration. Here is the point the molar numbers hide until you convert them to mass: a millimole of punicalagin weighs far more than a millimole of a small procyanidin, so equal *loudness* looks like wildly unequal *grams*.

AT EC_{50} = 0.75 MMOL/L	MOLAR MASS (G/MOL)	MASS CONCENTRATION
Punicalagin (ellagitannin, pomegranate)	1,084.7	0.81 g/L
Procyanidin B2 (flavan-3-ol dimer, grape)	578.5	0.43 g/L
Epigallocatechin gallate (tea)	458.4	0.34 g/L

Same molar activity, nearly a 2.4-fold spread in grams. This is exactly why a tannin number printed as milligrams-per-liter on a label can mislead, and why the index refuses to work in mass: the mouth hears *activity*, not weight. It is also why degree of polymerization — how many flavan-3-ol units are strung into a condensed tannin — is a design lever and not a footnote. A lone monomer barely grips; string it into a dimer and it grips; run the chain out to a mean of eight or ten units and each molecule carries enough stacking faces and

hydroxyls to bridge several PRP chains at once, dropping its EC_{50} and lifting its IFI-A. Length is loudness, up to the point where the tannin grows too large to stay in solution and simply falls out on its own.

The reference anchor and the scorecard ceiling frame the whole axis:

READING	IFI-A	EC_{50} ÷ REFERENCE	POTENCY VS REFERENCE
Reference anchor	1,000	1.00×	1.00×
Phoenix · SS-0096 (A)	1,400	0.71×	1.40×
Candidate at double loudness *(illustrative)*	2,000	0.50×	2.00×
Per-axis scorecard ceiling	5,000	0.20×	5.00×

The six grips of this volume

The six organisms we hold on this axis today are the plants whose entire character the tongue reads through tannin — and they split cleanly across the two tannin families, which is half of why they taste so distinct. Grape, tea, blueberry, and sorghum grip with **condensed** tannins, the polymerized flavan-3-ols; pomegranate and raspberry grip with **hydrolyzable** ellagitannins. The construct counts, the axes each plant expresses, and the engineering difficulty are the canonical figures the whole library resolves against.

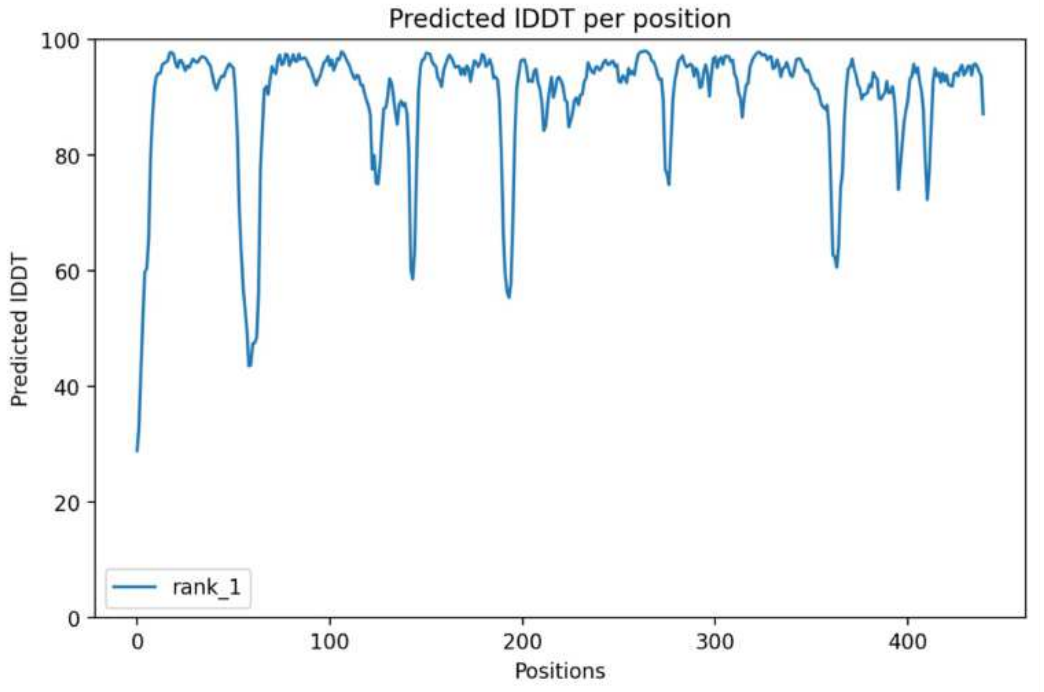
ORGANISM	CONSTRUCTS	AXES EXPRESSED	DOMINANT TANNIN CLASS	DIFFICULTY
Grape	1,610	B · A · M	Condensed — proanthocyanidins (via ANR)	Hard
Tea	280	U · B · A	Condensed — flavan-3-ols (catechins, EGCG)	Hard
Blueberry	120	A	Condensed — proanthocyanidins + anthocyanins	Moderate
Pomegranate	120	A	Hydrolyzable — ellagitannins (punicalagin)	Hard
Sorghum	120	A	Condensed — proanthocyanidins	Frontier
Raspberry	40	A	Hydrolyzable — ellagitannins	Moderate

Two things are worth reading off that table slowly. The first is that grape carries ten times the constructs of any other plant here — 1,610 against the next-largest 280 — because grape is where astringency most *matters* commercially and where the quality of the grip, not just its size, decides everything from a cheap red to a cellar wine. The second is that four of the six carry astringency as their *only* expressed axis, while grape and tea carry it braided to bitterness, and tea to umami on top. That braid is not incidental. Many of these same polyphenols that grip the PRPs also reach the bitter receptor TAS2R38, so on the two plants with the deepest tannin chemistry, astringency and bitterness travel together — which means on grape and on tea you cannot design the one without accounting for the other. That coupling is the first and most important of this axis's honest limits — the reason grape and tea are the hardest grips in this book to design cleanly. Summed, these six

hold **2,290** constructs of designed grip, every one scored against the single reference, every one reproducible to the tolerance the next section describes.

Designing a grip on purpose

The reason we can promise a grip and not merely hope for one is that we invert the old order of breeding. For ten thousand years the method was to select the phenotype and pray the genotype followed — grow the vine, taste the fruit, keep the seed of the one whose tannin pleased you, and inherit whatever accident of chemistry came attached. We run it the other way. We model the pathway, find the single step that limits the trait, score every edit that could lift it, and write the winning edits in — so that the grip is a specification before it is ever a harvest.



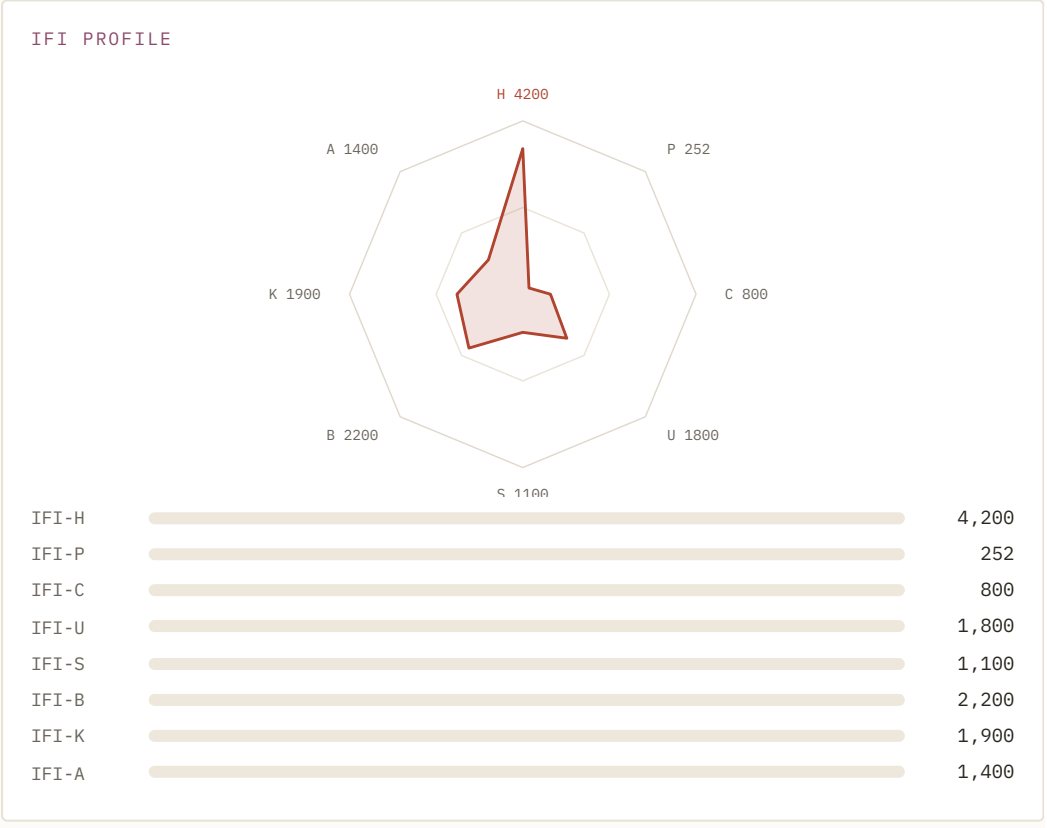
The structural-model method, drawn here on the capsaicin acyltransferase: the same lens of a protein language model scoring every substitution by predicted effect, turned on the tannin enzymes — ANR, the flavan-3-ol reductases, the galloyl transferases — in every astringency host.

The engine that does this is organism-agnostic, and that is the whole leverage of the platform. A protein language model scores every possible single-residue substitution in a target enzyme by its predicted effect on catalytic efficiency and fold stability; a genome-scale flux-balance model confirms which step is the true rate limiter rather than an upstream one; CRISPR writes the chosen edits. On the heat axis that pipeline produced **two** substitutions in the capsaicin acyltransferase — **S39L** and **L345G** — that no breeding program ever found, and lifted peak output to a batch-to-batch coefficient of

variation of **≤7%** against the **42%** of wild-type. For astringency the target enzymes are different — the method is identical. The condensed-tannin route runs through **ANR**, anthocyanidin reductase, which reduces anthocyanidins to the 2,3-*cis* flavan-3-ols (epicatechin) that polymerize into proanthocyanidins; the grape work expresses its tannin precisely there, at ANR and the reductases beside it. The hydrolyzable route runs through the galloyl transferases and the oxidative coupling that builds the ellagitannin core. The relevant carbon arrives, in every one of these plants, down the same phenylpropanoid trunk — PAL → C4H → 4CL — that feeds the capsaicin branch; the tannin plants simply turn off that trunk earlier, into the flavonoid pathway, through chalcone synthase and the flavan-3-ol reductases. Five of the platform's seven modules cross from pepper to vine to tea unchanged. Only the terminal enzymes are plant-specific, and they are exactly the enzymes the model is built to score.

What we design at those enzymes is not only *how much* tannin but *what kind*: the ratio of catechin to epicatechin, set at the LAR/ANR branch, and the mean degree of polymerization, set by how long the chains extend before they terminate. Short oligomers give a bright, forward, quickly-resolving grip; long polymers give the slow, spreading, architectural grip of a wine built to age. Two plants can carry the same IFI-A and feel entirely unalike because one speaks it in dimers and the other in decamers. The number sets the loudness; the chemistry sets the voice.

That the method reaches astringency at all is proved by the one cultivar that reaches *every* axis. Phoenix was designed gene by gene to register on all eight axes at once, and its astringency is not an afterthought but a precisely specified **1,400** — a real, co-engineered grip riding alongside a heat of 4,200 and a bitterness of 2,200:



Read that profile as the math of the last section, run across all eight axes at once, and astringency sits squarely in its middle:

AXIS	IFI	$EC_{50}(C) \div EC_{50}(REF) = 1000/IFI$	POTENCY = IFI/1000
Heat (H)	4,200	0.238	4.20×
Zero-Bitter (B)	2,200	0.455	2.20×
Kokumi (K)	1,900	0.526	1.90×
Umami (U)	1,800	0.556	1.80×
Astringency (A)	**1,400**	**0.714**	**1.40×**
Sweetness (S)	1,100	0.909	1.10×
Cool-Burn (C)	800	1.250	0.80×
Pungency (P)	252	3.968	0.25×

Each axis is anchored to its own reference compound at IFI = 1,000; scores across axes reflect potency relative to each axis's own defined reference, not a single common scale.

The precision in that last column is the part that is hardest to see and most important to hold, and astringency is the axis where it earns its keep most dramatically. Set the target at A: 1,400. At wild-type's 42% coefficient of variation, one standard deviation is 0.42 × 1,400 = **588**; batches scatter across roughly **812 to 1,988** at one sigma, and a two-sigma run — the ordinary spread you would actually see across a season — swings from **224 to 2,576**. That is the difference between a grip you can barely find and a grip that strips the mouth raw, from the *same seed*. Hold the platform's ≤7% instead and one sigma is 0.07 × 1,400 = **98**; batches land between **1,302 and 1,498**, two sigma between **1,204 and 1,596**. The target holds. This matters more here than on any other axis because of the dose-response geometry: EC_{50} is by definition the steepest point of the sigmoid, the concentration where a given error in dose produces the *largest* error in response, and because astringency sums over a meal, that error does not average out — it accumulates. On an axis this nonlinear and this cumulative, a 42% swing is not a tolerance. It is the difference between architecture and an assault, and we would rather set the dial deliberately than inherit whatever an accident left.

Where the grip goes wrong

A family of scientists keeps the line between a fact and a claim bright and clean, and the same discipline requires us to be just as clear about where this axis is hard, where it fights us, and where it fails. Four honest limits, each of which we design around rather than past.

The first is the **bitterness coupling** already named. The multidentate polyphenols that precipitate PRPs also, many of them, reach TAS2R38 and read as bitter — so on the plants with the deepest tannin chemistry, grape and tea, lifting astringency drags bitterness up with it. The two cannot be designed in isolation; a grip engineered without minding the bitter axis arrives green and harsh. The library's answer is to co-design, pulling the Zero-Bitter axis — TAS2R38 attenuation, the 20-to-65% range — in the same construct that lifts the grip, so the structure rises while the bitterness is held. It is more work and it is the only honest way to do it.

The second is **the taster, not the tannin**. Because astringency is a reaction with your saliva, it depends on *your* saliva. People differ in PRP gene copy number and in salivary flow rate, and a low-flow, low-PRP mouth has less protein film to lose and rebuilds it more

slowly, so the identical tannin reads as sharper and lingers longer there than in a high-flow mouth. The index anchors IFI-A to a standardized saliva and a calibrated panel precisely so the *number* is stable — but we say plainly that the number is the compound's loudness, not a promise about one particular person's evening.

The third is **the matrix**. A tannin does not meet your PRPs alone; it meets them through whatever else is in the food. Fat, protein, polysaccharide, and ethanol all compete for the tannin before your saliva can, binding a share of it and softening the grip — which is the whole reason cheese tames a tannic red, cream gentles a strong tea, and a sauce can carry a heat that the bare fruit could not. IFI-A measures the intrinsic loudness of the compound; the dish decides how much of that loudness the mouth actually hears. A number is a property of the molecule. A meal is a negotiation.

The fourth is **over-design** — the failure that is most tempting and most important to refuse. Because higher degree of polymerization and higher concentration both lift IFI-A, it is easy to keep pushing, and there is a point past which more grip is simply worse: the tannin crosses from *structure* into *harsh*, precipitates so much saliva that the mouth cannot recover between bites, and turns the pleasure of resistance into punishment. The design target on this axis is always a *window*, never a maximum. The grip is a thing that is better for being resisted a little — and ruined by being resisted completely. We tune toward the window. We do not chase the ceiling.

What the polyphenol is also for

Here the mechanism folds back into the ethos, and it does so almost literally. The polyphenols that make a tannin — the flavan-3-ols, the ellagitannins, the proanthocyanidins, the very molecules of the last several pages — are among the most studied compounds in human nutrition, and we will say exactly this much about that and not one word more. A plant grown to carry a fuller, more structured polyphenol load is carrying *more of its own chemistry*; we think that is worth something; and we will not tell you it cures anything, because it does not, and we are not in the business of health halos. There are no cures here, no detox, no claim dressed as a benefit. What there is, is a plant grown to hold the most it can — the most flavor, the most structure, the most of the chemistry it raised to defend itself — met by your body at the table, as food, with intention, the way food was meant to be met.

What is singular about this axis is that the thing you taste and the thing the nutrition literature studies are *the same molecule*. On heat, the capsaicin that burns and the capsaicin that is studied are one compound, and we can say that cleanly. On astringency it is cleaner still, because the grip you feel *is* the polyphenol load — the degree of polymerization we tune for mouthfeel is the same structure the body meets, the punicalagin that defends the pomegranate seed is the punicalagin on your tongue. We would rather set that dial deliberately than inherit whatever an accident left. That is the whole ethos, and on this axis it is nearly tautological: the compound of the grip and the compound of the nourishment are one compound, grown on purpose, to be as full of itself as a plant can be.

The road ahead, and the one index beneath it

There is more road here than we have walked. The six organisms of this book are the ones we hold today; the astringency axis reaches much further.



Cacao in cross-section — a plant whose entire quality turns on a procyanidin grip, and the most obvious next library on this axis.

Cacao is the obvious next library — a plant whose whole quality turns on a procyanidin grip we could design from the bean rather than wrestle out in the roasting and conching, where today the astringency is blunted by brute process instead of being built right from the start. The hydrolyzable-tannin fruits beyond pomegranate and raspberry — the oak-aged register the wine world borrows from barrels, the quince and the medlar and the persimmon that the eating world mostly gave up on precisely because their young grip was untamed — are an open frontier for anyone willing to set the window instead of suffering the extreme. And every intersection this axis touches is a volume of its own: astringency against bitterness is the coffee-and-cacao problem; astringency against umami is the tea triangle, the savory-and-structured balance a great oolong already hints at; astringency against sweetness is the whole art of the dessert wine and the balanced berry, grip and sugar tuned against each other until each makes the other legible.

The reason any of this is tractable — the reason the axis can mint books forever without the library fracturing — is a single shared index beneath all of it. The matrix that generates this whole shelf is forty-two organisms against eight axes, and astringency, being a *dimension* and not a property, holds a tannin cell in nearly every row: in principle it can be written into anything that grows a polyphenol, which is to say nearly anything that grows. We pick the books worth making. The axis mints more forever.

And every tannin, every organism, every cultivar and construct named in these pages is tagged against that one index, so the numbers hold across the whole library to the decimal. The grape we built here is the same entity, carried in more depth, in the volume given wholly to **Grape & Wine**; the tea of these pages opens fully in the volume on **Tea**; the cacao we could only point toward is grown and built in **Cacao & Chocolate**; and the bitterness that shadows astringency on every deep-tannin plant is the subject of **Zero-Bitter**. Look up *punicalagin* or *proanthocyanidin* or *IFI-A* in the master cross-book index and every chapter across the library that touches it

answers at once — a single encyclopedia assembled from sixty-odd lenses on one world. A book like this is the vertical cut: astringency, followed through every plant that speaks it. The organism books are the horizontal cuts: one plant, followed through every axis it carries. Where they cross — this book's grape and the grape book's astringency — they tell the same truth in the same numbers, because they draw from the same index. That is what lets the whole shelf read as one library, and it is why the grip you have followed here will meet you again, consistent to the decimal, wherever tannin appears in the rest of the shelf.

Why we love the reticent one

The last word belongs to the sensation itself, and to the reason we love it. Astringency is the most *reticent* of the eight — the one that gives nothing away at first, that arrives late and builds slow and lingers after everything else has gone, that asks you to meet it with fat and salt and time and a second sip rather than handing you pleasure on contact. We can now say why, exactly: because it is a precipitation, not a signal; because protein takes seconds to come out of solution, and sums across a meal, and outlasts the swallow until the mouth can rebuild what it lost. The poetry and the mechanism turn out to be the same sentence.

It is, in that way, the most grown-up of the flavors: the taste of patience, of structure, of the thing that is better for being resisted a little. Our family came to this work loving exactly that — the grip of a good wine, the spine of a strong tea, the clean dry edge that tells you a thing has architecture and not just sweetness. We measured it so we could keep it. We learned its chemistry — the PRP and the polyphenol, the stack and the hydrogen bond, the EC_{50} and the degree of polymerization — so we could grow it on purpose and to the window rather than the extreme. And we wrote it down here, all these thousands of words of it, so that the next person who feels their mouth draw in at a glass of young red or a cup of first-flush tea will have, where there used to be only poetry, a number — and behind the number, a whole living science of the one sensation the tongue does not taste, but feels. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.