THE SCOVILLE SPLICE LIBRARY · VOLUME XVIII

Grape & Wine

Every tannin, a number. *The pucker, by design.*

The definitive account of the grape as an instrument of astringency — the receptor-level mechanics of tannin, the pathway that builds it, and the 1,610 constructs with which we have learned to write the pucker, the color, and the scent of wine by design.

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



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Opening essay · The taste that isn't a taste

The first grape our family ever argued about was a black one, warm off a dusty vine in late August, and the argument was not whether it was good — it was good — but *why* the second handful made the roof of the mouth go quiet and dry, as if the fruit had reached in and gently wrung out a cloth. Sweet, we could name. Sour, we could name. This third thing had no clean word, and so for most of human history it borrowed one: we called it astringent, from the Latin *adstringere*, to bind fast, to draw tight — a word about rope and surgeons' sutures long before it was a word about wine.

That borrowing turns out to be exactly, almost eerily, correct. What a tannin does in the mouth is bind fast. It is not a flavor arriving at a taste bud. It is a mechanical event — a protein in your saliva pulled out of solution and precipitated, the lubrication it was providing suddenly gone, and the bare friction of tissue on tissue reported upward as *dryness, grip, pucker*. Astringency is the only major sensation on our index that is not, strictly, a taste at all. It has no sweet-receptor, no bitter-receptor of its own. It is touch, read through chemistry.



The research greenhouse at first light — where a late-summer question about one dusty grape became a program of 1,610 constructs.

For ten thousand harvests the vigneron worked this sensation entirely by feel. Chew a seed; is it green or nutty? Rub a skin between finger and thumb; does the pigment stain or slide? Taste the ferment at dawn and again at noon and decide, on the strength of a tired palate, whether the grip is *ripe* or merely *hard*. It is one of the great bodies of human skill, and we revere it the way you revere any art practiced that long against that much uncertainty. But it could never say *how much*. It could never say that this Nebbiolo carries a tannin that binds salivary protein at one-third the concentration of that Pinot, or that the difference a winemaker feels between "green" and "silky" is, underneath, a measurable shift of six flavan-3-ol subunits in a chain and nine percentage points of galloylation. The feeling was true. The number was missing.

The grape, of all the organisms in our library, is the one where that missing number cost the most. It is the most measured plant on earth and the least *designed* one — a fruit with thousands of years of connoisseurship lavished on it, whole academic disciplines and auction houses and regional laws built around predicting what a vintage will do, and underneath all of it a chemistry that the grower could coax and pray to but never set. A pepper's heat you can at least breed toward with brute selection; a grape's tannin is woven so tightly into its color, its scent, its sugar, and its season that pulling on one thread moves five others, and the vigneron's art has always been as much about *managing the entanglement* as about any single quality. This book is the record of learning to cut the threads one at a time — and of discovering, with a reverence that has not worn off, how much the old art already knew about a chemistry it could not see.

The Iyengar Flavor Index supplies the number. On the astringency axis — **IFI-A**, anchored not to a taste receptor but to the precipitation of salivary proline-rich proteins, the very proteins the tannin binds — a grape's grip becomes a single value, loudness made legible.

$$\text{IFI-A}(t) = 1000 \cdot \text{EC}_{50}(\text{ref}) \,/\, \text{EC}_{50}(t)$$

EC_{50} here is the tannin concentration at which astringency reaches half of its maximum — equivalently, the concentration that precipitates half of a standard salivary protein in the bench assay. Invert it against a fixed reference tannin, scale by a thousand, and a table grape, a Cabernet, and a thirty-year Barolo can be read on one page. We did not build IFI-A to retire the vigneron's thumb. We built it because you cannot *design* what you can only feel — and the grape, it turned out, was asking to be designed.

This is the book of that work: the receptor, the molecule, the pathway, the berry, the 1,610 constructs, and the wine at the end of them. We are a family of scientists who happen to love what grows. What follows is written the way you'd write about something you love and want to understand all the way down.



PART I OF VI

Reading the Pucker

salivary PRPs · the tactile mechanism · IFI-A and the grain of a tannin

Chapter 1 · Astringency is not a taste — *the protein in the saliva.*

Open your mouth and run your tongue across your teeth. The slide you feel — the ease of it — is a film: a two-to-ten-micron layer of water, mucins, and small proteins secreted continuously by the salivary glands, among them a remarkable family called the **proline-rich proteins**, the PRPs. In human saliva, PRPs are not a minor component. They are roughly seventy percent of the protein by mass in the parotid secretion — an extravagant investment of the body's protein budget in molecules whose main job, as far as anyone can tell, is to sit in the mouth and wait for tannins.

They wait because tannins are ancient plant weapons. A leaf full of condensed tannin is a leaf that binds and precipitates the digestive proteins of whatever tries to eat it; the PRPs are the mammalian counter-move, a sacrificial decoy — proteins the body floods into the mouth precisely so the tannin will bind *them* instead of the enzymes further down. When you drink a young Tannat, you are watching a four-hundred-million-year-old arms race play out across your own tongue, and the dry, drawn-tight feeling is the sound of your side spending ammunition.



A single berry, backlit. Nearly all of its tannin lives in two places you cannot see from here: the skin, three or four cells deep, and the seed.

The PRPs are built for the job in a way that took us some time to properly admire. They are **intrinsically disordered** — they have no folded structure, no compact globular shape, just open, flexible chains strung with an unusual abundance of the amino acid proline. Proline is the oddity of the twenty: its side chain loops back and bonds to its own backbone nitrogen, making a rigid five-membered pyrrolidine ring and a permanent kink in the chain. That ring is the binding face. A tannin's flat aromatic rings — its catechol and galloyl groups — stack against the proline rings by hydrophobic and π-stacking association, while the tannin's many hydroxyls hydrogen-

bond to the exposed backbone carbonyls that the disordered, kinked chain leaves hanging open. An ordered protein hides its backbone; a PRP offers it up. The grape evolved a polymer to bind proteins; the mammal evolved the most bindable protein it could.

The binding itself proceeds in three stages, and knowing them is knowing why astringency *feels* the way it does — slow to arrive, cumulative over sips, slow to leave:

1. **Association.** Tannin and PRP meet and form soluble complexes, one tannin bridging one or a few protein chains through hydrogen bonds and hydrophobic contact. Nothing is felt yet.
2. **Growth.** As more tannin binds, the complexes enlarge and their surfaces turn hydrophobic; water is excluded; the particles begin to associate with one another.
3. **Precipitation.** Cross-linked aggregates exceed their solubility and fall out of solution. The salivary film loses the proteins that gave it body. Lubrication collapses. Oral surfaces drag, and the brain — which has free nerve endings in the mucosa reporting friction and mechanical strain — calls it *astringency*.

Because the process is a precipitation and not a receptor binding a ligand, it has the kinetics of a precipitation: it builds across repeated exposures as the salivary protein pool is drawn down, it does not switch off the instant the stimulus leaves, and it takes a full minute or two of fresh saliva to rebuild the film. This is why the fourth sip of a tannic wine grips harder than the first, why astringency *lingers* where sweetness has long since gone, and why a trained panel scores it not at a single instant but as a time-intensity curve. We will return to that curve; it is the shape we learned to design.

The salivary defense is not one protein but a graded arsenal, and which members a tannin recruits is part of what sets the *character* of the grip. It is worth naming them, because the next three chapters quietly assume them:

SALIVARY PROTEIN	SHARE OF PAROTID PROTEIN	TANNIN AFFINITY	NOTE
Acidic PRPs (aPRP)	~30%	moderate	also bind calcium; first line
Basic PRPs (bPRP)	~23%	**highest**	proline-richest; the IB-5 / P-B peptides
Glycosylated PRPs (gPRP)	~17%	moderate	sugar chains add lubrication
Histatins	small	high	histidine-rich; strong tannin binders
Statherin / mucins	balance	low–moderate	the film's bulk lubricant

The basic PRPs and histatins are the hungriest — the first spent and the most missed — and a tannin that preferentially strips them reads as a harder, drier grip than one that merely thins the mucin film. This is why astringency is not one sensation but a family of them: *drying, puckering, roughing, grainy, chalky* are not loose synonyms but reports of which proteins went down and how far the film collapsed.

And it is why astringency is so readily *modulated* by everything else in the glass, a fact every winemaker exploits and we have had to model carefully. **Ethanol** raises the tannin's solubility and slows precipitation, softening grip — part of why a 15% wine can taste rounder than its tannin load deserves, and part of why the lower-alcohol wines of Chapter 12 need their tannins designed finer to compensate. **Polysaccharides** — grape-skin rhamnogalacturonans and yeast-wall **mannoproteins** — coat tannins and compete for them, blunting astringency; lees aging works partly this way. **Low pH** sharpens grip; **higher viscosity and residual sugar** cushion it; **temperature** shifts the whole precipitation equilibrium. The IFI-A we publish is measured under fixed conditions precisely so that these modulators are held constant — but the winemaker lives in the space where they all move at once, and a designed tannin is only useful if we understand the matrix it will finally be tasted in.

One consequence matters enough to state plainly here, because it governs the whole book. **Astringency has no dedicated receptor, so it cannot be turned off at the tongue.** You cannot engineer a human mouth to stop feeling grip, the way one might imagine silencing a bitter receptor. The only place to intervene is upstream, in the tannin itself — its size, its chemistry, its quantity. The grape is the only lever. That is the entire reason this is an organism monograph and not a receptor one.

Chapter 2 · The mathematics of the pucker — *EC_{50}, and the structure that sets it.*

To put a number on grip we needed an EC_{50}, and astringency, being a precipitation rather than a receptor response, made us earn it. Our bench assay is a **turbidimetric titration**: a fixed quantity of a standard basic PRP — we use a synthetic analogue of the salivary peptide **IB-5**, one of the hungriest tannin-binders in human saliva — is titrated against increasing tannin, and the solution's turbidity, the cloud of precipitating complex, is read by light scattering at 650 nanometers. The tannin concentration producing half-maximal turbidity is EC_{50}: the concentration at which the tannin has pulled half of what it is ever going to pull out of solution. We cross-calibrate that bench number against a twelve-person sensory panel scoring astringency intensity on a defined scale, so that every IFI-A we publish is anchored to both a protein falling out of a cuvette and a human being telling us, blind, how much the sample dries the mouth.

The reference — the tannin we set to **1,000** — is deliberately humble: a grape-seed procyanidin fraction of mean degree of polymerization 4 and zero galloylation, the kind of ordinary, slightly green tannin a seed will give up in a careless press. Against that anchor the index runs from the near-nothing of a ripe table grape to the architectural tannins we now build on purpose.

Behind every IFI-A is a second instrument reading the tannin's actual structure, because a magnitude without a structure is a number you cannot design toward. We characterize every fraction three ways. **Quantity** we measure by the methylcellulose-precipitable-tannin assay and the classic Adams–Harbertson protein-precipitation method — how much tannin, in milligrams of epicatechin equivalent per liter, the sample holds. **Chain length and subunit composition** we read by **phloroglucinolysis** (acid-catalyzed cleavage in the presence of phloroglucinol): the interflavan bonds break, the extension subunits are captured as phloroglucinol adducts and the terminal subunits released free, and the ratio of the two, by HPLC, gives us mDP directly, along with the exact percentages of catechin, epicatechin, epigallocatechin, and galloylated units. **Grip itself** is the PRP-precipitation EC_{50} of the last chapter. The three together are the fingerprint; the IFI-A is its loudness.

A worked number makes the formula concrete. Our reference procyanidin reaches half-maximal PRP precipitation at an EC_{50} of about 0.80 grams per liter. A skin-dominant engineered fraction — mDP 11, 6% galloylation, 40% prodelphinidin — reaches half-max at 0.31 g/L. Then IFI-A = 1000 × 0.80 / 0.31 ≈ **2,580** — the magnitude of the Velvet line you will meet in Part V, derived from nothing but a

structure and a cuvette. The gap between seed and skin tannin, which a taster feels as the whole difference between green and ripe, is just as legible in the fingerprint:

SOURCE	TYPICAL MDP	% GALLOYLATION	% PRODELPHINIDIN	CHARACTER
Seed tannin	2–16	up to ~30%	~0%	small, galloylated, bitter, green
Skin tannin	10–85	low (<5%)	up to ~50%	long, prodelphinidin-rich, fine, ripe

Nearly every lever this book describes is a way of moving a grape's tannin from the top row toward the bottom one — or, with lines like Cathedral, somewhere no natural grape has ever sat.

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

A tannin that reaches half-maximal precipitation at one-quarter of the reference's concentration binds four times as hard per unit and scores **IFI-A 4,000**. The normalization ceiling for the scorecards, shared across all eight axes, is 5,000; above it, as we will see, a tannin stops being more astringent and starts being merely insoluble.



A cut berry: the clear flesh that becomes sugar and acid, the pigmented skin and the pale seed that between them hold almost the whole of the tannin. Grip is a geography problem before it is a chemistry one.

What sets EC_{50} is not how *much* tannin is present — that is concentration, the winemaker's extraction lever — but what *kind*. Three structural variables do almost all of the work, and the platform fits them to a single phenomenological law that has held across thousands of fractions:

$$EC_{50}(t)^{-1} \approx k \cdot (\text{mDP})^{0.6} \cdot (1 + 2.1 \cdot f_G) \cdot (1 + 0.8 \cdot f_{pD}) \cdot e^{(-\text{mDP} / 22)}$$

Read it left to right and it is the whole physics of grip:

- **mDP — mean degree of polymerization**, the average number of flavan-3-ol subunits per chain. More subunits means more binding faces, so potency rises with mDP — but only to a power

of about 0.6, sub-linearly, and the trailing exponential term bends the curve back down above the high teens, where the chains grow too large and hydrophobic to stay in solution long enough to bind protein. Astringency peaks, in our hands, around mDP 12–16 and then declines into a dusty, chalky *insolubility* that the palate reads as thinness, not grip. Bigger is not louder forever.

- **f_G — the galloylation fraction**, the share of subunits esterified with a gallic-acid group. Galloyl rings are flat, hydrophobic, and superb at stacking on proline; each point of galloylation is worth more than a point of anything else, which is why the coefficient is 2.1 — the steepest in the law. Seed tannins, heavily galloylated, punch far above their modest size.
- **f_pD — the prodelphinidin fraction**, the share of subunits carrying the trihydroxylated (epigallocatechin-type) B-ring rather than the dihydroxylated (epicatechin-type) one. Extra hydroxyls add hydrogen-bonding capacity and lift potency modestly, but — and this is the crucial subtlety of the next chapter — they shift the *character* of the grip far more than its magnitude.

STRUCTURAL LEVER	WHERE IT LIVES	EFFECT ON IFI-A	EFFECT ON GRAIN
mDP ↑ (to ~14)	skin > seed	↑ strongly	coarser, then fuller
mDP ↑↑ (>18)	over-polymerized	↓ (insoluble)	chalky, thin
Galloylation f_G ↑	seed	↑↑ (steepest)	green, hard, bitter edge
Prodelphinidin f_pD ↑	skin	↑ modestly	finer, softer, "velvet"

This is the equation the old vigneron was solving in the dark with a thumb and a seed between the teeth. Seeing it written out does not diminish the art; it tells you what the art was *about*. And it hands us, for the first time, four independent dials — quantity, length, galloylation, hydroxylation — any of which we can now set in the living vine before a single berry is picked.

Chapter 3 · The grain — *the two numbers a tannin needs.*

One number is not enough to describe a tannin, and the reason is the deepest thing we learned about this axis. **IFI-A tells you how loud the grip is. It does not tell you whether you want to be in the room with it.** A Nebbiolo and a badly made seed-heavy press wine can post nearly the same IFI-A; one is a cathedral and the other is chewing a tea bag. Loudness and quality are orthogonal, and a grape monograph that pretended otherwise would be worthless to the only people it is really for — the ones who make wine.

So every tannin in this book carries two marks: its **IFI-A**, the magnitude from Chapter 2, and its **grain**, a quality descriptor we define from the distribution underneath the mean. Grain is the difference between a tannin that arrives as a single coarse wall and one that arrives as thousands of fine points — same force, utterly different sensation. In the lab, grain resolves into three measurable things:

- **The mDP *distribution*, not its mean.** A tannin pool with mDP 9 can be a tight population clustered near 9 (fine, even, "silky") or a broad smear from 2 to 30 (a few bitter monomers up front, a few insoluble giants behind — "coarse," "disjointed"). We quantify it as the polydispersity of the chain-length histogram from phloroglucinolysis, and we have learned to breed the histogram narrow.
- **The galloylation-to-prodelphinidin balance.** Galloylation drives grip *green and hard*; prodelphinidin drives it *fine and velvet*. The ratio f_G : f_pD is, more than anything else, what a taster means by "the grain of the tannin." Seed-dominated, high-galloyl, low-prodelphinidin tannin is the green grip of an under-ripe or over-pressed wine. Skin-dominated, low-galloyl, high-prodelphinidin tannin is the cashmere of a great one.
- **The time-intensity shape.** The same precipitation can be front-loaded (immediate grip, quick fade) or back-loaded (slow build, long drying finish). We read it off the sensory time curve as rise time and persistence, and it tracks mDP and the degree of pigment-binding.

The great gift of having a structure behind every grip is that the winemaker's lexicon — a vocabulary refined over centuries and until now wholly subjective — resolves, term by term, into measurable chemistry. We keep a working map, and we tune to it:

TASTER'S WORD	STRUCTURAL CORRELATE
green, hard, aggressive	high galloylation, low mDP, seed-dominant, broad histogram
grippy, firm, structured	high mDP, moderate galloylation, tight histogram
fine, silky, velvety	high prodelphinidin, low galloylation, tight mid-high mDP
chalky, dusty, drying	over-polymerized (mDP > 18), near the solubility edge
hollow, soft, flabby	low total tannin, low mDP — the deficiency, not a virtue
dry, long, lingering	high pigment-binding, back-loaded time curve

Set those two numbers against each other — IFI-A on one axis, grain on the other — and every tannin on earth occupies a point on a plane. The natural grape is confined to a diagonal band across that plane: loud tannins come coarse, fine tannins come quiet, and the famous regions are famous precisely because they sit at the best points nature allows along that band. The ladder below is that band made explicit — the measured native profiles of the varieties a winemaker already knows by feel, read at last as numbers. It is the reference backbone of this book, and every engineered line in Part V is defined by where it departs from it:

VARIETY	NATIVE IFI-A	MDP	% GALLOYL	% PRODELPHINIDIN	GRAIN
Flame Seedless (table)	120	3	5%	low	faint
Pinot Noir	880	9	3%	42%	silky
Gamay	1,050	7	4%	35%	soft
Grenache	1,180	8	5%	30%	supple
Merlot	1,620	11	6%	33%	round
Sangiovese	1,950	13	9%	22%	firm, savory
Syrah	2,180	12	7%	38%	polished
Cabernet Sauvignon	2,600	14	10%	28%	structured
Nebbiolo	3,420	16	8%	35%	huge, fine
Sagrantino	3,750	17	11%	30%	powerful
Tannat	3,950	18	12%	32%	massive

Read the galloylation column down against the IFI-A column and the diagonal is right there in the data: the loudest natural tannins, Tannat and Sagrantino, carry their power on high galloylation and high mDP, and pay for it in a grain that needs years or a great kitchen to tame; the finest, Pinot, buys its silk with modest magnitude and a high prodelphinidin fraction. Nebbiolo is the marvel precisely because it sits a little *above* the band — large and, for its size, improbably fine — which is why Barolo is spoken of the way it is, and why it was the natural profile we most wanted to understand before we tried to exceed it. What the platform does, mechanically, is lift tannins further *off the diagonal* — into the upper-left corner nature forbids,

where a tannin is both very loud and very fine. Cathedral is a flag planted in that corner, past even Nebbiolo. Everything in Part II is the machinery that let us walk there.



The grain of a tannin, captured at magnification during fraction QC. The histogram of chain lengths behind a single IFI-A number is where "green" and "silky" actually live.

Here is the move that the whole platform exists to make, stated once so the rest of the book can lean on it: **in the natural grape, magnitude and grain are chained together, and we have unchained them.** A vine cannot ordinarily give you a very high tannin that is also very fine — high tannin means long chains and seed contribution, which drag in galloylation and coarseness; fineness means skin-dominant, prodelphinidin-rich tannin, which tends to come in gentler amounts. Nature offers a trade-off curve, and every classic wine region is a particular hard-won point on it. By setting the enzymes that choose length, galloylation, and B-ring hydroxylation independently — the subject of Part II — we can place a tannin anywhere on the plane of *loud × fine*, including the corner nature keeps locked: enormous and silky at once. That corner is a grape we named **Cathedral**, and you will meet it in Part V.

We want to be careful, in a book for winemakers, not to oversell the number. Grain, even doubly quantified, is still a compression of something a great palate reads in full. The index is an instrument, not an oracle. But an instrument is what the craft never had, and it changes what is sayable. A winemaker can now ask us, in the vineyard planning meeting, for *IFI-A 2,600, grain fine, f_G under eight percent, histogram tight at eleven* — and receive a design targeting those specifications, with batch-to-batch variation held by design to under seven percent. That sentence was not utterable five years ago. The rest of this book is how it became one.



PART II OF VI

The Molecule

the flavonoid pathway · ANR at the fork · polymerization, transport, and the bitter monomer

Chapter 4 · The flavonoid pathway, drawn — *from phenylalanine to the fork.*

Every tannin, every anthocyanin, every drop of color and grip in every wine ever made begins at the same molecule the whole platform begins at: **phenylalanine**, and the general phenylpropanoid entry that our capsaicin work made familiar. The reverence we feel here is partly that: the pepper and the grape, the burn and the pucker, run their first three steps down the identical corridor before parting. The platform was never about peppers. The pathway said so first.

The general phenylpropanoid steps, shared by essentially every vascular plant:

STEP	ENZYME	SUBSTRATE → PRODUCT
1	**PAL** — phenylalanine ammonia-lyase	L-phenylalanine → *trans*-cinnamic acid (+ NH_3)
2	**C4H** — cinnamate 4-hydroxylase (CYP73A)	cinnamic acid → *p*-coumaric acid
3	**4CL** — 4-coumarate:CoA ligase	*p*-coumaric acid → *p*-coumaroyl-CoA

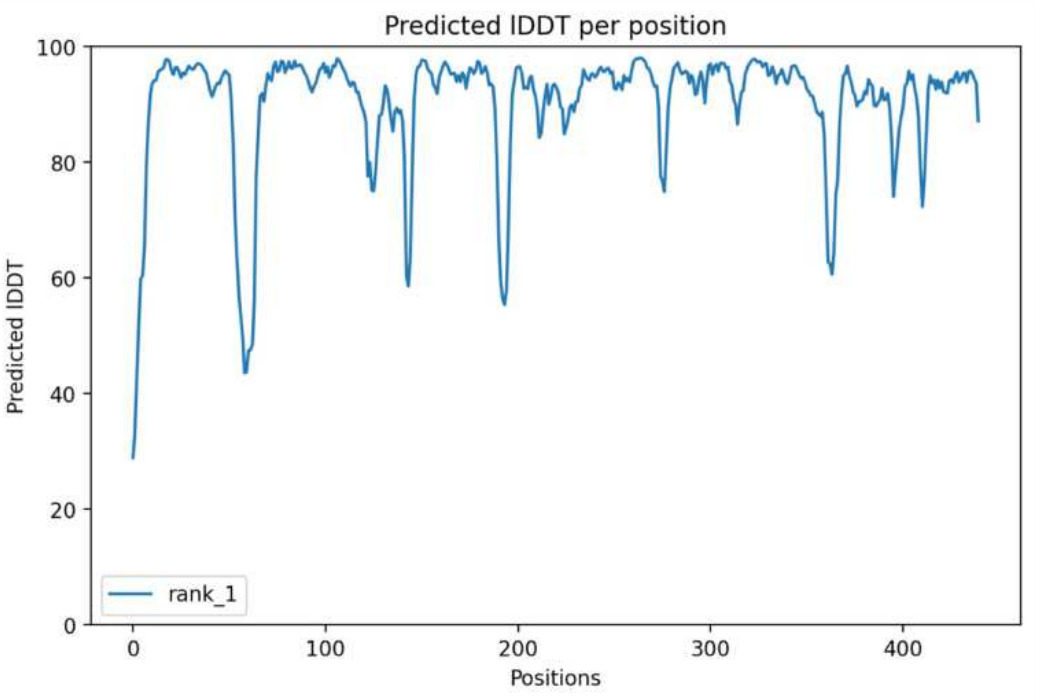
From *p*-coumaroyl-CoA the road forks toward the flavonoids, and here the grape asserts its own chemistry:

STEP	ENZYME	SUBSTRATE → PRODUCT
4	**CHS** — chalcone synthase	*p*-coumaroyl-CoA + 3 malonyl-CoA → naringenin chalcone
5	**CHI** — chalcone isomerase	naringenin chalcone → naringenin (a flavanone)
6	**F3H** — flavanone 3-hydroxylase	naringenin → dihydrokaempferol (DHK)
7a	**F3′H** — flavonoid 3′-hydroxylase	DHK → dihydroquercetin (DHQ)
7b	**F3′5′H** — flavonoid 3′,5′-hydroxylase (CYP75A)	DHK/DHQ → dihydromyricetin (DHM)
8	**DFR** — dihydroflavonol 4-reductase	DHK/DHQ/DHM → leucoanthocyanidins (flavan-3,4-diols)
9	**LDOX/ANS** — leucoanthocyanidin dioxygenase / anthocyanidin synthase	leucoanthocyanidin → anthocyanidin (cyanidin, delphinidin)

Step 7 is quietly one of the most consequential in the grape. **F3′H** hydroxylates the B-ring once; **F3′5′H** hydroxylates it twice. That single extra hydroxyl is the branch between the procyanidin world (catechin, epicatechin — two B-ring hydroxyls, eventually red cyanidin pigments) and the prodelphinidin world (gallocatechin, epigallocatechin — three hydroxyls, eventually blue-purple delphinidin pigments). Remember from Chapter 3 that prodelphinidin content is the *grain* dial — the finer, velvet character — and

remember it is also the *color* dial, blue versus red. In the grape, the texture of the tannin and the hue of the wine are set by the **same enzyme** at the **same step**. Nature bundled them; we will spend Chapter 8 learning when to keep them bundled and when to prise them apart.

Two more branches leave this same trunk, and a grape monograph should name them because they share the enzymes — and the carbon — with the tannin we are designing. From dihydroflavonols, **flavonol synthase (FLS)** draws off **quercetin, kaempferol, and myricetin**, the pale-yellow flavonols that the berry makes as a UV sunscreen in its skin and that, in wine, act as **co-pigments** — stacking face-to-face with anthocyanins to deepen and stabilize color, a quiet partner in the hue work of Chapter 8. And from the CHS node, a single enzyme swap — **stilbene synthase (STS)** using the same *p*-coumaroyl-CoA and malonyl-CoA substrates — makes **resveratrol**, the stilbene the vine throws up against fungal attack and the most famous molecule in the grape's pharmacology. Every one of these branches competes for the same upstream flux, which is why the flux-balance model of Chapter 5 matters: push hard on tannin and you draw carbon away from pigment, flavonol, and stilbene, and a thoughtful design budgets the whole phenylpropanoid output rather than maximizing one line of it. The reactions, too, are compartmentalized — the early steps on the cytoplasmic face of the endoplasmic reticulum, organized into a loose **metabolon** that channels intermediates hand to hand, the later storage in the vacuole — and that physical organization is part of why dosing a regulator moves the whole branch in concert, the subject two chapters on.



The platform's structure-confidence pipeline, shown on the pepper Pun1/AT3 acyltransferase. The identical pLDDT and PAE workflow modeled every grape flavonoid enzyme — ANR, LAR, DFR, F3′5′H — before a single variant was synthesized.

And then, at the leucoanthocyanidin, comes the fork that this whole book turns on — the branch point between **color** and **tannin**, between the anthocyanin that makes a wine red and the flavan-3-ol that makes it grip. On one side, LDOX carries the leucoanthocyanidin on to anthocyanidin and, by glucosylation, to pigment. On the other side, two reductases divert the flux into the flavan-3-ol monomers

that will polymerize into the condensed tannins. Those two reductases — **LAR** and **ANR** — are the most important enzymes in the grape for everything this book cares about, and the next chapter belongs entirely to the second of them.

Chapter 5 · ANR, the enzyme at the fork — *how the vine chooses its grip.*

If you asked us to point at the single protein where the design of a wine's tannin is most decided, we would point at **anthocyanidin reductase — ANR**, the product of the gene the first plant geneticists to find it named *BANYULS*, after the sweet, dark, tannic wine of the French village where the mutant vine's seed coat first gave the phenotype away. ANR is the enzyme that makes the grape's defining tannin subunit, and it is the enzyme at the center of our 1,610 constructs.

The two reductases at the fork do related but distinct chemistry, and the distinction is the design space:

ENZYME	SUBSTRATE	PRODUCT	STEREOCHEMISTRY	ROLE IN THE CHAIN
LAR — leucoanthocyanidin reductase	leucoanthocyanidin (flavan-3,4-diol)	(+)-catechin / (+)-gallocatechin	2,3-*trans*	terminal unit (chain-stopper)
ANR — anthocyanidin reductase	anthocyanidin (cyanidin / delphinidin)	(−)-epicatechin / (−)-epigallocatechin	2,3-*cis*	extension unit (chain-builder)

Read the right-hand column and the whole architecture of a tannin falls out of it. **LAR makes catechin, the 2,3-*trans* isomer, which tends to sit at the end of a chain and stop it. ANR makes epicatechin, the 2,3-*cis* isomer, which is the dominant *extension* subunit — the link that keeps the polymer growing.** The ratio of their activities, LAR : ANR, is therefore the ratio of chain-stoppers to chain-builders, and that ratio sets the mean degree of polymerization — the mDP that Chapter 2 told us is the loudest single lever on grip. Push ANR up or LAR down and chains run longer; the tannin grows larger and, up to the solubility ceiling, more astringent. Reverse it and chains stay short, monomeric, and — the warning of Chapter 7 — bitter.

There is a second gift in ANR's chemistry, and it is the one the task of making *fine* tannin depends on. ANR acts on the *anthocyanidin* — on cyanidin to give epicatechin, and on **delphinidin to give epigallocatechin**, the prodelphinidin subunit that Chapter 3 named as the velvet dial. So ANR does not merely set how long the chain is; working on whichever anthocyanidin the F3′5′H branch has handed it, it also sets how much of the fine, trihydroxylated subunit the chain contains. One enzyme, two of our four dials: length, through the LAR:ANR balance, and grain, through its delphinidin-derived product. No wonder the *banyuls* mutant, losing it, loses both its tannin and its dark seed at once.

ANR's chemistry is subtler than a single reduction, and the subtlety is useful. It is a double-bond reductase that also, in the process, **epimerizes** — it takes the 2,3-*trans* configuration of its anthocyanidin substrate and delivers the 2,3-*cis* flavan-3-ol, which is why its product is *epi*catechin and not catechin, and why the chains it builds have the stacking geometry that polymerizes well. The enzyme is strictly **NADPH-dependent**, consuming reducing power the berry must also supply, so that tannin yield is quietly coupled to the fruit's redox budget — another term the flux model tracks. Its expression is beautifully timed: in the grape, *VvANR* runs hard through the green Stage I, in both the developing seed coat and the skin, exactly when Chapter 11 will show the tannins being laid down, and quiets after véraison — so that the window to influence tannin *amount* closes early, while the window to influence its *ripening structure* stays open to harvest. The grape carries a single functional *VvANR* against **two** leucoanthocyanidin reductases, *VvLAR1* and *VvLAR2*, and that asymmetry — one chain-builder, two chain-stoppers — is itself a design surface: the two LAR paralogs are expressed in different tissues and at different times, so that trimming one (as the VvLAR2 dosage edit of Chapter 15 does) reshapes the chain-length histogram without touching the other's role. Where ANR is the accelerator, the two LARs are independent brakes, and we learned to ride them separately.

ANR is a member of the NADPH-dependent reductase-epimerase-dehydrogenase superfamily — a Rossmann-fold protein binding its nicotinamide cofactor across a classic dinucleotide-binding groove, with a substrate pocket that must accommodate the flat, planar anthocyanidin cation and deliver hydride to it with the correct face selectivity to yield the 2,3-*cis* product. That geometry is exactly the kind our structure pipeline was built to read. We modeled VvANR and both grape LAR paralogs — VvLAR1 and VvLAR2 — with the same AlphaFold-class workflow shown above on Pun1, scoring every residue by pLDDT confidence and every domain pair by predicted aligned error, and then let a 650-million-parameter protein language model, ESM2, score every possible single-residue substitution in the active-site shell for its predicted effect on catalytic efficiency and fold stability. A genome-scale flux-balance model of grape-berry metabolism predicted, before we touched a vine, that the flavan-3-ol branch itself — not an upstream phenylpropanoid step — would be the primary control point for tannin yield in the cultivars we cared about. We were, in other words, able to know which letters of *VvANR* to change, and why, from a screen before we grew anything. The three edits that came out of it are Chapter 15's subject; that they exist at all is ANR's.

Chapter 6 · Polymerization, transport, and the vacuole — *the part the vine still keeps.*

Honesty is a house value, so we will say plainly where the vine is still ahead of us. We can make the subunits — catechin, epicatechin, their gallocatechin cousins — with precision. We can set the ratio that governs chain length. But the actual *polymerization* of condensed tannin, the covalent linking of those subunits into chains, remains the one step in this pathway that no one, including us, can draw with full confidence. It is the open problem of grape biochemistry, and we would rather tell you that than pretend the map is finished.

What is known is enough to engineer by, if not enough to satisfy. The flavan-3-ol monomers are made in the cytosol and must be moved into the **vacuole**, the great acidic storage compartment where tannins and anthocyanins are kept, and where — the best current evidence says — much of the polymerization happens, driven by the vacuole's low pH and by oxidative coupling. Three machines carry and condense them:

- **GST (glutathione S-transferase) — VvGST4.** Despite the name, it is not acting as a classic detoxifying transferase here; it appears to work as a cytoplasmic *carrier*, a ligandin that binds the flavonoid and ferries it to the tonoplast membrane. Knock it down and both anthocyanin and proanthocyanidin collapse — the subunits are made but never reach the vacuole. It is a bottleneck, and therefore a lever.
- **MATE transporters** — the multidrug-and-toxic-compound-extrusion family, including the grape *anthoMATE* proteins (VvAM1, VvAM3) for acylated anthocyanins and MATE-type carriers implicated in proanthocyanidin precursor transport. These are the doors in the tonoplast; they set the rate at which the vacuole fills.
- **Oxidative coupling** — the still-blurry condensation step, likely involving **laccases (VvLAC)** and **peroxidases** in the vacuole, driving the linkage of subunits and, over time, the cross-linking that keeps raising mDP even after the fruit is picked.

Governing all of it is a regulatory circuit of real beauty, the **MBW complex** — a ternary partnership of a **MYB** transcription factor, a **bHLH** factor, and a **WD40** scaffold protein that together switch the pathway's genes on. In the grape, the proanthocyanidin branch has its own dedicated MYB activators:

REGULATOR	TYPE	CONTROLS
VvMYBPA1	R2R3-MYB	proanthocyanidin branch — activates LAR, ANR, and upstream genes; expressed through early berry and seed
VvMYBPA2	R2R3-MYB	proanthocyanidin branch in skin
VvMYBPAR	R2R3-MYB	proanthocyanidin-specific activation
VvMYBC2-L1 / L3	R2R3-MYB	repressors — the brakes on the circuit
VvMYC1, VvMYCA1	bHLH	partner factors for both PA and anthocyanin branches
VvWDR1 / VvWDR2	WD40	the scaffold completing the complex

The circuit's logic rewards study. The **MYB** chooses *which* branch — PA or anthocyanin — by which target promoters it recognizes; the **bHLH** supplies activation strength; the **WD40** is the structural chaperone that holds the pair productive. Activators and repressors compete for the same bHLH partners, so the pathway's output is not a simple switch but a *balance* — VvMYBPA1 pulling flux into tannin, the VvMYBC2 repressors damping it, the ratio setting the tone. This is why dosage is such a clean lever: nudging a single activator's abundance shifts the whole competitive equilibrium, and the enzymes, transporters, and condensation machinery downstream all move together because they all read the same complex. It is also why the system responds to **epigenetic** state — promoter DNA methylation and histone marks at the MYBA and MYBPA loci modulate how loudly the circuit runs, and part of the vintage-to-vintage swing a vineyard suffers is the circuit reading the season's stresses through exactly these marks. A designed line fixes the tone and so fixes the swing; that is where much of the seven-percent CV comes from.

We should name the open problem precisely, because candor about it is part of the science. We can make the subunits, set their ratio, load them into the vacuole, and tune the regulatory tone over all of it — but the **terminal condensation**, the actual forging of the interflavan C4→C8 and C4→C6 bonds into a polymer, has no cleanly identified "proanthocyanidin polymerase." The best current picture is non-enzymatic or semi-enzymatic oxidative coupling in the vacuolar environment, perhaps guided by laccase-type oxidases, perhaps templated on the vacuolar membrane, proceeding partly *after* the cells have done their work — which is why mean polymerization keeps creeping upward in the skin for weeks and even, slowly, in the finished wine. We engineer the supply and the conditions and let the condensation find its own equilibrium, and the results hold to spec. But we would be poor scientists to pretend we had drawn the last arrow when we have not, and the family rule is that we would rather show you the edge of the map than paint over it.



Trays of regenerating lines under controlled light. Most of the grape program is not editing a single enzyme but dosing a regulator — turning VvMYBPA1 up by a measured amount and letting the whole proanthocyanidin branch answer.

The regulators are why the program is as large as it is. A single enzyme edit changes one reaction; a measured change in **VvMYBPA1 dosage** moves the entire branch — supply of subunits, transport, the lot — in concert, and most of the elegant results in Part V come from nudging a regulator by a known amount rather than hammering an enzyme. It is also why we can be honest about the polymerization gap and still deliver tannins to spec: we do not need to understand the final condensation perfectly if we can set the subunit supply, the length-ratio, the transport rate, and the regulatory tone that the condensation reads from. We steer the river without yet mapping every eddy. The vine keeps a secret; we have learned to work beautifully around it.

Chapter 7 · The monomer problem — *why great grip and bitterness are enemies in the same molecule.*

There is a cruelty built into the flavan-3-ol, and any honest book about designing grape tannin has to put it at the center rather than the footnotes. **The same molecules that make astringency, in their small forms, make bitterness** — and bitterness, unlike astringency, *does* have a receptor, which means it cannot be waved away as mere mouthfeel. It is a taste, read loud and clear, and it is the thing that most often separates a tannin you want from one you don't.

The chemistry is specific. Flavan-3-ol **monomers** — catechin, epicatechin — and the small **oligomers**, dimers and trimers, are bitter: they are the right size and shape to fit the pocket of the bitter receptor **TAS2R** (and the broadly-tuned **TAS2R38** our index anchors the bitterness axis to), and the palate reads them as the hard, lingering bitterness of an over-extracted red or a green seed. As the chain grows past three or four subunits, the molecule becomes too large to engage the receptor well, and its character crosses over: it stops being *bitter*, a taste, and becomes *astringent*, a precipitation. Somewhere around the tetramer, the grape's own chemistry hands the sensation from one system to the other.

> Short chains (mDP 1–3): bitter, TAS2R-active — **IFI-B high** Long chains (mDP ≥ 5): astringent, PRP-precipitating — **IFI-A high**

The crossover is gradual, and mapping it is part of what the Zero-Bitter program sells. Human bitterness is read not by one receptor but by a family of about twenty-five **TAS2R** subtypes, broadly tuned and overlapping, and the flavan-3-ols engage several — catechin and epicatechin are detectably bitter at roughly 0.4–1 millimolar, well within the concentration a seed-heavy red reaches, and their galloylated forms are bitterer still. As subunits add, the molecule's growing size and conformational bulk price it out of the receptor pockets, and by the pentamer the bitterness has largely surrendered to astringency:

MDP	DOMINANT SENSATION	RECEPTOR / MECHANISM	AXIS
1 (monomer)	bitter	TAS2R (several subtypes)	IFI-B
2–3 (oligomer)	bitter, faint grip	TAS2R, onset PRP binding	B → A
4–5	grip with bitter edge	crossover	A ≈ B
6–12	astringent, clean	PRP precipitation	**IFI-A**
>18	chalky, thin	over-precipitation, insoluble	A falling

Two complications keep the program honest. First, **ethanol enhances the bitterness** of the small flavan-3-ols even as it softens their astringency — so the same monomer pool that is tolerable in grape juice can turn hard in a 14% wine, and a Zero-Bitter line must be designed against the alcohol it will finally be tasted at. Second,

bitterness and astringency are perceptually entangled; a wine the taster calls "bitter" is often one whose astringency arrived without enough fruit or body to frame it, and part of the fix is the kokumi and sweetness framing of the multi-axis work rather than the monomer count alone. We drain the monomer pool because it is the cleanest single lever — and we hold, always, a deliberate remainder, because a wine with no bitterness at all has lost its spine along with its sting.

This is the crossover that the grape's **IFI-B** axis — the Zero-Bitter program — exists to exploit. We want the grip without the bitterness; we want the flux that would otherwise pool as bitter monomers to run *into* the chain instead, emerging as astringency, where we can place it with craft. That is a composition problem, and it is worth being precise about what it is *not*. We are not editing the human bitter receptor — the grape never touches the eater. We are changing the **ligand**: engineering the berry so that fewer bitter monomers and small oligomers accumulate, so that the TAS2R38 in your mouth simply finds less to bind. The index records the result as a lower IFI-B, a designed 20–65% reduction in predicted bitterness, but the mechanism lives entirely in the fruit.

The lever is the one Chapter 5 gave us: the **LAR : ANR balance** and the regulatory tone over it. Push toward ANR-driven extension, hold LAR's chain-stopping in check, keep VvMYBPA1 supplying the branch, and the monomer pool drains into polymer. The green, bitter edge of an under-ripe seed tannin — which is monomer-and-galloyl-rich — gives way to a longer, cleaner, astringency-forward tannin. In the Zero-Bitter grape lines, our designs target a monomeric flavan-3-ol fraction reduced by half while holding total tannin and total grip constant: the same force on the palate, with the bitterness drawn off like foam from a ferment.

There is a limit, and we respect it in the design. A little monomer, a little bitterness, is *structure* — it gives a wine its spine and its savor, and a tannin stripped of all of it tastes flabby and sweetly simple, a fruit drink with grip. The goal is never zero. It is **placement**: the bitterness set low and deliberate, the astringency set where the winemaker asked, the two decoupled at last from the accident of chain length that in a natural grape forces you to take them together. The monomer problem is not solved by abolishing the monomer. It is solved by finally being allowed to choose how much of it you keep.



PART III OF VI

Color & Scent

anthocyanins and the MYBA locus · the green note and the pepper note · thiols, terpenes, and petrol

Chapter 8 · Color — *the pigment that shares a pathway with the grip.*

Pour a young red and tip the glass against white: the rim is a violet so saturated it looks wet even dry, and that color is, molecule for molecule, the grip's own sibling. We ended Chapter 4 at the fork where leucoanthocyanidin chooses between tannin and pigment; this chapter follows the pigment road, because in the grape you cannot design one without disturbing the other, and a winemaker who is given a tannin spec will, the same afternoon, want to talk about color.

The pigments are the **anthocyanins** — anthocyanidins dressed in sugar. The step that makes them is a single glucosyltransferase:

STEP	ENZYME	SUBSTRATE → PRODUCT
10	**UFGT** — UDP-glucose:flavonoid 3-O-glucosyltransferase	anthocyanidin → anthocyanidin 3-O-glucoside (a stable, soluble pigment)
11	**AOMT** — anthocyanin O-methyltransferase	peonidin, petunidin, **malvidin** 3-glucosides (methylated, more stable hues)
12	**acyltransferases** → acylated anthocyanins (still more stable, bluer)	

Which anthocyanidin arrives at UFGT is set, again, by the B-ring hydroxylation of Chapter 4: F3′H flux gives cyanidin-type pigments, red; **F3′5′H** flux gives delphinidin, petunidin, and malvidin — the blue-purple, more stable, *Vitis vinifera* signature. In a great dark red, malvidin-3-glucoside dominates, and it is no coincidence that the same F3′5′H branch that bluens the color also, through ANR's action on delphinidin, finens the tannin. **Color and grain ride the same enzyme.** Breed a vine bluer and you tend to breed its tannin silkier; this is a gift when you want both and a constraint when you want only one, and much of our color-program work (180 of the 1,610 constructs) is precisely about uncoupling the two where a winemaker needs, say, a deep color with a firmer, more savory grain than the delphinidin route would naturally give.

The whole switch sits under one of the most elegant loci in crop genetics, the **VvMYBA** cluster on chromosome 2 — the master regulator of UFGT. Its natural history is worth telling, because it taught us how reversible the grape's color is. **Every white wine grape is a broken red one.** The common ancestor of cultivated grapes was dark; white cultivars arose when a Gypsy-type retrotransposon, **Gret1**, inserted into the promoter of *VvMYBA1* and silenced it, and a second, independent pair of mutations knocked out the neighboring *VvMYBA2* — two hits, both needed, both carried forward through millennia of vegetative propagation. Chardonnay and Riesling are, at this locus, mutants in which the red switch is simply off. That the whole of white winemaking rests on a jumped piece of selfish DNA is the kind of fact that makes us love this plant; that the switch is a clean, known, bistable thing is what makes color one of the more tractable dials in the program.

The pigment itself is a small, telling family. Grapes build five anthocyanidins, all as their 3-*O*-glucosides and, in *Vitis vinifera*, further acylated and methylated, and the balance among them is the hue:

ANTHOCYANIDIN	B-RING	HUE	WHERE IT LEADS
Cyanidin	2-OH	red	procyanidin side; paler reds
Delphinidin	3-OH	blue-red	prodelphinidin side; depth
Peonidin	methylated cyanidin	red	warmer reds
Petunidin	methylated delphinidin	blue-red	–
Malvidin	di-methylated delphinidin	**blue-purple, most stable**	the signature of great dark reds

Color, infuriatingly and wonderfully, is not fixed by the molecule alone; it is an equilibrium. The anthocyanin flips between a red **flavylium** cation at low pH, a colorless hemiketal as pH rises, and blue quinonoidal forms — so the *same* pigment load looks brighter in a low-pH wine and washed-out in a high-pH one, which is one more reason the acid work of Chapter 11 and the color work of this chapter cannot be designed apart. **Co-pigmentation** — the flavonols of Chapter 4 stacking against the flavylium and shielding it — holds more of it in the colored form, and **sulfur dioxide**, the winemaker's universal preservative, reversibly *bleaches* anthocyanin by adding to the flavylium, so a young wine's color is always partly hidden under its SO_2 and returns as the free pigment condenses into the SO_2-resistant polymeric pigments of aging. Designing color, in other words, is never designing a number; it is designing a molecule's whole behavior across the pH, the co-pigment pool, and the long slow condensation that Chapter 16 follows to its garnet end.

What the pigment does *after* fermentation closes the loop back to astringency, and sets up Chapter 16. In wine, anthocyanins and tannins do not stay apart. They condense — directly, and through acetaldehyde ethyl bridges — into **polymeric pigments**, and they form the stable **pyranoanthocyanins**, vitisin A and vitisin B, from reactions with pyruvate and acetaldehyde thrown off by the yeast. These combined molecules are what carry a mature wine's color from violet toward garnet and hold it there for decades — and, crucially, a tannin bound up in a polymeric pigment **is a tannin that no longer grips as hard.** Color stabilization and tannin softening are the same aging reaction seen from two sides. When we design a grape's anthocyanin load and its tannin together, we are designing not only how the young wine looks and feels but the *rate and grace* with which it will let go of its grip over twenty years. That is the longest-horizon thing we do, and we will come back to it.

Chapter 9 · The green note and the pepper note — *methoxypyrazine, rotundone, and the aromas we dial.*

A grape is not only texture and color; it is scent, and two aroma molecules in particular behave so much like switches — present or absent, loved or hated, and biosynthetically single-gene enough to design — that they deserve a chapter before the subtler aromatics of the next. Both are *potent* past the point of intuition: they are felt at concentrations where there are only a handful of molecules per breath, which makes them extraordinary levers, a few parts per trillion changing the whole character of a wine.

The **green note** is **methoxypyrazine**, chiefly **3-isobutyl-2-methoxypyrazine, IBMP** — the unmistakable smell of green bell pepper, of a snapped stem, of Sauvignon Blanc gooseberry and of Cabernet before it is ripe. Its odor threshold is around **2 nanograms per liter**, roughly two parts per trillion; a wine at 15 ng/L reeks of capsicum, a wine at 8 ng/L is pleasantly herbaceous, and the gap between them is a rounding error of matter. IBMP is made by the methylation of a hydroxypyrazine precursor by an O-methyltransferase, **VvOMT3**, and it accumulates in the green berry and then **declines through ripening and especially with sunlight on the fruit** — which is the whole reason warm vintages and leaf-pulling reduce the green character. For a vine grower it is a thing to be managed by canopy and prayer. For us it is a dial: VvOMT3 expression up for a wine that wants to keep a cool-climate herbal lift in a hot site, or down for a site that cannot ripen the pyrazine away on its own.



The research plot at dusk. Methoxypyrazine falls with light and heat on the berry; for ten thousand years the only dial on the green note was where you planted and how you pruned.

The **pepper note** is **rotundone**, and it is the one that most astonishes people who smell the pure compound: a single sesquiterpene ketone that *is* the smell of freshly ground black pepper, the defining top note of a great Syrah and of Grüner Veltliner, Duras, and a handful of others. Its threshold is near **16 ng/L**, and — a lovely quirk of human biology — a meaningful fraction of people are specifically anosmic

to it, unable to smell it at all, so that the same Syrah is peppery to one taster at the table and merely dark-fruited to another. Rotundone forms by the oxidation of the sesquiterpene **α-guaiene**, catalyzed by a cytochrome P450 of the grape skin, and it builds *late* and in *cool* conditions — the mirror image of methoxypyrazine's light-driven decline. The anosmia is the quiet lesson in it: a designed aroma is not a sensation we control but a molecule we place, and who smells it, and as what, stays gloriously the drinker's own.

The biosynthesis of the green note is worth one more layer, because it is the cleanest single-gene story on the aroma shelf. The methoxypyrazines begin from an amino acid — valine or leucine, condensed with a dicarbonyl — to make a **hydroxypyrazine**, which is odorless; it is the final *O*-methylation by **VvOMT3** that turns it into the volatile, screaming-green IBMP. So the whole green character hangs on one methyltransferase's activity, and the berry's natural decline of IBMP through ripening is partly a decline in that enzyme and partly photochemical breakdown under sunlight — which is why a half-hour's measured leaf removal at the right phenological moment can halve the pyrazine in the finished wine. The quantitative lever is steep: every doubling of VvOMT3 output moves IBMP by roughly a factor that crosses the perceptual threshold in a single step, so the dial is sensitive and must be set with a light touch. In our warm-site aromatic whites and Cabernets, the VvOMT3 knock-down of Chapter 15 holds the pyrazine *down* where a hot season would otherwise have burned it off unpredictably — not to erase the character but to make its absence, or its measured presence, a choice rather than an accident of the weather.

COMPOUND	NOTE	ODOR THRESHOLD	VARIETAL HOME	OUR LEVER
IBMP (methoxypyrazine)	green bell pepper	~2 ng/L	Cabernet, Sauvignon Blanc, Carménère	VvOMT3 dosage
Rotundone	black pepper	~16 ng/L	Syrah, Grüner Veltliner, Duras	α-guaiene P450 route

What we will not do with these is pretend they are free. An aroma is a signature of place as much as of plant — the green of a Loire Sauvignon and the pepper of a Northern Rhône Syrah are *terroir*, written partly in climate, and a grower may rightly want to keep them chancy and vintage-bound rather than fixed by a gene. We offer the dial; we do not insist anyone turn it. The grape program holds about 110 constructs across all of its aroma work, and the ethic of the aroma constructs is lighter-handed than the tannin ones: nudge, do not nail. Scent is where a wine keeps its weather.

Chapter 10 · Thiols, terpenes, and petrol — *the aromas that hide until the ferment.*

The aromas of the last chapter were switches. The ones in this chapter are subtler, and more interesting to design, because many of them do not exist in the intact grape at all. They sit in the berry as **odorless bound precursors** — glycosides, cysteine and glutathione conjugates — and are only *released* during crushing and fermentation, when the yeast's enzymes snip the aromatic free. Designing them means designing a *potential*, a cellar of scent locked in the fruit that the ferment will open. There is something we find almost moving in that: the grape carrying its best smells folded shut, trusting the winemaking to unfold them.

The thiols are the tropical, pungent, electric aromas of Sauvignon Blanc and, increasingly prized, of other whites and even rosés: **3-mercaptohexan-1-ol (3-MH)**, grapefruit and passionfruit; its acetate **3-MHA**, more passionfruit still; and **4-mercapto-4-methylpentan-2-one (4-MMP)**, box tree and blackcurrant bud. Their thresholds live in the **nanograms per liter**, and in the grape they are almost entirely bound — 3-MH exists as a cysteinylated and glutathionylated conjugate that the yeast's carbon-sulfur **β-lyase** cleaves during fermentation to liberate the volatile thiol. The design target, therefore, is not the thiol but the **conjugate pool and the glutathione supply** in the berry, plus the yeast's lyase capacity — a two-organism problem, grape and *Saccharomyces*, that we treat as one system. On the grape side we tune the **glutathione** reservoir and the conjugate-forming machinery so the precursor is abundant; on the yeast side, the lyase encoded by genes like *IRC7* governs how much of that reservoir is actually cut free during a given ferment. A rich precursor pool wasted on a low-lyase yeast makes a dull wine from a promising grape, so the clean-living discipline we bring to the vine extends to pairing it with the right ferment — the two organisms designed as one chain from sunlight to glass.

The monoterpenes are the floral, Muscat, rose-and-citrus aromas of Muscat itself, Gewürztraminer, and Riesling: **linalool, geraniol, nerol, α-terpineol, citronellol**, with thresholds in the tens to hundreds of micrograms per liter. Unlike the thiols, a good share is present free in the ripe berry, but much again is **glycosidically bound**, awaiting acid or enzymatic hydrolysis in fermentation and aging to release a second wave of floral lift. The terpene supply traces to the plastidial **MEP pathway** and the terpene synthases; a famous single mutation in **VvDXS** (1-deoxy-D-xylulose-5-phosphate synthase) is most of what separates aromatic Muscat-type grapes from neutral ones — one letter, roughly, standing between a floral grape and a quiet one.

The norisoprenoids are the strangest and, to us, the most beautiful, because they are **debris** — fragments of the carotenoid pigments the berry made for photosynthesis and then tore down as it ripened,

cleaved by carotenoid-cleavage dioxygenases into C13 ketones of staggering potency:

COMPOUND	NOTE	THRESHOLD	ORIGIN
β-damascenone	baked apple, rose, stewed fruit	~50 ng/L	carotenoid cleavage
β-ionone	violet	~90 ng/L	carotenoid cleavage
TDN (1,1,6-trimethyl-dihydronaphthalene)	**kerosene / petrol**	~2 µg/L	carotenoid-derived, builds with age
vitispirane	camphor, eucalyptus	~ µg/L	carotenoid-derived

TDN is the one wine drinkers argue about: the petrol note that aged Riesling famously develops, loved by its devotees and alarming to the uninitiated, rising from a precursor over years in bottle and amplified by sun on the vine. That a wine should smell, gorgeously, of kerosene because a grape spent a hot afternoon dismantling its own sunscreen is the kind of fact that keeps us humble about ever "finishing" this plant. We can raise or lower the carotenoid pool and bias the cleavage, and so set how much petrol a Riesling will grow into — but we design these the way the last chapter prescribed, with a light hand, because the whole genre of age-worthy aromatic white is a bet on time, and we are not in the business of taking the suspense out of it. We are in the business of making sure the potential is *there*, folded shut in the fruit, for the cellar to open.



PART IV OF VI

The Berry & The Vine

the double sigmoid · véraison · the ripeness paradox and the lower-alcohol frontier

Chapter 11 · The double sigmoid — *how a berry becomes everything at once.*

To design a grape you have to know when, in its short life, each thing you care about is written — because almost nothing about a berry is set at a single moment, and most of what matters is laid down early and then merely *changed*. The berry's growth traces one of the cleaner curves in botany, a **double sigmoid**: two S-shaped growth phases with a flat lag between them, and the whole chemistry of wine hangs on which phase each compound belongs to.

Stage I — the green berry (roughly the first 60 days after flowering). The berry grows fast, first by cell division and then by expansion, and it is hard, green, and sour. This is when the **tannins are built.** Proanthocyanidin synthesis runs at its height in both skin and seed; VvMYBPA1 is at its peak; the flavan-3-ol subunits are made and polymerized and largely *finished* by the end of this stage. Astringency is, in the deepest sense, a *young* property of the grape — the tannin is almost all there before the fruit has any sugar or color at all. Organic acids — **tartaric** and **malic** — accumulate to their maximum. Methoxypyrazine is high. The berry is building its defenses and its skeleton while it is still inedible.

The lag phase. Growth stalls. The seeds harden and mature. Little changes in size; a great deal is being readied.

Véraison — the turn. Then, over a few days, the berry changes everything at once: it softens, it begins to color as the MYBA switch flips UFGT on and anthocyanin floods the skin, and it begins to load sugar. Véraison is the hinge of the whole curve and the most dramatic event in the vineyard year — a block of vines going from green to purple as if a dial were turned.

Stage II — ripening (véraison to harvest). The berry swells again by cell expansion and fills with **sugar** — glucose and fructose imported through the phloem, climbing toward 200–260 grams per liter. **Malic acid falls** as the warming berry respires it away, while tartaric, more stable, largely stays; total acidity drops and pH rises. Anthocyanin accumulates. Aromas and their bound precursors develop. And the tannin — already made — does not so much increase as **change**: it polymerizes further, oxidizes, binds to the new anthocyanins, and *softens*. This is the "tannin ripening" a winemaker tastes for, the slow grind of green grip toward ripe grip, and it is mostly a change of structure, not of amount.

The sugar loading of Stage II is itself a piece of machinery we have had to learn in order to retune it in Chapter 12. As the berry softens, its phloem unloading switches from **symplastic** (cell-to-cell, through plasmodesmata) to **apoplastic** (into the cell-wall space and then across the membrane), and hexose transporters of the **VvHT** family

together with **SWEET**-type transporters pull glucose and fructose into the flesh cells against a steepening gradient. The berry is, in this phase, essentially an **accumulator with no respiratory brake** on sugar — whatever the leaves photosynthesize and send, it banks — which is exactly why warm, sunny, long seasons drive sugar so relentlessly high. Reading the whole curve as a table is the clearest way to hold which clock governs what:

COMPOUND / PROPERTY	STAGE I (GREEN)	VÉRAISON	STAGE II (RIPENING)
Berry size	rising fast	lag	rising again (expansion)
Tannin (PA)	**built, peaks**	fixed in amount	structure ripens, softens
Malic acid	high	high	**falls** (respired)
Tartaric acid	high	stable	stable
Sugar	low	onset	**climbs to 200–260 g/L**
Anthocyanin	none	**onset (UFGT on)**	accumulates
Methoxypyrazine	high	declining	low
Aroma precursors	low	building	develop

The two heavy black rows — tannin built-and-fixed in Stage I, sugar climbing through Stage II — are the whole story of the next chapter. One is written young and merely matured late; the other is banked late and without limit. Nature runs them on one timeline. We came to believe they did not have to be.



A berry in cross-section: the clear flesh that will carry the sugar and acid, the colored skin and the seed that already hold a tannin finished weeks before. In the grape, texture is laid down young and ripened slowly; sugar comes late and fast.

Here is the fact the next chapter is built on, and it is the central inconvenience of modern winegrowing: **the clocks are not synchronized.** Tannin structure ripens on one schedule, governed by the slow post-véraison chemistry of the skin and seed. Sugar accumulates on another, faster, schedule, driven by photosynthesis and temperature. Aroma ripens on a third. In a cool, long season they come due together, and you get the classic balanced wine of a classic region. In a hot season — and the seasons are getting hotter — **sugar races ahead of everything else**, hitting pickable Brix while the tannins are still green and the aromas still underdeveloped, and the grower is forced to choose: pick early for structure and aroma and make a thin, hard, sour wine, or hang the fruit for phenolic ripeness and make a sweet-fruited, soft, but *enormous* wine at

sixteen percent alcohol. For two decades this has been the quiet crisis of the wine world. The grape itself bundled these clocks together. We think we can take them apart — and that is the next chapter, and one of the things we are proudest of in the whole 1,610.

Chapter 12 · The ripeness paradox — *decoupling the grip from the sugar.*

The ripeness paradox is simple to state and has been, until now, nearly impossible to solve: **a winemaker wants ripe tannins and modest alcohol, and the grape insists on tying the two together.** Phenolic ripeness — the soft, fine, resolved tannin of Chapter 11's Stage II — arrives only after weeks of hang time, and those are the same weeks in which sugar climbs toward the high teens of potential alcohol. You cannot, in a natural vine, order one without the other. Every elegant, structured, low-alcohol red of the old record was a gift of a cool climate that let the slow clock finish before the fast one ran away. As those climates warm, the gift is being withdrawn, and the industry's workarounds — pick early and acidify, or pick late and dealcoholize by spinning cone or reverse osmosis — all amount to fixing in the cellar what went wrong in the fruit, and all of them take something out of the wine along the way.

The platform's answer is to go to the fruit, to the clocks themselves. There are two independent interventions, and together they are about fifty of the grape constructs — a small number for an outsized effect, because they act on timing and regulation rather than building new chemistry:

- **Advance the tannin clock.** By holding VvMYBPA1 tone up and biasing the LAR:ANR balance toward the long, fine, ANR-extended chains of Chapter 5 *earlier* in Stage II, our model predicts the tannin would reach its ripe, resolved, low-bitterness structure weeks ahead of its natural schedule — so that phenolic ripeness is designed to arrive at a lower Brix, while the sugar clock is still only partway up its curve.
- **Slow the sugar clock.** By tuning sugar import and the berry's accumulation kinetics — moderating the apoplastic phloem-unloading and the **VvHT** and **SWEET** hexose transporters that fill Stage II — we lower the sugar ceiling the berry runs toward, so that a fully phenolically-ripe grape tops out nearer 200 g/L than 260. The carbon the leaves fix is not lost; it is simply not all banked as sugar, and the vine partitions more of it to the structural and phenolic ripening we have advanced.

Both interventions have to respect the **acid** side of the ledger, because a lower-sugar wine that is also flabby and high-pH has solved nothing. The malic acid that Stage II respires away falls fastest in exactly the warm conditions that drive sugar, so the hottest sites lose their freshness as they gain their alcohol — a double penalty. The decoupling lines therefore pair the slowed sugar clock with a measured **retention of malic acid**, moderating its respiratory breakdown so the fruit arrives at harvest with the natural acidity a cooler season would have preserved. Our model predicts a grape

that would reach ripe, fine tannin, developed aroma, *and* sound acid at a potential alcohol the grower sets — the balance that a great cool vintage used to hand out by luck, available by design.

The arithmetic of why this matters is the most consequential equation in commercial winemaking, and it is almost insultingly simple:

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ABV (% v/v) ≈ S (g/L) / 16.8
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About 16.8 grams of sugar per liter become one percent of alcohol. A natural grape ripened for phenolic maturity at 255 g/L makes a **15.2% wine.** A decoupled line that reaches the *same* ripe, fine tannin at 200 g/L makes a **11.9% wine** — a difference of more than three percentage points of alcohol, the difference between a wine that tires the table by the second glass and one that invites a third, achieved not by stripping alcohol out afterward but by never making it. Our design targets a tannin held to spec — IFI-A and grain on target — at a potential alcohol the grower sets.

There is a nourishment note here, and we will make it the way we make all of them: quietly, as an intention and not a claim. A wine carrying its structure and its scent at twelve percent rather than fifteen is a wine the body meets more gently, and the choice to make one is, among other things, a choice about care — about food and drink as things you give yourself with attention. We are not going to tell you a number on a bottle is good for you. We are going to tell you that we built the vine so that the grower who wants to make a lighter, more nourishing wine no longer has to fight the fruit to do it, and leave the rest, as it should be, to the table.



PART V OF VI

The 1,610 Constructs

how the grape library is organized · the flagship lines · the edits, with ΔΔG

Chapter 13 · The 1,610 — *how the grape library is organized.*

Of the roughly 19,500 product SKUs across the whole Scoville Splice platform, **1,610 are grape-program designs** — the sixth-largest organism program, and the deepest investment the company has made in a single fruit outside the peppers themselves. Grape is rated **Hard** in our difficulty ledger, and it earns the rating three times over: a large, redundant flavonoid gene family with multiple paralogs for nearly every step; the woody, slow, clonally-propagated biology of a perennial vine that turns every generation of a test into a question of years; and a polymerization step we have already confessed we cannot fully draw. That the program is as large as it is reflects not indecision but the width of the design space — grape is one of the few organisms where we work three axes at once, and the library is organized by them.

CONSTRUCT FAMILY	AXIS	COUNT	WHAT IT DOES
Astringency program	**IFI-A**	620	tannin structure — mDP, galloylation, prodelphinidin, grain; the core of the book
Zero-Bitter program	**IFI-B**	340	drains the bitter monomer pool into polymer; 20–65% bitterness attenuation
Multi-axis program	**IFI-M**	310	A×B (grip without bitter), A×K (grip with body), A×S (ripe grip, modest sugar)
Color program	anthocyanin	180	MYBA-locus and UFGT work; hue, depth, polymeric-pigment stability
Aroma program	volatile	110	methoxypyrazine, rotundone, thiol-precursor, terpene, norisoprenoid dials
Ripeness-decoupling	timing	50	the two clocks of Chapter 12 — phenolic ripeness at lower Brix
Total		**1,610**	

The three primary axes — **B, A, M** — are not an accident of what grape happens to contain; they are a statement about what grape is *for*, on the index. It is the organism where astringency, the hardest axis to define and the only non-receptor one, reaches its fullest expression, and where the richest **multi-axis** work on the platform happens. The grape's M-program is the proving ground for the whole idea that axes compose: astringency combined with kokumi (the CaSR-mediated body and continuity that we map a wine's

"texture" and "weight" onto) to give grip that feels *upholstered* rather than bare; astringency decoupled from bitterness, the A×B pairing that is really the Zero-Bitter story told from the mouthfeel side; astringency held against a designed, modest sweetness for the off-dry and the ripeness-decoupled styles. A grape scorecard, unlike a pepper's, is rarely about one loud axis. It is about a *shape* — a quiet H, P, C; a present S; a moderate, deliberate B and K; and an A placed exactly where the winemaker drew the line.

The multi-axis constructs draw on the platform's dual and triple libraries — the **M2** dual-axis set of 8,400 constructs and the **M3** triple-axis set of 2,800 — from which the grape's 310 M-program entries are the pairings a wine actually needs. The CaSR-mediated **kokumi** axis deserves a word, because "body" is the most abused term in wine and the index lets us finally ground it: kokumi substances — γ-glutamyl peptides, and in wine the long-chain polysaccharides, mannoproteins, and the polymeric tannins themselves — act on the calcium-sensing receptor to lengthen and thicken a flavor's *continuity*, the sense that the wine fills the mouth and holds rather than arriving and vanishing. A great red's "weight" is, to a first approximation, its IFI-K, and the A×K construct family builds the particular luxury of a grip wrapped in body, where the tannin's drying edge is upholstered by continuity so that the wine feels, in the old tasting word, *seamless*. These are the constructs we are proudest of, because they are the ones that turn a tannin from a quantity into a texture — and texture, far more than power, is what the people who make great wine are really chasing.



The bench where the grape library was assembled and sequenced — 1,610 constructs, each verified before it ever reached a vine.

Every construct is held to the platform's locked quality standard — **batch-to-batch coefficient of variation at or under 7%** on its target axes, against the 30–45% a natural vineyard swings from vintage to vintage and block to block. That number is the whole quiet argument of the company restated for grape: not that a designed tannin is *better* than a great vigneron's, but that it is *the same every year*, which a vineyard, at the mercy of its weather, can never promise.

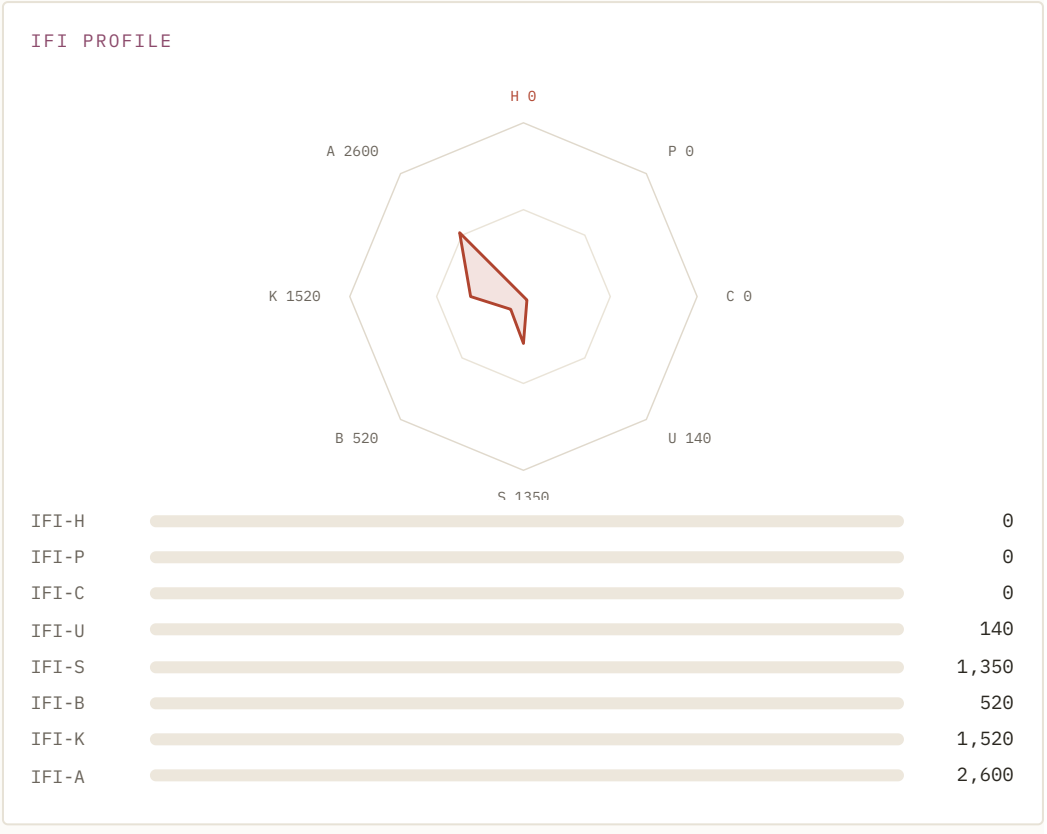
Holding a *perennial* to seven percent is harder than holding an annual pepper there, and it is worth being honest about why, because the difficulty is the proof the number means something. A vine is clonally propagated, so a line is genetically fixed — that part helps — but it is also long-lived, grafted, and exposed to a decade of seasons in which the epigenetic tone over the MYB circuit (Chapter

6) can drift with drought, heat, and crop load. Every grape construct is therefore validated not in one harvest but across **replicated plantings in multiple seasons and sites**, with the tannin fingerprint — mDP by phloroglucinolysis, galloylation, prodelphinidin, and PRP-precipitation EC_{50} — re-measured each year, and a line is not called live until its target axes hold inside the seven-percent band across that spread. It is slow, unglamorous work, the opposite of the single dramatic edit, and it is most of what "Hard" means in the difficulty ledger. Three patents and twenty-one peer-reviewed papers (Zenodo, CC BY 4.0) stand behind the methods; the grape-specific work runs across six of them. And one thousand of the platform's roughly nineteen thousand five hundred catalogue SKUs are live and purchasable today — the grape lines among them are the ones the next two chapters name.

Chapter 14 · The flagship lines — *six grapes we built on purpose.*

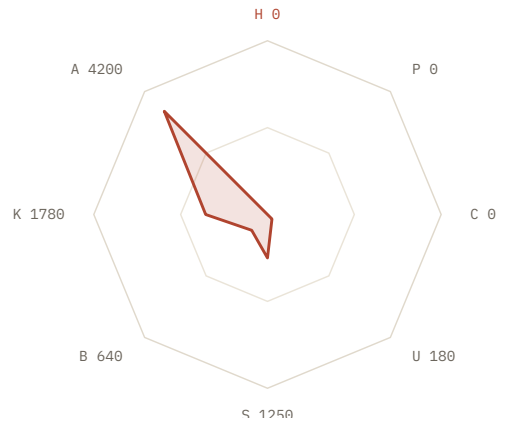
The grape program's flagships are not, as the superhot peppers' are, a ladder of a single escalating axis. They are a *fan* — six points placed deliberately around the plane of loudness and grain that Chapter 3 opened, each answering a specific thing a winemaker could never previously ask a vine for. We name them, as we name everything, for what they are. We do not assign them the SS-series registration numbers of the pepper catalog; these are design lines, expressions of the grape program, and they are referred to by name.

Velvet is the line that most directly breaks the old trade-off: a red-wine grape carrying a serious, Cabernet-weight tannin — IFI-A 2,600 — at a *fine* grain nature reserves for far gentler wines, built by pushing the ANR-extended, prodelphinidin-rich chains of Chapter 5 and holding galloylation under eight percent. Designed to grip like a structured wine and feel like a soft one.



Cathedral is the corner of the plane nature keeps locked: an enormous tannin — IFI-A 4,200, Tannat-and-beyond territory — held at a *fine, even* grain, the age-worthy monument that in a natural grape would be unbearably coarse at that magnitude. It is the clearest single proof that loudness and grain are now independent. We did not build it because every wine should be this big. We built it because no vine could, and we wanted to see the corner.

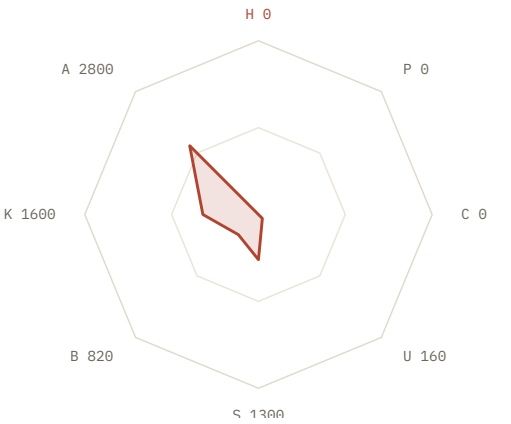
IFI PROFILE



IFI-H	0
IFI-P	0
IFI-C	0
IFI-U	180
IFI-S	1,250
IFI-B	640
IFI-K	1,780
IFI-A	4,200

First Growth is restraint, not spectacle: a classically-proportioned claret-style grape, IFI-A 2,800 at a firm, traditional grain with the deliberate thread of structural bitterness a great Médoc keeps — the line for a maker who wants the canonical shape, made reproducible. Its scorecard keeps a higher, deliberate B than Velvet's — the Médoc's savory grip is not a flaw to be engineered out but the style's own signature, held exactly where tradition placed it:

IFI PROFILE



IFI-H	0
IFI-P	0
IFI-C	0
IFI-U	160
IFI-S	1,300
IFI-B	820
IFI-K	1,600
IFI-A	2,800

Noon is the ripeness-decoupled line of Chapter 12 made flesh: a fully phenolically-ripe red tannin, IFI-A 2,400, grain fine, designed to reach its ripe structure at **200 g/L sugar — a potential alcohol of 11.9%.** Grip and perfume of a serious wine, at the weight of a light one. It is, quietly, the line that matters most to where wine is going, because it is the one that answers the warming season not by subtracting alcohol in the cellar but by never banking it in the fruit —

and it is the clearest place the nourishment ethic of this library touches a grape, a serious wine a person can meet gently and drink slowly.

Ink is the color-stability flagship: deep malvidin-dominated anthocyanin with an anthocyanin-to-tannin ratio tuned for maximal polymeric-pigment formation, IFI-A a moderate 2,500 built to *soften gracefully and hold color* over two decades in bottle rather than to impress on release.

Clear is the white: the MYBA switch left off, a near-absent tannin (IFI-A 150) with the monomeric bitterness drawn right down, built as the clean, low-grip canvas for the thiol and terpene aromatics of Chapter 10 to sing against.

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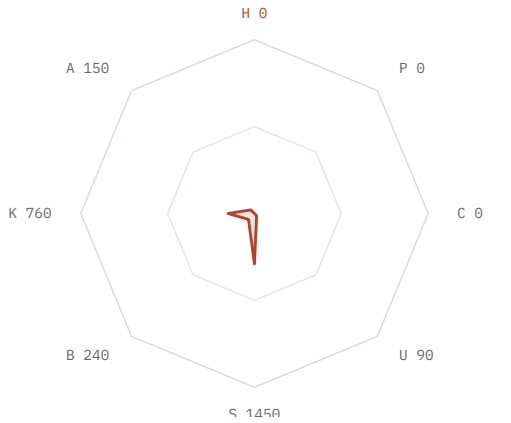
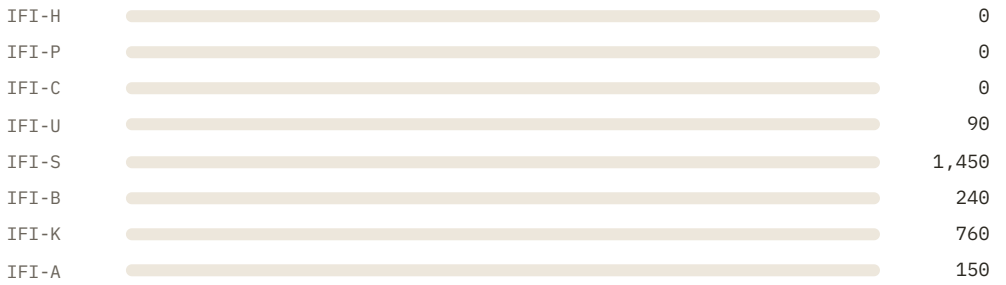




LINE	STYLE	IFI-A	GRAIN	SIGNATURE
Velvet	red	2,600	fine	structured tannin, soft feel
Cathedral	red	4,200	fine	the locked corner — huge and silky
First Growth	red	2,800	firm	the canonical claret, made reproducible
Noon	red	2,400	fine	ripe grip at 11.9% potential alcohol
Ink	red	2,500	fine	color-stable, ages with grace
Clear	white	150	—	low-grip canvas for aromatics

Beneath the names are specifications, and a winemaker plans from the specification, not the poetry. Each line is a defined target in the structural variables of Part I, reached by the edits of Chapter 15 drawn from the A, B, M, color, and decoupling families of the 1,610:

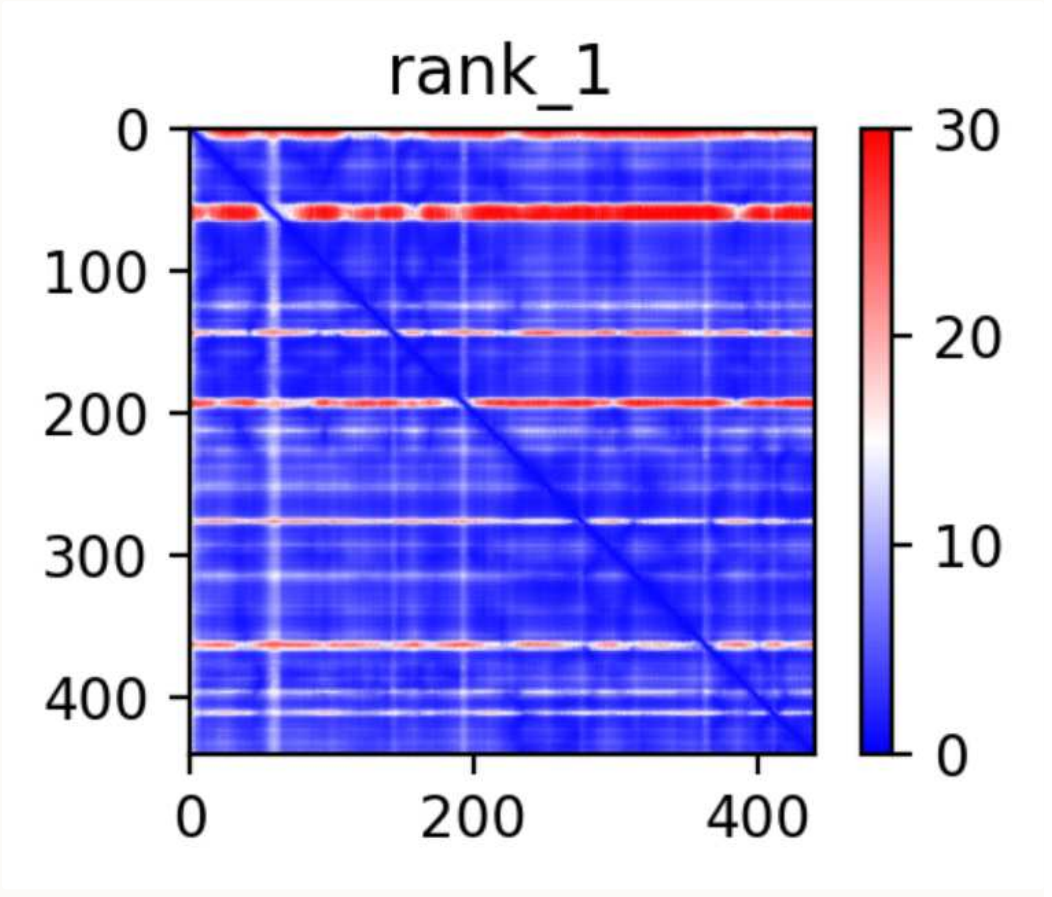
LINE	LEAD EDITS	MDP TARGET	% GALLOYL	% PRODELPHINIDIN	POTENTIAL ABV
Velvet	ANR↑, F3′5′H+, LAR2−	11	<8%	40%	13.5%
Cathedral	full stack (ANR↑↑, LAR−, MYBPA1↑↑)	14	<6%	38%	14.0%
First Growth	ANR↑ moderate, galloyl held	13	10%	28%	13.5%
Noon	decoupling + ANR↑, malic retained	11	7%	40%	**11.9%**
Ink	color-program + ANR↑, anthocyanin↑	10	6%	36%	13.5%
Clear	MYBA off, monomer-drain	2	low	—	12.5%

Compare the Cathedral row to the Tannat row in Chapter 3's ladder — mDP 14 against 18, galloylation under 6% against 12%, and a tighter histogram throughout — and you can read, in four numbers, how a tannin can out-grip Tannat on the IFI-A scale while feeling finer than Cabernet: more of its magnitude is carried by chain length and prodelphinidin, less by the coarse, green, bitter galloylation that nature uses to reach that power. That substitution — length and fineness *for* galloylation — is, in one line, the whole engineering thesis of the grape program.

The fan is the point. A pepper program climbs; a grape program *places*. And placement — exactly here on the loudness axis, exactly there on the grain axis, held to within seven percent every single harvest — is the thing a winemaker has wanted since the first jar of must went wrong in a warm cellar and no one could say quite why.

Chapter 15 · The edits — *the letters we changed, and the energy they cost.*

Behind the six lines is a smaller set of molecular edits, each one scored, modeled, and energy-accounted before it was ever written into a vine. We run the same pipeline the whole platform runs — the one shown earlier on the pepper Pun1 acyltransferase: an AlphaFold-class structure prediction scored by per-residue pLDDT confidence and by the predicted-aligned-error map that tells us which domains are positioned reliably against which; then **ESM2**, a 650-million-parameter protein language model, scoring every possible single-residue substitution in the enzyme for its predicted effect on catalytic efficiency and fold stability; then a **genome-scale flux-balance model** of grape-berry metabolism to confirm that the step we mean to edit is the real rate limiter and not a bystander; and only then CRISPR, writing the chosen letters.



The predicted-aligned-error map from the structure pipeline, shown on Pun1/AT3. The same PAE analysis fixed the domain geometry of grape ANR and both LAR paralogs, telling us which active-site substitutions we could trust the model about before we made them.

We report every point substitution with its **ΔΔG** — the change in folding free energy, in kilocalories per mole, where a negative number means a *more* stable protein. Stability matters as much as activity in a perennial that must express an edited enzyme faithfully across years and seasons; an edit that raises catalytic efficiency but destabilizes the fold is an edit that drifts, and drift is the enemy of the seven-percent CV. The grape program's core edits:

EDIT	TARGET	EFFECT	ΔΔG (KCAL/MOL)
VvANR F142L	ANR active-site shell	k_cat ↑ toward epicatechin; extension-unit supply ↑ → mDP ↑	−1.6
VvANR S89A	ANR cofactor pocket	NADPH turnover ↑; stabilizes the Rossmann fold	−1.2
VvLAR2 dosage −40%	LAR2 (chain-stopper)	terminal-unit supply ↓ → mDP ↑, grain finer	expression
VvMYBPA1 +2×	PA master regulator	whole proanthocyanidin branch flux ↑; anthocyanin unchanged	regulatory
VvGST4 L91F	flavonoid transport carrier	vacuolar loading ↑; condensation efficiency ↑	−1.1
VvF3′5′H bias +15%	B-ring hydroxylation	prodelphinidin fraction ↑ → grain finer, hue bluer	−0.7
VvOMT3 −60%	methoxypyrazine O-MT	IBMP green note down for warm-site aromatics	expression

Three things in that table are worth reading twice. First, the two **VvANR** substitutions, F142L and S89A, are the grape's analogue of the pepper's *Pun1* double variant — single letters in the enzyme at the very fork of the pathway, chosen by the model to raise extension-unit supply and stabilize the fold at once, and they are the molecular root of the long, fine chains that make Velvet and Cathedral possible. Second, several of the most powerful entries are not point mutations at all but **dosage and regulatory** changes — VvLAR2 down, VvMYBPA1 up — because in a pathway this regulated, the loudest lever is often the transcription factor, not the enzyme. Third, the edits **compose**: Cathedral is not one of these but most of them at once — ANR up, LAR down, MYBPA1 up, GST4 and F3′5′H trimming grain — stacked and balanced so that the mDP histogram lands tight and high and the galloylation stays low, which is the only way a tannin gets to IFI-A 4,200 without turning coarse.

A word on how the pipeline earns the confidence to make so few, so precise. The structure step accepts a model only where **pLDDT** confidence clears 80 across the catalytic shell and the **predicted-aligned-error** map shows the substrate-binding and cofactor domains fixed against each other — because an edit proposed into a region the model is unsure of is an edit we do not trust. **ESM2**, at 650 million parameters, scores every single-residue substitution's change in a sequence-likelihood that correlates with both activity and stability, and we keep only substitutions that improve the predicted catalytic efficiency *and* carry a favorable ΔΔG, the intersection that the table reports. The **flux-balance** model — a genome-scale stoichiometric reconstruction of grape-berry metabolism — then runs each candidate *in silico* across the whole network to confirm the edit moves the flux we mean and does not starve pigment, flavonol, or the berry's own energy; many promising

enzyme edits die here, correctly, for moving an assay number without moving a glass. Only the survivors are written, and in the grape they are written into **embryogenic callus** — the slow, exacting tissue-culture route by which an edited vine is regenerated, grafted, and grown out over the years a perennial demands before a single cluster can be tasted. The method is patient because the plant is patient; three patents and the grape share of twenty-one peer-reviewed papers stand behind it; and its whole purpose, stated once more, is to let us change as little as possible to move exactly the thing we meant.

We will end the chapter where we began the method: in restraint. The model proposes thousands of substitutions; we make very few. The flux-balance step exists precisely to stop us editing enzymes that would change a number on an assay without changing anything in a glass, and most of what the pipeline does is tell us, with real confidence, which edits *not* to bother making. Designing a grape is, more than anything, the discipline of changing as little as possible to move exactly the thing you meant to move. The letters we leave alone are as much the design as the ones we change.



PART VI OF VI

The Cellar & The Frontier

fermentation and aging · the designed tannin over time · the law, and the table

Chapter 16 · The cellar — *what a designed tannin does once the yeast arrives.*

A grape is a proposal; wine is the argument. Everything we have designed into the fruit now has to survive crush, ferment, and years — and a designed tannin behaves, in the cellar, according to rules worth a winemaker's close attention, because some of our interventions change the fruit and some change the whole arc of the aging wine.

Extraction comes first. The tannin of Chapter 11 lives in skin and seed, and it must be pulled into the must during maceration — the days the fermenting juice spends on its skins. Extraction follows a kinetics close to first-order approach to a ceiling:

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C(t) = C∞ · (1 − e^(−k·t))
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where C∞ is the extractable tannin the fruit holds and k the rate set by temperature, alcohol (which rises as the ferment proceeds and pulls seed tannin harder), and cap management. The winemaker's whole maceration art is choosing where on that curve to stop. A designed grape changes the terms kindly: because we set C∞ — the amount — and the *grain* in the fruit, the cellar no longer has to chase grain through punishing extraction choices, over-extracting for structure and picking up green seed tannin along the way. The structure is already in the skins at the grain you asked for; maceration becomes a matter of *how much*, not *what kind*.

A designed maceration protocol, written as we would write any recipe in this library:

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MACERATION · the designed cap — Velvet, 1 tonne
— 5-day cold soak, 8 °C, to extract skin anthocyanin and fine tannin ahead of alcohol
— inoculate selected S. cerevisiae; ferment 24–28 °C
— 2 gentle pump-overs daily; NO hard seed extraction (grain is already set in the fruit)
— press at dryness, before alcohol strips coarse seed tannin
— malolactic with O. oeni; rack off gross lees
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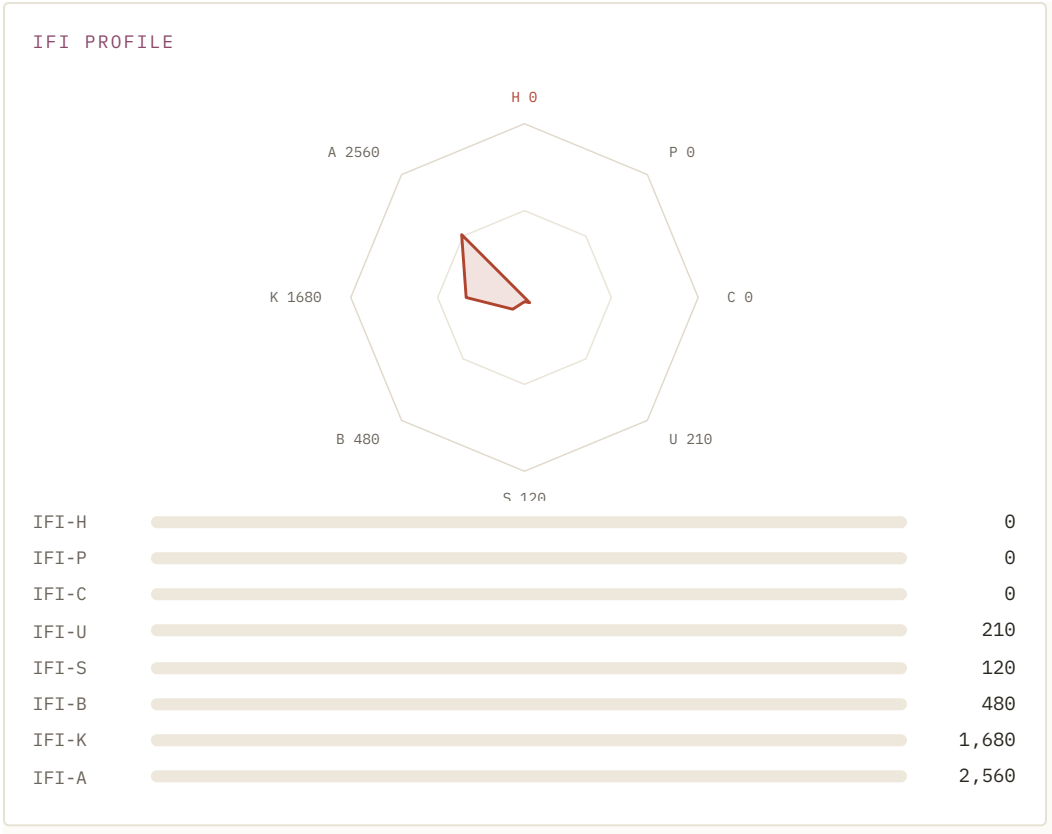
Method. 1) Chill and hold the must five days to lead with the fine skin fraction. 2) Warm and inoculate; keep the cap wet and cool. 3) Taste daily against the IFI-A target, not the calendar. 4) Press the moment the designed grip is in the wine — the fruit will not punish a timid press the way a natural grape does, because there is no green seed tannin waiting to be over-extracted. 5) Let malolactic soften the acid and build the kokumi-mapped texture. The wine that results reads, young, close to the fruit's own scorecard, now dry:



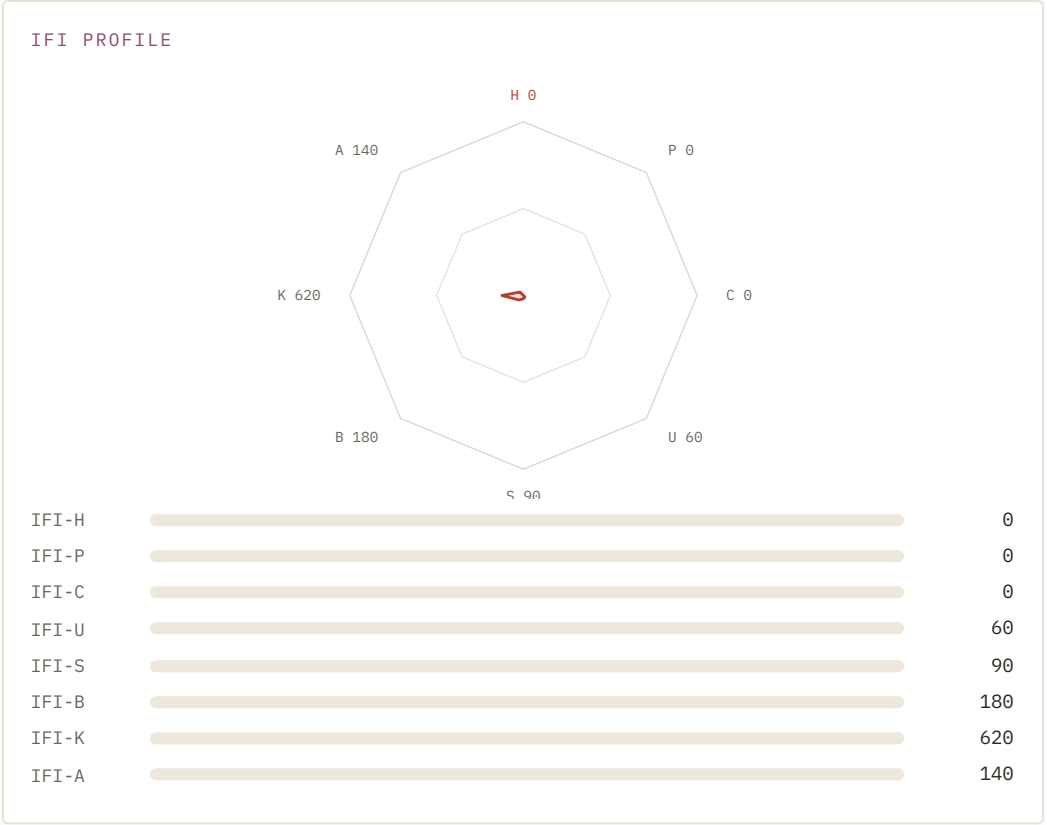
Note the S has fallen to near-nothing — the sugar is alcohol now — while the A has held and the K, the body, has *risen* with malolactic and lees contact. The grip we designed into the grape is intact in the glass.

Between press and bottle sit the cellar's own chemistries, and a designed grape meets each of them differently. **Malolactic fermentation**, run by *Oenococcus oeni*, converts the sharp malic acid to softer lactic and throws off a little buttery diacetyl — but recall that the ripeness-decoupled lines were engineered to *retain* malic in the fruit, so for those the winemaker chooses how much of that retained acid to convert and how much to keep as freshness, a dial the grape handed back to the cellar. **Sulfur dioxide**, added in measured doses, protects against oxidation and spoilage and, as Chapter 8 noted, temporarily binds and bleaches anthocyanin; a color-stable line like Ink is designed to move its pigment quickly into the SO_2-resistant polymeric forms so that less color is lost to the necessary preservative. **Micro-oxygenation and lees contact** steer the tannin's early polymerization and lend mannoprotein body — the yeast walls releasing the very polysaccharides that soften grip and build kokumi — and **oak**, where it is used, contributes its own hydrolyzable **ellagitannins**, a chemically distinct tannin that scavenges oxygen and nudges the grape tannins toward gentler aging, along with vanillin, whiskey-lactone coconut, and the toast notes of guaiacol and eugenol. A designed grape does not render the cellar's art obsolete; it hands that art a known, reproducible starting material, so that every one of these interventions becomes a deliberate choice rather than a correction. That is the whole relationship we want with the winemaker: not to replace the judgment, but to remove the guesswork beneath it.

The white wines ask the opposite of the reds, and the Clear line is made for the asking. Where a red maceration is an extraction problem, an aromatic white is a *protection* problem — keeping grip near zero, oxygen away from the delicate thiols, and acid intact so the aromatics ring:

PROTECTIVE WHITE · Clear, aromatic style — 1 tonne
— whole-cluster press; minimal skin contact to hold tannin near zero
— cold settle 24 h at 10 °C; rack clean juice off the gross solids
— cool ferment 13–16 °C with a high-β-lyase yeast to free the bound thiols
— NO malolactic — preserve the natural acid and the aromatic lift
— brief lees stir for mannoprotein body; early, protective SO_2

Method. 1) Press whole clusters gently and keep the juice off its skins — Clear has almost no tannin to give, and we want none of it here. 2) Settle cold and rack bright, so the ferment runs clean. 3) Ferment cool, with a yeast chosen for the β-lyase of Chapter 10, to cut the grape's rich thiol-conjugate pool free as passionfruit and grapefruit. 4) Skip malolactic to keep the acid taut. 5) Rest briefly on the fine lees for a whisper of mannoprotein body, under protective sulfur. The wine lands light, dry, aromatic, and almost weightless on the palate — exactly the canvas the grape was designed to be:



Set this card beside Velvet's finished-wine card a page back and the two halves of the grape program face each other: the red, all designed structure and body, A 2,560 and K 1,680; the white, all aromatic lift and restraint, nearly bare of grip, its whole art in what it refuses to carry. One organism, two opposite masterpieces, both written in the fruit.

Aging is where the color chemistry of Chapter 8 pays out. Over months and years, the tannins and anthocyanins condense into **polymeric pigments** and **pyranoanthocyanins**, and the

consequence is the one Chapter 8 foreshadowed: a tannin bound into a polymeric pigment *grips less*. Astringency softens as color stabilizes; they are one reaction.

PIGMENT SPECIES	FORMED FROM	COLOR EFFECT	ASTRINGENCY EFFECT
Direct polymeric pigment	tannin + anthocyanin	stabilizes red, shifts toward garnet	tannin softens
Ethyl-bridged polymer	flavanol–(acetaldehyde)–anthocyanin	bluer, then eventually sediments	softens, then precipitates out
Vitisin A	anthocyanin + pyruvate	orange-red, highly stable	–
Vitisin B	anthocyanin + acetaldehyde	stable	–

This is why **Ink** was designed as it was — its anthocyanin-to-tannin ratio tuned to feed exactly these reactions, so that it sheds its youthful grip slowly and holds its color for twenty years rather than browning in five. And it is why a designed grape is not only a young-wine instrument: by setting the fruit's pigment and tannin load together, we are setting the *rate and grace of the wine's whole life* — the single longest-horizon act of design in the library, a sentence written in the fruit that takes two decades in a cellar to finish being read.

Chapter 17 · The law and the table — *what a designed vine is allowed to be, and what it is for.*

We will end where an honest book about this work has to: at the law, and then at the table, because a designed grape enters a world more bound by tradition, appellation, and regulation than almost any food on earth, and because none of the chemistry means anything until someone pours it.

The regulatory frame is real and we do not wave it away. Wine is defined, in the US by the **TTB** (the Alcohol and Tobacco Tax and Trade Bureau, which approves every label through its Certificate of Label Approval) and internationally by the **OIV** (the International Organisation of Vine and Wine), as the fermented product of *Vitis vinifera* grapes, grown within defined appellations — American Viticultural Areas here, the PDO and PGI systems of the EU abroad — many of which specify permitted grape varieties and traditional practices by law. A designed vine sits athwart several of these frames at once, and the questions are genuine:

- **Gene-edited versus transgenic.** Our grape work is overwhelmingly *edited* — changes to the vine's own genes, its own promoters, its own dosage — rather than the insertion of foreign DNA. That distinction is the hinge of the regulation. In the US, the USDA's **SECURE rule** exempts from regulation certain categories of edit that could have arisen through conventional breeding; several of our single-letter and dosage changes fall inside it, and each is assessed individually. In the EU, the older **GMO Directive (2001/18)** swept gene-edited crops in with transgenics, but the **New Genomic Techniques** proposal advanced in 2023–2024 would carve out certain targeted edits — a moving frame we track rather than predict.
- **Appellation and tradition.** A PDO that permits only named varieties, propagated traditionally, may simply not admit a designed vine, however fine its wine — and we think that is often *correct*. The traditional appellations are a cultural inheritance, not merely a technical standard, and the right posture toward them is respect, not a lawyer. A designed grape's home is the vast world of wine *outside* the protected names, and the grower's choice to plant one is a choice to work in that open country.
- **Permitted practice.** The OIV maintains a long, specific code of what may be done to a wine — which additions, which treatments, which clarifications — and a designed grape changes none of that downstream code; it changes only the fruit that enters it. A wine from a Noon vine is made by wholly conventional, permitted cellar practice. The novelty is entirely in the vineyard, which is the honest place for it to be.

There is a piece of history here that we offer not as an argument but as a perspective, because it genuinely shaped how we think about this work. **The entire wine world already rests on a radical genetic intervention, and has for over a century.** When *phylloxera* — a root-feeding aphid from North America — destroyed the vineyards of Europe in the late nineteenth century, the craft was saved by **grafting**: nearly every *Vitis vinifera* vine grown for fine wine since has been grown on the roots of American *Vitis* species, *riparia* and *rupestris* and their hybrids, a chimera of two continents' genomes joined at the graft union below the soil. The Cabernet of the most tradition-bound estate in Bordeaux is a vinifera top grafted onto an American rootstock, and no one calls that wine anything but Cabernet. We do not cite this to wave away the real and reasonable questions a designed vine raises. We cite it because the line between "natural" and "intervened" in the grape was crossed, out of necessity and with enormous care, a hundred and fifty years ago — and the craft absorbed it and remained itself. We mean to be as careful, and as honest, as the people who did.

- **Disclosure.** Our position is the plain one: label it, truthfully, and let the drinker choose. Nothing about this work needs hiding, and a designed wine that pretended to be something else would betray the whole ethic of the company.

A smaller regulatory grace note, and a nourishment one: most fine-wine fining — the clarification that pulls harsh tannin and haze from a young wine — has traditionally used animal products, isinglass from fish, gelatin, egg white, casein. A grape built to the grain you want needs far less fining, and what it needs can be done with **bentonite clay or pea protein** — so a designed wine is, almost incidentally, far easier to make **vegan**, and the plant-reverent, vegetarian-first ethic that runs under this whole library reaches even into the cellar's clarifying tanks.

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



The vine at the end of the day. The law will take its time; the grape keeps its own.

And the nourishment through-line, stated once, the way we always state it — as an intention, never a claim. The grape is one of the richest sources in the whole plant kingdom of the polyphenols the body is glad to meet: the flavan-3-ols and anthocyanins that *are* this book's tannins and pigments, the stilbene resveratrol the vine makes to defend its skin. We make no health claim and we never will; we are not a pharmacy and a glass of wine is not a medicine. What we will say is that designing a grape's phenolics is, unavoidably, designing *what the fruit can carry* to the body that eats or drinks it —

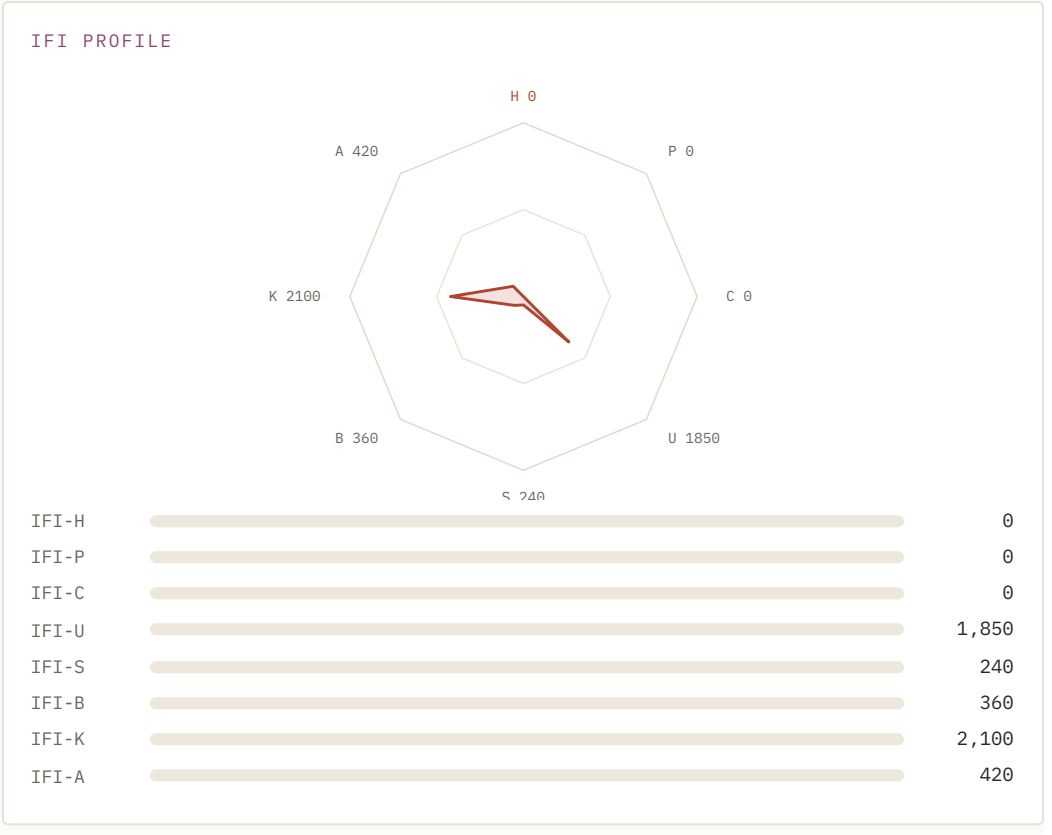
and that a lighter, cleaner, more considered wine, grown on a vine that was asked to hold the best of itself, is among the gentler pleasures a person can give themselves with intention. That intention is the whole of the ethic. The chemistry is only how we keep the promise.

Chapter 18 · The table — *where the number goes quiet again.*

We built an index so that a tannin could be read. We will close by setting the index down, because the last word on a grape is never a number — it is a meal, and a glass, and people at a table, which is where this family's whole question started and where it is always trying to get back to.

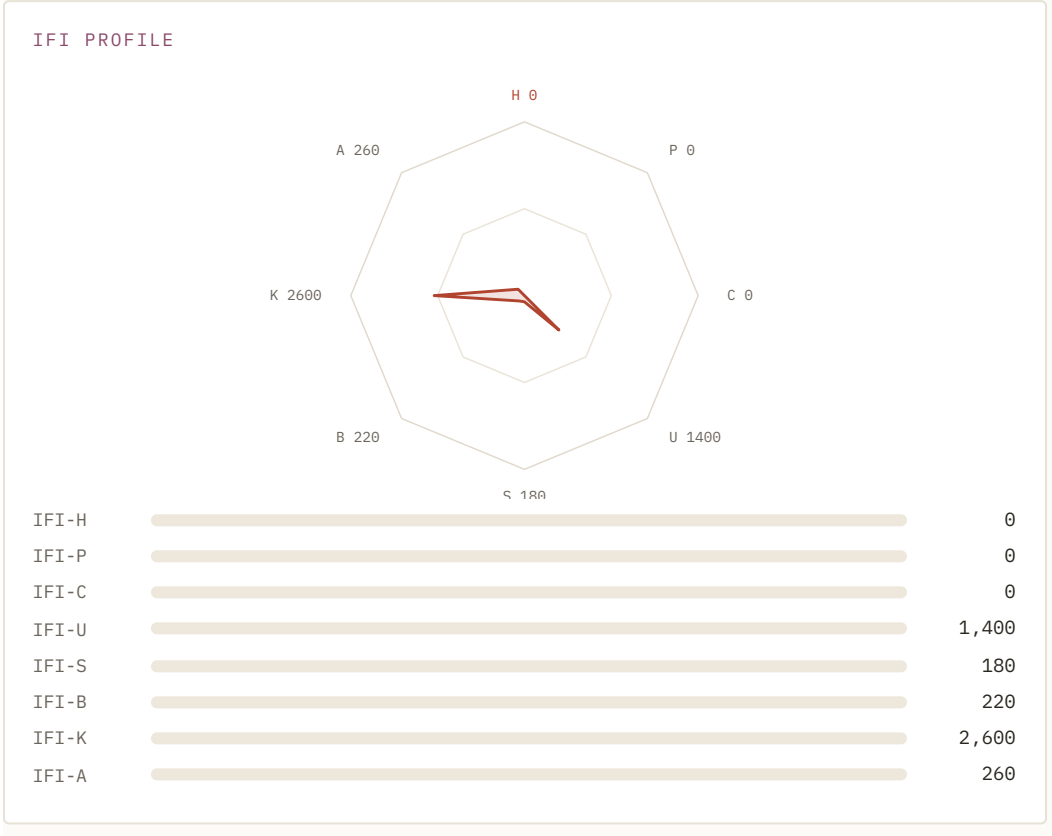
So: a few pairings, scored the way we score everything, not to tell you what to like but to show the index doing its quiet work underneath a good dinner. A wine's grip, placed by design, is a *structural* ingredient at the table — it is what cuts fat, what frames protein, what lets a dish and a glass hold each other up. A fine high-A red against a rich plate is not a coincidence a sommelier memorized; it is two astringency-and-fat equations solving each other, and now we can write both sides.

Velvet, against a wild-mushroom and walnut ragù over soft polenta — the plant-forward pairing we reach for first, and the one that most delights us, because the engineered grip meets the mushroom's deep savor and the walnut's own gentle tannin and the three of them build a structure no one of them had alone. The dish, scored:



Read the two scorecards together — Velvet's A 2,560 and K 1,680 against the ragù's U 1,850 and K 2,100 — and you can see the pairing *work* on the page: the wine brings the astringency and the dish brings the umami and the kokumi, and the two cards interlock along the axes each one leaves open. That is what a sommelier's intuition looks like once it has been written down.

Cathedral, against a slab of aged hard cheese and a few walnuts — the pairing that proves the oldest rule on the table, that a huge tannin wants fat and protein to spend itself against. The cheese's richness and salt meet the IFI-A 4,200 grip head-on; each disarms the other; and because Cathedral's grain is fine, what would be a brawl with a coarse tannin becomes an embrace. The plate, scored:



The cheese posts a towering K 2,600 — pure kokumi, pure continuity — and that is exactly the axis Cathedral's enormous A needs to lean on; the wine's grip and the plate's body hold each other up where neither alone would be comfortable. It is the same solving-of-two-equations as the ragù, written louder. A taster feels it as *rightness*; the index shows it as two cards whose open axes are each other's full ones.

For a maker who keeps meat and fish, and we write their table with exactly the same respect as the plant one: **First Growth against a slow-braised short rib** — the firm, canonical tannin cutting the braise's richness the way a great Médoc has done for two centuries, now reproducible vintage to vintage. **Clear, its low grip and high aromatics against a plate of raw oysters or a line-caught fish crudo with citrus** — where you want almost no tannin at all, only the thiol lift and a clean acid, and the designed white gives exactly that and nothing it doesn't need. **Noon, the 11.9% red, against a long vegetarian lunch that wants a wine to last three glasses** — the whole reason we decoupled the clocks, poured out at last.

This is the end of the instrument's usefulness and the beginning of the point. The EC_{50} and the mDP, the ANR fork and the MBW complex, the 1,610 constructs and the seven-percent CV — all of it was only ever in service of the moment the number disappears, when the glass meets the plate and the designed grip does its silent structural work and no one at the table has to know a thing about proline-rich proteins to feel the wine and the food reach for each other and hold. We measured the pucker so that we could build it well. We built it well so that it could vanish, which is what a tannin does at its finest, into the simple rightness of a good thing eaten

slowly, with people you love, grown by a family that reveres the plant it comes from — and grown, always, toward the fullest flavor, and the fullest nourishment, the vine can carry.