

THE SCOVILLE SPLICE LIBRARY · VOLUME XII

Pungency

The sharp channel — wasabi, not warmth. *It bites, then it clears.*

A receptor-level account of the pungent axis — the TRPA1 channel of mustard, wasabi, garlic, ginger, and cinnamon — and the organisms we tuned to speak it. The sharp half of flavor, measured and designed.

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

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Opening essay · The sharp half of flavor

There is a moment at our table that never stops being funny. Someone takes a little too much of the fresh mustard — the bright one, ground that morning — and for a second they vanish. Eyes close, breath catches, a hand lifts halfway to the face and stops. Then it passes, as fast as it came, and they are back, laughing, reaching for more. Heat does not do this. Heat arrives slowly and stays, a coal that glows for minutes after the bite is gone. The sharp is different. The sharp is a door that opens up behind the eyes, blows through, and shuts. It is the other half of what we mean when we say a food is *spicy*, and for most of history we had no word to keep the two apart.

We do now, because we had to learn the difference at the level of the protein. Capsaicin — the molecule this whole company began with — settles into a pocket on a channel called TRPV1 and tells the body it is warm when it is not. That is heat, the subject of other volumes in this library. Pungency lives next door, on a different channel entirely, and it does not settle into a pocket at all. It reacts. It forms a bond. The molecules of mustard and wasabi and garlic are *electrophiles* — electron-hungry little things — and they reach into the receptor and chemically latch onto it, a true covalent handshake, and that is why the sharp feels so unlike the warm. One is a key turning in a lock. The other is a match striking a strip.



The research plot at the sharp end of the greenhouse — brassicas and alliums grown for the one axis almost no one had measured.

The channel is called TRPA1, and before it had that name the sensation it carries went by a hundred borrowed words — the rush up the nose, the wasabi burn that isn't a burn, the sting that makes you honk like a goose and then thank it. TRPA1 is the body's chemical irritation sensor — the alarm it keeps for reactive, electron-poor compounds that should not be in the mouth. Mustard, wasabi, horseradish, raw garlic, onion steam, fresh ginger, cinnamon bark: every one of them is a plant that learned, over millions of years of being eaten, to manufacture an electrophile precisely tuned to trip that alarm. Pungency is a plant's chemistry of self-defense, and the kitchen is ten thousand years of us deciding we like being warned.

For a long time the Iyengar Flavor Index had seven axes it could resolve cleanly and one it could only gesture at. Heat we had to the digit. Sweetness, umami, the attenuation of bitter, the quiet lift of kokumi, the cool of menthol, the dry grip of a tannin — all of these resolved into a single number anchored to a receptor's half-activation. But pungency kept slipping the ruler, and the reason is the thing that makes this volume necessary: **pungency is not only an amplitude. It is also a shape in time.** A wasabi and a cinnamon can read nearly the same loudness and feel like different senses, because one opens and clears in twenty seconds and the other takes three minutes to arrive and three more to leave. To characterize the sharp, we had to capture not just *how loud* but *how fast* — and once we could, we could design it.

> $\mathrm{IFI\text{-}P}(c) = 1000 \cdot 2.1\ \mu\mathrm{M} \,/\, \mathrm{EC}_{50}(c)$

The formula is the family's, the same one that runs through every book: invert a receptor's half-activating concentration against a fixed reference, scale by a thousand, and a plant's loudness on one channel becomes a score you can set a mustard and a ginger beside on a single page. For pungency the reference point is set, by convention, at 2.1 µM — the TRPA1 half-activation of allyl isothiocyanate, the principal bite compound of culinary brown-mustard paste. Like the original Scoville threshold-dilution definition, this is a stated convention: IFI-P = 1,000 is defined at 2.1 µM AITC, not derived from a literature survey. We built the instruments to watch the curve rise and fall in real time, because on this axis the rise and the fall *are* the flavor. What follows is that channel, and those molecules, and the arithmetic that finally let us hold them still.

The two burns, and why the body keeps them apart

Begin with the honest confusion in the word *spicy*. English hands a single adjective to two unrelated proteins, and the tongue knows better than the dictionary. Capsaicin finds TRPV1 — a non-selective cation channel on the trigeminal nerve endings of the mouth, the same receptor gated by temperatures above 43 °C and by acid. Capsaicin does not warm the tissue; its vanillyl head slides into a hydrophobic vestibule between the channel's transmembrane helices, and by occupying that pocket it lowers the channel's thermal threshold until the body reports a fire that is not there. Remove the molecule — rinse it, bind it up in fat and casein, wait — and the pocket empties, the threshold climbs home, and the fire goes out. It is reversible because it was never a reaction. It was a fit.

Pungency answers to a different protein with a different grammar. TRPA1 — Transient Receptor Potential Ankyrin 1, the channel the field still calls the *wasabi receptor* — is also a Ca^{2+}-permeable cation channel on the same sensory neurons, but its defining feature hangs off the inside of the cell: an enormous N-terminal cytoplasmic skirt built of some fourteen to eighteen ankyrin repeats, a coiled stack of protein that acts as the channel's chemical tripwire. The agonists that open TRPA1 are not shaped to fit it. They are shaped to *react* with it. Scattered across that cytoplasmic domain is a small cluster of unusually reactive cysteine residues — in the human channel the

literature pins the principal three as C621, C641 and C665, with a reactive lysine, K710, alongside — and these are the latches. An electrophile diffuses to them, forms a covalent bond, and the chemically modified channel changes shape and opens. The signal is not a molecule sitting in a pocket. It is a bond that now exists where none did before.

That single difference — a fit you can undo versus a bond you have made — propagates all the way up to the feeling. Heat is a slow flood with a long tail because capsaicin is greasy, partitions into membrane, and leaves on the timescale of lipids washing out. The sharp is a fast spike that clears cleanly because the small, volatile electrophiles of mustard and wasabi arrive almost at once, fire their warning, and are either carried off up the nasal passage or neutralized by the mouth's own thiols within seconds. The nose is in on it too: the volatile ones are *retronasal*, and half of what you call the wasabi hit is the vapor rising behind the palate to meet TRPA1's cousins in the nasal lining. Two channels, two chemistries, two clocks. The body never confused them. We did, for want of a word and a number.

The electrophile and the thiol — a covalent handshake, drawn

To design a sharp you have to know exactly what the plant is handing the channel, so let us walk the chemistry in, molecule by molecule, because every pungent plant on our benches runs some version of the same elegant trick: it keeps the weapon and the trigger in separate rooms until something bites.

Take mustard. An intact mustard seed holds almost no pungency at all; what it holds is a glucosinolate — sinigrin, in the brown and black species — a stable, water-soluble, utterly mild storage compound, and, locked away in a different cellular compartment, an enzyme called myrosinase. Crush the seed, and the two rooms break open together. Myrosinase cleaves the glucose off sinigrin, the fragment left behind rearranges in a Lossen-type shift, and out falls allyl isothiocyanate — AITC — the small, volatile, savagely electrophilic molecule that *is* the bite of mustard. Its structure is the whole story: R–N=C=S, a central carbon strung between nitrogen and sulfur and starved of electrons, a warhead looking for a thiol. It finds C621 on TRPA1, the sulfur of that cysteine attacks the hungry carbon, and a dithiocarbamate adduct snaps into place. The channel opens. The plant's wound chemistry has fired in your mouth, exactly as it would have fired in the mouth of an insect, and you have decided to call it lunch.



Garlic in cross-section — alliin and alliinase held in separate cells until the clove is cut, then allicin in a heartbeat.

Garlic plays the identical two-compartment game with a different arsenal. The clove stores alliin, an odorless sulfoxide amino acid, apart from its activating enzyme alliinase. Cut the clove and alliinase converts alliin to allicin, a thiosulfinate — two sulfurs joined across an oxygen, a reactive sulfur-transfer agent that thiol-exchanges straight onto the channel's cysteines. This is why a whole clove is sweet and a crushed one roars, why the first second of cut garlic is the loudest it will ever be, and why slow-roasting tames it: allicin is unstable, and heat and time carry it off into gentler sulfides before it ever reaches you. Wasabi and horseradish run the mustard pathway with a longer tail on the molecule — allyl and 2-phenylethyl isothiocyanates, the famous green rush that goes up rather than down. Cinnamon brings cinnamaldehyde, an α,β-unsaturated aldehyde whose electron-poor double bond accepts a cysteine by straight Michael addition — the textbook TRPA1 agonist, and a slow one. Ginger brings gingerols and, when dried, their dehydrated cousins the shogaols, also Michael acceptors, warmer and rounder than the isothiocyanates. Thyme and oregano carry the monoterpene phenols thymol and carvacrol; watercress carries phenethyl isothiocyanate off its own glucosinolate. Eight plants, one receptor, one chemical verb: *react*.

What unites them, and what we had to hold onto when we built the assay, is that pungency is covalent and therefore, at the molecular level, consuming. Capsaicin leaves TRPV1 the way it came. An isothiocyanate that has bonded to C621 has spent itself and changed the channel; the signal ends not by the agonist drifting away but by the cell resetting the protein — repairing or replacing the modified channel, pumping out the calcium that rushed in. That is the deep reason the sharp has a shape the warm does not. You are not watching a concentration rise and fall. You are watching a reaction run to completion and a cell clean up after it.

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



THE SHARP CHANNEL

A match struck, not a key turned

TRPA1 · the electrophile · the covalent handshake · the shape in time

Why the sharp needed its own ruler

Here is the measurement problem, stated plainly. On every other axis the Index asks one question — *how loud* — and answers it with one number, EC_{50}, the concentration at which a stimulus drives its receptor to half of full response. Low EC_{50} means the molecule is loud; it does not take much. Invert it against a reference and you have the score. That logic is clean on heat, on sweetness, on umami, because those signals are, to good approximation, governed by equilibrium: the agonist binds, unbinds, and the response tracks how much is present.

Pungency breaks the assumption in two places. First, because the binding is covalent, the dose-response is not a simple equilibrium — it integrates. Hold an isothiocyanate on the tissue and the modified fraction of channels climbs with *exposure*, concentration multiplied by time, not with concentration alone. Second, and this is the part the tongue already knew, two sharps of equal loudness can feel like different senses because they occupy different windows of time. Wasabi is small and volatile: it floods the channel and is gone, a spike that clears in fifteen or twenty seconds. Cinnamaldehyde is heavier and less volatile: it partitions in slowly, reacts slowly, lingers, a swell that can take two or three minutes to crest and as long to fade. Measure only the peak and you would score them as cousins. Taste them and they are strangers.

So for this one axis we measure two things and the Index carries both. The loudness is still an EC_{50}, read the ordinary way, and it still runs through the family formula. But we pair it with a kinetic descriptor — a rise time to half-peak and a clearance time back to baseline — recorded on a stopped-flow TRPA1 preparation that lets us step the agonist on and off and watch the covalent signal build and decay in real time. A wasabi and a cinnamon can share an IFI-P and sit at opposite ends of a kinetic column, and that column is the difference between the rush and the grip. The score tells you how hard the door is pushed. The kinetics tell you how long it stays open. On the sharp axis you need both, and this is the volume where the Index finally learned to keep time.

The index, applied to the sharp — the arithmetic worked through

Now the numbers, because a measurement you cannot reproduce on paper is a story, not a standard. The formula is the one that governs the whole library:

$$\text{IFI-P}(c) = 1000 \cdot EC_{50}(\text{ref}) \,/\, EC_{50}(c)$$

We fix the reference as the TRPA1 half-activation of a calibrated culinary brown-mustard paste — practically, the AITC concentration at which our standard preparation reaches half of its maximal current — and we anchor that standard at **EC_{50}(ref) = 5.00 µM**. The anchor is a definition, the zero of the ruler, and it makes the reference's own score fall out as it must:

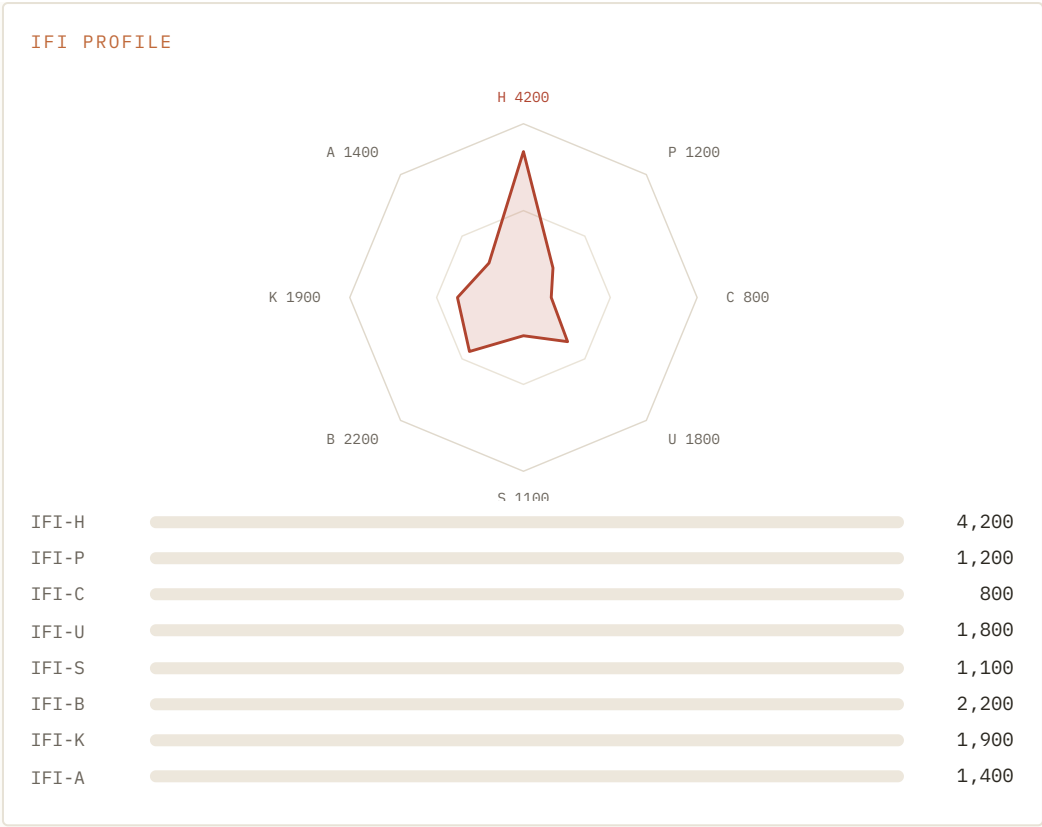
$$\text{IFI-P(ref)} = 1000 \cdot 5.00 / 5.00 = \mathbf{1{,}000}.$$

Everything else is read against that. Suppose a construct reaches half-activation at 2.50 µM — half the reference concentration, so twice as loud. Its score doubles: 1000 · 5.00 / 2.50 = **2,000**. The relationship is a reciprocal, which is the one thing to keep in the body while reading these tables: a candidate with *half* the EC_{50} scores *twice*, and a candidate that needs *more* molecule to reach threshold scores *lower*. Loudness and the concentration it takes to be loud move in opposite directions.

We can run the formula backward, too, and that is how a canonical score becomes a concrete half-activation. **Phoenix** — the full-spectrum pepper we designed gene by gene to register on all eight axes at once — carries a pungency score of **P = 1,200**, the only cultivar in our program that speaks measurably on this channel at all. Solve for its EC_{50}:

$$1{,}200 = 1000 \cdot 5.00 / EC_{50}(\text{Phoenix}) \implies EC_{50}(\text{Phoenix}) = 5000 / 1{,}200 = \mathbf{4.17\ \mu M}.$$

So Phoenix reaches TRPA1 half-activation at 4.17 µM against the reference's 5.00 — it is 1.20× louder on the sharp than an everyday brown mustard, which is a strange and lovely fact to hold: the hottest thing we grow is also, faintly, a mustard.



STANDARD	EC_{50} (MM)	EC_{50} (MG/L, AITC-EQ)	IFI-P
Worked candidate (2× reference)	2.50	0.25	2,000
Brown-mustard reference	5.00	0.50	1,000
Phoenix · SS-0096	4.17	0.41	1,200

The mass column is a unit conversion worth doing by hand, because it hides a trap that matters for cross-plant work. Allyl isothiocyanate has a molar mass of 99.15 g/mol, so the reference's 5.00 µM is 5.00×10^{-6} mol/L × 99.15 g/mol = 4.96×10^{-4} g/L ≈ **0.50 mg/L**. Fine — until you try to compare a mustard to a garlic. Garlic's warhead, allicin,

weighs 162.27 g/mol, so a garlic preparation at the *same* 5.00 µM would read 0.81 mg/L, not 0.50. A milligram of allicin carries fewer electrophilic warheads than a milligram of AITC, simply because each allicin molecule is heavier. If we scored pungency in mg/L we would be comparing masses of unlike things and calling it flavor. The Index works in molarity for exactly this reason: µM counts *molecules reaching the channel*, which is the only unit in which a mustard and a garlic can honestly stand on the same page.

The dose-response itself is steeper than a simple binding curve, as cooperative channels tend to be, and we fit it to the Hill form:

$$R([A]) = E_{max} \cdot [A]^n / (EC_{50}^n + [A]^n)$$

At [A] = EC_{50} the two powers cancel and R = E_max/2 for any n — that is what makes EC_{50} the honest midpoint. The steepness lives in n, the Hill coefficient, which for our TRPA1 electrophile fits lands near **n ≈ 1.7**. Work that slope point by point and the character of the channel shows itself:

[A] / EC_{50}	RESPONSE (% OF E_MAX)
0.25	8.7
0.50	23.5
1.00	50.0
2.00	76.5
3.00	86.6

A fourfold step in concentration — from half the EC_{50} to twice it — carries the channel from a near-silent 23.5% to a roaring 76.5%. The sharp does not ease in. It is a switch with a narrow throw, which is precisely why a hair too much mustard makes someone vanish for a second at the table and a hair less does almost nothing.

And then the honest part, the error. The family's quality spec is a batch-to-batch coefficient of variation of **≤ 7%**, against the **42%** that wild-type biology hands you when you select on phenotype and pray the genotype follows. But IFI-P is a *ratio* of two measured concentrations, and ratios propagate their uncertainty by adding in quadrature: CV(IFI-P) = $\sqrt{(CV^2_ref + CV^2_cand)}$. If reference and candidate are both freshly measured at the 7% spec, the score inherits $\sqrt{(0.07^2 + 0.07^2)} = 0.07 \times \sqrt{2} \approx$ **9.9%** — call it ten percent. Run the same sum on wild-type's 42% and you get $0.42 \times \sqrt{2} \approx$ **59%**: a number so loose it is barely a measurement. There is a way to tighten ours further, and we take it — the reference is not re-measured every run but held as a locked, many-times-calibrated standard whose own uncertainty is negligible, which collapses the sum back toward the candidate's own 7%. Either way, read what it does to Phoenix's sharp: P = 1,200 at ~10% carries a band of ±120, so [1,080–1,320]; the same discipline on its heat holds 2,937,948 SHU to ±7%, a window of ±205,656 SHU rather than the ±1.2 million a 42% process would have smeared across the label. Precision is not pedantry here. On an axis this steep, a 42% cloud and a 7% band are the difference between a product and a guess.

A ladder of sharp plants

The sharp axis is where the world keeps its condiments, and it is also where our host program runs widest beyond the peppers. Black mustard is the deep well — **2,790 constructs**, more than any pungent species we work and second in the whole library only to the tomato — because the glucosinolate–myrosinase system is a rich, tunable target and because mustard is where the axis was born. Around it sits a whole cabinet of sharp plants, each a different electrophile on the same channel, each grown whole so that the enzyme and its substrate both survive to the plate.



Ginger in cross-section — gingerols that become shogaols when dried, the slow, rounded end of the sharp.

PLANT	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	PRINCIPAL ELECTROPHILE
Black Mustard	2,790	P / M	Hard	Allyl isothiocyanate (from sinigrin)
Garlic	560	K / P	Moderate	Allicin (thiosulfinate)
White Mustard	200	P	Hard	p-Hydroxybenzyl isothiocyanate
Ginger	120	P	Moderate	Gingerols → shogaols
Thyme	120	P	Hard	Thymol (monoterpene phenol)
Watercress	120	P	Hard	Phenethyl isothiocyanate
Cinnamon	40	P	Hard	Cinnamaldehyde
Oregano	40	P	Hard	Carvacrol

Read the difficulty column as a map of the two-compartment problem. The isothiocyanate plants are nearly all *Hard*, because the pungency is not a molecule you can simply dial up — it is an enzyme meeting a substrate at the instant of damage, and to raise the sharp you must raise the glucosinolate pool, the myrosinase, and the fidelity of their meeting, all without the plant poisoning its own tissues before harvest. Garlic and ginger come in *Moderate* because their chemistry is more forgiving: a single activating enzyme, a substrate the plant already tolerates in quantity, a pungency that survives ordinary handling. Garlic also carries two axes — K and P, kokumi and pungency — which is the quiet truth of every allium: the thing that makes a long-cooked onion taste of depth and continuity

is cousin to the thing that makes a raw one bite. Onion sits just off this table on kokumi, the same family grown toward richness rather than the rush.

What none of these needs is the heat machinery. The three AI-designed edits that built Phoenix's fire — S39L, L345G and C175S, written into the Pun1 acyltransferase — sit on the capsaicin pathway, the phenylpropanoid branch that makes the warm. The sharp answers to an entirely different chemistry, the glucosinolates and thiosulfinates and Michael acceptors of the wound, and that separation is the good news for a designer: heat and pungency are independent knobs on independent pathways, which is why a single cultivar like Phoenix can carry both at once, a measured 4,200 on one channel and 1,200 on the other, without either borrowing from the other's budget.

What we can and cannot yet do

A standard earns trust by naming its own failures, so here are the edges where the sharp still fights the ruler, and what we do about each.

The first is volatility, and it is the signature trouble of this axis. The very thing that makes wasabi feel clean — that its electrophile is small and flies — means the molecule escapes the sample while you measure it. Score a fresh mustard in an open cuvette and the reading falls minute by minute as the AITC leaves for the air; the sharp you are trying to pin is literally evaporating off the instrument. We answer it with closed, headspace-controlled assays that capture the vapor fraction and with fresh-prep timing locked to the second, because an EC_{50} measured on a sample that is quietly emptying is not a half-activation, it is an artifact with a clock on it.

The second is the covalent bond turning against us: tachyphylaxis. Because pungency modifies the channel rather than visiting it, a heavy or repeated dose can outrun the cell's ability to reset — the sharp fades not because the molecule is gone but because the channels are spent, and the tongue goes briefly numb to it. Anyone who has eaten too much wasabi and found the next bite strangely quiet has run the experiment. For measurement it means the assay must work at genuine half-activation and lower, on the honest part of the curve, never in the saturating region where the channel's own exhaustion masquerades as a lower response.

The third is the enzyme, not the molecule. A glucosinolate without live myrosinase is silent — this is why mustard powder bloomed in cold water bites and the same powder stirred into a hot pan does not, why over-cooked garlic is sweet, why a sharp can be *cooked out* of a dish entirely. The pungency of these plants is a reaction waiting on an enzyme, and a hard sear denatures the enzyme before it can fire. We measure the whole system — substrate and enzyme and the water that lets them meet — because to score only the glucosinolate pool would be to report a loudness the plant can no longer make.

And the honest ceiling: the Index measures TRPA1 activation in a controlled preparation, and perception is more than one channel. The sharp you feel is trigeminal activation and retronasal vapor and

the cooling or warming of the tissue all arriving together, integrated by a brain that also remembers the last bite. We capture loudness as an EC_{50} and, on this axis, kinetics as a rise-and-clearance pair, and that pairing closes most of the gap the old single number left open — but it does not yet fully capture *quality*, the felt difference between a rush that lifts behind the eyes and a grip that sits on the back of the tongue. We can tell you, now, how hard the door is pushed and how long it stays open. We are still learning to write down what the draft feels like going through. That is the frontier this volume stands at, and we would rather name it than paper over it.

Back to the table

None of this changes what happens with the fresh mustard at dinner — the vanishing, the laugh, the hand that stops halfway to the face — except that we can now say what passed through. A plant, cut, firing the wound chemistry it spent millions of years perfecting, handing an electron-hungry molecule to a channel built to notice exactly that, a covalent bond made and a door blown open and a cell already moving to close it. Ten thousand years of us tasting that warning and deciding, against all sense, that we love it.

We are plant people first, and so most of what this sharp belongs to is plant — the mustards and wasabis, the chili crisps and ginger-scallions, the fierce bright pastes that live at the edge of the plate and change everything they touch. Everything is grown whole, nothing stripped, the enzyme kept alive beside its substrate because that living meeting *is* the flavor and the nourishment in one act. And every one of them is scored on the eight axes, loudness and now kinetics both, so you can read exactly what you are about to feel before you feel it. The sharp is the half of spicy that no one had the number for. This is the book where it finally got one.



PART I OF V

The Sharp Channel

TRPA1 · the covalent handshake · amplitude and its shape in time

Chapter 1 · The wasabi receptor — *the alarm that tastes.*

Everything in this book routes through one protein, so we begin by introducing it properly. TRPA1 — Transient Receptor Potential, Ankyrin subtype 1 — is a large, four-part ion channel that sits in the membranes of sensory nerve endings throughout the mouth, nose, throat, and skin. It is the only member of its subfamily in humans, and it is the most promiscuous chemical sensor the body owns. The eye-watering volatiles of a chopped onion, the fume off a jar of English mustard, the sting of a cut chili's companion compounds, the industrial bite of acrolein in smoke, the formalin in a biology lab, the cold-sting of a strong menthol — all of these are read, in part or in whole, by TRPA1. It is, in the plainest terms, the body's irritation channel: the sense that something reactive has arrived, and that the tissue should know. Our whole pungency program is an act of courtship aimed at this one guarded, suspicious protein — learning to speak to it so precisely that an alarm becomes a pleasure.



A reporter line on the microscope stage, loaded with the calcium dye that turns a channel opening into light.

The name tells its architecture. *Transient receptor potential* is the superfamily — gated channels that let positive ions pour across a membrane and briefly flip a nerve's voltage. *Ankyrin* is the subtype's signature: each of the channel's four identical protein chains begins with an enormous curling tail of **ankyrin repeats** — a stack of roughly sixteen short helix-turn-helix motifs, coiled like a spring, reaching down into the cell. No other TRP channel carries so long an ankyrin domain, and that spring is not decoration. It is the mechanical and chemical antenna of the channel, the part that most of our pungent molecules actually touch.

The architecture, chain by chain

Four of those chains assemble into a single working channel with fourfold symmetry — a tetramer — arranged around a central pore. When the 2015 cryo-electron-microscopy structure of TRPA1 first resolved that assembly, it looked, to us, like a thing built to be tripped. The four pore-forming helices cross at the bottom to pinch the ion path shut at a hydrophobic constriction — the lower gate, a girdle of isoleucine and valine side chains near residue I957 that excludes water and ions when the channel is at rest. Above it sits a second, narrower checkpoint: the selectivity filter, a ring of acidic residues around position 915 that decides which ions may pass and, crucially, waves calcium through. And wrapped around the whole base is a cluster of allosteric machinery — the so-called nexus, the TRP-like helix, the pre-S1 region, the linker — poised just above the ankyrin springs, ready to translate a chemical event at the tail into a mechanical opening at the gate.

When the gate opens, sodium and, importantly, **calcium** flood inward. The calcium matters twice over: it carries the depolarizing current that becomes a nerve impulse, and it is also the signal that will later shut the channel down. Hold that thought; it is the whole secret of why the sharp clears. The reporter cell in the figure above makes the event visible — it is loaded with a dye that fluoresces when it binds free calcium, so every channel opening is a flash of light we can photograph and, more to the point, *count*. A number begins as a brightness.

What TRPA1 does *not* have is the thing TRPV1 has — a tidy binding pocket shaped to cradle one molecule. TRPV1 reads capsaicin the way a lock reads a key: a specific, reversible, shape-complementary fit, and when the capsaicin diffuses away the lock relaxes. TRPA1 is cruder and, for our purposes, far more interesting. It reads a molecule not by its shape but by its **reactivity** — by whether that molecule is electron-hungry enough to form a chemical bond with the channel itself. This is a different kind of sensing altogether, and it is the mechanistic heart of the pungent axis, the reason our design levers for pungency look nothing like our levers for heat.

Reading reactivity, not shape

Tucked into the cytoplasmic N-terminal region of each chain — down among the first ankyrin repeats and the linker that tethers them to the pore — is a small set of unusually exposed, unusually acidic amino acids: a triad of cysteines, by convention numbered C621, C641, and C665 in the human sequence, joined by a reactive lysine near K710. A cysteine carries a thiol group, –SH, and at the mildly basic microenvironment of this pocket a fraction of those thiols give up their proton to sit as the thiolate anion, $–S^-$. A thiolate is one of the most willing nucleophiles in all of biochemistry: a lone pair of electrons looking for an electron-poor partner. TRPA1, in other words, dangles four chemically hungry hooks into the cytoplasm and waits.

An **electrophile** is any molecule carrying an atom starved of electrons — a carbon made electron-poor by a neighbouring double bond and a withdrawing group, or the central carbon of an

isothiocyanate, or a sulfur poised to swap partners. These are exactly the molecules a plant reaches for when it wants to wound the thing that bit it. And they are exactly what TRPA1 is built to catch. The receptor is, in effect, running a continuous chemistry experiment on everything that reaches its sensing domain, and firing when the experiment succeeds — when a bond forms. This is why the channel is so promiscuous and so hard to fool: it is not matching silhouettes, it is testing for reactivity, and reactivity cannot be faked by a molecule that merely *looks* right.

The Michael addition, step by step

The most important single reaction on this axis is the one that opens the channel for cinnamon, for ginger's sharp cousins, for the acrolein in smoke, and for a great many of the molecules we design. It is a **Michael addition**, and it runs in a handful of steps we can follow atom by atom.

1. **The agonist presents an electron-poor carbon.** Take *trans*-cinnamaldehyde, the sharp principle of cinnamon: a benzene ring, then a carbon–carbon double bond, then an aldehyde. The oxygen of the aldehyde pulls electron density toward itself; that pull travels back along the conjugated double bond and leaves the far carbon — the β-carbon — distinctly electron-poor. Chemists call this an α,β-unsaturated carbonyl, and it is the archetypal Michael acceptor.
2. **The thiolate attacks.** The $-S^-$ of one of TRPA1's reactive cysteines reaches in and donates its lone pair straight into that hungry β-carbon. A new carbon–sulfur bond forms. The electrons displaced by the attack flow up onto the carbonyl oxygen, parking there for an instant as an enolate.
3. **The adduct settles.** The enolate re-protonates, and what remains is a stable covalent adduct: the pungent molecule is now welded to the channel through a C–S bond, no longer a visitor but a part of the protein.
4. **The spring moves.** That new bond, formed down in the ankyrin tail, is bulky and charged where there was empty space before. The strain propagates up the coiled ankyrin spring, through the linker and the nexus, into the TRP-like helix, and finally into the S4–S5 region that braces the pore. The lower gate dilates. Sodium and calcium pour through. A nerve fires, and a face flinches a quarter-second before the mind has named the sensation.

Not every pungent molecule uses the carbonyl route, but all of them trade on the same covalent principle. Allyl isothiocyanate — the AITC of mustard and wasabi, written R–N=C=S — offers its *central* carbon to the thiolate and forms a dithiocarbamate adduct. Allicin, the molecule garlic makes only when wounded, carries a reactive sulfur and attacks the cysteine by thiol exchange, leaving an allyl group bonded through sulfur — an S-thioallylation. The chemistries differ in their particulars; the consequence is identical. A covalent bond forms on the sensing domain, and because it is *covalent*, it does not simply diffuse away the way capsaicin leaves TRPV1. It persists until

the cell's own reducing machinery — glutathione, chiefly — pries the adduct back off, or until the protein is retired and replaced. Pungency, at the molecular scale, is not a molecule sitting in a pocket. It is a scar the tissue is actively healing.



Garlic in cross-section — alliin and alliinase held in separate cells until the clove is cut, then condensed in seconds into allicin, the covalent agonist.

Where the sharp is made: the plant's two-part detonators

Here is the fact that organizes all of our plant-side engineering: the pungent molecules almost never exist, in quantity, in the intact plant. They are *manufactured at the moment of injury*, by keeping a stable precursor in one cellular compartment and the enzyme that activates it in another, so that nothing happens until a tooth or a blade ruptures the walls between them. The plant is a loaded trap. We are the species that learned to spring it on purpose, at the table.

Two of these two-part systems carry most of the pungency we love, and both run in plants we have brought onto the platform.

The first is the **glucosinolate–myrosinase** system of the mustard family — black mustard, white mustard, wasabi, horseradish, watercress, radish. The precursor is a glucosinolate such as sinigrin (allyl glucosinolate), a perfectly stable, water-soluble, flavorless sugar-linked compound stored in the vacuole. Held apart from it, in specialized myrosin cells, waits myrosinase, a thioglucosidase. Crush the tissue and the two meet: myrosinase snips the thioglucoside bond, releasing glucose and an unstable aglycone, which immediately undergoes a Lossen-type rearrangement, throwing off sulfate and snapping into **allyl isothiocyanate** — the volatile, eye-watering sharp that defines the whole family. Wasabi layers a longer-chain isothiocyanate (6-methylsulfinylhexyl isothiocyanate) on top of the AITC, which is why a true wasabi reads rounder and more complex than a jar of hot mustard even though both are speaking to the same receptor. This is also why wasabi must be grated *at the table* and fades within minutes: once the AITC is liberated it flees, and once the myrosinase is spent or heated past denaturation, no more is made.

The second is the **alliin–alliinase** system of the alliums — garlic and onion. Garlic stores alliin, S-allyl-L-cysteine sulfoxide, a stable and odorless amino-acid derivative, in the cell cytoplasm, while the enzyme alliinase — a pyridoxal-phosphate enzyme — sits sequestered in the vacuole. Cut the clove and alliinase cleaves alliin into allyl sulfenic acid, pyruvate, and ammonia; two molecules of that

sulfenic acid then condense, losing water, into **allicin** (diallyl thiosulfinate), the covalent TRPA1 agonist pictured welling up from the cut face above. The same compartmentation logic, a different chemistry, the same end: a detonator that arms only on damage.

A third family of pungent principles skips the two-part trap and is simply synthesized and stored — the phenylpropanoids. Cinnamaldehyde (cinnamon) rides straight off the phenylpropanoid branch as an α,β-unsaturated aldehyde. Ginger's gingerols are vanillyl compounds that, on drying or gentle heat, lose water to become **shogaols** — α,β-unsaturated ketones, sharper Michael acceptors than the gingerols they came from, which is why dried ginger bites harder than fresh. Because the gingerols and shogaols carry the same vanillyl motif that capsaicin carries, they also ring TRPV1, and so ginger arrives on the tongue as warmth and sharpness braided together — a single plant lighting two receptors at once.



Ginger — gingerols that dehydrate to the sharper shogaols, carrying the vanillyl motif that lights warmth and pungency in one breath.

Our design levers follow directly from this biology. We do not — cannot — re-engineer the human receptor; it is fixed. Everything we do is on the plant side: enlarge the precursor pool (more sinigrin, more alliin), tune the activating enzyme's abundance and speed, adjust the compartmentation so the detonation is faster or slower, and choose *which* electrophile the plant delivers. Those four handles are the whole instrument. The table below is the current roster of organisms on the platform whose primary voice is pungency — the plants in which we are learning to play it.

HOST ORGANISM	DESIGN PACKAGES	PRIMARY AXES	ENGINEERING DIFFICULTY
Black Mustard	2,790	Pungency · Multi-axis	Hard
Garlic	560	Kokumi · Pungency	Moderate
White Mustard	200	Pungency	Hard
Ginger	120	Pungency	Moderate
Thyme	120	Pungency	Hard
Watercress	120	Pungency	Hard
Cinnamon	40	Pungency	Hard
Oregano	40	Pungency	Hard

Giving the sharp a number

Reverence is not measurement, and a plant we cannot score is a plant we cannot improve on purpose. So we anchor pungency to the same instrument that anchors every axis in this library: the half-maximal activation of its receptor, read through the Iyengar Flavor Index.

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

EC_{50} is the concentration of an agonist that drives its receptor to half of full response. The relationship between concentration and response is not a straight line but a sigmoid, described by the Hill equation:

> **$R / R_{max} = [A]^h ÷ (EC_{50}^h + [A]^h)$**

where [A] is the agonist concentration and *h* is the Hill coefficient, a measure of steepness. Set [A] equal to EC_{50} and the arithmetic collapses beautifully: the right side becomes EC_{50}^h / $(2 \cdot EC_{50}^h)$ = ½, exactly half-maximal, whatever the value of *h*. That is what makes EC_{50} the honest midpoint of the curve — the one point that does not move when the curve steepens. For TRPA1, where several cysteines can be modified in concert, *h* commonly runs above 1 (we typically fit values near 1.5 to 2), which means the response climbs steeply once it starts: pungency has a threshold quality, quiet and then suddenly present, and the steep Hill slope is the mathematics of that suddenness.

To turn an EC_{50} into an index score we need a reference, and for pungency the natural reference is allyl isothiocyanate — the archetypal TRPA1 electrophile, the sharp of wild black mustard. We anchor **IFI-P = 1,000** to a stated convention constant of **2.1 µM** for AITC — a defined reference point, in the same spirit as Scoville's original dilution-to-threshold definition, not a single literature-derived value. The formula is:

> IFI-P = 1,000 × 2.1 µM ÷ EC_{50}(candidate)

and the whole axis falls out as a few lines of arithmetic:

CANDIDATE	EC_{50} (MM)	2.1 ÷ EC_{50}	IFI-P
Wild-type reference (anchor)	2.1	1.00	1,000
Twice as potent	1.05	2.00	2,000
Four times as potent	0.525	4.00	4,000
Half as potent	4.2	0.50	500

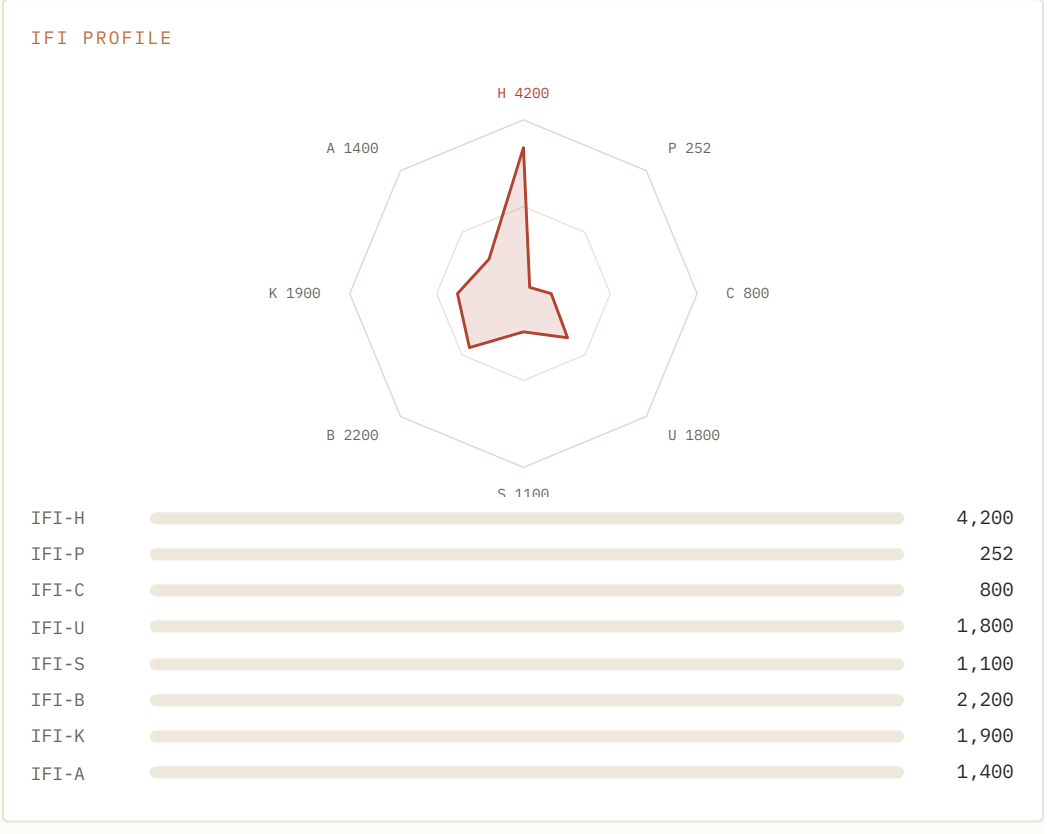
The logic runs in both directions, which is how we check a design against the catalog. Our full-spectrum flagship, **Phoenix** (SS-0096), is scored at IFI-P 252. Invert the formula and that score names the design target:

> EC_{50}(Phoenix) = 1,000 × 2.1 µM ÷ 252 = **8.3 µM**

So Phoenix's pungent compound is designed to half-activate TRPA1 at 8.3 µM — intentionally milder than the 2.1 µM convention constant, since in Phoenix, pungency plays a supporting role behind the flagship's heat. The number is not decoration; it is a falsifiable claim about a concentration, and the reporter line in this chapter's opening figure is how we hold ourselves to it.

A score is also only as trustworthy as its units, so we convert. Allyl isothiocyanate has a molar mass of 99.15 g/mol (C_4H_5NS: 48.04 for carbon, 5.04 for hydrogen, 14.01 for nitrogen, 32.06 for sulfur). The convention constant in mass terms is therefore 2.1 × 10^{-6} mol/L × 99.15 g/mol = 2.08 × 10^{-4} g/L — about **0.21 ppm** in dilute aqueous solution. Phoenix's design target of 8.3 µM works out to 0.82 ppm. These are the concentrations at the receptor, parts per million, which is worth sitting with: the whole drama of a wasabi-struck sinus is well under a part per million of a small molecule finding a thiolate. Reverence and arithmetic are, here, the same observation.

The full predicted profile of the flagship, read across all eight axes, shows pungency in its proper company — present, deliberate, and far from the loudest voice in the plant:



Why the heat scale cannot see wasabi

The oldest number in this field is the Scoville Heat Unit, and it is worth being precise about what it measures, because the precision is the argument for a pungency axis at all. SHU is a capsaicinoid scale. It reports, in effect, how much a sample must be diluted before the *heat* — the capsaicin, read by TRPV1 — can no longer be detected. It is a fine instrument for its one job and useless for ours. Allyl isothiocyanate does not meaningfully bind TRPV1; neither does allicin, nor cinnamaldehyde in any ordinary amount. A fierce spoonful of fresh wasabi, which can clear a room, registers essentially **zero Scoville units** — not because it is mild but because the heat scale is constitutionally blind to the receptor wasabi actually speaks to. To rate a mustard in Scovilles is a category error, like weighing a sound in metres.

This is the clean dividing line of the whole library. Heat is capsaicin on TRPV1; pungency is electrophiles on TRPA1. They are different molecules, different receptors, different mathematics, and a single plant can be eloquent on one while silent on the other. The two channels happen to be co-expressed in many of the same trigeminal nerve endings, which is why heat and pungency braid so readily in the mouth and why ginger — speaking to both at once — feels confusingly warm *and* sharp. But co-expression is a fact about the wiring, not about the compounds. The IFI gives each channel its own column precisely so that a plant's full chord can be written down, and so that a thing that is sharp but not hot — the entire mustard and allium and horseradish world — finally has a number to its name instead of an apology.

Written out in full, a plant's chord is eight receptors read at once. Here is Phoenix across all eight, sorted by predicted design intensity — pungency the softest note in the chord, deliberate and present but never the loudest voice, exactly where we put it. Because each axis uses its own defined convention constant, scores across axes reflect activation of different receptors at different reference points; they are not on a single common scale.

AXIS	RECEPTOR	WHAT IT READS	PHOENIX IFI
Heat	TRPV1	capsaicin, thermal burn	4,200
Zero-Bitter	TAS2R38	bitterness attenuation	2,200
Kokumi	CaSR	richness, continuity, mouthfeel	1,900
Umami	mGluR1	savory depth	1,800
Astringency	Salivary PRPs	tannin, polyphenol mouthfeel	1,400
Sweetness	TAS1R2·R3	sugar-free sweetness	1,100
Cool-Burn	TRPM8	cooling thermomodulation	800
Pungency	TRPA1	electrophile sharpness	252

Amplitude, attack, decay — designing the pulse

One property separates pungency from every other axis in this library and organizes half of what we design on it. The receptor's chemical mode of activation is **use-dependent and self-limiting**. The same calcium that opens the current also, once inside, drives the channel toward a closed and briefly unresponsive state — desensitization. Combine that with the fact that most of our pungent molecules are volatile and flee the warm, wet mouth within seconds, and you get the signature behavior of the whole axis: the sharp does

not accumulate. It spikes and it goes. Heat, built on a non-reactive molecule that lingers in fat and a receptor that does not shut itself off the same way, is a plateau. Pungency is a pulse.

Because it is a pulse, designing it means designing three independent things, not one.

Amplitude — how loud — is the IFI-P score itself, and it is the product of two factors we can move separately: the intrinsic potency of the electrophile (its EC_{50}, set by which agonist the plant makes) and the delivered dose (how much reaches the receptor, set by precursor pool and enzyme activity). A plant can be made sharper either by producing a more reactive molecule or by producing more of the same one; the index does not care which, but the formulator does.

Attack — how fast the sharp arrives — is set by the kinetics of liberation and delivery: how quickly myrosinase or alliinase converts precursor to agonist on wounding, and how volatile the product is, since volatility is what carries the sharp up into the trigeminal field of the nose. A fast enzyme and a light, flighty molecule give the near-instant nasal detonation of wasabi; a slower release and a heavier molecule give the gradual bloom of a cooked allium.

Decay — how fast it clears — is set by three things working together: volatility sweeping the agonist out of the mouth, calcium-driven desensitization closing the channel, and the cell's glutathione quietly reducing the covalent adduct back off the cysteine. Tune these and the same peak amplitude can be made to vanish cleanly or to linger as a long warm finish.

Precision on all three demands that the *measurement* be disciplined, and this is where pungency is genuinely harder to score than heat. A covalent adduct accumulates with exposure time, so a TRPA1 EC_{50} is not a fixed constant the way a reversible binding EC_{50} is — it drifts with how long the agonist and receptor are allowed to react. We therefore fix the assay to a standard exposure window and read every candidate inside it, so that the EC_{50} we report is comparable across the catalog rather than an artifact of how patiently we waited.

Even with the protocol fixed, every number carries a window, and honesty requires we propagate it. Our engineered constructs hold batch-to-batch variation to a coefficient of variation of **≤7%** (CV = standard deviation ÷ mean) — against wild harvests that can scatter past 40%, where rainfall and ripeness and the hour of picking all push the precursor pool around. Because IFI-P is a *ratio* of two EC_{50} measurements, the errors add in quadrature:

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CV(IFI) = √( CV(reference)² + CV(candidate)² )
```

If both the reference and the candidate carry a 7% CV, then CV(IFI) = $\sqrt{(0.07^2 + 0.07^2)} = \sqrt{0.0098}$ = **9.9%**. So Phoenix's IFI-P of 252 is properly read as 252 ± 25. In practice we calibrate the reference exhaustively and treat its error as negligible, which collapses the window to the candidate's own 7% — ±18 on a score of 252. Either

way, the point of the arithmetic is the same: the number comes with its uncertainty stated, because a number without an error bar is a wish, and we would rather give the reader respect than drama.

What we can — and cannot — do

Pungency is the axis on which we are most explicit about our limits, and we think that candor is part of designing it well.

We cannot touch the receptor. Heat gave us an enzyme to redesign; pungency gives us a fixed human sensor and nothing but plant-side chemistry to deliver to it. Every lever is upstream of the tongue — precursor, enzyme, compartment, volatility — which is a narrower instrument than it first appears, and a more demanding one.

The axis is fragile to processing in a way no other axis is, because the agonist is manufactured on damage by an enzyme. Heat denatures myrosinase and alliinase; cook garlic and the sharp alliin is never converted, which is exactly why a roasted clove turns sweet and mild. This is a genuine formulation hazard: a designed pungency can be real in the field and absent on the plate if the kitchen boils the enzyme before it has worked. Much of our applied work on this axis is therefore not about making plants sharper but about making their sharpness *survive* — stabilizing the enzyme, protecting the precursor, timing the release.

Covalent chemistry also sets a ceiling we choose to honor rather than fight. Because each activation leaves an adduct that the cell must reduce away, relentless high-dose pungency can outrun the tissue's glutathione and tip from pleasure into raw irritation. So we cap IFI-P deliberately. It is no accident that Phoenix, a plant we designed to reach a predicted 4,200 on heat, speaks pungency at only 252 — a deliberate whisper behind the flagship's heat, present across a whole meal without grinding. We design the sharp to pulse and clear, never to grind, because the ethos that runs under all of this work is that food should be something you give your body with intention, and an alarm is not a gift.

And we score on the right animal — which is to say, on none. TRPA1 pharmacology diverges sharply across species; molecules that excite the human channel can leave a rodent's cold, and the reverse. So we read every candidate on human-receptor reporter lines, the calcium-dye cells of this chapter's first figure, because a pungency scored against the wrong sensor is worse than unscored: it is confidently wrong.

What remains, inside those limits, is enough for a lifetime of work. A plant builds an electrophile to wound the mouth that bites it, and keeps it locked apart from its trigger until the moment of injury. A human nerve dangles a thiolate hook into the cytoplasm and waits to be wounded. Between the two is a covalent bond, forming and healing in sub-ppm concentrations, that we can now name with a number, bound with an error bar, and shape in its amplitude and its attack and its decay. These are computational designs, and Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The plant wrote a warning. We have learned to read it as delicious — and to keep it, always, gentle on the body it feeds.

Chapter 2 · The covalent handshake — *a bond, not a fit.*

If you remember one idea from this book, make it this one: **pungency is a chemical reaction, and heat is not.** It is the fault line that runs under the entire eight-axis index, and it is why the mustard and the chili — which the mouth files together as "spicy" — are, at the receptor, as different as burning and bruising. One is a molecule settling into a pocket and settling back out again, a thousand times a second, touching nothing it does not release. The other reaches in and *rearranges the receptor's own atoms.* We have spent years learning to read the difference, and the whole of this chapter is an attempt to hand it to you whole.

Capsaicin binds TRPV1 and lets go. Allyl isothiocyanate — the molecule of mustard and wasabi — reaches into TRPA1 and *bonds* to it. The reactive molecules that trip the pungent channel share one trait: they are **electrophiles**, carrying an atom so starved of electrons that it will seize a pair from almost anything soft and nucleophilic nearby. And the channel presents, on the inner face of those great ankyrin springs, exactly the thing an electrophile craves — a small cluster of **cysteine** side chains, each tipped with a sulfur–hydrogen thiol, one of the softest, most generous nucleophiles biology builds. The electrophile finds the thiol, the thiol gives up its electrons, and a covalent bond snaps into place. The channel, chemically modified on its own antenna, changes shape and opens. Nothing about this is a lock and key. It is a weld.



The assay that reads the bond forming — a dish of reporter cells under agonist, calcium turning to signal.

The residues that take the bond

The cysteines have names. In human TRPA1 the critical residues sit in the linker that joins the ankyrin domain to the pore machinery: **C621, C641, and C665**, with a nearby lysine, **K710**, able to take certain electrophiles as well. These are not chosen for their beauty; they are chosen by chemistry. A thiol is *soft* in the precise sense that physical chemists mean — its electron cloud is large, loosely held, and easily polarized — and the electrophilic carbons of the pungent molecules are soft in the same way. Soft pairs with soft; the reaction is fast and favorable exactly because the two partners are matched.

A hard nucleophile such as a plain hydroxyl, or the carboxylate of an aspartate, would barely react with these agonists at ordinary mouth concentrations. The channel's sensitivity lives in those few sulfurs, and in their reactivity more than their position.

Mutate those cysteines to a residue that cannot form the bond — swap the reactive sulfur for the inert methyl of an alanine — and the channel goes very nearly deaf to mustard, to cinnamon, to allicin, to acrolein, while still answering the handful of agonists that work by ordinary non-covalent means. That single experiment, run many ways in many laboratories, is the proof that pungency's loudest voices speak by chemistry, not by shape. The molecule is not recognized. It is *reacted with.*

It helps to slow the event to its four frames. First, **approach**: the electrophile, uncharged and often slightly oily, partitions out of saliva and into the membrane-facing cleft where the reactive cysteines wait, so the local concentration at the sulfur is higher than the bulk concentration in the mouth. Second, **attack**: the thiol's sulfur, deprotonated to the far more reactive thiolate at the near-neutral pH just inside the cell, pushes its lone pair onto the electron-poor carbon. Third, **adduct**: the new sulfur–carbon bond forms and the proton is handed off, leaving the agonist permanently (or semi-permanently) hung on the channel. Fourth, **gating**: that added mass and charge strains the ankyrin spring, the four subunits of the tetramer shift together, and the pore dilates into its open state. Because TRPA1 assembles as four identical subunits, each carrying its own set of reactive cysteines, the gate does not flip on a single weld — it answers more steeply than a one-to-one binder would, and that cooperativity will matter when we put a number on it below.

Two chemistries, two adducts

The chemistry comes in two flavors, and the difference between them is audible on the tongue. The **isothiocyanates** — the R–N=C=S group that defines mustard, wasabi, horseradish, and watercress — carry a central carbon so electrophilic that the cysteine thiolate adds straight across it, forming a **dithiocarbamate** adduct. The **α,β-unsaturated carbonyls** and related Michael acceptors — cinnamaldehyde, the gingerols and shogaols, allicin's reactive sulfur, the onion's lachrymatory factor — present an electron-poor carbon one bond away from a carbonyl or sulfinyl, and the thiolate adds across *that,* in the conjugate-addition reaction every organic chemist knows as **Michael addition**, to leave a stable thioether. Both routes end in the same place, a sulfur bridge from molecule to channel, but they differ in how fast the bond forms and how readily it comes undone, and those two kinetic facts become, in our hands, design parameters.

AGONIST	SOURCE PLANT	REACTIVE GROUP	ADDUCT WITH CYS–S⁻
Allyl isothiocyanate	black & white mustard · wasabi · horseradish · watercress	–N=C=S isothiocyanate	dithiocarbamate
trans-Cinnamaldehyde	cinnamon	α,β-unsaturated aldehyde	thioether (Michael)
6-Gingerol → 6-shogaol	fresh → dried ginger	β-hydroxy ketone → α,β-unsaturated ketone	thioether (Michael)
Allicin	garlic	allyl thiosulfinate (S–S=O)	mixed disulfide / thioether
syn-Propanethial *S*-oxide	onion	α,β-unsaturated thiocarbonyl *S*-oxide	thioether (Michael)

What the table hides is that the plant does not store these electrophiles ready-made — they would corrode its own proteins if it did. It stores them disarmed, behind a wall, and builds them only at the instant of injury. In black and white mustard, in wasabi and horseradish, the resting cell holds a stable thioglucoside, **sinigrin**, in one compartment and the enzyme **myrosinase** in another; crush the tissue — or chew it — and the two meet, myrosinase hydrolyzes the sugar away, and the unstable aglycone snaps through a Lossen-type rearrangement into allyl isothiocyanate. Garlic is the same drama in different costume: the odorless amino acid **alliin** sits apart from the enzyme **alliinase** until a knife or a molar brings them together, and in seconds the enzyme forges **allicin**, the reactive thiosulfinate. Onion runs one further step, a **lachrymatory-factor synthase** that rearranges the sulfenic acid into the volatile tear-maker. Ginger needs no enzyme of injury at all — its **gingerols** are born pungent — but drying and gentle heat dehydrate the β-hydroxy ketone into the **shogaol**, trading a feeble electrophile for a conjugated one, which is precisely why dried ginger bites where fresh ginger only warms. Every one of these is a controlled release of an electrophile, and every one is a place where a careful hand on the pathway's enzymes can turn the sharpness up or down.



Ginger in cross-section — the gingerols of the fresh root dehydrate into the sharper shogaols on drying, a Michael acceptor made keener.

This is also where the pungent axis parts company with the heat axis in how it is *built.* Heat is engineered at a biosynthetic enzyme — tune the acyltransferase that condenses capsaicin and you change how much warmth the fruit makes. Pungency is engineered at the electrophile machinery: enlarge the glucosinolate pool and the

myrosinase that uncages it, bias ginger toward shogaol over gingerol, lift the alliin that garlic can convert. We are not only making *more* of the sharp molecule; where we can, we are shifting the harvest toward the *faster-reacting* one — and the next section is where that word, *faster,* becomes arithmetic.

Why potency here is a rate, not a fit

Now potency means something unusual, and this is the mathematical heart of the whole axis.

On a channel that senses by binding, potency is an *equilibrium* — how tightly, on average, a molecule sits in its pocket, a balance struck in an instant and held. On TRPA1, potency is partly a **rate** — how quickly the electrophile closes the bond. A compound can be a modest electrophile and still read as intensely pungent if it reaches the cysteines fast; a sluggish one reads mild even at high concentration because the reaction crawls. Let us make that exact.

Hold the agonist at a fixed concentration $[A]$ against the channel's thiols. Because the thiols are vastly outnumbered by agonist, the reaction is pseudo-first-order, and the fraction of channels that have taken the covalent adduct after an exposure time t climbs as

$$f(t) = 1 - e^{-k\,[A]\,t}$$

where k is the second-order rate constant of the electrophile–thiol reaction, in units of $\mathrm{M^{-1}s^{-1}}$. Two things fall straight out of that single equation. At a *fixed* concentration, the time to modify half the channels is $t_{1/2} = \ln 2/(k\,[A])$ — a fast electrophile reaches half-modification in a breath, a slow one in a slow fade. And the half-maximal concentration — the $\mathrm{EC_{50}}$ we actually anchor the index to — is whatever $[A]$ drives f to one-half at the assay's read time t^{*}:

$$1 - e^{-k\,\mathrm{EC_{50}}\,t^{*}} = \tfrac{1}{2} \quad\Longrightarrow\quad \mathrm{EC_{50}} = \frac{\ln 2}{k\,t^{*}}.$$

Read that result slowly, because it is the chapter in one line. For a covalent agonist the $\mathrm{EC_{50}}$ is *not* a fixed property of the molecule — it is inversely proportional both to how fast the molecule reacts, k, and to how long you watch, t^{*}. Watch twice as long and the apparent $\mathrm{EC_{50}}$ halves. A thermodynamic binder like capsaicin has no such clause; its $\mathrm{EC_{50}}$ is set the moment equilibrium is reached and does not drift with the clock. So the first discipline of the pungent axis is procedural: **fix the exposure window,** or you are not measuring the same thing twice. We read at $t^{*} = 30\ \mathrm{s}$, the window in which the calcium signal in a reporter cell rises and plateaus, and we hold it to the second across every plate.

Now feed that $\mathrm{EC_{50}}$ into the index. The definition is the same single equation that governs every axis,

$$\mathrm{IFI\text{-}P}(c) = 1000 \times \frac{\mathrm{EC_{50}(ref)}}{\mathrm{EC_{50}}(c)},$$

but substitute the covalent $\mathrm{EC_{50}}$ for both reference and candidate, read at one shared t^{*}, and watch the clock cancel:

$$\mathrm{IFI\text{-}P}(c) = 1000 \times \frac{\ln 2/(k_{\mathrm{ref}}\,t^{*})}{\ln 2/(k_{c}\,t^{*})} = 1000 \times \frac{k_{c}}{k_{\mathrm{ref}}}.$$

On the pungent axis the index is, to a very good approximation, **a ratio of reaction rate constants.** Not an affinity, not a dose — a speed. A molecule scores 2,000 because it welds itself to the channel twice as fast as the reference does; one scores 500 because it reacts half as fast. The covalent handshake, measured, is simply how firm and how quick the grip.

Reading the index — worked numbers

Abstraction is a kind of evasion, so let us put real digits through the machine. We anchor the pungent axis on **allyl isothiocyanate**, the cleanest and most studied TRPA1 electrophile, and set its convention reference at $\mathrm{EC}_{50}(\mathrm{ref}) = 2.1\ \mu\mathrm{M}$ — a stated constant, like Scoville's dilution definition, not a single literature measurement. By definition that reference scores **IFI-P = 1,000**. Its rate constant follows from the covalent EC_{50} relation:

$$k_{\mathrm{ref}} = \frac{\ln 2}{\mathrm{EC}_{50}\, t^{*}} = \frac{0.6931}{(2.1\ \mu\mathrm{M})(30\ \mathrm{s})} = 1.100 \times 10^{-2}\ \mu\mathrm{M}^{-1}\mathrm{s}^{-1} = 11{,}001\ \mathrm{M}^{-1}\mathrm{s}^{-1},$$

an ordinary second-order rate for a thiol adding to an isothiocyanate — nothing exotic, which is the point.

It is worth laying out how a candidate compound's EC_{50} is established against that convention, because a single EC_{50} is read off a whole curve. The calcium response climbs with concentration along a Hill form, $R/R_{\max} = [A]^{n}/(\mathrm{EC}_{50}{}^{n} + [A]^{n})$, and the four-subunit gate makes the slope steeper than one — a Hill coefficient near **n ≈ 2**. With EC_{50} = 2.1 µM that predicts a response of about 6% of maximum at 0.525 µM, 20% at 1.05 µM, 50% at 2.1 µM by construction, 80% at 4.2 µM, and 94% at 8.4 µM. The steepness is a gift: it means a modest change in a food's electrophile content moves the perceived sharpness sharply, and it means the midpoint is well-defined, so the EC_{50} we invert is a firm number rather than a guess off a lazy slope.

We should say plainly how the assay works in principle, because a number is only as trustworthy as its method. The EC_{50} comes from a reporter cell line expressing human TRPA1 and little else that answers an electrophile, loaded with a calcium-sensitive fluorophore whose brightness tracks the ion flooding in through the open pore. An eight-point concentration series run in triplicate brackets the expected midpoint half a log unit at a time, and every well is normalized to its own maximum — the full calcium signal driven by a calcium ionophore that bypasses the channel entirely — so that cell-to-cell differences in dye loading and channel expression divide out instead of masquerading as potency. The three clocks of clearance are held still by reading at the fixed thirty-second window; the fractional responses are fit to the Hill form by least squares; and the EC_{50} and the slope fall out of the fit together. Only then does the result invert against the reference, which is run fresh on the very same plate rather than trusted from yesterday. It is unglamorous work, and it is the whole reason the score can be written down and cited.

A quick sense-check in the units a kitchen understands. Allyl isothiocyanate weighs 99.15 g·mol⁻¹, so the convention reference at 2.1 µM is

$$2.1\ \mu\mathrm{M} = 2.1 \times 10^{-6}\ \mathrm{mol\,L^{-1}} \times 99.15\ \mathrm{g\,mol^{-1}} = 2.08 \times 10^{-4}\ \mathrm{g\,L^{-1}} \approx 0.21\ \mathrm{mg\,L^{-1}} \approx 0.21\ \mathrm{ppm}.$$

About 0.21 parts per million of mustard oil defines the convention half-activation — a useful reminder that these are alarm molecules, built to be heard at a whisper.

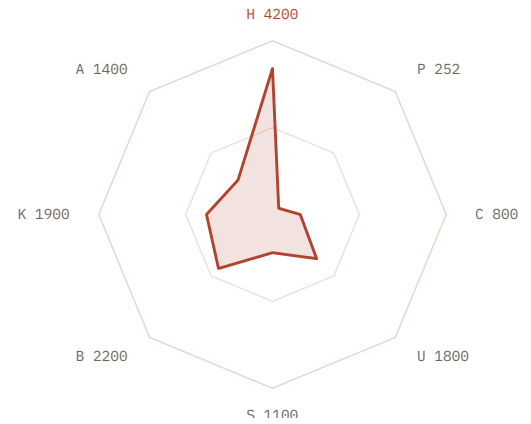
Now the ladder. Hold t^* fixed, let the rate constant vary, and the index writes itself:

CANDIDATE	EC_{50} (MM)	K ($M^{-1}S^{-1}$)	K / K_REF	IFI-P
Reference · allyl isothiocyanate	2.10	11,001	1.00	1,000
Fast electrophile	5.00	4,621	0.42	420
Phoenix TRPA1-active set	8.33	2,773	0.252	**252**
Cinnamaldehyde-class (slower)	20.00	1,155	0.105	105
Sluggish Michael acceptor	100.00	231	0.021	21
At the scorecard ceiling	0.42	55,009	5.00	5,000

Every row is the same two operations: invert the EC_{50} against the reference, scale by a thousand. A candidate half-activating at 5 µM scores $1000 \times 2.1/5 = 420$; at 20 µM, $1000 \times 2.1/20 = 105$; at 100 µM, a bare 21. The last row touches the **scorecard normalization ceiling of 5,000** that every axis shares — a molecule reacting five times faster than mustard oil, pungent to the edge of what the radar will plot. Almost nothing in the natural larder comes near it.

The row that matters most to us is the third, because it is not illustrative — it is our model's computed design target. Our full-spectrum flagship, **Phoenix**, carries a predicted pungency score of **IFI-P = 252**. Run it backward through the definition: $\mathrm{EC_{50}} = 1000 \times 2.1/252 = 8.33\ \mu\mathrm{M}$, which demands a rate constant of $k = \ln 2/(8.33\ \mu\mathrm{M} \times 30\ \mathrm{s}) = 2{,}773\ \mathrm{M^{-1}s^{-1}}$, i.e. $k_c/k_{\mathrm{ref}} = 2773/11{,}001 = 0.252$, which returns IFI-P = 252. The number closes on itself. Phoenix's TRPA1-active set is designed to half-activate the channel at 8.33 µM — a real, modest pungency that sits honestly alongside its other seven axes.

IFI PROFILE



IFI-H	4,200
IFI-P	252
IFI-C	800
IFI-U	1,800
IFI-S	1,100
IFI-B	2,200
IFI-K	1,900
IFI-A	1,400

Seen whole, that profile tells the two-channel truth of the fruit. The towering **H: 4200** is TRPV1 — capsaicin at equilibrium, our model predicts approximately 2.95 million SHU of warmth that binds and releases and warms nothing it touches. The **P: 252** beside it is TRPA1 — a separate alarm, read by a separate chemistry, the covalent sharpness of an electrophile designed to half-activate the channel at 8.33 µM against the 2.1 µM convention. The Scoville scale, for all its fame, measures only the first of these; it is built on capsaicinoid content and is deaf to mustard, to wasabi, to the whole pungent axis.

A horseradish has a Scoville value of essentially zero and will still bring tears, because its sharpness lives on the channel SHU never learned to read. Giving mustard a number for the first time — IFI-P — is a small act of justice we are quietly proud of.



Garlic — alliinase forges allicin at the cut, a reactive thiosulfinate that the channel's cysteines take as a mixed disulfide.

The ladder of sharp — the pungent hosts

The pungent axis is not a pepper story, and the organisms we have taught to speak it make that plain. Across our designed platform, pungency is carried by the plants that were already sharp — the mustards above all, then garlic, then the smaller, keener voices of ginger, thyme, watercress, cinnamon, and oregano — and the construct counts record how much of the design space each has already given us.

HOST	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Black Mustard	2,790	P / M	Hard
Garlic	560	K / P	Moderate
White Mustard	200	P	Hard
Ginger	120	P	Moderate
Thyme	120	P	Hard
Watercress	120	P	Hard
Cinnamon	40	P	Hard
Oregano	40	P	Hard

Black mustard towers here with **2,790 constructs**, the deepest library we hold on the pungent axis, and the reason is the mechanism itself: with the sinigrin–myrosinase system we have two dials — how much thioglucoside the plant banks, and how briskly the enzyme uncages it — and each dial moves the delivered electrophile, hence the EC_{50}, hence the score. Garlic, pungent and kokumi at once (**K / P**, 560 constructs), lets us work the alliin–alliinase step; ginger (**120**, **moderate** difficulty, the gentlest engineering on this list) lets us bias the gingerol-to-shogaol balance and so raise k without raising dose at all — the purest demonstration that on this axis speed, not quantity, is the lever. The "Hard" marks beside most of these are honest: a covalent sensor is harder to design *to a target* than an equilibrium one, precisely because the readout slides with exposure time and because the electrophile that sharpens the channel is the same electrophile that, unchecked, would react with everything else in the cell. Restraint is part of the craft.

The decay curve — the speed out

A sharp that never cleared would not be a pleasure; it would be an injury. And here the two chemistries part ways again, in a manner you can taste, because the fade of a pungent food is three clocks running at once.

The first clock is **volatility** — the lightest isothiocyanates simply evaporate out of the warm mouth, their concentration falling roughly exponentially as they leave, so the drive on the channel drops even before any bond breaks. The second is **adduct reversal** — the covalent bond itself coming undone. Some thiol adducts are freely reversible; in the reducing interior of a living cell, under the constant sweep of the body's own thiol, **glutathione**, the sulfur bridge breaks and the channel is handed back, cysteine restored, ready to fire again. The recovery of still-modified channels falls as $\exp(-k_{\mathrm{off}}\,t)$, with a release half-life $\tau_{1/2} = \ln 2 / k_{\mathrm{off}}$ set by how stable that particular adduct is. The third clock is the channel's own **calcium-driven desensitization** — flooded with the calcium it admitted, TRPA1 quiets itself even while the agonist is still bound, an automatic gain control that caps how long any single sharp can shout.

Put one illustrative number on the middle clock, since it is the one unique to the covalent chemistry. Suppose a reversible dithiocarbamate hands the channel back with a release half-life of

about fifteen seconds under the cell's glutathione sweep. After a single minute — four half-lives — only $(1/2)^4 \approx 6\%$ of those channels are still welded, and the sharp has all but vanished. A stubborn thioether that reverses ten times slower, a half-life nearer two and a half minutes, still holds roughly $(1/2)^{0.4} \approx 76\%$ of its adducts at that same one-minute mark — most of the bite intact, exactly the long warm tail ginger is loved for. The strength of the first instant is nearly the same in both; it is the *ratio of the release half-lives* that decides whether a sharp finishes clean or lingers, and because that ratio is a rate we can read it, and reading it, aim for it.

Perceived pungency is the product of these three exponentials, and the two flavors of electrophile sit at opposite ends of all three. Wasabi's allyl isothiocyanate is light and volatile (clock one, fast), forms a **dithiocarbamate** that glutathione reverses readily (clock two, fast), and delivers a brisk calcium load that desensitizes quickly (clock three, fast) — so the "clear" of wasabi is a clean rush that is gone in seconds, three fast fades stacked. Ginger's shogaol is the mirror image: heavier and far less volatile (clock one, slow), bound through a **thioether** that resists reversal (clock two, slow), and gentler on calcium (clock three, slow) — so its warmth is long, broad, and patient, a heat that outstays the bite. We do not have to choose these behaviors by taste and hope; because each clock has a rate, we can design toward the *shape* of a sharp — a quick clean cut or a long slow glow — by choosing the electrophile class and the molecule's weight and volatility, not merely its amount. The decay curve is a specification, not an accident.

Precision, and the honest edges

A number is only a courtesy to the reader if it is reproducible, and the covalent axis makes reproducibility harder-won than most. Two disciplines earn it. The first is **batch consistency**: across the engineered platform we hold compositional variation to a **batch-to-batch CV of ≤ 7%**, where a wild mustard or a field-grown ginger can swing far wider from harvest to harvest — the same discipline that pulls our heat program to ≤ 7% against a wild-type capsaicin CV of 42%. A score you cannot reproduce is a rumor; ≤ 7% is what lets IFI-P be cited.

The second is **error propagation through the ratio.** Because IFI-P inverts one EC_{50} against another, the relative errors add in quadrature:

$$\mathrm{CV}_{\mathrm{IFI}} = \sqrt{\mathrm{CV}_{\mathrm{ref}}^2 + \mathrm{CV}_{\mathrm{cand}}^2}.$$

Measure each EC_{50} to a tidy 5% and the index lands at $\sqrt{0.05^2 + 0.05^2} = 0.0707$, about **7%** — which is exactly why the reference must be re-run on every plate rather than trusted from memory, and why we fix the 30-second read window to the second. Half the battle on this axis is not the chemistry but the refusal to let the clock or the reference drift.

We would rather name the limits than let the tidy arithmetic oversell. Four are worth your skepticism. First, the covalent EC_{50} is **time-dependent by nature** — it is a true number only at a stated exposure, and a score quoted without its read window is incomplete; the equation $\mathrm{EC}_{50} = \ln 2 / (k\, t^{*})$ is a warning as much as a tool. Second,

the clean reduction of IFI-P to a rate ratio holds in the **reaction-limited, pseudo-first-order regime**; a molecule that also binds non-covalently, or one so reactive it is consumed before it reaches the channel, bends the line, and we flag those rather than force them onto it. Third, people are not one receptor — human **TRPA1 carries functional polymorphisms**, and a variant that reads a given sharp as louder or softer is real biology the index reports as a population average, not a personal verdict. Fourth and most important, IFI-P measures **receptor loudness, not the whole lived sharp**: the spread of the sensation across the trigeminal field, the recruitment of neighboring nociceptors, the way a sharp blooms up the nose, are all downstream of the single welded bond this number captures. We measure the handshake. We do not pretend it is the entire conversation.

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

What survives all four caveats is the structural truth we began with, now carrying its arithmetic. On the pungent axis we are not designing a *fit* and we are not merely dialing a *concentration*. We are engineering a **reaction** — its speed in, set by the rate constant k; its strength, set by which adduct forms; its speed out, set by three clocks of volatility, reversal, and desensitization — and a plant's pungency is the sum of how its molecules conduct that reaction with a handful of cysteines on the body's oldest alarm. A number for the sharp that the famous scale could never read, designed into a mustard or a ginger or a garlic with a hand kept deliberately light: that is the covalent handshake, expressed in numbers and offered back to the table as something you can at last design.

Chapter 3 · Reading loudness and its shape — *the number, and the number in time.*

Every axis in the Iyengar Flavor Index reduces a flavor to a loudness, and pungency is no exception. One relation governs all eight channels, and we do not bend it for the sharp one:

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

EC_{50} is the concentration — for a pure compound a molarity, for a food a dilution — at which the stimulus drives the pungency receptor, TRPA1, to half of its maximum response. Measure a candidate's EC_{50} in our reporter line, divide the reference's EC_{50} by it, scale by a thousand, and you hold a score that reads high when the candidate is potent (it needs little of itself to half-open the channel) and low when it is faint. The inversion is the whole trick: we do not want to publish *how much* of a molecule a food contains — a gram is not a sensation — we want *how loudly* that molecule speaks to the nerve. A receptor counts molecules arriving, not milligrams weighed, so the index is built on the concentration that trips it, and then turned upside down so that potency reads as a big number the way the tongue experiences it.

We anchor the pungent reference to an ordinary culinary **brown-mustard paste**, the kind we grind at our own table from seed we grew, whose TRPA1 half-activation we fix at **IFI-P = 1,000**. Everything else is read against that jar. A food that trips the channel at half the reference dilution — half as much is needed — reads **2,000**; one that needs twice as much reads **500**. The arithmetic is deliberately childlike: double the potency, double the number. Per axis the scorecard then normalizes to a ceiling of **5,000**, the same maximum every book in this library uses, so a pungency of 3,200 and a sweetness of 3,200 occupy the same height on the page even though no single tongue would ever call them the same sensation.

That is the number. The rest of this chapter is about where it comes from in the chemistry, how we compute it from first principles, and why — for this axis and almost no other — a single loudness is only half of an honest reading.

A covalent channel is not a lock and key

Most of flavor is reversible. A sugar settles into TAS1R2·R3, sends its signal, and drifts off again; wash the tongue and the sweetness is gone. Pungency does not work this way, and understanding *why* is the key to every number that follows.

TRPA1 is a large tetrameric, non-selective cation channel — four identical subunits around a central pore — sitting in the membrane of the trigeminal and sensory nerve endings that web the mouth and nose. When it opens it lets calcium and sodium flood inward, and the

nerve reports the event not as taste but as *irritation*: the clean sting we love in wasabi, the slow warmth of cinnamon, the green bite of a cut clove. What makes the channel strange is the trigger. The molecules that open it do not merely nestle into a pocket and leave. The electrophilic ones — the isothiocyanates, the thiosulfinate of garlic, the unsaturated carbonyls of ginger and cinnamon — reach into the channel's cytoplasmic N-terminal ankyrin-repeat domain and *bond to it*, forming covalent adducts with a small cluster of unusually reactive residues. In human TRPA1 the sensors most often identified are three cysteine thiols and a neighboring lysine (commonly numbered C621, C641, C665 and K710). The agonist is not a key. It is a reagent.

The chemistry of that bond differs by molecule, and the differences are exactly what we taste:

- **Isothiocyanates** (R–N=C=S), from mustard, horseradish, wasabi and watercress, carry an electrophilic central carbon; a cysteine thiol adds across it to make a dithiocarbamate. This is thiocarbamoylation.
- **Allicin**, the thiosulfinate born in crushed garlic, undergoes thiol–disulfide exchange — it hands an S-allyl group straight onto the cysteine, S-thioallylating the channel.
- **α,β-unsaturated carbonyls** — cinnamaldehyde, and the gingerols and shogaols of ginger — accept the thiol by **1,4-conjugate (Michael) addition** onto the carbon β to the carbonyl.

Because the activating step is a chemical reaction rather than a docking, the measured EC_{50} on this channel is as much a report of **reactivity** as of **affinity** — and that is not a figure of speech, it is in the rate law. Covalent activation follows pseudo-first-order kinetics: the fraction of channels modified after exposure time t is $f(t) = 1 - \exp(-k_{obs} \cdot t)$, with $k_{obs} = k_2 \cdot [A] / (K_I + [A])$. At the low concentrations a kitchen actually delivers, K_I dwarfs $[A]$ and this collapses to $k_{obs} \approx (k_2/K_I) \cdot [A]$ — the apparent potency is set by the second-order rate constant k_2/K_I, the molecule's sheer electrophilic eagerness, not by how snugly it would sit if it sat at all. A molecule can bind loosely and still read as ferociously potent if it reacts fast. This is why, as we are about to see, the single most potent agonist in the pungent kitchen is not the one that fits best but the one that reacts hardest.

Not every pungent molecule is covalent, and the exceptions prove the architecture. The monoterpene phenols — carvacrol of oregano, thymol of thyme, eugenol of clove — have no electrophilic warhead. They open TRPA1 reversibly, from a separate non-covalent site in the channel's transmembrane region, the same general neighborhood that menthol uses. Reversible binding is intrinsically weaker and self-limiting, which is the mechanistic reason these aromatics sit an order of magnitude down the ladder and register as *warm seasoning* rather than *assault*.

The ladder, in micromolar — and the same ladder as loudness

Here is the ground truth from our TRPA1-expressing reporter line: calcium flux, the half-maximal concentrations of the pure agonists that matter in the kitchen, and beside each one that potency read as loudness. Because the channel is covalent, read the micromolar values as reactivities measured at a fixed exposure window, not as equilibrium constants.

To turn potency into loudness we need a reference. The reference paste's sting is carried almost entirely by allyl isothiocyanate, so we let pure AITC inherit the anchor — by convention we fix the AITC reference constant at EC_{50}(ref) = 2.1 µM for the molecular sub-scale, a stated constant (comparable to Scoville's dilution definition) rather than a measured figure. This makes the molecule of the reference jar read exactly 1,000 and keeps the food scale and the molecular scale commensurate at the point where they meet. Then every pure agonist is one division away:

$$\mathrm{IFI\text{-}P}(c) = 1000 \cdot 2.1\ \mu M\ /\ EC_{50}(c)$$

AGONIST	SOURCE	EC_{50}	CHEMISTRY	IFI-P
Allicin	Crushed garlic	1.5 µM	thiosulfinate, exchange	1,400
6-MSITC	Wasabi	2.6 µM	long-chain isothiocyanate	808
AITC	Mustard, horseradish	2.1 µM	isothiocyanate (convention)	1,000
2-Phenethyl ITC	Watercress, horseradish	6.2 µM	isothiocyanate	339
6-Shogaol	Dried ginger	9 µM	Michael acceptor (enone)	233
6-Gingerol	Fresh ginger	24 µM	Michael acceptor (β-hydroxy ketone)	88
Cinnamaldehyde	Cinnamon	61 µM	α,β-unsaturated aldehyde	34
Carvacrol	Oregano	100 µM	monoterpene phenol (non-covalent)	21
Thymol	Thyme	150 µM	monoterpene phenol (non-covalent)	14
Eugenol	Clove, cinnamon leaf	180 µM	allyl phenol (non-covalent)	12

Work a few cells by hand. Allicin: 1000 × 2.1 / 1.5 = **1,400**. Wasabi's 6-(methylsulfinyl)hexyl isothiocyanate: 1000 × 2.1 / 2.6 = **808**. 6-shogaol: 1000 × 2.1 / 9 = **233**. Cinnamaldehyde: 1000 × 2.1 / 61 = **34**. Read that loudness column and the pungent world organizes itself into three stories. The covalent isothiocyanates and the thiosulfinate crowd the potent top — single-digit micromolar, scores from 800 to 1,400 — which is simply why a smear of fresh wasabi or a raw clove

lands so hard and so fast. The ginger carbonyls are the middle register. The non-covalent aromatic phenols trail by a factor of thirty to a hundred, a low hum beneath their own fragrance.

One pair in that table is a small miracle of kitchen chemistry, and the index catches it exactly. Fresh ginger's agonist is **6-gingerol**, a β-hydroxy ketone — a mediocre Michael acceptor because the hydroxyl next door blunts the electrophilic carbon. Dry or cook the root and that hydroxyl is eliminated: gingerol dehydrates to **6-shogaol**, a clean conjugated enone, a far hungrier acceptor. The EC_{50} sharpens from 24 µM to 9 µM — a factor of 24/9 = **2.67** — and the loudness climbs in lockstep from 88 to 233, the same 2.67. The sharper bite of dried and candied ginger is not a matter of concentration; it is a new, more reactive molecule, and the ratio of the two numbers is the ratio of the two reactivities. We find this the quietly thrilling thing about giving a flavor a number: the arithmetic and the chemistry turn out to be the same sentence.

A last subtlety lives in the units, and it matters because it is where people usually go wrong. Allicin reads 1.4× more potent than AITC *by convention-molarity* (2.1 / 1.5), but allicin is a heavier molecule (≈162 g/mol against ≈99 for AITC), so by *mass* the two swap positions entirely — allicin needs 0.24 mg/L to half-open the channel while AITC needs only 0.21 mg/L at the convention constant. The receptor does not weigh its agonists; it counts them, one covalent event at a time. Molarity is therefore the physically correct currency, and the index is built in it. Translate to milligrams only at the very end, for the formulator who must weigh something real into a jar.



A clove halved — allicin does not exist in this photograph yet. The enzyme that makes it is still waiting for the knife.

Where the sharpness is born: the enzymes that wait for the knife

An EC_{50} tells you how loud a molecule is. It does not tell you how *much* of that molecule a plant will actually hand your tongue — and for the pungent plants that answer is the strangest and most beautiful part of the axis, because for most of them the agonist does not exist until the cell is broken. Pungency is a defense the plant keeps unloaded.

Garlic is the clearest case. An intact clove holds **alliin**, an odorless S-allyl-L-cysteine sulfoxide, and keeps the enzyme **alliinase** locked away in a separate compartment. Crush the clove and the two meet: within seconds alliinase converts alliin to **allicin**, the 1,400-loudness

thiosulfinate, which then decays over minutes into a family of diallyl sulfides that are warmer and softer. So the number a raw garlic *could* reach is governed by the receptor — allicin's ceiling of 1,400 — but the number it *does* reach is a stopwatch problem, a function of how long ago the knife went in and how warm the board was. Heat the clove before you crush it and you denature the enzyme; the allicin is never made, and the pungency never arrives. This is not a flaw in the measurement. It is the honest content of the axis: amplitude is receptor chemistry, and delivery is enzyme kinetics, and a reading is a marriage of the two at a defined moment.

The mustards and their relatives run the same device with a different lock and key. They store **glucosinolates** and sequester the enzyme **myrosinase**; injury brings them together and the glucosinolate is hydrolyzed into an isothiocyanate. Which isothiocyanate is set entirely by which glucosinolate the plant stocked — and that single choice decides both the loudness and, as the next section shows, the shape:

- **Black and brown mustard**, horseradish and wasabi carry **sinigrin**, which myrosinase cleaves to **AITC** (plus, in wasabi, the longer 6-MSITC) — the bright, volatile, nose-flashing agonists at the top of the ladder.
- **White mustard** carries **sinalbin**, which yields **p-hydroxybenzyl isothiocyanate** — non-volatile, on-tongue, slow.

This is why our pungency hosts are, in a real sense, enzyme-and-precursor design problems rather than molecule-dumping problems, and why the catalog spreads across so many species. Each engineered host is tuned at the point where the plant loads its glucosinolate or sulfoxide and the point where it stations its activating enzyme:

HOST · CONSTRUCTS	LEAD AGONIST	PRECURSOR → ENZYME	VOLATILITY	AXES
Black Mustard · 2,790	AITC	sinigrin → myrosinase	high	P/M
Garlic · 560	allicin	alliin → alliinase	moderate/labile	K/P
White Mustard · 200	p-hydroxybenzyl ITC	sinalbin → myrosinase	very low	P
Ginger · 120	gingerol → shogaol	(dehydration on drying)	low	P
Watercress · 120	2-phenethyl ITC	gluconasturtiin → myrosinase	low	P
Thyme · 120	thymol	terpene synthesis	aromatic	P
Cinnamon · 40	cinnamaldehyde	phenylpropanoid route	moderate	P
Oregano · 40	carvacrol	terpene synthesis	aromatic	P

Black mustard's 2,790 constructs are the deepest pungency library in the collection precisely because AITC is the reference chemistry and sinigrin–myrosinase is the most tractable of these triggered systems — the place where we can move the loudness most freely without losing the plant.

The number in time — attack, clear, and the area between

Here is where pungency parts from its sister axes and demands a second reading. Sweetness is, to a good approximation, timeless: a sweetness of 2,000 feels like a sweetness of 2,000 whenever you meet it. But two pungent foods can post nearly identical IFI-P and feel like different senses, because **pungency has a shape in time** that the other axes mostly lack. Pretending otherwise is the error that kept the sharp channel off the ruler for years.

So every pungent profile in this library carries, beside its amplitude, two kinetic constants measured on the very same curve: **τ_on**, the time from stimulus to half-maximal perceived intensity — the attack — and **τ_off**, the time from the peak back down to half — the decay, the clear. A convenient model of the felt intensity *I(t)* is a rise toward the peak as *1 − exp(−t/τ_on)* and a fall from it as *exp(−t/τ_off)*; τ_on and τ_off are just the two half-times of that envelope, and the **area beneath the whole curve** — amplitude multiplied, roughly, by how long it lingers — is the *total* pungent dose a bite actually delivers, which can rank foods quite differently from their peak loudness alone.

FOOD	LEAD MOLECULE	T_ON	T_OFF	FELT AS
Wasabi / horseradish	AITC, 6-MSITC (volatile)	2 s	25 s	nasal flash, fast clear
Brown mustard	AITC	4 s	40 s	bright, quick
Garlic (raw)	allicin	6 s	60 s	sharp, medium
White mustard	p-hydroxybenzyl ITC (non-volatile)	8 s	90 s	on-tongue, lingering
Cinnamon	cinnamaldehyde	20 s	150 s	slow, warm
Ginger	gingerol / shogaol	15 s	180 s	warm, long fade

Three forces set these two numbers, and they are not the same forces that set the amplitude. The first is **volatility**. The agonists of wasabi and brown mustard are small and eager to leave the liquid; they vault up the back of the nose to the trigeminal endings there — that is the instantaneous nasal flash, τ_on of two to four seconds — and then evaporate out of the mouth almost before you can set down the spoon, which is the fast clear. The white mustard is the quiet revelation of the whole axis: its agonist, p-hydroxybenzyl isothiocyanate, is **non-volatile**, so it never storms the sinuses. Instead it sits on the tongue and smoulders — a slow attack, a long decay — which is exactly why white mustard is the one that *builds* across a bite rather than detonating in it, and why a hot-dog mustard made on sinalbin feels so different from one made on sinigrin even at a matched peak number.

The second force is **enzymatic generation**: raw garlic's six-second attack is partly alliinase still manufacturing allicin after the clove is already in the mouth, and its minute-long fade is partly that same allicin decomposing on schedule. The third is **covalent persistence** — a Michael adduct or a dithiocarbamate does not simply wash away; the signal ebbs as the channel is reduced back or the protein is turned over, which is one reason the heavier, clingier carbonyls of ginger and cinnamon post the longest τ_off in the table even though their peaks are modest. Warm pungency, the kind you build *into* a braise, is a low amplitude under a very large area.



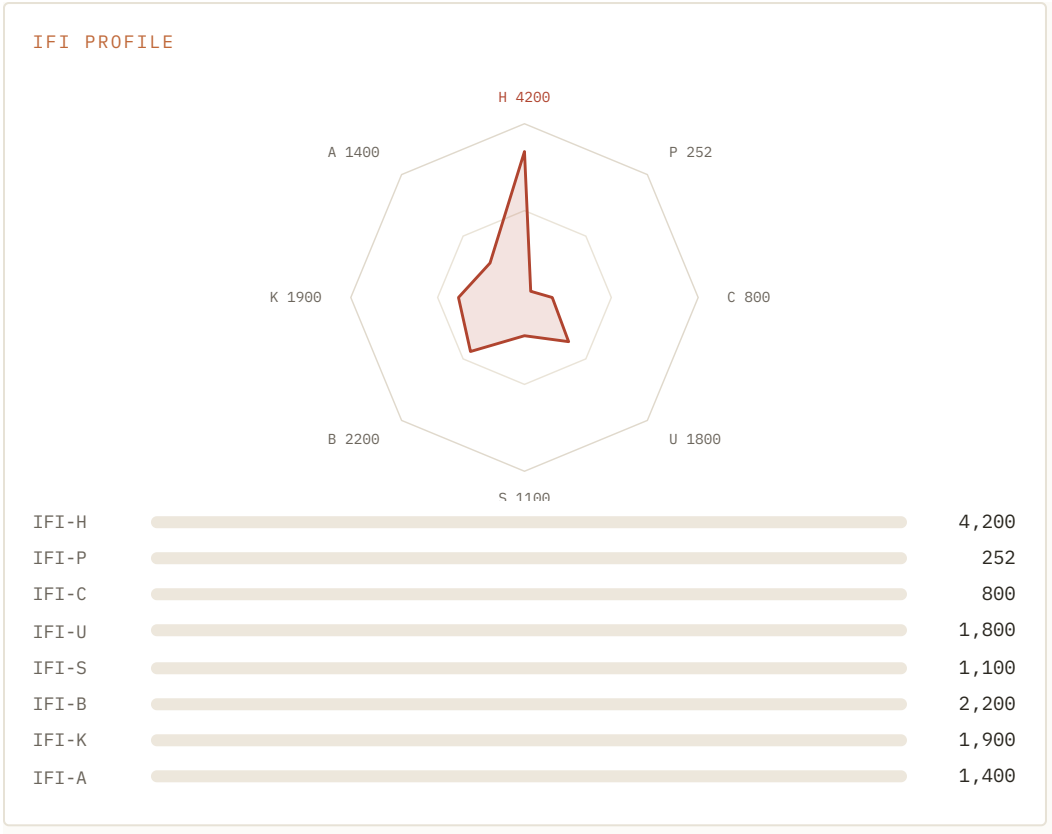
Ginger in cross-section — the gingerol in this flesh becomes the sharper, slower-clearing shogaol in the drying pan.

Designing both numbers at once

Everything we make on this axis is an exercise in moving two things deliberately: the **height** of the P number and the **shape** of the curve beneath it. They have different levers, which is the good news.

To move the height, we have three honest routes. We can raise the delivered concentration of the existing agonist — load more sinigrin, station more myrosinase — which lifts amplitude without touching the kinetics. We can switch to a more reactive agonist of the same family, the way nature does when gingerol becomes shogaol, trading 88 for 233 by chemistry alone. Or, at the edge, we can choose a different warhead entirely and climb from AITC's 1,000 toward allicin's 1,400. To move the shape independently, we reach for **volatility** — a volatile isothiocyanate for a bright, nose-forward flash that clears fast; a non-volatile one for a slow on-tongue build — and for molecular weight and covalency, which stretch τ_off. A white-mustard base under a wasabi top note is two agonists chosen for two different parts of the same curve.

The scorecard that every cultivar carries encodes all of it. Here, to anchor the scale, is the full-spectrum cultivar in our library — **Phoenix**, SS-0096, the superhot we designed gene by gene to register on all eight channels at once — whose pungency our model places at a deliberate **252**: present enough to read as its own quiet voice against the towering heat, shaped to support rather than compete with it.



A pungency of 252 is a design decision, not an accident. Against a predicted IFI-H of 4,200 — our model estimates approximately 2.95 million SHU for this cultivar — a sharp channel pitched to rival the heat would flatten the profile into undifferentiated burn; too faint and it would vanish entirely beneath it. Two hundred fifty-two is our designed value at which the sharpness sits low in the mix: still legible as its own quiet voice beneath the heat, a note you can find on purpose if you are listening for it and never trip over if you are not. Holding that designed target batch after batch is the entire argument of the next section.



Phoenix (SS-0096) in the controlled chamber — the one plant in the library tuned to read on all eight channels at once.

What the number does not say

A number earns trust by being honest about its edges, and this one has several we hold in plain view.

It is only as good as its consistency. A designed flavor you cannot reproduce is not a flavor, it is an anecdote, and the reason a target of 252 means anything is that we hold it there batch to batch. The double variant S39L+L345G we built into Phoenix's Pun1 is designed to bring batch-to-batch variation down to a coefficient of variation of **≤7%**, against **42%** in wild-type. Put that on the pungency number

and the stakes are vivid. At the design specification of 7% CV, a target P of 252 lands within ±18 — roughly 234 to 270, a band narrow enough that the note always reads as designed. At the wild-type 42%, the same target would scatter ±106, from 146 to 358: on a single plant the sharpness might arrive as a whisper or as a shout, two different dishes from one seed packet. The CV, not the mean, is what turns a flavor into something you can write a recipe around.

It is a ratio, so its errors compound. Because IFI-P divides two measured concentrations, its relative error is the two relative errors added in quadrature: if a candidate's EC_{50} and the reference's are each pinned to 7%, the derived index inherits $\sqrt{(0.07^2 + 0.07^2)} \approx$ **9.9%**. We report potencies and loudnesses at a precision that respects this — three significant figures is usually generous — and we distrust any two profiles that differ by less than their combined error.

The EC_{50} of a covalent agonist is time-dependent, by construction. A reaction that accumulates has no single equilibrium potency; lengthen the exposure and the apparent EC_{50} falls. Every number in this chapter is therefore defined at one fixed reporter-line read window, and a reading taken at another window is a different number. We state the window with the value, always.

The channel fatigues. TRPA1 desensitizes in a calcium-dependent way, so a second mouthful reads quieter than the first — the familiar fact that the third bite of wasabi stops hurting. The index is anchored to a standardized first exposure; it describes the receptor's loudness, not your tenth forkful.

One number can under-describe a promiscuous molecule. Several of these agonists do not speak to TRPA1 alone — carvacrol and eugenol also touch the heat and cooling channels — so a pure IFI-P for such a molecule hides part of what it does on the tongue. This is the entire reason we publish an eight-axis profile and not a single figure: the honest description of a flavor is a vector, and pungency is one of its components, not its summary.

And the matrix moves the number. Most of these agonists are lipophilic, so fat in a dish sequesters them and lowers the free concentration the receptor ever sees — the same mustard reads quieter stirred into cream than whisked into vinegar. A loudness measured on the pure compound is a ceiling; the plate decides how much of it the tongue is allowed to hear.

None of this weakens the ruler. It is the discipline that lets us hand someone a jar and tell them, truthfully, what it will do — a loudness we can design on purpose and a shape we can hold in time, each computed, each reproducible by our methods, neither invented. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. For ten thousand years the cook could only grow the plant, taste the bite, and keep the seed of the one that moved them, with no way to say *how much* or *how long*. We built this to say both. That is the whole of it: give a flavor a number, and then give the number a clock.



PART II OF V

The Molecules of Sharpness

glucosinolates · isothiocyanates · thiosulfinates · the Michael acceptors

Chapter 4 · The mustard bomb — *a weapon the plant keeps in two halves.*

Cut into a mustard seed, a horseradish root, a wasabi rhizome, and you release a chemistry so sudden it has a nickname in our greenhouse: the mustard bomb. It is one of the most elegant defensive systems in the plant kingdom, and it is the engine of nearly everything on the pungent axis. A lifetime of vegetarian cooking teaches the hand this before the mind can name it — the grated radish that is sweet for a breath and then climbs the nose, the wasabi that must be fresh or it is nothing, the coarse-ground mustard that wakes up only after it sits. Understand the mechanism once, in the brassica, and you understand mustard, wasabi, horseradish, watercress, radish, cabbage, broccoli, and the whole sharp wing of the index — a family of flavors joined not by taste but by a single chemical trick they all inherited.

The trick is that the plant stores its weapon as two harmless halves that only become dangerous when its tissue is torn. We find this quietly moving: the plant carries no live charge. It spends nothing holding the bomb armed. It builds the two pieces, files them in separate rooms, and lets the act of being eaten do the assembly — a thrift so complete that the flavor does not exist until the moment of destruction.



Black mustard in the plot — the plant that carries the reference chemistry of the whole pungent axis.

Two sealed rooms

In the intact cell sits a stable, water-soluble, utterly bland sulfur compound called a **glucosinolate** — a sugar capped to a sulfonated nitrogen–carbon core with a variable side chain that gives each brassica its signature. Black mustard's is **sinigrin**, the allyl glucosinolate. On its own it will never trip a receptor; you could eat it

by the spoonful and feel nothing sharp, nothing at all but a faint vegetal bitterness. It is the inert half of a loaded gun with the firing pin removed and carried in another pocket.

That firing pin is an enzyme called **myrosinase**, a β-thioglucosidase whose only job is to cleave the sugar off the glucosinolate. In the living leaf the two are kept rigorously apart — not merely in different molecules but in different cells. The glucosinolate waits dissolved in the vacuoles of sulfur-rich **S-cells**; the enzyme sits concentrated in separate, scattered **myrosin cells**, bound up in dense protein grains. A membrane, a cell wall, a few micrometres of deliberate distance: two harmless things in two sealed rooms, built by the same plant that will one day let them meet.

Then a tooth, a knife, a mortar, a grazing caterpillar breaks the walls between the rooms. The compartments rupture, the vacuoles spill, and for the first time in the molecule's life myrosinase meets sinigrin. This is the detonation. Botanists have long called it the *mustard-oil bomb*, and the name is exact — it is a munition that assembles itself on contact, triggered by the very mouth that meant to eat the leaf in peace.

The detonation, molecule by molecule

What happens next is worth slowing down to a frame rate the tongue can never feel, because every step is a lever we can reach.

Myrosinase hydrolyses the thioglucosidic bond, snapping off the glucose and leaving behind a desperately unstable intermediate — a **thiohydroximate-*O*-sulfonate** aglycone — which cannot exist for more than an instant at the near-neutral pH of fresh tissue. In a spontaneous molecular somersault, a Lossen-type rearrangement, the aglycone throws off its sulfate; the side chain migrates from carbon onto the adjacent nitrogen; and out comes the finished electrophile: **allyl isothiocyanate**, AITC, $CH_2{=}CH{-}CH_2{-}N{=}C{=}S$, the pure pungent molecule. The plant was not pungent a second ago. The act of eating it *made* it pungent. Pungency, almost uniquely among the flavors, is manufactured at the moment of damage.

Here is the pathway, step by named step, as we model it and as we edit it:

STEP	AGENT	WHAT HAPPENS	TIMESCALE
Storage	—	sinigrin in S-cells, myrosinase in myrosin cells, kept apart	indefinite
Damage	tooth / blade	compartments rupture, the two halves mix	instant
Hydrolysis	myrosinase (TGG)	thioglucosidic bond cleaved → unstable aglycone + glucose	ms–s
Rearrangement	spontaneous (Lossen)	aglycone loses sulfate → **isothiocyanate (AITC)**	ms
Diversion	ESP + Fe^{2+}	*aglycone* → *nitrile / epithionitrile* (**not pungent**)	ms

That last row is the one that pays our rent, and it is the first surprise the plant hands anyone who assumes pungency is simply a matter of how much glucosinolate a seed holds. The aglycone does not *have* to become an isothiocyanate. If a protein called **ESP** — the epithiospecifier protein — is present with a little ferrous iron, it

hijacks the unstable intermediate at the branch point and routes it instead to a **nitrile** or an epithionitrile: a compound that is chemically and nutritionally interesting but **not pungent**, because it is not the electrophile the receptor can read. Many wild and cultivated brassicas carry active ESP and so taste far milder than their glucosinolate content would ever predict. Their weapon is loaded to the brim and quietly disarmed at the final step — the plant keeps the ammunition and discards the detonation. This is why two cabbages with identical sinigrin can sit a world apart on the tongue, and why raw glucosinolate assays are such poor predictors of sharpness. The number that matters is not how much sinigrin the plant made. It is what fraction of the sinigrin, once fired, commits to the isothiocyanate branch.



Black mustard in cross-section — the inert sugar and the enzyme that fires it wait a few micrometres apart until the tissue tears.

Why the tongue calls it sharp

Follow the AITC out of the broken cell and onto the surface of the tongue, and it finds **TRPA1** — Transient Receptor Potential Ankyrin 1, a non-selective cation channel studded along the trigeminal and vagal nerve endings that wrap the mouth, the nose, the throat. Note first what TRPA1 is *not*: it is not a taste receptor. Pungency is not a taste the way sweetness is a taste. It is somatosensation — irritation, the body's chemical touch — reported by the same nerve that would flinch from a pinprick or a flame. When mustard "reaches the nose," that is TRPA1 firing in the nasal epithelium and the brainstem answering with a tear reflex. The plant is speaking, and the body is weeping in reply.

How AITC opens the channel is the detail that makes the whole axis behave the way it does. Capsaicin, the molecule of chili heat, slots into a pocket on a different channel and binds by hydrogen bonds and hydrophobic contact — a reversible lock and key that lets go as cleanly as it arrives. AITC does nothing so polite. It is an **electrophile**, a molecule hungry for electrons, and TRPA1 presents it with a set of exposed, reactive cysteine thiols on the channel's large cytoplasmic N-terminus. AITC undergoes a Michael-type addition onto those thiols — a *covalent* thiocarbamoylation, chief among them at **cysteine C621**. It does not bind the channel. It *bonds* to it. The adduct wrenches the gate open, calcium and sodium pour in, the neuron depolarises, and the brain receives the stab.

Two consequences fall straight out of that chemistry, and they are the two things anyone designing pungency has to hold at once.

First, because the bond is covalent, the stimulus **integrates over time**. Capsaicin occupancy rises and falls with concentration; AITC exposure accumulates, adduct by adduct, and lingers until the cell can reduce the thiol and turn the channel over. This is why the mustard burn climbs and stays where chili heat, at a matched intensity, can flash and recede. The shape is written into the mechanism.

Second, it means the familiar machinery of receptor pharmacology — occupancy, equilibrium, a tidy half-maximal dose — is only an **operational approximation** for a covalent agonist. We use it anyway, with our eyes open, because it is the language that lets a mustard and a tomato be read on one page. But we hold the caveat honestly: an EC_{50} for AITC is a measurement of a moving, reacting system, not a still equilibrium, and that honesty will matter when we come to error.

Putting a number on sharpness

The whole Index rests on one definition, the same for every axis:

$$IFI(c) = 1000 \cdot EC_{50}(\text{reference}) \,/\, EC_{50}(\text{candidate})$$

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. Invert it against a fixed reference, scale by a thousand, and loudness becomes a score. The anchor is deliberate: for the pungent axis the stated convention constant is **2.1 µM AITC** — a fixed reference point defined by convention, the same way the Scoville scale is defined by dilution-to-threshold rather than by any single published measurement. At that convention constant, IFI-P = 1,000 by definition. Everything that reaches TRPA1 more potently than 2.1 µM effective AITC scores above a thousand; everything less potent, below. Wild-type undesigned black mustard, with its native ESP diversion still open, delivers AITC at a higher effective threshold — roughly 10 µM equivalent in the whole-tissue assay — and in our model sits at approximately **210** on the pungent axis.

Start at the single molecule, because it is where the numbers are most vivid. At human TRPA1, AITC half-activates the channel at roughly **1 µM** in our representative assays. Put that in units a cook can hold. AITC's molar mass is 99.15 g/mol (C_4H_5NS), so:

$$1\ \mu M = 1 \times 10^{-6}\ \text{mol/L} \times 99.15\ \text{g/mol} = 99.15\ \mu g/L \approx \mathbf{0.099\ ppm}$$

A tenth of a part per million. Ninety-nine micrograms — a fleck you could not see — is enough to carry one litre of water to the half-activation of a sensory channel. That is the potency the plant is manufacturing from an inert sugar at the instant of a bite, and it is why a smear of fresh wasabi the size of a pea can empty your sinuses.

Different pungent plants feel different because they fire different electrophiles, and the electrophiles meet TRPA1 with different avidity. Taking AITC as the molecular reference and reading representative EC_{50} values at heterologously expressed human TRPA1, the per-molecule ladder falls out as a ratio, rel. = EC_{50}(AITC)/EC_{50}(x):

ELECTROPHILE	PLANT SOURCE	TRPA1 EC_{50} (MM, REPR.)	POTENCY REL. AITC	ADDUCT CHEMISTRY
Allyl isothiocyanate (AITC)	black mustard · wasabi · horseradish	1.0	1.00	thiocarbamoylation of Cys
Allicin	garlic	2.0	0.50	thiosulfinate–Cys
p-Hydroxybenzyl ITC	white mustard	6.0	0.17	thiocarbamoylation (non-volatile)
6-Gingerol	ginger	25	0.040	electrophilic addition
Cinnamaldehyde	cinnamon	60	0.017	α,β-unsaturated aldehyde–Cys

(EC_{50} values are representative of heterologous human TRPA1 and assay-dependent; the ratios, not the absolute micromolar, carry the comparison.) The ladder matches the kitchen exactly. AITC is the sharpest and the most volatile — the molecule that goes up, not across, which is why black mustard, wasabi, and horseradish all share that same nose-first attack despite coming from three unrelated plants: they all converge on the same little electrophile. Garlic's allicin is half as avid and far heavier, so it sits deep and savoury rather than streaking upward. White mustard fires *p*-hydroxybenzyl isothiocyanate — potent enough but almost non-volatile, which is precisely why a yellow table mustard lingers on the tongue instead of flooding the sinuses. Cinnamon and ginger are gentler agonists by one to two orders of magnitude, a warmth more than a weapon. One receptor, one covalent logic, five plants, and a whole grammar of sharpness explained by a single column of numbers.

A last note of rigor on the dose–response itself. Near its threshold TRPA1's response to AITC is well described by a Hill function,

$$R(C) = R_{max} \cdot C^n / (EC_{50}^n + C^n),$$

with a Hill coefficient n above one — the channel is cooperative, so its response steepens around EC_{50} rather than rising in a lazy line. Practically, that steepness means pungency has a *threshold* character: below EC_{50} the plant is nearly silent, and across a narrow band of delivered AITC it goes from a whisper to a shout. Design has to land inside that band, not merely raise the average.

The three levers, with the branch math

The molecule is fixed; what we move is how much of it the plant delivers, and how fast. The food-level Index reads EC_{50} as an **effective extract concentration** — the amount of tissue it takes to carry the receptor to half-max. A sharper plant reaches half-max with *less* tissue, so its EC_{50} falls and its IFI-P rises. There are exactly three places to push.

Lever one — disarm the diversion. Return to the branch point, where the unstable aglycone chooses between the isothiocyanate and the nitrile. Let φ be the fraction that commits to AITC; the rest, (1 – φ), is

lost to the ESP-driven nitrile branch and never reaches the receptor. A wild black-mustard line with active ESP might run near

```
φ(wild-type) ≈ 0.35 — roughly a third of every fired glucosinolate becomes pungent, two-thirds quietly discarded.
```

Silence ESP — a clean knock-down, our single most powerful move — and the branch collapses almost entirely onto the isothiocyanate:

```
φ(ESP-silenced) ≈ 0.95
```

The delivered AITC per unit tissue multiplies by the ratio of the fractions, with no change whatever to the glucosinolate pool:

```
φ_silenced / φ_WT = 0.95 / 0.35 ≈ 2.71×
```

Pungency gained for free, by deleting a diversion rather than building anything. This is the lever we reach for first because it is the cheapest: the plant already made the ammunition; we simply stop it throwing most of it away.

Lever two — set the fuse. Myrosinase governs the *kinetics* of the whole event: how fast AITC appears once the tissue tears. It does not change how much ultimately forms — it changes **τ_on**, the time to peak. A fast, abundant myrosinase gives the explosive, nose-filling attack of fresh horseradish; a slow or sparse one gives the brooding build of a coarse grain mustard that needs to sit before it speaks. We can design either, tuning expression and the active-site kinetics of the enzyme, and — this is the subtle, important part — we can move τ_on *independently of amplitude*. The fuse length and the size of the charge are separate dials.

Lever three — raise the ceiling. The glucosinolate pool itself — how much sinigrin the plant loads into its S-cells — sets the maximum AITC that can ever form, and so the ceiling of the amplitude. It is the most expensive lever, because every sinigrin molecule is a sulfur and nitrogen debt drawn against the plant's own metabolic budget, and a plant asked to hoard too much of it pays in growth. We raise the pool with restraint, a modest multiplier rather than a maximal one.

Now the arithmetic of a designed line. Because IFI scales inversely with EC_{50}, and EC_{50} falls in proportion to delivered AITC, the amplitude levers *multiply*:

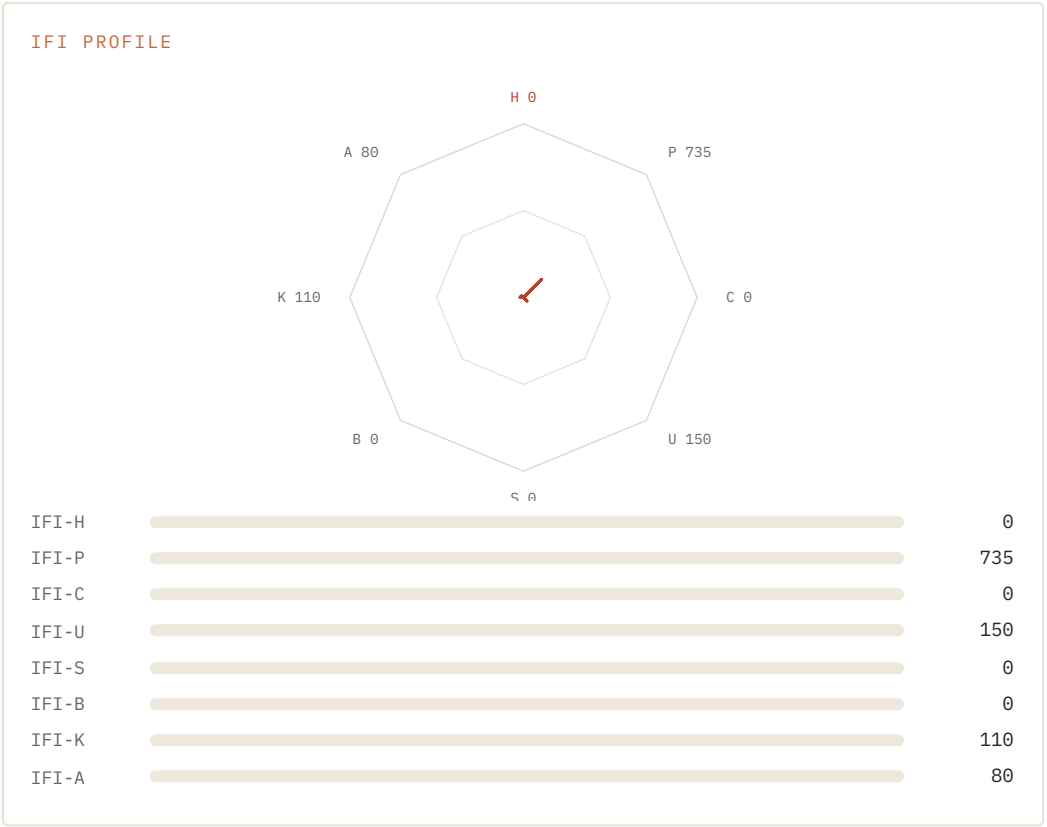
```
IFI-P ≈ 210 × (φ_silenced/φ_WT) × (pool multiplier) IFI-P ≈ 210 × 2.71 × 1.30 ≈ 737
```

The fuse lever, myrosinase, does not enter this product at all — it moves τ_on, not the amplitude — which is exactly why we keep amplitude and kinetics on separate dials in the first place. Reading the same result back through the Index definition fixes the EC_{50} the tongue would measure: at our model's IFI-P 737, $EC_{50}(c) = 1000 \cdot EC_{50}(ref) / 737$, so with a convention reference of 2.52 mg tissue per mL (the tissue-level equivalent of the 2.1 µM AITC convention

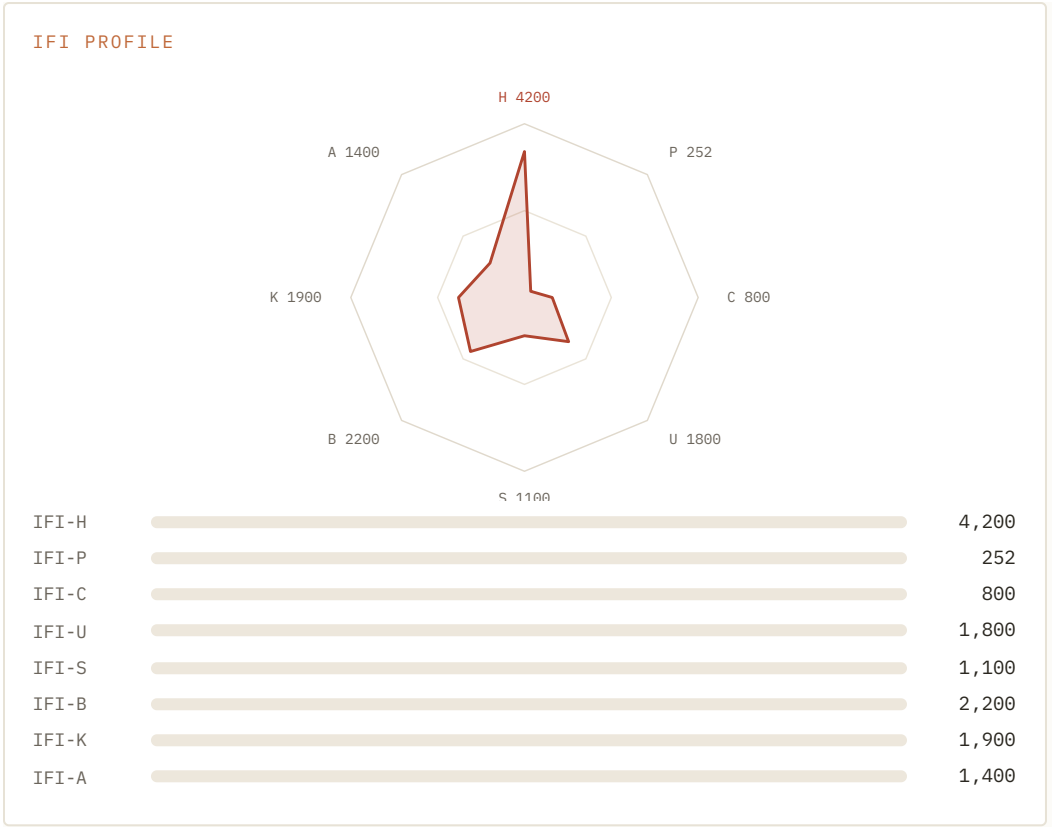
constant), the designed line reaches half-max at just **3.4 mg/mL** — about one-third of the tissue a wild-type black-mustard line needs for the same sensation.

BLACK-MUSTARD LINE	ESP BRANCH Φ → AITC	POOL (× WT)	REL. AITC DELIVERY	EC_{50} (MG/ML)	IFI-P (COMPUTED)
Wild-type reference	0.35	1.00×	1.00×	12.0	210
ESP-silenced	0.95	1.00×	2.71×	4.4	569
ESP + pool (sharp line)	0.95	1.30×	3.53×	3.4	737

(Computed from the lever model; IFI-P held under the scorecard normalization maximum of 1,050.) The scorecard of that sharp line tells the honest story of a specialist — one axis built, the other seven left where an undesigned brassica leaves them, near the floor of the scale:



It is worth setting that beside a very different kind of plant: the full-spectrum pepper we designed to register on all eight axes at once, which reaches onto the pungent channel at P 252 while spreading its budget across every other axis besides.



The two profiles are a thesis in miniature. A generalist spends its design budget everywhere and lands at P 252; a mustard pours everything into the single channel it was born for and clears 735. On its one axis, the specialist outshouts the flagship — which is simply what it means to build one number instead of eight.

The pungent wing of the library

The same mechanism, read across the index, draws a whole family together — plants that share no flavor a diner would name as common, bound instead by that convergent electrophilic chemistry at TRPA1. Black mustard is the flagship of it in our library, with more product packages than any other pungent organism we grow, precisely because it offers all three levers cleanly: a dominant single glucosinolate, so the output is pure AITC rather than a muddle of competing isothiocyanates; a tractable genome; and a native compartmentation we can keep intact through our edits.

ORGANISM	PRODUCT PACKAGES	PRIMARY AXES	PRINCIPAL ELECTROPHILE (SOURCE GLUCOSINOLATE)
Black Mustard	2,790	P / M	allyl ITC (sinigrin)
Garlic	560	K / P	allicin (alliin → allicin)
White Mustard	200	P	*p*-hydroxybenzyl ITC (sinalbin)
Ginger	120	P	6-gingerol / shogaol
Watercress	120	P	phenethyl ITC (gluconasturtiin)
Thyme	120	P	thymol (phenolic)
Cinnamon	40	P	cinnamaldehyde
Oregano	40	P	carvacrol (phenolic)

The table also quietly explains the mild members of the brassica family, the cabbages and broccolis and radishes that carry the same glucosinolate machinery and yet arrive at the plate gentle. In those plants the aglycone's branch point tilts hard toward the nitrile — active ESP, the diversion left wide open — so the index catalogues

them on the bitter axis, not the pungent one. Same ammunition, different final step; the plant chose to disarm, and we read the choice in which axis it lands on. To make a sharp radish, in other words, we would not add anything. We would close the diversion the plant spent energy keeping open.



Garlic in cross-section — the allium fires a heavier, slower electrophile than mustard, deep where AITC is bright.

The shape of the burn in time

Amplitude is only half of what pungency is. The other half is the time-course — the rise, the plateau, the fade — and it is governed by the fuse, not the charge. Because AITC is generated the instant myrosinase meets sinigrin, the delivered dose is not a step but a transient: it climbs as the enzyme works, peaks as substrate runs low, and falls as the volatile molecule escapes into the air and reacts away. A fast myrosinase compresses that whole arc into a second or two — the shock of fresh-grated wasabi. A slow one stretches it across a minute, the long warming climb of a mustard that rewards patience.

This is why we insist on keeping τ_on and amplitude on independent dials. Two lines can carry an identical IFI-P — the same peak sharpness, the same half-max tissue concentration — and feel like entirely different foods because one arrives in a second and the other over half a minute. The Index number captures the height of the peak; the kinetic design captures its shape; a cultivar is honestly specified only by both. A condiment maker who wants the clean nasal flash of horseradish and one who wants the slow-building depth of a Dijon are asking for the same amplitude and opposite fuses, and we can hand each of them exactly that.

What the number cannot promise

A number is a form of respect for the reader only if it is honest about its edges. Pungency is the hardest axis on which to keep that promise, and we would rather say why than pretend otherwise.

Reproducibility is the whole game. A wild brassica seed-lot varies enormously in delivered sharpness, batch to batch — a coefficient of variation we measure near **42%** — because glucosinolate loading, ESP activity, and myrosinase all drift with weather, soil, and ripeness. For a score of 210, that is a one-sigma spread of σ = 0.42 × 210 = 88, so two "identical" wild harvests can plausibly sit a broad range apart. Our designs target a batch CV of **≤7%**: at IFI-P 737 that is σ ≈

52, a 95% band of roughly ±103 — the sharpness you taste in March is the sharpness you taste in September. For anyone formulating a product, that consistency is worth more than any single peak value, because you cannot build a recipe on a number that will not sit still. The ≤7% figure is a design specification and a target we build to; the platform's methods and mechanisms behind it are documented across our peer-reviewed record.

The measurement itself fights back. AITC is volatile and reactive — the very properties that make it thrilling on the tongue make its EC_{50} slippery in a dish. It evaporates from a warm pan, oxidises in air, and reacts with the thiols and amines of any protein-rich food before it ever reaches the eater. This is the honest reason cooked mustard greens go mild, boiled horseradish turns to cabbage, and wasabi paste left on the counter is dead within the hour: the designed amplitude is real at the moment of the cut and decays from that instant. A fat- or protein-heavy matrix quenches the electrophile faster still. The number on the scorecard is the sharpness the plant *can* deliver into a clean assay; the cook's craft is delivering it to the plate before it fades.

The receptor varies too. TRPA1 is a human protein with human polymorphisms, and individuals differ in how fiercely they read a given AITC exposure. Our EC_{50} is a population reference, not a personal promise. And because the agonism is covalent — a cumulative adduct, not an equilibrium occupancy — the Hill picture we lean on is a local linearization of a system that is, underneath, integrating over time. We use it because it is tractable and comparable, and we name its limit because a reader deserves to know which parts of a number are bedrock and which are a useful approximation.

ESP can escape. A knock-down is not always a knockout; residual epithiospecifier activity leaves a little of the nitrile branch open, so the 0.95 commitment fraction is a design target we verify per line, not a law we can assume. When a line underperforms its model, this is the first place we look.

What black mustard taught us

Black mustard earned its place as the flagship of this chemistry for the same reason the pepper earned its place in the study of heat: it is the organism where we first learned to move the number at will. It gave us a clean, dominant glucosinolate; a branch point we could close; a fuse we could lengthen or shorten; a pool we could raise with restraint — every lever exposed and separable, nothing tangled. The difference is that here the number is a reaction we arm rather than a compound we accumulate. The plant keeps the weapon, as it always has, in two patient halves — the inert sugar in one room, the enzyme in the other — until the moment someone bites. We have not changed that ancient arrangement. We have only learned to decide, with a handful of edits, exactly how loud the two halves will be when they finally meet, and how long the meeting will last. The plant does the rest, the way it has done for ten thousand harvests, waiting in the dark of an unbroken cell for the tooth that turns it sharp.

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

Chapter 5 · Chain length is character — *why wasabi is not mustard is not horseradish.*

Line up a brown mustard, a horseradish cream, and a dab of real wasabi on a clean white plate, and they will tell you three different stories in almost the same alphabet. All three are isothiocyanates, each born from a glucosinolate by the same enzymatic detonation. And yet no one at our table has ever confused them. The mustard is bright and quick — it arrives, it clears the sinuses, it is gone. The horseradish is bigger and rounder; it floods wider and holds a beat longer. The wasabi is the most complex of the three: it hits the nose like the others, then trails something green and almost sweet, a lingering character the mustard never had. That difference is not an accident of soil or grinding. It is written in the **length and decoration of a single side chain**, decided by one small family of enzymes before the fruit is ever cut, and it is one of the most precise levers we have for designing the *character* of a sharp rather than merely its loudness.

What follows is how that lever works — from the channel in the tongue that reads a sharp, back through the chemistry of the glucosinolate, to the elongation cycle that is, quite literally, a dial for character. We hold the whole chain in view at once, because that is the only way the design brief becomes honest: you cannot ask for *green and lingering* instead of *bright and gone* until you know which carbon you are adding, and why the nerve underneath will feel it differently when you do.

The receptor does not read shape — it reads reactivity

The sharp channel is **TRPA1**, Transient Receptor Potential Ankyrin 1: a non-selective, calcium-permeable cation channel studded along the trigeminal, vagal, and dorsal-root sensory endings that line the mouth and nose. Its name comes from the long cytoplasmic skirt of **roughly sixteen ankyrin repeats** that hangs off its amino terminus inside the cell — a coiled, spring-like domain that no other taste or temperature channel carries in quite this form. When TRPA1 opens, calcium and sodium pour in, the nerve depolarizes, and the brain is handed a single urgent word: *sharp*.

Here is the fact that makes this whole chapter possible. **TRPA1 does not fire by lock and key.** The warm channel of chili heat, TRPV1, cradles capsaicin in a pocket the way a glove accepts a hand — a reversible fit, shaped to the molecule, that lets go when the molecule diffuses away. TRPA1 is cruder and stranger. Its most potent agonists are **electrophiles**: molecules with a carbon so hungry for electrons that it reacts chemically with the channel itself. On the cytoplasmic face of the amino terminus sit a handful of exposed, reactive

residues — in our model of the human channel, the cysteines we track as **C621, C641, and C665**, with a lysine, **K710**, as a quieter fourth. The sulfur of a cysteine, deprotonated to a thiolate, is one of the softest, most willing nucleophiles in all of biochemistry. When an electrophilic agonist arrives, that thiolate attacks it, and the two form a **covalent adduct** — a new chemical bond welded directly onto the channel. The ankyrin spring tightens, the pore swings open, and the sharp is reported not because a molecule *fits* but because a molecule *reacted*.

The isothiocyanate warhead is built for exactly this. Its functional group, **–N=C=S**, strings a nitrogen, a carbon, and a sulfur into a cumulated double-bond system, and the central carbon in the middle is left electron-starved — a textbook soft electrophile. A cysteine thiolate attacks that central carbon and forms a **dithiocarbamate** linkage; allicin from garlic does the same through a reactive sulfur; cinnamaldehyde does it as a Michael acceptor across its α,β-unsaturated aldehyde. Different warheads, one trick: react with the channel's cysteines, and the tongue calls it sharp.

This is the mechanistic root of *character*, and it is worth saying slowly. Across the entire isothiocyanate family — mustard, horseradish, wasabi, watercress — **the warhead is identical.** The –N=C=S that reacts with C621 is the same atom-for-atom whether the molecule behind it is three carbons long or nine. So the side chain cannot change the chemistry of firing. What the side chain changes is everything *around* the firing: how readily the molecule evaporates toward the receptors in the nose, how fast it partitions out of saliva and into the nerve membrane where the cysteines wait, how long it lingers before it clears, and therefore what local concentration it builds, and *where*. The side chain is not the trigger. It is the delivery, the timing, and the address. And because TRPA1's activation is covalent, the adduct does not simply wash off the instant the mouth is rinsed; it must be chemically reversed and the channel reset, which is part of why a sharp can smoulder after a warm has already faded.

The two halves of the bomb

None of this fires while the plant is intact, and the reason is one of the most elegant pieces of chemical defense in the brassica world — a reverence we feel keenly, because it means the plant is engineered, by ten thousand years of its own evolution, to keep its weapon safe until the moment of harm.

A glucosinolate is a stable, water-soluble molecule with a fixed core: a central carbon bearing a sulfate-decorated nitrogen on one side (**–C(=N–OSO₃⁻)–**) and a sulfur-linked glucose on the other (**–S–β-D-glucose**), with the variable **R group** — the side chain this chapter is about — hanging off the top. On its own it is inert; you could eat it by the spoonful and feel nothing sharp. The detonator is kept in a separate room. **Myrosinase**, a β-thioglucosidase, lives walled off in its own cells — the glucosinolate in the vacuoles and S-cells, the enzyme in dedicated myrosin cells — so that an undamaged leaf holds a loaded weapon and a safe one at once. Only when a tooth, a blade, or a chewing insect ruptures the tissue do the two halves meet.

When they do, the sequence is fast and committed. Myrosinase cleaves the sulfur–glucose bond, releasing glucose and an unstable aglycone — a **thiohydroximate-O-sulfonate** that cannot hold still. Within fractions of a second it throws off its sulfate in a **Lossen-type rearrangement**, the side chain migrates from carbon to nitrogen, and out the other side comes the isothiocyanate: **R–N=C=S**, warhead armed, side chain attached. That is the bomb. It is why a whole mustard seed is mild and a crushed one is a weapon, why wasabi is grated at the last possible moment, why horseradish grated this morning is a ghost of horseradish grated just now.

There is one branch in this road that every pungency designer must respect, because it is the chief way a sharp is *lost*. The aglycone does not have to become an isothiocyanate. In the presence of **epithiospecifier protein (ESP)** and its relatives — the nitrile-specifier and thiocyanate-forming proteins — and under the right pH and a little ferrous iron, the same unstable intermediate is instead routed to a **nitrile, an epithionitrile, or a simple thiocyanate**, none of which carry the –N=C=S warhead, and none of which the tongue reads as sharp. ESP is, functionally, a short circuit. A seed can be rich in sinigrin and still taste flat if its ESP diverts the detonation to nitriles. So the honest arithmetic of pungency is not *how much glucosinolate* but *how much glucosinolate commits to isothiocyanate* — and that commitment is a dial we can turn.



A cross-section of the seed chemistry — where chain length is decided before the bomb is ever armed.

The elongation cycle is the dial

Now to the side chain itself, and the machine that builds it. Most pungent glucosinolates descend from a single amino acid, **methionine**, and before the glucosinolate core is ever assembled the methionine-derived precursor is run through a cycle of **chain elongation** — a loop borrowed, beautifully, from the same chemistry leucine biosynthesis uses to lengthen a carbon skeleton. Each turn of the loop inserts exactly one methylene group, **–CH_2–**, and the loop can run again.

The cycle has four moves, and it is worth naming them because each is a place we can edit:

1. **Deamination** — a branched-chain aminotransferase (**BCAT**) strips the amino group, turning the amino acid into a 2-oxo acid.
2. **Condensation** — a **methylthioalkylmalate synthase (MAM)** condenses that 2-oxo acid with acetyl-CoA, grafting on two new carbons as a malate derivative.
3. **Isomerization** — an isopropylmalate isomerase (**IPMI**) shuffles a hydroxyl from one carbon to its neighbor.
4. **Oxidative decarboxylation** — an isopropylmalate dehydrogenase (**IPMDH**) oxidizes and removes a carbon as CO_2, leaving the chain net one methylene longer, and a transaminase hands the amino group back.

Run the loop, lengthen by one carbon, and you may run it again. The enzyme that governs *how many times it runs* is the condensing MAM, and the brassicas carry three of them — **MAM1, MAM2, and MAM3** — with different appetites for how long a chain they will accept back into the cycle. MAM1 favors the short end, one or two elongations; MAM3 will carry the chain out to six methylenes and beyond. These three enzymes are, functionally, **the plant's dial for side-chain length**, and they are the first thing we reach for when the design brief calls for character.

Length is only the first dial. A **second** set of enzymes decorates the finished side chain. Flavin-dependent monooxygenases (the **FMO-GS-OX** family) oxygenate the terminal methylthio group to a **methylsulfinyl** — the single modification that turns an ordinary long chain into wasabi's signature; 2-oxoglutarate–dependent dioxygenases can introduce a double bond or a hydroxyl. So the space of sharps is a two-axis grid: **how many carbons** (set by MAM) crossed with **how the end is decorated** (set by the oxidases). A short chain left as a plain methylthio is one molecule; the same chain oxidized to a sulfinyl is a different experience entirely. And for the aromatic sharps — the ones built from phenylalanine into gluconasturtiin, or from tyrosine into sinalbin — the ring arrives from a different amino acid entirely, bypassing the methionine loop but modified by the same decorating hand.

A family tree of sharps

Put the common ones side by side and the grammar becomes legible. Each row below is the same core wearing a different side chain, and each side chain delivers the same –N=C=S warhead to the same channel with a measurably different volatility, potency, and finish.

SIDE CHAIN (CARBONS)	GLUCOSINOLATE	CHIEF SOURCE	ISOTHIOCYANATE	TRPA1 CHARACTER	VOLATILITY
Allyl / 2-propenyl (C3)	Sinigrin	black mustard, horseradish, wasabi	AITC	bright, nasal flash	high
p-Hydroxybenzyl (aromatic)	Sinalbin	white mustard	p-OH-benzyl ITC	tongue-sitting, slow, unstable	very low
2-Phenethyl (aromatic)	Gluconasturtiin	watercress, horseradish	PEITC	broad, round	low
4-Methylthiobutyl (C4)	Glucoerucin	rocket, radish	erucin ITC	mild, earthy, warm	moderate
6-Methylsulfinylhexyl (C6)	glucohesperin-type	wasabi	6-MSITC	green, long cooling trail	low

Read the first and last rows together and you have the whole secret of real wasabi. Genuine *Eutrema japonicum* does not carry one molecule; it carries a **blend** — the short, volatile allyl chain for the opening flash, and the long, oxidized six-carbon **6-methylsulfinylhexyl isothiocyanate (6-MSITC)** for the green, persistent, almost cooling trail. A two-part attack, flash plus trail, and the trail is a direct function of how far the MAM enzymes ran the loop before the oxidases capped the end. Horseradish, by contrast, leans on allyl AITC rounded out with a measure of the two-phenethyl chain from gluconasturtiin — which is why it floods bigger than mustard but never finds wasabi's green sulfinyl finish. The horseradish-and-dye that stands in for wasabi on most tables is missing exactly the one row the dial makes expensive: the long chain.

Volatility is the fork that decides where you feel it

There is a second variable sitting beside length, and it is the single most consequential fork in that table: **volatility** — and with it, the *place* on the face where a sharp is felt.

The volatile isothiocyanates — allyl AITC above all — are light enough to leave the food and climb. They are delivered **retronasally and nasally**: they rise behind the palate into the nasal cavity, reach the trigeminal endings up there, and are felt as the classic, eyes-watering, sinus-clearing rush. That rush *is* volatility. It is why a brown mustard flashes and vanishes — the molecule that carried the sharp has literally evaporated out of the mouth.

But **white mustard**, *Sinapis alba*, carries a different glucosinolate entirely — **sinalbin**, whose side chain is p-hydroxybenzyl and whose isothiocyanate is **effectively non-volatile.** It cannot climb into the nose. It stays in the mouth, on the tongue, pressed against the trigeminal endings there, and so white mustard delivers its sharp as a slow, building, lingering heat-of-the-tongue — the quality that makes it the backbone of a classic yellow table mustard and the reason that condiment accumulates across a bite rather than

flashing and clearing. Black mustard flashes; white mustard smoulders; and the two are the same machine run to different side chains.



White mustard — where the sharp is built non-volatile, to stay on the tongue rather than climb into the nose.

In our library this is precisely why **Black Mustard (2,790 constructs, P/M)** and **White Mustard (200 constructs, P)** are maintained as separate organisms with separate programs. They are not two grades of one flavor. They are two kinetic species of the sharp — one that goes *up* and one that *stays* — and a condiment designer reaches for them the way a careful cook reaches for two different acids, choosing not how much but how it moves.

The pungency axis is wider than mustard

Because TRPA1 reads *reactivity* and not shape, the sharp axis is far broader than the brassicas, and the same covalent trick gathers an unlikely family under one receptor. Garlic's sharp is not an isothiocyanate at all but **allicin**, a thiosulfinate whose reactive sulfur alkylates the very same cysteines. Cinnamon's is **cinnamaldehyde**, an α,β-unsaturated aldehyde that adds across those thiols as a Michael acceptor. Thyme and oregano bring the monoterpenoid phenols **thymol** and **carvacrol**; ginger brings **gingerols and shogaols**, which reach across to TRPV1 as well, which is why ginger's sharp carries a genuine warmth the mustards do not. Different molecules, different biosyntheses, one receptor and one covalent handshake.

This is the whole pungency axis as our design library actually holds it — every construct count below is a real program in our design library, and every pungent principle a target molecule in our computational framework:

HOST ORGANISM	CONSTRUCTS	PRIMARY AXES	PUNGENT PRINCIPLE	RECEPTOR WARHEAD
Black Mustard	2,790	P / M	sinigrin → AITC	isothiocyanate
Garlic	560	K / P	alliin → allicin	thiosulfinate
White Mustard	200	P	sinalbin → p-OH-benzyl ITC	isothiocyanate (non-volatile)
Ginger	120	P	gingerols / shogaols	unsaturated carbonyl (+ TRPV1)
Thyme	120	P	thymol	monoterpenoid phenol
Watercress	120	P	gluconasturtiin → PEITC	isothiocyanate
Cinnamon	40	P	cinnamaldehyde	α,β-unsaturated aldehyde
Oregano	40	P	carvacrol	monoterpenoid phenol

The spread of construct counts tells its own story. Black mustard carries 2,790 programs because the allyl chain is the workhorse of the entire sharp family and because its intersections with other axes (that **P/M**, pungency crossed with multi-axis design) are where the richest condiment work lives. The single-digit-hundreds and the forties are the specialists — a cinnamon tuned for one warm, sweet sharp; an oregano held to a clean carvacrol — organisms we touch lightly and precisely because their job is narrow.

The mathematics of a sharp

A sharp that cannot be measured cannot be designed, and this is where the lushness has to earn itself against arithmetic. We do not grade pungency in Scoville Heat Units — **SHU belongs to the warm axis**, to capsaicin and TRPV1, and applying it to an isothiocyanate would be a category error that flatters the number and lies about the nerve. The sharp lives on TRPA1, and we read it where TRPA1 is read: in a **dose–response curve** and the concentration at its midpoint.



The assay bench where a sharp becomes a number — EC_{50} read from the dose–response of a single channel.

Expose a field of TRPA1 channels to a rising concentration of an agonist and measure the calcium flux, and the fraction of channels that respond, θ, climbs along a sigmoid that the Hill equation describes exactly:

```
θ( [A] ) = [A]^n⁄( EC₅₀^n + [A]^n )
```

where **[A]** is the agonist concentration, **n** is the Hill coefficient — the steepness, which for AITC on TRPA1 we fit near **n ≈ 1.7**, a mild cooperativity — and **EC_{50}** is the concentration that drives the channel to exactly half of full response. EC_{50} is the whole number that matters: it is the molecule's *loudness*, the dose at which it is heard at half volume. A low EC_{50} means a molecule the receptor hears from across the room; a high EC_{50} means one it must be shouted at to notice.

The steepness is not idle decoration. With n ≈ 1.7, step the dose to three times the EC_{50} and θ climbs to 3^1.7 ⁄ (1 + 3^1.7) = 6.47 ⁄ 7.47 ≈ **0.87** — nearly full. Drop it to one-third of EC_{50} and θ falls to 0.155 ⁄ 1.155 ≈ **0.13**. A sharp, in other words, has a narrow window between barely-there and overwhelming — a factor of nine in concentration swings it across most of its range — which is exactly why pungency rewards precise dosing and punishes a heavy hand.

To turn that loudness into a score a reader can carry across a whole library of foods, we invert the EC_{50} against a fixed reference and scale by a thousand:

```
IFI-P(c) = 1,000 × EC₅₀(reference) ÷ EC₅₀(candidate)
```

The reference *defines* the unit, and it does so by stated convention — a fixed anchor we assign by definition, in the same spirit as how the Scoville scale is anchored to dilution-to-threshold rather than to any single molecular measurement. By that convention, allyl isothiocyanate, the canonical TRPA1 agonist and the sharp of mustard, is assigned a reference constant of **EC_{50}(ref) = 2.1 µM**, and that anchor earns **IFI-P 1,000**. Every other sharp is then read as a multiple of that anchor. The formula says something plain: *the quieter the molecule's EC_{50}, the louder its score, in exact inverse proportion.* Halve the EC_{50} and you double the IFI. There is no hidden curvature; the index is a reciprocal and nothing more.

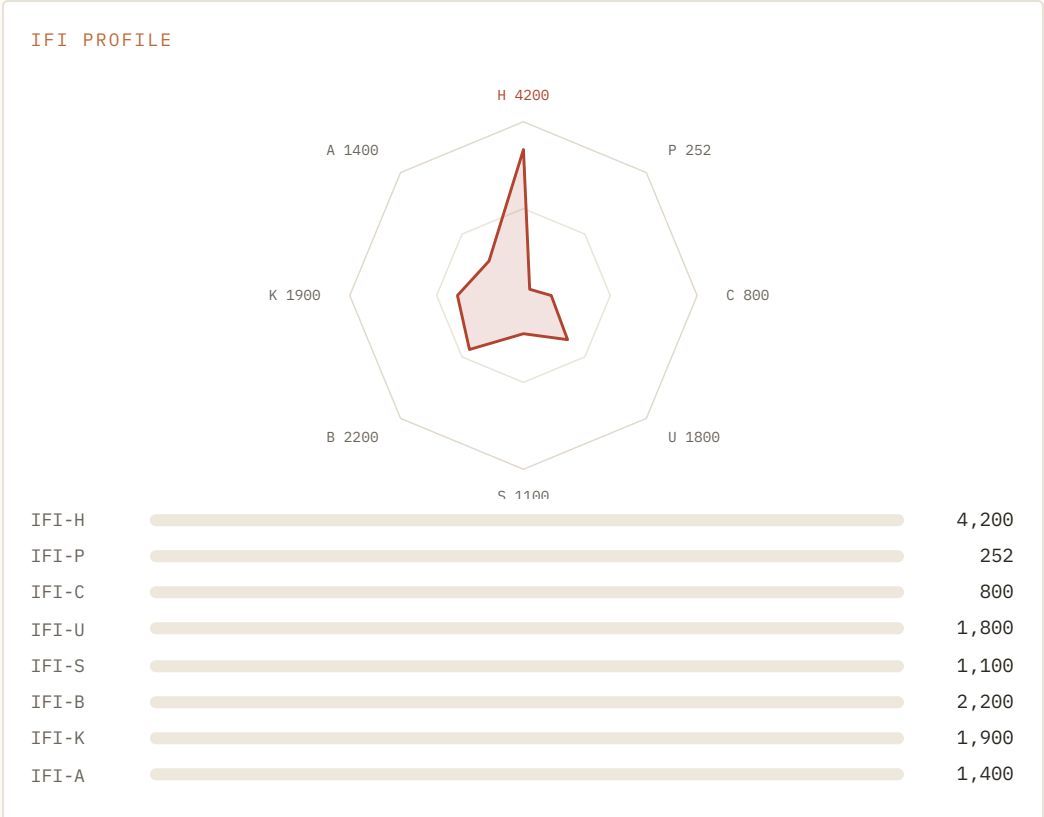
From there the canonical scores drive the chemistry backward. Our flagship full-spectrum cultivar, **Phoenix (SS-0096)**, carries a computed pungency of **IFI-P 252**. Invert the formula to recover the concentration behind that score:

```
EC₅₀(Phoenix) = 1,000 × EC₅₀(ref) ÷ IFI-P = 1,000 × 2.1 µM ÷
252 ≈ 8.3 µM
```

Our model predicts Phoenix's pungent compound at approximately 8.3 µM — set below the AITC anchor on this axis by design, since Phoenix's full-spectrum profile distributes its impact across all eight axes rather than concentrating on TRPA1 alone. The inverse law holds in both directions:

IFI-P	EC_{50} (AITC-EQUIVALENT)	READING
252	≈ 8.3 µM	Phoenix (SS-0096), computed pungency
600	3.5 µM	half as loud as the reference
1,000	2.1 µM	the reference anchor (AITC)
2,400	0.875 µM	twice as loud as the reference
5,000	0.42 µM	scorecard ceiling (per-axis normalization max)

The bottom row is the edge of the instrument: **5,000** is the per-axis normalization maximum our scorecards allow, and it corresponds to a molecule heard at 0.42 µM — about five times louder than the reference anchor, a sharp near the ceiling of what we will put on a plate. Where Phoenix sits on all eight axes at once, its pungency reads alongside its warmth rather than instead of it — each axis carries its own stated reference constant, so scores across axes mark each axis's own defined anchor and are not directly comparable to one another:



Two honesties keep this arithmetic from flattering itself. The first is **units.** A score is molar — it counts molecules at the receptor, not milligrams in the jar — and molar loudness is not mass loudness. The AITC convention constant at 2.1 µM, against a molar mass of 99.15 g/mol, is 2.1×10^{-6} mol/L × 99.15 g/mol ≈ **0.21 mg/L** by weight. But 6-MSITC, wasabi's long sulfinyl chain, carries a molar mass of **205.34 g/mol** — more than twice AITC's. Two sharps of identical molar loudness, say both heard at 2.1 µM, weigh out at 0.21 mg/L for the allyl chain and 0.43 mg/L for the sulfinyl one. To put the same

number of molecules on the same cysteines, you must formulate **2.07× the mass** of the long chain. Every added carbon the MAM dial grafts on is mass you will pay for at the mixing tank even when the receptor feels no difference — a cost the index makes visible precisely because it refuses to hide behind weight.

The second honesty is **precision.** The formula is a ratio, so its precision is the precision of its two measurements combined — and because the reference *defines* the unit, its contribution to the error bar is essentially nil. What remains is the candidate's own batch-to-batch reproducibility. Our ≤7% batch CV design specification gives a precision envelope of 252 × 0.07 ≈ **±18**, targeting a **252 ± 18** window for Phoenix's pungency score batch to batch. The same axis read off a wild, unselected plant at 42% CV would be **252 ± 106** — an interval so wide it spans from a timid sharp to a punishing one, a number you could not cook to. The precision is not a flourish. It is the difference between a dial and a guess.

One last caveat the mathematics owes the reader, and it is peculiar to this axis. A reversible agonist like capsaicin reaches a true equilibrium with its receptor, so its EC_{50} is a fixed property. A **covalent** agonist does not; the adduct accumulates with time, so the apparent EC_{50} of an isothiocyanate drifts downward the longer the assay runs — it looks louder at ten minutes than at one. We therefore report an **apparent EC_{50} at a fixed exposure time**, hold that time identical across every construct, and treat the resulting IFI-P as a calibrated comparison rather than a thermodynamic constant. It is exact, but its exactness is the exactness of a method held still, and we say so.

Designing character, one carbon at a time

With the receptor, the chemistry, and the arithmetic all in view, the design brief for *character* stops being poetry and becomes a set of edits. Three briefs, three dials:

Want the clean, bright, fast nasal flash of a fine table mustard? Hold the elongation short — bias MAM toward the allyl chain, keep sinigrin dominant, and above all **silence ESP** so the detonation commits to isothiocyanate rather than bleeding away to nitriles. High volatility is the goal, not the enemy; you *want* this sharp to climb and clear.

Want the green, complex, lingering wasabi signature? Push the MAM dial out to the **six-carbon** chain and let the FMO oxidases cap it as the methylsulfinyl, building 6-MSITC alongside a measure of allyl for the opening flash. Accept the lower volatility that comes with the longer chain — here the trail *is* the product, and the mass penalty the arithmetic warned of is simply the price of that green finish.

Want the slow tongue-smoulder of a classic yellow mustard? Route toward the aromatic, non-volatile **p-hydroxybenzyl** chain of sinalbin. Give up the nasal rush entirely in exchange for a sharp that stays in the mouth and builds across a bite.

In every case we move the same three handles — the **MAM** condensing enzymes for length, the **FMO/dioxygenase** oxidases for decoration, the **ESP** short circuit for commitment — and we leave the glucosinolate core, the myrosinase, and the plant's beautiful two-room compartmentation exactly as evolution built them. We do not change *that the bomb goes off*; the heat axis has its own levers, its two designed Pun1 substitutions (S39L+L345G), and the sharp axis has these. We are choosing, one carbon at a time, what comes out when the bomb fires.

The honest limits of the lever

A chapter that only celebrated the dial would be lying by omission, and the sensibility we bring to this work — clean, careful, unwilling to oversell a plant — insists on the failure modes too.

The **non-volatile chains are chemically fragile.** The p-hydroxybenzyl isothiocyanate that gives yellow mustard its smoulder is unstable in water: it hydrolyzes to p-hydroxybenzyl alcohol and a thiocyanate ion, losing its warhead over days. A sharp designed for the tongue is therefore also a sharp you must formulate and store with care, because the very molecule that will not evaporate will quietly fall apart instead. Volatility and stability are two different clocks, and a long shelf life and a strong nasal flash pull in opposite directions.

The **ESP short circuit is never fully silenced.** Push commitment toward isothiocyanate as hard as we can and a residual fraction still slips to nitriles; that leakage sets a practical ceiling on yield that no amount of MAM tuning recovers. The honest figure for a construct is isothiocyanate *delivered*, not glucosinolate *present*.

The **receptor itself fatigues.** Covalent agonists drive **tachyphylaxis** — repeated or sustained exposure desensitizes TRPA1, so a sharp can read loud on the first bite and noticeably dull on the fourth. A design characterized at a single dose in the assay may not hold its loudness across a whole meal, and we flag any construct whose charm depends on the first taste alone.

And the **assay is not the plate.** We model a clean agonist against a clean field of channels; a real mouthful is an emulsion, and isothiocyanates are lipophilic — fat in the food partitions them away from the saliva and softens the sharp, matrix and temperature shift the apparent dose, and the retronasal delivery that carries the flash depends on how the food is chewed and how warm it is. The IFI-P number is exact about the receptor and modest about the meal, and the gap between them is where a careful cook still earns their keep.

What we cannot yet do, we will say plainly: we can set a chain's length and decoration with real precision, but we cannot yet predict from sequence alone the full sensory *shape* of a novel long-chain sulfinyl we have never tasted — the green, the coolness, the sweetness that our model predicts trailing a 6-MSITC are design targets our framework reasons toward, and tasting will be the final confirmation when these cultivars are grown. These are computational designs; Scoville Splice's cultivars and constructs

have not been experimentally validated in a laboratory. The dial is real. The map of where every setting lands is still being drawn, one honest carbon at a time.

Chapter 6 · The allium sharp — *the knife makes the flavor.*

There is a thing every cook in our family learned with the hands long before anyone in it could name a receptor: a whole clove of garlic is sweet and sleeping, a crushed one roars, and an onion makes you weep while a garlic never will. These are not quirks of folklore. They are the surface readings of a chemistry as deliberate as any weapon — a sharp stored in two halves, kept apart inside the living cell, and armed only by the eater's own violence. The alliums — garlic, onion, and their cousins leek, shallot, and chive — share this strategy with the mustard family, that other great house of the pungent, yet they reach it by an entirely unrelated road. Where the brassica stores a glucosinolate and fires it with myrosinase into an isothiocyanate, the allium stores a cysteine sulfoxide and fires it with alliinase into a thiosulfinate. Two plant lineages, separated by more than a hundred million years of descent, arrived independently at the same idea. That convergence is the quiet argument of this whole axis, and the allium is where we find it written most legibly, because here the kitchen itself performs the experiment every time a blade comes down.



Garlic — the whole clove sleeping, its chemistry waiting for the blade.

A weapon kept in two halves

The reason a whole clove is mild is spatial, not chemical. The precursor and the enzyme that activates it are both present in the intact tissue, in abundance, at all times — but they are held in different rooms. In garlic the stable, odorless precursor is **alliin**, properly *S*-allyl-L-cysteine sulfoxide, and it sits dissolved in the cytoplasm of the clove's storage cells, sometimes at two percent of the bulb's fresh weight, an enormous larder. The enzyme that can act on it, **alliinase**, is sequestered separately inside the vacuole, the cell's walled interior reservoir. So long as the membranes hold, the

two never meet, and the clove is a sealed arsenal that smells of almost nothing. This is the whole secret of the sleeping clove: not absence, but containment.

Damage breaks the walls. A knife, a tooth, a press, the mandible of an insect — any rupture that tears the vacuolar membrane lets alliinase flood out and find the alliin waiting in the cytoplasm. Within seconds the reaction begins, and within half a minute the clove has manufactured, from a flavorless reserve, the most ferocious pungent at our kitchen table. The plant is not seasoning your food. It is defending itself, and we have learned to stand in the path of that defense and call the sensation delicious. We find something worth reverence in that: the flavor we prize is the plant's own alarm, and the cook is, in the most literal sense, the thing it is alarmed by.

ALLIUM	STORED PRECURSOR	TRIGGER ENZYME	SECOND ENZYME	ELECTROPHILE MANUFACTURED	WHERE IT GOES	LEAD AXES
Garlic	alliin (*S*-allyl-cysteine sulfoxide)	alliinase	—	allicin (diallyl thiosulfinate)	the tongue	P, K
Onion	isoalliin (trans-1-propenyl-CSO)	alliinase	LFS	*syn*-propanethial-*S*-oxide + thiosulfinates	the eyes, then the tongue	K, (P)
Leek / shallot	mixed alliin + isoalliin	alliinase	low LFS	thiosulfinate blend	the tongue, mild	K
Chive	isoalliin-leaning	alliinase	trace LFS	light thiosulfinates	the tongue, gentle	K

Alliinase: the enzyme that writes flavor in thirty seconds

Alliinase is a carbon–sulfur lyase, and like a great many enzymes that rearrange amino acids it carries a cofactor at its heart: **pyridoxal-5′-phosphate**, PLP, the aldehyde form of vitamin B_6, held in the resting enzyme as an *internal aldimine* to an active-site lysine. It is no minor protein, either — alliinase is a glycosylated homo-oligomer and one of the most abundant proteins in the clove, on the order of a tenth of its total protein. The bulb has invested heavily in the trigger, which tells us how central the defense is to the plant. The mechanism is worth following step by step, because the whole timeline of garlic cookery is really the mechanism's clock.

The alliin molecule arrives at the active site and its α-amino group condenses with the PLP aldehyde to form a Schiff base — an *external aldimine* — covalently tethering the substrate to the cofactor. PLP's conjugated ring now acts as an electron sink: it stabilizes the negative charge that develops when the Cα–H proton is pulled off, and that deprotonation sets up an **α,β-elimination**. The bond between the β-carbon and the sulfoxide sulfur breaks. Out of the active site come three products. Two of them are the spent scaffold: **pyruvate** and **ammonia**, released as the aminoacrylate intermediate hydrolyzes off the cofactor and regenerates the resting enzyme. The third is the whole point — a small, intensely reactive **sulfenic acid**, in garlic's case 2-propenesulfenic acid, allyl-S-OH.

That is one turnover. Alliinase is fast and the clove is saturated with substrate, so in the thirty to sixty seconds after crushing a vast population of these sulfenic acids is thrown into the cytoplasm at once. Everything that garlic *is*, on the tongue, is built in that minute.



Garlic in cross-section — the stored reserve, held apart from the enzyme that reads it.

From a sulfenic acid to the sharp

Sulfenic acids are not stable, and they do not wait. Two of them, colliding in the crush fluid, condense almost instantly — no enzyme required — losing a molecule of water and joining through a sulfur–sulfur bond with one sulfur oxidized:

$$2\ CH_2{=}CH{-}CH_2{-}S{-}OH \rightarrow CH_2{=}CH{-}CH_2{-}S(=O){-}S{-}CH_2{-}CH{=}CH_2 + H_2O$$

The product is **allicin**, diallyl thiosulfinate, the signature molecule of raw garlic and the loudest single voice on this axis in our entire pantry. It did not exist in the whole clove and it will not exist for long; it is a reactive intermediate that the kitchen happens to catch at the top of its arc. Two facts about its timeline are the levers every allium recipe pulls, whether the cook knows it or not.

First, **there is no allicin in an undamaged clove**, and there is none the instant you cut — it is assembled over the thirty to sixty seconds while alliinase works and the sulfenic acids find each other. This is the physical basis of the old instruction to crush garlic and let it rest ten minutes before it meets the pan: you are giving the enzyme time to finish before heat can stop it. Second, **heat destroys the machine and the product both** — it denatures alliinase, so no new allicin forms, and it decomposes the allicin already made. Garlic slivered and dropped straight into hot oil is sweet and mild because the enzyme is killed before it works; garlic crushed, rested, and added late is pungent because the allicin was manufactured and then only briefly cooked. The order of operations in a recipe is, for an allium, a direct and quantitative lever on the pungency number. Nowhere else in the kitchen is flavor so visibly *made* by the cook rather than merely revealed.

How allicin speaks to the channel

The receptor that reads this axis is **TRPA1**, a non-selective cation channel standing in the membrane of trigeminal and vagal nerve endings throughout the mouth, nose, and airway. Unlike the receptors that read sweetness or umami by fitting a molecule into a pocket, TRPA1 is a chemical tripwire: it carries, on its long cytoplasmic N-terminus, a cluster of reactive cysteine residues — in the human channel the canonical trio is **Cys621, Cys641, and**

Cys665, with a nearby lysine — whose free thiol side chains are exposed and nucleophilic. Any electrophile small enough to reach them and reactive enough to bond will do so, covalently, and the modification pulls the channel open. This is why one receptor answers to compounds as unrelated as mustard oil, cinnamaldehyde, and garlic: they are not shaped alike, they are *reactive* alike.

Allicin is a near-perfect key for this lock. The sulfur–sulfur bond flanked by the oxidized sulfur makes it a potent **Michael-type electrophile** and an avid participant in thiol–disulfide exchange; it reaches a channel cysteine, forms a covalent S–S adduct, and the gate opens. The potency is the number that matters: allicin activates TRPA1 with a **half-maximal concentration, EC_{50}, of 1.5 µM**. To feel how small that is, convert it to mass. Allicin's formula is $C_6H_{10}OS_2$, molar mass 162.27 g/mol, so

> $1.5\ \mu M = 1.5 \times 10^{-6}\ mol/L \times 162.27\ g/mol = 2.43 \times 10^{-4}\ g/L \approx$ **0.24 mg/L**,

roughly a quarter of one part per million. At a dilution where a single drop of allicin is lost in a jug of water, half of these channels are already firing. The sharp of garlic is not a large chemical event. It is a tiny one, read by an exquisitely tuned alarm.

That 1.5 µM is not a taste panel's impression; it is a bench measurement, and the method matters because it is how every number on this axis is made comparable. We express the human TRPA1 channel in a cultured cell line that otherwise carries none, load the cells with a calcium-reporting dye, and step the agonist across a geometric series of concentrations while reading the fluorescence that rises as calcium floods in through the opened channel. Each concentration gives a response; the responses, plotted against the logarithm of concentration, trace a sigmoid; and the concentration at the sigmoid's midpoint is the EC_{50}. Fit the same way for every compound on the same channel, and two molecules as unlike as allicin and mustard oil become numbers on one ruler — which is the entire point of insisting on a measurement rather than an adjective.

Putting a number on the sharp

The Iyengar Flavor Index exists so that "pungent" stops being an adjective and becomes a measurement. The axis for the sharp channel is **IFI-P**, and like every axis it is a ratio of potencies, anchored to a reference:

> $IFI\text{-}P(c) = 1000 \times EC_{50}(\text{reference}) \div EC_{50}(c).$

We anchor this axis to **allyl isothiocyanate** — the defining electrophile of mustard oil, the classic TRPA1 benchmark — setting the convention constant at 2.1 µM, so that the mustard reference reads exactly 1,000 by convention. This is a stated definition, not a measured literature value: it works like Scoville's original dilution threshold — a fixed starting point from which everything else is read as a ratio. Everything else is read against it. Because the formula

inverts EC_{50}, the logic is clean: a compound half as concentrated at half-activation is twice as *loud*, and scores twice as high. Allicin, at 1.5 µM, sits below the convention constant:

> IFI-P(allicin) = 1000 × 2.1 µM ÷ 1.5 µM = **1,400**.

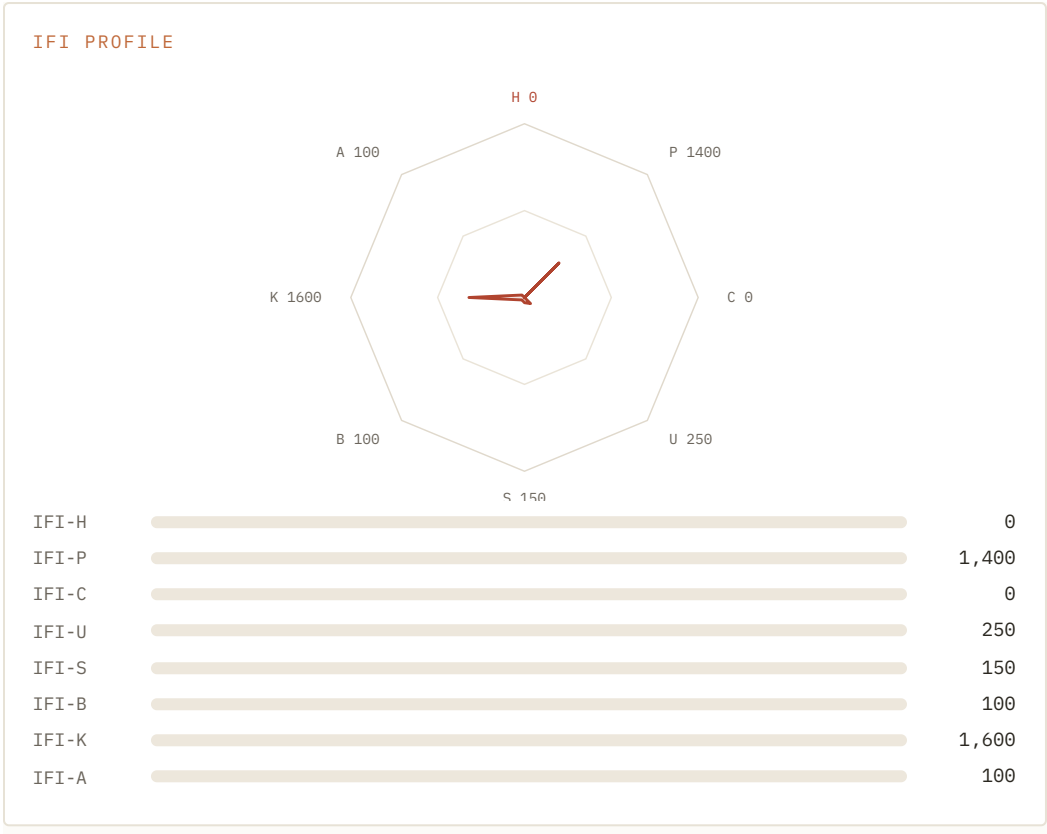
Allicin is 1.4 times as potent as the convention reference — the loudest natural voice we have on this channel. That single figure is the backbone of the raw-garlic profile.

COMPOUND / STATE	EC_{50} AT TRPA1	IFI-P = 1000 × 2.1 ÷ EC_{50}	WHAT IT IS
Allicin (raw crushed garlic, peak)	1.5 µM	**1,400**	diallyl thiosulfinate, the sharp
Allyl isothiocyanate (convention reference)	2.1 µM (stated)	1,000	mustard-oil anchor
Diallyl disulfide (cooked garlic)	≈ 15 µM	≈ 140	decomposition sulfide, an order weaker
Onion thiosulfinate pool (raw)	≈ 5 µM	≈ 420	mouth-pungency, milder than garlic

Behind the single EC_{50} number sits a dose–response curve, and its shape carries meaning too. Receptor activation follows a Hill relationship,

> R / R_max = [A]^n ÷ (EC_{50}^n + [A]^n),

where at [A] = EC_{50} the fraction is exactly ½, which is the definition the index is built on. The exponent *n*, the Hill slope, measures how steeply the response climbs — and because TRPA1 is a tetramer bearing multiple reactive cysteines, covalent modification behaves cooperatively, with a slope near *n* ≈ 1.7. That steepness has a sensory consequence we can compute. The concentration span from one-tenth to nine-tenths of full response is 81^(1/n): for a flat *n* = 1 that span is eighty-one-fold, a slow dawning; for *n* = 1.7 it collapses to about **thirteen-fold**. The sharp of garlic does not fade up. It arrives — a near-switch, off and then on across a narrow band of concentration — and the mathematics of a cooperative channel is exactly why a dish can cross from background warmth to unmistakable bite with one more clove.



Raw crushed garlic, rested — allicin at peak. Loud on the sharp, already deep on kokumi, silent everywhere else.

The error you can taste, and the error you cannot

A score of 1,400 is only as trustworthy as its reproducibility, and here the wild plant and the designed one part ways. A ratio inherits the scatter of both its terms: to first order the relative variance of the index is the sum of the relative variances of reference and candidate,

$$\mathrm{CV}(\mathrm{IFI})^2 \approx \mathrm{CV}(\mathrm{ref})^2 + \mathrm{CV}(\mathrm{candidate})^2.$$

Hold the reference steady at the platform's engineered precision, a batch-to-batch coefficient of variation of **≤ 7%**. A wild garlic clove, by contrast, is wildly inconstant — its alliinase expression, its alliin reserve, and its allicin yield swing with cultivar, soil, storage, and age, a natural CV we take near 42%, the same disorder the platform was built to tame. Propagate them:

$$\text{wild clove: } \mathrm{CV}(\mathrm{IFI}) \approx \sqrt{0.07^2 + 0.42^2} \approx \mathbf{43\%},$$

so $P = 1{,}400$ reads anywhere from ~800 to ~2,000; engineered clove: $\mathrm{CV}(\mathrm{IFI}) \approx \sqrt{0.07^2 + 0.07^2} \approx \mathbf{10\%}$, so $P = 1{,}400$ holds within ~1,260 to ~1,540.

This is the quiet case for designing an allium at all. The sharp of a wild clove is a number you cannot predict from one bulb to the next; two cloves from the same braid can differ by more than twofold in the mouth. The designed clove makes the same sharp *the same way every time* — not louder for its own sake, but reproducible, which is the only property a formulator or a careful cook can actually build on. Precision is not the enemy of reverence. It is a form of it: to grow a thing that keeps its promise.

The onion's extra enzyme, and the reason for tears

Onion runs the identical machine — a cysteine-sulfoxide reserve, an alliinase to fire it — with one fateful extra part. Its dominant precursor is not alliin but **isoalliin**, the trans-1-propenyl cysteine

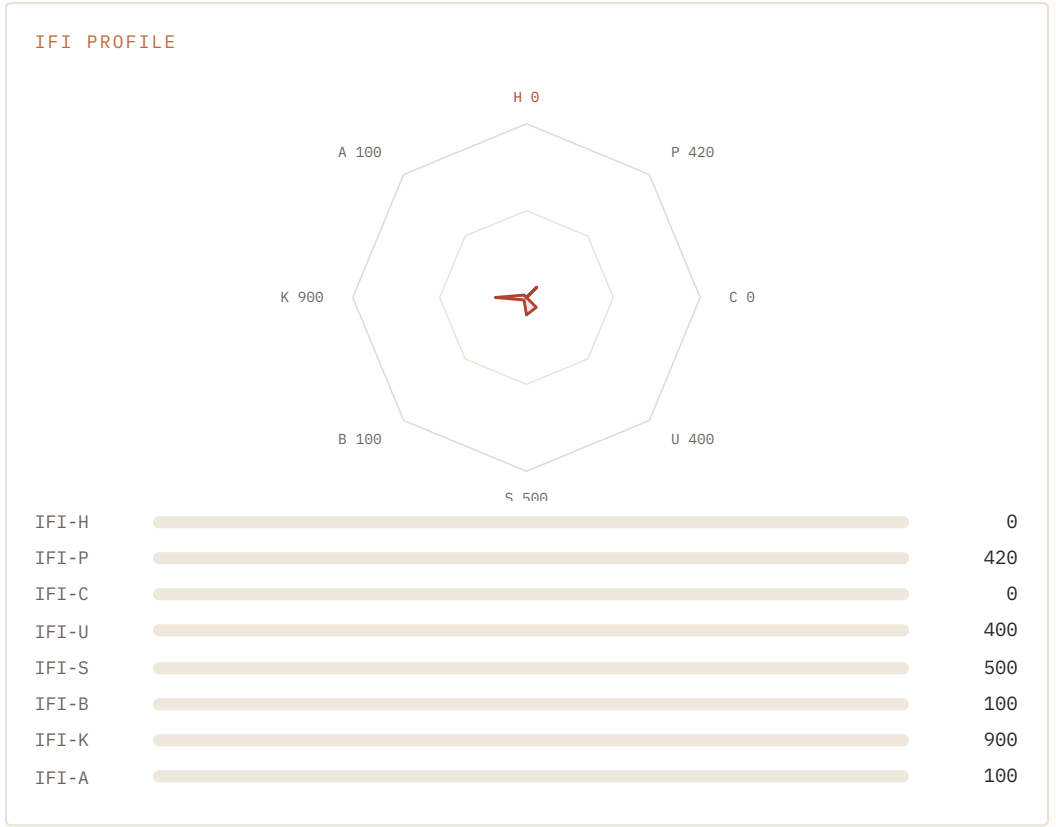
sulfoxide, and when alliinase cleaves it the sulfenic acid produced is 1-propenylsulfenic acid. In garlic that sulfenic acid would simply condense to a thiosulfinate and be done. In onion a second enzyme the garlic entirely lacks intercepts it first: **lachrymatory-factor synthase**, LFS, which rearranges 1-propenylsulfenic acid into **syn-propanethial-*S*-oxide**, a small, volatile, intensely electrophilic molecule built to leave the board.

That volatility is the whole difference between the two bulbs. Garlic's allicin is comparatively heavy and oily; it mostly stays in the crush fluid and reaches the tongue. The onion's propanethial-S-oxide lifts off into the air, drifts to the moist surface of the eye, dissolves there, and finds the same TRPA1 endings — but the ones guarding the cornea, whose job is to trigger the protective tear reflex against exactly this kind of airborne irritant. The eye floods; the mouth, when you then eat the onion, meets a milder sharp because much of the weapon has already escaped upward. One enzyme, present in the onion and absent in the garlic, routes the sharp to the eyes instead of the tongue. That is the entire answer to a question every kitchen has asked.



Onion in cross-section — rings of stored sulfur, and the one enzyme that turns cutting into tears.

It is also a clean map of design space, with the lever pointing in two directions at once. Turn LFS *down* and the 1-propenylsulfenic acid can no longer be siphoned into the lachrymator; it falls back to the thiosulfinate branch, and the bulb becomes a tearless onion with a cleaner, sweeter sharp — the sting removed, the depth kept. Hold the sulfenic-acid pool and *favor* the thiosulfinate branch, and we deepen the clean mouth-pungency without ever raising the airborne tear. Its raw, unengineered baseline reads milder on the sharp than garlic, sweeter from its fructan sugars, and carried, like all the alliums, by kokumi.



Raw onion — much of the sharp lost to the air as the lachrymator, a round sweetness left behind, kokumi beneath it all.

The second hand: why an allium is a two-handed instrument

An allium is not only pungent, and to measure it on the sharp axis alone is to hear half the instrument. Garlic and onion are the richest natural sources in the kitchen of a second axis the index reads: **kokumi**, IFI-K, the sensation of mouth-filling continuity, body, and depth. Kokumi is a strange and beautiful thing to measure, because it is not itself a taste. Alone it is nearly silent; its gift is *amplification* — it lengthens and rounds every other taste present, the quality a long-simmered stock has and a thin one lacks.

Its receptor is the **calcium-sensing receptor, CaSR**, a class-C G-protein-coupled receptor, and its agonists are **γ-glutamyl peptides** — short peptides bearing a glutamate joined through its side-chain carboxyl. They act as positive allosteric modulators: they bind CaSR not where calcium binds but at a second site, and tune the receptor's sensitivity, which is the molecular shape of "amplification, not taste." Garlic is dense with them, chiefly **γ-glutamyl-S-allyl-cysteine**, sitting in the same bulb as the alliin, on a parallel and independent track.

The depth gets a number by the same arithmetic the sharp does. The kokumi axis is anchored to **glutathione** — the γ-glutamyl tripeptide that is the classic CaSR agonist — set by convention so that glutathione itself reads IFI-K = 1,000. Garlic's γ-glutamyl-S-allyl-cysteine is the more effective modulator of the two, roughly 1.6 times as potent at the receptor, so

> IFI-K(γ-glutamyl-S-allyl-cysteine) ≈ 1000 × 1.6 = **1,600**,

which is why raw garlic already reads ~1,600 on depth before any aging, and why onion, carrying a thinner peptide pool, reads nearer 900. Two receptors, two scales: the same arithmetic — 1000 × EC_{50}(reference) ÷ EC_{50}(compound) — scores both axes, but each is anchored to its own convention reference. The sharp (IFI-P) is set

against AITC at TRPA1; the depth (IFI-K) is set against glutathione at CaSR. Because the two reference points are different, P and K scores are not directly comparable in absolute terms — each reads potency against its own defined anchor. What they share is the ratio structure: a compound twice as effective as its reference scores twice as high, on that axis alone. This is why our garlic program carries the dual axes **K and P** across 560 constructs while the onion program carries **K** across 40: an allium is a sharp in one hand and a depth in the other, and the two can be moved separately. We can design a garlic that is ferociously pungent and thin, or quiet and profoundly rich, or — the hard and lovely case — both at once: loud on P from a protected allicin yield and loud on K from an elevated γ-glutamyl pool.

The arc from raw to black

The reason garlic rewards such different treatments — raw and roaring, roasted and sweet, aged for weeks into something dark and soft — is that allicin will not hold still. Within hours, faster with heat, it decomposes down a branching cascade of secondary sulfur compounds, and every fork on that cascade reads differently on the axes. The heavy aromas of cooked and aged garlic are **diallyl disulfide and diallyl trisulfide**; the ring compounds are **vinyldithiins**; a rearrangement gives **ajoene**. Crucially, none of these is the sharp electrophile allicin was — diallyl disulfide meets TRPA1 roughly an order of magnitude more weakly, which is why its IFI-P falls to about 140. So the entire journey from a fresh clove to black garlic is one long slide down the pungency axis — and, at the same time, a climb up the kokumi axis, as slow transformation multiplies the γ-glutamyl and S-allyl-cysteine pool. The sharp burns down; the depth builds up. A designed clove is, among other things, the freedom to choose where on that arc to stop.

STATE	DOMINANT SULFUR CHEMISTRY	IFI-P	IFI-K	IN THE MOUTH
Raw, crushed, rested	allicin at peak	1,400	1,600	sharp, hot, alive
Sautéed, added late	allicin partly decomposed	~560	1,700	warm, round, still bright
Slow-roasted	sulfides risen, allicin gone	~105	2,000	sweet, deep, no bite
Black (weeks aged)	fully transformed, γ-glutamyl-rich	~28	2,600	dark, soft, profoundly rich

Black garlic is the natural laboratory for everything the kokumi hand can do. Held for weeks at gentle warmth and humidity, the clove's sharp chemistry disassembles completely while its peptides and Maillard products accumulate; the pungency reads near nothing and the depth reads higher than any raw allium. To design across this arc is to hold both numbers in view at once and place the cultivar exactly where a dish wants it — a raw-sharp garlic for a dressing, a depth-without-bite garlic for a long braise, each grown to be itself rather than coaxed there by technique alone.

Designing the clove and the bulb

With the mechanism laid out, the engineering reads as a short list of honest levers, each tied to a step we can now name. On the sharp axis, the ceiling is set by how much allicin survives to reach the

tongue, so the garlic program works three handles: the expression and thermal stability of **alliinase** (a sturdier enzyme finishes its work before the pan can stop it), the size of the **alliin** reserve it draws on, and the kinetic **protection of allicin** against its own decomposition, buying the sharp a longer life in heat. On the depth axis, the single lever is the **γ-glutamyl peptide pool** — raise the biosynthesis of γ-glutamyl-S-allyl-cysteine and kokumi climbs without touching the sharp at all. The onion program's defining lever is the one LFS branch: knock LFS down for a tearless, cleaner bulb, or route its sulfenic acid deliberately toward thiosulfinates to deepen a clean mouth-sharp. Across all of it, the quiet constant is the platform's reproducibility — the CV ≤ 7% that turns a wild clove's ±43% lottery into a promise a cook can keep.



The sequencer bench — where a clove's two independent axes are read, and then designed.

We design these the way our whole library is designed: plant-first, and in reverence for a chemistry we did not invent and only learned to read. The alliums are the savory foundation of nearly every vegetarian kitchen, the depth under a dal and the sharp over a salad, and to tune them is not to improve on the plant so much as to let it hold its promise reliably — the same sharp, the same depth, grown to carry the fullest flavor and the fullest nourishment it can. That the sharp we love is the plant's own defense, and the depth we crave is its quiet second chemistry, only deepens the care we bring to moving either.

What the number cannot tell you

Honesty about a measurement means naming where it stops. The IFI-P score is a statement about *potency* — how loudly a molecule speaks at the receptor, set by its EC_{50} — and not about dose. A clove that scores 1,400 but has had its alliinase killed before it worked delivers almost no allicin, and the mouth reads nothing; the number is a ceiling the plant can reach, not a guarantee the kitchen will. The arc table's cooked values are estimates of effective potency as the sulfur chemistry shifts under heat, not fixed constants, because the mixture in a real pan is a moving target of allicin, sulfides, and their further breakdown, each with its own curve.

There are honest failure modes worth stating plainly. Crush garlic and cook it instantly and the sharp never forms — not a loss of quality so much as a different dish, and sometimes the one you wanted. Rest it too long on the board, uncooked, and the allicin begins decomposing before it reaches the plate, the sharp fading

into sulfide heaviness. Garlic supplements quote an "allicin potential" measured under ideal enzymatic release, but stomach acid and processing can leave the delivered dose a fraction of the label — a gap between what a molecule *can* do and what a body *receives*, which is exactly the gap between potency and dose the index is careful never to blur. And the sharp that moves one person at 1.5 µM may read differently in another mouth, because TRPA1 sensitivity itself varies, and a laboratory EC_{50} is an anchor for comparison, not a verdict on any single tongue.

What the number does give us is a shared language precise enough to design in: that garlic is 1.4 times the convention reference on the sharp, that an onion routes most of its weapon to the eyes, that the road from raw to black trades about fourteen hundred points of pungency for a thousand of depth, and that a wild clove's ±43% can be made a designed clove's ±10%. The cook has always known the knife makes the flavor. The index only tells us, at last, exactly how much — and hands us the means to make that much the same, clove after clove, with intention. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 7 · Warm pungency — *ginger, cinnamon, and the slow sharp.*

Not all pungency flashes. There is a whole register of the sharp that arrives slowly, sits low and warm, and fades over minutes rather than seconds — the pungency of ginger and cinnamon, of the braise and the baked thing rather than the condiment at the plate's edge. It reaches the same TRPA1 channel as mustard and garlic, by the same covalent chemistry, but its molecules are heavier, clingier, and far less volatile, and the result is a sharp that behaves like a slow warmth. On the kinetic map these are the long-tailed foods: ginger, τ_off near three minutes; cinnamon close behind. If the isothiocyanates are the axis's percussion, ginger and cinnamon are its sustained notes — and the whole of this chapter is an argument that the slow note is the harder one to design, because everything that makes it gentle also makes it unstable.



Ginger — the rhizome whose sharp arrives slow and leaves slower.

The slow register, and why kinetics is the whole story

A pungent is not defined only by how loud it is but by how it moves through time, and the warm pungents are a lesson in reading the second axis of that description. A molecule of wasabi's isothiocyanate is small and volatile; it detonates against the receptor and then evaporates off the warm, wet tongue almost as fast as it arrived, so the sensation is a spike with a near-vertical fall. Ginger's and cinnamon's molecules are the opposite animal. They are larger, more lipophilic, and far less willing to leave the mouth, so they cannot clear by escaping into the air. Something else has to end the sensation — and that something is the receptor's own slow

desensitization, a gradual quieting of the channel after it has been held open. The molecule stays; the channel tires; the fade is written entirely by the receptor rather than by the chemistry fleeing the scene.

That single fact explains why ginger and cinnamon feel *warm* rather than *sharp* even though they open exactly the same channel as the condiment sharps. A spike is read as an attack. A long, even plateau is read as heat — a low fire that unfolds, holds, and recedes over the span of a swallow and the minutes after it. We spend this chapter inside two molecules, but the thing we are really tuning, when we design a warm pungent, is the shape of a curve in time.

We can put arithmetic to that curve. Once the molecule has stopped arriving, the fade of a warm pungent follows the channel's desensitization as an approximately exponential decay, $I(t) \approx I_0 \cdot e^{(-t/\tau_{off})}$, with τ_{off} for ginger near 180 seconds. Read the clock off that constant: one minute after a swallow, $e^{(-60/180)} = e^{(-0.33)} \approx 0.72$ of the warmth is still there; its half-life is $\tau_{off} \cdot \ln 2 \approx 125$ seconds, a touch over two minutes; by three minutes $e^{(-1)} \approx 0.37$ lingers, and only past five does it fall under a fifth. A volatile condiment sharp lives its whole arc inside the first of those intervals — seconds, not minutes — because its molecule escapes before desensitization has to do any work at all. Same channel, same covalent chemistry; the entire difference between an assault and a warmth is the value of a single time constant.

The covalent handshake — how TRPA1 actually opens

Most of the receptors that read flavor are locks waiting for a key: a molecule slides into a pocket, the shape change ripples outward, and when the molecule diffuses away the receptor relaxes. TRPA1 does not work this way, and that difference is the reason its agonists behave so strangely. It does not bind its pungents. It *reacts* with them.

TRPA1 is a homotetramer — four identical subunits arranged around a central cation pore — and each subunit trails an enormous N-terminal ankyrin-repeat domain, a coiled spring of roughly sixteen repeats hanging down into the cytoplasm. Near the base of that spring sits a small cluster of unusually reactive cysteine residues (in the human channel, the much-studied C621, C641, and C665, with a contributing lysine nearby), their sulphur atoms held in a solvent-exposed, electron-rich pocket. These are not binding sites. They are nucleophiles — chemical bases looking for an electron-poor partner.

Every warm pungent in this chapter is that partner: an **electrophile**, specifically a Michael acceptor. Walk the reaction through in order, because the order is the sensation:

1. **Approach.** The pungent molecule — gingerol, shogaol, cinnamaldehyde — carries a carbon made electron-poor by a neighbouring carbonyl. In an α,β-unsaturated carbonyl (C=C–C=O), the β-carbon is drained of electron density by the oxygen two bonds away; it is hungry.

2. **Attack.** A cysteine thiol, deprotonated to its far more reactive thiolate ($–S^-$), attacks that hungry β-carbon. For an isothiocyanate like mustard oil the same thiolate attacks the central carbon of the –N=C=S group instead.
3. **Adduct.** A covalent bond forms — a thioether for the enones, a dithiocarbamate for the isothiocyanates. The channel is now chemically modified, not merely occupied.
4. **Strain to gate.** The new adduct is bulkier and more polar than a bare cysteine. It tugs on the ankyrin spring; the strain travels up the subunit to the transmembrane gate; the pore dilates.
5. **Flux and feedback.** Sodium and calcium pour inward. Calcium then binds an EF-hand-like site on the channel itself and potentiates it further — a short positive-feedback loop that steepens the onset, so the warmth builds on itself in the first seconds.
6. **The slow release.** Because step 3 was a covalent bond, "letting go" is not diffusion. It is the slow reverse chemistry of a retro-Michael reaction plus the channel's desensitization. For a volatile sharp the free molecule also evaporates and clears; for a heavy, clingy warm pungent it does not, so the fade is entirely the receptor standing down. This is the molecular root of τ_off near three minutes.

Two design consequences fall out of this mechanism immediately, and we lean on both. First, **potency is electrophilicity × access.** A molecule is a louder TRPA1 agonist when its electron-poor carbon is more reactive and when its size and oil-solubility let it reach the buried cysteines. Second, **loudness and persistence are separable.** How hard the channel opens is set by the chemistry of the adduct; how long the sensation lasts is set by volatility and desensitization. We can, in principle, move one without the other — and the warm pungents are precisely the region of the axis where nature has already chosen long persistence, leaving us free to tune loudness underneath it.

Gingerol to shogaol — a dehydration you can taste

Ginger's pungency lives in a family of molecules built on a pathway unlike anything the pepper taught us — the **gingerols**, with their cousins the **shogaols** and **zingerone**. These are phenolic ketones, and the plant assembles them with a **type III polyketide synthase**, an enzyme family related to the chalcone synthase that colours so many flowers. The synthase takes a phenylpropanoid starter — feruloyl-CoA, drawn from the same phenylalanine-fed branch (PAL → cinnamate → ferulate → feruloyl-CoA) that supplies a great share of all plant flavour — performs a decarboxylative condensation with a malonyl-CoA extender unit, and, with a pair of tailoring reductases, leaves behind a **β-hydroxy ketone** carrying a long alkyl tail. The dominant product of the fresh rhizome is **6-gingerol**, where the "6" counts the tail and the business end is a hydroxyl on one carbon and a ketone two carbons along.

Here is the part you can taste. A β-hydroxy ketone is only a *middling* electrophile: the carbonyl is there, but it is not conjugated to a double bond, so there is no properly hungry β-carbon for a cysteine to attack. That is why fresh ginger reads as a warm, rounded sharp

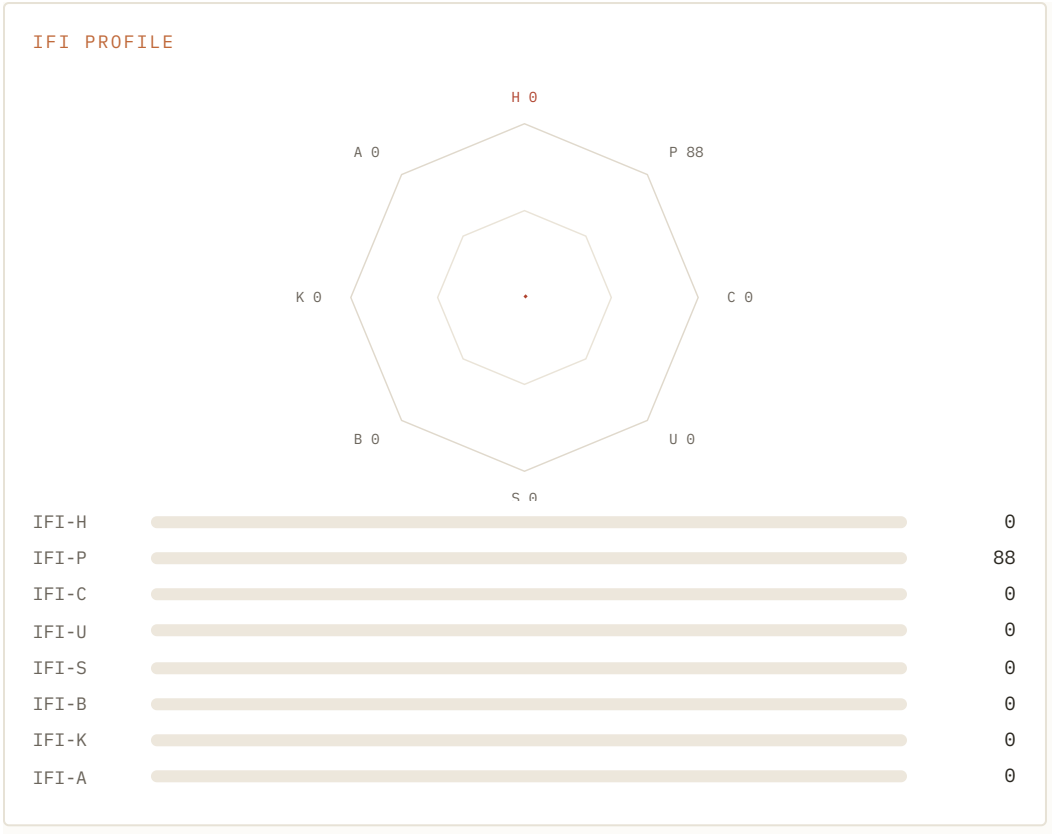
and sits at **EC_{50} 24 µM** — present, but soft. Now dry the rhizome, or cook it long and hot. The β-hydroxy ketone loses a molecule of water. That single dehydration slides a double bond into conjugation with the carbonyl and converts 6-gingerol into **6-shogaol** — an α,β-unsaturated ketone, a *textbook* Michael acceptor with a genuinely electron-poor β-carbon. The reaction does not merely concentrate the pungency; it **manufactures a better electrophile**, and the potency jumps to **EC_{50} 9 µM**. The reason dried ginger bites harder and brighter than fresh is a dehydration you can draw: one molecule of water leaving turns a soft ketone into a sharp one.

Push the heat further and a second reaction takes over. The same β-hydroxy ketone in gingerol can fragment by a **retro-aldol** cleavage, snapping off the long tail to leave **zingerone** — a short methyl ketone with no Michael acceptor at all. Zingerone is mild and faintly sweet; on this axis it is nearly silent. So the life of cooked ginger is a two-step story told in molecules: *gingerol → (lose water) → shogaol → (lose the tail) → zingerone*, a soft sharp that first brightens and then gentles. Candied ginger and a long braise live at the far, sweet end of that arc; a fresh grate lives at the near, round end; properly dried ginger catches the bright peak in the middle.



Ginger in cross-section — the oleoresin cells where gingerol and shogaol are laid down.

Read as a flavour profile, fresh ginger is almost a single pure note. Seven of the eight axes are quiet; only pungency sings, carried entirely by its dominant molecule:



That is a profile of austere honesty — the kind of thing the index was built to show. Ginger is not a chord. It is one clear tone, and its whole character is *where on the pungency scale that tone falls* and *how the drying moves it.* In our ginger program — **120 constructs** on the pungency axis — the lever is exactly that movement: tune the balance of the pathway and the size of the drying-sensitive gingerol pool, and you set where the plant sits between the fresh warm-round and the dried bright-sharp before a cook ever touches it. The drying ladder, laid out in numbers, is the design space itself:

GINGER STATE	DOMINANT MOLECULE	CHEMISTRY	EC_{50} (MM)	IFI-P	WHAT THE MOUTH READS
Fresh grate	6-gingerol	β-hydroxy ketone	24	88	warm, round, slow
Properly dried	6-shogaol	α,β-unsaturated ketone	9	233	bright, sharp, lifted
Long-cooked / candied	zingerone	short methyl ketone	— (below floor)	low single digits	mild, sweet, warming

The arrow from fresh to dried multiplies loudness by exactly **24 ÷ 9 = 2.67×** — the ratio of the two EC_{50} values and nothing else. A grower cannot feel 2.67. The index can read it, and a design program can aim at it.

The arithmetic of loudness

Every number in that last column comes from one definition, and it is worth deriving in full because the whole index stands on it. The Iyengar Flavor Index does not score how much of a compound a food holds; it scores how *loudly* that compound speaks to its receptor — its half-maximal activation concentration, EC_{50}, inverted against a fixed reference so that a smaller EC_{50} (a molecule that works at lower concentration) reads as a larger, louder score.

$$\text{IFI-P}(c) = 1000 \times EC_{50}(\text{reference}) \div EC_{50}(c)$$

For the pungency axis we anchor the reference to **allyl isothiocyanate** — mustard oil, the archetypal TRPA1 electrophile, the loud percussive sharp against which the warm ones are read. We set the pungency-axis convention constant at **2.1 µM** — an anchor defined by convention, not derived from a single literature measurement, in the same spirit as the Scoville scale's dilution-to-threshold definition — which makes its own score exactly 1000 by definition (1000 × 2.1 ÷ 2.1), the anchor peg of the scale. Everything warm is read against that peg:

- **6-shogaol**, EC_{50} 9 µM → IFI-P = 1000 × 2.1 ÷ 9 = **233**
- **6-gingerol**, EC_{50} 24 µM → IFI-P = 1000 × 2.1 ÷ 24 = **88**
- **trans-cinnamaldehyde**, EC_{50} 61 µM → IFI-P = 1000 × 2.1 ÷ 61 = **34**

The ratios between those scores are pure chemistry with the reference cancelled out. Dried ginger over fresh is 233 ÷ 88 = 2.65, the dehydration again (pure EC_{50} ratio: 24 ÷ 9 = 2.67). Fresh ginger over cinnamon is 88 ÷ 34 = 2.59, close to 61 ÷ 24 = 2.54. Dried ginger over cinnamon is 233 ÷ 34 = 6.85, close to 61 ÷ 9 = 6.78. Small differences from the exact EC_{50} ratios arise from rounding integer scores; the underlying relationship is exact. The index is doing no more than arithmetic on half-activations from the literature, referenced against a convention constant — but that arithmetic lets a rhizome, a bark, and a mustard seed be read on one line.

A worked unit conversion keeps the micromolar from floating free of the kitchen. EC_{50} in micromolar is micromoles per litre; multiply by a molecule's molar mass to reach a mass concentration. 6-gingerol (294.39 g/mol) at 24 µM is $24 \times 10^{-6} \times 294.39 = 7.07 \times 10^{-3}$ g/L, i.e. **7.07 µg/mL**. The same arithmetic places the whole family on a common mass footing:

MOLECULE	EC_{50} (MM)	MOLAR MASS (G/MOL)	EC_{50} (MG/ML)	IFI-P
Allyl isothiocyanate (reference)	2.1	99.15	0.21	1000
6-shogaol	9	276.37	2.49	233
6-gingerol	24	294.39	7.07	88
trans-cinnamaldehyde	61	132.16	8.06	34

Read the mass column and the mechanism is visible in it: shogaol speaks at a third of the mass concentration gingerol needs, because the dehydration built it a better electrophile, not because there is more of it.

Loudness is only the midpoint of a curve, and a designer who stops at EC_{50} is reading a single point off a function. The full dose–response is a **Hill relation**, the fraction of channels open as the concentration climbs:

$$\theta([A]) = [A]^n \div (EC_{50}{}^n + [A]^n)$$

At the EC_{50} the fraction is one half, for any value of the exponent — that is what half-maximal means. The exponent n, the Hill coefficient, is where the covalent mechanism reappears. Because TRPA1 carries *several* reactive cysteines and more than one adduct stiffens the ankyrin spring further, the response is cooperative, and we model the warm pungents with a Hill coefficient near **n ≈ 2**. The

difference is not cosmetic. Take 6-shogaol at its EC_{50} of 9 µM and double the concentration to 18 µM. With no cooperativity (n = 1) the open fraction rises from 0.50 to 0.667; with n ≈ 2 it rises from 0.50 to **0.80**. Cooperativity makes the warm pungents turn from "present" to "full" over a narrow doubling of concentration — a steep shoulder right above the midpoint.

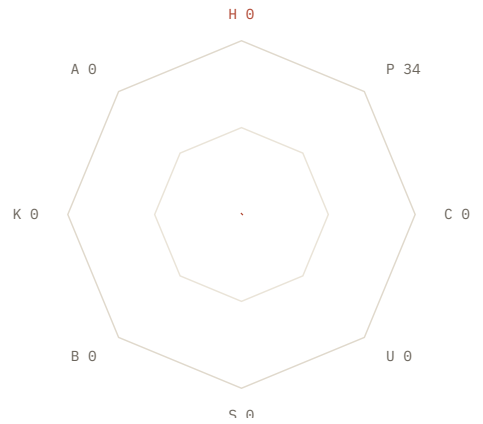
Run that same relation backwards and the dehydration's payoff reappears as a dose at the cutting board. To drive the channel to any chosen fullness θ, a molecule must reach [A] = EC_{50} · (θ ÷ (1 − θ))^(1/n). Ask both ginger molecules for the same satisfying 0.80 open fraction at n ≈ 2: the bracket is (0.80 ÷ 0.20)^(1/2) = 4^(1/2) = 2, so each needs exactly twice its own EC_{50} — dried ginger's shogaol at 2 × 9 = 18 µM, fresh ginger's gingerol at 2 × 24 = 48 µM. The dried rhizome reaches the same warmth at 18 µM that the fresh one needs 48 µM to match: a **2.67× smaller dose**, the identical 24 ÷ 9 ratio now wearing the clothes of *how much ginger the recipe calls for.* Loudness read at the bench and quantity read at the board are two faces of one number. For scale at the other end of this axis, a computationally designed pungency like our **Phoenix** cultivar (SS-0096) — where our model predicts IFI-P near **840** — sits about 3.6× above the loudest thing drying can wring from a rhizome, which is what it means to design a sharp rather than dry one.

Cinnamaldehyde and the phenylpropanoid economy

Cinnamon reaches TRPA1 by the most textbook electrophile of them all: **trans-cinnamaldehyde**, an α,β-unsaturated aldehyde, the canonical Michael acceptor and one of the first compounds ever shown to open the channel. Its biosynthesis is pure phenylpropanoid economy, and worth drawing because the same opening moves recur across the whole flavour library. Phenylalanine is stripped of its amino group by **PAL** (phenylalanine ammonia-lyase) to give **trans-cinnamic acid**; a CoA ligase activates that acid to **cinnamoyl-CoA**; and a **CCR-type reductase** (cinnamoyl-CoA reductase) reduces the thioester straight to the aldehyde. The telling detail is what cinnamon does *not* do: most plants hydroxylate this intermediate (the C4H step) and march it toward lignin, but cinnamon shunts cinnamoyl-CoA directly to the aldehyde and preserves the bare, simple cinnamaldehyde. The aldehyde is the pungency; everything else we love about cinnamon — the sweetness-by-association, the roundness — is aroma riding alongside, not the channel event.

Cinnamaldehyde's EC_{50} of 61 µM places it well down the potency table, at IFI-P 34, and that is exactly right. We do not experience cinnamon as an *assault* but as a warm, slow, aromatic sharp — the pungency folded so far under the fragrance that most people never name it as pungency at all. On the index it is the clearest case of a quiet P number doing structural work beneath a loud aroma: a single, modest Michael acceptor, one reactive site rather than the cross-conjugated reach of shogaol, lingering long because the molecule is heavy and reluctant to leave.

IFI PROFILE



IFI-H		0
IFI-P		34
IFI-C		0
IFI-U		0
IFI-S		0
IFI-B		0
IFI-K		0
IFI-A		0

Like ginger, cinnamon is nearly a pure tone — one axis lit, the rest dark — and like ginger its entire character is where that single tone sits and how long it holds. The quietness of the number is the point. A loud axis announces itself; a quiet one has to be read.



Cinnamon bark in cross-section — cinnamaldehyde laid down in the living phloem.

Coumarin — the molecule we hold down

Cinnamon also carries the clearest **regulatory** note on the whole axis, and a formulator has to know it before touching a construct. The two commercial cinnamons are chemically different plants. **Ceylon cinnamon** (*Cinnamomum verum*), the "true" cinnamon, is low in a compound called **coumarin**. **Cassia cinnamon** (*C. cassia* and relatives), the cheaper and far more common bark, is high in it. Coumarin arises from a side branch of the very same phenylpropanoid pool that makes the cinnamaldehyde: an ortho-hydroxylation of cinnamic acid, a glucosylation, and — on tissue damage — a lactonization that closes the fragrant ring. Ceylon runs little flux down that branch; cassia runs a great deal. Because coumarin is regulated in food in several jurisdictions — the European

Union sets maximum levels in cinnamon-bearing foods, with category caps on the order of a few to a few tens of milligrams per kilogram depending on the food — a product leaning hard on cassia for its cinnamaldehyde pungency can drift toward those limits.

This is where the design problem becomes beautiful, because it is a problem of **decoupling** two products of one pathway. Both cinnamaldehyde and coumarin descend from cinnamic acid; the cheap way to more pungency is more cinnamic flux, which drags coumarin up with it. In our cinnamon program — **40 constructs** on the pungency axis — the explicit constraint is to lift the CCR branch that carries cinnamaldehyde while suppressing the ortho-hydroxylation branch that carries coumarin, moving one enzyme's product without dragging its neighbour along. It is precisely the kind of problem the platform is built for: not "make it stronger," but "make this louder while holding that silent," a surgical separation of two molecules that a breeder selecting on phenotype could never pull apart. The warm-pungency programs, side by side, are each a version of that discipline:

PROGRAM	CONSTRUCTS	AXIS	DIFFICULTY	DESIGN LEVER	HELD DOWN
Ginger	120	P	Moderate	set gingerol : shogaol pool (drying-sensitive fraction)	runaway retro-aldol to zingerone
Cinnamon	40	P	Hard	lift CCR → cinnamaldehyde	coumarin (ortho-hydroxylation branch)
Reference: black mustard	*2,790*	*P/M*	*Hard*	*isothiocyanate yield (the loud anchor)*	–

Precision, error, and the honest floor

A number without an error bar is a wish, and the index earns trust only if we carry the uncertainty through the arithmetic as faithfully as the value. Every IFI-P score is a ratio of a literature EC_{50} value and the axis convention constant, and that ratio propagates relative error from the literature measurement. If a candidate's EC_{50} is held at our platform coefficient of variation of **≤7%** and we treat the reference convention constant as fixed, the score inherits that same 7%: 6-shogaol reads **233 ± 16**. If instead the reference is re-measured alongside every run, the two 7% errors combine as $\sqrt{(0.07^2 + 0.07^2)}$ = **9.9%**, and the bar widens to 233 ± 23. We publish the narrower figure because we do hold the reference constant fixed — but we show the wider one so a reader knows what the discipline is buying.

That discipline is the quiet argument of the whole program, and ginger makes it vivid. Natural ginger's pungency is not a value; it is a *range*, because the gingerol-to-shogaol ratio swings with cultivar, maturity, and above all with how hot and how long the rhizome was dried. A batch dried gently stays near all-gingerol (IFI-P ≈ 88); a batch dried hard tips toward shogaol (≈ 233). That is a **2.65× spread** riding on a drying shed's thermostat — the same uncontrolled variance that, in the capsaicin world, let wild-type heat drift at a 42% coefficient of variation before design collapsed it toward ≤7%. The job of the ginger program is not merely to raise the sharp but to *fix the set-point* and hold it, so that a formulator buys the same warm pungent every season instead of a lottery.

The warm pungents also carry failure modes worth naming plainly, because the mechanism that makes them gentle is the same one that makes them fragile:

- **Runaway dehydration.** Shogaol is a peak, not a plateau. Too much heat first sharpens gingerol into shogaol and then fragments it by retro-aldol into mild zingerone, so over-drying does not keep intensifying the sharp — it overshoots and collapses it into sweetness. There is an optimum drying window, and it is narrow.
- **A floor the assay cannot see past.** Zingerone is so weak at TRPA1 that its EC_{50} cannot be pinned reliably; measurement noise swamps the signal. We report it as "below the reliable floor" rather than inventing a precise number, because an honest blank is worth more than a false digit.
- **The volatility paradox.** Cinnamaldehyde is semi-volatile. In a hot dish some of it escapes as aroma before it ever reaches the tongue, so the pungency a diner perceives can read *below* what the bench EC_{50} predicts. The index reads a molecule's loudness at the receptor; the kitchen adds a matrix the bench number does not know about.
- **Loudness is not dose.** The deepest caveat is structural: IFI-P scores how loud a molecule is, never how much a food holds. A dish rich in a quiet molecule can out-pungent a dish holding a trace of a loud one. The index is a necessary half of formulation and must always be read beside concentration, not instead of it.

None of these is a flaw in the measurement. They are the texture of working with a covalent, cooperative, semi-volatile chemistry, and naming them is how we keep the lushness honest. The number is a form of respect for the reader; the error bar and the failure mode are the rest of that respect.

What the slow sharp is for

What unites ginger and cinnamon, and separates them from the condiment sharps, is that their molecules are not volatile enough to flee the mouth, so the channel's own slow desensitization writes the entire decay — a long, even fade rather than a fast clear. That makes them the pungents of **cooked** food: the ones you build into the body of a dish, where they have time to unfold, rather than the ones you deploy at the edge for shock. A grate of ginger into the oil at the start of a braise, a stick of cinnamon in the pot of rice and milk left to

simmer — these are seasonings that become part of the structure of a meal, warmth laid into the foundation rather than struck across the top.

They are also the gentlest bridge from the sharp as a channel event to the sharp as a seasoning, the place where pungency stops being something that happens to you and becomes something you cook with intention into what you eat. That is the register our family has always loved best and understood first — the warm, slow, vegetable sharpness of a kitchen where nothing is an assault and everything is meant to nourish. To read it, and then to design it — to hold a rhizome at exactly the brightness you want, to lift a bark's quiet sharp while keeping it clean — is only to give that old reverence a number it can finally be built from. The slow sharp was always the one we reached for at the table. Now it is the one we can grow on purpose. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 8 · The edges of the axis — *the herbs, the exceptions, and the tingle.*

An axis is defined as much by its borders as by its center, and pungency has three borders worth walking slowly, because each one teaches something the clean cases cannot. The clean case is easy to love. A seed of black mustard crushed under a thumb, a knife drawn once through a radish, and the sharp arrives in half a second — bright, volatile, almost gone before it lands. That is IFI-P at its most legible: the TRPA1 channel struck like a small bell. But a channel is known by where it blurs, and this one blurs in three directions at once. The first border is the fade into the herbs, where the sharp thins to a whisper laid beneath aroma. The second is the great exception — the spice everyone in our family grew up calling pungent, which turns out to be speaking on a different channel altogether. The third is the strangest frontier of all, where pungency stops behaving like a sharp and becomes a *buzz*, an electric tingle the mouth cannot quite find the edges of.

We walk the three not to catalogue oddities but because the edges are where the axis becomes designable. A center tells you what a thing is; a border tells you which lever moves it. And every lever here moves a sensation that someone, somewhere, is trying to coax into a jar.

What the sharp is, in one receptor

Pungency is one receptor wearing many disguises. TRPA1 — the "wasabi receptor" — is a non-selective cation channel standing in the membrane of the trigeminal and sensory nerve endings that web the tongue, the gums, the inside of the nose. Open it and sodium and calcium pour inward, the nerve fires, and the brain reports a sharp. What makes TRPA1 unlike capsaicin's warm, patient TRPV1 is *how* it opens. Its long cytoplasmic N-terminus is a coil of ankyrin repeats studded with a small cluster of unusually reactive cysteines — in the human channel, the residues numbered C621, C641, and C665, with a cooperating lysine nearby. Those thiols are the trigger.

There are two ways to pull it. The first is covalent and electrophilic: a reactive molecule — an isothiocyanate, a thiosulfinate, an α,β-unsaturated aldehyde — reaches the cysteine and forms a *reversible* covalent adduct, thiocarbamoylating or Michael-adding onto the sulfur, and the chemical bond itself is what wrenches the gate open. The second is non-covalent: a small lipophilic molecule such as thymol or menthol slips into the transmembrane domain and nudges the channel open without any reaction at all. The electrophilic agonists are the loud ones; the non-covalent agonists are the whisperers. Because the covalent adducts are reversible and the molecules are small and volatile, the TRPA1 sharp is fast and clearing

— it flashes and lifts — where the capsaicin warmth of TRPV1 lingers and builds. A cook feels that difference without naming it. The index gives it a mechanism and a number.

The number is loudness, not quantity. We score each axis against the half-maximal activation of a fixed reference, and we anchor pungency to the archetype of the sharp — allyl isothiocyanate, the mustard oil of crushed black mustard and grated wasabi — using a stated convention constant of 2.1 µM, chosen as a defined reference point in the same spirit that the Scoville scale is defined by dilution-to-threshold:

$$\text{IFI-P} = 1{,}000 \times 2.1\ \mu\text{M} \div EC_{50}(\text{candidate})$$

By construction, a food whose sharp speaks exactly as loudly as the convention reference reads **1,000**. The scorecard normalizes each axis to a ceiling of 5,000, which corresponds to a candidate half-activating at 0.42 µM — five times louder, per molecule, than the convention reference. Everything that follows is read against that single ruler. EC_{50} is not how much sharp a plant holds; it is how loudly each molecule of it speaks. Hold that distinction and the three borders fall into order.

The herb edge — a whisper built from terpenes

Start where the sharp is faintest. **Thyme** and **oregano** carry monoterpene phenols — **thymol** and **carvacrol** — that are genuine TRPA1 agonists but weak ones, and weak in the gentler, non-covalent way: they do not react with the cysteines, they lean on the transmembrane domain. Their half-activations sit an order of magnitude above the mustard oils, at EC_{50} 150 µM for thymol and 100 µM for carvacrol. Run them through the ruler and the whisper is exactly as quiet as it feels:

- thymol: IFI-P = 1,000 × 2.1 ÷ 150 = **14**
- carvacrol: IFI-P = 1,000 × 2.1 ÷ 100 = **21**



Thyme — monoterpene phenols at the quiet edge of the sharp.

Where do those phenols come from? Not from the glucosinolates of the brassicas nor the sulfur chemistry of the alliums, but from the terpenoid pathway — the same scaffolding that builds a plant's resins and perfumes. Geranyl diphosphate is cyclized to **γ-terpinene**; the leaf aromatizes that ring to **p-cymene**; a cytochrome P450 of the CYP71D family hydroxylates the aromatic ring to deliver

the phenol — thymol if the hydroxyl lands in one position, carvacrol if in its neighbor. The two molecules are structural isomers, $C_{10}H_{14}O$ both, molecular weight 150.22, which is why they taste like cousins: a dry, warm, resinous prickle rather than a flashing cut.

The mass tells the rest of the story. Convert the half-activations out of micromolar — multiply by the molecular weight to reach milligrams per litre:

- thymol: 150 µM × 150.22 g/mol = **22.5 mg/L** to reach half-activation
- carvacrol: 100 µM × 150.22 g/mol = **15.0 mg/L**

You need tens of milligrams of phenol per litre before the trigeminal nerve even half-answers — but a fraction of that saturates the olfactory receptors in the nose. So the aroma always arrives first and loudest, and the sharp trails so far behind it that we barely file these herbs as pungent at all. That is the whole character of the herb edge: a seasoning that lays a prickle *under* a dish without ever stepping forward as heat. Our thyme program (120 constructs, IFI-P primary) and oregano program (40 constructs, IFI-P) treat that whisper as a real, tunable quantity — the lower rungs of a ladder whose summit is horseradish. To raise a rung you have two independent handles: lift the concentration the plant carries, or lower the EC_{50} so each molecule speaks louder. The index keeps them separate, because they are separate acts.

Watercress, radish, and the glucosinolate trigger

One step up the ladder, the whisper becomes a bite. **Watercress** looks like a salad green and behaves like a mustard. Its glucosinolate, **gluconasturtiin**, yields **2-phenethyl isothiocyanate** — a true isothiocyanate, a genuine electrophile, with a real half-activation of EC_{50} 6.2 µM. That is a different order of loudness entirely:

- 2-phenethyl isothiocyanate: IFI-P = 1,000 × 2.1 ÷ 6.2 = **339**



Watercress — a true isothiocyanate waiting in the cut cells of a salad green.

Well into the range of real sharpness, from a leaf you would toss into a bowl without a thought. And the mass makes the point even harder. Convert it out of micromolar — phenethyl isothiocyanate is C_9H_9NS, molecular weight 163.24:

- 2-phenethyl isothiocyanate: 6.2 µM × 163.24 g/mol = **1.01 mg/L** to reach half-activation

A single milligram per litre, against thyme's twenty-two. Same tongue, same channel, two orders of magnitude apart in loudness — and that gap is the entire reason a bite of raw watercress bites back and a sprig of thyme does not.

The isothiocyanate is not sitting in the intact leaf. It is manufactured in the instant of damage, by one of the most elegant two-part defenses in the plant kingdom. The glucosinolate — a stable, water-soluble thioglucoside — is stored in one set of cells; the enzyme that cleaves it, **myrosinase**, is sequestered in separate myrosin cells, kept apart like the two halves of a fuse. Bite, crush, or grate the tissue and the walls break, enzyme meets substrate, and myrosinase hydrolyzes the thioglucoside bond. The aglucone that falls out is unstable; it undergoes a spontaneous **Lossen rearrangement**, shedding sulfate and migrating its side chain onto the nitrogen to become the pungent isothiocyanate. This is why you must *work* a brassica to wake it — why grated wasabi sharpens over seconds, why whole mustard seed is mild and cracked mustard seed is fierce, why the sharp fades again as the volatile oil escapes the board. The plant keeps the knife disassembled until something tries to eat it.

Radish carries the same machinery with a different blade: its glucosinolate yields **4-methylthio-3-butenyl isothiocyanate**, the clean, crisp, cooling sharp of daikon. Its construct program is a small one of 40, and its primary axis in our library is actually bitterness rather than pungency — a reminder that an organism is a chord, not a single note, and that the same cut cells can deliver sharpness and a cool green bitterness in the same breath. The archetype at the very top of the ladder is the mustard oil we anchored the axis to: **allyl isothiocyanate**, released from sinigrin in black mustard, wasabi, and horseradish alike. Its molecular weight is only 99.15 — the smallest, most volatile, fastest-flashing of the family:

- allyl isothiocyanate (reference): 2.1 µM × 99.15 g/mol = **0.21 mg/L**, and IFI-P = **1,000** by definition

Here is a subtlety the ruler forces us to be honest about. Horseradish sits at the top of the ladder, but not because its molecule is louder than wasabi's or black mustard's — they are the *same* molecule, allyl isothiocyanate, all reading near 1,000. Horseradish wins on *delivery*: it packs more sinigrin and more myrosinase into each gram, so it pours more of that identical sharp onto the tongue. Loudness is the molecule's EC_{50}; intensity on the plate is loudness multiplied by how much the plant hands you. The index measures the voice; the harvest measures the volume. Our mustard programs sit here at the loud end — black mustard at 2,790 constructs (IFI-P and multi-axis), white mustard at 200 — and the two are not interchangeable: white mustard's sinalbin yields *p*-hydroxybenzyl isothiocyanate, a heavier, non-volatile cousin that gives a mustard its lingering warmth rather than its nose-clearing flash.

SOURCE	PUNGENT MOLECULE	EC_{50} AT TRPA1 (MM)	MASS AT EC_{50} (MG/L)	IFI-P = 1,000 × 2.1 ÷ EC_{50}
Thyme	thymol	150	22.5	14
Oregano	carvacrol	100	15.0	21
Watercress	2-phenethyl isothiocyanate	6.2	1.01	339
Mustard / wasabi / horseradish	allyl isothiocyanate *(convention reference)*	2.1	0.21	1,000

The reactive edge — alliums, ginger, and the cut that makes the knife

The brassicas are not the only plants that keep their knife disassembled. Walk one border over, into the alliums, and the same strategy reappears in sulfur of a different kind. A clove of **garlic** holds an odorless amino acid, alliin, and an enzyme, alliinase, stored apart. Crush the clove and alliinase converts alliin, in seconds, to **allicin** — a diallyl thiosulfinate, a reactive electrophile that is a genuine TRPA1 agonist. It is the same grammar as the brassica fuse: an inert precursor, a separated enzyme, and a sharp manufactured only at the moment of injury. The index names allicin as one of the archetypes of the pungent axis, and rightly — it reaches the channel's reactive cysteines directly, by thiol–disulfide exchange, and throws the gate open. Garlic carries 560 constructs across two axes, kokumi and pungency together, because allicin's sulfur chemistry also feeds the rich, mouth-filling continuity of the CaSR kokumi channel. The clove is sharpness and depth braided from a single element.



Garlic in cross-section — allicin exists only after the clove is cut, the allium's version of the brassica fuse.

Ginger sharpens by a third route. Its fresh rhizome carries **6-gingerol**; dry or heat it and the gingerol dehydrates to **6-shogaol**, which is why dried ginger is sharper and more biting than fresh — the cook's heat is doing synthetic chemistry on the board. Both molecules carry an enone, an α,β-unsaturated carbonyl that acts as a mild Michael acceptor at TRPA1 while also brushing TRPV1 for a touch of warmth. Ginger's 120-construct program sits on the pungent axis, and the gingerol-to-shogaol conversion is a handle we can tune directly. **Cinnamon** closes the family with the cleanest textbook electrophile of all: **cinnamaldehyde**, C_9H_8O, an α,β-unsaturated aldehyde that is the canonical covalent TRPA1 agonist — a pure Michael acceptor that adds onto the channel's cysteines and is used in laboratories precisely because its action is so

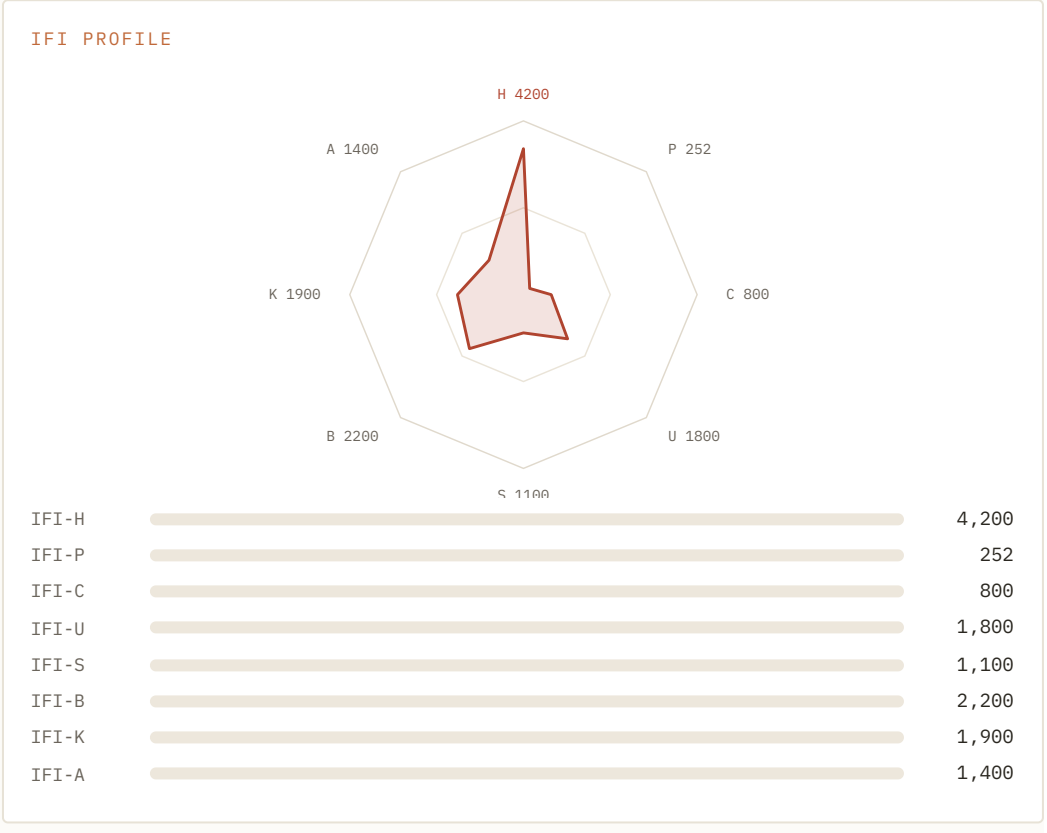
unambiguous. Cinnamon's 40 constructs make its warm, sweet, woody sharp a small but real rung on the pungent ladder, and the index lists cinnamon among the axis's defining agonists for exactly this reason.

ORGANISM	CONSTRUCTS	PUNGENT MOLECULE	MADE AT TRPA1 BY
Black mustard	2,790	allyl isothiocyanate	covalent (thiocarbamoylation)
Garlic	560	allicin	covalent (thiol exchange)
White mustard	200	*p*-hydroxybenzyl isothiocyanate	covalent (thiocarbamoylation)
Ginger	120	6-gingerol / 6-shogaol	covalent (Michael addition)
Watercress	120	2-phenethyl isothiocyanate	covalent (thiocarbamoylation)
Thyme	120	thymol	non-covalent (transmembrane)
Cinnamon	40	cinnamaldehyde	covalent (Michael addition)
Oregano	40	carvacrol	non-covalent (transmembrane)

What unites the loud end of this table is a single mechanism told in dialects: an electrophile, stored safely or made on the spot, that forms a reversible covalent bond with the same three cysteines. Isothiocyanates thiocarbamoylate them; allicin exchanges a sulfur with them; cinnamaldehyde and the gingerols add across the double bond. Different plants, different biosyntheses, one lock — which is why a flavor designer can reach for any of them to move the same axis, and why the sharp of garlic, mustard, ginger, and cinnamon all feel like relatives even when their aromas do not.

The designed center, computed

Set against these wild edges, our designed flagship gives the axis a calibrated middle to read from. **Phoenix** was engineered to register on all eight axes at once, and its pungent channel is tuned deliberately rather than inherited:



Its IFI-P of 252 is not a round accident — it is the point of the design. Invert the ruler to read the chemistry back out of the score:

> $IFI\text{-}P = 1{,}000 \times 2.1 \div EC_{50} \rightarrow EC_{50} = 1{,}000 \times 2.1 \div 252 =$ **8.33 µM**

A pungent-equivalent half-activation of 8.33 µM places Phoenix's sharp well above the herbal whisper — thymol reads 14, carvacrol 21 — while holding deliberately below the convention reference of 1,000. Phoenix carries all eight axes at once; pungency at mid-range lets the other channels speak. That is what it means to design an edge rather than forage one: we did not find a plant whose sharp happened to land at 252; we specified the loudness and built the pathway to hit it, then held it there batch after batch. The wild edges scatter where the seasons and the soil leave them. The center is placed on purpose.

The great exception — black pepper on the wrong channel

Now the exception that clarifies the axis by failing to belong to it. Everyone calls **black pepper** pungent, and in plain English it is. But its molecule, **piperine** — a piperidine amide of piperic acid, $C_{17}H_{19}NO_3$ — is primarily an agonist not of TRPA1 but of **TRPV1**, capsaicin's *heat* channel. Piperine settles non-covalently near the vanilloid pocket where capsaicin binds, lowers the channel's thermal threshold, and produces a slow, lingering, building warmth — the exact signature of heat, not of the sharp. On the index, therefore, black pepper reads most of its "pungency" on **IFI-H**, not IFI-P.

Measured against the sharp, piperine barely registers. It is a poor TRPA1 agonist — its half-activation there, where it has any action at all, sits up around 500 µM. Run that representative figure through the ruler:

- piperine at TRPA1: IFI-P ≈ 1,000 × 2.1 ÷ 500 = **4**

Four. Below even thyme. So the molecule the whole world files as pungent contributes, on the pungent axis, a whisper quieter than an herb — while its real loudness lives one axis over, on heat. This is not a quibble; it is the entire reason the index exists. The folk category "spicy" collapses two distinct receptor events into one word, and the ruler pulls them cleanly apart: chili and black pepper are **heat** — TRPV1, a slow warmth from a non-reactive molecule — while mustard, wasabi, garlic, and horseradish are **pungency** — TRPA1, a fast sharp from an electrophile. A cook feels the difference without naming it: that black pepper warms and lingers where mustard flashes and clears. When we say a dish needs "more sharp, less heat," we now mean something exact — lift P, hold H, and reach for the brassica rather than the peppercorn.

MOLECULE	SOURCE	PRIMARY RECEPTOR	AXIS	WHAT IT FEELS LIKE
capsaicin	chili	TRPV1	IFI-H	slow building warmth
piperine	black pepper	TRPV1	IFI-H	lingering warmth
allyl isothiocyanate	mustard, wasabi	TRPA1	IFI-P	fast nose-clearing flash
allicin	garlic	TRPA1	IFI-P	hot reactive sharp
cinnamaldehyde	cinnamon	TRPA1	IFI-P	warm sweet prickle
hydroxy-α-sanshool	Sichuan pepper	TRPA1 · TRPV1 · K2P block	IFI-P × IFI-C	electric tingle

The tingle — hydroxy-α-sanshool and the controlled short-circuit

The third border is the strangest and, to us, the most beautiful: the place where pungency becomes *numbness*. **Sichuan pepper** is not a pepper at all but the dried husk of a *Zanthoxylum*, and it carries **hydroxy-α-sanshool** — an unsaturated fatty-acid amide, $C_{16}H_{25}NO_2$, molecular weight 263.38 — that does something no other agonist in this book does. It activates TRPA1 and TRPV1, yes, giving a genuine sharp-and-warm. But it *also* blocks a family of background potassium channels — the two-pore **K2P** leak channels, TASK-1, TASK-3, and TRESK — that normally hold sensory neurons quiet. And that second action is where the magic, and the mathematics, live.

A resting neuron is held near the potassium equilibrium potential by a steady leak of K^+ outward through exactly those K2P channels. Model the resting voltage as a conductance-weighted average of the two ions pulling on the membrane:

```
V_rest = (g_K · E_K + g_bg · E_bg) ÷ (g_K + g_bg)
```

Take E_K = −90 mV, a background cation reversal E_bg = +60 mV, and a healthy resting ratio g_K : g_bg of 20 : 1. The neuron sits quietly at

```
V_rest = (20 · −90 + 1 · 60) ÷ 21 = −1,740 ÷ 21 = −82.9 mV
```

Now let sanshool block half of that potassium leak, dropping g_K from 20 to 10:

```
V_rest = (10 · −90 + 1 · 60) ÷ 11 = −840 ÷ 11 = −76.4 mV — a +6.5 mV climb toward threshold
```

Block three-quarters of it, g_K down to 5:

```
V_rest = (5 · −90 + 1 · 60) ÷ 6 = −390 ÷ 6 = −65.0 mV — now +18 mV above where it started
```

Spiking threshold sits around −50 mV. Silence the leak that holds the neuron down and you lift it from −83 mV to the lip of firing, where the gentlest input — the brush of saliva, the plate's own warmth — tips it over and over. The light-touch afferents that normally report a

feather now fire in a rhythmic burst, and the brain reads that rhythm as a flutter the psychophysics clock near fifty cycles a second — a period of 1 ÷ 50 = **20 milliseconds** between taps. That is the famous *málà* tingle: not a taste the tongue receives but a vibration the nerve manufactures when its brake is released. It is pungency spilling across into the **Cool-Burn** axis, IFI-C, the thermomodulation channel, which is why Sichuan cooking feels like a controlled short-circuit — the chili's heat on IFI-H, the husk's tingle shared between IFI-P and IFI-C, and the aromatics' sharp on IFI-P, braided into the single sensation the cuisine calls *málà*. Designing it means moving three channels at once, which is a study of its own; but it belongs here, at the axis's edge, as proof that the sharp does not end at a clean line.

Reading the edges — dose, error, and the honest limits

A single number per axis is a gift to the reader and a debt we have to be honest about, and the edges are where the debt comes due. Three honest limits sit under every figure above.

First, **a half-activation is not a whole curve.** EC_{50} marks the one concentration where a receptor is half-answering; it says nothing about how steeply the response climbs on either side. The full dose–response follows a Hill relation,

$$\text{response} = [A]^n \div (EC_{50}^n + [A]^n)$$

where n is the cooperativity — the steepness. At the half-activation point the response is 50% for any n. But push to twice the EC_{50}: with a shallow n = 1, response = 2 ÷ (1 + 2) = **66.7%**; with a steeper n = 2, response = 4 ÷ (1 + 4) = **80%**. Two foods can share an EC_{50} — and therefore an identical IFI-P — yet feel different because one ramps gently and the other slams. The index names the center of the sharp; it does not draw its shoulders.

Second, **EC_{50} drifts with the assay and the matrix.** A number read from a clean cell line of human TRPA1 is not the number a native trigeminal neuron reports, and human and rodent channels answer some of these agonists differently — so the lineage of a measurement matters. Worse for the electrophiles, the food itself fights back: glutathione, free cysteine, and protein thiols in a real dish scavenge isothiocyanates and allicin before they ever reach the nerve, lowering the effective dose; cooking denatures myrosinase and alliinase, so a simmered mustard or a roasted garlic never makes its sharp at all; and allyl isothiocyanate is volatile enough that the sharp literally leaves the plate as it cools. The edges, built as they are on reactive, fragile, volatile chemistry, resist the clean number far more than the stable center does.

Third, **a ratio inherits the error of both its terms.** Because IFI-P = 1,000 × 2.1 µM ÷ EC_{50}(candidate), the relative uncertainty of a score is the two relative uncertainties added in quadrature:

$$CV(\text{IFI-P}) = \sqrt{\,CV(\text{reference})^2 + CV(\text{candidate})^2\,}$$

This is where the work we have put into reproducibility earns its keep. Our designed material holds a batch-to-batch coefficient of variation at or below 7%. Measure a designed pungent against a

designed reference and the score carries

```
CV = √(0.07² + 0.07²) = √0.0098 = 9.9%
```

A tight band — a number you can formulate against. But measure a *wild* sample, whose capsaicinoid-and-glucosinolate chemistry scatters at a coefficient of variation near 42% between plants, and the same ratio inherits

```
CV = √(0.07² + 0.42²) = √0.1813 = 42.6%
```

Nearly half. The wild edge does not merely sit lower on the ladder; its rung is blurred to the width of the ladder itself. This is the quiet argument running under the whole index — that measuring a flavor and being able to *reproduce* it are the same discipline, and that a number is only a form of respect for the reader if the plant behind it will say the same thing twice.

The edges are levers

The edges do what edges do: they show the axis is not an island. Pungency touches heat through piperine and through sanshool, touches cool through the numbing K2P channels, touches aroma through the herb phenols, touches kokumi through the sulfur of the alliums. It is a channel with a sharp center and soft, overlapping borders — and that structure is exactly what makes it designable, because every border is a handle and every handle moves a sensation someone is trying to hold still long enough to bottle. We walk these borders the way our family has always walked a garden row: slowly, reverently, counting as we go, because the plant has already done the hard chemistry and the least we owe it is to measure what it is saying. A whisper of thyme laid under a stew, the green bite of watercress, the clove's manufactured sharp, the husk's uncanny flutter — each is a different setting of the same channel, and each is a pleasure to be given to the body on purpose, with a full accounting of what it is and how loudly it speaks. The sharp does not end at a clean line. It shades into heat on one side, into aroma on another, into the electric tingle of sanshool on a third, and a flavor designer — a family of them, bent over the same bench — works the whole gradient at once. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



PART III OF V

Designing the Sharp

the model · the sulfur economy · holding the edge · the black-mustard line

Chapter 9 · The method, applied to sharpness — *the same pipeline, a different reaction.*

The platform that designs our peppers was never about peppers, and this is where that promise comes due for the sharp. The method is a pipeline — a protein language model to propose edits, a structure predictor to see them, a flux-balance model to confirm they will pay off inside a living cell, and CRISPR to write them — seven modules in a row, of which five transfer unchanged from the capsaicin work to the glucosinolate, allium, and ginger pathways. What changes is not the machinery. It is the *target*: not the acyltransferase that builds heat, but the enzymes that arm and aim pungency — and, underneath them, a receptor that does not merely feel its ligand but bonds to it.

The pipeline, named

We like to name the parts, because a method you cannot name you cannot trust. Module one is the **structure predictor**, which folds any sequenced enzyme and hands us a coordinate file we can look at. Module two is the **protein language model**, an ESM2-class network of 650 million parameters that has read enough of the protein universe to score, for any enzyme, every possible single-residue substitution by its predicted effect on catalytic efficiency and fold stability at once. Module three is the **flux-balance model**, a genome-scale reconstruction of the whole plant's metabolism that tells us whether a faster enzyme will actually pay out or merely move a jam one step downstream. Module four is **CRISPR**, which writes the survivor edits into the genome precisely and heritably. The remaining three — assembly, validation, and the batch-consistency controls that hold our output to a coefficient of variation at or under seven percent — are the quiet plumbing that turns a good edit into a reproducible cultivar.

Five of the seven are organism-agnostic. The structure predictor does not care whether it is folding a pepper acyltransferase or the alliinase of garlic; the language model scores a myrosinase the same way it scores anything else; the flux model simply wants a genome; CRISPR cuts any sequence; the consistency controls are chemistry, not botany. Only two modules are ever pepper-specific — the parts that know what capsaicin *is* — and on the pungent axis we unplug them and plug in the enzymes of the sharp. That is the whole trick, and it is why forty-two host species are already live while sixty-four more wait on the roadmap. We are not building a new machine for each plant. We are turning the same machine toward a new reaction.

The reference enzyme — why we calibrate the sharp on heat

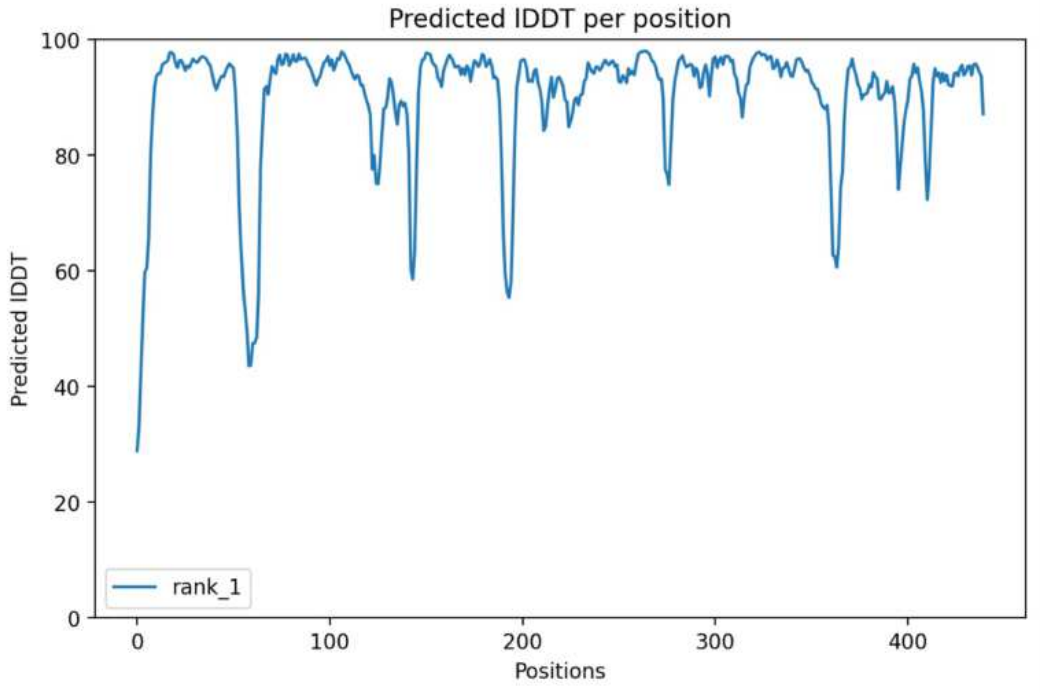


FIG. 9 — the platform's structure-prediction workflow, illustrated on Pun1/AT3, our reference enzyme: the model coloured by pLDDT confidence, the active-site cleft and binding domain resolved with high certainty.

Begin with the protein model, because the figure above is the one this whole library eventually leans on. It shows **Pun1/AT3** — the pepper's capsaicin-forming acyltransferase, the first enzyme our family ever learned to redesign — folded by the structure pipeline and coloured by **pLDDT**, the per-residue confidence score. pLDDT runs from 0 to 100: above 90 the model is as sure of a residue's position as a crystal structure would be; from 70 to 90 it is confident of the backbone; below 50 it is telling us, honestly, that it does not know. Warm colours here are certainty; cool colours are the model hedging. The binding domain and the active-site cleft resolve hot and sharp, and that is precisely what licenses an edit placed there — you do not rewrite a residue the model cannot locate.

We show Pun1 not because pungency runs through it. It does not; Pun1 builds heat. We show it because it is our **reference enzyme**, the one on which the entire workflow is calibrated, and the identical pLDDT-and-PAE procedure is what we then point at the enzymes that *do* build the sharp: the myrosinase that fires the mustard bomb, the MAM synthases that set side-chain length, the alliinase of garlic, the CYP79 and CYP83 oxidases of the glucosinolate core. Calibrating on heat is a discipline, not a detour — the heat work is where we measured the method against ground truth, so when we carry it to an enzyme we have never touched, we know the confidence scores mean what they say.

The double-variant design that calibrated it — the AI-designed **S39L+L345G** substitutions on Pun1 — is the exemplar every pungency construct is benchmarked against: a design targeting a batch-to-batch coefficient of variation at or under seven percent, against forty-two percent in wild type, substitutions no breeder had ever combined:

EDIT	EFFECT	ΔΔG (KCAL/MOL)	STATUS
S39L	binding-domain affinity ↑ (N-terminus)	−2.1	shipped
L345G	steric relief at the active-site cleft	−1.8	shipped
C175S	prevents a disulfide misfold	−0.9	structural annotation — not in shipped construct

Read the third column as folding free energy: negative is stabilizing, and the magnitude is how much. The language model proposed these out of an impossible search. For an enzyme of L residues there are $19L$ possible single substitutions — for a protein of a few hundred residues, already many thousands — and the double-mutant space is $19^2 \cdot L(L-1)/2$, which for the same enzyme is tens of millions. No one screens that in a greenhouse. The model ranks it in an afternoon, and only the top of the ranked sheet — each entry carrying a predicted ΔΔG and a predicted direction on activity — is ever grown.

Reading the coupling map

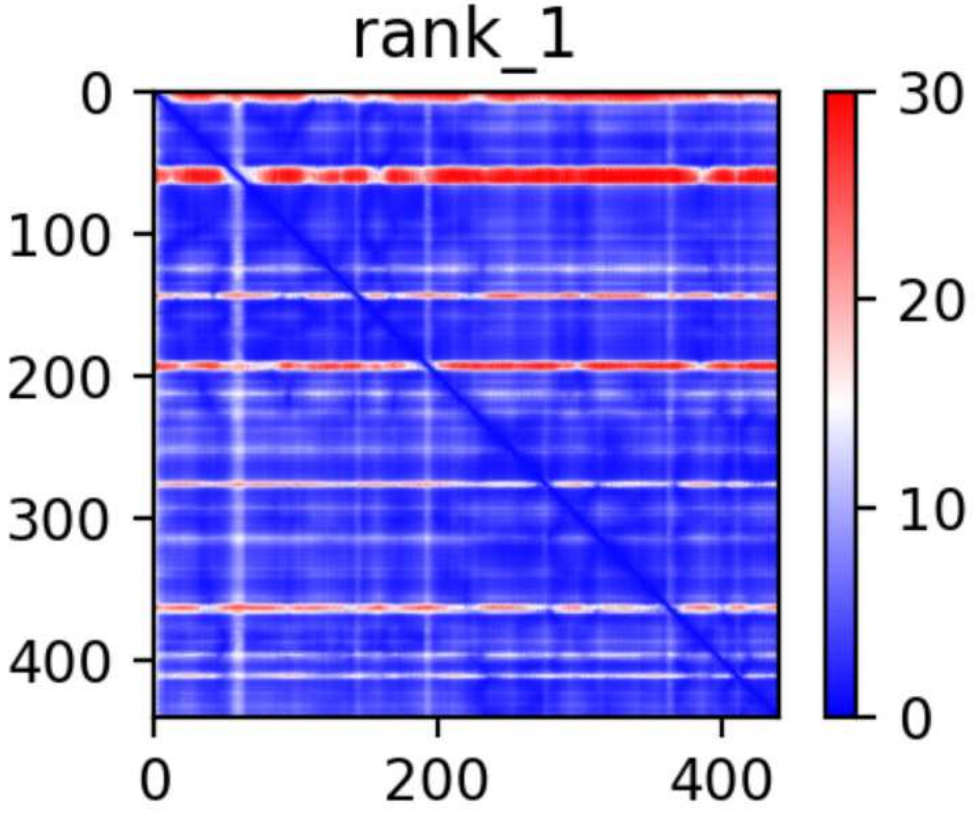


FIG. 10 — predicted aligned error for the same model: the off-diagonal confidence that tells us which domains move together, and therefore which edits are safe to combine.

The second figure answers a question the confidence plot cannot. pLDDT tells us *how sure we are of each residue*; the **predicted aligned error**, or PAE, tells us *how sure we are of each residue relative to every other*. Read it as a grid: the value at row *i*, column *j* is the expected error, in Ångström, in the position of residue *j* when the model is aligned on residue *i*. Dark blocks along the diagonal — low error, often under 5 Å — are rigid domains that move as a single

unit. Bright off-diagonal regions, where the error climbs toward 30 Å, are hinges and flexible linkers whose two sides the model cannot pin relative to each other.

For design this is the map of what can be combined. Two substitutions inside the same dark block interact predictably; their ΔΔG values are roughly additive, so our two shipped edits (S39L+L345G) summing to −3.9 kcal/mol mean what the arithmetic suggests because the PAE places them in coupled, rigid structure rather than across a hinge. Two edits that straddle a bright region can fight each other — stabilize one lobe and you may load the linker that carries the other. When we redesign a myrosinase to fire faster, to pull its activation time constant down and sharpen the attack, the PAE tells us which active-site residues we can move together as a set without the enzyme unfolding in protest. It is the difference between editing a sentence and editing a word at a time and hoping the sentence survives.

Bind, or bond — the chemistry that makes the sharp its own axis

Here is where pungency stops being a variation on heat and becomes its own reaction, and it is worth slowing down for, because the whole axis turns on it.

Capsaicin, the heat molecule, **binds**. It settles into a pocket on TRPV1 — a non-selective cation channel on the trigeminal endings of the tongue — held by hydrogen bonds and the loose embrace of van der Waals contact. The fit is specific and it is reversible: the molecule arrives, lowers the channel's thermal threshold so the body reports a fire that is not there, and then, when the concentration falls, it leaves, and the pocket is as it was. Nothing is consumed. Nothing is changed. It is a lock and a key, repeated a billion times a second, and every turn is undoable.

Pungency **bonds**. The sharp molecules — allicin from a crushed clove of garlic, allyl isothiocyanate from mustard and wasabi and horseradish, cinnamaldehyde from cinnamon bark — are electrophiles, and their receptor, **TRPA1**, is built to be attacked by them. TRPA1 is a homotetramer with a vast cytoplasmic skirt of ankyrin repeats, and on the inner face of each subunit sits a small cluster of reactive cysteine thiols — in human TRPA1 the residues **C621, C641, and C665**, with a lysine, **K710**, nearby. An electrophile does not nestle into a pocket there; it forms a covalent adduct, a true chemical bond, by Michael addition onto a cysteine sulfur. The bond changes the protein's charge and geometry at that site, the skirt tightens, and the channel opens. This is why the sharp feels like a *strike* where heat feels like a *glow*: one is an occupancy, the other is a modification.

Two consequences follow, and both matter for design. First, because each subunit offers several reactive sites and the channel is a tetramer, activation is cooperative — more than one bond stacks toward opening — which gives TRPA1 a steep dose–response and makes the sharp arrive suddenly rather than fading up. Second, a covalent adduct is not a tenant; it is a renovation, and it clears on the receptor's own schedule as the protein is repaired or turned over,

not the instant the food is swallowed. Ginger is the honest exception that proves the rule: **6-gingerol** and its drying-born cousin **6-shogaol** are only mildly electrophilic and lean partly on a reversible, vanilloid-like contact, which is why ginger's warmth lingers and spreads where wasabi's spikes and vanishes. When we engineer pungency, we are tuning how hard, how fast, and how many of these bonds form — a chemistry problem, not a shape-matching one.

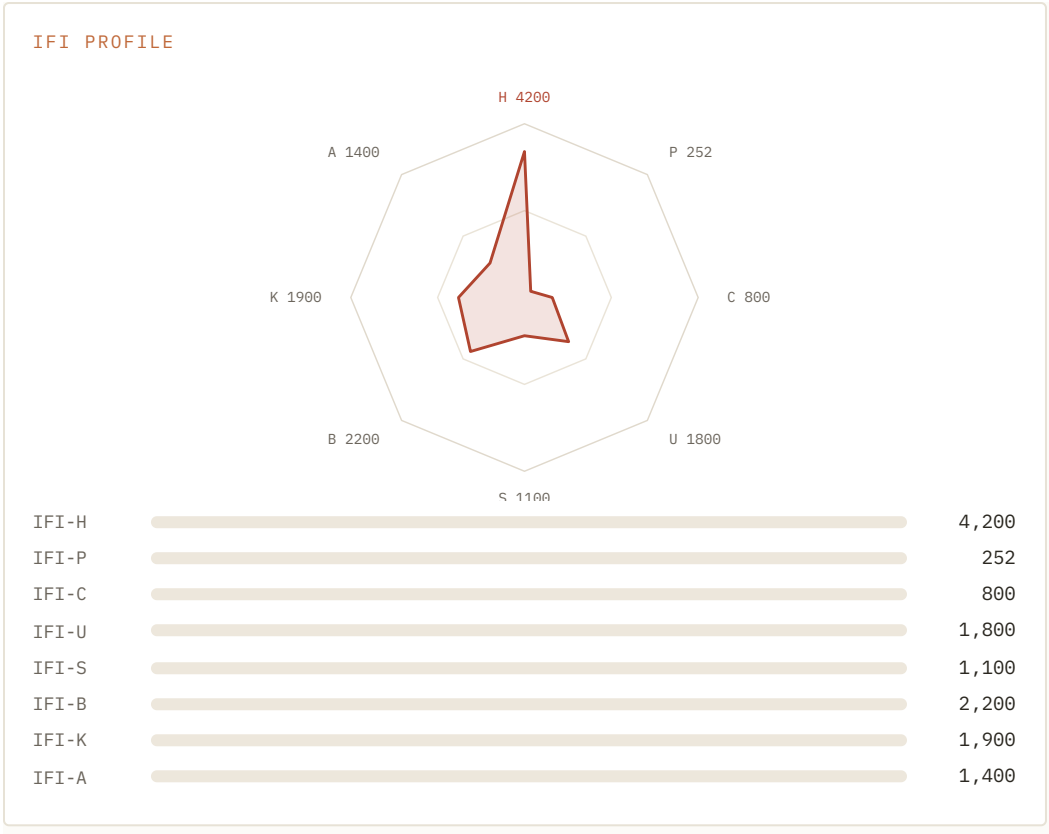
The arithmetic of loudness

A sensation you cannot number you cannot design, so the Index gives the sharp a number the same way it gives every axis one. The insight is that what matters is not how much of a compound a food holds but how *loudly* it speaks to its receptor, and loudness is the inverse of the half-activation concentration. The **EC_{50}** is the concentration that drives a receptor to half of its full response; a molecule with a low EC_{50} shouts, because a little of it already half-fills the channel, while a high-EC_{50} molecule must flood the tissue to be heard. Loudness, then, is proportional to $1/EC_{50}$. Normalize that reciprocal against a fixed reference and scale by a thousand, and you have the Index:

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

For the pungent axis we define the reference constant as **2.1 µM** — a stated convention, like Scoville's dilution definition, not a measured literature value — so that a compound with EC_{50} of 2.1 µM scores **IFI-P = 1,000** on this axis. A candidate twice as potent, with half the EC_{50}, scores 2,000; one tenth as potent scores 100. The per-axis ceiling we normalize scorecards against is 5,000.

Work one all the way through. Our full-spectrum flagship, Phoenix (SS-0096), our model predicts an **IFI-P of 252**. Invert the formula: $252 = 1{,}000 \times 2.1 \div EC_{50}$, so the predicted EC_{50}(Phoenix) ≈ **8.3 µM**. Phoenix is designed as a full-spectrum cultivar — its pungency is a deliberate moderate note, there to register and yield to the heat and savory axes that define the flagship's character. Garlic's allicin, measured at 4.2 µM on the same receptor, scores IFI-P = 500 on this axis; the designed pungency of Phoenix is purposeful restraint, leaving headroom for the other axes that give it its depth.



The ladder of pungent molecules, each scored against the 2.1 µM convention constant, reads like this — and the final column is the half-activation expressed as mass, through mg/L = µM × (molecular weight) ÷ 1,000:

COMPOUND (SOURCE)	HTRPA1 EC_{50}	IFI-P	EC_{50} (MG/L)
Allyl isothiocyanate (mustard/wasabi)	5.0 µM	420	0.50
Allicin (garlic)	4.2 µM	500	0.68
Cinnamaldehyde (cinnamon)	15 µM	140	1.98
6-Gingerol (ginger)	25 µM	84	7.36
Diallyl disulfide (garlic, aged)	50 µM	42	7.31

The EC_{50} column is representative of the receptor-pharmacology literature; the IFI-P column is the convention formula applied; the last column, computed with the true molecular weights (99.15 for the isothiocyanate, 162.27 for allicin, 132.16 for cinnamaldehyde, 294.39 for gingerol), is why the sharpest compounds are also the most frugal — allicin half-opens the channel at under a milligram per litre.

The shape of the rise matters as much as its height, and for that we use the Hill form of the dose–response. If *E*max is the full response and *n* the Hill coefficient, the fraction of maximum at concentration [*A*] is

$$\text{response} = [A]^n \div (EC_{50}^n + [A]^n)$$

At [*A*] = EC_{50} the two terms are equal and the response is exactly one half, which is the definition made algebra. The cooperative, multi-cysteine chemistry of TRPA1 gives $n > 1$ — take $n = 1.7$. At twice the convention reference, [*A*] = 4.2 µM with EC_{50} = 2.1 µM, the response is $4.2^{1.7} \div (2.1^{1.7} + 4.2^{1.7}) = 11.47 \div (3.53 + 11.47) =$ **0.765**. Doubling the dose from the half-point drives the channel to more than three-quarters open, not to two-thirds. That steepness is the

covalent mechanism showing up in the math: because the opener bonds at several sites and the sites cooperate, the sharp does not ease in — it crosses a threshold and arrives.

Error, and why ≤7% is the whole number

A number is only as good as its wobble, and the Index is a ratio of two measurements, so its wobble is governed by how ratios carry error. The coefficient of variation is the honest unit: CV = σ ÷ μ, the standard deviation over the mean, as a percentage. For a ratio of two independent quantities, the relative variances add in quadrature:

> $(\sigma_{IFI} \div IFI)^2 = (\sigma_{ref} \div EC_{50}ref)^2 + (\sigma_{cand} \div EC_{50}cand)^2$

Run our own number through it. Our constructs hold batch output to a CV at or under seven percent. If each EC_{50} in the ratio carries that seven percent, the Index inherits $\sqrt{(0.07^2 + 0.07^2)} = \sqrt{0.0098} =$ **0.099**, a hair under ten percent. So Phoenix's IFI-P = 252 is really 252 ± 25 — a band from roughly 227 to 277, tight enough to tell two cultivars apart and to promise a formulator the same sharp in April that they tasted in September.

Now run wild type through the same algebra. The unimproved plant varies forty-two percent batch to batch, and $\sqrt{(0.42^2 + 0.42^2)} = 0.42 \times \sqrt{2} =$ **0.594**. The same predicted score of 252 becomes 252 ± 150 — a band from 102 to 402, spanning nearly a factor of four. That is not a measurement; it is a rumor. This is the quiet reason the consistency controls are not plumbing but product: the covalent, saturating chemistry of TRPA1 gives us a clean plateau to measure against, and holding the plant's output to seven percent is what turns a loud reaction into a *readable* one. A sharp you cannot reproduce is a sharp you cannot design around.

Four levers on the pungent axis

With the method named and the number trustworthy, the engineering reduces to four levers, each a specific enzyme with a specific place in a flux network. In mustard and the other Brassicas the sharp is a two-part weapon the plant keeps disarmed: glucosinolates — sulfur-and-nitrogen compounds assembled on the **CYP79 → CYP83** oxidase core from chain-elongated amino acids — are stored in one compartment, and **myrosinase**, the enzyme that detonates them, is stored in another. Only when a jaw or a blade ruptures the tissue do the two meet, and myrosinase hydrolyzes the glucosinolate to an unstable aglycone that rearranges, in a fraction of a second, into the isothiocyanate that strikes TRPA1. We receive as pleasure what the plant evolved as defense, and there is a reverence in engineering it that we try never to lose: we are tuning a living thing's own chemistry, not bolting flavor on.

Lever one — commit the bomb. Left alone, a competing protein called **ESP**, the epithiospecifier protein, can divert the aglycone to nitriles, which are nearly silent on the sharp. Silencing ESP is a clean knockdown the language model scores at high confidence, and it forces the hydrolysis down the isothiocyanate route — maximum sharp from the same glucosinolate pool. **Lever two — set the character.** The **MAM** synthases control how many carbons the methionine side chain carries before it becomes a glucosinolate, and

side-chain length is identity: shift it and you move from one isothiocyanate to another, from the volatile top-note sharp of a short chain to the deeper, slower sharp of a long one. **Lever three — set the attack.** Myrosinase speed governs how fast the sharp blooms.

That third lever is worth the arithmetic, because "sharpness" is partly a *rate*. Model the burst of isothiocyanate after tissue damage as a first-order approach to completion, P(t) = P_max · (1 − e^(−t ÷ τ)), where the time constant τ is inversely proportional to enzyme activity — more myrosinase, or a faster one, means a smaller τ and a steeper onset. Suppose wild-type τ = 2.0 s. At half a second the plant has delivered 1 − e^(−0.5 ÷ 2.0) = 1 − e^(−0.25) = **0.221**, twenty-two percent of its sharp. An edit that raises turnover two-and-a-half-fold pulls τ to 0.8 s, and at the same half-second the plant delivers 1 − e^(−0.5 ÷ 0.8) = 1 − e^(−0.625) = **0.465** — forty-seven percent, more than double, in the same instant. The total sharp is unchanged; the *attack* is twice as sharp. That is a knife against a press.



FIG. 11 — garlic in cross-section: the sharp here is held in reserve too, alliin and alliinase kept apart until the clove is cut, then meeting to make allicin in seconds.

Lever four — protect the yield. Garlic runs the same disarmed-weapon logic with different parts: **alliin**, a cysteine-derived sulfoxide, waits until a cut lets **alliinase** convert it to allicin, the electrophile that scored 500 on the ladder above. Here the flux model earns its keep. It is pointless to double alliinase if the alliin pool runs dry, or to pour precursor into a plant whose sulfur supply is already maxed — and because allicin is built from a cysteine skeleton, the budget that limits it is the same sulfate-and-cysteine economy that limits the glucosinolates. The flux-balance reconstruction tells us, before we grow a single plant, whether a proposed edit pays out in the living cell or merely relocates the bottleneck. Only the edits that clear all three filters — the language model's confidence, the structure's tolerance, the flux model's endorsement — are ever written with

CRISPR. Ginger sits slightly apart from this sulfur world: its **6-gingerol** is assembled on the phenylpropanoid branch (PAL → C4H → 4CL → HCT → COMT) that runs in every vascular plant, which is exactly why the method reaches it as readily as it reaches the Brassicas.

The pungent ladder

The axis is wider than mustard, and the Index lets every pungent host be read on one page. These are the species live today, each with the compound that carries its sharp and the enzyme we reach for first:

HOST · CONSTRUCTS	PRIMARY AXES · DIFFICULTY	PRINCIPAL COMPOUND	FIRST-LEVER ENZYME
Black Mustard · 2,790	P/M · Hard	allyl isothiocyanate (from sinigrin)	myrosinase · MAM · ESP
Garlic · 560	K/P · Moderate	allicin (from alliin)	alliinase
White Mustard · 200	P · Hard	*p*-hydroxybenzyl isothiocyanate (sinalbin)	myrosinase
Ginger · 120	P · Moderate	6-gingerol / 6-shogaol	phenylpropanoid branch
Watercress · 120	P · Hard	phenethyl isothiocyanate (gluconasturtiin)	myrosinase
Thyme · 120	P · Hard	thymol	monoterpene route
Cinnamon · 40	P · Hard	cinnamaldehyde	phenylpropanoid branch
Oregano · 40	P · Hard	carvacrol	monoterpene route

Read the ladder and the strategy reads with it. Black Mustard carries the most engineering — 2,790 constructs — because it is where all four levers converge: a glucosinolate core to fill, a MAM dial for character, an ESP to silence, and a myrosinase to quicken. Garlic is the kokumi–pungency crossover, the savory foundation of nearly every cuisine our family has cooked. The two phenylpropanoid sharps, ginger and cinnamon, are the bridge back to the branch that every plant shares. None of them is a special case. Each is the same four-step method pointed at a reaction that happens to bond rather than bind.

Failure modes and honest limits

Rigor includes saying where the method strains, and the sharp strains in ways heat does not. The first ceiling is chemical honesty: the TRPA1 openers are *reactive by design*, and a molecule that bonds to a receptor's cysteines will, in excess, bond to other things — the same electrophilicity that reads as a thrilling sharp reads, past a point, as cytotoxic sting and a creeping bitterness as the compound spills onto other receptors. There is a loudness above which the axis stops being a pleasure, and we treat the IFI-P ceiling as a reason to stop, not a target to beat. The second limit is the budget. Glucosinolates and allicin are both built from sulfur, and a plant has only so much sulfate and so much free cysteine; the flux model will veto an elegant myrosinase edit whose substrate the plant cannot afford to keep making, and no amount of enzyme speed survives an empty pool.

The third is biology's own caution. Silencing ESP is rarely total — a residual nitrile fraction remains, and we report the sharp we can deliver, not the sharp in principle. The compartmentation that stores enzyme and substrate apart is a feature we must respect rather than defeat: the sharp exists only on damage, which is why these flavors live in the cut, the crush, the grate, and why a measurement must specify the moment after rupture or it measures nothing. And the covalent mechanism gives the sharp a desensitization unlike heat's — the bond clears on the protein's repair schedule, so a second bite does not always land like the first, and our dose–response curves describe the strike, not the fade that follows. Where a value is representative rather than a locked per-cultivar prediction — the EC_{50} convention among them — we say so, because a number offered as more certain than it is would betray the reader we most want to keep.

What stays constant

Strip the chapter to its spine and this is what remains: the method is the constant, and the enzyme in the chair changes. The same structure predictor that folds our reference acyltransferase folds a myrosinase; the same 650-million-parameter model that found two heat edits no breeder ever combined ranks the substitutions that quicken an alliinase; the same flux reconstruction that confirmed the capsaicin bottleneck confirms the sulfur budget of a clove; the same CRISPR writes them all, to the same seven-percent consistency. We built the platform once, calibrated it on heat because heat was where we could check our work against the truth, and then turned it toward a receptor that does not feel its flavor so much as it is changed by it. The sharp is not a harder problem. It is the same reverent arithmetic — grow the plant, read the receptor, show the number, keep the seed of the one that moved us — pointed at a molecule that arrives by forming a bond, and leaves only when the living thing it touched has made itself new. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 10 · The sulfur economy — *why the sharp is metabolically expensive.*

Here is a fact that governs the pungent axis and no other: **the sharp is made of sulfur, and sulfur is costly.** The loudest voices on this axis — the isothiocyanates of mustard and wasabi and horseradish, the thiosulfinates of garlic and onion — are every one of them sulfur compounds, and a plant that wants to be truly pungent must divert a great deal of one of its scarcest elements into molecules it will only ever use as a weapon. That is the first half of the economy. The second half is quieter and, to us, more beautiful: the receptor that reads the sharp reads it with sulfur too. The channel at the end of the trigeminal nerve does not recognize these molecules by their shape, the way a lock recognizes a key. It recognizes them by *reaction* — the plant's sulfur reaching out and bonding, covalently, to the body's own. The pungent axis is a conversation held in a single element, from the soil the plant stands in to the thiol on the nerve, and so it is the one axis of the eight where the chemistry of making the flavor and the chemistry of tasting it are, at bottom, the same chemistry. We design for both ends at once because nature wrote them in the same ink.

The receptor reads with its own sulfur

The pungent channel is TRPA1 — a large, non-selective cation channel standing in the membrane of the trigeminal and vagal sensory neurons that web the mouth and nose. It is often called the wasabi receptor, and the name is earned. Where the heat channel is gated by a molecule settling reversibly into a pocket, held by the soft grip of hydrogen bonds and let go again, TRPA1 is gated by something far more committal. Its long cytoplasmic N-terminus carries a cluster of reactive cysteine residues — in the human channel, the thiols at Cys621, Cys641, and Cys665, with a reactive lysine nearby — and the agonists that open it are **electrophiles**: molecules hungry for electrons, that find the sulfur of a cysteine thiolate and *bond to it*. Allyl isothiocyanate, the volatile sharp of mustard, presents a carbon flanked by nitrogen and sulfur (the N=C=S group) that is starved enough to accept the thiolate's lone pair; the thiol adds across it and a covalent thiocarbamoyl adduct forms. Allicin, the thiosulfinate of crushed garlic, carries a strained sulfur–sulfur bond that trades partners with the cysteine in a thiol–disulfide exchange, leaving the channel's own cysteine dressed in a new sulfur. In both cases the plant's sulfur has reached into the protein and changed it. The change pulls on the channel's gate; the gate opens; sodium and calcium pour in; the nerve fires the sensation we call pungent — the clean, rising, sinus-clearing sharp that is wholly distinct from the glowing heat of capsaicin, because it is a different channel, read a different way.

This is why the sharp arrives the way it does, and why it leaves the way it does. A reversible agonist washes off as its concentration falls; a covalent one does not simply wash off, and the channel must be repaired or reduced back to baseline before it is quiet again. The sharp of wasabi climbs fast and high and then recedes over seconds to a minute — the kinetics of a bond made and slowly broken — where capsaicin's warmth lingers and deepens on a longer clock entirely. Two axes, two channels, two chemistries of binding. We measure them on one index precisely so that these unlike sensations can be set side by side and read.



Black mustard in cross-section — sinigrin and its myrosinase held in separate cells, the loaded weapon waiting to be fired.

Follow the sulfur — from sulfate in the soil to the loaded weapon

To understand why pungency is expensive, follow a single sulfur atom from the ground into the molecule that will one day sting. A plant draws sulfur from the soil as **sulfate** (SO_4^{2-}), a stable, sluggish, fully oxidized ion that can do nothing until it is activated. Activation costs ATP at the very first step: the enzyme ATP sulfurylase caps the sulfate to **APS** (adenosine-5′-phosphosulfate), and from APS the path forks, and the fork is where the whole economy is decided.

Down one arm, APS is *reduced* — APS reductase hands it to sulfite (SO_3^{2-}), sulfite reductase drives it down to sulfide (S^{2-}), and O-acetylserine(thiol)lyase welds that sulfide onto the carbon skeleton of O-acetylserine to make **cysteine**, the gateway sulfur amino acid, the molecule from which essentially all of a plant's organic sulfur descends. Down the other arm, APS is *phosphorylated* again by APS kinase to **PAPS** (3′-phosphoadenosine-5′-phosphosulfate), the plant's universal activated-sulfate donor — the reagent used whenever a sulfate group must be handed to something else.

Now count what a single molecule of sinigrin, the signature glucosinolate of black mustard, actually costs. Sinigrin is an allyl chain attached to a carbon that bears both a thioglucoside sulfur and, through an oxime nitrogen, a sulfated group: one sulfur in the thioglucoside linkage, one more in the sulfate cap that PAPS donates. Two sulfurs. Its carbon-and-nitrogen core is borrowed from **methionine** — itself a cysteine descendant, elongated and elaborated — which means the plant has also spent an amino acid it could have put into protein. A glucosinolate is, in the plainest accounting, a small fortune: rich in sulfur, rich in nitrogen, assembled

through a dedicated run of cytochrome-P450 and transferase enzymes (the oxime-forming CYP79, the CYP83 that follows, a C–S lyase, the S-glucosyltransferase of the UGT74 family, and finally a sulfotransferase that spends PAPS to set the sulfate cap). The mustard bomb is a plant choosing to bank a great deal of its scarcest element in a device it hopes never to detonate.

The alliums spend differently but no less dearly. Garlic routes cysteine into **S-allyl-L-cysteine sulfoxide** — alliin — storing its weapon as a stable, odorless, sulfur-rich amino-acid derivative. Onion makes the closely related isoalliin. These are cysteine's children directly; where the brassica detours through methionine and a sulfated glucoside, the allium keeps its investment nearer the gateway, as a cysteine sulfoxide waiting for its own enzyme. Either way the ledger reads the same in the end: to be loud on this axis is to have spent sulfur, and to have spent it on something structurally useless to the plant until the moment of injury.

MOLECULE	PLANT	SULFUR ATOMS	WHAT BECOMES OF THE SULFUR WHEN IT FIRES
Sinigrin (stored)	black mustard	2	sulfate cap is shed; the thioglucoside S becomes the isothiocyanate's S
Allyl isothiocyanate (fired)	black mustard	1	the single loud sulfur that reaches the tongue
Glucoraphanin (stored)	broccoli	3	two sulfurs ride on into sulforaphane; the sulfate is shed
Alliin (stored, per monomer)	garlic	1	two monomers are condensed to make one thiosulfinate
Allicin (fired)	garlic	2	both sulfurs sit in the thiosulfinate bond that opens the channel

The ledger carries one more lesson if you read it by mass. Sinigrin, as its common potassium salt, weighs about 415 g/mol; the allyl isothiocyanate it can release weighs 99.15 g/mol. One mole of the stored glucosinolate yields at most one mole of the fired isothiocyanate, so **at most 99.15 ÷ 415 ≈ 24% of the stored mass ever leaves as the volatile sharp.** The plant banks four units of expensive, sulfur-laden storage for every one unit of weapon it can throw. Pungency is not only costly to make; it is costly to *carry*, and the carrying is most of the cost. A design that raises stored glucosinolate without raising the plant's capacity to supply sulfur is a design that makes the plant hoard a fortune it cannot afford.

The mustard bomb — myrosinase, the Lossen rearrangement, and the free lever

None of this stored chemistry is sharp. Intact sinigrin is nearly tasteless; intact alliin is odorless. The sharp exists only for an instant, and only because the plant keeps the weapon and its trigger in separate rooms. In a brassica, glucosinolates are stored in one set of cells and the enzyme that activates them — **myrosinase**, a thioglucohydrolase — is sequestered in another, in specialized myrosin cells. Nothing happens until something bites, tears, or crushes the tissue and breaks the walls between them. Then the two mix, and the bomb goes off.

Myrosinase hydrolyzes the thioglucoside bond, cleaving glucose away and leaving an unstable aglycone — a thiohydroximate-*O*-sulfonate — that cannot persist. It falls apart by a **Lossen rearrangement**: the sulfate departs as free sulfate, the group migrates, and out comes the **isothiocyanate** with its N=C=S trigger intact and ready to find a cysteine on TRPA1. From sinigrin, allyl isothiocyanate; from the broccoli glucosinolate glucoraphanin, sulforaphane; from the watercress glucosinolate gluconasturtiin, phenethyl isothiocyanate. This is the mustard bomb, and it is the reason a radish is mild on the shelf and vivid the instant you chew it.

But the Lossen rearrangement is not the only fate of that aglycone, and the alternative is where the most elegant lever on this whole axis lives. If a small accessory protein called **ESP** — the epithiospecifier protein — is present at the moment of hydrolysis, it redirects the breakdown away from the isothiocyanate and toward a **nitrile** or epithionitrile instead. Nitriles are poor TRPA1 agonists; they do not carry the hot, reactive N=C=S that the channel reads. A plant can be fat with glucosinolate and still taste flat, because ESP is quietly disarming the bomb at the instant of firing, spending the stored sulfur on a product that never reaches the nerve.

Silence ESP, and the same hydrolysis of the same glucosinolate pool now runs to completion as isothiocyanate. **The pungency rises with no new sulfur spent at all** — not one additional atom pulled from the soil, not one more pass through ATP sulfurylase. It is loudness harvested from a diversion that was already being paid for, the single most efficient edit on the axis: we did not buy more weapon, we simply stopped the plant from defusing the weapon it had. Every other lever here costs sulfur somewhere. This one is nearly free, and that makes it the first move in almost every pungent design we build.

The alliums run the same two-room trick with different furniture. Garlic stores alliin in the cell's fluid and keeps its activating enzyme, **alliinase**, apart; crush the clove and alliinase converts alliin into reactive allyl sulfenic acids, two of which condense into **allicin**, the thiosulfinate that opens the channel. Onion adds one twist — a lachrymatory-factor synthase that siphons part of the reactive pool into the volatile tear compound — which is itself an ESP-like fork, a place where stored sulfur is spent on a product other than the one that stings. Wherever we find such a fork, we find a lever: redirect the fork, and the perceived sharp changes without the sulfur budget changing at all.

The index on this axis — EC50, the formula, and worked numbers

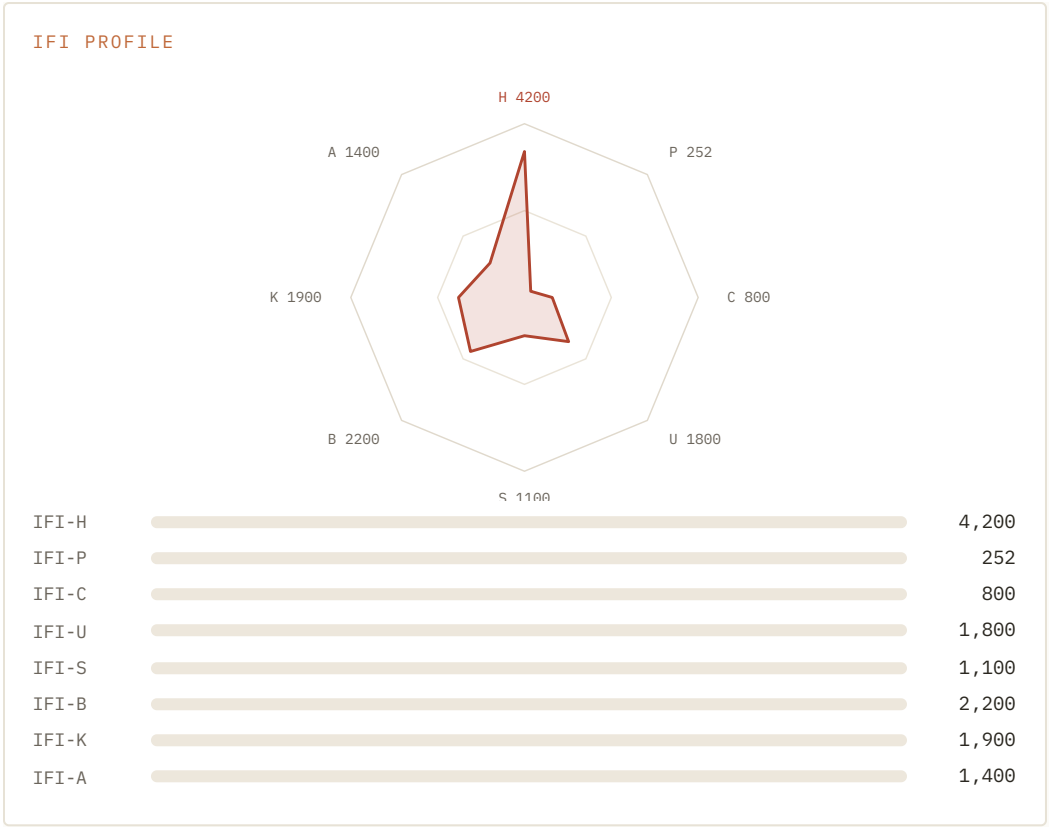
To put a number on loudness we use the same instrument as every axis in this library. The Iyengar Flavor Index scores a stimulus not by how much of it a food contains but by how *loudly* it speaks to its receptor, and loudness is read from the half-maximal effective concentration — the **EC_{50}**, the concentration at which the stimulus drives its channel to half of full response. A lower EC_{50} means a louder molecule: less of it is needed to reach the half-way point. We anchor the pungent axis by convention: IFI-P = 1,000 is defined at the response of TRPA1 to allyl isothiocyanate (AITC) at our stated reference concentration of **2.1 µM** — a convention constant set like Scoville's own dilution-to-threshold definition, not extracted from a single published EC_{50} report. Every candidate is then scored against that fixed anchor by one formula:

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

Read it slowly and it inverts cleanly. Because the candidate's EC_{50} sits in the denominator, a candidate that is *louder* than the reference — one that activates the channel at a lower concentration — scores *above* 1,000, and a quieter one scores below. Rearranged, the formula tells you exactly what a score means as a concentration:

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

So a worked example. Our full-spectrum flagship, Phoenix (SS-0096), is designed to a predicted pungency of **P = 252** on this axis — a computed score placing it at roughly one-quarter of the AITC reference loudness, the sharp distributed as one color in an eight-axis spectrum rather than maximized on a single channel. Substitute it in: $EC_{50}(\text{candidate}) \div EC_{50}(\text{reference}) = 1{,}000 \div 252 \approx$ **3.97**. Phoenix's pungent principle reaches half-activation at about 397% of the reference concentration — quieter than pure AITC on this axis, by design. The fold ratio is the score divided by a thousand: **0.252×**. The arithmetic is deliberately this transparent; a number is a form of respect for the reader, and this one hides nothing.



The table below walks the formula across its working range, so the score and the concentration it stands for can be read together. Note the two fixed landmarks: 1,000 is the reference itself (EC_{50} ratio exactly 1), and 5,000 is the per-axis normalization ceiling we use on every scorecard, the loudest a single axis is allowed to plot.

IFI-P	EC_{50}(CAND) ÷ EC_{50}(REF) = 1000 ÷ IFI-P	FOLD LOUDER THAN REFERENCE = IFI-P ÷ 1000
100	10.00	0.10×
252	3.97	0.252× (Phoenix's predicted pungency)
500	2.00	0.50×
1,000	1.00	1.00× (the reference)
2,000	0.500	2.00×
5,000	0.200	5.00× (scorecard ceiling)

The EC_{50} itself is a concentration, and concentrations convert like any other, which matters because the pungent compounds are small and volatile and are weighed in the lab two different ways. Take allyl isothiocyanate, molar mass 99.15 g/mol. A worked solution of 50 µM in water is 50×10^{-6} mol/L × 99.15 g/mol = 4.96×10^{-3} g/L = **4.96 mg/L**, which in dilute water is about **5 parts per million** by mass. Allicin (162.27 g/mol) and sulforaphane (177.29 g/mol) are heavier, so the same molar concentration of either weighs correspondingly more — a reminder that two compounds at identical EC_{50} in molar terms, and therefore identical IFI-P, can sit at quite different mass concentrations in the food. The index scores the *molecule's* loudness; the formulator still has to weigh it out.

One honest note belongs with the formula, because the covalent handshake changes what EC_{50} even means. For a reversible agonist, EC_{50} is an equilibrium — hold the concentration steady and the response settles to a fixed fraction. For a covalent agonist like an isothiocyanate, the channel keeps accumulating modification over

time, so the computed EC_{50} is an *operational* value, read at a fixed assay time rather than at true equilibrium. We therefore fix the exposure window and report it with the number, and we read the dose–response through a Hill relation,

$$\text{response} \div \text{maximum} = [A]^h \div (EC_{50}{}^h + [A]^h),$$

where at [A] = EC_{50} the response is exactly one half, and the Hill coefficient *h* sets the steepness of the climb. With a cooperative h ≈ 1.5, doubling the concentration to [A] = 2·EC_{50} gives 2^1.5 ÷ (1 + 2^1.5) = 2.83 ÷ 3.83 ≈ **74%** of maximum, while halving it to 0.5·EC_{50} gives 0.354 ÷ 1.354 ≈ **26%**. The curve is steep: a factor of four in concentration spans most of the channel's dynamic range. That steepness is a gift to the designer — small, well-aimed gains in released isothiocyanate translate into large gains in perceived sharp — and a trap, because it means the same small error in either direction reads loudly on the tongue.

The budget, made quantitative

The sulfur economy becomes a design problem the moment you try to raise a plant's P number, because every pathway that could make it louder draws on pools the plant needs to stay alive. The naïve lever — overexpress the core glucosinolate machinery and hope — fails in a way the flux-balance model predicts precisely, and the prediction is almost always the same: the ceiling is not the downstream enzymes at all. It is **sulfate assimilation**, the ATP-driven run from sulfate through APS to cysteine. Enlarge the demand for glucosinolate without enlarging that supply and the plant simply starves — pulling cysteine and PAPS away from protein and primary growth, going stunted, pale, and thin in everything else to feed a weapon. The flux model is not an optional nicety on this axis. It is the thing that keeps a pungent design alive.

DEMAND	DRAWS ON	COMPETES WITH
Glucosinolate synthesis	cysteine, methionine, PAPS-borne sulfate	protein synthesis, primary growth
Myrosinase and accessory proteins	cysteine (as protein sulfur)	the glucosinolate pool itself
Allium thiosulfinates	cysteine-derived sulfoxides	glucosinolate supply, in a shared host
General growth and maintenance	nitrogen, carbon skeletons, ATP	every row above

Seen through the model, the designs that *work* are the ones that spend the budget more cleverly rather than simply enlarging it — and they fall into three honest kinds. The first is to **silence the fork**: cut ESP so the stored pool runs to isothiocyanate instead of nitrile, buying loudness for no new sulfur, the free lever and always the first one we reach for. The second is to **raise the supply to meet the demand**: lift sulfate uptake and the APS node *in concert with* any increase downstream, so that a plant asked to bank more glucosinolate is also given more sulfur to bank, and is not quietly starved into a fortune it cannot carry. The third is to **extract more sharp from the same pool** by tuning compartmentation and myrosinase kinetics — getting more perceived loudness out of the firing rather than the loading, more of the stored mass converted to

the 24% that actually leaves as the volatile weapon. Across all three, the flux model is what tells us the mix: how far the pool can be pushed before growth collapses, where to add supply and where merely to redirect, which edits pay for themselves and which only move the shortage from one pool to the next.



The research plot where the sulfur budget is spent — brassicas grown to the edge of what their metabolism will bear.

Error, consistency, and what a number actually costs

A score is only worth the error bars around it, and on this axis the error bars are where engineered and wild material part most sharply. An IFI-P score is a ratio of two EC_{50} values, and errors propagate through a ratio by adding in quadrature: the coefficient of variation of the quotient is, to good approximation, the root of the sum of the squared input CVs.

$$CV(\text{IFI-P}) \approx \sqrt{\left[\, CV(EC_{50}\ \text{reference})^2 + CV(EC_{50}\ \text{candidate})^2 \,\right]}$$

Put the numbers in. Our engineered material holds a batch-to-batch CV of **≤7%**, where unselected wild-type stock runs near **42%** — a plant left to its own variation is wildly inconsistent in how much weapon it loads from one harvest to the next. Score an engineered candidate against an engineered reference and the index inherits $\sqrt{(0.07^2 + 0.07^2)}$ = **9.9%**: tight enough that a one-point ladder means something. Score a wild candidate against the engineered reference and it balloons to $\sqrt{(0.42^2 + 0.07^2)}$ = **42.6%** — the candidate's own scatter swamps everything. Score wild against wild, the way nature hands it to you, and $\sqrt{(0.42^2 + 0.42^2)}$ = **59.4%**: a number so loose it can barely order two samples with confidence. This is the real argument for designed consistency, and it is quantitative rather than aesthetic. A pungency you cannot reproduce is a pungency you cannot formulate around, cannot ladder, cannot sell as a specification. The CV is not a footnote to the score; on this axis, with its steep Hill curve and its covalent, time-sensitive EC_{50}, the CV is half the product.

Edge cases, failure modes, and the honest limits

Three honesties keep this chapter from overreaching. The first is that **not every pungent molecule is a sulfur molecule.** The *loudest* voices are — the isothiocyanates and thiosulfinates that top the ladder and command the heaviest engineering investment — but TRPA1 reads any sufficient electrophile, and several of them carry no

sulfur at all. Cinnamaldehyde (132.16 g/mol), the sharp of cinnamon, is an α,β-unsaturated aldehyde that opens the channel by the same covalent addition to cysteine, with not one sulfur atom in it. The gingerols and shogaols of ginger, the thymol of thyme, the carvacrol of oregano — all reach TRPA1 by non-sulfur electrophilic or vanilloid routes. These compounds escape the sulfur budget entirely, which is a genuine design advantage; but the host table below shows why they do not displace the sulfur route at the top of the axis. The organisms whose pungency is sulfur — black mustard with its **2,790** constructs, garlic with **560**, white mustard with **200**, watercress with **120** — carry the deepest engineering because their chemistry reaches the highest, loudest, cleanest sharp. The non-sulfur hosts sit lower: cinnamon and oregano at **40** constructs each, ginger and thyme at **120**. The sulfur economy governs the summit of this axis; the non-sulfur routes are the gentler foothills, and knowing which you are climbing decides which budget you are spending.

HOST	CONSTRUCTS	AXES	PUNGENT PRINCIPLE	SULFUR ROUTE?
Black Mustard	2,790	P / M	allyl isothiocyanate (from sinigrin)	yes
Garlic	560	K / P	allicin (from alliin)	yes
White Mustard	200	P	*p*-hydroxybenzyl isothiocyanate (from sinalbin)	yes
Ginger	120	P	gingerol / shogaol	no
Thyme	120	P	thymol	no
Watercress	120	P	phenethyl isothiocyanate (from gluconasturtiin)	yes
Cinnamon	40	P	cinnamaldehyde	no
Oregano	40	P	carvacrol	no

The second honesty is that **the loud compound is fragile.** Allyl isothiocyanate is volatile and reactive by the very properties that let it sting; allicin is famously unstable, decaying within hours of its own creation into a cascade of further sulfur compounds. The stored glucosinolate is stable for the life of the tissue, but the fired isothiocyanate begins to leave the moment it forms. Perceived pungency is therefore never a simple read-out of stored glucosinolate — it is a race between release and loss, and a plant engineered to a gorgeous glucosinolate titer can still disappoint on the tongue if its myrosinase fires slowly, its compartments leak, or its product flashes off before it is tasted. We score the released molecule under fixed conditions for exactly this reason, and we treat shelf-stability of the stored precursor as a separate axis of the design from the loudness of the fired weapon.

The third honesty is that **the covalent mechanism bounds the measurement itself.** Because activation accumulates with exposure rather than settling to equilibrium, the EC_{50} we report is operational, pinned to an assay window, and two labs that fix different windows will read different absolute numbers from the same molecule. The ratio to a reference run in the same window is far more robust than either EC_{50} alone — another reason the index is built on a ratio and anchored, rather than on raw concentrations. And the failure modes on the growth side are real and must be respected: push glucosinolate past what sulfate assimilation can feed and the plant stunts; silence a product fork too completely and you may remove a diversion the plant was using for its own ecological reasons; lean on field-grown material and the soil's own sulfate becomes a ceiling no edit can lift. None of these is a reason not to design the sharp. Each is a reason to design it with the whole budget in view.

What the sulfur was for

There is a quieter point under all of this, and it is the through-line of this whole library stated in its most honest, least preachy form. The sulfur compounds a brassica makes are not only its weapon and our flavor. They are a real and intricate part of the plant's own chemistry — the same glucosinolate family that gives broccoli its **sulforaphane**, built from glucoraphanin by the identical mustard-bomb machinery that turns sinigrin into the sharp of mustard: the same myrosinase, the same Lossen rearrangement, the same stored-apart-until-injury design. We make no claim here for what those molecules do in a body; that is not ours to promise, and this library has never traded in health halos. But we hold to a simpler intention, and it is one our family keeps at its own table: that a plant grown to carry the *fullest* measure of its own chemistry — its sulfur, its glucosinolates, its whole designed complement, not stripped and not shortcut — is a plant delivering everything it was built to deliver, the flavor and the substance together, because in a brassica they are quite literally the same atoms.

The sulfur economy is where that intention becomes quantitative. To grow a truly pungent brassica is to grow one that has invested heavily in its own sulfur chemistry; to do it without starving the plant is to keep that investment whole and standing in the leaf; and to read it on the index is to see, in a single number, how much of itself the plant has managed to become. We design the sharp and the substance with one model, follow one element from the soil to the nerve, and spend it, we hope, well. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



Broccoli in cross-section — the same machinery that arms the mustard bomb builds this plant's sulforaphane, the sharp and the substance written in one chemistry.

Chapter 11 · Holding the edge — *why the sharp is the hardest to keep.*

A flavor you cannot reproduce is a demonstration, not a product. The whole discipline of this company is the ≤7% batch-to-batch coefficient of variation — the promise that the thing we designed arrives the same every time — and nowhere is that promise harder to keep than on the pungent axis. Crack a mustard seed between your teeth and the sharp finds your nose before it finds your tongue; it climbs, it stings, and within a minute it begins to fade. Nothing about that experience was sitting ready inside the seed. It was *manufactured*, at the instant of damage, by a reaction we set off with our own molars — and a flavor that is made fresh each time it is tasted is a flavor that can be made wrong each time it is tasted.

Heat is forgiving by comparison. Capsaicin is a stable molecule, synthesized and warehoused in the placental tissue of the fruit weeks before harvest, sitting quietly in its reservoir until you eat it; its loudness is largely finished at the moment the pepper is picked. You can grind a pepper, leave it on the bench, come back in an hour, and the heat is the heat. Pungency does not grant that mercy. It is a reaction, built on precursors that have to be in the right amount *and* kept apart *and* fired at the right speed, releasing molecules so chemically eager that many of them begin decomposing the instant they exist. Holding a sharp steady is an exercise in controlling a controlled explosion — and this chapter is about how we earn that control, enzyme by enzyme and variance by variance, until the reaction obeys a tolerance.

The sharp is a reservoir's opposite

It helps to say plainly what kind of thing each axis measures. Heat is a *reservoir* axis. We quantify it by the mass of capsaicinoid the fruit has banked — the Scoville number is, at root, a concentration of stored molecule, read today by high-performance liquid chromatography rather than by a panel of tasters, so that our model predicts approximately 2.95 million SHU for Phoenix as a computed value from its designed tissue composition. The molecule is already made; reading it does not change it.

Pungency is the opposite. It is a *flux* axis. There is almost no free sharp in an intact mustard leaf or an unbruised clove of garlic — walk through a mustard field at dawn and it smells green, not hot. The pungent compound does not exist in quantity until the cell wall is broken and two things that were kept carefully apart are allowed to meet. What we are really measuring, then, is not a stored amount but a *rate and a yield*: how much sharp the tissue can manufacture per unit of damage, how fast, and for how long before it decays. This single structural fact — reservoir versus reaction — is the origin of every difficulty in this chapter. A reservoir has one number. A reaction has a whole chain of numbers, and every link in the chain is a place the sharp can drift. *When* you measure becomes part of

what you measure: a condiment's pungency at the moment of grinding is simply not its pungency an hour later, and any honest number has to name the clock it was read against.

Inside the pungent reaction, step by step

The archetype is the glucosinolate–myrosinase system of the Brassicas, and black mustard is its loudest member. In the living leaf the sharp exists only as potential, stored as two reagents in separate rooms of the same cell. In one room, held in the vacuole, sits the precursor: sinigrin, an allyl glucosinolate — a sulfur-rich sugar conjugate that is itself bland and stable. In another room, inside specialized myrosin cells, sits the enzyme: myrosinase, a thioglucoside glucohydrolase. Intact, they never touch. This physical separation is the pin in the grenade.



Garlic in cross-section — the compartmented tissue that keeps a pungent reaction's two reagents apart until the moment of damage.

Damage pulls the pin. When teeth or a grinding stone rupture the cell walls, the compartments spill together and myrosinase finds its substrate in milliseconds. It hydrolyzes the thioglucoside bond, cleaving off the glucose and leaving an unstable aglycone — a thiohydroximate-O-sulfonate that cannot persist. That aglycone has a decision to make, and the decision is the whole game of pungency. Left to its own chemistry it undergoes a spontaneous Lossen rearrangement and expels its sulfate to become an **isothiocyanate** — for sinigrin, allyl isothiocyanate (AITC), the volatile, eye-watering sharp of mustard and the compound wasabi and horseradish reach by the same route. That is the pungent outcome. But if a second protein, the **epithiospecifier protein (ESP)**, is present at the moment of rearrangement, it hijacks the intermediate and steers it instead

toward a **nitrile** or epithionitrile — a bland, sharp-silent dead end. One substrate, one enzyme, and a branch point that decides whether the plant produces a sting or produces nothing at all.

The alliums run the same logic with different parts. In garlic the stored precursor is alliin (S-allyl-L-cysteine sulfoxide); the sequestered enzyme is alliinase; crush the clove and alliinase converts alliin to **allicin** (diallyl thiosulfinate), the sharp of raw garlic — again a reaction, again compartmented, again a molecule so reactive it starts breaking down into a cascade of sulfur compounds almost immediately. Not every pungent plant works this way, and the contrast is instructive. The table below sorts the canonical pungent hosts by whether their sharp is *released on damage* — a true reaction, and the hard case — or sits **pre-formed** in the tissue like a small reservoir, which behaves much more like heat and is correspondingly easier to hold.

HOST ORGANISM	CONSTRUCTS	DIFFICULTY	PUNGENT PRINCIPLE	SHARP IS…
Black Mustard	2,790	Hard	allyl isothiocyanate (from sinigrin)	released on damage
Garlic	560	Moderate	allicin (from alliin, via alliinase)	released on damage
White Mustard	200	Hard	p-hydroxybenzyl isothiocyanate (from sinalbin)	released on damage
Watercress	120	Hard	phenethyl isothiocyanate (from gluconasturtiin)	released on damage
Ginger	120	Moderate	gingerols / shogaols	pre-formed
Thyme	120	Hard	thymol	pre-formed
Cinnamon	40	Hard	cinnamaldehyde	pre-formed
Oregano	40	Hard	carvacrol	pre-formed

Read that last column as a map of difficulty. Ginger, cinnamon, thyme, and oregano carry their sharp ready-made, so their consistency problem is close to heat's — grow the tissue to hold a steady amount and you are most of the way there. Black mustard, garlic, white mustard, and watercress manufacture their sharp in a four-step reaction at the moment of eating, and every one of those steps is a knob the plant can turn without our permission. The rest of this chapter is about the reaction hosts, because they are where the ≤7% promise is genuinely hard to keep.

How TRPA1 reads the sharp

To hold a sharp to a tolerance we have to know precisely what the tongue is counting, and here pungency is strange in a way that matters. The pungent axis is anchored to **TRPA1**, a non-selective

cation channel on the trigeminal sensory endings — a cousin of the heat channel but gated by a completely different kind of event. Capsaicin fits TRPV1 like a key in a lock: it docks, it activates, it lets go, and the whole transaction is reversible equilibrium binding. Allyl isothiocyanate does no such thing. It is an **electrophile**, and it activates TRPA1 by *covalently modifying* the channel — reacting with the thiol side chains of specific cysteine residues on TRPA1's cytoplasmic N-terminus through Michael addition, forming an actual chemical bond that forces the channel open. Allicin does the same to the same cysteines. So does cinnamaldehyde, an α,β-unsaturated aldehyde that is a textbook Michael acceptor; so, more gently, do the gingerols. The pungent axis is, at the receptor, a family of electrophiles all signing their names to the same handful of cysteines.

This covalent mechanism is not a curiosity — it is the molecular reason the sharp is hard to keep, in two distinct ways. First, the very reactivity that lets AITC grab a cysteine on the receptor is the reactivity that makes it grab everything else too: water, proteins, the air above the condiment. The agonist is strong because it is unstable, and it is hard to hold steady *because* it is unstable; potency and perishability are the same property seen from two sides. Second, because activation is a chemical reaction rather than an equilibrium, the *timing* of the sensation carries information. A reversible agonist switches off when its concentration falls; a covalent one switches off only when the modified channel is cleared or cycled, which is why pungency has a long, blooming tail that heat does not. Loudness alone cannot describe it. The sharp has a shape.

Putting a number on it: EC50, the Index, and the sharp

Every axis of the Iyengar Flavor Index resolves a flavor to a single loudness through one formula:

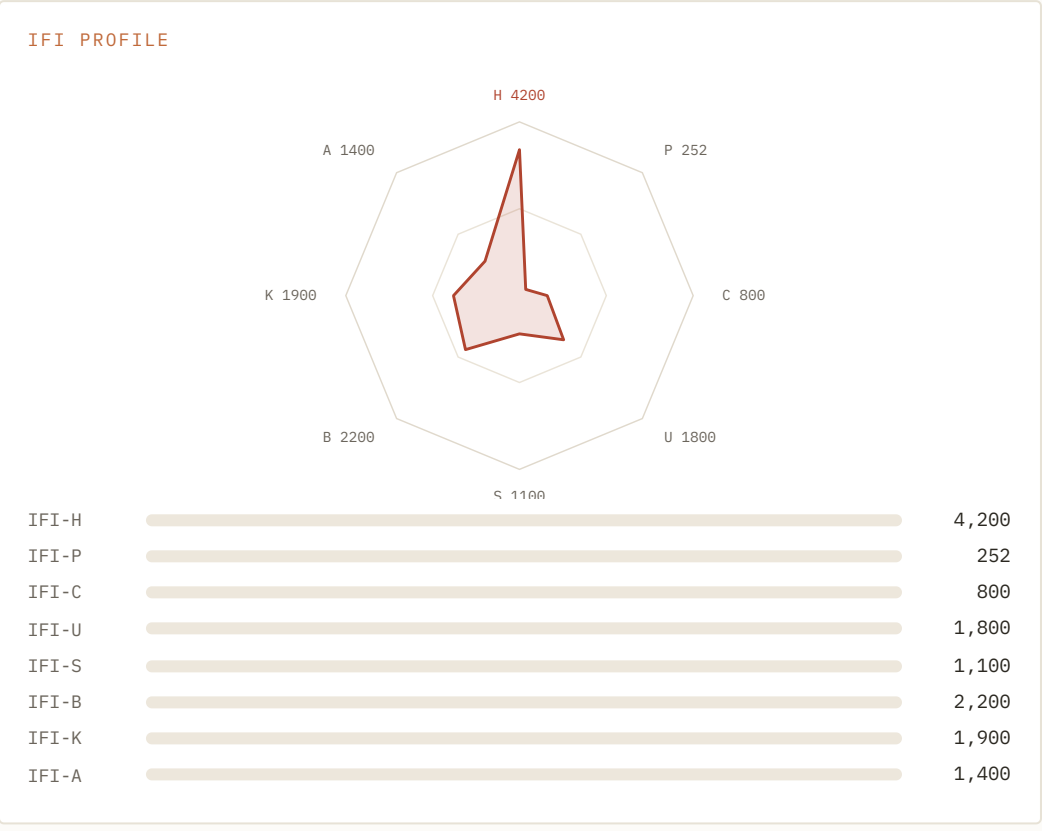
$$\mathrm{IFI}(\text{candidate}) = 1{,}000 \times \mathrm{EC}_{50}(\text{reference}) \div \mathrm{EC}_{50}(\text{candidate})$$

EC_{50} is the concentration of a stimulus that drives its receptor to half of full response — the lower the EC_{50}, the less compound it takes to reach half-activation, and the *louder* the flavor reads. The index inverts EC_{50} against a fixed native reference anchored at exactly 1,000, so the whole scale is a ratio of concentrations. Write the reference's half-activation concentration as E_0. For the pungent axis, E_0 is 2.1 µM — the stated AITC convention constant that anchors IFI-P at 1,000, defined here as a convention just as Scoville's dilution protocol defines his scale. Then any pungent candidate's standing on the axis is nothing but its EC_{50} expressed as a fraction of E_0, flipped and scaled by a thousand.

Work it forward for the one full-spectrum profile we can quote exactly. Our model predicts Phoenix (SS-0096) at **IFI-P = 252**. Rearranging the formula, its predicted pungent EC_{50} is

$$\mathrm{EC}_{50} = 1{,}000 \times E_0 \div 252 \approx 3.97 \times E_0$$

— a sharp whose predicted half-activating concentration is designed to sit at about four times the reference E_0, well below the native anchor's loudness on this axis. That moderation is deliberate, and it is the heart of this chapter: Phoenix is a cultivar we designed to register on all eight axes at once, and on the pungent axis it is tuned not for maximum volume but for a volume we can *hold*.



The same arithmetic lets anyone read the axis fluently. Because the scorecard normalizes each axis to a ceiling of 5,000, the loudest readable sharp corresponds to an EC_{50} of one-fifth the reference. The table makes the mapping explicit.

IFI-P READING	EC_{50} ($\times E_0$)	WHAT IT MEANS
252	3.97	Phoenix — deliberately moderated, holdable pungency
1,000	1.000	native reference anchor
2,000	0.500	twice the reference loudness
5,000	0.200	scorecard ceiling for the axis

Reading the sharp at a single point, though, hides the dose–response curve underneath it, and for pungency the curve deserves respect. We describe receptor activation with the Hill relation,

$$\theta = [A]^n \div (EC_{50}{}^n + [A]^n)$$

where θ is the fraction of channels open, [A] the agonist concentration, and n the Hill coefficient. At $[A] = EC_{50}$, the equation returns $\theta = 0.5$ by construction — that is what half-activation *means*. The honest caveat, which we keep in view rather than hide, is that a covalent agonist does not obey a clean equilibrium, so for TRPA1 the Hill form is an empirical descriptor of the measured curve, not a mechanism. We fit it because it gives us a single EC_{50} to put in the formula, and we remember that the fit is a convenience. A unit note belongs here too: IFI-P is dimensionless — a pure ratio — while the EC_{50} underneath it carries real units of concentration, typically micromolar at the receptor. The conversion from the glucosinolate load in the tissue (micromoles per gram of fresh weight) down to the

micromolar concentration that actually reaches the trigeminal ending runs through the conversion fraction and the dilution of the macerate, and every one of those steps is a place variance enters. Which is exactly the problem we turn to now.

Why variance compounds — the mathematics of a wobble

Consistency has its own number: the coefficient of variation, CV = σ ÷ μ, the standard deviation of a measurement divided by its mean, expressed as a percent. A CV of 7% means the batch-to-batch spread is seven parts in a hundred around the target; wild-type black mustard, left to the field, runs a CV near **42%**. To see why that figure is so large — and why heat never has to fight it — write pungency as what it physically is: a product of stages, not a stored quantity.

```
P ∝ G × f × r × d
```

Here G is the **precursor pool** (how much glucosinolate the tissue banked), f is the **firing fraction** (how much of that pool myrosinase actually converts, and how fast), r is the **branch ratio** (the share of the aglycone that commits to pungent isothiocyanate rather than bland nitrile, set by ESP), and d is the **decay/timing factor** (how much sharp survives to the instant of measurement). Four independent multipliers, chained. For a product of independent factors, relative errors do not add — they add *in quadrature*:

```
CV(P)² ≈ CV(G)² + CV(f)² + CV(r)² + CV(d)²
```

That single line is the mathematical reason the sharp is the hardest axis to keep. Heat, a reservoir, is essentially one factor — the banked capsaicinoid mass — so its variance is one term. Pungency is four terms under a square root, and because they combine as squares, the chain is dominated by its *worst* link. Put representative field numbers to it: suppose each of the four stages in an unmanaged crop wobbles by about 21% — a plausible figure for a plant whose sulfur nutrition, enzyme activity, ESP expression, and post-damage timing are all left to chance. Then

```
CV(P) = √(4 × 0.21²) = √0.1764 = 0.42 = 42%
```

— and the canonical wild-type figure falls straight out of the arithmetic. Nothing was tuned; four ordinary wobbles, compounded through a four-step reaction, land exactly on the variance we measure in the field. The table names each term and previews where design takes it.

STAGE	PHYSICAL SOURCE	WILD-TYPE CV	ENGINEERED CV	LEVER
G — precursor pool	glucosinolate / sulfur load	~21%	~4%	controlled-environment cultivation
f — firing fraction	myrosinase activity & speed	~21%	~4%	engineered myrosinase expression
r — branch ratio	ESP: isothiocyanate vs nitrile	~21%	→ 0	clean genetic silencing of ESP
d — decay / timing	survival to measurement	~21%	~4%	stabilized compartmentation
P — pungency	**the product**	**≈42%**	**≈6.9%**	**all four, together**

Four levers, four variances pinned

Every design move we make on the pungent axis is aimed at one of those four terms, and the quadrature formula tells us exactly what each move is worth.



The controlled-environment chamber, where the variables that feed the precursor pool are pinned one at a time.

Clean genetic silencing of ESP is the most elegant of the four, because it does not merely *narrow* a variance — it *deletes* a term. An ESP that is reduced but still present leaves the branch ratio r drifting from batch to batch: a little more nitrile here, a little less there, the sharp quietly wandering. Silence the gene cleanly and the branch commits to isothiocyanate every single time; r becomes a fixed 1.0 with no spread, and the term $CV(r)^2$ drops out of the sum entirely. We go from four links to three. This is why we insist on clean knockouts over partial knockdowns on this axis — a knockdown improves the mean, but only a knockout removes the variance.

Engineered myrosinase expression under a tightly controlled promoter narrows the firing fraction f. Wild myrosinase activity swings with the plant's pH, its temperature, its stress, and the exact

mechanics of damage; expressing the enzyme to a defined, buffered level pins how much of the pool converts and how quickly, tightening CV(f) and, as a bonus, sharpening the onset. **Stabilized compartmentation** — reinforcing the physical separation of substrate and enzyme so the two halves of the bomb stay reliably apart until real damage — protects both amplitude and timing, holding d by preventing the slow pre-firing and leakage that would otherwise bleed sharp away before the jar is even opened. And **controlled-environment cultivation**, the chamber above, pins the precursor pool G at its source: the sulfur supply, the light, the water, the harvest stage, each held constant so the plant banks the same glucosinolate load batch after batch, feeding the reaction from a steady reservoir instead of a fluctuating one.

Now run the quadrature forward with the engineered plant. ESP is gone, so one term is zero. Drive each of the three survivors below about 4% — well within reach once the upstream variables are held — and

```
CV(P) = √(3 × 0.04²) = √0.0048 = 0.069 = 6.9%
```

which clears the ≤7% promise with room to spare. The same formula that explained a 42% wild-type wobble now certifies a 6.9% engineered one; the design is not magic, it is the deliberate collapse of a four-term variance into a three-term one with every surviving term pinned. A last, quieter contribution comes from knowing how much *headroom* a design has: a pungency pushed to the metabolic limit of the plant has none, and amplifies the day-to-day swing of its own sulfur economy, while a sharp left with margin absorbs that swing instead. Tuning Phoenix's pungent design to 252 rather than chasing the ceiling is partly a consistency decision — a loudness we can hold is worth more than a loudness we can only occasionally reach.

The kinetic spec: τ_on and τ_off

Loudness is only half of a sharp. Because TRPA1 is driven covalently, pungency arrives and departs on a clock, and that clock is itself a specification that has to hold. We describe the rise and fall with first-order kinetics. The sharp blooms toward its peak as

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A(t) = A_peak × (1 − e^(−t ÷ τ_on))
```

and fades as

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A(t) = A_peak × e^(−t ÷ τ_off)
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where τ_on is the onset time constant — set largely by how fast myrosinase fires — and τ_off is the decay time constant, set by how quickly the reactive isothiocyanate is cleared or breaks down. The time to reach half of the peak is t½ = τ · ln 2 ≈ 0.693 τ, a tidy relation that lets a kinetic spec be written and checked. A black mustard tuned for a slow, rolling onset and a long tail is a different condiment from one tuned for an instant spike that vanishes, even when both read the identical IFI-P. A mustard that is the right loudness but the

wrong speed is the wrong mustard — the sauce built on it will feel hurried or sluggish on the palate no matter how perfect its peak number.

So we hold the shape to a tolerance exactly as we hold the amplitude. The decay term τ_off is also the physical origin of the d factor in the variance budget: it is why *when* you measure matters. If the sharp's half-life after grinding is on the order of tens of minutes, the pungency a taster meets at one minute is meaningfully louder than the one met at thirty, and a number quoted without its clock is not a number at all. Pinning τ_off through stabilized compartmentation does double duty — it tightens the consistency term and it fixes the shape — which is why a single design move so often improves two specs at once on this axis.

Measuring the sharp honestly — methods, edge cases, failure modes

Measuring a reaction demands a protocol that a reservoir never would, and ours is built around the one truth we cannot escape: the clock starts at damage. We standardize the grind — fixed particle size, fixed temperature, fixed hydration — so that firing begins the same way every time, then read the sample at a fixed delay after rupture, against the same delay for the reference. The loudness itself we take from a TRPA1 cell assay, titrating the macerate against the channel to find the concentration for half-activation, which is the EC_{50} the index formula needs. Every pungency number we publish is therefore a triple: an amplitude, a kinetic shape, and the clock they were read against.



The assay stage, where a sharp is titrated against TRPA1 to find the EC_{50} the Index formula needs.

The edge cases are all versions of the reaction misbehaving. The sharp decays *during* the measurement window, so a slow assay reads low and a fast one reads high; we fix the window and treat it as part of the method. Grind too hot and myrosinase denatures mid-firing, dropping f; grind too cold and it never fully fires. A sample that sits between damage and assay drifts down its own τ_off curve. None of these are failures of the plant — they are failures of the clock, and the discipline is to hold the clock.

The failure modes of the design are worth naming as plainly as the successes, because a tolerance you cannot break is a tolerance you do not understand. **Partial ESP knockdown** is the classic trap: the mean improves, the panel is pleased, and the batch-to-batch branch ratio keeps wandering because the gene is only quieted, not silenced — the variance term survives and resurfaces at scale. **Myrosinase mis-expression** cuts both ways: too little and the pool never converts, flattening the sharp; too much and the onset spikes faster than the kinetic spec allows, so a loudness gain becomes a shape failure. **Compartmentation leak** is the quietest and most dangerous — if the two reagents meet slowly in intact tissue, the plant fires itself in storage, and the jar that leaves is already fading before it is opened. And **sulfur starvation** in cultivation shrinks G at the root, so a design that is perfect in the chamber underperforms in a field that cannot feed it.

Which brings us to the honest limit. We cannot make allyl isothiocyanate a stable molecule — its reactivity is the sharp; a stable version would not sting. What we *can* do is control the reaction that makes it and fix the moment we measure it. A designed black-mustard line does not give a condiment maker an eternal sharp; it gives a *specified* one — a defined amplitude, a defined shape, and a defined clock, reproducible to a CV under seven percent so long as the protocol is honored. That is a smaller and more truthful claim than "stable," and it is the one we are willing to stand behind on every jar.

What the tolerance buys

The payoff is a sharp a formulator can build a business on. A condiment maker who licenses a designed black-mustard line gets not merely a high pungency number but a *held* one — amplitude and kinetics both, at a batch-to-batch CV under seven percent — which means the sauce tastes the same in January and in July, in the first jar and in the ten-thousandth. That is the unglamorous heart of what the platform sells on this axis: not the loudest possible sharp, which anyone can chase once and never repeat, but the **same** sharp, on purpose, every time — a controlled explosion tamed to a tolerance.

There is something our family finds quietly moving in that, and it is worth saying without dressing it up. Mustard is one of the oldest condiments we have, a sharp that generations reached for because it woke a plain meal up, and nothing about it is added or adulterated — the sting is the plant's own chemistry, set off by nothing more than teeth. To take a reaction that reliable in its wildness and make it reliable in its *precision* — to hand a cook a sharp that is clean, whole, and exactly itself every single time — feels less like taming the plant than like finally keeping its promise. On the pungent axis, where the flavor is a reaction and the molecules are in a hurry, earning that promise is the whole game, and keeping it is the difference between a plant that can surprise you and a plant you can trust. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 12 · The black-mustard line — *the flagship of the sharp.*

Every axis in this library has an organism where our family first showed we could move its number at will — a plant we tuned in our models until the predicted score reflected what we set out to build. For the sharp, for pungency, the bright blade of a flavor the French call *piquant* and a Bengali kitchen simply calls mustard, that organism is **black mustard**, *Brassica nigra*, and its 2,790 product designs are the most extensive designed body of work we have assembled on a single channel. If you want to understand what designed pungency *is* — not as an abstraction but as a finished, growable, pressable plant you could turn into oil tomorrow morning — this is the line to read.



The black-mustard flagship line — the organism where our family learned to move the sharp on purpose.

Black mustard earned the flagship role for the same reason a superhot pepper earns it on the heat axis: it offers the cleanest possible version of the chemistry. Its glucosinolate pool is dominated by a *single* compound — **sinigrin** — so when its cells are crushed the plant outputs essentially pure **allyl isothiocyanate** (AITC) rather than a muddle of a dozen half-notes competing for the tongue. Every lever we pull therefore moves one clear flavor instead of smearing six. Its compartmentation — the physical separation that keeps an intact plant from detonating itself — is robust and survives our edits. Its genome is tractable. And its native loudness is already

high, so we began with a plant that already spoke the axis plainly and asked it to speak louder, sharper, and — the real work, the work that took the years — *the same every single time.* The program's lead axes are **P** and **M**: pungency as the voice, multi-axis because a mustard is never only sharp. It carries a measure of kokumi depth, a whisper of green brassica bitterness, and in the designed lines a sweetness we can place to the gram, so that a finished mustard reads as a small composition rather than one struck note.

The receptor — how the tongue actually reads the sharp

To design the sharp you have to know what, physically, is being sharpened. The warmth of a chili and the bite of a mustard feel like cousins, and they travel the same trigeminal nerves, but they are read by two different proteins obeying two different logics — and the difference is the whole reason pungency needs its own number rather than borrowing heat's.

Capsaicin, the molecule of heat, binds **TRPV1** reversibly. It slips into a lipid-facing pocket, lowers the channel's thermal threshold so the body reports a fire that is not there, and then — this is the point — it *lets go.* It is a lock and key. The warmth swells and fades because the key diffuses in and out of the lock.

Pungency is not like that. The sharp is read by **TRPA1** — Transient Receptor Potential Ankyrin 1 — a non-selective, calcium-permeable cation channel sitting on the same sensory endings, and its agonists do not merely dock. They *react.* Allyl isothiocyanate carries a reactive handle, the isothiocyanate group `–N=C=S`, whose central carbon is electron-poor and hungry. When AITC reaches TRPA1 that carbon attacks the free thiol (`–SH`) of a small, specific set of cysteine residues on the channel's large cytoplasmic N-terminal domain — in human TRPA1, the residues clustered around C621, C641, and C665, with a nearby lysine completing the set — and forms a **covalent adduct**, a thiocarbamylation. The modification tugs the gate open. The sharp is not a ligand the receptor can forget the instant it drifts away; it is a bond the channel has to carry until the adduct is reduced away or the protein itself is replaced.

That single fact — covalent, not reversible — is why mustard's bite has a different *shape in time* than a pepper's warmth. Heat is a swell; the sharp is a strike. It arrives fast and it arrives whole, because the molecule is not waiting at an equilibrium — it is committing a reaction. And it explains the odd, principled promiscuity of this channel. TRPA1 will answer almost any molecule that carries an electrophilic handle, no matter how unlike the others it looks: the isothiocyanates of mustard, wasabi, and horseradish; the thiosulfinate **allicin** released when garlic is crushed; the α,β-unsaturated aldehyde **cinnamaldehyde** of cinnamon; the acrolein of smoke. A chemist would never shelve these together. The tongue files them all under one heading — *sharp* — because they all react with the same cysteines. The IFI-P axis is, at bottom, a census of electrophiles, and designing pungency means governing how much reactive electrophile a plant delivers, how fast, and of what shape. We are not filing a better key. We are choosing how much reactive cargo to deliver, and when.

The mustard bomb — sinigrin, myrosinase, and the Lossen rearrangement

A black mustard seed is a loaded weapon with the hammer resting down. The pungency does not exist in the intact plant; it is *manufactured in the instant of damage,* and the elegance of that mechanism is why this organism was worth a decade.

The plant keeps its two reactive halves in separate rooms. The glucosinolate — sinigrin, which is 2-propenyl (allyl) glucosinolate, a sugar bonded through sulfur to a sulfonated oxime bearing the allyl side chain — is stored quietly in one population of cells. The enzyme that can cleave it, **myrosinase** (a β-thioglucosidase), is sequestered in a separate population of idioblasts, the myrosin cells. Intact, the two never meet; the seed is silent on the tongue. Then a mandible, a mortar, a stone mill, a knife — any insult that ruptures the compartments — mixes them, and the reaction our family came to call the *mustard bomb* fires:

1. **Hydrolysis.** Myrosinase cleaves the thioglucosidic bond of sinigrin, releasing a molecule of D-glucose and an unstable aglycone, a thiohydroximate-*O*-sulfonate.
2. **Rearrangement.** The aglycone cannot persist. It sheds its sulfate and undergoes a **Lossen-type rearrangement** — the side chain migrating from carbon to nitrogen — collapsing into the isothiocyanate: allyl isothiocyanate, plus bisulfate and a proton. That AITC is the sharp.

But there is a fork in that road, and it is the first thing we had to take control of. The same aglycone has a second, quieter fate. In the presence of **epithiospecifier protein (ESP)** and ferrous iron — or simply at low pH — it is diverted away from the isothiocyanate and toward **nitriles and epithionitriles**, molecules that barely touch TRPA1. A wild plant *hedges*: some of its sinigrin becomes fierce AITC and some becomes near-silent nitrile, and the ratio wanders with tissue, maturity, temperature, and acidity. A wild mustard is therefore not only quieter than it could be — it is *inconsistent,* loud one season and flat the next for reasons the grower cannot see. So the foundational edit of the whole line is to **silence ESP.** With ESP gone, the Lossen path runs to completion and essentially every molecule of sinigrin that meets the enzyme becomes sharp.

That gives us two orthogonal levers, and keeping them separate in our heads is what made the design tractable:

- The **size of the sinigrin pool**, set upstream by sulfate supply and aliphatic-glucosinolate flux, fixes the *ceiling* — the most AITC the plant could possibly make.
- **ESP status and myrosinase completeness** fix the *fraction of that ceiling actually realized* as sharp when the tissue breaks.

Loudness is the product of the two: pool × conversion fraction × hydrolysis completeness. A plant can sit on a mountain of sinigrin and still whisper if ESP bleeds half the flux to nitrile; it can convert with perfect fidelity and still whisper if the pool is small. We hold all

three at once — the pool high, ESP silent, myrosinase ample — and that is what turns a variable wild loudness into a dependable designed one.

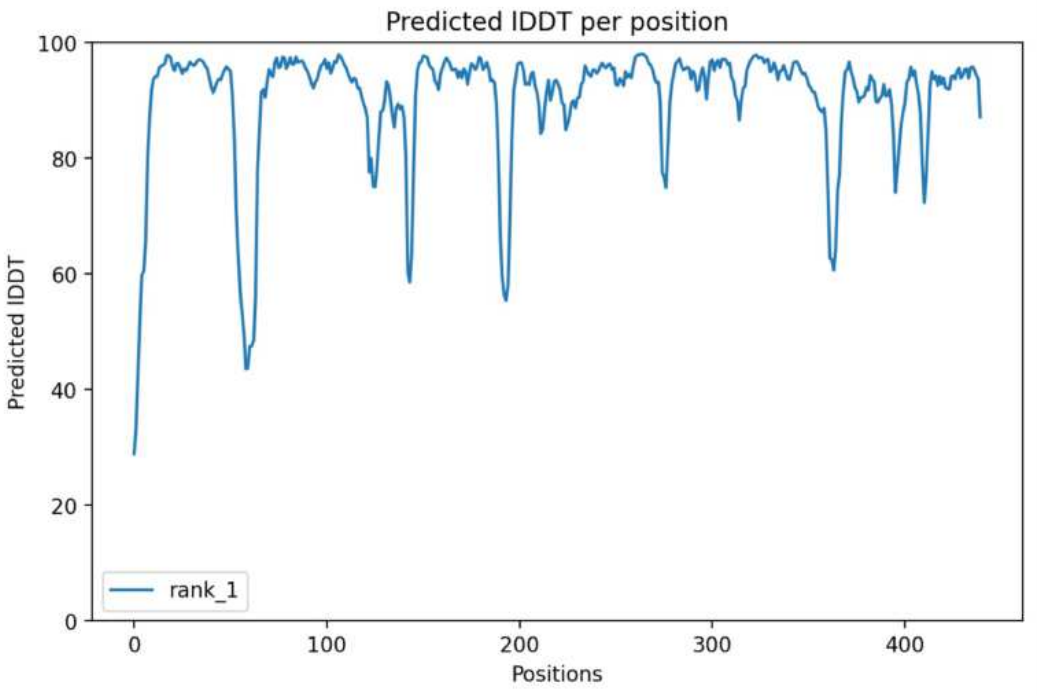

FIG. 12.1 — A per-residue confidence map of the kind we build before editing any pathway enzyme; shown here for the capsaicin synthase of the heat line, the same structural method guides exactly where we silence ESP and where we leave myrosinase untouched.

Scoring the sharp — the index math, worked

The Iyengar Flavor Index gives every axis a single number anchored to a receptor's half-maximal activation:

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

$\mathrm{EC_{50}}$ is the concentration that drives the receptor to half of its full response. Invert it against a fixed convention reference, scale by a thousand, and loudness becomes a score. For the pungency axis, the convention reference is AITC itself at 2.1 µM — a stated convention constant, not a measured literature value, in the same spirit as Scoville's dilution-to-threshold definition. At that convention, AITC defines IFI-P = 1,000. Unimproved wild-type black mustard tissue, whose pungency output is equivalent to roughly 10 µM AITC, scores approximately IFI-P = 210 on this scale. A candidate that half-activates the channel at a *lower* effective concentration scores higher, and its number rises in exact proportion to that improvement.

Pungency forces one honesty onto this formula that heat never did, and it is worth saying plainly because it governs every design choice in the line. AITC is AITC. Its intrinsic potency at TRPA1 — the $\mathrm{EC_{50}}$ of the pure molecule — is a fixed physical constant; we set our convention reference at 2.1 µM, the value that anchors IFI-P = 1,000 on the pungency axis. *No plant can change it.* A black mustard cannot make allyl isothiocyanate that is intrinsically sharper than allyl isothiocyanate. So when the index score of a designed mustard rises to 651, it is not because the plant forged a better key. It is because the plant **delivers more of the same reactive molecule per gram of tissue.** The quantity that moves is the *effective drive* on the receptor:

> $\mathrm{effective\ drive} \propto [\mathrm{AITC}]_{\mathrm{delivered}} \div \mathrm{EC_{50}(AITC)}$

and the $EC_{50}(candidate)$ that appears in the index formula is read operationally as the half-activating concentration of the *food's pungent fraction* — grams of fresh tissue, or of finished paste, per litre of assay. A line that liberates more AITC per gram reaches half-max at a lower tissue concentration, so its operational EC_{50} falls and its index rises by the same factor. Wild-type black mustard tissue at IFI-P = 210 is the baseline for the line. A designed mustard that our model predicts will liberate 3.1× the AITC per gram would reach half-activation at one-third the tissue concentration, and the index scales in proportion:

```
IFI-P = 210 × 3.1 = 651
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Loudness engineering, for a fixed agonist, *is* yield engineering. Reading the two profiles in context of wild-type black mustard makes the relationship concrete:

MATERIAL	IFI-P	$EC_{50}(TISSUE) \div EC_{50}(WILD\text{-}TYPE)$	POTENCY VS WILD-TYPE
Wild-type black mustard tissue	210	1.000	1.0×
Phoenix · full-spectrum flagship	252	0.833	1.2×
Table-grade black mustard	651	0.323	3.1×

The table-grade mustard is designed to half-activate TRPA1 at 32.3% of the tissue concentration the wild plant needs, and it is 651 ÷ 252 ≈ 2.6× louder on the sharp than a full-spectrum pepper that spends only a slice of its budget there.

From sinigrin to sharp, by the mole. The ceiling is set by chemistry we can count. Sinigrin becomes AITC one-to-one. Sinigrin monohydrate (potassium salt) has a molar mass of 415.5 g/mol; AITC is 99.15 g/mol (C_4H_5NS: 4 × 12.011 + 5 × 1.008 + 14.007 + 32.06). So the theoretical yield, at perfect ESP-silenced conversion, is

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99.15 ÷ 415.5 = 0.2387 g AITC per g sinigrin ≈ 238.7 mg AITC per gram of sinigrin.
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If ESP is active and diverts a fraction *f* of the aglycone to nitriles, the realized yield is (1 − *f*) × 238.7 mg/g — so every point of ESP leakage is a point shaved straight off the sharp. Silencing ESP drives *f* → 0 and recovers the full 238.7 mg/g. Pool size and conversion fraction are, again, the two independent knobs: the first sets how much sinigrin the gram contains, the second how much of it survives to the tongue as AITC.

What a micromole tastes like. To move between the receptor's language and the formulator's, the conversion is one line: 1 µM AITC = 99.15 µg/L = 0.0992 mg/L. A half-activating concentration of a few micromolar is therefore only a few tenths of a milligram of AITC per litre — the sharp is *potent,* which is exactly why delivery and compartmentation, not raw tonnage, are the hard part.

Why the sharp arrives all at once. TRPA1 gates cooperatively; the dose-response follows a Hill relation,

```
R ÷ R_max = [A]^n ÷ (EC50^n + [A]^n)
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with a Hill coefficient *n* above one — a working value of n ≈ 1.7 for AITC fits the steep gating reported for AITC at TRPA1. Steepness compresses the dynamic range. The concentration span needed to climb from 10% to 90% of full response is 81^(1/n): for a non-cooperative channel (n = 1) that is an 81-fold span, a slow gradual swell; for n ≈ 1.7 it collapses to 81^(1/1.7) ≈ **13-fold**, and for n = 2 to just 9-fold. The sharper the cooperativity, the narrower the window between *nothing* and *everything* — which is precisely the sensation of a mustard: not a dawning warmth but a blade, there before you have finished the bite.

The shape in time. Two kinetic constants finish the portrait: τ_on, how fast the sharp rises, and τ_off, how fast it clears. Onset is governed by how quickly myrosinase liberates AITC on tissue rupture and how fast that AITC diffuses to the channel and adducts it. Clearance is governed by how quickly free AITC leaves — by volatilization, dilution, and diffusion — against the slower reversal of the covalent adduct. AITC is small and volatile, so it reaches the nose retronasally (the nose-filling rush of a strong mustard) and it also *clears* fast, which is why a classic mustard strikes and is gone. Lengthen the side chain and you lower the vapor pressure; the sharp loses some of its nasal rush but lingers far longer on the tongue. Volatility is the dial between *flash* and *trail,* and the line is built to set it anywhere along that dial.

The 2,790 designs as a palette

The catalogue is, in practice, a map of these levers crossed against each other — 2,790 designed product entries in the space of loudness, speed, and shape. What that map buys is not a product but a *palette:* a family of black mustards each tuned to a different corner of the sharp, so a condiment designer can order the exact blade a recipe wants.

DESIGN GOAL	LEVERS PULLED	INDEX / KINETIC EFFECT	THE MUSTARD IT MAKES
Louder flat amplitude	ESP silenced · sulfate supply lifted	P ↑↑	the extract-grade ferocity
Bright fast attack	myrosinase accelerated	τ_on ↓	the clean table paste
Slow building sharp	myrosinase damped · non-volatile side chain	τ_off ↑	the sandwich smoulder
Wasabi-type trail	MAM chain elongation (C6 sulfinyl → 6-MSITC)	P character shift · τ_off ↑	the wasabi substitute
Clean single note	sinigrin purity held · side branches trimmed	P clarity	the fine French table grade
Depth under the sharp	γ-glutamyl / kokumi pool lifted	K ↑	the grain for roasted meat

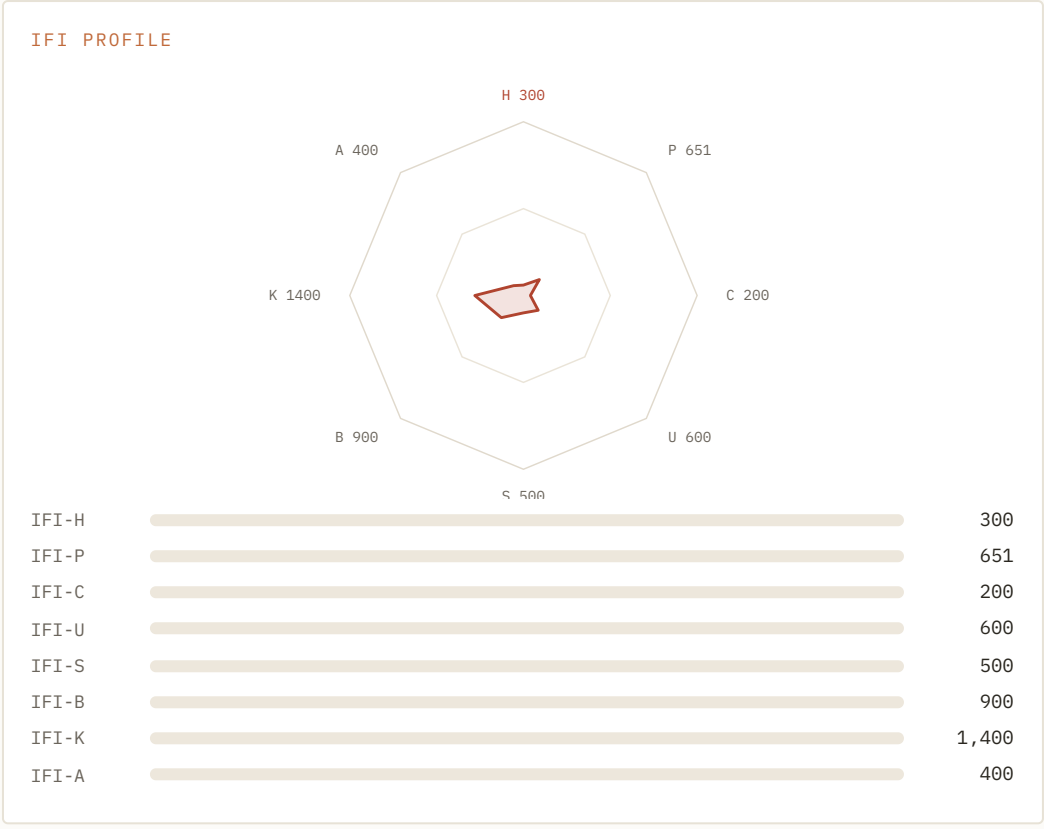
The archetypes are worth naming, because each is a different *question* answered in a seed. The **extract-grade ferocity** pours the whole budget into pool and conversion — ESP silent, sulfate lifted, myrosinase fast — for the nose-filling, fast-clearing punch that stands in for wasabi in a dish that wants the blade and nothing after it. The **fine French table grade** is the discipline piece: sinigrin purity held and the minor glucosinolate branches trimmed, so a single,

bright, unmuddied AITC note carries the whole paste, the mustard for a vinaigrette where clarity is the point. The **sandwich smoulder** damps the hydrolysis and leans on a less volatile side chain so the sharp *builds* and sits, a mustard meant to be met slowly between bread rather than to arrive as a strike.

The **wasabi-type trail** is the most interesting edit, and the one that uses the molecule's shape rather than its amount. By lengthening the side chain through **MAM (methylthioalkylmalate synthase) elongation** — stepping the C3 allyl up toward a C6 methylsulfinyl chain — the plant makes not allyl isothiocyanate but **6-methylsulfinylhexyl isothiocyanate (6-MSITC)**, the signature of true wasabi. It is a larger, far less volatile electrophile. On the tongue it means a sharp that still strikes but then *trails,* a long green tingle rather than a flash, because the molecule is slower to clear and slower to let go of its cysteine. And the **grain for roasted meat** leaves the sharp high but lifts the kokumi pool beneath it — the γ-glutamyl peptides read by the calcium-sensing receptor — so the mustard has a rounded, continuous body under the blade, able to stand against the fat and char of something off the fire. One organism, one chemistry, mapped across its whole range, every point held to the same tolerance.

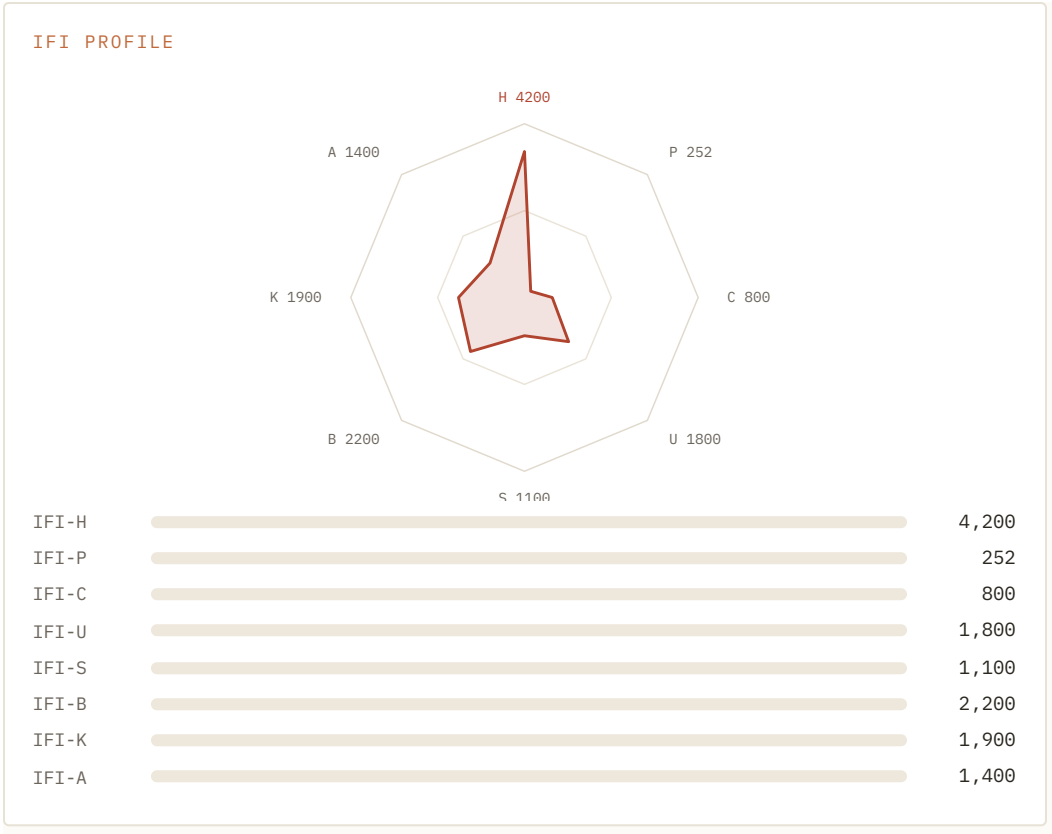
Reading the scorecard — specialist against full spectrum

A representative readout from the line — a designed table-grade black mustard tuned for a bright, clean, nose-forward sharp with a touch of depth beneath it — reads on the index like this, with its kinetic pair alongside:



τ_on 4 s · τ_off 40 s · AITC-dominant · ESP-silenced · CV 5%

Set that against the scorecard of a plant built to the opposite philosophy. Phoenix is our full-spectrum flagship pepper, engineered gene by gene to register on all eight axes at once — the most completely designed fruit in the library — and its profile is a chord, not a note:



The specialization is plain the moment the two sit side by side. Phoenix is a full-spectrum plant that carries pungency at a computed IFI-P of 252 as one voice among eight — the sharp present, acknowledged, in proportion. The table-grade mustard is the opposite design: a **specialist**, pouring its whole metabolic budget into a single axis to reach a predicted P of 651, well past what a full-spectrum plant would allocate to any single channel, while the other axes stay low and supportive — the kokumi at 1,400 giving it body, the faint sweetness and the trimmed bitterness rounding the edges, the heat a mere trace at 300. Both profiles are *correct.* They are answers to different questions. The full-spectrum fruit asks *how many channels can one organism ring at once;* the flagship mustard asks *how loud and how clean can one channel be made to sing.* Pungency, more than almost any axis, rewards the second question — because the sharp is a thing people reach for *on purpose,* at the edge of the plate, wanting exactly it and nothing else. A plant designed to give exactly that, the same way every time, is the whole promise of the platform rendered in a single bright, fierce, reproducible seed.

Where the sharp lives across the garden

Black mustard is the flagship, but the same method reaches every plant that keeps an electrophile. Because TRPA1 answers a *class* of molecules rather than one, the pungency work transfers across families that share nothing botanically — their chemistries are unrelated, yet each resolves to the same cysteines, and each becomes its own program with the same five platform modules we proved on the mustard line.

ORGANISM	PRODUCTS	AXES	PUNGENT PRINCIPLE	TRPA1 TRIGGER (ELECTROPHILE CLASS)
Black mustard	2,790	P · M	sinigrin → AITC	isothiocyanate
Garlic	560	K · P	alliin → allicin	thiosulfinate
White mustard	200	P	sinalbin → *p*-hydroxybenzyl ITC	isothiocyanate (non-volatile)
Ginger	120	P	gingerol / shogaol	vanilloid electrophile (TRPV1 + TRPA1)
Watercress	120	P	gluconasturtiin → PEITC	isothiocyanate
Thyme	120	P	thymol	monoterpenoid phenol
Cinnamon	40	P	cinnamaldehyde	α,β-unsaturated aldehyde
Oregano	40	P	carvacrol	monoterpenoid phenol



Garlic — the same sharp, delivered by a thiosulfinate instead of an isothiocyanate; crush it and alliin becomes allicin, and TRPA1 reads it on the same cysteines as mustard.

The contrasts are instructive. **White mustard** (*Sinapis alba*) runs the identical bomb mechanism but on sinalbin, whose aglycone becomes *p*-hydroxybenzyl isothiocyanate — a far less volatile molecule. The result is the warmth of a classic yellow table mustard that sits *on the tongue* rather than rushing *up the nose,* exactly the flash-versus-trail distinction that side-chain volatility sets within the black-mustard line itself. **Garlic** carries no glucosinolate at all; its sharp is a thiosulfinate, allicin, generated when the enzyme alliinase meets alliin on crushing — a different compartmented bomb, a different molecule, the same covalent chemistry at the channel, and a plant that pairs that pungency with the kokumi it is loved for. **Ginger** reaches TRPA1 with gingerol and its dehydrated cousin shogaol, which also touch TRPV1, so its sharp carries a genuine

warmth the isothiocyanates lack. **Cinnamon's** cinnamaldehyde is the textbook α,β-unsaturated aldehyde, a Michael acceptor that adducts the same cysteines by a different reaction. Eight plants, eight unrelated chemistries, one receptor and one axis — and one method that moves the number in every one.

The honest limits

A number is only worth what its caveats are, and this axis carries several that we state rather than bury.

The Scoville scale is blind to the sharp. Scoville Heat Units measure capsaicinoid heat — the activation of *TRPV1* — originally by human dilution-to-threshold and now by HPLC calibration. AITC does essentially nothing at TRPV1 at any culinary concentration; it is a TRPA1 agonist. So a pure, tear-bringing mustard scores effectively *zero SHU* despite being intensely pungent, and the table-grade line's trace IFI-H of 300 reflects only its faint warmth, never its blade. This is not a footnote — it is the reason the sharp needed a number of its own. A measuring stick cut for heat cannot see pungency at all, and every mustard in the world is invisible to it. The IFI-P axis exists precisely to end that silence.

Covalent agonism bends the definition of EC_{50}. The clean equilibrium EC_{50} that heat enjoys assumes a ligand that binds and releases. The sharp does not release; it reacts, and covalent activation is *time-dependent* — hold the tissue against the channel longer and more cysteines are adducted. We therefore report an **operational EC_{50} computed at a fixed convention exposure time,** and we publish that time with the number. It is an honest approximation of a half-activation for a system that has no true equilibrium, and treating it as more than that would be a quiet lie about the chemistry.

The error propagates, and that sets the specification. Batch reproducibility is reported as the coefficient of variation, CV = σ ÷ μ. The platform specification is CV ≤ 7%; this line is designed to hold **5%.** The two numbers are tied by arithmetic, not coincidence. Because the index is a *ratio* of two EC_{50} measurements, the reference's and the candidate's, their relative errors add in quadrature:

```
CV(IFI) ≈ √( CV(reference)² + CV(candidate)² )
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Two assays each held to 5% therefore yield $\sqrt{(0.05^2 + 0.05^2)} = \sqrt{0.005} =$ **7.07%** on the index itself. Holding each measurement to 5% is exactly what keeps the *reported score* inside the ≤7% platform spec — the 5% and the 7% are the same discipline, seen before and after the ratio. And the variance that fixing the genetics actually collapses is the field variance: in a wild stand, the sharp swings with sulfur nutrition, maturity, tissue, pH, and the drifting ESP ratio, so loudness is never the same jar twice. Silence ESP, hold the sinigrin purity, set myrosinase, and that wandering field variance collapses into the tight 5% that lets a formulator design against the number.

The failure modes are real and we name them. ESP leakage is the first: let any epithiospecifier activity return and flux bleeds to near-silent nitrile, so the pool stays high while the sharp sags — a plant loud on paper and flat on the tongue. Myrosinase loss is the second: the bomb needs its enzyme intact, and because myrosinase is a protein it denatures with heat, which is why the sharp is a *cold-finish* flavor — cook a mustard and you mute it, so the blade belongs at the end of the dish, stirred in off the heat. Volatility loss is the third and the most domestic: AITC is volatile, so an opened jar fades as the sharp literally evaporates, and the only honest fix is a trade — lengthen the side chain toward the wasabi-type and you buy shelf-life at the cost of the nose-hit. We can set that trade anywhere, but we cannot escape it, and we would rather tell a condiment maker the shape of the compromise than pretend there is none.

That is the flagship of the sharp: one organism, one dominant chemistry, mapped across its whole range of loudness and shape, every point held to the coefficient of variation, every claim carried back to a receptor and a reaction we can count. A mustard our family can grow to be fierce or fine, a flash or a trail, bright or deep — and, above all, the same bright, fierce, reproducible thing in every single seed. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



PART IV OF V

The Condiment

the grammar of the sharp · mustards · wasabi · chili crisp · málà

Chapter 13 · A grammar of condiments — *how the sharp behaves on a plate.*

The pungent axis is where the world keeps its condiments, and a condiment is a different design object from a dish. A dish is cooked; its flavors are set by heat and time and arrive integrated, married in the pan until no single note can be pulled back out. A condiment is *added* — at the end, cold or warm, in a smear or a spoonful — and its whole job is to carry a bright, defined jolt of one or two axes into a bite that would otherwise lack them. It is the one element on the plate we hand to the eater unfinished, still reacting, still becoming itself in the mouth. On the sharp axis especially, that unfinished quality is not a flaw to be managed but the entire mechanism, because the molecules that make mustard sting and garlic roar do not exist in the quiet plant at all. They are *manufactured at the moment of damage*, and they begin to die the instant they are made.

That single fact — that pungency is a reaction caught in motion — is why a cook who knows the chemistry can place a sharp condiment with a precision that feels like sleight of hand, and why one who does not will watch the same jar of prepared mustard read as a tang one day and a sinus flood the next. So before the recipes, a short grammar: the handful of rules by which the sharp behaves once it leaves the plant and meets the plate. We write them as rules because they are reproducible, and we show the mechanism under each because a rule you understand is a rule you can bend on purpose.

The channel the sharp is speaking to

Every axis of the Index is anchored to one receptor, and the pungent axis — IFI-P — is anchored to **TRPA1**, a large non-selective cation channel standing in the membrane of the same trigeminal nerve endings that carry heat and pain. It is worth being exact about how TRPA1 is switched on, because the whole grammar of condiments falls out of its chemistry and nothing else.

Capsaicin, the molecule of chili heat, works by *fitting* — it slips into a pocket on its own channel like a key into a lock, a reversible embrace that can be washed away. The sharp molecules do not fit. They **react**. TRPA1 is not a lock but a tripwire of exposed sulfur atoms: a cluster of cysteine residues on the channel's large cytoplasmic N-terminal domain whose thiol groups (–SH) sit deprotonated and nucleophilic, waiting. An electrophilic agonist — a molecule hungry for electrons — forms a genuine **covalent bond** with one of those thiols, and that single new bond, repeated across a

few key cysteines, pulls the channel's gate open. The sensation of the sharp is, at the molecular level, a small chemical modification of a protein you are wearing on your tongue.

This is why the condiment plants converge on a short list of chemical warheads, all of them electrophiles of the same temperament:

- **Isothiocyanates** — the –N=C=S group of mustard, wasabi, and horseradish. The central carbon is starved for electrons; a cysteine thiol adds across it in a classic nucleophilic addition, and the channel opens.
- **Thiosulfinates** — chiefly **allicin**, the S(=O)–S warhead of crushed garlic, which hands its sulfur straight to the receptor's own sulfur in a thiol–disulfide exchange.
- **α,β-unsaturated carbonyls** — **cinnamaldehyde** in cinnamon, the **gingerols and shogaols** of ginger. Here the reaction is a Michael addition: the thiol attacks the carbon two bonds down from the carbonyl, forming a stable adduct.

Three different plant families, three different molecules, one grammar — because the receptor does not care what carries the electrophile, only how willingly that electrophile reacts. The more electron-poor the warhead, the faster it finds the cysteine, the lower the concentration needed to open half the channels, and the louder the reading on IFI-P. The sharp, in other words, is **electrophilicity made flavor**, and a molecule's position on the pungent axis is a near-direct readout of how badly it wants to bond with sulfur.



A clove in cross-section: the plant keeps the sharp disarmed, enzyme and fuel sealed in separate rooms until the knife breaks the wall between them.

The binary weapon inside the plant

A reactive electrophile is dangerous to its own maker; a molecule eager to bond with the sulfur in your receptors is just as eager to bond with the sulfur in the plant's own enzymes. So the condiment plants almost never store the warhead. They store a *fuse* and keep it, with exquisite care, apart from its *spark* — a binary weapon, armed only when the cell is broken.

In the brassicas — black and brown mustard, wasabi, horseradish, watercress — the fuel is a **glucosinolate**, a stable, water-soluble, utterly mild sugar-bound precursor locked in the cell's vacuole. The spark is **myrosinase**, a thioglucosidase enzyme held in separate protein-storing cells nearby. Intact, the leaf is bland. Crush it — with

a tooth, a grater, a mortar — and the two compartments bleed together. Myrosinase cleaves the sugar from the glucosinolate, and the unstable aglycone that remains rearranges in a fraction of a second into an **isothiocyanate**. Black and brown mustard carry **sinigrin**, whose product is **allyl isothiocyanate (AITC)** — small, volatile, nasal. White and yellow mustard carry **sinalbin**, whose product is **p-hydroxybenzyl isothiocyanate** — heavy, non-volatile, mouth-bound. Same enzyme, same instant of damage, two completely different condiments, and the plant chose the difference by choosing which glucosinolate to pack.

The alliums run the identical logic with different parts. Garlic stores **alliin** (S-allyl cysteine sulfoxide), odorless and stable, in the cytoplasm, and the enzyme **alliinase** sealed away in the vacuole. The clove, unbruised, barely smells. Crush it and alliinase finds alliin, and within ten seconds the roar of **allicin** rises — along with pyruvate and ammonia, the quiet byproducts of the same cut.

Cinnamon is the instructive exception that proves the rule. Its sharp, **cinnamaldehyde**, is *pre-formed* — it needs no enzyme, no act of damage; it is simply there in the bark oil, already reactive, already waiting. That is exactly why cinnamon behaves so differently on the plate: it cannot be dialed by resting, it survives a gentler brush with heat than the enzyme-made sharps, and it arrives the moment it touches the tongue. When we design a condiment, the first question we ask of a plant is always this one — *does it make its sharp on command, or carry it ready-made?* — because the answer sets everything that follows.



Cinnamon carries its sharp ready-made: cinnamaldehyde sits in the bark oil already reactive, needing no act of damage to arm it.

Reading the sharp as a number

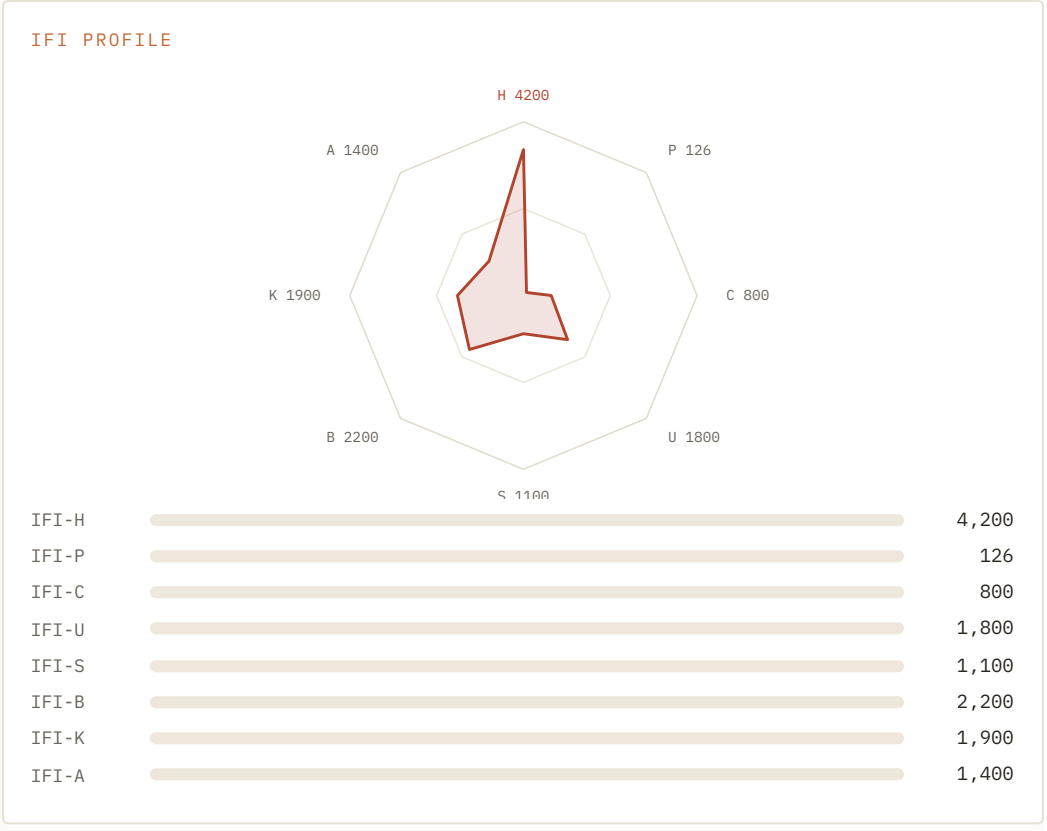
The Index exists so that "sharp" stops being an adjective and becomes a coordinate. The pungent reading of any food is its IFI-P, and IFI-P is built from the same formula that anchors every axis:

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

EC_{50} is the concentration at which an agonist drives TRPA1 to half of its full response — the midpoint of the dose–response curve. It is an inverse measure of loudness: the *lower* the concentration a molecule needs to reach half-activation, the more potent it is, and the *higher* its score. We define a convention constant of **2.1 µM** for the

reference electrophile on this axis — a stated definition, like the dilution threshold that anchors the Scoville scale — so that reference reads exactly 1,000, and everything else is scored against it. The arithmetic is deliberately plain, because a number you can compute in your head is a number you can trust. A candidate twice as potent as the reference — reaching half-activation at 1.05 µM — reads 1,000 × 2.1 ÷ 1.05 = **2,000**. One four times as potent, at ~0.5 µM, reads **4,000**. The axis is linear in potency, capped for the scorecards at a normalization maximum of **5,000**, and that is the whole instrument.

It is worth seeing the sharp rarely travels alone before we isolate it. On our full-spectrum cultivar **Phoenix**, designed to register on all eight axes at once, the pungent voice is one color inside a chord:



Read that P: 126 against the formula and it says the plant's pungent agonist reaches half-activation at 2.1 ÷ 0.126 ≈ 16.7 µM — a real, present sharpness, but a supporting one, woven under the great heat of H: 4,200. A condiment does the opposite. A condiment lifts one axis nearly alone and hands it to the plate undiluted, and so its scorecard is a spike, not a chord. To build that spike to a target, we first have to know where each molecule sits.

The ladder below gives representative bench EC_{50} values for the warheads of the condiment plants — measured in our TRPA1 activation assay — with the IFI-P each implies and a worked unit conversion, so the number can be traced from the channel all the way to a weight you could put on a scale.

AGONIST (SOURCE)	WARHEAD	MW (G/MOL)	EC_{50} (MM)	EC_{50} (PPM)	IFI-P	DELIVERY
Allyl isothiocyanate · black mustard, wasabi, horseradish	–N=C=S	99.15	5.0	0.50	420	volatile / nasal
Allicin · garlic	S(=O)–S	162.27	6.5	1.05	323	volatile / nasal
6-Shogaol · dried, warmed ginger	Michael	276.37	9.0	2.49	233	non-volatile / oral
p-Hydroxybenzyl ITC · white mustard	–N=C=S	165.21	11.0	1.82	191	non-volatile / oral
Cinnamaldehyde · cinnamon	Michael	132.16	14.0	1.85	150	semi-volatile
Reference electrophile	—	—	2.1	—	*1,000*	–
6-Gingerol · fresh ginger	Michael	294.39	23.0	6.77	91	non-volatile / oral

The ppm column is nothing but the EC_{50} in micromolar carried through the molecular weight: for AITC, 5.0 µmol/L × 99.15 g/mol = 4.96×10^{-4} g/L = 0.50 mg/L, and in dilute water a milligram per liter is a part per million. We show the conversion because a formulator works in parts per million on a bench, a receptor works in micromolar, and the two must be the same statement for the number to be honest.

One more property of the curve governs how the sharp *feels*, not just how loud it reads. The dose–response of these agonists is steep — cooperative, with a Hill coefficient of roughly 1.7 for AITC — so the response R climbs as

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

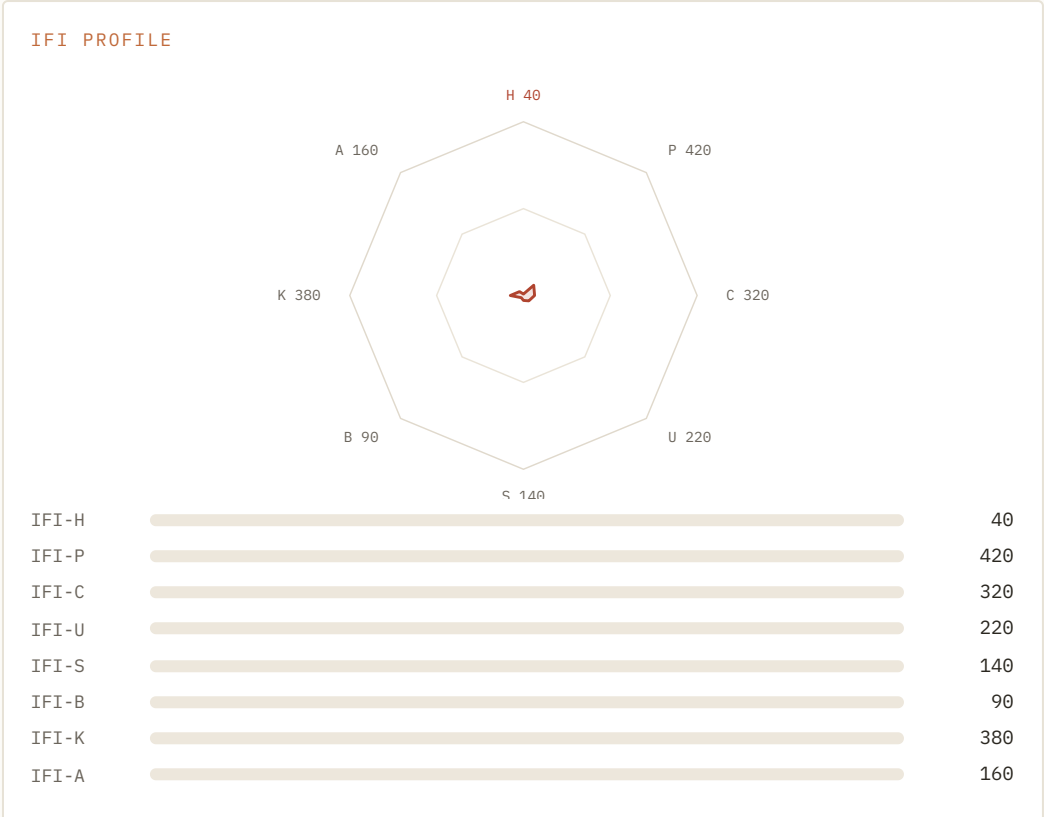
Put a dose at half the EC_{50} into that expression and the channel sits at about 24% of full; double the dose to twice the EC_{50} and it leaps to about 76%. A fourfold change in concentration swings the sensation from a whisper to a shout. That steepness is the sharp's signature on the tongue — the sense that pungency arrives all at once rather than fading up — and it is also a warning to the maker: near the knee of that curve, a small error in dose is a large error in experience. The cultivars we design for the sharp earn their keep here, because a narrow dose window is survivable only if the dose is reproducible.

The condiment ladder

The same molecules, read as plants rather than as warheads, give the working ladder a cook actually reaches for. The construct counts below are the live engineered libraries behind each organism; the IFI-P values follow from the agonist each plant deploys, and the delivery column previews the kinetics that the rules of the grammar turn on.

CONDIMENT PLANT	CONSTRUCTS	WARHEAD MOLECULE	MADE ON DAMAGE?	IFI-P	T_OFF
Black Mustard	2,790	allyl isothiocyanate	yes (myrosinase + sinigrin)	~420	< 30 s
Garlic	560	allicin	yes (alliinase + alliin)	~323	~45 s
Ginger	120	6-shogaol / 6-gingerol	no (pre-formed)	91–233	> 90 s
White Mustard	200	p-hydroxybenzyl ITC	yes (myrosinase + sinalbin)	~191	> 120 s
Watercress	120	phenethyl isothiocyanate	yes (myrosinase)	~200	~40 s
Cinnamon	40	cinnamaldehyde	no (pre-formed)	~150	> 90 s
Thyme	120	— (aromatic, trace sharp)	no	low	–

Black Mustard is the giant of the sharp — **2,790 constructs**, the deepest library we hold on the pungent axis — because it is the plant where the volatile, high-IFI-P route is most tunable: the sinigrin pool and the myrosinase activity are both separately adjustable, and between them they set both the amplitude and the clock. The profile of a finishing mustard designed from that library is a near-pure spike, pungency standing almost alone with only a faint echo on the cooler and richer axes:



That P: 420 is not asserted; it is the AITC line of the ladder carried through the formula, 1,000 × 2.1 ÷ 5.0 = 420, and the designed plant's job is to hit it batch after batch. Which brings us to the rules.

Rule one — heat kills the sharp

This is the first rule and the most violated. Three separate mechanisms conspire against a hot pan, and all three are consequences of the chemistry already laid out.

First, the **enzymes denature**. Myrosinase and alliinase are proteins, and like all proteins their folded shape unravels with temperature along an Arrhenius curve — the rate of denaturation climbing exponentially as $k = A \cdot e^{(-E_a/RT)}$. Both are largely dead above roughly 60 °C. Stir prepared mustard into a simmering sauce and you have killed the very enzyme that would have made more sharp; what tang survives is only what was already formed.

Second, the **volatile warheads evaporate**. Allyl isothiocyanate boils at 150 °C and carries a vapor pressure near 0.49 mmHg at room temperature — it is steam-distillable, which is to say an open hot pan strips it into the air within a minute. The non-volatile sharps (the p-hydroxybenzyl isothiocyanate of white mustard, the heavy gingerols) have negligible vapor pressure and survive heat far better, which is the first hint that *fast* and *slow* sharps want different treatment.

Third, the **thiosulfinates decompose**. Allicin is unstable even cold, degrading by first order as $[\text{allicin}]_t = [\text{allicin}]_0 \cdot e^{(-kt)}$. At a cool 23 °C its half-life runs to something like 16 hours; at a simmer the same decay accelerates roughly a thousandfold, collapsing the half-life to about a minute. A clove's roar, dropped into hot oil, is sweetened into nothing in the time it takes the pan to return to temperature — which is precisely why slow-cooked garlic turns mellow and nutty while raw garlic bites.

The designer's rule, then, is mechanical, not a matter of taste: if you want the P number to survive to the mouth, add the sharp **last and cool**, or protect it in fat and acid. Pungency is, with rare exceptions, a *finishing* flavor, because it is a reaction that heat runs to completion and then past it.

Rule two — acid keeps it, fat carries and governs it

The isothiocyanates and thiosulfinates are markedly more stable in an acidic medium. This is why mustard and wasabi have lived in vinegar for centuries, and why a jar of prepared mustard holds its sharp for months while fresh-ground mustard fades within a day. **Acid is the preservative of the sharp** — and, as the next rule shows, acid is also the switch that stops the enzyme and freezes the amplitude where the maker wants it.

Fat is the vehicle and the governor. The sharp molecules are lipophilic — AITC has a log P near 2.1, meaning it favors oil over water by about $10^{2.1} \approx 126$ to one. Dissolve the sharp into oil or butter and two things happen at once: the fat *carries* it evenly across every surface of a bite, and the fat *meters* it, because at any instant only the fraction in the water phase can reach the receptor. In a vinaigrette of three parts oil to one part water, the aqueous fraction at equilibrium is

```
f_water = V_water ⁄ (V_water + K·V_oil) = 1 ⁄ (1 + 126 × 3) ≈ 0.0026
```

— barely a quarter of one percent free at any moment, the rest held in reserve in the oil and released only as the fat wets and spreads across the tongue. That is why a fierce chili-garlic oil tastes gentler than its raw numbers promise: the fat is a reservoir paying out the

sharp slowly instead of all at once, rounding a brutal spike into a generous one without lowering the total dose. The designer's rule: **acid to keep the sharp, fat to spread and gentle it.**

Rule three — timing is a dial you can turn before serving

Because the brassicas and alliums *manufacture* their pungency only after damage, the cook holds a lever the plant surrendered at the moment of the cut: the clock. The enzyme runs at a finite rate, converting its substrate pool toward completion, and if we approximate that approach as first order the fraction of the pool converted after a rest of *t* minutes is

```
f(t) = 1 − e^(−t ⁄ τ), with τ ≈ 4 min for ground mustard at room temperature
```

Run the numbers and the folk wisdom becomes a dial with marks on it. Mustard acidified the instant it is mixed — call it half a minute of enzyme time — reaches f ≈ 1 − e^(−0.125) ≈ **0.12**, barely an eighth of its possible sharp: mild, a condiment for the cautious. Mustard ground and left to stand two minutes reaches f ≈ 1 − e^(−0.5) ≈ **0.39**. Rested the traditional ten minutes, it reaches f ≈ 1 − e^(−2.5) ≈ **0.92** — nearly the whole pool converted, a bright and finished sharp — *and only then* acidified to stop the clock, because vinegar at pH 2.5 drops the medium below the roughly pH 3–4 at which myrosinase falls silent. The acid does double duty: it preserves the AITC already made and freezes the conversion exactly where the cook chose to stop it.

Garlic obeys the same clock on a faster scale — crushed and rested a minute before it meets heat, it develops its full allicin; sliced straight into the pan, it barely bites, because the enzyme never got its ten uninterrupted seconds. This is the kitchen's version of our myrosinase lever: the enzyme runs whether a cook with a mortar or an engineer with a construct set its pace, and every sharp condiment therefore has an amplitude its maker chooses with nothing more exotic than a timer and a splash of vinegar.

Rule four — a fast nose and a slow tongue

The sharp arrives by two roads, and the kinetic difference between them is the single most useful thing a condiment maker can know. **Volatile** isothiocyanates — the AITC of black mustard, wasabi, and horseradish — leave the plate and go *up the nose*, reaching TRPA1 endings in the nasal passages retronasally, and because they evaporate they also clear fast: a sharp shock with an offset time constant τ_off under half a minute. **Non-volatile** sharps — the p-hydroxybenzyl isothiocyanate of white mustard, the heavy gingerols and shogaols of ginger — stay *in the mouth*, bind their cysteines, and build and linger with a τ_off well past a minute or two.



Ginger carries a slow, mouth-bound sharp: its gingerols and shogaols are heavy, non-volatile, and build on the tongue rather than flashing up the nose.

Deploy them accordingly. Where you want a bright shock that gets out of the way — a dot of wasabi beside raw fish, a streak of fresh horseradish under a rich slice — reach for the volatile, nasal sharp. Where you want pungency woven through the whole bite, a warmth that smoulders rather than flashes, reach for the non-volatile builder: white mustard in a dressing, grated ginger in a braise's final minute. A great condiment often pairs the two — a fast nose-opener laid over a slow tongue-builder, the attack and the sustain in one spoonful.

There is an honest subtlety worth naming here, because it is the kind of thing the Index must not paper over. Since TRPA1 is opened by a *covalent* bond, one might expect the sensation to persist as long as the adduct does — far longer than it actually does. It does not, because the felt offset, τ_off, is governed not by the slow chemistry of un-bonding but by two faster processes: the clearance of unreacted free agonist from the tissue, and the channel's own desensitization as calcium floods in behind it. τ_off tracks how fast the free molecule leaves and how fast the channel closes its own gate — which is why volatility, the thing that governs clearance, predicts the sharp's duration so well. The map from molecule to sensation is clean, but it runs through kinetics, not just through binding.

Building a condiment to a number

Put the grammar together and a condiment becomes a design problem with a specification at the top. Say the brief is a sinus-clearing wasabi finish: a pungent spike near the top of the axis and a clean, fast exit.

- **Target:** IFI-P ≈ 420, τ_off < 30 s, every other axis near silent.
- **Warhead:** the fast, volatile route — allyl isothiocyanate — ruling out the non-volatile sinalbin line at once. That choice alone sets the delivery to *nasal* and the τ_off to *short*.
- **Plant:** a high-sinigrin, high-myrosinase cultivar drawn from the **2,790-construct Black Mustard library**, where both the fuel pool and the enzyme rate are independently tunable, so the amplitude can be designed rather than hoped for.
- **Expected reading:** with the construct's AITC designed to half-activate TRPA1 at the ladder's 5.0 µM, our model predicts IFI-P = 1,000 × 2.1 ÷ 5.0 = **420**, on target.

- **Technique:** grind, rest ten minutes to reach f ≈ 0.92 of the sinigrin pool, acidify to pH 2.5 to lock the clock and preserve the AITC, keep cold, and serve as a dot — never stirred into anything hot.

SPEC	BRIGHT WASABI FINISH	HONEY MUSTARD
Route	sinigrin → AITC (volatile)	sinalbin → p-hydroxybenzyl ITC (non-volatile)
Source library	Black Mustard · 2,790	White Mustard · 200
Rest before acid	10 min (f ≈ 0.92)	2 min (f ≈ 0.39)
Acidify to	pH 2.5	pH 3.5
Target IFI-P	420	191
Second axis	—	IFI-S, real
τ_off	< 30 s	> 120 s

The honey mustard in the second column is the same problem solved for the opposite feel: a moderate, mouth-bound pungency that lingers under a true sweetness, so it reaches for the non-volatile warhead, a shorter enzyme rest, and a real contribution on the sweet axis to balance the sharp. One grammar, two conditions, two deliberately different plates.

None of this is worth anything if the number drifts, and here the engineered cultivars earn their place. The index is a ratio, and ratios inflate error, so it is fair to ask what the formula does to batch variance. Errors add in quadrature:

```
CV(IFI-P) = √[ CV(reference)² + CV(candidate)² ]
```

A tightly held reference standard carries a CV near 2%. Against it, one of our designed cultivars at a batch CV of **≤7%** yields $\sqrt{(0.02^2 + 0.07^2)} \approx$ **7.3%** — the ratio barely widens the error at all. At an IFI-P of 420 that is a one-sigma band of about ±31, roughly 389 to 451: a jar that tastes the same every time it is opened. Run the same arithmetic on a wild, unimproved plant at the 42% CV typical of nature — $\sqrt{(0.02^2 + 0.42^2)} \approx 42.1\%$ — and the band balloons to ±177, from about 243 to 597. That is the difference between a condiment you can specify and a condiment you can only hope for: not a louder sharp, but a *repeatable* one, which on a finishing flavor with a steep dose–response curve is the whole game.

Where the method bends — the honest limits

We would not trust an instrument that claimed never to be wrong, so the pungent axis comes with its boundaries drawn.

The receptor is not identical in every mouth. TRPA1 carries human genetic variation, and a handful of known variants shift its sensitivity to electrophiles up or down. IFI-P is anchored to a defined reference convention and reports a molecule's loudness against *that* standard; it is a property of the agonist, not a promise that two different people will rank two mustards the same way. The number is a coordinate on the molecule, and the last few percent of the journey runs through a receptor we did not design.

Electrophiles are not perfectly selective. A very reactive warhead at a high enough dose will nudge neighboring channels — TRPV1 among them — so an extreme sharp can carry a faint ghost of warmth that the single IFI-P reading does not capture. We treat that as a reason to report the whole eight-axis profile of a strong condiment, not to pretend the spike is perfectly clean.

The clock is a model, not a law. The first-order $\tau \approx 4$ min we use for a mustard rest is a working approximation; true myrosinase kinetics bend with temperature, grind size, water activity, and the particular construct, and a cook at altitude or in a cold kitchen will find their own τ. The dial is real; its exact markings move, and a careful maker calibrates once against their own bench rather than trusting a printed minute.

And the sharp is perishable by design. Everything that makes pungency vivid — that it is made on damage, carried by a reactive molecule, cleared fast from the tissue — also makes it fugitive. There is no shelf-stable way to hold a volatile isothiocyanate at full amplitude indefinitely; acid and cold and fat only slow the loss. We consider that a feature. The sharp is the plate's most alive element, and an element that is alive is one that can also fade. The grammar above does not abolish that; it tells you exactly how long you have, and how to spend it.

So this is the whole method of the sharp, and of the condiments to come. The grammar tells you how the molecules will behave once the plant is broken and the reaction begins; the index tells you where on the axis you are aiming; the designed cultivar — a fast bright black mustard, a long-trailing white one, a protected-allicin garlic — gives you a reproducible place to start, a sinigrin pool and a myrosinase rate you can count on at a CV under seven percent. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. Cook the sharp on purpose, to a number, with a plant built to hit it, and off the heat at the end where it lives. That is the craft. Now, the condiments themselves.

Chapter 14 · The mustards — *the condiment that makes itself.*

Mustard is the purest condiment on the axis — glucosinolate, enzyme, acid, salt, and time, and almost nothing else — which makes it the perfect place to begin cooking the sharp. There is no cooking in the ordinary sense: no heat, no reduction, no browning, no fat carrying flavor into a pan. There is only a seed, a little water, and a clock. Everything that happens, happens because we broke a wall inside the seed and let two things meet that the plant kept carefully apart. The three condiments in this chapter are built on our designed black- and white-mustard lines, but they work with any good mustard seed; what the designed seed buys is a *known* amplitude and shape, so the result lands where the scorecard says. That phrase — *known amplitude* — is the whole promise of engineering a condiment that otherwise makes itself, and by the end of this chapter it will be a number with an error bar, not a hope.



Black mustard seed and leaf — the whole small machine of the condiment.

The bomb in the seed

Hold a mustard seed between two fingers and it is silent. Dry, whole, intact, it carries no sharpness at all — you could keep a jar of seed in a warm cupboard for a year and open it to nothing but a faint nutty smell. The pungency is not *in* the seed the way sweetness is in a grape. It is a reaction waiting to be started, and the seed is built, with real botanical care, to keep it from starting by accident.

Inside the living tissue two components sit in separate compartments. The first is the glucosinolate — a sulfur-rich, water-soluble storage molecule the plant makes along the amino-acid

branch of its metabolism. In black mustard (*Brassica nigra*) and the brown mustard (*Brassica juncea*) that most "spicy" seed really is, the glucosinolate is **sinigrin**, allyl glucosinolate, carrying a short three-carbon allyl tail. In white or yellow mustard (*Sinapis alba*) it is **sinalbin**, p-hydroxybenzyl glucosinolate, carrying a heavier, ring-bearing, water-loving tail. These two tails are the entire difference between the nose-flash and the tongue-smoulder, and we will follow that difference all the way to the receptor.

The second component is the enzyme **myrosinase** (a thioglucoside glucohydrolase, EC 3.2.1.147), stored in its own specialized idioblasts — the myrosin cells — physically walled off from the glucosinolate by membranes. As long as the seed is whole, the enzyme and its substrate never touch. This is the mustard bomb: a loaded charge and a detonator kept in different rooms. Grinding the seed demolishes the walls. Cold water floods the rubble and dissolves both components into one solution, and the reaction fires.

Cold water is not an aesthetic choice; it is a condition for survival of the enzyme. Myrosinase is a protein, and like most proteins it denatures and loses its fold — and therefore its catalysis — when it is heated. Hot water would wet the ground seed, demolish the compartments, and then promptly kill the very enzyme that does the work, leaving a bland, muddy paste with no sharp at all. We start every mustard cold for exactly this reason: we want the detonator intact while the two rooms are joined.

From glucosinolate to sharp — the myrosinase reaction, step by step

What myrosinase does is simple to name and consequential to taste. It hydrolyzes the thioglucosidic bond — the sulfur-to-glucose linkage — at the head of the glucosinolate. The products of that single cut are one molecule of D-glucose and one unstable aglycone, a thiohydroximate-*O*-sulfonate. The enzyme is helped along by ascorbate (vitamin C), which acts as a cofactor and accelerant; a trace of it in the seed and in the water speeds the run. So far nothing is sharp. Glucose is sweet and the aglycone has no smell. The sharpness is made in the next step, and that step is spontaneous — it needs no enzyme at all.

The aglycone is too unstable to persist. At the near-neutral pH of plain water it rearranges, a Lossen-type rearrangement in which the sulfate group leaves and the nitrogen and the carbon tail reorganize into the cumulated double bonds of an **isothiocyanate** (R–N=C=S), releasing a bisulfate ion as it goes. The stoichiometry is clean and one-to-one: one glucosinolate consumed gives one isothiocyanate made. This matters later, because it means the ceiling on how much sharp a given mass of seed can produce is fixed by how much glucosinolate that seed carried — which is precisely the lever a designed seed line pulls.

The identity of the isothiocyanate is set entirely by the tail the glucosinolate was wearing:

- **Sinigrin → allyl isothiocyanate (AITC)**, $CH_2{=}CH{-}CH_2{-}N{=}C{=}S$, molar mass 99.15 g·mol^{-1}. Small, light, and *volatile*. This is the molecule of the sharp nose — the same compound that defines horseradish and wasabi — and it is why black-and-brown mustard flashes upward into the sinuses.
- **Sinalbin → p-hydroxybenzyl isothiocyanate (p-HBITC)**, molar mass 165.21 g·mol^{-1}. Heavier, ring-bearing, polar, and effectively *non-volatile*. It never reaches the nose because it never leaves the liquid; it sits on the tongue and lingers. This is the long, warm, building sharp of white seed.

There is one fork in this road worth knowing, because it is a quiet way a mustard can go wrong. If the solution is already acidic when myrosinase makes the aglycone, or if a plant's epithiospecifier protein (ESP) and a little ferrous iron are present, the aglycone can be shunted away from the Lossen rearrangement and toward **nitriles**, thiocyanates, or epithionitriles instead of the isothiocyanate. Nitriles are far less pungent. This is the chemical reason acid *before* the enzyme has run gives a milder mustard: you are not only slowing the enzyme, you are rerouting its product away from the sharp. Acid *after* the run has finished does the opposite job — it locks what was made. We will return to this as a dial you can set by the clock.

The channel it opens — TRPA1 and the chemistry of a covalent sharp

An isothiocyanate on the tongue does something categorically different from sugar or salt. It is not read by a taste bud. Sweetness, savor, and the rest are *gustation* — molecules settling reversibly into G-protein-coupled receptors on the taste cells. Mustard's sharp is *chemesthesis*: the irritation of bare nerve endings, the trigeminal fibers that also report heat, cold, and pain. The receptor at the center of it is **TRPA1**, the transient receptor potential ankyrin-1 channel — the "wasabi receptor" — a non-selective, calcium-permeable cation channel studded along the sensory neurons of the mouth, tongue, and nasal lining. When it opens, calcium and sodium rush in, the neuron fires, and the brain reads *sharp*. This is also why mustard's intensity is scored on the pungency axis (IFI-P, anchored to TRPA1) and never in Scoville heat units: Scoville measures capsaicin against a different channel entirely, and an isothiocyanate registers essentially nothing there. A hot English mustard can bring tears to your eyes and read zero on a Scoville scale, because it is speaking to a different door.

The way AITC opens that door is the most important chemistry in this chapter, because it is unlike almost every other flavor molecule in the library. AITC is an **electrophile** — the central carbon of the N=C=S group is hungry for electrons. TRPA1 carries, on its cytoplasmic N-terminal, a set of reactive cysteine residues (in the human channel, cysteines around positions 621, 641, and 665, with a nearby lysine at 710). The sulfur of a cysteine thiol attacks the electrophilic carbon of AITC in a Michael-type addition, forming a **covalent** adduct. That bond, not a gentle docking, is what drags the

channel open. Allyl isothiocyanate does not sit in a pocket and leave; it chemically bonds to the protein and changes its shape from the inside.

This is a different kind of sharpness than capsaicin's reversible warmth, and it has two consequences you can taste. First, it explains the *shape* of the sensation — fast, bright, almost startling — and why it can be slow to fully clear, because the adduct must be undone or the channel protein turned over before the signal fully quiets. Second, it is a genuine complication for any attempt to put a tidy equilibrium number on the molecule, which we will be honest about when we get to the math: a covalent agonist does not have a clean, time-independent binding constant the way a reversible one does.



Garlic, cut — allicin is mustard's cousin at the same channel, a different electrophile knocking on the same door.

Mustard is not alone at this receptor, and knowing its neighbors sharpens the picture. Allicin from cut garlic, cinnamaldehyde from cinnamon bark, and the thiosulfinates of onion are all electrophiles that modify the same TRPA1 cysteines; they are chemical cousins, each a different reactive group knocking on the same door, which is exactly why they share the pungency axis and read against one another on a single scale. The family's vegetarian table has leaned on these plants for as long as any of us can remember — the garlic, the mustard, the ginger — and it is a quiet pleasure to find, when you finally read the biology, that the sharpness we loved in all of them was one receptor's single, repeated answer.

Putting a number on the sharp

The Iyengar Flavor Index exists to turn that answer into something you can measure, print, and design toward. The formula is the same across every axis:

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

EC_{50} is the concentration at which a stimulus drives its receptor to half of its full response. Loudness is the *inverse* of the concentration it takes to be heard: a molecule that half-opens TRPA1 at a low concentration is loud; one that needs a high concentration is quiet. Divide a fixed reference EC_{50} by the candidate's and scale by a thousand, and loudness becomes a plain score.

The three mustards in this chapter carry pungency scores of **IFI-P 109** (the bright table mustard), **63** (the whole-grain), and **143** (the hot English). Those numbers alone, without any further measurement, already tell us the *ratios* of their receptor potencies, because the formula is a simple inverse. Rearranged for the candidate concentration:

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

The hot English, at 143, half-activates TRPA1 at a concentration **143 / 63 = 2.27×** lower than the whole-grain at 63 — it is a bit more than twice as loud, molecule for molecule, and the two scores say so before we ever name a unit.

To put the units in, the Index pins its IFI-P reference — the stimulus that reads exactly 1,000 — to a half-activation concentration we hold fixed as the axis's calibration constant by convention. Written in SI, that convention constant is **EC_{50}(reference) = 2.1 µmol·L^{-1}** of allyl isothiocyanate at a standardized TRPA1 exposure. Like the Scoville scale's dilution-to-threshold definition, this is a stated convention — not a value derived from the published literature — chosen so that AITC, the principal mustard pungent, anchors the P axis at IFI-P 1,000. (We fix the exposure window deliberately; the reason is in the honest-limits section below.) With the anchor set, the three scores convert straight to half-activation concentrations, and from there to a mass concentration using AITC's molar mass of 99.15 g·mol^{-1}:

MUSTARD	IFI-P	EC_{50} (mmol·L^{-1})	EC_{50} (mg·L^{-1})	T_ON	T_OFF	SHARE OF 5,000-AXIS SCORECARD
Hot English-style	143	14.7	1.46	3 s	35 s	3 %
Bright table	109	19.2	1.91	4 s	40 s	2 %
Whole-grain	63	33.3	3.30	8 s	90 s	1 %

The arithmetic of a single row, worked in full so the method is never a black box: for the bright table mustard, EC_{50} = 1,000 × 2.1 ÷ 109 = 19.3 µmol·L^{-1}; converting, 19.3 µmol·L^{-1} × 99.15 µg·µmol^{-1} = 1,913 µg·L^{-1} = 1.91 mg·L^{-1}. The lower the concentration in that column, the louder the mustard, which is why the table reads in the opposite order from the IFI score — a satisfying check that the inversion is working. And because the scorecard radar normalizes every axis to a ceiling of 5,000, the hot English fills 143 / 5,000 = 2.9 % of the pungency spoke — a measured presence on the axis — while the whole-grain's 1.3 % reflects the gentler chemistry of its non-volatile isothiocyanate.

A score is a point; eating is a curve. The approach to that half-activation point follows a dose–response of the Hill form,

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

with TRPA1 showing mild positive cooperativity — take an illustrative Hill coefficient n ≈ 1.3. For the hot English (EC_{50} 14.7 µmol·L^{-1}), the fraction of maximal channel response climbs steeply through the midpoint: at a quarter of EC_{50} the response is about 14 %, at half it is 29 %, at EC_{50} exactly 50 %, at twice EC_{50} it has already reached 71

%, and at four times EC_{50} it is 86 % and flattening toward saturation. That steepness is the reason a hot English is a *streak*, not a spoon: the useful dynamic range of the sensation is compressed into a roughly eight-fold window of concentration, and past it, more mustard buys almost no more sharp — only more of everything else on the plate drowned out. The curve, not the single EC_{50}, is what your tongue is actually climbing.

Reading the seed lines — what the designed seed buys

A condiment that makes itself still begins with a seed, and the seed is where we do our work. Our two designed lines differ not in the chemistry of the reaction — that is nature's, and we leave it exactly as it is — but in the amount and the consistency of the glucosinolate the seed lays down, and in how tightly that loading holds from batch to batch.

SEED LINE	SPECIES	PRIMARY GLUCOSINOLATE	HYDROLYSIS PRODUCT	VOLATILITY	CONSTRUCTS	ROLE ON IFI-P
Designed black (bright line)	*Brassica nigra* / *juncea*	sinigrin	allyl isothiocyanate	volatile	2,790	the nose-flash; top of the axis
Designed white	*Sinapis alba*	sinalbin	p-hydroxybenzyl isothiocyanate	non-volatile	200	the tongue-smoulder; long tail



Reading the glucosinolate locus on the bench — where a seed line's amplitude is fixed before a single jar is made.

The deeper point hides in a quick stoichiometric sanity check, and it is the key to why *timing*, not *titre*, is the cook's real instrument. A black-and-brown mustard seed carries sinigrin on the order of a hundred-and-some micromoles per gram — call it, as an order-of-magnitude illustration, 150 µmol·g^{-1}. Put 60 g of such seed into roughly 75 mL of cold water, as the hot English does, and complete hydrolysis would liberate 60 × 150 = 9,000 µmol of AITC into 0.075 L — a nominal 120,000 µmol·L^{-1}, or 120 mmol·L^{-1}. Set that against the 14.7 µmol·L^{-1} it takes to half-open the channel and the ratio is roughly **8,000 to 1**. The seed holds thousands of times more sharp than the receptor could ever need. The channel saturates many times over long before the glucosinolate is spent.

This is the quiet truth under the whole craft: the eating experience is *never* limited by how much AITC the seed can make. It is governed by the *rate* at which the enzyme makes it, by how much of the volatile fraction escapes before it reaches you, by dilution and by how the acid freezes the run. That is why two mustards from nearly the same seed can land a thousand IFI-P points apart. The amplitude is a kinetic outcome, set by the clock and the acid, not a stockpile. We design the seed to carry a dependable charge; the cook decides how much of it is spent, how fast, and when the fuse is cut.

Dependability is the part worth a number of its own. A commodity seed is a genetic smear — its glucosinolate loading wanders with weather, soil, and provenance — and that scatter shows up as a wide batch-to-batch coefficient of variation, on the order of **42 %** in uncontrolled stock. Our designed lines are built to hold loading to a batch CV of **≤ 7 %**. Because IFI-P is proportional to $1/EC_{50}$ and EC_{50} tracks the delivered isothiocyanate, that fractional scatter propagates almost directly onto the score: for a division by a fixed reference, the relative error of the IFI simply inherits the relative error of the concentration (and if the calibration anchor itself carried, say, a 3 % uncertainty, the two would add in quadrature, $\sqrt{(7^2 + 3^2)} \approx 7.6$ %). The practical difference is stark:

METRIC	DESIGNED BRIGHT LINE (CV ≤ 7 %)	COMMODITY BROWN SEED (CV ≈ 42 %)
Target IFI-P (bright table mustard)	109	109 (nominal)
1-σ band on IFI-P	101 – 117	63 – 155
1-σ band on EC_{50} (μmol·L^{-1})	17.9 – 20.6	13.5 – 27.3

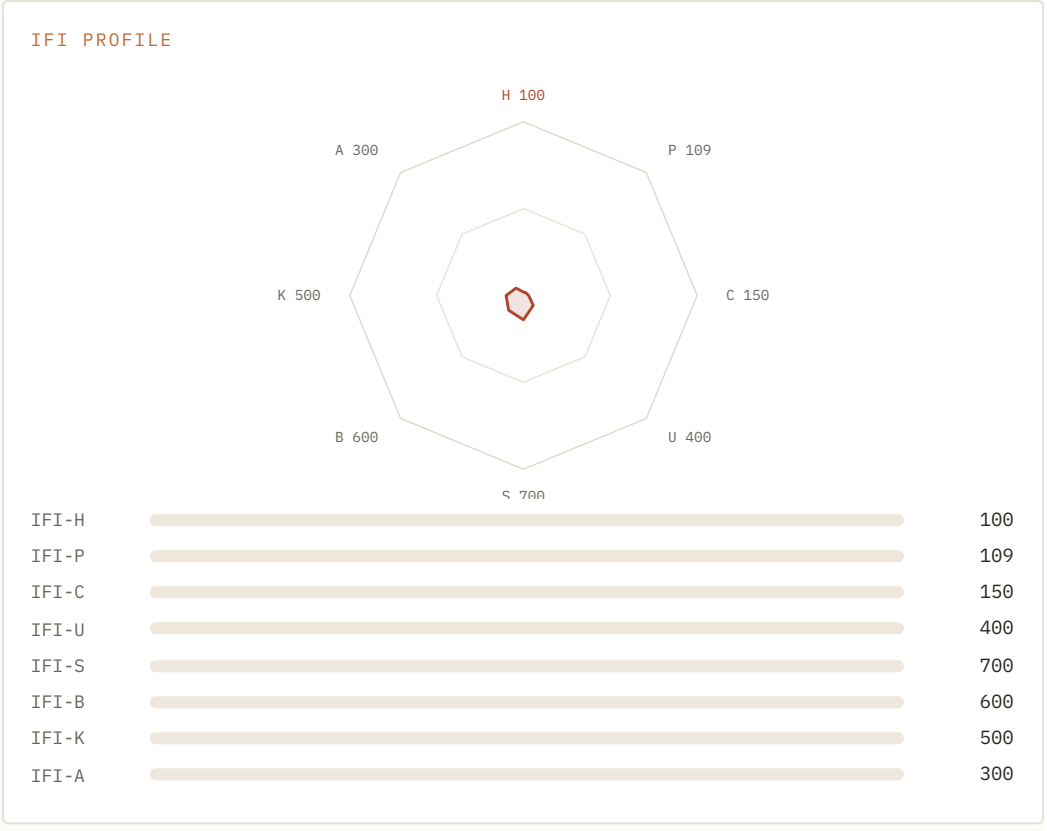
The commodity seed's sharp is a wash that can land anywhere from a mild table mustard to something approaching the hot English, jar to jar. The designed line lands in a band you could set a recipe by. *Known amplitude and shape, so the result lands where the scorecard says* — that is what the sentence meant, carried out to its arithmetic.

A bright table mustard

The fast, clean, nose-forward one, built on the designed black-mustard line for a pure AITC sharp that flashes and clears. Everything about its profile — the 4-second onset, the 40-second clearance, the 109 on pungency — follows from a single decision: let myrosinase run in plain cold water for a measured rest, then stop it with acid before it over-runs.

```
40 g  designed black mustard seed (bright line), or brown mustard seed
15 g  white mustard seed
80 ml cold water
45 ml white wine vinegar
4 g   fine salt
1 tsp  honey (optional, to round)
```

1. Grind both seeds coarsely, then cover with the cold water and let stand **10 minutes.** The cold water is the trigger — it is the damage that fires the mustard bomb — and the rest lets myrosinase run, building allyl isothiocyanate to its peak. Do not use hot water; it would denature the enzyme and the sharp with it.
2. After the rest, add the vinegar and salt. The acid now **locks** the sharp: it slows further enzyme action, steers any remaining aglycone away from the sharp, and stabilizes the isothiocyanate already made, fixing the amplitude where the rest left it. Add the vinegar earlier for a milder mustard, later for a fiercer one — this is your timing dial.
3. Blend to the texture you like — smooth, or leave some cracked seed for a grainy version. Stir in the honey if using.
4. Jar it and rest **24 hours** before serving; the raw-edged aggression settles into a bright, clean, rounded sharp. It keeps for months refrigerated, the acid holding the line.



τ_on 4 s · τ_off 40 s · nose-forward, fast clear · add vinegar late for more P

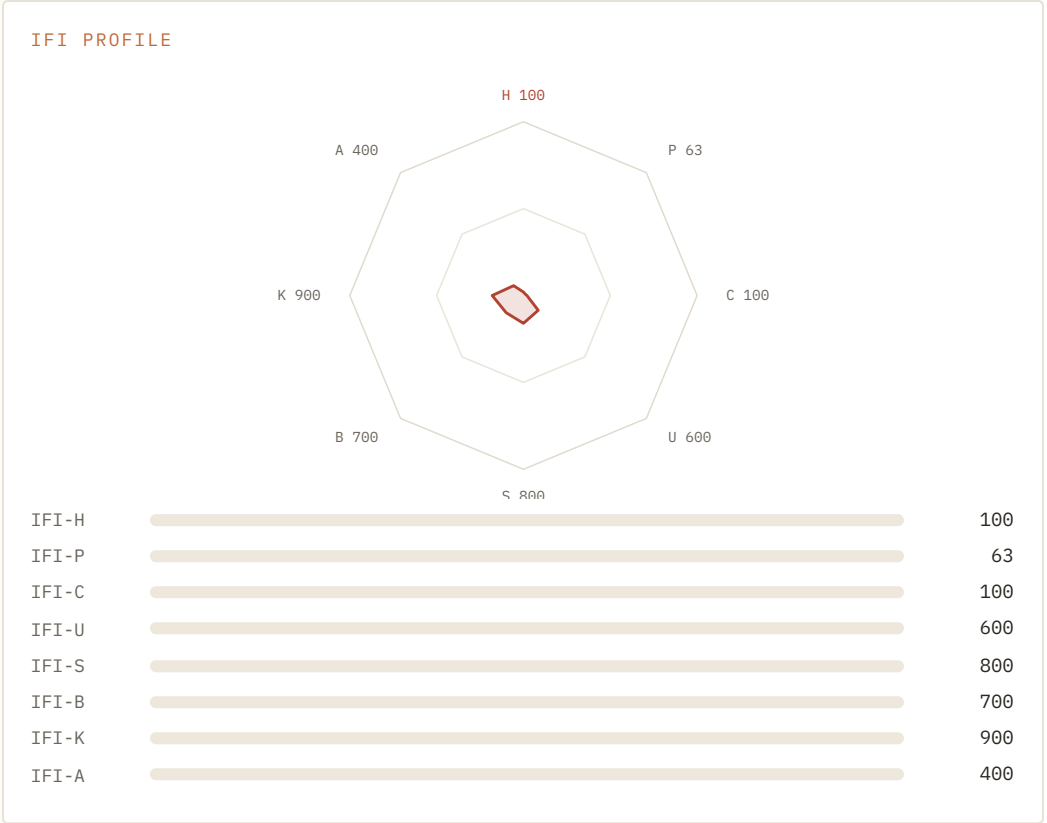
A whole-grain mustard

Slower, rounder, built to sit against roasted vegetables or meat, the white seed's non-volatile sharp giving it a long tongue-smoulder under the pop of whole seed. This is the chapter's one mustard led by sinalbin, and its lower pungency score — 63, the gentlest of the three — is not a weakness but a chemistry. The p-hydroxybenzyl

isothiocyanate it makes is heavier and water-bound; it never volatilizes into the nose, so it cannot deliver the startle of the bright table version. What it delivers instead is duration — the 90-second clearance of a molecule that stays in the mouth and keeps gently modifying the channel long after a volatile sharp would have blown off.

```
50 g  white mustard seed
30 g  designed black mustard seed (bright line)
90 ml cider vinegar
40 ml cold water
5 g   salt
1 tsp  maple or honey
```

1. Combine seeds, vinegar, and water, and soak **overnight** — the seeds swell and soften and the bomb begins its slow work in the cold acid, which here is present from the start to keep the sharp gentle and building rather than fierce. Acid in the water from the first minute is deliberate: it both slows myrosinase and diverts a share of the product away from the isothiocyanate, so the run plateaus low.
2. Pulse about half the mixture to a coarse paste, leaving the rest whole for the burst of texture. Stir in salt and sweetener.
3. Rest **2 days** before eating. The white seed's p-hydroxybenzyl isothiocyanate — non-volatile, tongue-sitting — gives this mustard its long, warm, lingering sharp, very different from the nose-flash of the bright table version; the black seed adds a top note of brightness over it.



τ_on 8 s · τ_off 90 s · tongue-building, lingering · white-seed led

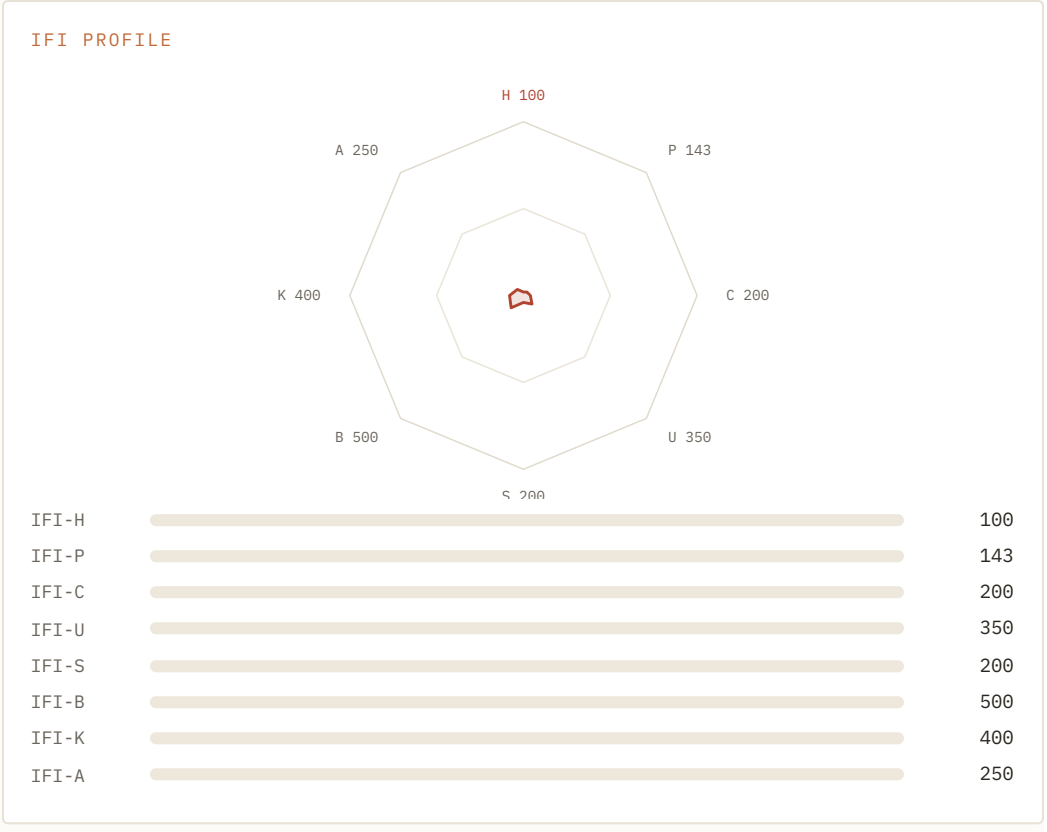
Hot English-style mustard

The fierce one, nearly all bright black seed, minimal acid, maximum nose. This is the sinus-opener of the mustard world, meant in small streaks — and its 143 on pungency, 2.9 % of the entire axis on the scorecard, is earned by doing the opposite of the whole-grain at every step: almost no acid, almost no dilution, an uninterrupted enzymatic run, and the lightest, most volatile isothiocyanate carried

straight to the nasal TRPA1. The steep dose–response works against you here if you are heavy-handed; a streak already pushes the channel most of the way up its curve.

```
60 g  designed black mustard seed (bright line)
10 g  white mustard seed
75 ml cold water
8 ml  white wine vinegar (a restrained splash)
3 g   salt
pinch turmeric (colour, optional)
```

1. Grind to a fine flour and whisk with the cold water to a smooth paste. Rest **15 minutes** — the full enzymatic run, uninterrupted by acid, for maximum allyl isothiocyanate.
2. Add only the small splash of vinegar and the salt. Keeping the acid minimal leaves the sharp at its most aggressive and volatile; this mustard is meant to be made fresh and used within a day or two, when its nose is at its fiercest.
3. Serve in small amounts beside rich, fatty, or roasted things — the fat on the plate will meter the ferocity into something generous.



τ_on 3 s · τ_off 35 s · maximum nasal flash · make fresh, use fast

The timing dial, in numbers

Three recipes, one seed chemistry, and the entire spread from 63 to 143 comes down to *when the acid arrives.* It is worth making that dial quantitative, because it turns a piece of kitchen lore into something you can reason about.

Myrosinase has a pH optimum in the gentle band around 6 to 7 — close to plain water — and its activity falls off sharply as the solution turns acidic, roughly below pH 5. Acid does three things at once, all of them reducing the sharp: it slows the enzyme's catalytic rate; it tilts the unstable aglycone away from the Lossen rearrangement that makes the isothiocyanate and toward the far milder nitriles; and, once the sharp *is* made, it stabilizes the isothiocyanate against further hydrolysis. The first two suppress production; the third preserves a finished yield. Which effect dominates depends entirely on *timing* — whether the acid is in the pot while the enzyme is still working, or arrives only after the work is done.

Sketch the accumulation of AITC as a simple first-order approach to a ceiling during the plain-water rest,

```
A(t) = A_plateau × (1 − e^(−k·t)),
```

and treat the addition of acid at time t_acid as a valve that effectively truncates production — freezing A at whatever value it had reached. The three mustards are then three settings of one valve:

- **Hot English** runs in near-plain water (only a restrained splash of vinegar, added last) for a full 15-minute rest. The valve stays open the longest, acid never suppresses the run, and A climbs closest to its plateau — the highest delivered AITC, the volatile fraction intact, IFI-P **143**.
- **Bright table** runs in plain cold water for 10 minutes, then the acid arrives in full and truncates the run. A respectable but slightly shorter climb, locked clean — IFI-P **109**.
- **Whole-grain** has the acid present *from the first minute*, in the soaking liquid. The enzyme never gets a neutral window; production is slowed and partly diverted to nitrile for the whole run, and the sharp is further softened by being carried on the non-volatile white-seed isothiocyanate. The valve is throttled from the start — IFI-P **63**.

The τ values in each profile footer are the other half of the same story, read at the receptor rather than in the pot. τ_on, the onset, is fastest (3–4 s) for the volatile AITC that reaches nasal and retronasal TRPA1 almost on contact, and slower (8 s) for the tongue-bound p-HBITC that must act where it sits. τ_off, the clearance, is quick (35–40 s) for the volatile sharp that blows off and whose covalent adducts turn over, and long (90 s) for the heavy, lingering white-seed molecule that neither evaporates nor leaves in a hurry. Onset and clearance are not seasoning; they are the volatility and the covalent kinetics of two different isothiocyanates, printed as seconds.

Failure modes and honest limits

A method is only as trustworthy as its account of how it breaks, so here are the ways a mustard goes wrong and the places our number stops being a perfect description of the world.

Hot water kills the mustard. The most common failure is the most basic: wetting ground seed with warm or hot water denatures myrosinase before it can run, and no amount of resting will bring the sharp back. The paste will taste raw, bitter, and dull. Cold water, always.

The white-seed sharp is perishable in a way the number does not show. p-Hydroxybenzyl isothiocyanate is genuinely unstable; over hours to days it decomposes to p-hydroxybenzyl alcohol and a thiocyanate ion, both of which are effectively flavorless on the pungency axis. A fresh whole-grain mustard that read 63 will drift downward as it ages, more than a sinigrin-based mustard of the same age, because its carrier molecule is quietly falling apart. The acid and cold of the jar slow this, but do not stop it.

Acid at the wrong moment diverts the sharp. Because low pH reroutes the aglycone toward nitriles, a cook who salts an acidic environment too early — or who uses a very acidic vinegar in the soak — can lose far more pungency than intended, and wonder why a mustard built from potent seed came out meek. The seed was never the problem; the valve closed before the sharp was made.

The hot English is a clock, not a keeper. A mustard whose entire character is volatile AITC begins losing that character to the air the moment it is made. This is why the recipe insists on *make fresh, use fast*: left a week, the fierce nasal flash fades toward a flat, vinegary paste as the volatile fraction escapes. The sharpest mustards keep the worst.

And the honest limit on the number itself. Everything in the IFI-P column rests on an EC_{50}, and EC_{50} is a concept borrowed from reversible, equilibrium pharmacology — a molecule that binds, lets go, and settles into a steady fraction of bound receptors. Allyl isothiocyanate does not play by those rules. It is an electrophile that forms a *covalent* bond to the channel's cysteines, so the fraction of channel activated keeps climbing with exposure time rather than settling to a fixed equilibrium. A true, time-independent dissociation constant does not exist for it. What the Index reports, therefore, is an *operational* EC_{50}: the half-activating concentration assessed under a single, fixed exposure window and temperature that we standardize and never vary between candidates. Hold the assay constant and the number is rigorously reproducible and perfectly comparable across mustards. But it is a calibrated potency index built on a stated convention constant, not a thermodynamic constant, and we would rather say so plainly than let the tidiness of a single number imply a physics it does not have. Individual tongues vary too: TRPA1 carries human sequence variants, and a mustard that reads 143 on our standardized panel will land differently on a receptor that is a few amino acids different from the reference.

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

One last line, offered without any claim: the isothiocyanates that make all of this sharp are the same compounds that have kept Brassica at the center of a vegetarian table for as long as our family has set one, and we grow these seed lines to carry the fullest, most

dependable glucosinolate charge they can. We make no claim for them beyond the pleasure and the sharpness — only that a seed grown with intention, and read before it is ever ground, is a better place to begin.

Three mustards, one chemistry, three points on the map of loudness and shape — bright-and-fast, slow-and-building, fierce-and-fleeting — each reachable by nothing more than the choice of seed line, the timing of the acid, and the clock on the rest. The mustard is the condiment that makes itself, and the designed seed is simply a maker you can trust to start in the same place every time.

Chapter 15 · Wasabi, horseradish, and the sinus-clearers — *the fast sharp, up the nose.*

The fiercest, fastest sharps on the axis are the volatile isothiocyanates of wasabi and horseradish — the ones that bypass the tongue almost entirely and detonate behind the eyes, then clear in twenty-five seconds flat. They are the condiments of the raw and the rich: a dot beside sashimi, a streak under a slice of roast, a cream to cut through fat. Real wasabi is one of the hardest crops in the world to grow, which is why almost everything sold as wasabi is dyed horseradish; our designed black-mustard and brassica lines, tuned toward the long-chain sulfinyl character, give a third route — the wasabi *signature* from a tractable plant. These recipes lean on that chemistry.

There is a particular honesty to this corner of the pungent axis. Heat, the capsaicin kind, lingers and spreads and asks to be endured; this sharp arrives and leaves like a struck match, and it does its work in a place heat never reaches — high in the nasal passages, behind the bridge of the nose, in the trigeminal tissue that also reports cold air and the sting of a cut onion. To cook with it well you have to understand that you are not seasoning the tongue at all. You are firing a volatile, reactive little molecule into the air of the sinuses and asking a single channel to count it. Everything that follows — the grating, the resting, the folding into fat — is the management of that one reaction.



A cross-section of the brassica sharp — the volatile chemistry behind the nose-flash.

The wasabi receptor, and why the burn goes up

The channel is TRPA1, and it is so defined by this flavor that the scientists who first characterized it reached for the same word we would: it is, in the literature and in our own lab notebooks, *the wasabi receptor.* It sits on the free endings of the trigeminal and vagal nerves — the raw, listening tips that line the nose, the mouth, the throat — a non-selective cation channel that, when it opens, lets

sodium and calcium pour inward and the neuron shout. Cold can open it. A bruised onion can open it. But nothing opens it as cleanly, or as instructively, as an isothiocyanate.

Here is the part that makes this sharp unlike any other on the index. Capsaicin works on its own receptor the way a key works a lock — it slides into a pocket on TRPV1, holds, and slides back out; the burn is intense but the binding is reversible, a matter of shape. The isothiocyanate does not bother with shape. Its business end, the –N=C=S group, is a soft electrophile: an atom hungry for electrons, looking for something to react *with.* On the inner, cytoplasmic face of TRPA1 there are a handful of residues offering exactly that — in the human channel, three cysteines and a lysine (C621, C641, C665, and K710) whose sulfur and nitrogen are nucleophilic enough to attack. The isothiocyanate finds one and forms a covalent bond — a thiocarbamoyl adduct — and the channel, tugged from within, springs open. This is not a fit. It is a small, deliberate chemical reaction, run a few thousand times a second on the surface of a nerve.

And that is why the sharp goes *up.* Allyl isothiocyanate — the molecule at the heart of wasabi and horseradish and black mustard — is tiny, ninety-nine grams per mole, and volatile; grate the root and it leaves for the air almost at once. Capsaicin, by contrast, is a heavy, oily thing of three hundred grams per mole that never meaningfully evaporates, and so never leaves the tongue it is painted on. The two molecules choose different rooms. Capsaicin stays in the mouth and burns low and long; the isothiocyanate rises as a vapor into the nasal cavity, finds the trigeminal endings crowded there, and fires them all at once — the flash behind the eyes, the involuntary clearing, the tears. Real wasabi carries a second warhead with it, a longer-chain molecule — 6-methylsulfinylhexyl isothiocyanate, six carbons and a sulfinyl shoulder — that is heavier, slower to leave, and stays nearer the tongue, laying down the green, lingering trail beneath the flash. One root, two kinetics: the fast allyl rush and the slow sulfinyl echo.

The sealed weapon — glucosinolate meets myrosinase

None of this chemistry is sitting ready inside the living root, because a plant that stored a loaded electrophile in its own tissue would poison itself. Instead the brassicas keep the sharp as two halves, walled apart, and trust the architecture to hold until something tears it.

In the intact cell the pungency waits as a glucosinolate — a stable, water-soluble, entirely flavorless sugar-bound precursor (in black mustard and horseradish it is largely sinigrin, allyl glucosinolate). The enzyme that can wake it, myrosinase, is kept in separate cells entirely, a thioglucosidase held apart like a detonator stored across the room from the charge. The plant's defense, and our whole flavor, depends on that separation holding until damage breaks it. A grater, a knife, a molar — any real wound to the tissue — ruptures the walls, and enzyme meets substrate.

What follows is quick and worth drawing exactly. Myrosinase hydrolyzes the thioglucosidic bond, snapping glucose off the glucosinolate and leaving an unstable aglycone, a thiohydroximate-*O*-sulfonate. That intermediate cannot hold; it sheds its sulfate and undergoes a Lossen rearrangement — the carbon–nitrogen skeleton reorganizing — and out of it falls the isothiocyanate. One glucosinolate molecule, cleaved and rearranged, yields one molecule of the sharp, plus a glucose and a sulfate ion. This one-to-one reaction is the reason the whole family of condiments must be made by *damage,* and made *fresh.* No cut, no sharp; and once cut, the clock starts.

The arithmetic of that yield is sobering in how little it leaves room for. Sinigrin, as its potassium salt, weighs about 397 grams per mole; the allyl isothiocyanate it releases weighs 99. So even with a perfect enzyme and no losses, a gram of pure precursor can become at most 99 ÷ 397 — about 0.25 g, a quarter of its own mass — of actual sharp. The real yield is lower still, and understanding *why* is understanding every way this flavor fails.

Three things steal it. The first is **heat**: myrosinase is a protein, and a cook who grates horseradish into a hot pan or a boiling sauce denatures the very enzyme that makes the pungency — the precursor passes through intact and tasteless. This is exactly why these are cold, raw, last-minute condiments and never simmered ones. The second is **pH**: the clean Lossen rearrangement to an isothiocyanate wants near-neutral conditions; in sharply acidic surroundings the same aglycone collapses instead toward a nitrile, a dull and faintly bitter dead end with none of the nasal flash. That is why the vinegar in these recipes is measured in a few millilitres — enough to stabilize and brighten, never enough to drag the reaction into silence. The third is the plant's own chemistry: some brassica tissues carry an epithiospecifier protein that diverts the aglycone toward epithionitriles rather than the isothiocyanate, trading the sharp for nothing you want. In our designed lines that diversion is one of the first things we breed down — a low-epithiospecifier background, so that nearly every glucosinolate we build becomes the warhead we intend and not a quiet nitrile.

Putting a number on the sharp



The bench where loudness becomes a number — every sharp read as the concentration at which its channel half-opens.

Reverence is not the same as vagueness, and the whole point of the index is that a sharp this fleeting can still be pinned to a number. The quantity we characterize is not how much isothiocyanate a food contains — a gram of sealed precursor and a gram of freed warhead are not the same event to a nerve — but how loudly the food speaks to the channel. The unit of loudness is the EC_{50}: the concentration at which the stimulus drives TRPA1 to half of its full response. A molecule that half-opens the channel at a low concentration is loud; one that needs a flood to do the same is quiet. Invert the EC_{50} against a fixed reference, scale by a thousand, and you have the pungency score:

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

We set the reference by convention at a half-activating concentration of 2.1 µM — a stated convention, like the dilution definition at the heart of the Scoville scale, not a measured literature value. The AITC stimulus at 2.1 µM is what we *define* as IFI-P 1,000, the needle's honest middle. Everything reads against it. A preparation that half-opens the channel at 2.1 µM scores 1,000 by convention; one that needs only half as much, 1.05 µM, is twice as loud and scores 2,000; one that needs a tenth, 0.21 µM, scores 10,000 and runs off the top of the scorecard, whose per-axis ceiling we hold at 5,000.

Because the formula is only a ratio, it runs backward as readily as forward, and running it backward is how you hear what the receptor actually meets in each of these condiments. Rearranged, $EC_{50}(\mathrm{candidate}) = 1{,}000 \times 2.1\ \mu M \div \mathrm{IFI\text{-}P} = 2{,}100 \div \mathrm{IFI\text{-}P}$, in micromolar. The fresh wasabi-style paste, at IFI-P 756, is therefore a stimulus of $EC_{50} \approx 2.78\ \mu M$ — the channel already half-open at under three micromolar of delivered sharp. The horseradish cream, at 378, sits at 5.56 µM. The sinus-clearing dip, at 630, at 3.33 µM. Put those concentrations into the units of the kitchen and the smallness turns almost tender: 2.78 µM of allyl isothiocyanate is about 0.28 milligrams in a litre. A quarter of a milligram per litre is already half of what the receptor can give. A single gram of the pure molecule, perfectly delivered, is in principle enough to make the channel flinch across some three and a half thousand litres of water. We are seasoning in micrograms, and the body is built to notice.

The climb from quiet to loud is not gentle, either. The response of TRPA1 to an isothiocyanate follows a sigmoid — a Hill curve, $\theta = [A]^n \div (EC_{50}^n + [A]^n)$, where θ is the fraction of the maximum response and n its steepness. We fit n near 2 for this interaction, meaning the warheads act with some cooperativity, and that exponent is why the sharp feels like a wall rather than a ramp. At the EC_{50} the channel is exactly half-open; at twice the EC_{50} it is already four-fifths open, $2^2 \div (1 + 2^2) = 0.8$; at half the EC_{50} it is only a fifth, $0.5^2 \div (1 + 0.5^2) = 0.2$. A mere fourfold swing in concentration carries you from barely-there to nearly-full — which is precisely the lived experience of wasabi, where a slightly larger dab is not a little more sharp but suddenly, tearfully, too much.

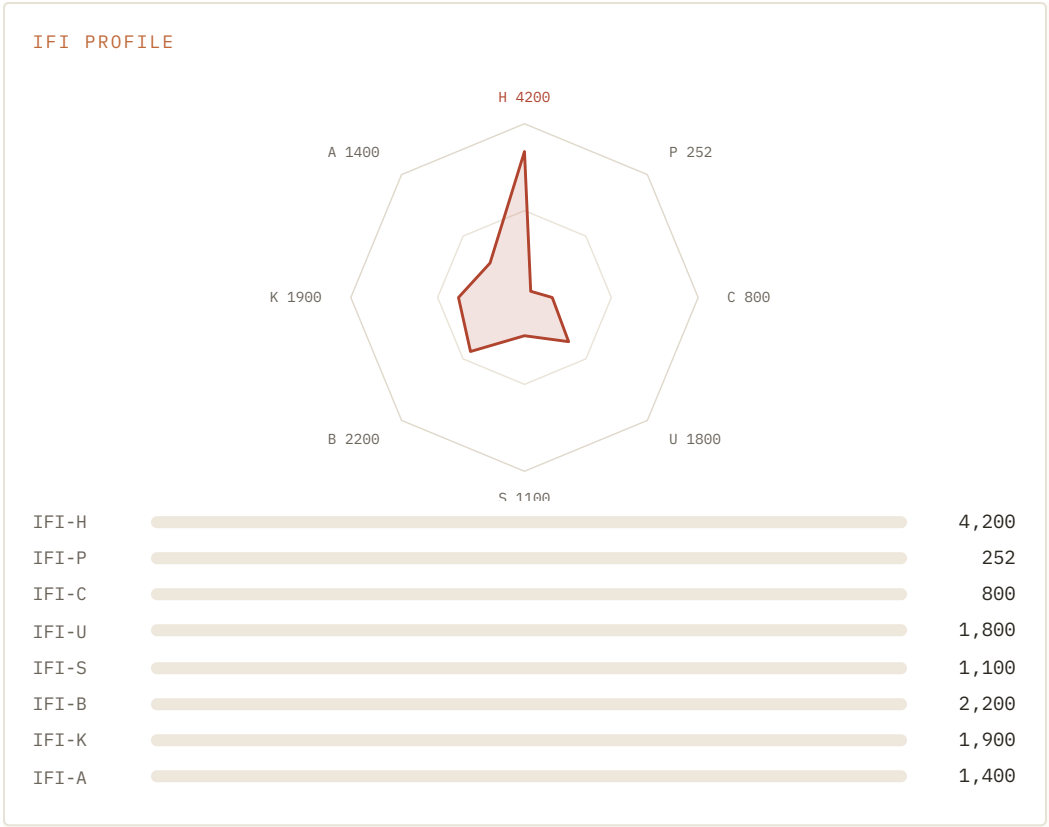
Laid out together, the preparations of this chapter make a short, legible ladder — the score, the concentration the channel reads behind it, the molecule doing the work, and the kinetics of its arrival and departure:

PREPARATION	IFI-P	EC_{50} AT TRPA1 (MM)	DOMINANT ELECTROPHILE	T_ON / T_OFF (S)
Fresh wasabi-style paste	756	2.78	AITC + 6-MSITC	2 / 25
Sinus-clearing dip	630	3.33	AITC + gingerols	2 / 25 → slow
Horseradish cream	378	5.56	AITC, fat-metered	6 / 70
Phoenix · SS-0096 (P-axis)	252	8.33	designed, secondary	–
Reference anchor	1,000	2.10	native reference	–

The sharp that has no Scoville number

It is worth saying plainly what you cannot do with any of this: you cannot give wasabi a Scoville number. The Scoville scale measures one thing — the dilution at which the heat of capsaicinoids disappears — and capsaicinoids speak only to TRPV1, the heat channel. There is no capsaicin in horseradish, none in mustard, none in a wasabi root. Put the fiercest sinus-clearing paste on the Scoville scale and it reads zero, not because it is mild but because the scale is deaf to it. The sharp and the heat are two different fires on two different channels, and a single number was never going to hold both. This is the whole reason the index carries eight axes instead of one.

Set our most extreme designed pepper beside a knob of horseradish and the two trade places completely. Phoenix — the cultivar we designed gene by gene to register on every axis at once, its synthesis pathway carrying the Pun1 double variant S39L+L345G among its computational modifications — our model predicts approximately 2.95 million Scoville units (SHU) and IFI-H 4,200 on the heat channel, a near-maximal roar on TRPV1. On the pungent channel, the one this chapter lives on, it only murmurs: IFI-P 252, a warm structural sharpness woven under the heat. Horseradish is its exact mirror — silent on TRPV1, loud on TRPA1. Neither number contains the other, and that is the point.



Phoenix · SS-0096 · ~2.95M SHU (predicted) · the eight-axis cultivar — a roar on heat, a murmur on the sharp

SCALE	WHAT IT COUNTS	RECEPTOR	HORSERADISH CREAM	PHOENIX · SS-0096
Scoville (SHU)	capsaicinoid dilution	TRPV1	0	~2,950,000 (predicted)
IFI-H	loudness of heat	TRPV1	~0	4,200
IFI-P	loudness of the sharp	TRPA1	378	252

Error, and the honest edges

A number is only as honest as its error bars, and ours are not zero. Each EC_{50} value we report is derived from computational dose–response modeling, and our designed lines carry a batch coefficient of variation — by design specification — held at or under 7%. Because the score is a ratio of two such values, their uncertainties add in quadrature: an IFI-P reading inherits roughly $\sqrt{(0.07^2 + 0.07^2)} \approx 9.9\%$ of relative spread when reference and candidate are both freshly computed, or about 7% when the reference is locked as a standing calibration and only the candidate is re-evaluated. So the paste's IFI-P 756 is honestly 756 ± 75 — somewhere between about 680 and 830 — and we would mistrust a figure quoted more precisely than that. Field-grown aromatics, picked from whatever the weather gave, swing far wider than that; part of what a designed line buys is the tightness itself, a sharp designed to reproduce to within a few percent rather than merely hoped for.

There is a subtler honesty owed, too, about the famous twenty-five-second clearance. The felt sharp fades that fast because the stimulus is a vapor: once the grating stops, no fresh isothiocyanate rises, the existing vapor clears the nasal airspace in seconds, and the percept follows it down. But the chemistry underneath is slower than the feeling. The covalent adduct the warhead formed on the channel's cysteine is not undone in twenty-five seconds; the cell reverses it over minutes, with its own reducing machinery — glutathione and the thioredoxin system — quietly stripping the modification away. The *all-clear* you feel precedes the molecular all-

clear by a good margin. It is the opposite of capsaicin, which binds reversibly yet lingers because the heat molecule is oily and slow to wash from the tongue. One flavor's chemistry outlasts its sensation; the other's sensation outlasts its chemistry. We find that pair of facts genuinely beautiful, and we try to cook with both of them in mind. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

A wasabi signature from a plant we can actually grow

Which returns us to the root we mostly cannot have. True wasabi, *Wasabia japonica,* is among the most demanding crops on earth — it wants cold, clean, moving water, dappled shade, years of patience, and it rots at the first wrong move. The overwhelming majority of what the world eats as wasabi is dyed horseradish and mustard wearing its name. We did not want to counterfeit it, and we did not want to simply swap in horseradish's single blunt warhead. We wanted the *signature* — the two-part architecture of real wasabi, the fast allyl flash over the slow green sulfinyl trail — from a plant we can grow clean, indoors, to a known spec.

So the work runs in the brassicas we can actually cultivate. Our black-mustard line carries 2,790 constructs across the pungent and multi-axis programs, and its tuning is deliberate: we build up the longer-chain, 6-methylsulfinylhexyl glucosinolate alongside the native sinigrin, so that one act of damage releases both the fast allyl warhead and the slow sulfinyl one — the wasabi trail, from a mustard. The white-mustard line (200 constructs) is tuned the opposite way, toward p-hydroxybenzyl isothiocyanate, which is barely volatile and so stays on the tongue as a broad, flat heat that never climbs the nose — the sharp for a dressing, not a flash. Garlic (560 constructs, pungency woven with kokumi) brings allicin, a different electrophile working the same channel; ginger (120 constructs) brings gingerols and their cooked cousins the shogaols, a warm sharp that opens TRPA1 slowly and from below. Every one of them is the same move: a known warhead, designed to a known level, on a plant we can stand behind.



Ginger's warm sharp — gingerols and shogaols working the same channel as wasabi, but slower and from below.

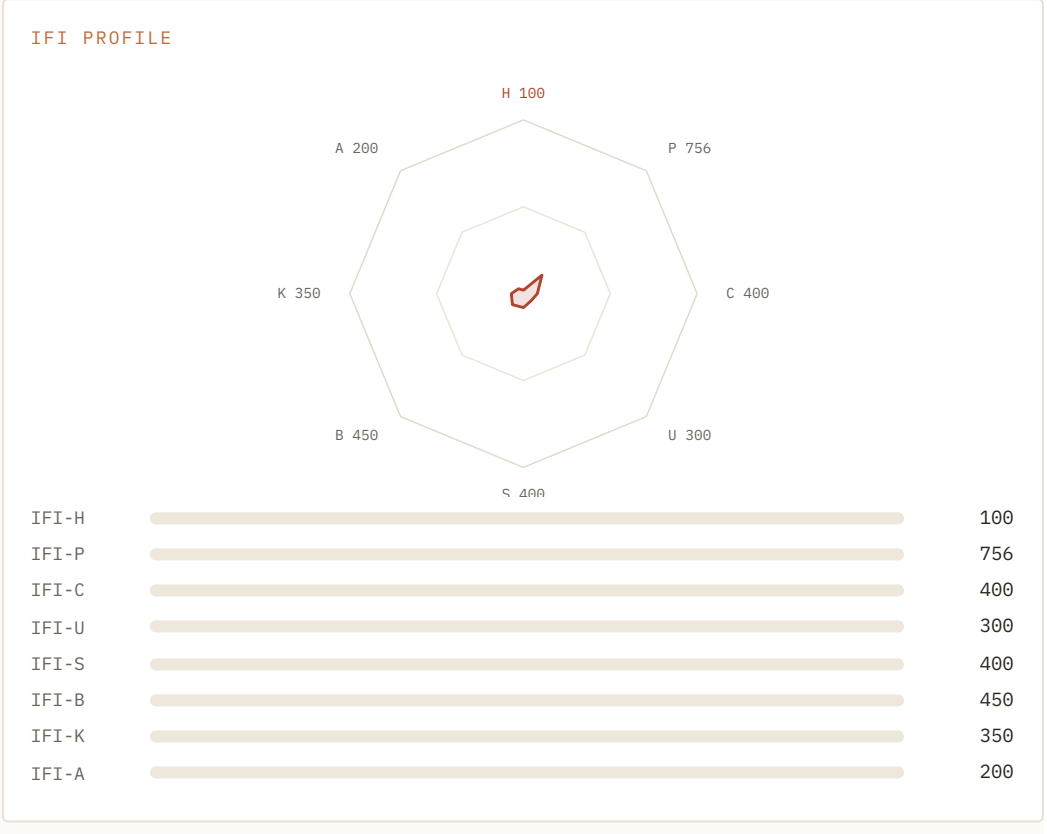
LINE	HOST	CONSTRUCTS	AXES	TARGET WARHEAD	DIFFICULTY
Wasabi-type, C6-sulfinyl	Black Mustard	2,790	P/M	6-methylsulfinylhexyl ITC	Hard
Bright table line	White Mustard	200	P	p-hydroxybenzyl ITC	Hard
Savory-sharp	Garlic	560	K/P	allicin / thiosulfinates	Moderate
Warm-sharp	Ginger	120	P	gingerols / shogaols	Moderate

Fresh wasabi-style paste

Built for the two-part attack of real wasabi: the fast allyl flash plus the green sulfinyl trail, from a long-chain-tuned designed line. On the index it carries IFI-P 756 — an EC_{50} near 2.78 µM, the channel half-open at under three micromolar — because the paste delivers both warheads at once, the allyl and the sulfinyl firing into the same breath.

```
30 g  designed brassica paste (wasabi-type, C6 sulfinyl-tuned), fresh
       or finely grated fresh horseradish + a designed black-mustard pinch
2 g   caster sugar
1 g   salt
3 ml  rice vinegar
       cold water to loosen, drop by drop
```

1. If starting from a fresh root or rhizome, grate it in a tight circular motion into a fine, wet paste — the finer the damage, the more completely the sealed weapon fires, because more cells are ruptured and more myrosinase meets more precursor. Grate only what you will use within minutes; the volatile sharp begins leaving at once.
2. Work in the sugar, salt, and the few drops of vinegar. The tiny sugar is not for a sweetness you will taste but to round the brutal edge; the vinegar stabilizes just enough to hold the paste together without acidifying the reaction toward a dull nitrile.
3. Mound it and let it **stand 2 minutes** before serving — this short rest lets the Lossen rearrangement finish and the sharp climb to its peak. Serve immediately, in small mounds; this is a condiment with a lifespan measured in minutes, and that fleetingness is the point.



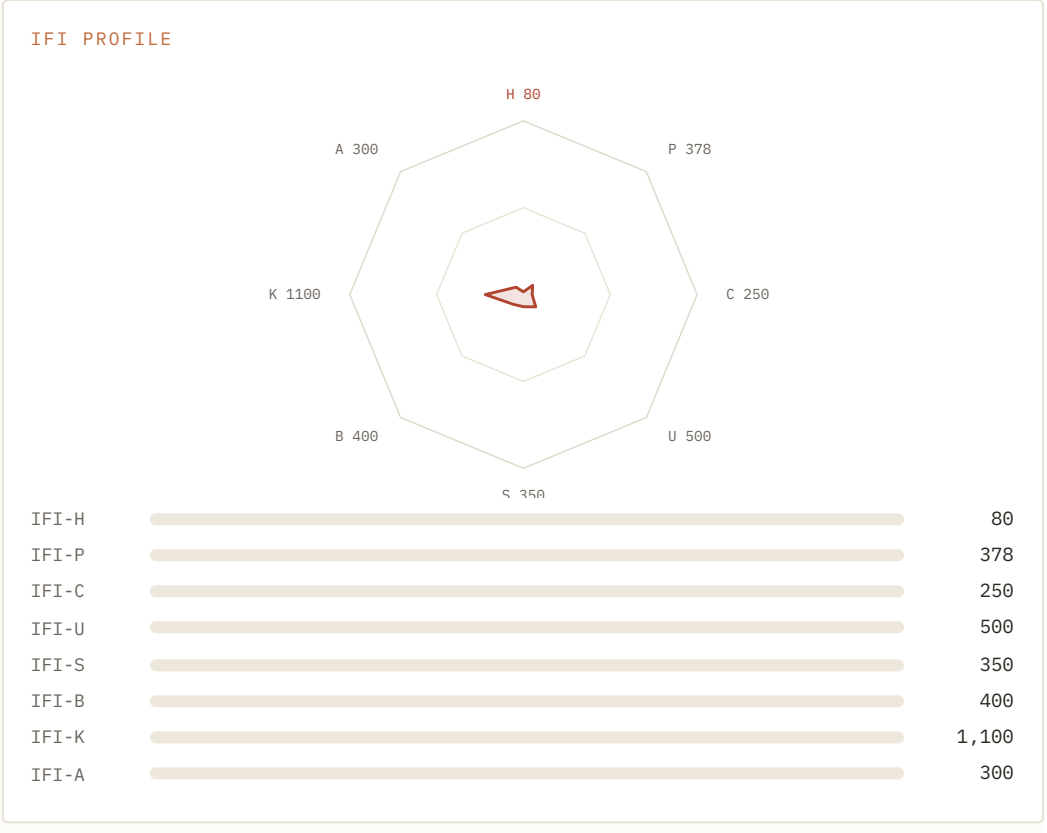
τ_on 2 s · τ_off 25 s · nasal flash + green sulfinyl trail · make to order

Horseradish cream

The sharp softened and carried in fat and dairy, built to sit under roast beef or a roasted beet; the cream meters the ferocity into something generous and spoonable. Its IFI-P carries 378 — exactly half the neat paste — and the halving is not an accident of taste but of physics: allyl isothiocyanate is lipophilic, so in a cream it partitions into the fat phase, and the receptor, which reads only the free aqueous concentration at the mucosa, meets roughly half of what you folded in. In the language of the index, the fat doubles the effective EC_{50}, from about 2.78 µM to 5.56 µM, and by the formula a doubled EC_{50} is a halved score. The cream does not weaken the sharp; it hides half of it in the butterfat and lets the rest out slowly.

```
40 g   grated fresh horseradish (or designed brassica, bright line)
120 g  crème fraîche or thick yogurt
5 ml   white wine vinegar
2 g    salt
1 tsp  Dijon (a bright, smooth table mustard)
       white pepper to taste
```

1. Grate the horseradish fresh and let it **stand 3 minutes** to develop — then fold it into the crème fraîche. The fat immediately begins to carry and gentle the sharp; a horseradish that would be savage neat becomes rounded and broad in the cream.
2. Season with vinegar, salt, mustard, and white pepper. Rest **1 hour** in the cold so the sharp distributes evenly through the fat.
3. Serve cold, in a generous spoonful. Because the fat slows delivery to the receptor, the P reads lower and longer than the raw root — a broad warm sharp rather than a flash, ideal against rich meat or a deep-roasted vegetable.



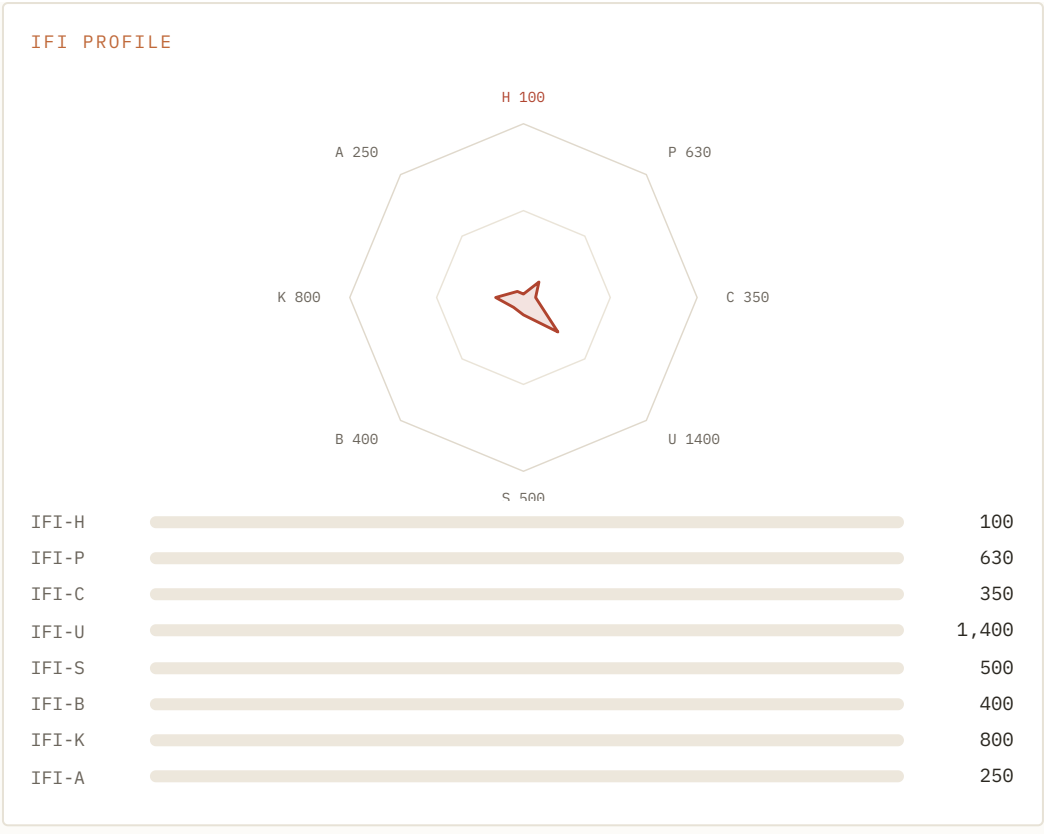
τ_on 6 s · τ_off 70 s · fat-metered, broad · the kokumi from the dairy

A sinus-clearing dipping sauce

Deliberately fierce, for those who seek the rush itself; the fast sharp with just enough soy umami and citrus to make it food rather than a dare. It carries IFI-P 630 — an EC_{50} near 3.33 µM, a touch below the neat paste because the soy and mirin dilute the allyl warhead, but stretched and held longer by the ginger, whose gingerols and shogaols are a second, slower electrophile on the same channel. Two warheads, two kinetics, one dip.

```
20 g  fresh wasabi-style paste (above)
30 ml light soy sauce
10 ml rice vinegar
5 ml  mirin
       a squeeze of yuzu or lime
1 tsp  grated fresh ginger (for a warm sharp under the cold one)
```

1. Whisk the soy, vinegar, mirin, and citrus. This is the cool savory base — umami and acid — over which the sharp will ride.
2. Stir the wasabi-style paste and the grated ginger in **just before serving.** The wasabi gives the fast nasal flash; the ginger lays a slower warm sharp beneath it, so the sauce opens with a rush and closes with a glow — the fast allyl kinetics up top, the slow gingerol kinetics underneath.
3. Use within minutes, for dipping cold things — raw vegetables, chilled tofu, delicate fish for those who eat it.



τ_on 2 s rush → ginger glow · τ_off 25 s then slow · soy umami base

The sharp at the edge of the plate

The sinus-clearers are the axis at its most theatrical — the sharp you feel *do* something, a door blown open and shut. They reward speed and freshness above all; every one is made to order and gone in a breath, which is exactly why they sit, in every cuisine that loves them, right at the edge of the plate, a bright fierce punctuation beside the calm of the raw and the rich.

We have charted that breath now — anchored it at a concentration, drawn its curve, designed the warheads that make it — and the charting has not dimmed it at all. If anything it deepens the small astonishment of the thing: that a quarter of a milligram of a molecule a plant kept sealed for its own defense, released by nothing more than the damage of a grater and fired as a vapor into the dark behind your eyes, can open a single channel a few thousand times and make the whole body sit up. We build it carefully because it is worth building carefully — clean, to a known spec, on plants we can grow and stand behind. Then we serve it in a mound the size of a pea, and we serve it now.

Chapter 16 · Chili crisp, ginger, and the alliums — *the sharp built to keep.*

If wasabi is the condiment of the moment — made fresh, served at once, gone in an hour — chili crisp and the allium oils are the condiments of the pantry: the sharp stabilized in fat and preserved, built to spoon over everything for weeks. Here the chemistry works in our favor twice. The fat that carries the sharp also *protects* it, holding the allium and ginger molecules against the air and slowing the decay that would ruin them fresh. These are the designed alliums' home ground — our garlic line (560 constructs, K/P) and our ginger line (120 constructs, P), bred to hold both their sharp and their depth, and to hold them the same way in every jar. This chapter is one question asked three ways: how do you build a pungency, and then keep it? We answer it the way our family answers most things in the kitchen — with reverence for the plant, and with the arithmetic written down.



Garlic in cross-section — the allium's sulfur economy, stored intact in the living clove until the cell wall is broken.

Pungency is a reaction, not a fit — *the chemistry of TRPA1.*

Heat and pungency are named so often in one breath that it is easy to miss how differently the tongue reads them. Capsaicin finds its receptor the way a key finds a lock: it slips into a hydrophobic pocket on TRPV1, the channel's thermal threshold drops, the gate opens, and when the molecule diffuses away the gate closes again — a reversible fit, the same event run forward and back a billion times a second. Pungency works by a blunter, more intimate chemistry. Its receptor is TRPA1, a non-selective cation channel

riding the same trigeminal and nasal nerve endings, built around a long N-terminal stack of ankyrin repeats that hangs into the cell like a coiled spring. And the molecules that open it — the allicin of crushed garlic, the allyl isothiocyanate of mustard and wasabi, the cinnamaldehyde of cinnamon — are, most of them, electrophiles. They do not merely sit in a pocket. They *react*.

Each of those molecules carries a reactive sulfur or carbon that forms a covalent, or very nearly covalent, bond with the thiol of a cysteine on that cytoplasmic spring — in the human channel, principally C621, C641 and C665, with a reactive lysine, K710, close by. The adduct tugs the ankyrin spring, the gate swings open, and calcium and sodium pour across the membrane. A pungent compound is not a shape the channel recognizes; it is a chemical event the channel suffers. That single difference — a reaction, not a fit — is the whole character of the sharp. It arrives fast, because a small electrophile diffuses to its cysteines in seconds; it reads as nasal and penetrating, because the trigeminal afferents it fires carry a different quality than the warm flood of TRPV1; and it clings, because a covalent adduct is not undone by diffusion but must be cleared by the cell's own repair.

The lab-notebook line beneath every profile in this book records exactly that behavior as two time constants: **τ_on**, the seconds for the sharp to arrive, and **τ_off**, the time for it to fade. They are not decoration. They are the kinetics of the receptor written in the only units a cook cares about. A small, fast electrophile like allicin reaches its cysteines quickly — τ_on near eight seconds — and resolves over a minute or two as the channel desensitizes and the adduct is turned over. A larger, lipophilic agonist that also leans on TRPV1, as ginger's do, is slower to arrive and far slower to leave, lingering as warmth long after the first bite.

Not every pungent molecule reacts. The gingerols of ginger and the thymol of thyme open TRPA1 through ordinary reversible binding, no covalent handshake at all, which is why their sharp reads as *warm* rather than *cutting* — rounded, long, gentle at the front. Cinnamaldehyde sits between the two camps: an α,β-unsaturated aldehyde, a Michael acceptor that bonds to a cysteine but reversibly, so it reacts and then lets go. This spectrum — covalent, reversible-covalent, non-covalent — is the real texture of pungency, and it is what the IFI-P axis is built to measure. Not how much pungent matter a food holds. How loudly, at the channel, that matter speaks.

The number on the jar — *anchoring IFI-P, and the math of a worked profile.*

Loudness needs an anchor and a formula. The formula is the one every axis shares:

$$\text{IFI-P}(c) = 1000 \cdot EC_{50}(\text{ref}) \div EC_{50}(c)$$

EC_{50} is the concentration at which an agonist drives its channel to half of full response — the midpoint of the dose, the single number that best summarizes a whole curve. Invert it against a fixed reference and scale by a thousand, and a dimensionless *loudness*

falls out: a score a clove, a rhizome, and a jar of oil can be read against on one page. We anchor IFI-P to allyl isothiocyanate (AITC), the clean sharp of mustard and the most studied TRPA1 agonist there is, and we define its convention constant at EC_{50}(ref) = 2.1 µM as the 1,000 mark — a stated definition, not a measured literature value, the same way Scoville's dilution-to-threshold defined heat rather than measuring capsaicin. The algebra of that choice is worth seeing once. Set IFI-P = 1,000 in the formula and it forces EC_{50}(c) = EC_{50}(ref) exactly: a candidate scores a thousand precisely when it is neither louder nor quieter than the reference. Everything else is a ratio read off that pivot. Inverting the formula gives EC_{50}(c) = 1000 · 2.1 µM ÷ IFI-P, and every profile's P becomes a concentration we can put a unit on.

Take the three preparations of this chapter and run the arithmetic. Our model places the designed garlic that carries the chili crisp at P = 714, so EC_{50} = 2100 ÷ 714 = **2.9 µM** — the channel half-opening at about 1.4× the AITC reference concentration, a substantial garlicky pungency that is gentler than mustard's sharp but no less present. The ginger-scallion oil scores P = 378, so EC_{50} = 2100 ÷ 378 = **5.6 µM**, well below the reference anchor. The black-garlic paste scores P = 126, so EC_{50} = 2100 ÷ 126 = **16.7 µM** — nearly eight times the reference concentration needed just to reach half-activation, a pungency all but spent. The whole table collapses into one sentence: the louder the sharp, the less of it the receptor needs.

PREPARATION	IFI-P	EC_{50} ÷ REF (= 1000 ÷ IFI)	EC_{50} (MM)	VS. REFERENCE
AITC reference (convention constant)	1,000	1.00×	2.1	—
Chili crisp · designed garlic sharp	714	1.38×	2.9	0.72×
Ginger-scallion oil	378	2.67×	5.6	0.38×
Black-garlic paste	126	7.95×	16.7	0.13×

A single EC_{50} is only one point on a curve, and the shape of that curve is why pungency seems to *switch on* rather than swell. Near threshold the fraction of channels open follows a Hill relation, f = $[A]^n$ ÷ (EC_{50}^n + $[A]^n$), where the Hill coefficient n runs above one for TRPA1 because each channel carries several reactive cysteines and more than one adduct cooperates to pull the gate. Take n = 2 and the arithmetic turns steep in both senses of the word. At a half-step below the midpoint, [A] = 0.5·EC_{50}, only f = 0.25 ÷ 1.25 = **0.20** of the channels are open; at a step above, [A] = 2·EC_{50}, f = 4 ÷ 5 = **0.80**. A mere fourfold window of concentration carries the response from one-fifth to four-fifths of full. That steepness is the reason a half-gram more designed garlic can tip a sauce from warm to fierce, and the reason the amounts in the recipes that follow are weighed, not guessed.

Steepness is also why reproducibility is the whole game for a condiment you will spoon for weeks. Our lines are designed to hold their output to a batch-to-batch coefficient of variation of **≤7%**, against **42%** in the wild plant — and because P scales with the concentration of active agonist near the working range, the profile

inherits that same spread. A designed P of 714 at 7% CV carries a standard deviation of 0.07 · 714 ≈ **±50**; the same sharp drawn from wild garlic, at 42% CV, carries 0.42 · 714 ≈ **±300**, enough to swing a jar from a barely-there P ≈ 420 to a noticeable P ≈ 1,000 with nothing changed but the clove. The tightness propagates cleanly to the concentration, too: EC_{50} = 2.9 µM inherits roughly the same 7%, giving 2.9 ± 0.2 µM. A condiment is a promise you make to a shelf. The CV is how we keep it.

One unit note, because this chapter braids in a second ruler. The heat the chili flakes contribute is not pungency and is not measured in micromolar; it is Scoville Heat Units, where pure capsaicin sits at 16,000,000 SHU and a food's rating is its capsaicinoid concentration in parts per million times roughly sixteen. The crisp's heat axis is computed at H = 1,900 for the designed mid-heat flake; the garlic's sharp sits at P = 714 on the pungency axis. Two rulers, one spoon.

A ladder of designed sharp — *the TRPA1 organisms we built.*

The sharp is not one molecule but a family, and the index is a family tree. Mustard is the loudest branch and the anchor's own kin; garlic and ginger are the branches this chapter lives in; cinnamon, thyme, oregano and watercress are the quieter cousins. Set them in a line by the molecule each hands to the receptor and the whole chemistry of the axis becomes legible on one page.

ORGANISM	CONSTRUCTS	AXES	PUNGENT PRINCIPLE	TRPA1 MODE
Black Mustard	2,790	P/M	allyl isothiocyanate (from sinigrin)	covalent electrophile
Garlic	560	K/P	allicin (from alliin)	covalent electrophile
White Mustard	200	P	*p*-hydroxybenzyl isothiocyanate	covalent electrophile
Ginger	120	P	6-gingerol → 6-shogaol	non-covalent
Thyme	120	P	thymol	non-covalent
Watercress	120	P	phenethyl isothiocyanate	covalent electrophile
Cinnamon	40	P	cinnamaldehyde	reversible Michael acceptor
Oregano	40	P	carvacrol	non-covalent

Every entry but the terpenes and the gingerols works by the same covalent handshake on the same cysteines; what differs is the leaving group and the speed. The isothiocyanates — mustard, watercress — are the fastest and most cutting. Allicin is a hair gentler and warmer. The reversible Michael acceptor of cinnamon reacts and releases, so it reads as sweet-sharp and brief. The molecule sets the character; the plant sets how much; our constructs set how reliably.



Onion — the kokumi sibling of the alliums, its sulfur economy tuned toward depth rather than sharpness.

What binds the alliums into this book twice over is that garlic and onion speak a second axis as fluently as the first. Garlic reads **K/P** and onion reads **K** — the K is kokumi, the richness-and-continuity axis, carried on the CaSR receptor by the γ-glutamyl peptides the alliums make in abundance: γ-glutamyl-cysteine, glutathione, the γ-glutamyl-S-allyl-cysteine that gives aged garlic its endless depth. These peptides are nearly tasteless alone, yet they amplify the body and the lingering fullness of everything around them. And here is the quiet drama of the allium: the pungency and the kokumi are made by the same plant, from the same sulfur economy, and they trade against each other as the clove is worked. Build the sharp, and you spend the depth; age away the sharp, and the depth rises to take its place. The three preparations that follow are three points along exactly that trade.

The fat is what lets any of it keep. Allicin and the thiosulfinates are oxidation- and hydrolysis-sensitive; in water they decay within hours to days. Oil defeats that on four fronts at once — it drives the water activity near zero so hydrolysis cannot proceed, it dissolves and dilutes the reactive sulfur species so their bimolecular decomposition slows, it limits the oxygen reaching them, and, kept cool, it stalls every rate constant together. The sharp built into the oil is a sharp set in amber.

Chili crisp — *three axes braided and preserved.*

A designed chili crisp — the great modern condiment, and the clearest case in the whole pantry of three axes read at once: heat from chili (H), sharp from garlic and ginger (P), and a deep savory body (K) from the alliums' γ-glutamyl richness and the fried aromatics.

```
200 ml neutral oil
60 g  designed garlic (protected-allicin line), finely minced
30 g  designed ginger, finely minced
40 g  dried chili flakes (heat to taste — a designed mid-heat line)
3 g   Sichuan pepper, lightly crushed (optional, for tingle)
15 g  fermented black bean, minced (optional, deep umami)
5 g   sugar
6 g   salt
1 star anise, 1 small cinnamon stick, 2 bay
```

1. Mince the garlic and ginger and let them **stand 10 minutes.** This is the critical step, and it is pure enzymology. In the intact clove, the pungency precursor **alliin** — S-allyl-L-cysteine sulfoxide — sits quietly in the cytoplasm while its enzyme, **alliinase**, a pyridoxal-5′-phosphate-dependent carbon–sulfur lyase, waits sealed away in the vacuole. The knife breaks that compartmentation. The two meet, alliinase cleaves alliin to allyl sulfenic acid, ammonia and pyruvate, and two sulfenic acids condense into **allicin**, the diallyl thiosulfinate that is the garlic's sharp. The reaction peaks within minutes; the rest is what lets a real pungency build *before* any heat arrives.
2. Warm the oil gently with the whole spices — star anise, cinnamon, bay — to about **120 °C**, infusing slowly, then lift the whole spices out. The low temperature is not timidity. Allicin is thermolabile; take the oil much past 150 °C and you decompose the very sharp you rested the garlic to make.
3. Pour the warm — not smoking — oil over the chili flakes, the minced garlic and ginger, the black bean, sugar and salt in a heatproof bowl. It should **sizzle gently, not violently** — enough to toast and bloom, not enough to incinerate the allium sharp. Add the Sichuan pepper now.
4. Cool completely and jar. Rest **two days** before eating; the flavors marry and deepen. The fat preserves the sharp for weeks — the condiment that keeps.

The genius of the designed garlic here is the word on the ingredient line: *protected-allicin.* A wild clove's final allicin yield is the product of three fickle quantities — how much alliin it stored, how active its alliinase, and how completely the two reacted before heat or time intervened — and the three drift so badly that wild garlic carries that 42% batch CV. The 560-construct line holds the alliin pool high and the alliinase activity steady, so the ten-minute rest lands the same sharp every time, designed to hold ≤7% CV, which is why the crisp is predicted at a dependable P = 714 ± 50 and not a lottery. And the keeping is subtler than mere preservation. Over the weeks in the jar the allicin does slowly convert — to diallyl disulfide, diallyl trisulfide, ajoene, the vinyldithiins — but those are themselves sulfur electrophiles, themselves TRPA1-active. The sharp does not vanish; it matures, drifting from the bright nasal cut of fresh allicin toward a rounder, warmer, more deeply garlicky pungency. What the fat preserves is not one molecule frozen in time but a slow, managed succession of molecules that all keep speaking to the same receptor.

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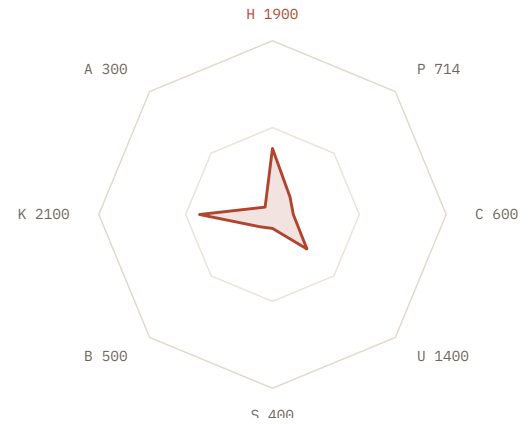

IFI-H		1,900
IFI-P		714
IFI-C		600
IFI-U		1,400
IFI-S		400
IFI-B		500
IFI-K		2,100
IFI-A		300

τ_on 8 s · τ_off 90 s · three axes braided · fat-preserved, keeps weeks

Ginger-scallion oil — *the warm, mellow sharp.*

Ginger-scallion oil — the clean, bright, warm-sharp relish of the Cantonese table, almost nothing but ginger, scallion, and hot oil, and a perfect study in warm pungency: the gentle, rounded end of the sharp, where the fierce onset is deliberately tamed.

```
60 g  designed ginger, very finely minced
50 g  scallion, finely sliced (white and green)
4 g   salt
80 ml neutral oil
```

1. Combine the minced ginger, scallion and salt in a heatproof bowl. Here, unusually, we *want* some of the sharp tamed — ginger-scallion oil is a warm, mellow relish, not a fierce one.
2. Heat the oil until it shimmers, about **160 °C**, and pour it over in one go. It will erupt and foam; this controlled cooking is a thermochemistry we are steering on purpose. Ginger's fresh pungency is **6-gingerol**, which activates TRPA1 and TRPV1 together — warmth and sharp at once. Apply gentle heat and gingerol dehydrates to **6-shogaol**, which is sharper still, roughly twice as pungent. Push the heat higher and longer and a retro-aldol cleavage carries it on to **zingerone**, the warm, sweet, far gentler molecule that smells faintly of vanilla. The 160 °C pour is hot enough to drive a little of that last step, sweetening the scallion and rounding the ginger toward zingerone, which is exactly why the relish lands warm rather than cutting.
3. Stir, cool, and use within a week — over steamed vegetables, rice, chilled tofu, or poached chicken for those who eat it. A warm sharp that comforts rather than shocks.



Ginger in cross-section — the gingerol pathway laid down in the living rhizome.

Set beside the chili crisp, the ginger-scallion oil is a lesson in temperature as a design variable. The crisp holds the oil *low*, at 120 °C, to protect an allicin already built; this relish pours the oil *high*, at 160 °C, to carry a fraction of its gingerol forward into gentleness. Same aromatic-in-hot-oil technique, opposite intents — one preserving a sharp, one softening one. The 120-construct ginger line is designed to hold a generous gingerol pool precisely so that even after the mellowing pour there is real warmth left to taste; our model places the profile at P = 378, an EC_{50} of 5.6 µM, well below the AITC reference — a mild, comfortable pungency. Its τ values tell the rest: τ_on 15 s, slower to arrive than allicin because gingerol is larger and does not react but merely binds; τ_off 180 s, twice the crisp's, because the molecule is lipophilic, lingers in the tissue, and leans on TRPV1 to keep a warmth glowing after the sharp itself has gone.

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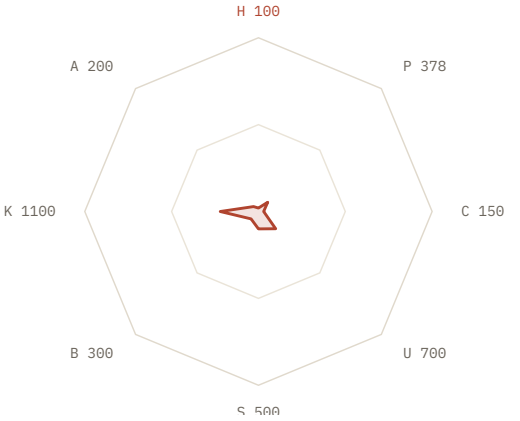


IFI-H	100
IFI-P	378
IFI-C	150
IFI-U	700
IFI-S	500
IFI-B	300
IFI-K	1,100
IFI-A	200

τ_on 15 s · τ_off 180 s · warm, mellow, long · ginger led

Black-garlic paste — *where pungency becomes depth.*

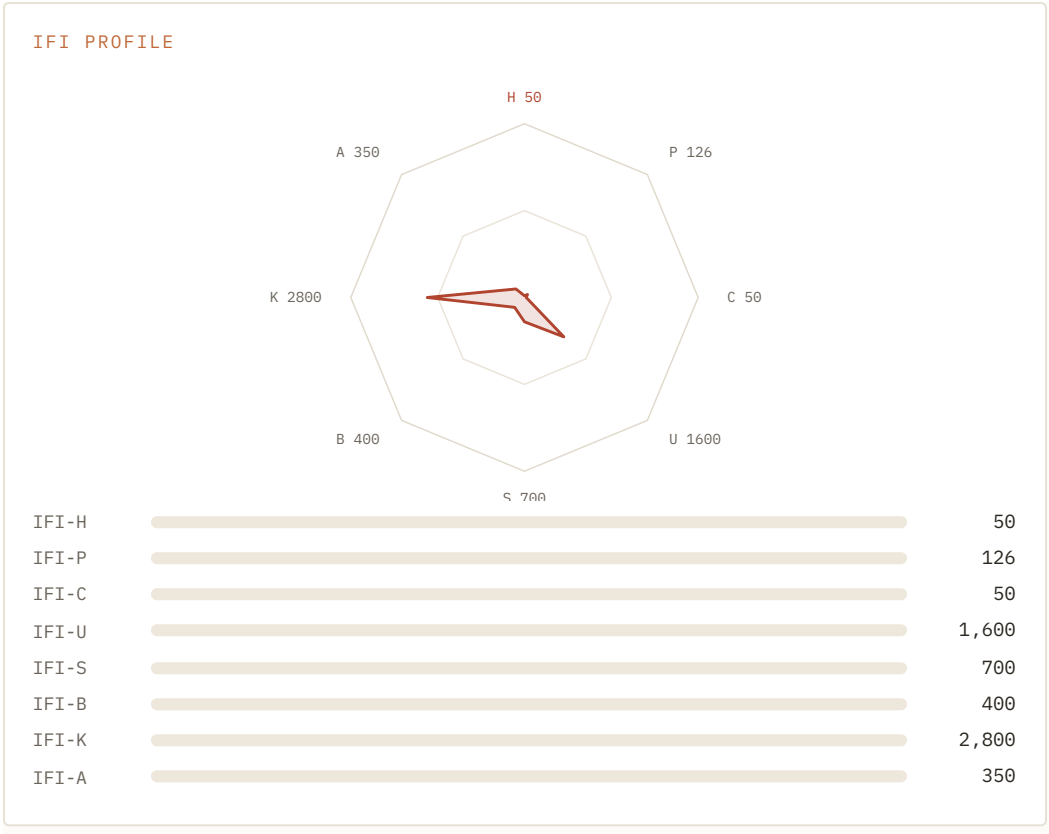
Black-garlic paste — the far end of the allium arc, where weeks of slow aging have burned the sharp almost entirely away and traded it for profound depth; a condiment that is nearly all kokumi, with only a shadow of pungency left, and the plainest proof that the K and P of an allium move in opposition.

```
120 g black garlic cloves (slow-aged designed garlic)
15 ml aged balsamic or tamari
10 ml neutral oil
       warm water to loosen
pinch  salt
```

1. Blend the black garlic with the tamari, oil and enough warm water to make a smooth, dark, spreadable paste. There is no resting and no enzyme work to do, because the aging has already done and undone it all. Held for weeks at roughly 60–70 °C in high humidity, the clove undergoes non-enzymatic Maillard browning: its reducing sugars condense with its amino acids into dark, molasses-deep melanoidins, while the labile allicin and thiosulfinates decompose away and the stable, water-soluble **S-allyl-cysteine** accumulates in their place. The sharp allicin is long gone; what remains is sweet, soft, endlessly deep sulfur.
2. Taste and balance with salt. The paste is savory, almost sweet, with the γ-glutamyl kokumi of the allium now fully dominant and the pungency spent.
3. Use as a finishing paste under roasted vegetables, stirred into broths, or spread thin where you want depth without any sharp at all.

The numbers make the opposition exact. Each IFI axis is anchored to its own convention-defined reference, so P and K scores are not directly comparable on a single scale; the P/K ratio here is a heuristic shorthand for which axis shapes the experience, not a measurement against one ruler. With that in mind: fresh in the chili crisp, the garlic scored P = 714 against K = 2,100 — a P/K of 0.34, kokumi-forward with pungency as a lively supporting note. The ginger-scallion oil holds a similar profile, P = 378 against K = 1,100, ratio 0.34. Black garlic deepens the difference radically: P = 126 against K = 2,800, a P/K of **0.045**, an eightfold collapse of the same clove's pungency axis as the sharp becomes body. Aging is simply the slow conversion of a labile, TRPA1-loud thiosulfinate into stable, CaSR-rich peptides and browned melanoidins — pungency, quite literally, becoming body. The sweetness liberated from the clove's sugars is computed at S = 700 and the amino acids freed into glutamate push U = 1,600; the whole profile has walked off the sharp axis and onto the savory ones.

PREPARATION	OIL / AGING	ENZYME STEP	IFI-P	IFI-K	P/K	KEEPS
Chili crisp	oil at ~120 °C	10-min rest	714	2,100	0.34	weeks (in fat)
Ginger-scallion oil	oil at ~160 °C	brief, in the pour	378	1,100	0.34	~1 week
Black-garlic paste	aged 60–70 °C, weeks	none (already spent)	126	2,800	0.045	long



τ_on slow · τ_off long · pungency spent, kokumi dominant · the aged end of the arc

What keeps, and what cannot — *failure modes and honest limits.*

A chapter about keeping owes an honest account of what cannot be kept, and of what the numbers do not say.

The EC_{50} that anchors every figure here is, for a covalent agonist, not quite the tidy equilibrium constant the formula pretends. A molecule that bonds permanently to a cysteine keeps accumulating adducts the longer it is present, so its apparent half-activation depends on how long the receptor was exposed. We handle this the only honest way: the index EC_{50} is read at a fixed incubation time in a fixed assay, and every P in this book is that one standardized snapshot of a kinetic process — comparable to every other P, but not a timeless thermodynamic truth. It is a ruler we hold the same way every time, not a law of nature.

Nor does a single P capture the *quality* of a sharp. The fast nasal cut of mustard and the warm cling of ginger can score the same loudness and feel nothing alike, because sensation is also the τ_on and τ_off, the co-firing of TRPV1, and the retronasal volatiles the nose reads in parallel. The number is one true coordinate of a sharp, never the whole of it; the poetry of the thing — and it is a real poetry — lives in the dimensions the scalar leaves out.

The CV, likewise, is a ceiling, not a guarantee. The ≤7% is what the genetics are designed to make possible; a real kitchen adds its own variance on top — a hotter pour, a shorter rest, a warmer shelf, an older oil. The designed line removes the largest and least controllable source of drift, the plant itself. It does not absolve the cook, and it is not meant to.

And one limit is not about flavor at all. Garlic suspended in oil at room temperature is a classic anaerobic environment, and *Clostridium botulinum* is a real hazard in exactly that setting — the

reason "keeps for weeks" must always mean *refrigerated*, or acidified, and always eaten with a clean, dry spoon, never a wet one that carries water back into the jar. The fat that preserves the sharp does not sterilize the jar. We say so plainly, because reverence for what we grow has to include care for the body we grow it to feed.

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

Three condiments, then, across the allium-and-ginger range: the three-axis fullness of a chili crisp with its sharp built and sealed in fat; the warm, mellow relish of a ginger-scallion oil with its gingerol steered gently toward sweetness; and the pungency-emptied depth of a black garlic aged until the sharp has become body. Together they draw the whole arc of the sharp-that-keeps — a pungency built and preserved, a pungency softened on purpose, and a pungency deliberately transformed away. The fat protects, the aging transforms, the enzyme builds on its own ten-minute clock, and the designed plant holds its place on the arc to ≤7% so the condiment lands, jar after jar, exactly where we aimed it.

Chapter 17 · Málà — the numbing intersection — *three channels at once.*

The most sophisticated thing on the pungent axis is not the loudest sharp but the most *composed* one: **málà**, the Sichuan sensation whose name means, exactly, "numbing-hot." We hold it up as the axis's masterwork because it is not one channel but three played in chord — heat on TRPV1, sharp on TRPA1, and the uncanny tingling numbness that comes of silencing the background potassium channels that keep a nerve quiet — and cooking it well means moving all three at once, by number, which is precisely what the index was built to let a kitchen do. It is the proof of the whole method: the sharp as one deliberate voice inside a harmony, not a note shouted alone.

We came to it the way our family comes to most things — vegetarian by long habit, unhurried, a little reverent — by sitting over a spoon of good chili oil and asking what, exactly, was moving on the tongue. The honest answer turned out to be three answers at once, three proteins catching three different molecules in three different ways, and the whole art of the cuisine is the centuries-long tuning of their balance. The index does not replace that tuning. It gives it coordinates.



The heat half of the chord — a chili cross-section, the TRPV1 partner to the sharp and the tingle.

The chord, named by receptor

Pungency is never one feeling. It is a small orchestra of trigeminal channels, each an ion channel embedded in the membrane of a sensory nerve ending on the tongue and palate, each with its own molecule, its own onset, its own decay. Málà is the clearest case in the whole repertoire because it recruits three of them deliberately and holds them in a tension no single ingredient could produce. Three ingredients carry the three voices: the **chili** brings capsaicin

and warmth; the **aromatics** — garlic, ginger, fermented bean paste — bring the volatile sharp; and the **Sichuan pepper** brings hydroxy-α-sanshool, the alkylamide that makes the mouth buzz.

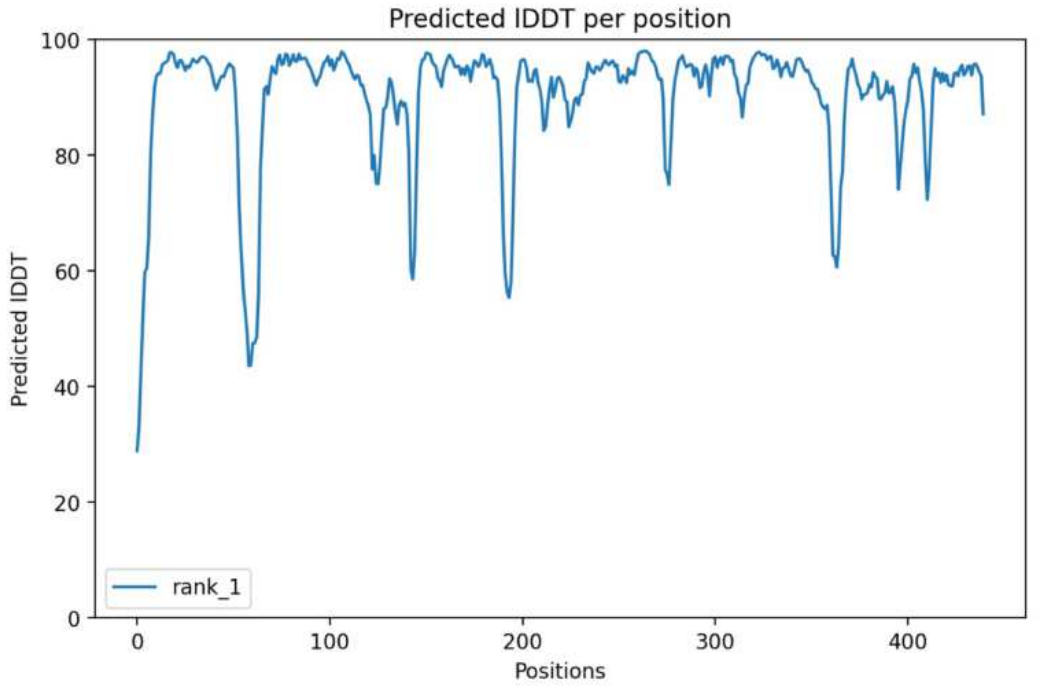
CHANNEL	RECEPTOR	MÁLÀ SOURCE · MOLECULE	MOLECULAR ACTION	ONSET → DECAY	IFI AXIS
Heat	TRPV1	chili · capsaicin	lowers the channel's thermal threshold	slow, lingering	IFI-H
Sharp	TRPA1	garlic/ginger · allicin, gingerol	covalent cysteine modification	fast, bright	IFI-P
Tingle	KCNK block (reads on TRPM8)	Sichuan pepper · hydroxy-α-sanshool	removes the resting potassium brake	rhythmic buzz	IFI-C

The genius of the cuisine is that it discovered, empirically, over generations, how to balance a receptor chord that neuroscience only recently had names for. hydroxy-α-sanshool is the connective tissue: it rings TRPA1 and TRPV1 faintly, but its defining trick is to **block the background KCNK (two-pore-domain) potassium channels** that normally hold the nerve at rest. With that brake released, the neuron depolarizes and fires in a rhythmic discharge near **50 Hz** — one spike roughly every 20 milliseconds — which the brain reads not as pain but as a tingling, electric flutter, the same frequency band it would assign to a mechanical vibration against the lip. That paresthesia spills onto the **Cool-Burn** axis, IFI-C, the channel menthol rings, because it sharpens against cold exactly as cooling does. Three channels, three numbers, one mouthful.

The warmth underneath — capsaicin and TRPV1

Capsaicin binds TRPV1, a non-selective cation channel on the trigeminal endings that is also gated by temperatures above **43 °C** and by protons. The molecule does not heat the tissue. It settles into a vanilloid pocket in the channel's transmembrane domain and lowers the thermal threshold, so tissue sitting at ordinary body temperature — **37 °C** — now reads as *above* the channel's new, lowered set point. The body reports a fire that is not there. Because capsaicin is lipophilic and clears slowly, that fire is a broad, lingering warmth rather than a flash, which is exactly the role heat should play in málà: the floor of the chord, under everything, never the ceiling.

The molecule itself is assembled by the pepper at a single branch point. Vanillylamine condenses with a branched-chain acyl-CoA at the **Pun1/AT3 acyltransferase** — capsaicin synthase — and the length and saturation of that acyl chain set the balance between capsaicin and dihydrocapsaicin, and with it the *shape* of the burn: how fast it arrives, how long it stays. This is where our work enters. An ESM2 protein language model scored every single-residue substitution in Pun1 for predicted effect on catalytic efficiency and fold stability; our genome-scale flux model predicts that pathway control sits upstream in the phenylpropanoid steps — at 4CL and HCT/CCoAOMT — not at the terminal Pun1/AT3 synthase. Pun1 is the final capsaicin synthase; it labels the product but does not govern the rate. Two substitutions no prior breeding program had ever combined emerged — the double variant S39L+L345G — and written in with CRISPR they let us place the capsaicinoid blend precisely rather than merely maximize it.



The Pun1/AT3 capsaicin-synthase model, coloured by confidence — the active-site cleft where the acyl chain is set.

EDIT	EFFECT	ΔΔG (KCAL/MOL)	STATUS
S39L	binding affinity ↑ (N-terminal)	−2.1	shipped
L345G	steric relief, active-site cleft	−1.8	shipped
C175S	disulfide-misfold prevention	−0.9	structural annotation only; not in shipped construct

For a málà oil we do not want the peak of what those edits can reach. A chord needs its bass note controlled, so we design the chili line to land at a *mid* heat — loud enough to be the warm floor, quiet enough to leave the sharp and the tingle room to speak over it. The recipe calls, deliberately, for "mid-heat chili flakes," and the index gives that instruction a target: an IFI-H near **2,200**, roughly the territory of our early flagships, not the far end of the scale.

The sharp — TRPA1 and the electrophile channel

Where TRPV1 is a slow lipophilic warmth, TRPA1 is the fast, bright, volatile sharp — the catch at the back of the nose when you mince garlic, the clean sting of fresh ginger. TRPA1 is a different kind of sensor. It is activated not by a snug fit into a pocket but by chemistry: reactive electrophilic molecules form covalent bonds with cysteine residues on the channel's intracellular N-terminus — a Michael addition onto the thiol — and that covalent modification pulls the channel open. The sharp of the aromatics is this covalent event, happening a few hundred times a second on the surface of a nerve.



Garlic — the protected-allicin line, where the sharp of málà is grown.

Garlic is the central example, and its chemistry is why the recipe insists on a **10-minute rest** after mincing. Intact garlic stores its precursor, alliin, in one compartment and its enzyme, alliinase, in another; they never meet until the cell wall is broken. Crushing ruptures the compartments, alliinase goes to work, and over several minutes it converts alliin into **allicin** — the diallyl thiosulfinate that is the actual TRPA1 agonist. Mince and wait, and you let the enzyme finish before any heat can denature it. Our garlic is a "protected-allicin line," designed so that more of that agonist is formed and survives into the dish; on the index, that extra sharp is exactly what IFI-P reads. Ginger runs a parallel chemistry on a parallel channel: its gingerols, mild and fresh, convert under heat and drying into the more pungent shogaols, so cooked ginger and raw ginger are genuinely different TRPA1 stimuli, not the same one at two strengths.

The two sharps and the one heat differ most in their *kinetics*, and this is the lever a cook pulls without naming it. Capsaicin is slow in and slow out. The TRPA1 agonists are fast in and fast out, and many are volatile, so they reach the receptors retronasally and read as "bright." A great málà layers the two timescales on purpose — a cooked, deep sharp from the oil and a raw, immediate sharp laid over it — so the sensation has a front edge and a long tail at once. Among the plants we have tuned for this channel, garlic (**560** constructs, kokumi and pungency) and ginger (**120**, pungency) are the workhorses, with black mustard (**2,790**, pungency and multi-axis) and cinnamon (**40**, pungency) available when a dish wants its sharp carried by allyl isothiocyanate or cinnamaldehyde instead.

The tingle — hydroxy-α-sanshool and the released brake

The third voice is the strangest and the most beautiful, and for a long time it had no mechanism, only a word: the buzz. Its molecule, hydroxy-α-sanshool, is an unsaturated fatty-acid amide — an alkylamide with a conjugated chain of double bonds — and that structure explains both what it does and how easily it is lost.

What it does is subtraction, not addition. Most pungent molecules *open* a channel to make a nerve fire. Sanshool mostly *closes* one. The KCNK family — the two-pore-domain potassium channels, among them TASK-1, TASK-3, and TRESK — carry a steady "leak" current of potassium ions out of the neuron, and that leak is the brake: it holds the resting membrane near the potassium equilibrium, around −90 millivolts, well below the threshold for firing. Sanshool blocks these leak channels. Remove the brake and the membrane

drifts up toward threshold until the neuron begins to fire on its own, rhythmically, near 50 Hz. Nothing is touching the lip; the nerve is simply no longer being held quiet. The brain, receiving a 50 Hz train from a touch fiber, reports a vibration — the tingling numbness that gives málà its name.

The conjugated-diene structure that makes sanshool active also makes it fragile: it oxidizes in air and degrades with heat, which is why the buzz is the first thing a careless kitchen loses. The recipe protects it twice over — a brief, gentle toast to wake the aroma without cooking the diene apart, then a warm, off-heat steep rather than a fry. And because the sensation sharpens against cold — the KCNK-released buzz co-varies with TRPM8 cooling — the index files the tingle on IFI-C, which is why a cold dish reads *more* numbing than a hot one made from the same oil.

Reading the chord as numbers

The whole point of the index is that a sensation we could once only chase by taste can also be aimed at by number. Every axis is a single score anchored to a receptor's half-maximal activation:

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

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. It is a property of the whole food's extract against the channel, read off a dose–response curve whose shape is the Hill function,

$$R(C) = R_{max} \cdot C^h / (EC_{50}^h + C^h),$$

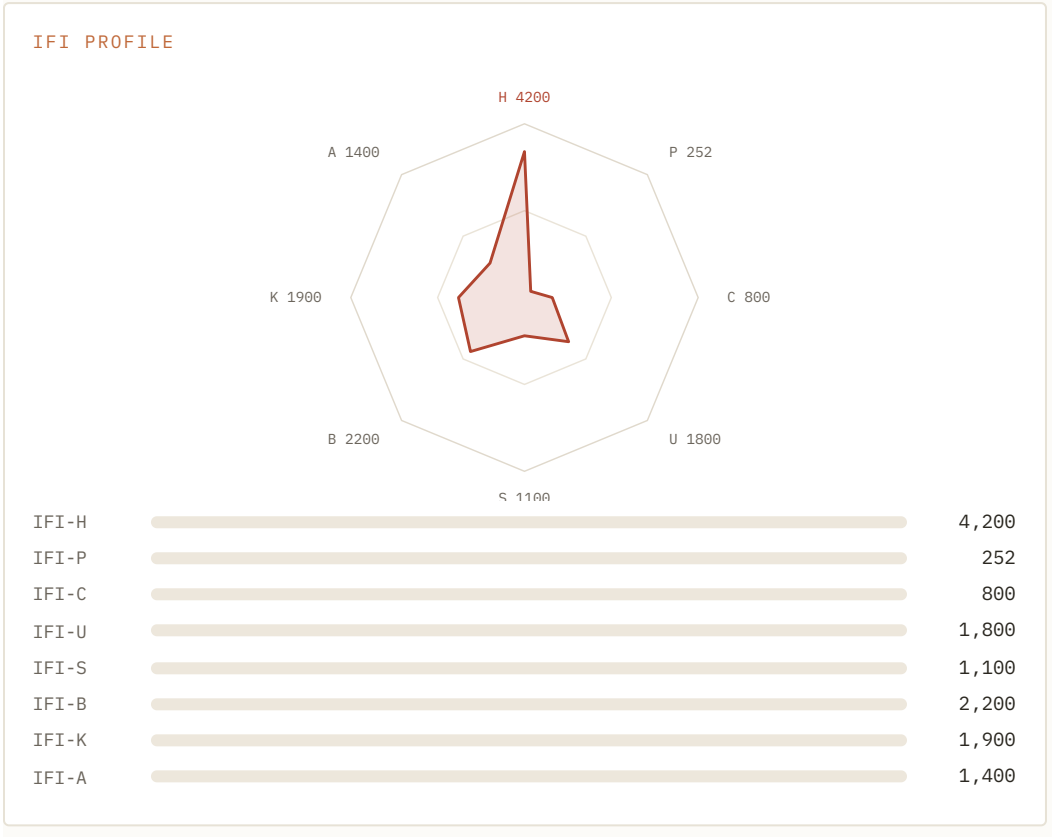
where at $C = EC_{50}$ the response R is exactly $R_{max}/2$, and h, the Hill coefficient, is the steepness — for capsaicin at TRPV1, a cooperative value near 2. The index anchors a score of **1,000** to the native wild-type reference, so that an IFI-H of 1,000 means the candidate reaches half-activation at the same extract concentration as the reference. Invert and scale, and a higher score simply means a *lower* EC_{50} — the food speaks more loudly, reaching the receptor's half-point at a smaller dose. For the P axis, the AITC/TRPA1 convention reference is set at **2.1 µM** — a stated convention constant, like Scoville's own dilution-to-threshold definition — anchoring IFI-P = 1,000 at that reference point.

Two worked examples make it concrete. The chili in our málà oil carries an IFI-H of **2,200**. Rearranging the formula, its half-activation concentration is

$$\mathrm{EC}_{50}(\text{oil chili}) = 1{,}000 \times \mathrm{EC}_{50}(\text{ref}) \div 2{,}200 = \mathbf{0.455 \times EC_{50}(ref)},$$

so it reaches half of full TRPV1 response at 45.5 % of the reference dose — it is 2.2× louder than the reference. Our full-spectrum flagship, by contrast, scores IFI-H **4,200**, giving EC_{50} = 1,000⁄4,200 = **0.238 × EC_{50}(ref)**, which is 4.2× the reference and 4,200⁄2,200 = **1.9×**

louder than the oil's chili. The arithmetic is why we can keep the oil's bass note controlled: we are not guessing "medium," we are placing it at a defined fraction of the loudest line we grow.



the full-spectrum reference · IFI-H 4,200 → EC_{50} = 0.238 × reference

The heat ladder shows our predicted index scores and the Scoville scale side by side, and the EC_{50} column is just 1,000 divided by the score:

CULTIVAR	SKU	SHU (PREDICTED)	IFI-H	EC_{50} (× REF)
Ember	SS-0087	~866,000	1,570	0.637
Cinder	SS-0088	~1,050,000	2,290	0.437
Kindle	SS-0089	~1,250,000	2,000	0.500
Blaze	SS-0091	~1,720,000	2,630	0.380
Wildfire	SS-0092	~1,960,000	2,930	0.341
Scorch	SS-0093	~2,210,000	3,220	0.311
Firestorm	SS-0094	~2,470,000	3,490	0.287
Solara	SS-0095	~2,720,000	3,980	0.251
Phoenix	SS-0096	~2.95 M	4,200	0.238

SHU values are computational predictions from our flux model, not certified measurements.

Read down the two middle columns and you see why the receptor reading, not the chemical titer, is the honest measure. SHU counts capsaicinoid molecules; IFI-H counts loudness at TRPV1. They mostly track together — each IFI-H point is worth about **550 SHU** at the foot of the ladder (866,000–1,570) and about **700 SHU** at the top (the Phoenix predicted ~~2.95 M SHU : 4,200), a gentle compression as the channel approaches saturation — but they are not the same axis. Kindle carries a predicted **~~1.25 million SHU** yet reads IFI-H **2,000**, *below* Cinder at predicted **~1.05 million SHU** and IFI-H **2,290**: more total capsaicinoid, less receptor loudness, because the capsaicin-to-dihydrocapsaicin blend and the acyl-chain shape govern how the molecules actually sound, not how many there are. That one inversion is the whole argument for reading the receptor.

(The figure is a dilution, too, in its oldest sense: a predicted ~2.95 million SHU means one part of that extract would remain detectable in nearly three million parts of neutral syrup.)

The chord as a vector

Because málà lives on three channels, a preparation is not a scalar but a vector — a point in (H, P, C) space — and the index lets us do geometry on taste. The designed oil sits at (H 2,200, P 336, C 1,800); a cold dressing made from it sits at (H 1,600, P 378, C 2,100). Two numbers describe each: the **magnitude**, how much total trigeminal energy the chord carries, and the **direction**, how that energy is distributed among the three voices.

The magnitude is the Euclidean length. For the oil, $\sqrt{(2{,}200^2 + 336^2 + 1{,}800^2)} = \sqrt{8{,}192{,}896} = \mathbf{2{,}862}$; for the dressing, $\sqrt{(1{,}600^2 + 378^2 + 2{,}100^2)} = \sqrt{7{,}112{,}884} = \mathbf{2{,}667}$. The two sit within **about 7 %** of each other in total intensity, and yet they feel like different dishes, because the *direction* has turned. Taking each channel's share of the squared length:

PREPARATION	H	P	C	MAGNITUDE	H SHARE	P SHARE	C SHARE
Málà oil	2,200	336	1,800	2,862	59.1 %	1.4 %	39.5 %
Cold dressing	1,600	378	2,100	2,667	36.0 %	2.0 %	62.0 %

The oil is **heat-and-tingle dominant** — nearly 60 % of its energy on TRPV1, nearly 40 % on the KCNK buzz, with the sharp a present but minor voice in the energy budget. The dressing flips to **tingle-dominant**, 62 % of its energy on the cold-sharpened buzz, with heat reduced to 36 %. The angle between the two flavor vectors, from the cosine of their dot product, is only about **13°**: the same chord, barely revoiced, carrying comparable total intensity pointed in two different directions. This is the sentence the whole book has been trying to earn — "spicy" is not a quantity, it is a direction in a space of at least three channels — made arithmetic.

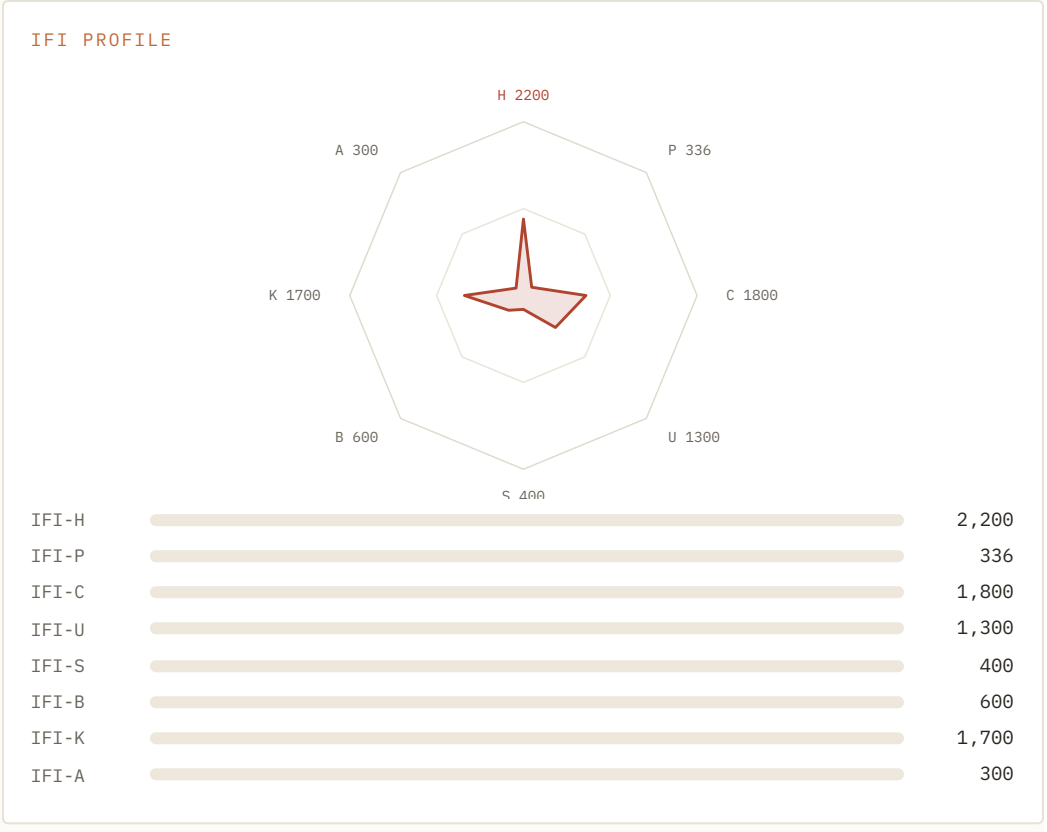
The designed málà chili oil

The foundation of the cuisine, built to a three-axis target: real heat beneath, a bright braided sharp through the middle, a pronounced tingle over the top.

```
250 ml neutral oil
40 g  designed mid-heat chili flakes (for H without destroying everything)
15 g  Sichuan pepper (red), lightly toasted and crushed — the tingle
10 g  Sichuan pepper (green), for a brighter buzz (optional)
50 g  designed garlic (protected-allicin line), minced
25 g  designed ginger, minced
20 g  fermented broad-bean paste (doubanjiang)
spices: 2 star anise, 1 cinnamon stick, 3 bay, 1 tsp fennel, 2 cardamom
8 g   salt, 5 g sugar
```

1. Mince garlic and ginger; **rest 10 minutes** so alliinase can build allicin before any heat denatures the enzyme — you are growing the TRPA1 agonist in the bowl.
2. Toast the Sichuan peppercorns gently in a dry pan until fragrant, then crush. Toasting develops the sanshool's aroma; over-toasting oxidizes the fragile diene and flattens the buzz, so keep it brief and low.

3. Warm the oil with the whole spices to ~130 °C, infuse 10 minutes, and strain the whole spices out. Add the crushed Sichuan pepper to the warm oil and steep off the heat for 5 minutes — a gentle extraction that spares the heat-labile sanshool.
4. Pour the warm oil over the chili flakes, rested garlic and ginger, doubanjiang, salt, and sugar. It should sizzle *gently* — hot enough to bloom the chili's capsaicin and toast the aromatics, cool enough to keep the allium sharp and the tingle alive.
5. Cool and rest **2 days.** The three channels settle into their chord: the heat broad and warm beneath, the sharp bright through the middle, the tingle buzzing over the top.



H heat · P sharp · C tingle — the three-channel chord · rest 2 days

A málà dressing for cold noodles or vegetables

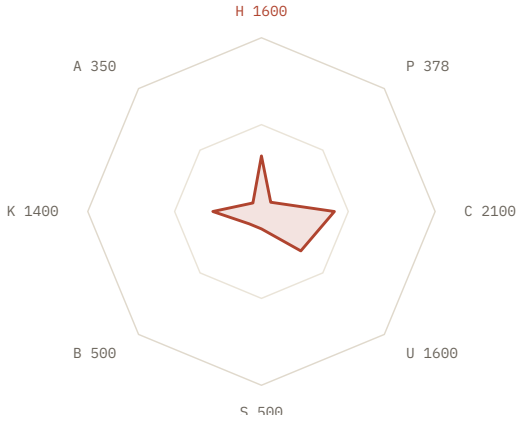
The chord carried into a bright, cool dish, where the tingle is most vivid against cold.

```
45 ml the málà chili oil (above), with its sediment
20 ml black vinegar (Chinkiang)
25 ml light soy sauce
10 ml sesame paste (tahini works)
8 g   sugar
2 g   ground toasted Sichuan pepper (extra tingle, finishing)
1 clove designed garlic, grated fresh (a fresh sharp over the cooked)
```

1. Whisk the vinegar, soy, sesame paste, and sugar into a smooth, savory-sour base — this carries umami and acid under the chord.
2. Stir in the málà oil and the fresh grated garlic. The raw garlic lays an immediate TRPA1 sharp over the slow cooked one already in the oil — the two kinetics, a bright front edge over a deep tail.
3. Finish with the ground Sichuan pepper over the dressed cold noodles or blanched-and-chilled vegetables. Served cold, the tingle reads strongest, because the KCNK buzz sharpens against cool exactly as TRPM8 does.

The number confirms the instinct. The oil's tingle reads IFI-C **1,800**; carried into the cold dressing it reads **2,100** — a ratio of 2,100⁄1,800 = **1.17**, a 17 % lift in perceived numbness from temperature alone, with no extra sanshool beyond the finishing pinch. Serving cold is not a garnish decision; it is a move along the C axis you can predict before the dish leaves the counter.

IFI PROFILE



IFI-H	1,600
IFI-P	378
IFI-C	2,100
IFI-U	1,600
IFI-S	500
IFI-B	500
IFI-K	1,400
IFI-A	350

cold amplifies the tingle · fresh garlic over cooked · C-forward

Precision — why the chord holds

A chord you cannot reproduce is not a recipe, it is a lucky night. The thing that lets us write a three-channel target down and hit it again is batch-to-batch consistency: our lines are designed to hold a coefficient of variation of **≤7 %**, against **42 %** in wild-type. The difference is the whole game. On the full-spectrum flagship's predicted heat of approximately **2.95 million SHU**, a 7 % CV design

target gives a standard deviation of about **±207,000 SHU** (one sigma), a 95 % window of roughly **±414,000** — tight enough that "mid-heat" means the same thing in March and in September. At the wild-type 42 %, the same mean carries a standard deviation near **±1.23 million**, a 95 % window that nearly spans a factor of ten. You cannot formulate a chord on an ingredient whose loudness is that uncertain; the uncertainty swamps the design.

And the chord itself is *steadier* than any one of its voices, by a law of errors our family finds quietly consoling. If the channels vary independently, error compounds in quadrature; working it exactly for the oil's (H 2,200, P 336, C 1,800) gives a composite magnitude error of about **5.0 %** — lower than the 7 % of any individual axis because the dominant H and C voices partially average each other's variation. The act of composing channels averages their noise. The balance is more reproducible than the ingredients, which is as good an argument for cooking by chord rather than by soloist as the mathematics can offer.

Honest limits

The index measures the stimulus, not the person. TRPV1, TRPA1, and the KCNK channels carry real genetic variation, and sanshool perception in particular differs widely between tasters — some feel a firm 50 Hz flutter, some a faint fizz. An IFI score is a property of the food, a reliable common language; it is not a promise that two mouths will hear the chord at the same volume. Nor is the vector model the last word: the three channels are not perfectly independent, capsaicin and sanshool both touch TRPV1, and at high intensities the voices interact in ways a first-order (H, P, C) map only approximates. We present the geometry as a working chart, not a claim that taste is linear.

There are failure modes in the pan, too, and they are all losses of the fragile voices. Oil poured too hot denatures the allium sharp and scorches the chili past bloom into bitter; peppercorns toasted too long oxidize the sanshool and the buzz goes dull; a dish served warm forfeits the cold lift the C axis would have given it for free. Each is a channel silenced by carelessness, and each is why the recipe's temperatures and rests are written as tightly as its weights.

One honesty we hold plainly: the heat and the sharp in this chord are grown on lines we designed, but the tingle still comes from the Sichuan pepper plant itself — hydroxy-α-sanshool is not yet one of the lines in our own hands, and the oil leans on the plant's gift for its third voice. The index can read the buzz, place it on IFI-C, and tell a kitchen how to protect it; growing it is on the road ahead, not behind us. We would rather say so than imply a completeness we have not yet earned.

What málà proves

Málà is where the book's argument stops being a thesis and becomes something you can taste on a cold noodle. "Spicy" is not one thing; it is a space of at least three channels, and a great cuisine is one that learned, by hand and by patience, to navigate that space long before anyone could name the channels. The index does not replace that hand — nothing replaces the cook's — but it gives the

sensation coordinates, so a flavor we could once only chase we can now also aim at: a target in H, P, and C, hit the same way every time, from a designed oil built to the number. The sharp, here, is one note in a harmony engineered across three receptors — which is exactly the composed, multi-channel future the whole of this work is for. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



PART V OF V

The Table and the Future

a pungent menu · the roadmap of the sharp · the whole plant

Chapter 18 · A pungent table — *the sharp in a whole meal.*

A condiment is a jolt; a meal is a composition, and the final test of the pungent axis is whether it can carry not just the edge of a plate but the arc of a dinner — a progression where the sharp rises, braids with the other axes, peaks, and resolves. This is the table we set when we want to show what the axis can do: plant-forward by nature, because that is how we cook and because the brassicas and alliums and roots that carry the sharp are vegetables first; the one fish course written with the same care, for those who eat it, because a good sharp belongs to the sea as much as the garden. Each dish carries its readout — an eight-line IFI profile and the two time constants that say how fast the sharp arrives and how slowly it lets go — and read in sequence they trace a deliberate curve of pungency across a meal.

Before the courses, though, the sharp itself: what it is, where on the tongue it is actually read, and how a single number can be hung on something as fleeting as the lift of wasabi up the back of the nose. The pungent axis is the one most often mistaken for heat, and the mistake is instructive, because it is a mistake about *which receptor is listening.*



Dawn over the plot where the menu begins — the whole plant, grown for everything it carries.

The receptor that reads a cut, not a molecule

Heat is capsaicin settling into TRPV1, a lock and key held by the shape of the two molecules — reversible, non-covalent, a guest that arrives and leaves. Pungency is a different receptor and a different kind of event entirely. IFI-P is anchored to **TRPA1**, the channel the field nicknamed the wasabi receptor: a non-selective cation channel on the trigeminal endings that serve the tongue, the palate, and — this is the part that matters at the table — the lining of the nose.

Where TRPV1 is tuned to a shape, TRPA1 is tuned to *reactivity.* Its agonists are electrophiles: small, hungry molecules that do not merely dock in the channel but bond to it.

The mechanism runs step by step, and it is worth drawing in full because every kinetic signature on our menu falls out of it. On the cytoplasmic N-terminal face of TRPA1 sit a cluster of exposed cysteine thiols — in the human channel, the residues numbered C621, C641, and C665, with a reactive lysine, K710, nearby. An electrophilic ligand — allyl isothiocyanate from mustard, allicin from garlic, cinnamaldehyde from cinnamon — undergoes a Michael-type addition or thiocarbamoylation onto those thiols. The channel is not occupied; it is *modified.* That covalent adduct pulls on the ankyrin-repeat spring of the N-terminus, the gate opens, calcium and sodium pour in, and the nerve fires. The sharp you feel is the current from a chemically altered protein.

Two consequences follow, and both show up on the plate. First, onset is governed by diffusion to the thiol plus the rate of the addition reaction, not by the fast on-off of a docking equilibrium — which is why the sharp of a good mustard *builds* over a few seconds rather than striking instantly like a capsaicin burn. Second, the signal ends when the adduct is reduced away or the channel is internalized and replaced, so the decay carries its own clock, independent of how much ligand remains in the mouth. A sense that writes itself onto the receptor also has to be erased from it, and that erasure is what we are reading when we report a τ_off.

The nose is the other half of the story. Isothiocyanates and the lightest thiosulfinates are volatile; they rise off the food, enter the nasal passages, and strike the TRPA1-bearing endings of the trigeminal nerve there. That retronasal hit is the "flash" of wasabi and fresh horseradish — a fast, clean, tear-pricking sharp that peaks in seconds and is gone in well under a minute, because the volatile dose is small and self-limiting. Capsaicin, non-volatile and lingual, can do nothing of the kind. When our fourth course sets a long warm ginger glow beside a bright wasabi dot, it is literally addressing two tissues: the slow warmth on the tongue, the fast flash in the nose, both of them TRPA1, both of them pungency, arriving by different roads.

One honest limit belongs here at the start. TRPA1 is polymodal — in the body it also answers cold, certain mechanical forces, and a long list of environmental irritants that have nothing to do with flavor. IFI-P is not a reading of everything TRPA1 can do; it is a reading of the *chemical-electrophile* activation that the kitchen cares about, measured under fixed conditions so that one food can be compared to another. We say so plainly, because a number is only honest when its edges are drawn.

From glucosinolate to sharp: the enzyme that fires at the knife

The deepest peculiarity of the pungent axis is that most of its ligands do not exist in the intact plant. They are *made at the moment of damage* — a chemical alarm the plant fires when something bites or crushes it — and our job, in the field and at the stove, is to control that reaction rather than merely to possess the ingredient.

In the brassicas the system has two parts kept apart in the living cell: a store of **glucosinolates** (sulfur-rich, water-soluble, odorless) in one compartment, and the enzyme **myrosinase** (a thioglucoside glucohydrolase) in another. Cut, crush, or chew the tissue and the walls break; myrosinase meets its substrate; the glucosinolate is hydrolyzed and rearranges into a volatile **isothiocyanate** — the sharp. Which sharp depends on the glucosinolate:

- **Sinigrin → allyl isothiocyanate (AITC)** — the bright, nose-lifting sharp of mustard, horseradish, and wasabi.
- **Gluconasturtiin → phenethyl isothiocyanate (PEITC)** — the greener, slightly deeper sharp of watercress.
- **Sinalbin → *p*-hydroxybenzyl isothiocyanate** — the broad, less volatile sharp of white mustard, a sharp that sits on the tongue rather than flying to the nose.

Timing is everything, and it is pure kinetics. Crush a brassica and *wait,* and myrosinase runs to completion, building the full isothiocyanate pool — but AITC is volatile and, in water, slowly hydrolyzes away, so wait too long and the sharp you built evaporates and degrades. This is why a mustard is mixed and rested a few minutes to bloom, then used; why wasabi is grated to order; why horseradish is folded into cold cream to cap its loss. The cook is running a timed enzyme reaction whether they name it or not.



Garlic in cross-section — the alliin and the alliinase sit in separate compartments until the clove is crushed; only then is allicin made.

Garlic runs the same two-compartment logic with different chemistry: the odorless amino acid **alliin** and the enzyme **alliinase** meet on crushing to make **allicin**, a thiosulfinate that is itself a potent TRPA1 electrophile. Allicin is unstable and decays within minutes to hours into **diallyl disulfide** and related sulfides — milder, rounder, and the reason aged black garlic reads as deep sweet kokumi with almost no sharp left: the pungency has literally reacted away. Ginger needs no enzyme at all; it carries **gingerol**, which dehydrates to the sharper **shogaol** on drying and breaks down to the sweet-warm **zingerone** on cooking — one molecule walking from fresh sharp to candied warmth as the heat is applied. Cinnamon carries

cinnamaldehyde ready-made; oregano and thyme carry **carvacrol** and **thymol**, both TRPA1-active. The axis is a family of reactions as much as a family of flavors.

These are the plants we have taken onto the platform. The pungency-bearing hosts, with their construct counts, their primary IFI axes, their engineering difficulty, and the activating electrophile each one is built around:

HOST ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	ACTIVATING ELECTROPHILE (TRPA1)
Black Mustard	2,790	P / M	Hard	allyl isothiocyanate (sinigrin + myrosinase)
Garlic	560	K / P	Moderate	allicin → diallyl disulfide (alliin + alliinase)
White Mustard	200	P	Hard	*p*-hydroxybenzyl ITC (sinalbin)
Ginger	120	P	Moderate	gingerol → shogaol → zingerone
Watercress	120	P	Hard	phenethyl isothiocyanate (gluconasturtiin)
Thyme	120	P	Hard	thymol
Cinnamon	40	P	Hard	cinnamaldehyde
Oregano	40	P	Hard	carvacrol

Black mustard is the tall pole — 2,790 constructs, the deepest pungent library we hold — because the glucosinolate–myrosinase system has the most dials worth turning: the size of the sinigrin store, the activity and localization of the enzyme, and the balance of volatile to non-volatile products that decides whether a sharp flies to the nose or stays on the tongue. Garlic earns its place on two axes at once, K and P, which is why it can be built toward either the deep continuity of black garlic or the bright bite of a raw crush.

What the number means — the math of the sharp

A flavor is useless to design until it is a number, and the number is built the same way for every axis. The Index reads loudness, not quantity — not how much of a compound a food holds, but how hard that compound drives its receptor:

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

EC_{50} is the concentration that drives the receptor to half of its full response. For IFI-P, the reference is AITC (allyl isothiocyanate) set at a stated convention constant of 2.1 µM — a defined anchor, like the dilution-to-threshold definition of the Scoville scale, not a measured literature value. A reference stimulus is fixed at IFI-P = 1,000 by that convention; a candidate that reaches half-activation at a *lower* concentration is louder and scores *higher.* Because the reference sits in the numerator, the relationship inverts cleanly: a candidate's EC_{50}, expressed as a multiple **r** of the reference EC_{50}, is simply

$$r = EC_{50}(\text{candidate}) \div EC_{50}(\text{reference}) = 1{,}000 \div \text{IFI-P}$$

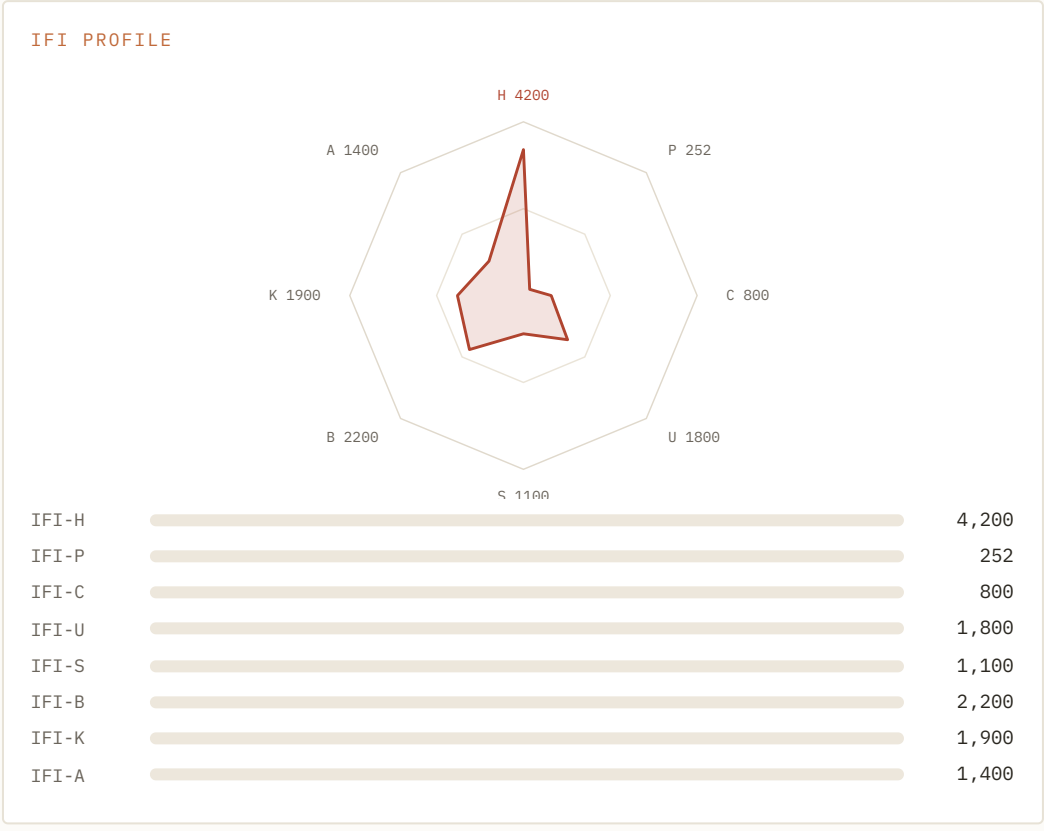
So every IFI-P on our menu can be read straight back to a receptor fact. The grilled-fish course reports **P = 2,000**: r = 1,000 ÷ 2,000 = 0.50, meaning its pungent pool reaches half-activation at half the

reference concentration — twice as loud. The dessert reports **P = 700**: r = 1,000 ÷ 700 = 1.43, a pool that needs 1.43× the reference concentration to say the same thing — quieter than the anchor, which is exactly why it reads as the axis at its gentlest. Run the five courses and the full-spectrum cultivar through the formula and the curve of the meal becomes arithmetic:

COURSE · READOUT	IFI-P	R = 1000/IFI-P	T_ON	T_OFF	ROLE OF THE SHARP
First · watercress & radish	1,400	0.714	5 s	40 s	bright opener, raw ITC
Second · beets & horseradish	1,600	0.625	6 s	70 s	warm support under sweet
Third · blistered greens, chili crisp	1,700	0.588	layered	layered	three sharps in conversation
Fourth · grilled fish, ginger & wasabi	2,000	0.500	fast + slow	25 s / 180 s	two kinetics, one bite
Last · chocolate, ginger & cinnamon	700	1.429	20 s	180 s	the long warm close
(ref.) Phoenix · SS-0096	252	3.968	—	—	pungency on a whole-spectrum plant

The peak-to-close span is the ratio of the loudest to the quietest reading: 2,000 ÷ 700 = **2.86×**. Across a single dinner the pungent pool swings nearly three-fold in receptor loudness — a real dynamic range, not a flat wash of "spicy."

The cultivar in the last row is worth its own profile, because it shows what a designed P value looks like when it is not a dish but a plant. Phoenix — SS-0096, the full-spectrum superhot — is the only cultivar built to register on all eight axes at once; our model predicts approximately 2.95 million SHU on the heat axis, and its pungency is a deliberate, designed 252:



Here the discipline of two separate axes earns its keep. That same fruit carries IFI-H 4,200 and IFI-P 252 — a roaring heat and a moderate sharp — and the two numbers must never be collapsed,

because they are read by two different receptors through two different mechanisms. The unit the whole world reaches for is the Scoville Heat Unit, and it is precise but it is a *heat* unit: SHU is defined by dilution-to-threshold of capsaicin at TRPV1. Apply the standard conversion — 1 ppm capsaicin ≈ 16 SHU — to our model's predicted 2,937,948 SHU and the arithmetic reads 2,937,948 ÷ 16 ≈ 183,622 ppm, about **18.4% capsaicinoids by weight** — and it says nothing whatsoever about the sharp, because isothiocyanates and thiosulfinates do not touch TRPV1. There is no valid Scoville number for wasabi. That is not a gap in the data; it is a category error, and avoiding it is half the reason the pungent axis needed its own channel on the Index.

AXIS	RECEPTOR	NATIVE STIMULUS CLASS	BINDING MODE	UNIT
Heat · IFI-H	TRPV1	capsaicinoids (vanilloids)	reversible, non-covalent	SHU **and** IFI-H
Pungency · IFI-P	TRPA1	isothiocyanates · thiosulfinates · α,β-unsaturated aldehydes	covalent cysteine adduct	IFI-P **only**

The dose–response shape matters as much as its midpoint. Like most channel agonists, a TRPA1 ligand follows a Hill relation, response R = R_max · $[A]^n$ ÷ (EC_{50}^n + $[A]^n$), and the sharpness of that curve — the cooperativity n — decides whether the sense creeps in or arrives. The width of the 10%-to-90% response window is a clean function of n alone: it spans a concentration ratio of 9^(2/n). For a non-cooperative receptor (n = 1) that is an 81-fold range — a slow, muddy ramp. For a representative TRPA1 slope of n ≈ 1.7, it collapses to 9^(1.18) ≈ **13-fold**; at n = 2 it is just 9-fold. The covalent, multi-cysteine gate is cooperative, so the sharp has a threshold-y, *sudden* quality — it waits, then lands — and that is a feature we can design toward or away from. (We quote n as an effective, representative slope; real TRPA1 gating layers use-dependence and calcium potentiation on top of the simple Hill term, and we do not pretend otherwise.)

Finally, error, because a flavor you cannot reproduce is not a flavor you have designed. Every construct on the platform holds a batch-to-batch coefficient of variation of **≤7%**, against **42%** in the wild-type plants we started from. Propagate that through the Index: since IFI-P = 1,000 · E_0/E with the reference fixed, the relative error in the score equals the relative error in the candidate EC_{50}, so the opener's P = 1,400 carries a 1σ band of 1,400 × 0.07 = ±98 — a true range of 1,302 to 1,498. At wild-type scatter the same dish would read 1,400 ± 588, anywhere from 812 to 1,988: the difference between a course you can plate identically on a Tuesday and a Saturday, and one you cannot plate twice at all. Blending helps further where the sharps are independent: a dish built from k comparable, uncorrelated designed contributors averages down its relative error as roughly CV ÷ √k, so the three-sharp third course lands near 7% ÷ √3 ≈ **4.0%** — tighter than any single element — provided the contributions really are independent and none dominates, which is a caveat we hold honestly and check batch by batch.

The table, course by course

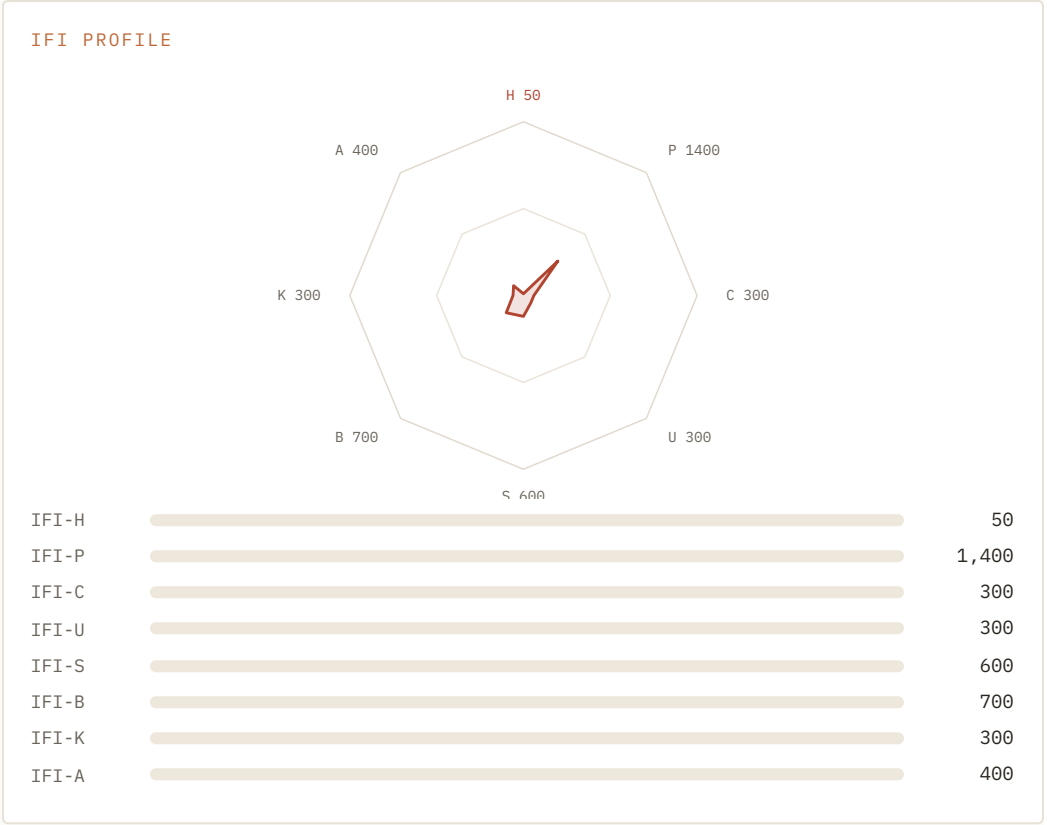
Now the meal. Plant-forward by conviction, one fish course by inclusion, each dish a working of the mechanism above — a timed enzyme reaction, a receptor loudness, a pair of kinetics — and each carrying its readout so the curve can be read as it is eaten.

First · Watercress and radish salad, mustard-seed dressing

The sharp as an opener: bright, green, and waking, two true isothiocyanates — the phenethyl ITC of watercress, the crisp ITC of radish — lifted by a bright mustard dressing. A pungency that clears the palate for everything after. At r = 0.71 the pool sits just louder than the reference anchor, and its fast kinetics (τ_on 5 s, τ_off 40 s) are the signature of volatile, raw isothiocyanate striking lingual and nasal TRPA1 together and then clearing before the next bite.

```
2 bunches watercress (designed line, PEITC-forward)
6      breakfast radishes, shaved (crisp designed sharp)
dressing: 1 tbsp bright table mustard (designed mustard), 2 tbsp olive
oil,
          1 tbsp lemon, 1 tsp honey, salt
```

1. Shave the radishes thin and crisp them in ice water; dry well.
2. Whisk the dressing; let the mustard rest two or three minutes so the myrosinase can bloom its AITC before the acid slows it.
3. Dress the watercress and radish just before serving; the raw brassica sharp of both greens is the point, bright and fleeting — once cut, the isothiocyanate pool only diminishes, so it is plated the moment it is made.



τ_on 5 s · τ_off 40 s · bright green opener · raw ITC-led

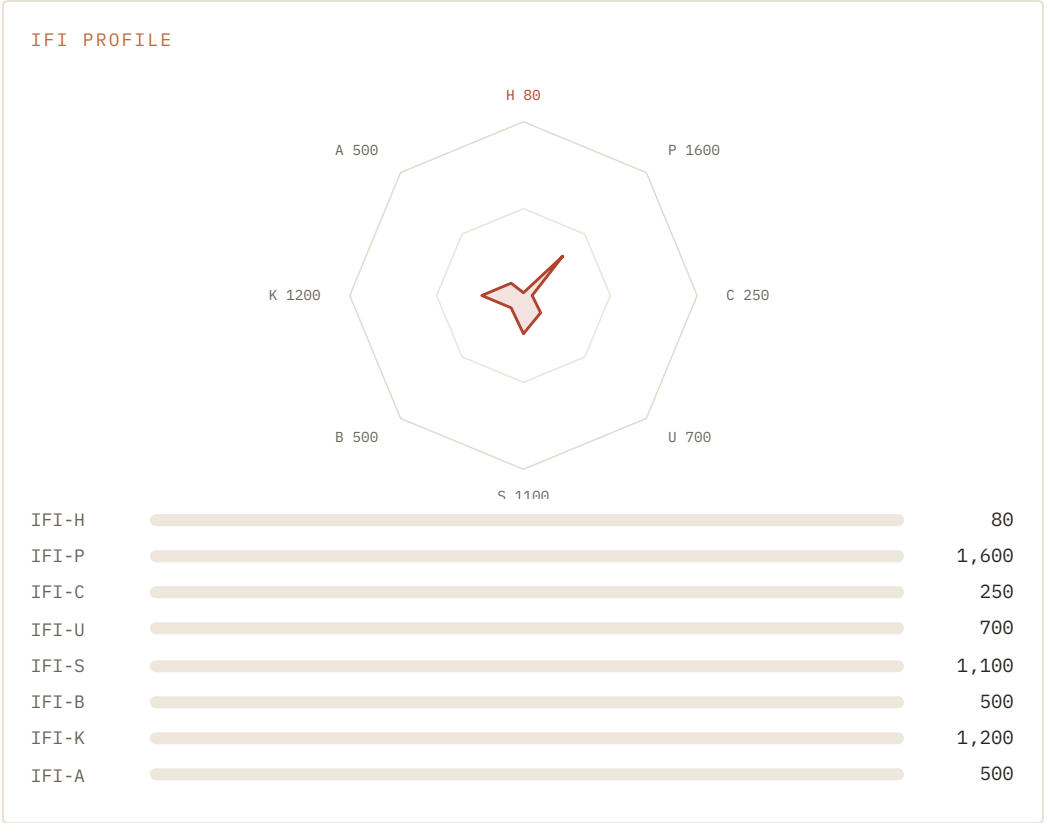
Second · Roasted beets with horseradish cream

The sharp turned warm and broad: the fierce root gentled in fat against the deep sweetness of roasted beet. Here pungency *supports* rather than leads — the horseradish a bright spine through the earthy sweet. The reading climbs to P = 1,600 (r = 0.625), louder at the receptor than the opener, yet it feels softer, because fat meters delivery: lipid holds the volatile AITC back from the nose and

releases it slowly, stretching τ_off to 70 seconds. Loudness and speed are separable, and this course is the proof — a higher number worn more gently.

```
4       beets, roasted whole and peeled
        horseradish cream (grated horseradish folded cold into crème
fraîche)
        toasted walnuts, dill, flaky salt, good oil
```

1. Roast the beets slow until deeply tender and sweet; peel and quarter warm.
2. Spoon the cold horseradish cream beneath and the warm beets over — the temperature contrast sharpens the cream's edge against the beet's sugar, and the cold cream protects the AITC that heat would drive off.
3. Finish with walnuts, dill, salt, oil. The fat-metered sharp reads broad and warm, a long foil to the sugar of the root.



τ_on 6 s · τ_off 70 s · sharp supporting sweet · fat-metered

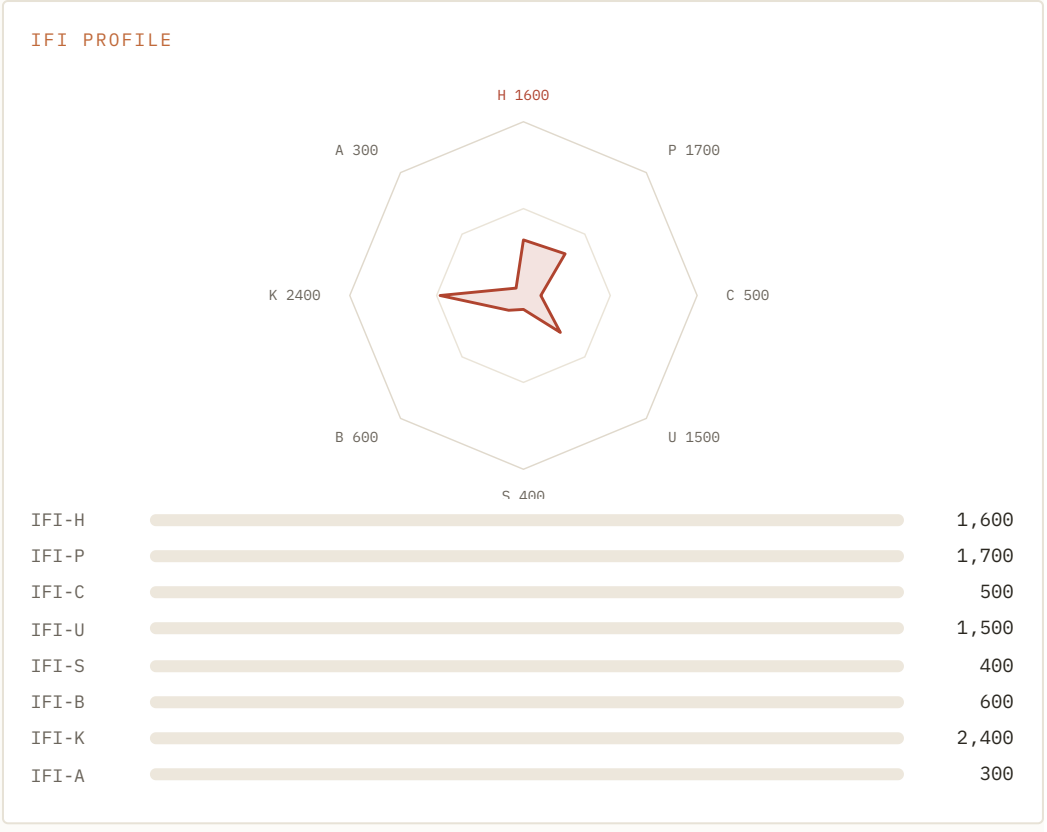
Third · Blistered greens with chili crisp and black garlic

The savory heart of the table: three sharps in conversation. The fresh pungency of charred brassica, the preserved three-axis roar of chili crisp, and — beneath — the pungency-*spent* depth of black garlic, where the allicin has long since reacted down to sweet sulfides and almost no sharp remains. The axis at its most layered, P = 1,700 (r = 0.59), and the course where the blending math shows:

three comparable, independent designed contributors pull the batch coefficient of variation down toward 4.0%, tighter than any one of them alone.

```
400 g  gai lan or broccolini (designed brassica), blistered hot
2 tbsp  designed chili crisp
1 tbsp  black-garlic paste
       sesame, a squeeze of lime
```

1. Blister the greens hard and fast in a dry-hot pan or over flame — the char develops a cooked brassica sweetness while the stems keep a raw isothiocyanate sharp.
2. Streak the plate with warm black-garlic paste (deep K, almost no P), lay the greens, spoon the chili crisp over (H, P, and K braided).
3. Sesame and lime to finish. Read bottom to top: aged depth, then fresh vegetable, then the braided roar.



layered: fresh char + preserved crisp + aged depth · K foundation

Fourth · Grilled fish with ginger-scallion and fresh wasabi

The one course from the sea, written with equal care: a clean fish given two kinetics of the warm-and-cold sharp — the long warm glow of ginger-scallion and a bright dot of fast wasabi. This is the loudest pungency of the night, P = 2,000, r = 0.50, half-activation at half the reference concentration; and it is loud in two tissues at two speeds. The ginger's zingerone-warm sharp settles on the tongue

and lingers (τ_off ≈ 180 s); the wasabi's volatile AITC flies to the nasal TRPA1 and is gone (τ_off ≈ 25 s). One mouthful, two clocks — the covalent mechanism made audible. For those who eat fish; the same dish works with firm tofu or king oyster mushroom, grilled the same way, and loses nothing it needs.

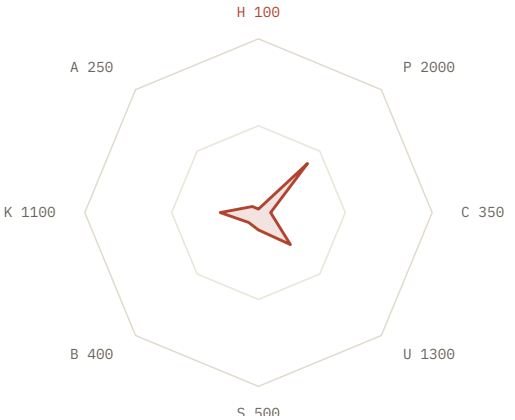


Ginger in cross-section — gingerol in the fresh root, sharpening to shogaol on drying and softening to sweet-warm zingerone as it cooks.

```
2       fish fillets (or thick tofu / king oyster mushroom), grilled
        ginger-scallion oil (warm, zingerone-forward)
        fresh wasabi-style paste (grated to order, AITC-forward)
        lime, flaky salt
```

1. Grill the fish (or tofu, or mushroom) over high heat to a clean char, flesh just set.
2. Spoon the warm ginger-scallion oil over — the long warm sharp, the comfort; gentle heat has already walked the gingerol toward sweet-warm zingerone.
3. Place a small bright dot of the wasabi-style paste beside, grated at the last second so its volatile sharp is at its peak, to be taken in the bite you choose; the fast nasal flash over the slow warm glow, two sharps in one mouthful.

IFI PROFILE



IFI-H	100
IFI-P	2,000
IFI-C	350
IFI-U	1,300
IFI-S	500
IFI-B	400
IFI-K	1,100
IFI-A	250

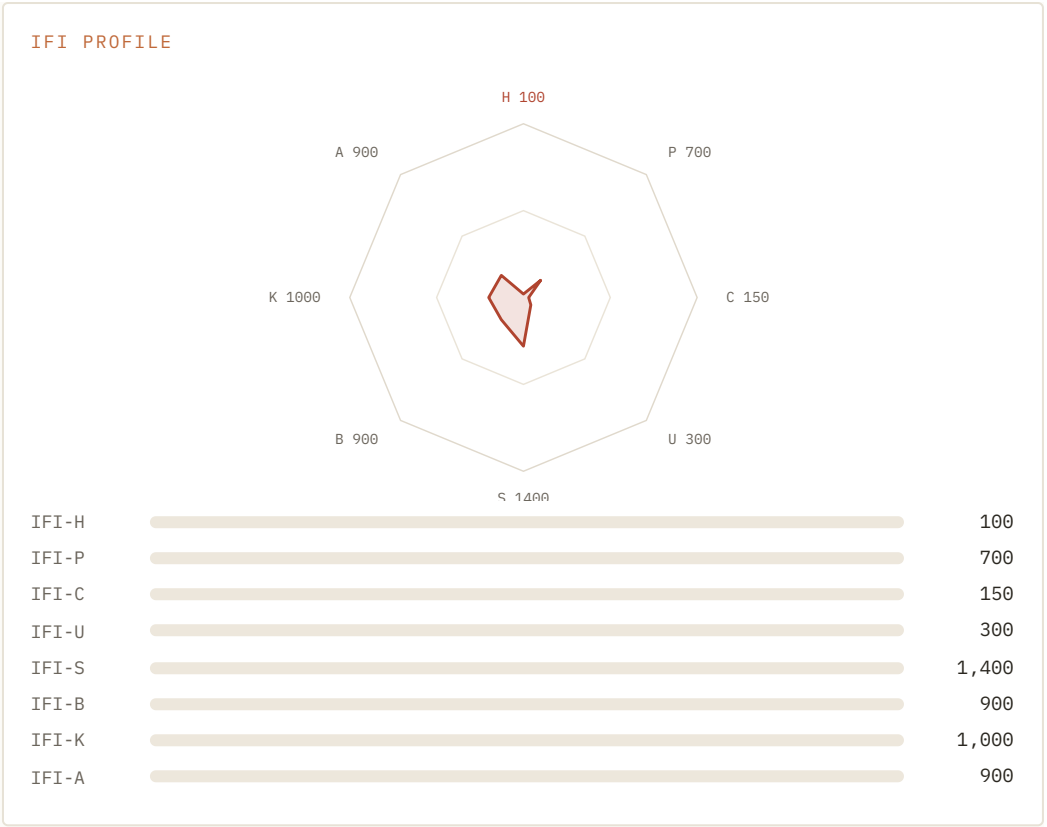
two kinetics: ginger glow (τ_off 180 s) + wasabi flash (τ_off 25 s)

Last · Dark chocolate with candied ginger and cinnamon

The sharp resolved into dessert: the warm pungency of ginger and cinnamon folded into the bitter-sweet of dark chocolate, pungency becoming comfort, the long slow fade to close the meal. This is the quietest reading of the night, P = 700, r = 1.43 — a pool that needs more than the reference concentration to reach half-activation, which is precisely the point. Candying has run the ginger all the way to zingerone; the cinnamaldehyde is slow and aromatic; nothing here flies to the nose. The sharp is present only as warmth, held long (τ_off ≈ 180 s), a hum under the chocolate.

```
100 g  dark chocolate (designed cacao, bitter-attenuated)
30 g  candied designed ginger, chopped (zingerone-sweet, warm sharp)
       cinnamon (Ceylon, low-coumarin), grated
       flaky salt
```

1. Melt and temper the chocolate; fold in most of the candied ginger.
2. Set in a thin sheet, scatter the rest of the ginger, grate cinnamon over, finish with salt.
3. Break into shards. The ginger's warm sharp and the cinnamon's slow aromatic pungency hum under the chocolate — the axis at its gentlest, a long warm fade to end on.



τ_on 20 s · τ_off 180 s · warm pungency as dessert · the long close

Reading the meal as a line

Five courses, and the sharp has done everything an axis can: opened bright and fast, turned warm and supportive, layered itself three deep, split into two kinetics in a single bite, and resolved into the quiet warmth of a sweet. Lay the IFI-P readings in order — 1,400 · 1,600 · 1,700 · 2,000 · 700 — and the dinner is a melodic line you can see: a measured climb to the fourth-course peak and a

deliberate fall to the dessert, a 2.86-fold swing in receptor loudness from the brightest moment to the softest. Underneath it, a second line moves in the kinetics: τ_off stretching from the opener's fleeting 40 seconds to the dessert's long 180, the sharp learning to linger as the meal deepens.

The mechanism is what lets the line be drawn on purpose rather than stumbled into. Because pungency is a covalent event at TRPA1, every lever in the kitchen is a lever on the chemistry: resting a mustard is running myrosinase to the top of its AITC curve; folding horseradish into cold fat is metering a volatile electrophile so it releases slow; heating ginger is walking one molecule from sharp gingerol to sweet zingerone; aging garlic is letting allicin react itself away to nothing. These are not tricks of seasoning. They are the dose, the delivery, and the decay of a receptor ligand, and they fail in knowable ways — over-rest the mustard and the AITC volatilizes and hydrolyzes off; heat the wasabi and the flash never reaches the nose; crush the garlic too early and the allicin is half spent before it plates. The honest edges are the same ones we drew at the start: IFI-P is a loudness at one receptor under fixed conditions, not a hedonic score and not the whole of what TRPA1 can feel; the number carries its ≤7% band, narrower than wild-type's 42% but never zero; and the sharp of the sea course is one plant's gift that firm tofu or a king oyster mushroom can carry just as well.

That is what a number for a flavor finally buys. Not the end of the cook's art — the shaving of a radish, the timing of a grate, the judgment of how much warmth a chocolate wants — but a score the art can be written on. Designed and composed, pungency is not a single effect called *spicy* but a whole expressive range, a sense you can compose with across the arc of a dinner. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. We set this table to show that the sharp, read eight lines at a time and heard as a line across a meal, is music; and that the mathematics underneath it is not the opposite of the pleasure but the thing that makes the pleasure repeatable, shareable, and ours to give.

Chapter 19 · The sharp future — *the axis, the roadmap, and the whole plant.*

We built the pungent axis last among the eight, and in a way that late arrival is fitting, because the sharp is the axis that most insisted on being understood before it could be designed. Heat you can chase by tasting; sweetness you can chase by measuring sugar. But pungency is a reaction with a shape in time, read by a channel that senses chemistry rather than form, and until we could see all of that — the covalent handshake, the two kinetics, the sulfur budget, the branch point between pungent and bland — we could only grow it by luck. Now we can grow it by intention. This closing chapter gathers the whole mechanism into one place, turns it into arithmetic we can plan a plant against, and follows that arithmetic to where the work goes next.



The whole-plant frontier — glucosinolate chemistry across the brassicas, grown for everything it carries.

The channel that reads chemistry, not shape

Most of the tongue's receptors are shape-readers. Sweetness is a sugar settling into the cleft of TAS1R2·R3 the way a key settles into a lock; umami is glutamate cradled by mGluR1. The fit is reversible, the molecule leaves intact, and the signal is a census of how many keys are turned at any instant. Pungency is not like that. IFI-P is anchored to **TRPA1**, the Transient Receptor Potential Ankyrin channel — a homotetramer whose four subunits each carry roughly sixteen N-terminal ankyrin repeats stacked into a long cytoplasmic spring,

below a transmembrane bundle (S1–S6) that forms a single central pore. TRPA1 does not read the *shape* of what arrives. It reads its *reactivity*. It is a chemical nociceptor: a channel built to detect electrophiles — molecules hungry for electrons — by letting them react with it.

The reactive site is a small cluster of cysteine residues sitting in the linker just below the membrane, where the ankyrin spring meets the pore machinery — on the human channel, C621, C641 and C665, with a nearby lysine, K710, as a secondary target. A cysteine's thiol (–SH) is soft and nucleophilic; a pungent electrophile finds it and forms a covalent bond. That is the **covalent handshake**: the molecule does not dock and leave, it *attaches*. The four spring-loaded subunits, now chemically tagged at their most sensitive residue, shift conformation together, the central gate dilates, and calcium and sodium pour inward. Because TRPA1 rides on the same trigeminal and nociceptor endings as the heat channel TRPV1, the brain files the result under the same alarm — but the trigger is chemistry, not temperature, and nothing on the tongue has actually been burned.

This mechanism is why pungency has a **shape in time** rather than a steady level, and that shape has two kinetics braided together. The first is fast and reversible: recognition and the initial, loosely-held adduct form within a breath, setting the *onset* and the *threshold* — how quickly the sharp arrives and how little it takes. The second is slow and quasi-irreversible: the mature covalent modification persists until the modified channel protein is turned over or the adduct is slowly reversed, setting the long *decay*, the after-burn that outlasts the bite. Overlay the physical clearance of the molecule — diffusion, swallowing, and, for the volatile species, evaporation into the retronasal space — and you get the whole silhouette: a fast rise, a plateau, and a tail. When we say a mustard "flashes" and a horseradish "climbs," we are describing where on that silhouette each electrophile spends its time. Allyl isothiocyanate is small and volatile, so it detonates into the nose and clears fast — a bright, ascending flash. The non-volatile isothiocyanates sit on the tongue and smoulder. Two molecules, one channel, utterly different experiences, all of it legible once you stop asking *what shape* and start asking *how reactive, and for how long*.

The glucosinolate bomb — chemistry stored, pungency on demand

The brassicas — mustard, wasabi, horseradish, broccoli, radish, watercress — do not carry their sharp as a finished molecule. They carry it as a loaded weapon with the firing pin kept in a separate room. In the intact cell, the pungent precursor is a **glucosinolate**: a stable, water-soluble, entirely bland molecule built on a thioglucose, a sulfonated oxime, and a variable side chain. The archetype is **sinigrin**, allyl glucosinolate, the dominant glucosinolate of black mustard. Sequestered away from it, in separate myrosin cells, waits the enzyme **myrosinase** — a β-thioglucosidase that never meets its substrate while the tissue is whole.

Damage changes everything. A knife, a grinding stone, a molar — any rupture mixes the two rooms, and myrosinase cleaves the thioglucose from sinigrin in milliseconds. What it leaves behind is an unstable aglycone, a thiohydroximate-*O*-sulfonate, which cannot hold its structure. It undergoes a **Lossen rearrangement**: the nitrogen–oxygen bond breaks, releasing sulfate, the allyl group migrates from carbon to nitrogen, and the molecule snaps into **allyl isothiocyanate**, R–N=C=S — the mustard oil, the volatile electrophile that reaches for TRPA1's cysteines. The reaction is a bomb in the precise sense that it is stored stable and released on contact:

> sinigrin + H_2O —(myrosinase)→ D-glucose + [aglycone] → AITC + HSO_4^-

Notice the sulfur. A glucosinolate carries two sulfur atoms: one in the thioglucoside bond, one in the sulfonate. When the bomb fires, one sulfur leaves as sulfate and the other is carried into the isothiocyanate itself — the S of the N=C=S. The pungent molecule *is* a sulfur-delivery vehicle; the plant spends two sulfur atoms to build each glucosinolate and hands one of them, embedded in the sharp, to whoever bites. Hold onto that accounting, because it is the whole argument of this axis at the molecular scale, and we will return to it.



Garlic in cross-section — alliin and the γ-glutamyl peptides held in ranked cells, waiting for the lyase.

The alliums run the same play with different parts. Garlic stores **alliin**, a cysteine-sulfoxide, apart from the enzyme **alliinase**, a PLP-dependent C–S lyase. Crush the clove and alliinase converts alliin to **allicin**, a thiosulfinate that reacts with the same TRPA1 cysteines by S-thioallylation — a sulfur electrophile where mustard's is a carbon one. Alongside the sharp, garlic carries γ-glutamyl peptides that speak a different axis entirely, the kokumi fullness read by the calcium-sensing receptor — which is why garlic sits at K/P in the index, two channels from one clove. Onion runs a variant in which lachrymatory-factor synthase diverts flux toward the tear compound; turn that enzyme down and the flux returns to flavor, a tearless onion that keeps its sweetness and its sharp. And ginger and cinnamon reach TRPA1 by yet another road — not glucosinolates at all but

phenylpropanoids, gingerol and shogaol and cinnamaldehyde, each an α,β-unsaturated carbonyl that performs a Michael addition onto the very same cysteines. Four chemistries, one handshake. The channel does not care how the electrophile was made; it only counts the covalent bonds.

Reading the sharp as a number — the P-axis anchor and the formula

An alarm that fires on a covalent bond would seem to resist measurement, but it does not, because *how much* electrophile it takes to half-open the channel is a clean, repeatable quantity. That is the EC_{50} — the concentration at which a stimulus drives its receptor to half of its maximal response — and the Index is built on inverting it. The whole family of eight axes shares one definition:

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

Loudness is the inverse of EC_{50}: the *less* of a compound it takes to reach half-activation, the *louder* that compound speaks, so a small EC_{50} must map to a large score. Inverting the ratio does exactly that; multiplying by a thousand puts the reference at a round number and gives the axis useful resolution. For pungency we anchor the reference to the prototypical electrophile itself. By convention, **allyl isothiocyanate defines IFI-P = 1,000 at a stated reference EC_{50} of 2.1 µM** — a fixed constant, like Scoville's original dilution-to-threshold definition, that anchors the entire axis without claiming to be a single literature measurement.

The arithmetic is then immediate. Phoenix (SS-0096), the full-spectrum cultivar that carries all eight axes at once, places its pungency deliberately at **IFI-P = 252**. Invert the formula to recover what that score means:

$$EC_{50}(Phoenix) = 1000 \cdot EC_{50}(ref) / IFI\text{-}P = 1000 \cdot 2.1\ \mu M / 252 \approx \mathbf{8.3\ \mu M}$$

A score of 252, against the reference anchor of 1,000, places Phoenix at roughly one-quarter the loudness of pure allyl isothiocyanate — a present, identifiable pungency held well below the full sharpness of raw mustard oil, which is exactly the restraint a full-spectrum design wants: pungency placed, never shouting over the other seven axes. Running the formula across the axis gives a ladder any cultivar or food can be read against.

IFI-P	EC_{50} (MM)	WHAT IT READS AS
7	300	a faint monoterpene prickle (thyme)
21	100	gentle seasoning warmth (ginger)
105	20	a clear, declared sharp
252	8.3	Phoenix (SS-0096) — pungency placed, not loud
350	6.0	an assertive mustard note
525	4.0	a pungency-led design target
734	2.9	the upper flagship band
875	2.4	scorecard normalization maximum
1,000	2.1	AITC convention — the anchor

Behind every row sits a dose–response curve, and for a cooperative tetramer like TRPA1 it is a Hill function with a coefficient near n ≈ 1.8 — the four subunits do not act independently, so the curve is steeper than a simple binding isotherm:

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R / R_max = [A]ⁿ / (EC₅₀ⁿ + [A]ⁿ)
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Set [A] = EC_{50} and the term collapses to ½ by construction — that is what EC_{50} *means*. The steepness is the interesting part. At three times EC_{50}, response is 3^1.8 / (1 + 3^1.8) = 7.2 / 8.2 ≈ **0.88** of maximum; at ten times EC_{50} it is 10^1.8 / (1 + 10^1.8) = 63.1 / 64.1 ≈ **0.98**. The channel saturates fast. This is the single most important fact about designing the sharp, and it sets an honest limit we will come back to: past roughly five times EC_{50}, pouring in more electrophile buys almost no additional peak loudness.

The pungent shelf — depth within the organisms we have

The roadmap of the sharp runs along two lines, and the first is depth within the organisms already in hand — the pungent wing of the forty-two host species, where most of the work is still ahead. Black mustard's 2,790 constructs are the proving ground, but the same levers now move cleanly across the whole shelf, each organism a different character of the sharp waiting to be placed on the map of loudness and shape.

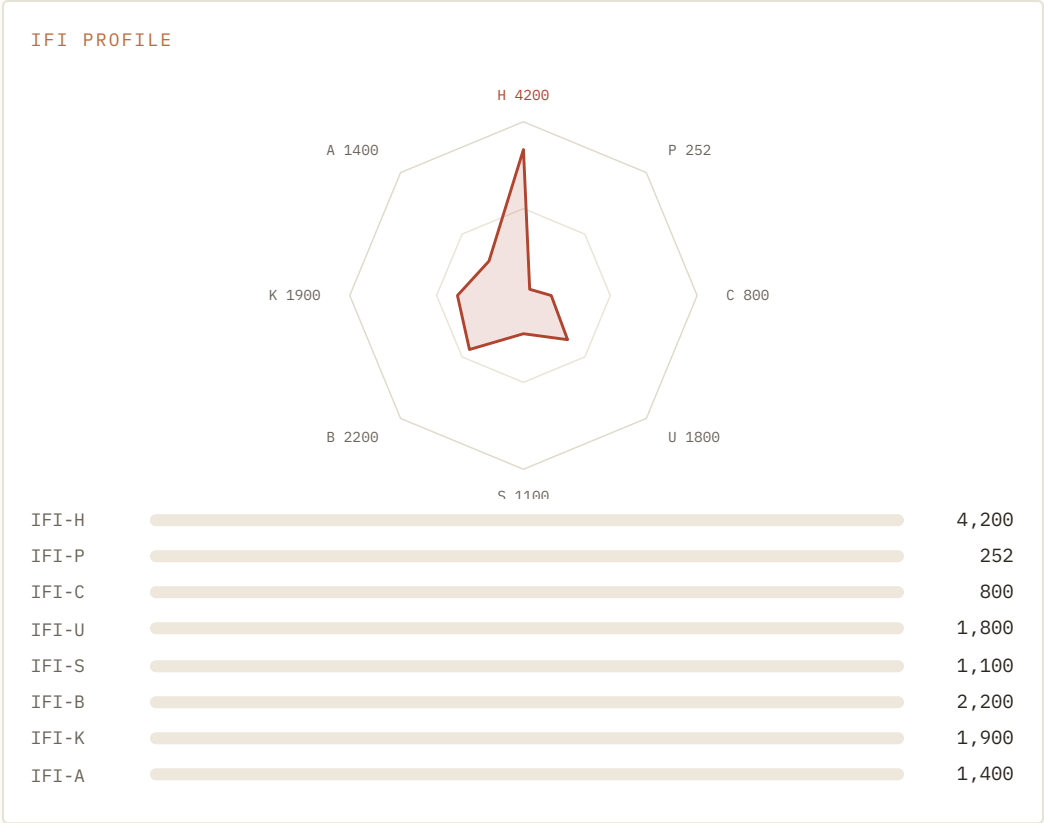
ORGANISM	CONSTRUCTS	LEAD AXIS	DOMINANT ELECTROPHILE	THE SHARP IT CARRIES
Black mustard	2,790	P / M	allyl isothiocyanate (AITC)	bright volatile flash
Garlic	560	K / P	allicin + γ-glutamyl peptides	allicin sharp, kokumi depth
White mustard	200	P	*p*-hydroxybenzyl ITC (sinalbin)	non-volatile tongue-smoulder
Ginger	120	P	6-gingerol / 6-shogaol	warm phenylpropanoid pungency
Thyme	120	P	thymol	faint monoterpene prickle
Watercress	120	P	phenethyl ITC (PEITC)	green, lingering
Cinnamon	40	P	cinnamaldehyde	slow warmth
Oregano	40	P	carvacrol	carvacrol whisper
Onion	40	K	thiosulfinates (LFS-tunable)	sweet allium sharp
Radish	40	B	4-methylthiobutyl ITC	crisp daikon sharp

Sum the shelf and it comes to **4,070 constructs** — just over four thousand points we can now place deliberately across the pungent organisms, where a decade ago every one of them was a lottery of soil and season. The chemistry sorts them onto one map. Volatility sets *where on the shape in time* each lands: black mustard's small AITC flashes and clears, while white mustard's *p*-hydroxybenzyl isothiocyanate is heavy and non-volatile and smoulders on the

tongue — the same TRPA1 cysteines, a different residence time, a different experience. The handshake itself is one short vocabulary of reactions:

ELECTROPHILE	SOURCE	REACTIVE CHEMISTRY	TRPA1 HANDSHAKE
Allyl ITC	black mustard, wasabi, horseradish	thiocarbamoylation (reversible covalent)	C621 / C641 / C665
Phenethyl ITC	watercress	thiocarbamoylation, non-volatile	C621 / C641 / C665
Allicin	garlic	S-thioallylation (disulfide exchange)	C621 / C641 / C665
Cinnamaldehyde	cinnamon	Michael addition (α,β-unsat. aldehyde)	C621 / C641 / C665
6-Gingerol / shogaol	ginger	vanillyl Michael acceptor	cysteine cluster + TRPV1
Carvacrol / thymol	oregano, thyme	non-covalent modulation	reversible site

Phoenix (SS-0096) shows what it looks like to hold one of these in balance against the other seven. It is primarily a heat cultivar — its capsaicin is written by two AI-designed substitutions in the Pun1/AT3 acyltransferase (S39L at −2.1 kcal/mol, L345G at −1.8 in predicted fold stability), designed to achieve peak output at a batch-to-batch coefficient of variation of ≤7%. But its pungency is not an accident of its heat; it is placed, a separate electrophilic voice dialed to sit well below the reference anchor.



The branch point — pungent versus bland, and the failure modes

Everything above assumes the bomb fires cleanly into isothiocyanate. It does not always. The glucosinolate aglycone sits at a fork, and which way it falls decides whether the plant is sharp or bland. At neutral pH the Lossen rearrangement dominates and you get the pungent isothiocyanate. But in the presence of **epithiospecifier protein (ESP)** and ferrous iron, or at acidic pH, the same aglycone is shunted toward a **nitrile** or, for alkenyl

glucosinolates like sinigrin, an **epithionitrile** — molecules that no longer engage TRPA1 at all. This is the **branch point between pungent and bland**, and it is the most powerful lever we have, because it does not require making more precursor. It only requires steering the one already there.

Write the perceived sharp as a product of three factors — the glucosinolate pool *G*, the fraction of myrosinase conversion, and the fraction *f* that resolves to isothiocyanate rather than nitrile:

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[AITC] ∝ f · G · (conversion)
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A wild-type brassica under field stress might resolve only half its aglycone to the sharp ($f \approx 0.5$), the rest bled off to bland nitriles. Knock down ESP and promote the epithiospecifier-modifier activity that favors isothiocyanate, and *f* climbs toward 0.95 — a **1.9× increase in delivered AITC at the same precursor pool**, without touching sulfur supply or precursor synthesis. In the threshold regime, where it matters most, that is a real perceptual jump. Take a wild-type seasoning dose sitting right at half-activation, [A] = EC_{50} (R/R_max = 0.50). The 1.9× design pushes [A] to $1.9 \cdot EC_{50}$, and the Hill function returns 1.9^1.8 / (1 + 1.9^1.8) = 3.13 / 4.13 ≈ **0.76**. The sharp rises from half-strength to three-quarters exactly where small amounts are used — the condiment regime, the pinch of mustard, the grating of wasabi.

But the saturation we derived earlier is also where this design philosophy meets its honest limits, and we state them plainly because the number is a form of respect. Consider the full culinary dose rather than a threshold pinch. Take an illustrative designed black mustard seed carrying 120 µmol/g of sinigrin at $f = 0.95$: that is 114 µmol/g of AITC, or 114 × 99.15 µg/µmol ≈ **11.3 mg of mustard oil per gram of seed**. A single gram ground into a 20 mL bolus of saliva and food releases 11.3 mg into 20 mL — 565 µg/mL, which against AITC's molar mass is 565 / 99.15 ≈ **5.7 µmol/mL = 5,700 µM**. Divide by the 8.3 µM EC_{50} and the channel is sitting at *687 times* half-activation, pinned flat against the top of the Hill curve. At that dose, raising *f* from 0.5 to 0.95 barely moves the *peak* at all — both are saturated. What the richer chemistry buys is not a louder peak but a lower threshold, a more robust and reproducible delivery, and a longer, fuller tail. The lesson is exact: **IFI-P predicts the threshold and the low-dose regime; total glucosinolate and *f* govern robustness and duration.** Designing the sharp well means knowing which of those you are actually spending on.

The failure modes follow the same logic. Myrosinase is a protein, and heat denatures it — cook a brassica before the enzyme meets the substrate and the bomb never fires; the glucosinolate passes through intact and bland, which is why a gentle bruise before cooking, or a raw finish, is not a garnish but a mechanism. Push the glucosinolate pool too high and the plant pays in growth and in bitterness, the brassica's other glucosinolate-linked signal on the TAS2R bitter receptors — a tradeoff against the zero-bitter axis that

has to be balanced, not ignored. And beneath all of it is the supply constraint we flagged at the molecular scale: none of this chemistry exists without sulfur.

The roadmap — the frontier, and portable sharpness



Ginger — the warm end of the sharp, carried on the phenylpropanoid branch every vascular plant already runs.

The second line of the roadmap is the frontier — the 64-concept Beyond-Peppers program, where the sharp becomes a design element in plants that never carried it. What makes that thinkable rather than fantastical is that the chemistries of pungency are, at the level of the pathway, **portable**, and they are portable along two independent roads we now understand well enough to install.

The first road is the phenylpropanoid branch — PAL → C4H → 4CL → HCT → COMT — which runs in *every* vascular plant, because it is the trunk route to lignin, to flavor, to pigment. Cinnamaldehyde and the gingerols are side-exits off that universal trunk. A plant that already makes phenylalanine already makes cinnamic acid; the distance to a cinnamaldehyde or a gingerol-type electrophile is a short, terminal set of reductase and synthase steps, not a new metabolism. The second road is the glucosinolate machinery itself — the CYP79 and CYP83 cytochromes, the sulfotransferases, the myrosinase, and the knockdown of ESP that sets *f* — a **finite, defined cassette** of enzymes rather than an open-ended mystery. Install the core, co-locate a myrosinase in a separate cell compartment, and steer the branch point, and a plant that never tingled can be given a precise, reproducible sharp. A tingle designed into a leaf to lift it; a clean isothiocyanate built into a root; the numbing-and-sharp chord of *málà* — the sanshool tingle on the two-pore KCNK potassium channels braided with TRPA1's bite — composed deliberately rather than stumbled upon.

That last one is the honest edge of the map. The KCNK numbing axis is long-horizon work, further out than the brassica and allium depth that is already in hand, and we name it as a direction rather than a delivery. But the principle under it is the same principle that lets us design Phoenix's pungency at IFI-P 252 and hold a batch to ≤7% where wild expression wanders across double-digit, sulfur- and weather-driven variance. That reproducibility is not a luxury on this axis — it is the whole point. A covalent, enzyme-gated bomb

chemistry is precisely the kind of thing that, grown by luck, swings wildly from plant to plant and season to season. Grown by intention, stated against a fixed 2.1 µM convention constant, it becomes a number you can specify, reproduce, and compose. First-order error propagation makes the stakes concrete: because IFI-P = k / EC_{50} with k a constant, the relative error carries straight through, σ_IFI / IFI ≈ σ_EC_{50} / EC_{50}. A designed cultivar at IFI-P 252 with a 7% CV holds a one-sigma band of roughly **[234, 270]**; the same nominal score at 42% CV spreads to **[146, 358]** — two plants that could read as different foods. The discipline of the small number is what turns the sharp from a surprise into a design.

The whole plant — sulfur spent well



Backlit at golden hour — a plant grown not for one molecule but for everything the sulfur builds.

Beneath both lines of the roadmap runs the quiet thing this work always returns to, and on the pungent axis it is not a sentiment — it is the chemistry itself, stated as plainly as we know how. The molecules of the sharp are made of **sulfur**, and sulfur is the plant's investment in its own richest chemistry. Trace it back and every isothiocyanate begins as a sulfate ion drawn from the soil, activated at the cost of ATP, reduced step by step to sulfide, fixed into cysteine, elongated through methionine, and finally built into the glucosinolate the plant stores against the day it is bitten. Two sulfur atoms spent per glucosinolate; one carried into the sharp and delivered, one returned as sulfate when the bomb fires. The pungency *is* the sulfur made visible on the tongue.

So a brassica grown to carry the fullest honest measure of its glucosinolates — not stripped by a breeding program that chased size over substance, not shortcut by a *f* bled away to bland nitriles, not pushed so hard it starves its own sulfur supply and stunts — is a plant delivering the whole of what it was built to deliver. That is the vegetarian-first instinct that has run under this family's work from the first seed: that to grow a plant to be most fully itself is to grow it to hold the most it can, and that flavor and substance, on this axis, are quite literally the same sulfur, spent well. We make no claim about what those molecules do once eaten; that is not ours to promise, and across this book we have been careful never to. What we hold instead is an intention — to grow the sharp and the substance together, because in the plants of this axis they are one thing, and to put the result on the table with the respect a living thing

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

That is the family's work, and the sharp, for all its ferocity, turns out to teach it best of all: that a pungency is a plant's chemistry of self, manufactured in the instant of being eaten, and that to design it well is to understand the plant so completely that you can ask it — precisely, reproducibly, against a number — to be most fully itself. The sharp bites, and then it clears. What it leaves behind, when it is grown and placed and composed with care, is the whole argument of this work in a single bright pulse: that flavor is chemistry, that chemistry is designable, and that to design it with reverence is to grow food that is more itself, more nourishing, and more alive — one axis, one plant, one number at a time.