

THE SCOVILLE SPLICE LIBRARY · VOLUME XI

Cool-Burn

Cool without cold. *The thermometer feels nothing.*

The receptor-level account of the thermal axis — how a leaf teaches the tongue to feel a cold that never arrives, and how we design that signal gene by gene across mint, lavender, eucalyptus, camphor, and the plants we taught to cool.

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

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Opening essay · The cold that isn't there

Crush a mint leaf between two fingers on a hot afternoon and something impossible happens: the air goes cool. Not the fingers — a thermometer pressed to the skin reports the same number it read a second ago, flatly, without drama. What cooled was the *message*. A handful of small molecules lifted off the bruised leaf, found nerve endings that have spent your whole life watching for winter, and told them a lie so convincing the body answered it as truth. You feel December in July. Nothing has changed but the message.

Our family has followed that one sentence — *nothing has changed but the message* — across forty-two plants, because it is the clearest thing taste has ever taught us. Flavor is not a property of food. Flavor is a conversation between a molecule and a receptor, a lock and a key struck a billion times a second on the wet surface of the tongue, and the tongue reports whatever the key tells it. Capsaicin tells the heat sensor a fire burns where there is none. Menthol tells the cold sensor a frost has come where there is none. The two are mirror images of a single trick, and this chapter is about the second one — the cool, and the strange warmth that so often rides beside it, the pair we have learned to call the thermal axis.

On the Iyengar Flavor Index this axis is **IFI-C**, Cool-Burn, and its receptor is **TRPM8** — the transient receptor potential channel, melastatin subfamily, member 8, the body's principal thermometer for everything below comfortable. Its warm shadow is **TRPV1**, the same channel heat and capsaicin speak to, which is why the axis carries two names in one. Cold and warmth are not two systems but a single argument the skin and tongue are always having, and menthol, icilin, eucalyptol, camphor, the long synthetic coolants — each one leans on that argument from its own side. Some only cool. Some cool, then turn and warm. A few, at the wrong dose, cool and *burn* at once, which is the paradox the axis is named for and the one we find most beautiful.



Dawn in the research greenhouse — the cooling work began over a glass of iced mint tea and never quite let go.

The channel that mistakes menthol for winter

To understand how a leaf fakes a frost, you have to meet the thing it lies to. TRPM8 is a protein that lives folded into the membrane of a sensory nerve ending, four copies of it gathered around a central pore like four hands cupped to hold water. Each copy threads the membrane six times — six helices we label S1 through S6 — and the four S5–S6 segments lean together to line the channel itself, a gate that is either shut or, for a few milliseconds at a time, open. When it opens it is not fussy about what it lets through: sodium, potassium, and above all calcium pour down their gradients into the cell. That inrush is the whole event. Calcium entering a cold-sensing nerve is the raw, wordless syllable the brain will later translate into the word *cold*.

What makes TRPM8 a thermometer and not merely a pore is that its gate is temperature-sensitive by design. At a comfortable mouth temperature near 34 °C it sits mostly closed, opening only now and then. Begin to cool the tissue and the balance tips: the open probability climbs as the temperature falls through its threshold near 26 °C and keeps climbing down toward 8 °C, where the channel is as loud as cold alone can make it. The protein does this because its gating is coupled, allosterically, to two other things at once — the voltage across the membrane and the binding of certain small molecules. Cold, voltage, and ligand are three levers on one gate, and the channel adds them up.

Menthol is a ligand that pulls the same lever cold does. It slips into a pocket cradled inside the voltage-sensing bundle, the S1–S4 helices, and seats itself against a single aromatic residue deep in the second helix — a tyrosine the structural work pins at position 745 in the human channel. Mutate that one residue and menthol loses most of its grip while cold still works, which is how we know the pocket is real and specific. Seated there, menthol does something quietly profound: it shifts the channel's activation curve so that the gate now opens at temperatures it used to ignore. The arithmetic of the gate has not changed; its set point has. A mouth at 34 °C, which should read as neutral, now reads to the brain as though it were sitting near 15 °C, because the channel is firing as if it were. The leaf did not cool anything. It moved the thermostat.

Not every coolant speaks through that exact pocket. Icilin, a synthetic super-agonist far louder than menthol, needs intracellular calcium to work and leans on residues one helix over, which is why its cold comes on with a deep, almost aching intensity the mint never reaches. Eucalyptol — 1,8-cineole, the breath of eucalyptus and a quieter note in many mints — is a weaker agonist at the same channel, cooling with a camphoraceous lift riding on top. Each molecule is a differently shaped key for a lock that was only ever built to read the weather, and the body, having no way to tell a key from a cold front, believes all of them.

The warm shadow, and why cool so often turns to burn

If TRPM8 were the whole story, this axis would be called Cool and nothing more. It is called Cool-**Burn** because the cold channel has a twin it cannot stop arguing with. TRPV1 — the receptor capsaicin inflames — is the body's sensor for heat above roughly 43 °C, and it sits in the same nerve endings, often the same cells, as the cold

channel. The two report to the brain along neighboring wires. Warmth is simply the other direction of the one thermal question the tongue is always asking, and several of the molecules we call coolants are not pure in their answer.

Camphor is the clearest case. It reads, at first, as cool and medicinal, and then it turns and warms, because camphor is at heart a TRPV1 agonist that also stirs the sharp channel TRPA1 — it speaks to the warm side while brushing the cold. Menthol itself is not innocent: at low dose it is almost pure TRPM8, a clean cold, but push the concentration high enough and it begins to touch TRPV1 and TRPA1 as well, and the clean cool acquires a bite underneath it. This is the literal mechanism of the paradox in the chapter's name. A mint that is merely strong is pleasant; a mint that is *too* strong crosses a threshold where the warm channels wake, and the mouth reports cold and burn in the same breath — the frost that stings. The failure mode and the signature are the same event seen at two doses.

We find this honest rather than inconvenient. The thermal axis is not two clean numbers but a single leaning quantity, and a profile that pretends otherwise is lying by omission. When we design a cool, we are really designing *where on the cold-to-warm argument* a food should sit, and how steeply it should tip if someone takes a second bite.

Writing cool as a number

A leaf can only ever tell a grower *that* it cools. To design cooling — to ask a plant for four minutes of it instead of forty seconds, or for a cool with no bite under it — we had to learn to say *how much*. The Index does this with one definition and one line of arithmetic, and the definition is the load-bearing half.

The thing we measure is not how much menthol a leaf holds. It is **EC_{50}** — the concentration of a molecule at which its receptor reaches half of its maximum response. A low EC_{50} means the receptor answers loudly to very little; the molecule is a persuasive speaker. A high EC_{50} means the receptor barely stirs until the molecule is lavished on it; the speaker is a mumbler. EC_{50} measures *loudness*, not *quantity*, and loudness is the thing a cook actually tastes.

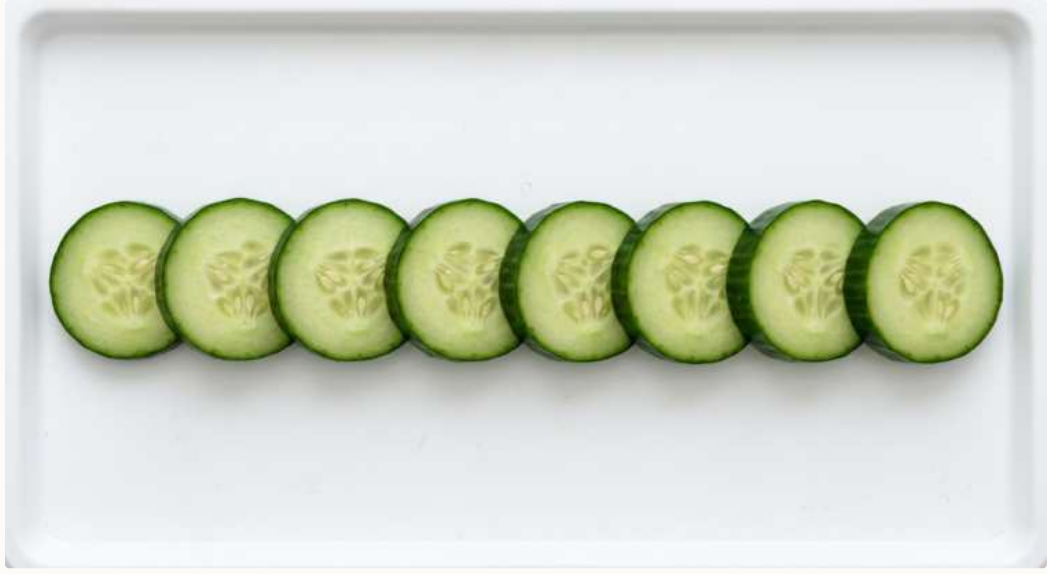
From loudness the score follows by inversion. Because a smaller EC_{50} means a louder molecule, we put EC_{50} in the denominator, fix a reference, and scale:

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

We anchor the reference to **(−)-menthol** itself — the molecule the whole axis is named for. By construction it sits at exactly **1,000**: substitute menthol for the candidate and the ratio collapses to 1,000 × 1 = 1,000, the fixed point every other cool is read against. And we cap the per-axis scorecard at **5,000**, the ceiling shared across the whole Index, so that the rare molecule loud enough to saturate the channel pegs the scale instead of running off the page.

The inversion is the idea, so it is worth watching it move. Suppose a candidate speaks to TRPM8 exactly half as softly as menthol — it needs twice the concentration to reach half-activation, so its EC_{50} is double. Then EC_{50}(reference) ÷ EC_{50}(candidate) = ½, and IFI-C = 1,000 × ½ = **500**: half as cool. Now suppose a candidate is twice as loud — half the EC_{50}. The ratio is 2, and IFI-C = **2,000**. Keep going: a molecule a fifth of menthol's EC_{50} scores 1,000 × 5 = **5,000** and meets the cap exactly; one a tenth of menthol's EC_{50} would compute to 10,000 and is clipped back to 5,000, pegged. The whole living range of cool, from a cucumber's hush to a synthetic coolant's roar, folds onto one short scale.

CANDIDATE EC_{50} (RELATIVE TO MENTHOL)	EC_{50}(REF) ÷ EC_{50}(CAND)	IFI-C
5 × menthol (speaks faintly)	0.2	200
2 × menthol	0.5	500
equal to menthol (the reference)	1.0	1,000
½ × menthol	2.0	2,000
⅕ × menthol	5.0	5,000 (cap)
⅒ × menthol	10.0	5,000 (pegged)



A cucumber in cross-section. It reads cool to the mouth, yet almost none of that cool is TRPM8 — the Index is careful to separate the channel's cold from the body's other colds.

A measurement, start to finish

The formula is clean; the measurement behind it is where the real care lives, so let us walk one all the way through as a lab would.

To find a candidate's EC_{50} we do not take a single reading. We expose the channel to a ladder of concentrations and watch the response climb, then fit the climb to a dose–response curve. The curve that describes a cooperative, four-subunit channel is the Hill equation:

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

Here R is the response, R_max its ceiling, [A] the coolant concentration, and *n* the Hill slope — a measure of how sharply the channel switches on. Set [A] equal to EC_{50} and the equation returns R_max ÷ 2 for any *n* at all; that is simply what EC_{50} *means*, the half-way point, falling out of the algebra. The Hill slope sets the steepness around it. For a channel that must gather more than one menthol before it opens cleanly, *n* sits near 2, and that number has a tangible consequence: with a slope of 2, a ninefold swing in concentration carries the channel from a tenth of its range to nine-tenths of it. The same journey on a non-cooperative receptor, slope 1, would take an eighty-one-fold swing. Cooperativity is why a good mint has a *threshold* — why a little does almost nothing and a little more does almost everything.

Now the units, because a receptor speaks in molarity and a confectioner buys in milligrams. Say a representative half-activation for menthol lands near 30 µM. Menthol's molar mass is 156.27 g/mol, so:

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30 µM = 30 × 10⁻⁶ mol/L × 156.27 g/mol = 4.69 × 10⁻³ g/L ≈ 4.7 mg/L
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In dilute water that is about 4.7 parts per million — a vanishingly small amount of matter to move a thermostat the size of a winter. The score does not change with the units; 4.7 mg/L and 30 µM are the same loudness written in two alphabets, and the ratio in the formula cancels the alphabet away.

Finally, the honesty of the number — its error. Every EC_{50} is a fit to noisy data, and the Index score is a *ratio* of two such fits, the candidate's over the reference's. When you divide two measurements, their relative uncertainties add in quadrature:

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CV(ratio) ≈ √( CV(candidate)² + CV(reference)² )
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We hold our engineered material to a batch-to-batch coefficient of variation of **≤ 7%** — against the **42%** a wild-type leaf swings across a season, sun to shade, morning to noon. Carry two measurements each at the 7% bound through the ratio and the score inherits $\sqrt{(0.07^2 + 0.07^2)} = \sqrt{0.0098} \approx \mathbf{0.099}$, call it ten percent. So an IFI-C we report as 2,000 is honestly 2,000 give or take roughly 200 — a real interval, not a decoration. A leaf at 42% could not tell 2,000 from 3,000, which is the whole reason ten thousand years of growers could keep the mint that cooled best and still never say by how much.

The pathway that builds the cool

A number is only ever a shadow of a molecule, and the molecule, in the plant, is built. The clean cool of a good peppermint is (−)-menthol, and (−)-menthol is the end of a short, insistent cascade that begins with the same building block every plant uses to make its scents: geranyl diphosphate, a ten-carbon unit the plant folds into a ring to make (−)-limonene. From limonene the route runs downhill through a run of oxidations and reductions — the ring hydroxylated by a cytochrome P450, then walked through isopiperitenol,

isopiperitenone, isopulegone, pulegone, and menthone before a final, stereo-careful reduction sets the last hydrogen and yields (–)-menthol. The committed, flux-controlling heart of that cascade is the **five-gene menthol module** we write and tune; everything upstream of it is machinery the plant already runs for a hundred other smells.

Two details of that pathway are where the art lives. The first is stereochemistry. The receptor's pocket is chiral — handed — and it reads (–)-menthol, the (1R,2S,5R) hand, with real affinity while all but ignoring its mirror image, (+)-menthol. A cool built from the wrong hand, or a sloppy racemic mix of both, spends half its mass on a near-silent isomer. The plant's reductases are themselves handed, so a clean cool is, quite literally, a clean stereochemistry: you tune the enzymes that set the last two carbons and you are tuning how much of the harvest speaks to the channel at all.

The second is what happens at the fork. The intermediate pulegone can divert toward menthofuran, and both pulegone and menthofuran are the harsh, off, slightly medicinal notes that ride under a cheap mint — and pulegone is a compound we would rather a body not take in quantity besides. To design a *clean* cool is to push flux down the main stem toward (–)-menthol and starve the branch that makes the off-note, so that what reaches the tongue is cold and almost nothing else. There is a quiet ethic in that for us: a cool with no harshness under it is not only a better flavor, it is a cleaner thing to give a body. We grow the signal, and we grow out what the signal does not need.



Dew on an engineered fruit at first light — the cold that is only ever a message, resting for a moment on a plant tuned to carry it.

The cooling plants, read on one scale

No single plant owns the cool. Peppermint is the loud, familiar center of it, but spearmint, eucalyptus, camphor laurel, lavender, and even the cool note hiding inside an orange each carry a differently shaped piece of the axis, and the point of the Index is that they can all be read on one page. The library we have built for this axis, counted in engineered constructs, looks like this:

ORGANISM	CONSTRUCTS	PRIMARY AXES	ENGINEERING DIFFICULTY
Peppermint	2,360	C / M	Hard
Lavender	160	C	Hard
Orange	120	B / C	Hard
Eucalyptus	80	C	Hard
Spearmint	80	C	Hard
Camphor	40	C	Hard

Every one of them is marked *Hard*, and the honesty of that label matters. Monoterpene pathways are longer than they look, they lean on temperamental P450 enzymes, and — as the whole stereochemistry of menthol insists — they demand that the handedness come out right or the yield is wasted on a silent isomer. These are not the easy wins. Peppermint carries the deepest library, 2,360 constructs, precisely because it is the plant where the clean cool is both most wanted and most worth the difficulty.

What each contributes is a different seat on the thermal argument. The chemistry sorts them better than the botany does:

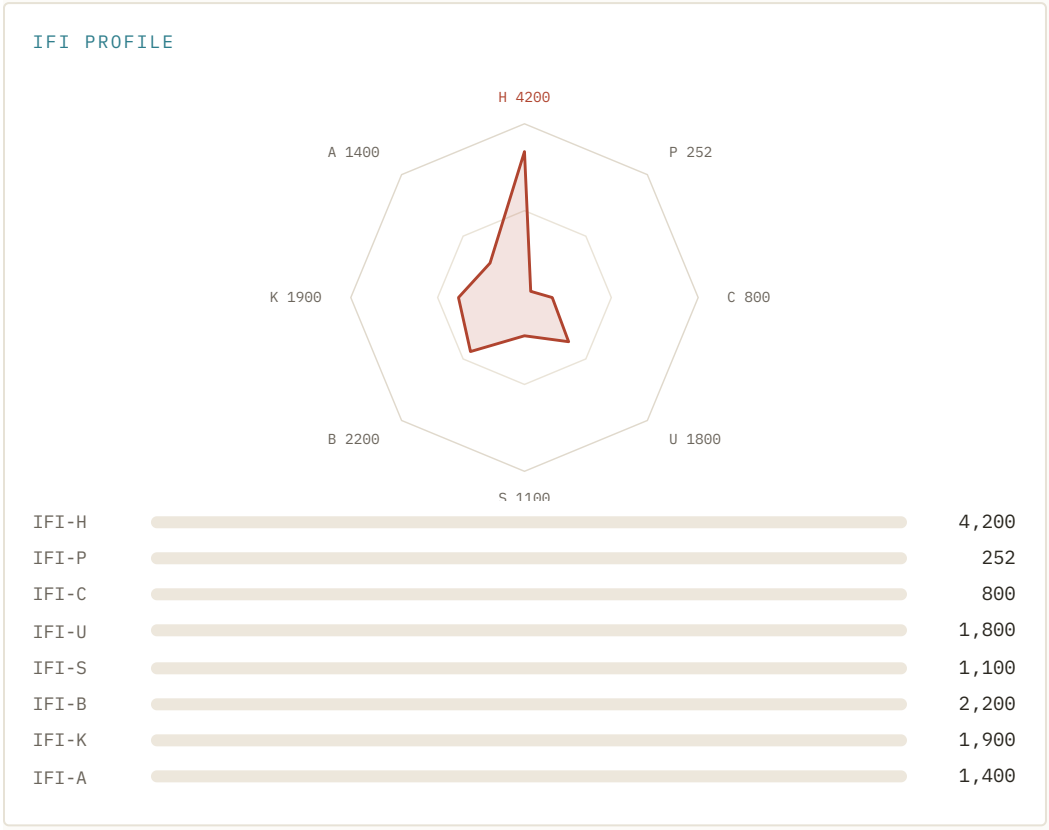
MOLECULE	CLASS	PRINCIPAL TARGET	SENSORY SIGNATURE
(−)-menthol	monoterpene alcohol	TRPM8 (agonist)	the clean, familiar cool; fades quickly
1,8-cineole (eucalyptol)	bicyclic ether	TRPM8 (weaker agonist)	cool with a camphoraceous lift
camphor	bicyclic ketone	TRPV1 / TRPA1	the warm shadow made plain
(−)-carvone	monoterpene ketone	weak at TRPM8	spearmint's sweet, herbal, cool-adjacent note
icilin	synthetic	TRPM8 super-agonist (Ca^{2+}-dependent)	deep, aching cold; no mint at all
WS-type coolants	carboxamides	TRPM8	long, lingering cool with no mint flavor

That last row is the confectioner's open secret and a hint of where design goes next: the synthetic carboxamide coolants speak straight to TRPM8 with none of mint's aroma, which is how a product can cool without tasting of peppermint. We are more interested in teaching a plant to carry a long, clean cool natively — a cool grown rather than dosed — but the row belongs on the page, because the Index reads loudness wherever it comes from.

Even fire carries a frost

The most beautiful proof that cool and burn are one axis is a fruit built to be the hottest thing we have ever grown. **Phoenix** — SS-0096, the first cultivar designed to register on all eight axes at once — our model predicts approximately 2.94 million SHU and a

predicted IFI-H of 4,200. It is, by any ordinary reckoning, pure fire. And yet when we read its full predicted profile, the cold channel is not silent:



Eight hundred on the cool axis, inside a pepper with a predicted heat of nearly three million SHU. That is not an accident and it is not a contradiction — it is the thermal axis told the truth. A fruit can be designed to lean hard toward the warm end and still carry a real, designed cool riding underneath, so that the first impression and the finish disagree, so that fire arrives with a frost folded inside it. The cool-and-burn the chapter is named for is, in Phoenix, not a dosing mistake but a composed effect: two ends of one argument, written into a single bite on purpose. The number 800 is small next to 4,200, and that is exactly the point — on one scale, you can see precisely how much winter a fire is designed to carry.

Honest limits

We would rather name the edges of this work than let a reader walk off them.

The first limit is time. TRPM8 desensitizes — the same calcium that carries the cold signal also, as it accumulates inside the nerve, quiets the channel that let it in. This is why a mint that blazes cold for twenty seconds is often dull by the fortieth, and it is the confectioner's genuine problem, not a flaw in the measurement. An EC_{50} captures loudness at an instant; it does not, on its own, capture *duration*. A long cool is a separate design target — a matter of delivery, of molecules that clear the pocket slowly, of starving the desensitization — and a high IFI-C does not guarantee it. A number that is loud is not automatically a number that lasts.

The second limit is the warm paradox, and it is a ceiling as much as a feature. Push any agonist high enough and the warm channels wake; a cool pursued past its clean range turns to sting. Designing a strong cool therefore means designing a *margin* — staying far enough below the dose where TRPV1 and TRPA1 join in that the frost never acquires its bite unless we mean it to.

The third limit is the gap between a dish and a mouth. We fit EC_{50} on a receptor in a controlled assay, where concentration is exact and temperature is held. A real mouth is warm, wet, moving, and full of saliva that partitions a coolant away from the very nerves it is meant to reach; perceived cool depends on how a lozenge dissolves, how a drink is chilled, how long the molecule lingers. The Index measures the channel's cold faithfully. It does not promise that two foods at the same IFI-C will feel identical across every delivery, and we say so plainly.

And there is a fourth, gentler limit, the one the cucumber taught us: not everything that reads cool to a person is cool to the channel. A cucumber's hush is mostly its water and its temperature and a green aldehyde in its aroma, with very little TRPM8 in it at all. The Index is deliberately narrow — it scores the channel's cold, the menthol kind, the lie told to the winter sensor — and it leaves the body's other colds to be measured by their own honest means rather than folded in to flatter a number.

What we do not do is make a claim on anyone's medicine. We design the signal, and the signal is cool, and cool is a kindness the mouth has always known how to receive — the relief of it, the clean relief, is real, and it is also not a cure, and we will not dress it as one. Ten thousand years of growers kept the mint that cooled the best and threw out the rest, never once able to say *how much*, never able to part the clean cool from the off-note riding beneath it, never able to ask a plant to do a thing it had not already half-learned. We can ask now, and we can design toward the answer, and we can write it down to within about ten percent. The leaf still tells its beautiful lie. We have only learned, at last, to hear exactly how loudly it is designed to lie — and to grow a plant that tells the truest version of it, cold and clean and almost nothing else, which is all we ever wanted the message to be.

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



PART I OF IV

The Receptor

TRPM8 · the cold channel · the warm shadow · chemesthesis, not taste

Chapter 1 · The Axis With Two Faces — *cool is not cold, and burn is not heat.*

Before anything else, the correction we have to make at every table where this comes up — and in our family it comes up often, because a cooling plant is the first thing a child asks after once they learn that a mint *isn't actually cold*: cool is not a taste. Sweet is a taste. Sour, bitter, salty, umami — each is read by a dedicated receptor on a dedicated population of cells, wired through the facial and glossopharyngeal nerves to the brainstem, each one a genuine report of *what a thing is made of*. Cool belongs to another sense entirely. It is **chemesthesis** — chemical sensation carried not by the taste nerves but by the **trigeminal nerve**, the fifth cranial nerve, the same wiring that reports a pinch, a scrape, grit under the eyelid, the sting of a cut onion, the warmth of a radiator held near the cheek. Heat lives there. Pungency lives there. And this axis, Cool-Burn, lives there most completely of all, because it is the one that borrows the nerve's own oldest language — temperature — and learns to speak it with nothing but molecules.

Three of our eight axes are chemesthetic: Heat, Pungency, and this one. They are not reports of flavor so much as reports of *temperature and touch the chemistry has forged* — a thermal weather the mouth insists is real. The tongue cannot tell the difference between a true chill and a convincing lie about one, and the whole of this chapter, and most of the craft, lives inside that single fact.

The trigeminal field, mapped

The anatomy is worth holding clearly, because it quietly explains almost everything the cool does. The trigeminal nerve splits into three divisions — ophthalmic, maxillary, mandibular — and its sensory cell bodies gather in the **trigeminal ganglion**, a dense knot of neurons just inside the skull. From there, bare and thinly-sheathed endings fan outward: the unmyelinated **C fibers**, slow and smoldering, and the lightly myelinated **Aδ fibers**, faster and sharper. They reach the lips, the gums, the deeper tissue of the tongue beneath the taste buds, the palate, the throat, the whole lining of the nose and the breathing passage behind it. These are the identical free nerve endings that carry pain and temperature from the skin. The mouth, in the end, is skin turned inward and made exquisitely sensitive — which is why what cools the back of your hand will, in the right dose, cool the back of your throat.

When a cooling molecule opens a cold-sensing channel on one of those endings, the ending fires exactly the volley it would fire if the tissue had truly gone cold. The brain, which keeps no independent

thermometer of its own, believes the volley without question. This is **labeled-line coding** at its purest: the cold line fires, the brain reads *cold*, and whether the tissue actually lost a single degree never comes up for review. A mint does not lower your temperature. It persuades the one nerve that would know.



Basil — like peppermint, spearmint, and lavender, a member of the mint family Lamiaceae, the aromatic lineage where the cooling terpenes run richest.

This is also why coverage, not concentration, is the thing that so often decides whether a cool *works*. Taste is confined to the taste buds — the tongue's upper surface and a scattering on the palate. Chemesthesis is everywhere the trigeminal nerve reaches. A strong peppermint cools a space you did not know had temperature: the sinuses opening on an inhale, the throat going wide and cold on a swallow, a coolness that seems to arrive from behind the eyes. When a confectioner complains that a cooling agent "doesn't bloom," what they mean, in our language, is that the molecules are not reaching enough of that trigeminal field. The fix is almost never more flavor. It is more *coverage* — more of the field recruited at once — and coverage is a volatility-and-delivery problem with a receptor sitting at the center of it.

Four sentinels, one thermometer

The axis has two faces because the thermal nerve posts more than one sentinel, and in real food they are never cleanly apart. Four channels together write the thermal weather the mouth reports, and Cool-Burn is the axis that scores the cold sentinel and its warm shadow as a pair.

RECEPTOR	AXIS	NATIVE TRIGGER	CHEMICAL KEY	THRESHOLD	WHAT YOU FEEL
TRPM8	IFI-C (cool)	cold	(−)-menthol, icilin, WS-23, eucalyptol	below ~26 °C	clean, fresh cool
TRPV1	IFI-H (heat)	heat, acid	capsaicin, protons	above ~43 °C	warmth → burn
TRPV3	warm shadow of C	warmth	camphor, carvacrol, eugenol	~32–39 °C	gentle, blooming warmth
TRPA1	IFI-P (pungent)	reactive electrophiles	allyl isothiocyanate, cinnamaldehyde, high-dose menthol	noxious cold / chemical	sharp prickling sting

Read that table as a map of the paradox in this book's title. **TRPM8** watches the cold side, firing as tissue drops below roughly 26 °C and firing just as readily when menthol arrives to counterfeit the drop. **TRPV1** watches the hot side, opening above roughly 43 °C and

when capsaicin forges *that*. Between them sit **TRPV3**, a warm sensor of skin and mouth that camphor and carvacrol wake, and **TRPA1**, the irritant channel, which menthol itself recruits once the dose runs high. A single molecule — menthol — can touch three of these four depending only on how much of it arrives. A whisper hits TRPM8 alone and reads as pure, clean cool. A shout spills onto TRPA1 and the cool acquires a sting, the thing a brutal peppermint does to the back of the throat. And camphor, the aromatic chill of a chest rub, is mostly a *warm* molecule in cold's clothing — a TRPV3 agonist that can even blunt TRPM8, reading as cooling only because its sharp volatility floods the whole trigeminal field at once and the brain scores the rush as fresh.

Every one of these four is **polymodal** — each answers to temperature *and* to chemistry *and* to voltage, summing its inputs rather than choosing between them. That is the deep reason cool and burn are, at the edges, the same gesture seen from two sides: they are run by sister proteins from one family, built to the same plan, posted to opposite ends of the same thermometer.

Inside the cold sentinel

To design on this axis we had to stop treating TRPM8 as a word and start treating it as a machine. It is a **homotetramer** — four identical subunits arranged around a central ion-conducting pore. Each subunit threads the membrane six times (the helices S1 through S6); the four S5–S6 segments line the pore itself, while the four S1–S4 bundles form a **voltage-sensor-like domain**, the allosteric engine of the channel. When the channel opens, it is not fussy about what it lets through: it is a non-selective cation channel, passing Na^+ and — the detail that matters most downstream — **Ca^{2+}**.

Menthol does not pry the pore open by force. It slips into a pocket inside that S1–S4 bundle, against a key tyrosine on the second helix, and by binding there it *tilts the channel's own arithmetic*. TRPM8's opening is governed by a balance of votes — cold casts one, membrane voltage casts another, the signaling lipid **PIP_2** (phosphatidylinositol 4,5-bisphosphate) is required as a standing cofactor, and menthol casts a vote of its own. Each agonist shifts the voltage dependence of opening toward the resting potentials a nerve actually sits at. Cold shifts it. Menthol shifts it. Together they shift it further than either alone — which is precisely why a cold drink with mint in it feels colder than either the cold or the mint has any right to make it. The sensations are not added. They are *multiplied*, through one shared allosteric gate.



The assay stage where a cooling response is read — the quiet instrument work behind a single EC_{50}.

Two more mechanistic facts earn their place in a designer's head. First, the counterfeits are not interchangeable. **Icilin** is a synthetic super-agonist — far more potent than menthol — but it is a conditional one: it demands a pulse of intracellular Ca^{2+} and the right residues in the pore to work at all, and in some species it barely works, which is a caution every time a panel tastes "cooler" than the chemistry predicts. The **WS-series carboxamides** (WS-3, WS-23) are odorless coolants that open TRPM8 cleanly while carrying almost no aroma and almost none of menthol's high-dose bite — the molecular basis for decoupling *the feeling of cool* from *the smell of mint*, which is half of modern oral-care design. Second, that Ca^{2+} the open channel admits is also its off-switch. Calcium entering through TRPM8 activates a lipase that consumes the very PIP_2 the channel needs, and the channel quietly runs down. This is **desensitization** — the reason the third mint is weaker than the first, and a failure mode that earns its own honest reckoning before the craft can be trusted.

The arithmetic of loudness

A sensation you cannot measure is a sensation you cannot design, and the whole Index exists to turn loudness into a number. The rule is one line:

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

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response — the midpoint of the dose–response curve, and the single most honest one-number summary of potency. It is small when a molecule is loud (a little goes far) and large when a molecule is faint. Inverting it is what makes the Index read the right way up: **a lower EC_{50} earns a higher score.** We scale by a thousand and anchor the reference so that **IFI-C = 1,000** is the half-activation of our wild-type cooling reference — peppermint's own menthol, read against TRPM8 under fixed assay conditions.

Fix that anchor at a working value so the arithmetic is concrete. Take the reference menthol EC_{50} as **80 µM** at the receptor. Then every score on the axis is just a ratio away from a concentration:

- A candidate that scores **IFI-C 1,000** has, by definition, EC_{50} = 80 µM — it equals the reference.

- A candidate at **IFI-C 2,000** must be twice as loud: EC_{50} = 1,000 × 80 ÷ 2,000 = **40 µM**.
- At the scorecard ceiling, **IFI-C 5,000**: EC_{50} = 1,000 × 80 ÷ 5,000 = **16 µM** — five times the potency of the reference, the loudest cool the card will draw.
- And a quiet cool riding alongside a dominant heat — the C **800** that our full-spectrum pepper carries — works out to EC_{50} = 1,000 × 80 ÷ 800 = **100 µM**, a cool a shade *softer* than reference menthol, exactly as a pepper's cool should be.

The dose–response curve those midpoints summarize is a **Hill function**: response = R_max × $[L]^n$ ÷ (EC_{50}^n + $[L]^n$). Set the ligand concentration [L] equal to EC_{50} and the term collapses to R_max ÷ 2 — half-maximal, which is the definition earning its keep. The exponent *n*, the Hill slope, runs near 2 for menthol on TRPM8, the fingerprint of a tetramer that opens best when more than one molecule has taken its seat. A steep slope (large *n*) is a cool with a threshold — nothing, nothing, then suddenly everything; a shallow slope is a cool that comes up like a dimmer. Both can share an EC_{50} and feel utterly different in the mouth, which is the first thing the single number cannot tell you, and the first thing a good designer checks behind it.

Because formulators think in weight, not molarity, the conversion has to be clean too. Menthol's molar mass is **156.27 g/mol**, so the reference 80 µM is 80 × 10^{-6} mol/L × 156.27 g/mol ≈ **12.5 mg/L** — about 12.5 parts per million of menthol-equivalent at the receptor. The ceiling at 16 µM is ≈ **2.5 mg/L**. The whole usable axis, reference to ceiling, lives inside a fivefold window of concentration — which is why precision, not brute dose, is the craft.

IFI-C	EC_{50} (mM)	MENTHOL-EQUIV (mg/kg)	RELATIVE POTENCY	READING
250	320	50.0	0.25×	a faint breath
500	160	25.0	0.5×	mild, background
800	100	15.6	0.8×	the cool inside a heat
1,000 (ref)	80	12.5	1.0×	reference cool
2,000	40	6.3	2.0×	loud, clean
4,000	20	3.1	4.0×	intense
5,000 (cap)	16	2.5	5.0×	scorecard ceiling

One more number keeps the rest honest: the error. Our platform holds batch-to-batch output to a coefficient of variation of **≤7%**, against the 42% that unselected wild-type populations drift across — and a score is only as trustworthy as that spread. The Index is a ratio, and errors in a ratio add in quadrature: for IFI-C = 1,000 × EC_{50}(ref) ÷ EC_{50}(candidate), the relative variance is $(\sigma/IFI)^2$ = $(\sigma_ref/EC_{50}_ref)^2$ + $(\sigma_cand/EC_{50}_cand)^2$. Run the reference as a tight internal standard each session — the discipline that makes the EC_{50} comparable at all — and its term nearly vanishes, so a score inherits essentially the candidate's own ≤7%. A reported **IFI-C 2,000** is therefore 2,000 ± ~140: a real band, not a point. Writing it as a bare

integer is a convenience. Reading it as one is a mistake, and the whole reason we fix the assay temperature, the reference, and the batch before we ever trust the third decimal of anything.

The plants we speak cool through

The cool does not come from a laboratory; it comes from a lineage of aromatic plants, most of them in the mint family, **Lamiaceae** — the same family as the basil on the previous page, and as peppermint, spearmint, and lavender. These are the plants whose glandular hairs brim with monoterpenes, and our work is to tune how loudly those terpenes land on TRPM8, and how far across the trigeminal field they bloom.

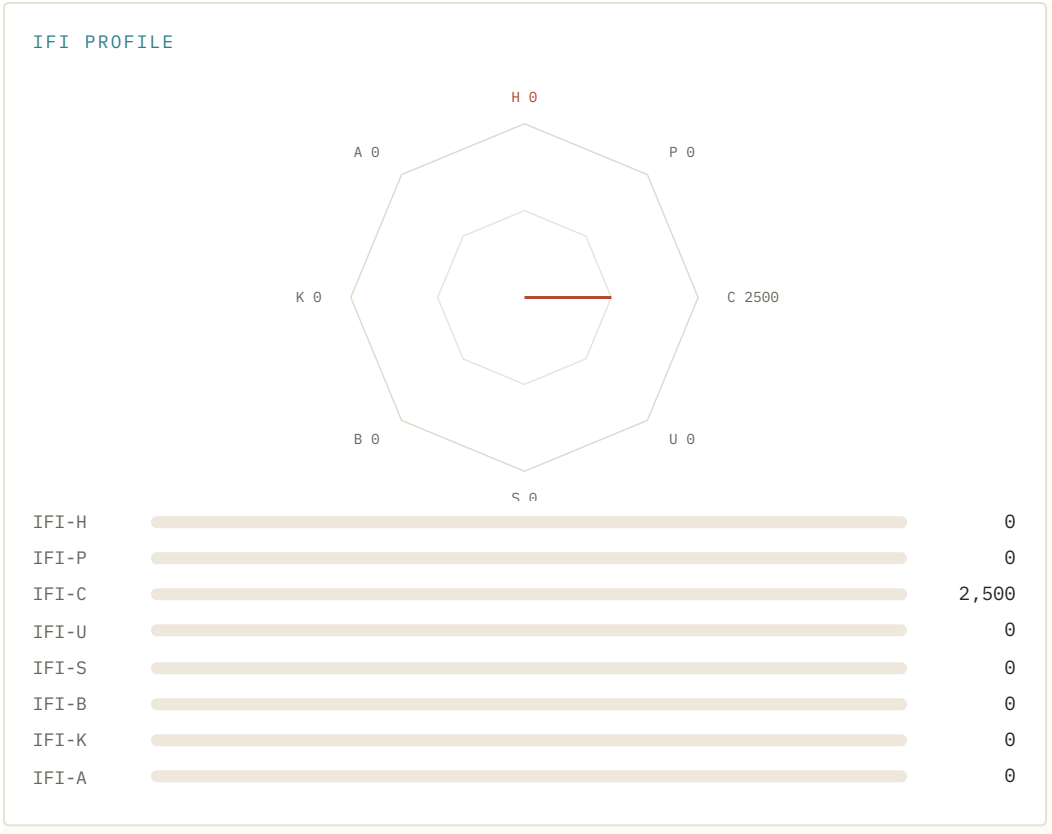
ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	COOLING SIGNATURE
Peppermint	2,360	C / M	Hard	(−)-menthol — the loud, clean reference cool
Lavender	160	C	Hard	linalool / camphor — soft, floral, warm-edged
Orange	120	B / C	Hard	a bright cool riding a bittersweet peel
Eucalyptus	80	C	Hard	eucalyptol (1,8-cineole) — airy, sinus-opening
Spearmint	80	C	Hard	carvone-led — rounder, sweeter, gentler cool
Camphor	40	C	Hard	the warm shadow — TRPV3 wearing cold's clothes

Peppermint carries the deepest construct library — **2,360** — for a reason: it runs the richest route to the loudest coolant, and nearly every lever on the axis can be rehearsed in it first. The route itself is the **five-gene menthol pathway**, a small committed cascade that walks a terpene backbone from geranyl diphosphate into (−)-limonene, then hydroxylates, oxidizes, and reduces it in turn — limonene cyclized, the ring hydroxylated by a cytochrome P450, the alcohol oxidized, the double bonds reduced step by step — until it arrives at menthone and, with a final reduction, at **(−)-menthol**. The stereochemistry is the whole game: menthol has three stereocenters and therefore eight possible stereoisomers, and only one of them, the natural (1R,2S,5R) (−)-menthol, fits the TRPM8 pocket with real authority. Its mirror image is a faint echo; its diastereomers are near-silent. A pathway that leaks the wrong isomer does not make a *weaker* cool so much as a muddier one, and much of what the 2,360 constructs are for is keeping that final reduction clean — pushing flux down the committed branch and starving the side-route to menthofuran that otherwise bleeds the yield and dulls the note.

Placing a plant on the edge

Our work, in the end, is to set a plant exactly where we want it on that edge — and the edge has three landmarks worth naming, because each is a different design and a different brief.

A **beverage mint** should sit deep in pure TRPM8 territory: loud cool, no sting, no warm tail. The brief we hand the bench reads, in target form, as a single spike —

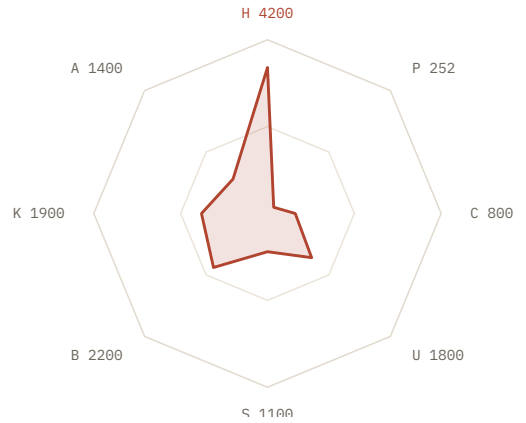


— an IFI-C near 2,500 (EC_{50} ≈ 32 µM, roughly twice the loudness of reference menthol) and silence everywhere else, because the one thing a beverage cool must not do is prickle. That means holding the menthol dose below the point where it begins to recruit TRPA1, and winning the extra loudness through *coverage and potency* rather than concentration — the WS-series logic of a cleaner key in the lock, not simply more keys.

A **warming winter confection** is the opposite brief: it rides the TRPV3 shadow on purpose, a cool that arrives fresh and then resolves into warmth as the camphor and carvacrol wake the warm sensor a half-second behind the cold one. On the axis it reads modest — an IFI-C near 900, EC_{50} ≈ 89 µM — because the *point* is not loudness but the turn from cold to warm, a two-channel choreography that a single number can only hint at.

And then the crossing itself. We have built peppers that cool and burn in the same bite — our full-spectrum cultivar, Phoenix (SS-0096), the one plant engineered to register on all eight axes at once, lives there deliberately:

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

Read the two numbers that matter here together. **H: 4200** is our model's predicted heat of approximately 2.95 million SHU — a TRPV1 signal near the top of anything grown. **C: 800** is a genuine, predicted TRPM8 cool, designed EC_{50} ≈ 100 µM, sitting right underneath it. Fire both sentinels at once and the mouth cannot arbitrate: the cold line and the hot line report simultaneously, and the brain, holding two true messages that contradict, experiences neither January nor July but the vertiginous *both*. That is the title of this book made flesh in a fruit — and it is only buildable because we can read the cold sentinel and the hot sentinel each as its own number, tune each to its own target, and hold each to its own ≤7% band.



Phoenix (SS-0096) in the controlled chamber — a single cultivar tuned to fire the cold sentinel and the hot sentinel in one bite.

Cool borrows sweetness

Cool does not act alone even once it leaves the trigeminal nerve; it reaches sideways into the taste senses and bends them, which is why the beverage and confectionery worlds care so much about getting it exactly right. Cooling the mouth — really, or by convincing counterfeit — measurably suppresses the perception of some tastes and sharpens others. Sourness reads brighter against cold. Carbonation's prickle, itself partly a TRPA1 and acid-sensing event, feels sharper when the cool primes the field. And — the cross-modal

fact that built an industry — **cool and sweet are bound together in the mind**, so a clean cool carries the *impression* of sweetness well past the point where the sugar has actually run out.

This is not loose synesthesia; it is measurable in threshold studies, where a fixed sugar concentration is judged sweeter in the presence of a cooling agent than without it, the perceived-sweetness curve lifting by a reliable margin. It is the reason a sugar-free mint does not taste deprived if its cool is built well: the cool collects on a debt of sweetness that memory and cross-modal wiring have already promised. For a family that has always cooked without reaching for more sugar than a dish needs, this is one of the quietly satisfying facts of the whole axis — that you can give the body the pleasure of sweetness by giving it, instead, a well-made cold. A designer who knows the binding builds the cool to collect the debt, and lets the sugar stay home.

The honest limits

A number this useful earns its keep only if we are honest about where it thins, and the cool axis has more thin places than most.

The cool fades as you use it. The Ca^{2+} that TRPM8 admits consumes the PIP_2 the channel runs on, and the cool desensitizes — the third mint weaker than the first, a sustained menthol dulling within seconds to a tenth of its opening strength. An EC_{50} measured on a rested channel overstates the cool of a mouth already two sips in. Where the use is repeated — gum, a long drink, a lozenge worked slowly — we design against the run-down, not just toward the peak, and we say so rather than quoting the fresh-channel number as if it lasted.

The reference is conditional, so we pin it. Cold and menthol are allosteric partners, which means menthol's EC_{50} genuinely *falls* as the tissue cools — a candidate measured at 22 °C will read louder at 15 °C and softer at 32 °C. There is no single true EC_{50} floating free of a temperature; there is only an EC_{50} at a stated temperature, run against a reference measured the same day under the same conditions. Every score on this axis is therefore a comparison, never an absolute, and the ≤7% we promise is a promise about *reproducibility*, not about a platonic constant that does not exist.

Loud is not the same as covering. Two cools can share an EC_{50} and part ways entirely in the mouth: one blooms across the sinuses and throat, the other sits as a bright point on the tongue and goes nowhere. Potency is what the receptor assay measures; coverage is a matter of volatility, delivery, and where in the trigeminal field the molecule physically arrives — and a score that captures the first is blind to the second. A faint-but-everywhere eucalyptol can read "cooler" to a panel than a loud-but-local menthol the number ranks above it.

Labeled-line is a working simplification. The clean story — cold line fires, brain reads cold — is true enough to design on and not the whole truth. Real thermal perception is partly combinatorial: the brain weighs the chorus of TRPM8, TRPV3, TRPA1, and TRPV1 together, and a strong enough signal on one channel colors how it

reads the others. The cool-burn crossing is the proof that the lines are not perfectly private. We build on labeled-line because it holds across the doses that matter; we stay alert to where it bends.

Menthol is two molecules at two doses. Below its TRPA1 threshold it is clean cool; above it, the same molecule turns on the irritant channel and the cool grows a sting. The window between loud-enough and too-much is real and narrow, and the whole reason the WS-series carboxamides exist is to widen it — to reach a high IFI-C without ever waking the sting. A design that chases loudness by dose alone will find the prickle; a design that chases it by potency walks around it.

None of this is a retreat from the number. It is the opposite — the care that makes the number worth trusting. We measure the cool because we revere it: because a cold that never touched the tissue, grown into a plant gene by careful gene and read receptor by receptor, is one of the cleaner pleasures we know how to give a body, and a pleasure you can only place with precision if you first learned to count it. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The axis is called Cool-Burn because at the edge, cool and burn are one gesture seen from two sides — and the gift of a number is that it lets us stand a plant exactly on that edge, and mean it.

Chapter 2 · TRPM8, in Full — *the channel that reads a winter.*

TRPM8 is a protein shaped like a gate, and like every good gate it is mostly a decision about when to be open. Four identical protein chains assemble into a single channel — a homotetramer — arranged around a central pore that, when it opens, lets sodium and calcium ions flood from outside the nerve cell to inside. That flood is the signal. Enough ions cross, the membrane's voltage tips past threshold, the nerve fires an action potential, and the brain receives a message it has no choice but to read as *cold*. Everything interesting about the cool axis is a question of what makes those four chains decide to open — and everything we design, we design by moving that decision.

We write this chapter the way we read the channel itself: from the outside in, from the shape of the protein to the arithmetic of its gate, and then back out to the tongue, where a bruised leaf tells a nerve a convincing lie about the weather. The wonder and the math are the same object seen from two sides. A winter you can feel in July is, underneath, a few millivolts moved on a single equilibrium, and we find both halves of that sentence equally beautiful.

Four chains and a gate

The channel was found in 2002, and the story of its finding is a lesson in the method. Two laboratories — one chasing the cold receptor by screening for menthol sensitivity, one by screening for cold sensitivity — arrived at the same gene, which is the cleanest possible proof that menthol and cold are two keys to one lock. The gene was already known to cancer biologists under another name, which is why the protein carries the awkward "melastatin" in its title, a vestige of a life it had before anyone knew it was the reason a winter wind stings. We find that history fitting. The cold sensor was hiding in plain sight, mislabeled, until someone asked the right question with the right molecule.

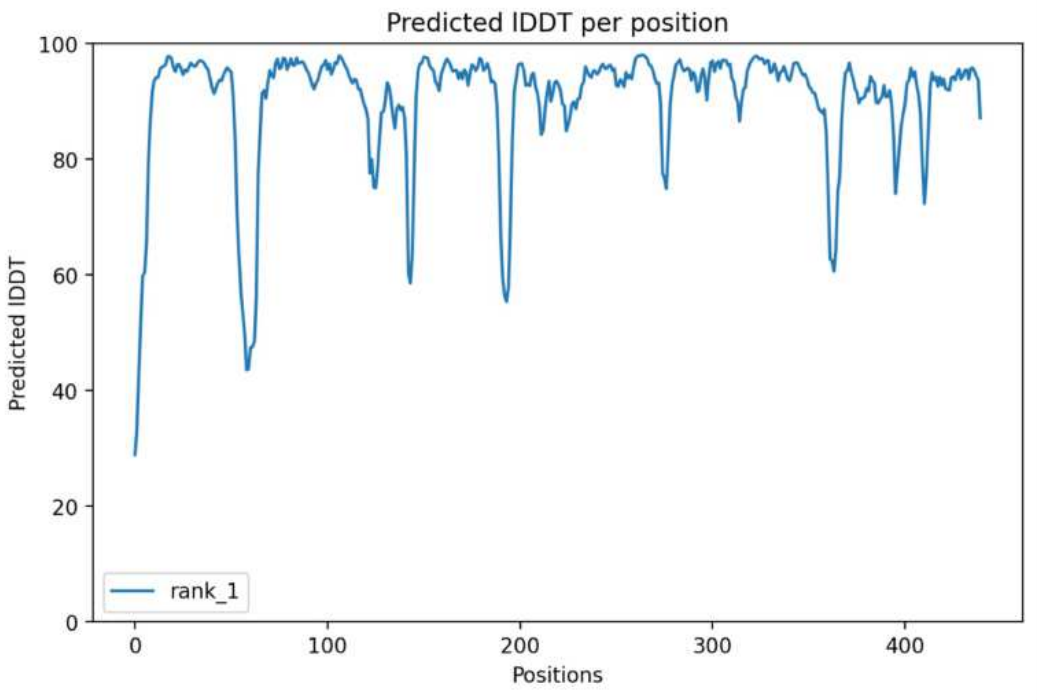
Each chain carries six membrane-spanning helices, numbered S1 through S6. The last two, S5 and S6, from all four subunits together line the pore itself — the physical hole the ions pass through, guarded by a selectivity filter that lets through sodium and, importantly, calcium, with a calcium-to-sodium permeability ratio of roughly three to one. That calcium is not incidental; it is the signal's own off-switch, the same ion that opens the channel's story and, a minute later, ends it. The first four helices, S1 through S4, fold into a compact bundle on the edge of each subunit called the **voltage-sensor-like domain**, the VSLD, and this bundle is the most important real estate in the whole protein for us, because it is where menthol binds. Below the membrane, a long **TRP helix** runs alongside the pore like a latch bar, mechanically coupling the sensor domains to the gate, and a large cytoplasmic skirt — the melastatin-homology regions, MHR1 through MHR4, plus a pre-S1 shoulder — holds the whole assembly together and anchors the lipids the channel cannot live without.

That the pore and the sensor are *separate* domains, bridged by the TRP-helix latch, is the architectural fact that makes the channel designable. A pore that opened only when a molecule was jammed physically into its throat could be tuned only by brute force. A pore opened at a distance, through an allosteric linkage, can be coaxed: move the equilibrium by a little energy applied anywhere on the machine — the lipid skirt, the voltage sensor, the pocket — and the gate downstream answers. The whole art of designing a cool is the art of finding the cheapest place to push.

The pocket, resolved

Cryo-electron microscopy of TRPM8 resolved the cooling-agent pocket directly, and it sits exactly where the pharmacology always said it should: inside the VSLD cavity, a pocket cradled by S1 through S4. A single tyrosine, **Y745** on helix S2, is the residue the field learned to watch first — mutate it and menthol loses most of its grip while cold sensing survives, the cleanest demonstration anyone could ask for that the molecule and the temperature are separate keys fitting the same lock from different angles. The superagonist **icilin** needs more. Its site overlaps menthol's but reaches further, and it requires a short run of residues — an asparagine, an aspartate, a glycine in the S2–S3 region, the stretch the field nicknamed the "icilin gap" — together with intracellular **calcium** as a co-requirement, which is why icilin's cold is calcium-dependent and menthol's is not. Two agonists, one pocket, different handshakes: it is the kind of detail that looks like trivia until you are trying to design a coolant that behaves like one and not the other.

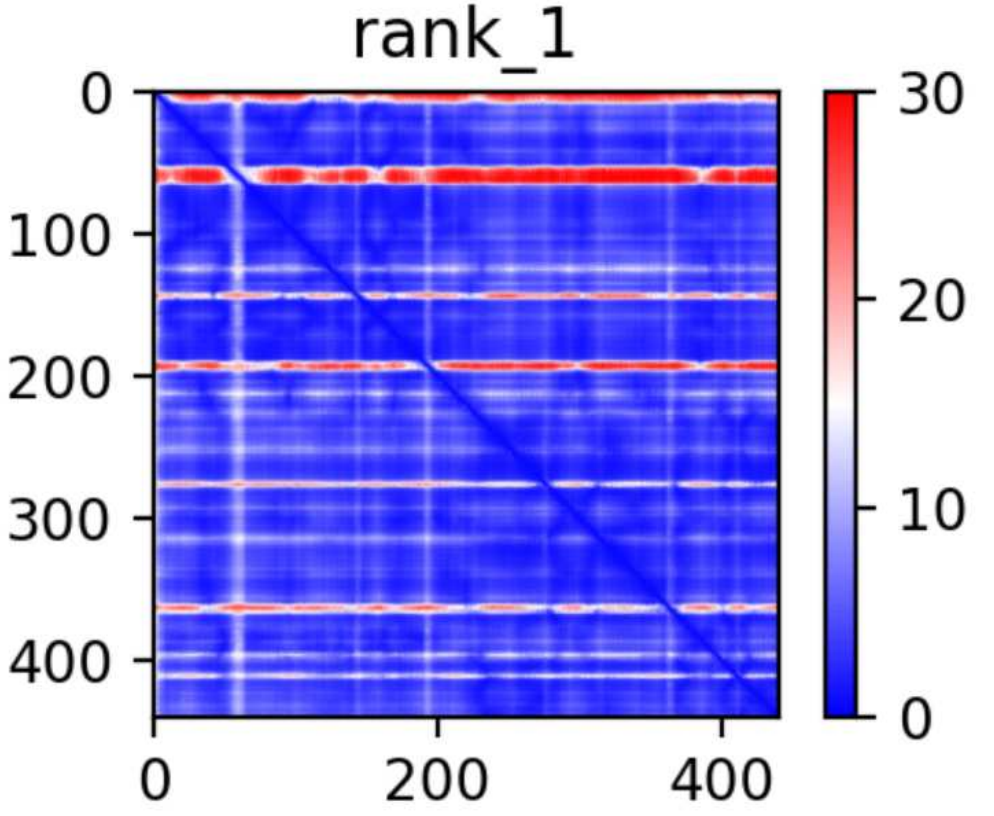
We work that pocket in the computer before we ever touch a plant. The same structural-modeling pipeline we run on every flavor enzyme — a predicted fold, scored residue by residue, the binding cavity resolved and the key docked into it — we run on TRPM8, so that a proposed coolant can be seated against Y745 and the icilin gap in silico and ranked before a single construct is built.



Our protein-modeling pipeline, shown here on the capsaicin acyltransferase Pun1/AT3, is the same one we run on TRPM8 and on every menthol-pathway enzyme — residues coloured by pLDDT, the binding pocket resolved at high confidence.

A model is only worth what its confidence is, so we read two numbers off every prediction before we trust it. The first is **pLDDT**, a per-residue score from 0 to 100 that estimates how sure the model is

of each atom's local position; the cradle of helices around the cooling pocket resolves in the high-confidence band, which is why we are willing to dock against it, while the floppy cytoplasmic tails score low and we treat them as sketches. The second is the **predicted aligned error**, the PAE — a residue-by-residue map of how much one part of the structure might shift relative to another. It is the map that tells us whether the VSLD pocket and the pore gate are pinned in a fixed geometry or only loosely related, and for an allosteric channel that coupling is the whole mechanism, so the PAE is the figure we stare at longest.



The predicted aligned error map — each pixel is the model's uncertainty about one residue's position relative to another; the low-error blocks are the rigid domains we are willing to design against.

But a binding pocket, resolved and scored, is only half the story, and the better half is the gating logic — the reason a channel with a menthol in its pocket is more *likely* to open rather than simply forced to.

Four inputs, one decision

TRPM8 is an allosteric machine with four inputs that all push on the same equilibrium between closed and open: membrane voltage, temperature, the cooling agent in the pocket, and a membrane lipid called **PIP2** (phosphatidylinositol 4,5-bisphosphate) that the channel requires as a permissive co-factor. The cleanest way to hold all four in your head is a two-state model. The channel is either closed or open; the probability of being open, Po, is set by a balance of free energies; and each input shifts that balance. The voltage dependence takes a Boltzmann shape —

```
Po = 1 / (1 + exp[ zF(V½ − V) / RT ])
```

— where $V_{½}$ is the voltage at which the channel is open half the time, z is the effective gating charge (near 0.85 for TRPM8, a channel only weakly voltage-sensitive), F is Faraday's constant, R the gas constant, and T the absolute temperature. At body warmth the thermal term RT/F is about 26.5 mV, and dividing by the gating charge gives a slope of roughly 31 mV for every e-fold change in the open-to-closed ratio. Those two constants — 26.5 mV of thermal voltage, 31 mV per e-fold — are the exchange rate of the whole machine, the number of millivolts a degree or a molecule has to be worth before the gate notices.

The four inputs act, in the model, in one currency: each one shifts $V_{½}$. Voltage is the axis itself. **Temperature** shifts $V_{½}$ toward physiological voltages as the tissue cools — the channel's defining trick. The **cooling agent** shifts $V_{½}$ the same direction, chemically faking the thermal shift. And **PIP2** is the permissive one, the lipid without which the other three have nothing to move: deplete it and $V_{½}$ runs back out to voltages the nerve never reaches, and the channel goes quiet no matter what sits in the pocket. Three inputs lean on the gate; the fourth decides whether the gate is listening at all.

At rest, in a warm mouth near 35 °C, $V_{½}$ for TRPM8 sits far to the depolarized side, out past +100 mV — call it +115 mV for a worked figure — so far that at the nerve's resting voltage of about −60 mV the channel is almost always shut. Put the numbers through the Boltzmann expression and the open probability at rest is

```
Po = 1 / (1 + exp[(115 − (−60)) / 31]) = 1 / (1 + exp[5.6]) ≈ 0.004,
```

about four parts in a thousand. The gate is, to a very good approximation, closed. Menthol's whole job is to move the +115.



The microscope stage where we read the gate itself — patch-clamp and calcium imaging turn an open channel into a current we can measure, one cell at a time.

The arithmetic of counterfeit cold

Menthol binds in the VSLD pocket and shifts $V_{½}$ powerfully in the negative direction — on the order of −80 to −100 millivolts at a saturating dose, enough to drag the half-open point down out of the unreachable range and into the voltages a resting nerve actually visits. Take −90 mV as the worked value. The channel that sat at +115 mV now sits at +25 mV, and the open probability climbs:

> `Po = 1 / (1 + exp[(25 − (−60)) / 31]) = 1 / (1 + exp[2.7]) ≈ 0.062.`

From four parts in a thousand to six in a hundred — a roughly seventeen-fold rise in the chance of being open, bought with a molecule and no change in temperature at all. Now let the mouth take a sip of something genuinely cold. Cooling shifts $V_{½}$ in the *same* direction menthol does, by very roughly 10 mV per degree, so a ten-degree chill is worth another −100 mV, and the two inputs add on one equilibrium:

> `Po = 1 / (1 + exp[(−75 − (−60)) / 31]) = 1 / (1 + exp[−0.48]) ≈ 0.62.`

CONDITION	$V_{1/2}$ (MV)	OPEN PROBABILITY PO	FOLD OVER REST
Warm mouth, at rest	+115	0.004 (0.4%)	1×
+ menthol (−90 mV)	+25	0.062 (6.2%)	~17×
+ a cold sip (−100 mV)	−75	0.62 (62%)	~170×

This is the whole of **synergy with real cold**, written as numbers. Menthol and a cold drink are not additively cool but multiplicatively cool, because they push the same $V_{½}$ and the Boltzmann curve is exponential in that shift: iced mint tea takes the channel from almost-shut to more-open-than-not, a hundred-and-seventy-fold change in the signal, and the tongue reports a cold that neither input could reach alone.

And it tells us precisely what menthol *is*. It is not a forgery of a molecule the cold would otherwise have delivered; it is a forgery of a **threshold**. If cold shifts $V_{½}$ at about 10 mV per degree, then menthol's −90 mV is worth about nine degrees of counterfeit winter.

A TRPM8 channel whose intrinsic set point would have waited for 26 °C now reaches the same open probability at 35 °C, because menthol has told it that 35 °C *is* cold. The counterfeit is not a lie about the leaf. It is a lie about the number at which the channel should begin to believe in frost.

The thermodynamics under that threshold is itself worth a moment of awe, because it is the deepest question the receptor poses. A channel that responds this steeply to temperature must have a gating step with an enormous enthalpy change. Treat the closed-to-open transition as an equilibrium and read its temperature dependence off the van 't Hoff relation:

$$\ln(Q_{10}) = (|\Delta H| / R) \times (1/T_1 - 1/T_2)$$

With an apparent $|\Delta H|$ of about 40 kcal/mol — tens of kilocalories, far larger than an ordinary conformational change — and a ten-degree step from 298 to 308 K, the arithmetic gives

$$\ln(Q_{10}) = (40{,}000 / 1.987) \times (1/298.15 - 1/308.15) = 20{,}131 \times 1.088\times10^{-4} \approx 2.19, \quad Q_{10} \approx 9,$$

so the open probability swings about nine-fold for every ten degrees — the steepness you feel as the almost-binary arrival of cold. Native TRPM8 currents are often measured steeper still, with Q_{10} values reaching the low twenties, which would demand an apparent enthalpy closer to 55 kcal/mol or a cooperativity the plain two-state picture understates. How a protein achieves a temperature dependence that large and that sharp is still argued — whether it lives in the folding of a specific domain, in the ordering of bound water, in the lipids — and we do not pretend the argument is settled. What is settled enough to design against is the *consequence*: menthol and cold are thermodynamically interchangeable inputs to the same gate, and a molecule that shifts $V_{½}$ by a known amount buys a known number of degrees of counterfeit cold.

A number for loudness

A channel that opens by a known arithmetic can be scored by one. The quantity we measure is not how much menthol a leaf holds but **EC_{50}** — the concentration of a coolant at which its receptor reaches half of its maximum response. A low EC_{50} means the channel answers loudly to very little; the molecule is a persuasive speaker. A high EC_{50} means the channel barely stirs until the molecule is lavished on it. EC_{50} measures *loudness*, not *quantity*, and loudness is the thing the mouth actually tastes. From it the Index score follows by one inversion, a fixed reference, and a scale factor:

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

We anchor the reference to **(−)-menthol** itself, the molecule the axis is named for, with a representative half-activation near **30 µM**. By construction menthol sits at exactly **1,000**: substitute it for the candidate and the ratio collapses to 1,000 × 1. A molecule twice as loud — half the EC_{50} — scores 2,000; one that needs twice the concentration scores 500. We cap the per-axis scorecard at **5,000**,

the ceiling shared across the Index, so the rare molecule loud enough to saturate the channel pegs the scale instead of running off the page.

To find an EC_{50} we do not take one reading; we climb a ladder of concentrations and fit the climb to the dose–response curve of a cooperative, four-subunit channel, the Hill equation, $R = R_{max} \times [A]^n \div (EC_{50}^n + [A]^n)$. The Hill slope *n* measures how sharply the gate switches on, and for a channel that gathers more than one menthol before it opens cleanly, *n* sits near 1.8. That number has a tangible consequence: with a slope of 1.8, the journey from a tenth of the response to nine-tenths spans only about an elevenfold change in concentration, where a non-cooperative receptor would need an eighty-one-fold swing. Cooperativity is why a good mint has a *threshold* — why a little does almost nothing and a little more does almost everything.

The receptor speaks in molarity; a confectioner buys in milligrams, so the units must cross. Menthol's molar mass is 156.27 g/mol, so the 30 µM reference is

> 30×10^{-6} mol/L × 156.27 g/mol = 4.69×10^{-3} g/L ≈ **4.7 mg/L**,

about 4.7 parts per million — a vanishing amount of matter to move a thermostat the size of a winter. It also shows why strong confections feel like a *wall* of cold rather than a dial: a mint at 0.2% menthol by weight carries roughly 2 g/L, near **12.8 mM**, more than four hundred times the EC_{50}, deep in the flat saturated top of the Hill curve where the channel is as open as the molecule can make it and adding more does nothing. The design space that matters is the steep middle, not the saturated top.

Finally, the honesty of the number — its error. Every EC_{50} is a fit to noisy data, and an Index score is a *ratio* of two such fits, so their relative uncertainties add in quadrature: $CV(\text{ratio}) \approx \sqrt{(CV_{candidate}^2 + CV_{reference}^2)}$. We hold engineered material to a batch-to-batch coefficient of variation of **≤ 7%**, against the **42%** a wild-type leaf swings across a season from sun to shade. Carry two measurements each at the 7% bound through the ratio and the score inherits $\sqrt{(0.07^2 + 0.07^2)} \approx$ **0.10** — ten percent. An IFI-C we report as 1,000 is honestly 1,000 give or take about 100, a real interval and not a decoration.

COMPOUND	BOTANICAL SOURCE	EC_{50} (MM, REPRESENTATIVE)	HILL N	IFI-C (RAW)	SCORECARD (CAP 5,000)
(−)-Menthol	Peppermint	30 (reference)	~1.8	1,000	1,000
Icilin	synthetic probe	0.36	~2.0	83,333	5,000 (pegged)
Linalool	Lavender	200	~1.0	150	150
1,8-Cineole (eucalyptol)	Eucalyptus	450	~1.2	67	67
Camphor	Camphor laurel	750	—	40	40 (also warms)
(−)-Carvone	Spearmint	1,100	~1.0	27	27

The icilin row is the lesson in the cap. At an EC_{50} near 0.36 µM it is more than eighty times louder than menthol, and the raw formula returns a number past eighty thousand — which is why the scorecard clamps it to 5,000 and we read the raw figure only as "saturating." The carvone row is the opposite lesson: spearmint cools gently not because it is weak mint but because its principal molecule speaks softly to this particular channel, an EC_{50} in the low millimolar, a loudness near 27. Same axis, the same arithmetic, four orders of magnitude between the top and bottom of what a plant can say about cold.

The plants we tuned to speak it

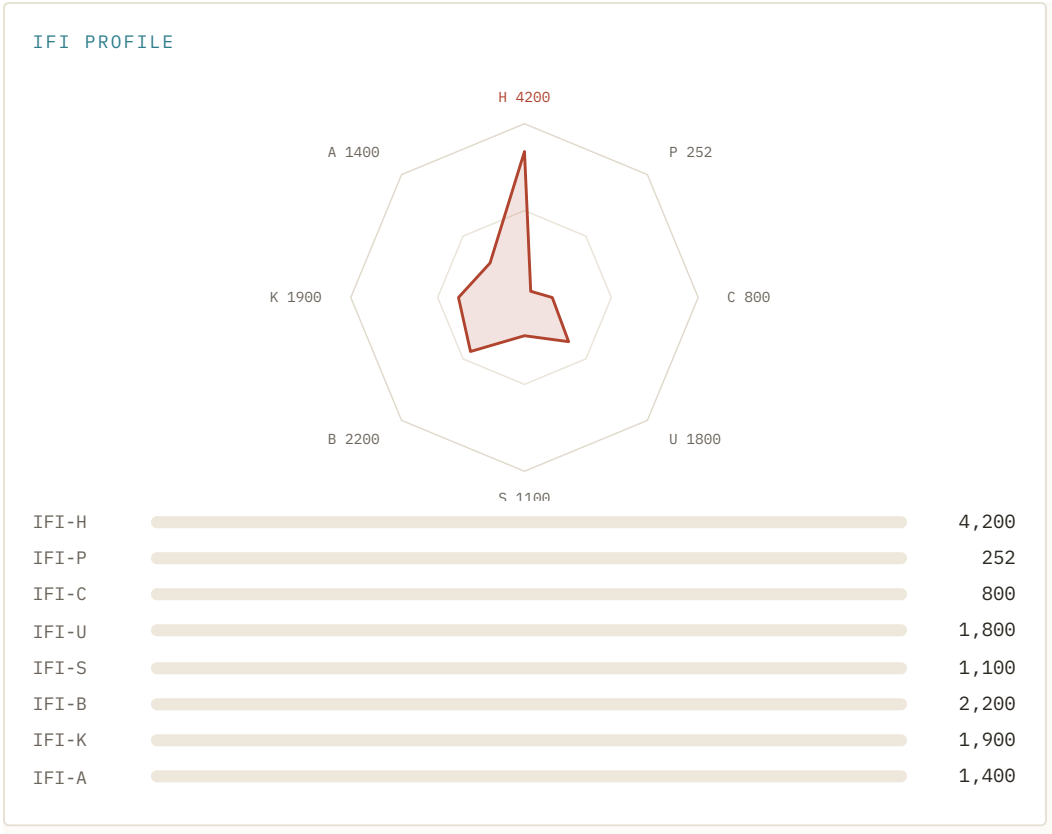
No single plant owns the cool, and the point of the Index is that the ones that carry a piece of it can all be read on one page. Counted in engineered constructs, the library we have built for this axis sorts by the molecule each host is tuned to make speak to TRPM8:

ORGANISM	CONSTRUCTS	PRIMARY AXES	THE MOLECULE IT SPEAKS
Peppermint	2,360	C / M	(−)-menthol
Lavender	160	C	linalool
Orange	120	B / C	a faint cool under the bitter
Eucalyptus	80	C	1,8-cineole
Spearmint	80	C	(−)-carvone
Camphor	40	C	camphor

Every one of them is hard-won — monoterpene pathways are longer than they look, they lean on temperamental cytochrome-P450 enzymes, and the receptor's pocket is *chiral*, reading the (1R,2S,5R) hand of menthol with real affinity while all but ignoring its mirror image. A cool built from the wrong hand spends half its mass on a near-silent isomer, so a clean cool is quite literally a clean stereochemistry. Peppermint carries by far the deepest library, 2,360 constructs, precisely because it is the plant where the clean, loud cool is both most wanted and most worth the difficulty — the place where moving the +115 is most worth doing well.

The pepper that still reads cold

The most beautiful demonstration that cool is a reading and not a flavor category is a fruit we built to be the hottest thing we have ever grown. **Phoenix** — SS-0096, the first cultivar designed to register on all eight axes at once — our model predicts approximately 2.95 million SHU and an IFI-H score of 4,200 on the heat axis. It is, by any ordinary reckoning, pure fire. And yet when we examine its full computational profile, the cold channel is not silent:



Eight hundred on the cool axis, inside a fruit our model predicts at close to three million SHU. Run the 800 back through the formula and it tells us exactly what the channel is doing: IFI-C of 800 means EC_{50}(candidate) = 1,000 × 30 µM ÷ 800 = **37.5 µM**, a cool-active principle designed to speak to TRPM8 about one-and-a-quarter times more softly than reference menthol. Not an accident, and not a contradiction — it is the thermal axis told the truth. The receptor the axis is named for carries two names, TRPM8 and its warm twin TRPV1, because cold and warmth are one argument the tongue is always having; a fruit can lean hard toward the warm end and still carry a real, predicted cool riding underneath, so that the first impression and the finish disagree on purpose. The number 800 is small next to 4,200, and that is exactly the point. Within its full eight-axis profile, you can see precisely how much winter a fire is designed to carry — each axis scored against its own reference, its own defined convention.

The second mint cools less

Two consequences of the gating model matter in the kitchen as much as the lab, and one of them is a problem we spend real effort fighting. The first we have already watched in the numbers: menthol and genuine cold push $V_{½}$ the same way, so their effects multiply, and iced mint is not a little cooler than warm mint but dramatically so.

The second is **desensitization** — the reason the second mint cools less than the first, and the cleanest example of calcium as the signal's own off-switch. Calcium floods in through the open channel; calcium activates the enzyme phospholipase C; PLC consumes PIP2, the permissive lipid TRPM8 cannot gate without; and with its co-factor depleted, the channel's enthusiasm fades even with menthol still seated in the pocket. In the language of the model, the pocket is still pushing $V_{½}$ negative, but the lipid that let the gate listen has been spent, and $V_{½}$ drifts back toward the unreachable range. The cool adapts over tens of seconds to a couple of minutes, which is exactly the timescale anyone feels across two strong mints in a row. It is molecular arithmetic you can taste.

This is why a high IFI-C does not, by itself, buy a *long* cool. Loudness is an instant; duration is a separate design target — a matter of how a lozenge releases its molecule, of coolants that clear the pocket slowly, of formulations that resist the PIP2 depletion rather than racing it. Anyone building a long-lasting confection is, whether they name it or not, fighting the same calcium that carried the first wave of cold, and solving that fight is a large part of what makes a cooling product feel expensive. We would rather teach a plant to carry a long, clean cool natively than dose a short one louder, but either way the enemy is the off-switch built into the signal.

Honest limits and a contingent pocket

We would rather name the edges of this work than let a reader walk off them. Four are worth stating plainly.

The first is the one we have just met: an EC_{50} measures loudness at an instant and says nothing, on its own, about *duration*. Desensitization is real, it is fast, and no single number captures it; a profile that reports only loudness is telling the truth about a moment, not the arc.

The second is the thermodynamics itself. We have treated the gate as a clean two-state equilibrium with an apparent enthalpy of tens of kilocalories per mole, because it is the model that lets us turn millivolts into degrees. But the real channel is steeper than that simplest picture, its Q_{10} larger than two states easily allow, and the physical seat of its enormous temperature sensitivity — domain, water, lipid — is genuinely unsettled. We design against the *consequence*, the interchangeability of cold and menthol as inputs to one gate, and we hold the deeper mechanism lightly because the field honestly does.

The third is that the pocket is not universal, which is both a limit and a gift. TRPM8 is not the same molecule across all animals: birds carry a version that reads cold perfectly well but is largely *indifferent to menthol*, a few residues in and around the binding pocket differing until the mint key no longer fits. This is the cold-side mirror of the old garden lore that capsaicin deters mammals but not birds — the pocket is a specific, evolved shape, not a law of nature, and the fact that our menthol fits our pocket so well is a contingency we inherited rather than a necessity. It is also a standing reminder that the fit between molecule and receptor is a *structure*, and structures are the thing we read, model, and in principle re-read.

The fourth is the gap between an assay and a mouth. We fit EC_{50} on a channel in a controlled cell, concentration exact and temperature held, read off a microscope stage one current at a time. A real mouth is warm, wet, moving, and full of saliva that partitions a coolant away from the very nerves it is meant to reach; perceived cool depends on how a lozenge dissolves and how a drink is chilled. The Index measures the channel's cold faithfully and does not promise two foods at the same IFI-C will feel identical across every delivery. And it is deliberately narrow: it scores *this* channel's cold — the menthol kind, the lie told to the winter sensor — and leaves the body's other

colds, the water and the aroma and the chill of the glass, to be measured by their own honest means rather than folded in to flatter a number.

What remains, when the limits are named, is a thing worth the reverence. For ten thousand years growers kept the mint that cooled the best and threw out the rest, never once able to say *how much*, never able to part the clean cool from the off-note riding beneath it, never able to ask a plant to move a threshold it had not already half-learned to move. We can ask now. We can seat a molecule against Y745 in the computer, read the shift it makes in V½, convert that shift into degrees of counterfeit winter and loudness into a score good to about ten percent — and then go to the greenhouse and grow a plant that tells the truest version of the lie, cold and clean and almost nothing else. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The leaf still fakes a frost. We have only learned, at last, to hear exactly how loudly it is lying, and to write the number down.

Chapter 3 · The Mathematics of a Cold That Isn't There — *loudness, not quantity.*

A grower measures mint in percent oil. A chemist measures menthol in milligrams. Neither number tells you how cold the thing will feel, and the gap between them is the whole reason the Index exists. Two cooling molecules at identical concentration can differ a thousandfold in how loudly they speak to the cold receptor; two mints at identical oil content can read as worlds apart if one carries clean menthol and the other carries menthol buried under pulegone and menthofuran — the harsh, slightly medicinal notes that ride under a cheap peppermint, and that we would rather a body not take in quantity besides. Quantity is not loudness. The Cool-Burn axis scores loudness, and loudness has a precise definition.

We spent a long time on that sentence, because it is the hinge the whole chapter turns on. A leaf can only ever tell a grower *that* it cools. The mint our family grew iced tea from, on the hot afternoons when this work first had hold of us, cooled beautifully and could not say by how much. To design cooling — to ask one plant for four clean minutes of it where another gives forty harsh seconds — we had to learn to write the cold down as a number, and a number you can trust enough to breed toward, formulate against, and hold steady batch after batch. This chapter is that number: where it comes from, how the arithmetic behaves, and exactly what it does and does not promise.



Golden hour in the research greenhouse, where a cool is first a living thing and only later a measurement.

The thermometer that menthol lies to

To write cold as a number you have to meet the thing that reads it. The receptor is **TRPM8** — the transient receptor potential channel, melastatin subfamily, member 8 — a protein folded into the membrane of a sensory nerve ending, four identical copies gathered around a central pore like four hands cupped to hold water. Each copy threads the membrane six times, six helices we label S1 through S6, and the four S5–S6 segments lean together to line the pore itself: a gate that is either shut or, for a few milliseconds at a

time, open. When it opens it is not fussy about what it passes — sodium, potassium, and above all calcium pour down their gradients into the cell. That inrush is the entire event. Calcium entering a cold-sensing nerve is the raw, wordless syllable the brain will later translate into the word *cold*.

What makes the channel a thermometer and not merely a pore is that its gate answers three levers at once, and answers them additively. The first is temperature: at a comfortable mouth near 34 °C the gate sits mostly closed, and as the tissue cools through a threshold near 26 °C the open probability climbs. The second is voltage across the membrane. The third is the binding of certain small molecules — and this is where menthol does its quiet, profound work. Menthol slips into a pocket cradled inside the voltage-sensing bundle, the S1–S4 helices, and seats itself against a single aromatic residue, a tyrosine the structural work pins at position 745 in the human channel. Mutate that one residue and menthol loses most of its grip while cold still works — which is how we know the pocket is real and specific, and not a story we told ourselves. Seated there, menthol shifts the channel's activation curve so the gate now opens at temperatures it used to ignore. The arithmetic of the gate has not changed; its set point has. A mouth at 34 °C, which should read as neutral, now fires as though it were sitting near 15 °C. The leaf cooled nothing. It moved the thermostat.

Two structural facts matter for the mathematics ahead, and we will lean on both. The first is that the four subunits cooperate — they do not open one at a time, independently, but help one another across the threshold. Cooperativity is why the channel's response to dose is *steep*, and steepness is what makes loudness a sharp, well-defined quantity rather than a smear. The second is that the open channel depends on a membrane lipid, PIP_2, pressed against its inner face. The same calcium that carries the cold signal also, once inside, switches on an enzyme that chews that lipid away — so the channel that let the calcium in is quieted by its own success. That self-quieting is **desensitization**, and it is the reason a cool is never a single number but a curve that rises and falls. We will give it its own term before the chapter is done.

One more character belongs on the stage, because the axis is named for the pair, not the single note. The cold channel has a warm twin, **TRPV1**, the receptor heat and capsaicin speak to, sitting in the same nerve endings along neighboring wires; a sharper third, **TRPA1**, rides beside them. Warmth and cold are not two systems but one argument the tongue is always having, and several molecules we call coolants are impure in their answer. Camphor, for one, reads cool and medicinal and then turns and warms, because it blocks the cold channel while stirring the warm one. Menthol itself is clean at low dose and begins, pushed high enough, to brush the warm channels too, so that the cool acquires a bite beneath it. Hold on to that: it is both the paradox the axis is named for and, at the wrong dose, its most instructive failure.

Reading the whisper: EC_{50} and the dose–response curve

Loudness has a precise definition, and it is **EC_{50}** — the half-maximal effective concentration, the amount of a cooling agent needed to drive the channel to half of its fullest response. A *small* EC_{50} means the channel answers a faint whisper of the molecule, which means the molecule is loud; a large EC_{50} means the channel barely stirs until the molecule is lavished on it, which means the molecule mumbles. We measure it the way pharmacology measures any agonist. We express the human receptor in cultured cells, load those cells with a dye that fluoresces when calcium enters, expose them to a graded ladder of the coolant, and read how brightly they answer at each dose. Plot response against the logarithm of concentration and the points trace a sigmoid — flat at the bottom where the dose is too low to matter, rising through a steep middle, flattening again at the top where every channel that can open already has.



The imaging stage, where cells carrying the human cold receptor are read dose by dose — fluorescence standing in for the calcium a cold nerve would have felt.

We fit that curve with the Hill equation:

$$\text{Response} = \text{Response(max)} \cdot [C]^n / (EC_{50}^n + [C]^n)$$

The EC_{50} falls straight out of the fit as the concentration at the curve's midpoint — set [C] equal to EC_{50} and the right-hand side collapses to one-half for any exponent at all, which is simply what the half-way point *means*. The exponent **n**, the Hill coefficient, tells us how steep the middle is, how cooperatively the four subunits act. For menthol on this channel n runs above one, often near two, and that number has a consequence you can taste. Anchor menthol's

EC_{50} at **80 µM** — the reference value our bench works from — and with n = 2 the fraction of maximum cool at any dose is $[C]^2 / (80^2 + [C]^2)$. Walk the ladder:

MENTHOL CONCENTRATION	$[C]^2 / (80^2 + [C]^2)$	FRACTION OF MAXIMUM COOL
20 µM (¼ · EC_{50})	400 / 6,800	5.9%
40 µM (½ · EC_{50})	1,600 / 8,000	20%
80 µM (EC_{50})	6,400 / 12,800	50%
160 µM (2× EC_{50})	25,600 / 32,000	80%
240 µM (3× EC_{50})	57,600 / 64,000	90%

Read the ends against each other. With a Hill slope of two, the channel climbs from a tenth of its range to nine-tenths across a mere ninefold change in concentration — from roughly 27 µM to 240 µM. The same journey on a non-cooperative receptor, slope one, would demand an eighty-one-fold swing. Cooperativity is why a good mint has a *threshold*: a little does almost nothing, a little more does almost everything, and a formulator who misjudges concentration by a little can miss the felt result by a lot. The steepness is not a footnote. It is the reason the cool arrives with a switch-like suddenness rather than a lazy ramp, and the reason dose has to be set with real care.

The formula, anchored to a memory

From loudness the score follows by one inversion. Because a smaller EC_{50} means a louder molecule, we put EC_{50} in the denominator, fix a reference, and scale by a thousand:

$$\mathrm{IFI\text{-}C}(\mathrm{candidate}) = 1{,}000 \cdot EC_{50}(\mathrm{reference}) \,/\, EC_{50}(\mathrm{candidate})$$

We anchor the reference to **(–)-menthol** itself. By construction it sits at exactly **1,000**: substitute menthol for the candidate and the ratio collapses to 1,000 · 80/80 = 1,000, the fixed point every other cool is read against. Everything louder than menthol scores above 1,000, everything softer below, and the scorecard caps at **5,000**, the per-axis ceiling shared across the whole Index, so the rare molecule loud enough to saturate the channel pegs the scale instead of running off the page and distorting it for everyone beneath.

Watch the arithmetic move, because it is the same three keystrokes every time. The synthetic carboxamide **WS-3** has an EC_{50} near 25 µM, so IFI-C = 1,000 · 80/25 = **3,200**: better than three times as loud as menthol, and odorless with it. Its cousin **WS-23**, at 35 µM, gives 1,000 · 80/35 = 2,285.7, which we carry as **2,290**. **Menthyl lactate**, a gentle menthol ester at 150 µM, gives 1,000 · 80/150 = **533**: softer than menthol at the front, and far longer-lasting behind it. And **icilin**, the synthetic super-agonist at 0.36 µM, computes to 1,000 · 80/0.36 ≈ **222,000**, a number so large the cap exists precisely to tame it; on the scorecard it sits pinned at 5,000 with its true value noted in the margin. Icilin is a standing reminder that the receptor can be driven far harder than any plant has ever driven it, because no leaf ever needed a cold that loud.

We chose menthol as the anchor deliberately, over the far louder icilin or the odorless WS compounds, for one reason: an index should be legible against the thing people already know. Everyone

carries a memory of menthol. Pin the axis to that memory and a score of 2,600 *means* something — more than twice as loud as the coldest mint you remember — in a way a score anchored to a laboratory super-agonist never could. Numbers, for us, are a form of respect for the reader, and respect begins with a reference the reader has already tasted.

The ladder, in two alphabets

Here is the axis laid out as a ladder, from the synthetic super-agonist that pegs the scale to the aromatic whisper that barely registers. We give each coolant's EC_{50} in two alphabets — the micromolar the receptor speaks, and the milligrams-per-litre a confectioner buys — because the Index is only useful if it crosses that border cleanly. The conversion is a single line, mg/L = µM · (molar mass ÷ 1,000); menthol's molar mass is 156.27 g/mol, so its 80 µM is 80 × 0.15627 = **12.5 mg/L**, about 12.5 parts per million in water. A vanishing amount of matter to move a thermostat the size of a winter.

COOLANT	CLASS	EC_{50} (MM)	EC_{50} (MG/L)	IFI-C	CHARACTER
Icilin	synthetic super-agonist	0.36	0.11	5,000 (capped; true ≈ 222,000)	glacial, calcium-dependent, flavorless
WS-3	synthetic carboxamide	25	5.6	3,200	odorless, long cool
WS-23	synthetic carboxamide	35	7.5	2,290	odorless, clean
(−)-Menthol	**monoterpene (anchor)**	**80**	**12.5**	**1,000**	**the reference cool**
Menthyl lactate	menthol ester	150	34.2	533	mild, very long-lasting
(+)-Menthol	enantiomer	240	37.5	333	weaker, slightly dusty
(−)-Menthone	monoterpene	400	61.7	200	minty, more warmth than cool
Linalool	monoterpenoid (lavender)	~2,000	~309	40	floral, barely cool
1,8-Cineole (eucalyptol)	monoterpenoid	~3,500	~540	23	respiratory, airy
(+)-Camphor	monoterpenoid	— (channel blocker)	—	—	warms rather than cools

Several things in that table reward a slow look. The first is the sheer *range*: from an EC_{50} of 0.36 µM to one of 3,500 µM is a factor of nearly ten thousand, which is why a scorecard has to normalize —

raw EC_{50} is unreadable across that span, but the inverted, anchored score folds it onto numbers a person can hold in one hand. The second is that the score does not care which alphabet you read it in. 12.5 mg/L and 80 µM are the same loudness written twice; the ratio in the formula cancels the units away, so a chemist and a confectioner arrive at the identical 1,000.

The third is the **enantiomer gap**, and it is the whole argument of handedness compressed into two rows. (−)-Menthol scores 1,000; its mirror image, (+)-menthol, scores 333 — a threefold difference in loudness from nothing but which way the molecule is turned in space. The receptor's pocket is chiral, built to read the (1R,2S,5R) hand and to all but ignore its reflection, and the plant's own reductases are handed to match. A cool built from the wrong hand, or a careless racemic mix of both, spends half its mass on a near-silent isomer. So a clean cool is, quite literally, a clean stereochemistry: tune the enzymes that set the final carbons and you are tuning how much of the harvest speaks to the channel at all. The last row is its own small lesson — camphor carries no EC_{50} because it does not open the channel; it blocks it, and warms the mouth through the heat receptor instead, which is why it sits on the axis with a dash where a loudness should be.

Adding coolants: the arithmetic of a blend

The Index does something subtler than rank molecules one against another: anchored to receptor loudness, it lets us *add* them. To a first approximation — below saturation, where the dose–response is still climbing and roughly linear in concentration — a formulation's cool is the sum of each coolant's concentration times its per-molecule loudness. Each component contributes not its full ladder score but that score scaled by how much of it is actually present at the nerve; a loud molecule used in a trace delivers less than a soft molecule used generously. Work a real lozenge.

COMPONENT	ROLE IN THE BLEND	EFFECTIVE CONTRIBUTION
(−)-Menthol	the familiar front, fast on	~900
Menthyl lactate	the long, soft tail	~300
WS-23	odorless coverage of the throat	~500
Combined front		**~1,700**

Menthol's intrinsic loudness is 1,000, and at the lozenge's working concentration it delivers nearly all of that — near 900 — as the bright opening note. WS-23's intrinsic loudness is 2,290, but it is used as a trace, so its generous-feeling 500 is only a fraction of what it could give; what it buys is reach, a clean cold in the back of the throat where the menthol thins out, and no mint flavor to argue with the rest. Menthyl lactate is the quiet one, intrinsic loudness 533, dosed to contribute near 300 — almost nothing at the front, everything at the finish. Below saturation these roughly add, and the combined front near 1,700 is how a formulator reasons about a blend: a little menthol for the familiar opening, a trace of WS-23 for coverage, a cushion of menthyl lactate for the tail, and the arithmetic turns that reasoning into a target.

But the curve has a ceiling, and the ceiling is where the arithmetic stops being kind. Past a point the channel is as open as it can get, and more molecule buys nothing. Push the same blend hard — imagine stacking menthol toward a 3,000 contribution and layering WS-3 near 3,200 on top of it, a nominal sum past 6,000 — and two things happen at once. The cool saturates and pins near the 5,000 ceiling, so the extra loudness is simply wasted; and the excess menthol, now far above its clean range, begins to wake the warm channels it was brushing all along. The frost acquires a sting. This is the paradox the axis is named for, seen as a design failure rather than a design: cool and burn in the same breath, the mouth reporting both because one molecule, over-dosed, is speaking to two receptors. A great cooling product is engineered to stay in the additive regime and well short of that margin — loud enough to be felt, far enough below saturation that the cold never turns on itself.

The second dimension: how long, not just how loud

Because desensitization is real, the honest version of the math carries a time term. The cool you feel is not a number but a curve, rising as the molecule arrives and falling as that inner-membrane lipid, PIP_2, is chewed away by the very calcium the cold let in. A serviceable model treats the felt cool as the molecule's loudness multiplied by a decaying availability, an exponential fade with a time constant **τ** set by how fast the pool empties:

```
Cool(t) ≈ IFI-C · e^(−t/τ)
```

A free, volatile molecule like menthol has a short τ — the cool is gone in under a minute, down to about a third of its peak by the time t reaches τ and a tenth again not long after. A bound, slow-clearing molecule like menthyl lactate has a long one. The Index itself does not fix τ; it is a property of delivery and clearance, not of the molecule's intrinsic loudness, so the seconds below are illustrative of the model rather than measured scores. But the shape they make is the real lesson. Integrate the curve over its whole life and the *total* cool delivered — the area under it — comes out to loudness times duration:

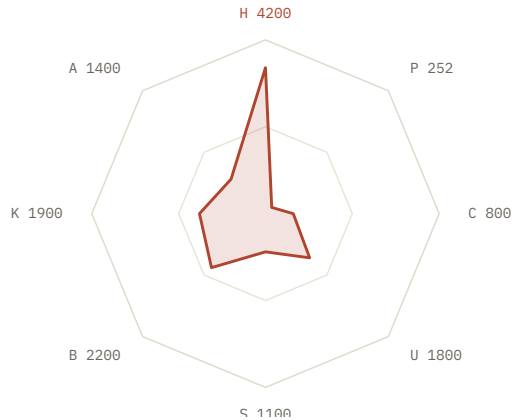
```
total cool ∝ ∫₀^∞ IFI-C · e^(−t/τ) dt = IFI-C · τ
```

Set menthol at IFI-C 1,000 with a short τ on the order of 40 seconds and it delivers about 1,000 × 40 = 40,000 "cool-seconds." Set menthyl lactate at its softer IFI-C 533 but a long τ on the order of 300 seconds and it delivers about 533 × 300 ≈ 160,000 — roughly four times the total cold, from a molecule that scores barely half the peak. That single comparison is the whole argument for why the soft, slow coolants exist and why a formulator reaches for them. A product designed in only the first dimension, how loud, chases a bright front that collapses in forty seconds. A product designed in both dimensions stacks molecules with different τ so their fading curves overlap into a plateau — the loud-and-fast opening the mouth notices, the soft-and-slow tail it keeps feeling. The craft of formulation, told in these terms, is the art of adding areas, not peaks.

The cool we designed into a fire

The most quietly convincing proof that this axis is real, and that its arithmetic buys intention, is a fruit we built to be the hottest thing our hands have ever grown. **Phoenix**, the first cultivar engineered to register on all eight axes at once, our model predicts at approximately 2.95 million SHU and a designed IFI-H of 4,200. By any ordinary reckoning it is pure fire. And yet its full profile does not leave the cold channel silent:

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



Phoenix under glass — a pepper our model predicts at nearly three million Scovilles, carrying a designed cool of 800 on the same scale as the mint.

Eight hundred on Cool-Burn, folded inside a pepper at nearly three million Scovilles. That number is worth reading carefully, because it quietly marks the difference between two kinds of score. The ladder's 1,000 is a *molecular* loudness — menthol's own, measured on the clean channel in a dish. Phoenix's 800 is an *organism* composite — the cold the whole fruit delivers to a real mouth, folding together which cooling molecules the plant makes and at what concentration it makes them. The two are read on the same scale on purpose, so that an 800 can sit legibly beside a 4,200, but they are not the same measurement, and we would mislead you to pretend a plant's axis score is just a molecule's loudness worn like a coat. What the 800 says is narrow and true: this fruit, as eaten, is designed to drive the cold receptor to a composed, intentional cool

— not an accident, not a contaminant, but a cold we dialed in because the mathematics let us ask for it by name while asking for the fire in the same breath.

That is the platform thesis the arithmetic was always serving. Cooling is not the private property of mints; it is an axis you can write into almost any organism once you can measure it. The library we have built around the cold receptor, counted in engineered constructs, runs from peppermint's deep 2,360 — the plant where a clean cool is both most wanted and most worth the difficulty — down through lavender at 160, orange at 120 (carrying a cool note beside its bitterness), eucalyptus and spearmint at 80 apiece, and camphor at 40, the warm contrarian of the set. Every one of them is marked *Hard*, and the honesty of that label matters: monoterpene pathways are longer than they look, lean on temperamental enzymes, and demand that the handedness come out right or the yield is spent on a silent isomer. The cool is never the easy win. It is only, now, a measurable one.

Honest limits

We would rather name the edges of this work than let a reader walk off them, so here are four.

The first is that a point EC_{50} captures loudness at an instant and says nothing, on its own, about duration. A molecule can be loud and brief; a high IFI-C does not promise a cool that lasts, which is why the time constant τ had to be carried as a separate design target. A number that is loud is not automatically a number that endures.

The second is that the additive arithmetic of a blend is a first approximation that breaks in two places. It breaks near saturation, where contributions stop summing and the ceiling clips them; and it breaks in the presence of an antagonist like camphor, which does not add a cool but subtracts one, blocking the channel other molecules are trying to open. A profile that reported the simple sum in either regime would be lying by convenience.

The third is the gap between a dish and a mouth. We fit EC_{50} on the receptor in a controlled assay, where concentration is exact and temperature is held. A real mouth is warm, wet, moving, and full of saliva that partitions a coolant away from the very nerves it is meant to reach; perceived cool depends on how a lozenge dissolves, how a drink is chilled, how long the molecule lingers. And the channel itself varies from person to person — small differences in the receptor's sequence make one mouth read a cold where another reads only a whisper. The Index measures the channel's loudness faithfully. It does not promise two foods at the same IFI-C will feel identical across every delivery and every person, and we say so plainly. Loudness, finally, is not the same as liking; the Index scores how loudly a molecule speaks, never whether the mouth is glad to hear it.

The fourth is simply the honesty of the number — its error. Every EC_{50} is a fit to noisy data, read in triplicate, and the Index score is a *ratio* of two such fits, the candidate's over the reference's. When you divide two measurements their relative uncertainties add in quadrature, $CV(\text{ratio}) \approx \sqrt{(CV(\text{candidate})^2 + CV(\text{reference})^2)}$. We hold our engineered material to a batch-to-batch coefficient of variation

of **≤ 7%**, against the roughly **42%** a wild-type leaf swings across a single season, sun to shade and morning to noon. Carry two measurements each at the 7% bound through the ratio and the score inherits $\sqrt{(0.07^2 + 0.07^2)} \approx 0.099$, about ten percent — so a WS-23 we report at 2,290 is honestly 2,290 give or take a couple hundred, a real interval and not a decoration. A leaf swinging at 42% could not tell 2,000 from 3,000, which is exactly why ten thousand years of growers could keep the mint that cooled the best and never once say by how much.

What the number is for

What the mathematics buys us, in the end, is *intention*. A grower who knows only percent oil can hope for a better mint. A designer who knows the EC_{50} ladder, the additive front, and the τ of each coolant can specify one: this loud, this long, this clean of the off-notes, in this plant, at this cost — and then grow it, and measure that it came out as asked, within the ≤7% variance that separates a flavor from a product. There is a quiet ethic in the cleanness of it, too. A cool with no harshness riding beneath it, the menthol grown generous and the pulegone starved out, is not only a better flavor; it is a cleaner thing to give a body, and that is reason enough to do the arithmetic well.

The number is not a replacement for the pleasure of a cold draught of mint on a hot day. The leaf still tells its beautiful lie — *nothing has changed but the message* — and we would not silence it for anything. We have only learned, at last, to hear exactly how loudly it is lying, and to build that pleasure on purpose, every batch, cold and clean and almost nothing else, which is all we ever wanted the message to be.

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

Chapter 4 · The Burn in Cool-Burn — *where the cool tips over.*

Everyone who has bitten too hard into a strong peppermint knows the moment the cool turns. For a breath it is pure cold — clean and wide and a little holy, the sensation of a window thrown open inside the mouth — and then, past some invisible line, the cold acquires an edge. A prickle gathers at the back of the throat. A sting arrives that is unmistakably *hot*, even as the tongue goes on insisting, truthfully, that it is cold. This is not a flaw in the mint, and it is not your imagination. It is the single most important phenomenon on the thermal axis, the reason we named the axis Cool-Burn rather than simply Cool, and it is governed by a fact we can now write down to the micromolar: menthol, loud enough, stops speaking only to the cold channel and begins to shout at the irritant one.



Peppermint — the leaf whose glandular trichomes hold the only common molecule that both cools and burns. The whole arc of this chapter lives in one plant.

The cold channel is **TRPM8**. The irritant channel is **TRPA1**, the sharp sentinel of the Pungency axis — the receptor that wasabi, raw garlic, and cinnamon bark set off. The warm channels stand on the far side: **TRPV3**, the skin and mouth's gentle warmth sensor, and **TRPV1**, the vanilloid heat channel that capsaicin gates. These four proteins are the entire thermal field of flavor, and the cool-burn is the story of how a single leaf molecule walks across it. The Iyengar Flavor Index gives that walk a coordinate — IFI-C, anchored on TRPM8 — and

this chapter is the account of what the coordinate measures, how we compute it, and where, exactly, the clean cold ends and the burn begins.

Two grammars: a pocket and a bond

The four channels are all TRP ion channels — four protein subunits arranged around a central pore, each subunit a bundle of six membrane-spanning helices, the last two of which line the gate. In the resting state the gate is shut and the cell holds its charge. Open the gate and sodium and calcium pour inward, the nerve ending depolarizes, and a message climbs the trigeminal nerve toward the brain. What differs between the channels — and what makes the cool-burn possible — is not the pore. It is the *grammar* by which each channel reads a molecule.

TRPM8 reads by **non-covalent recognition**: a lock and a key. Menthol slips into a pocket formed inside the voltage-sensor-like domain of each subunit — a cavity walled by residues on the S1–S4 helices, with a tyrosine (Y745 in the human channel) doing much of the holding. Replace that single tyrosine and menthol's grip all but vanishes while the channel still answers cold, which is how we know the cold and the chemical are read at neighboring but separable sites. The binding is reversible and fast; the menthol rests in the pocket, shifts the energy of gating, and lowers the temperature at which the channel opens — so a mouth at a comfortable 34 °C is told, chemically, that it has dropped below 26. The channel does not chill the tissue. It counterfeits the threshold, and the brain believes the counterfeit. Calcium entering through the open pore then recruits the membrane lipid PIP_2 away from the channel, and the cool begins, gently, to fade — the molecular root of why even a strong mint softens after the first thirty seconds.

TRPA1 reads by a completely different grammar — not a resting key but a **covalent bond made and held**. Its classic agonists are electrophiles: allyl isothiocyanate from mustard and wasabi, cinnamaldehyde from cinnamon bark, the thiosulfinates of cut garlic, acrolein from smoke. These molecules do not sit in a pocket; they chemically latch onto a cluster of cysteine residues on the channel's inner N-terminal face — in the human channel, cysteines at positions 621, 641, and 665 — forming a true adduct through Michael addition or disulfide chemistry. That is why wasabi's bite *builds and lingers*: a chemical bond is being made, atom to atom, and it does not simply wash off when the stimulus is diluted. The channel stays modified until the cell repairs it. A covalent agonist is a message written in ink; a non-covalent one is written in breath on glass.

Menthol touches TRPA1 in the second grammar's borderland — **non-covalently and biphasically**. At low concentration it can mildly *activate* the irritant channel; at higher concentration its action grows complex and, in some species and tissue preparations, turns inhibitory. We do not lean on the animal literature here, because the species disagree and the mouth is the only court with jurisdiction over flavor. The practical truth in a human mouth is simple enough and reproducible enough to design around: raise menthol past a

threshold and it recruits a TRPA1 sting on top of the TRPM8 cool, and the brain — which has no separate wire for "menthol" — fuses the two reports into one paradoxical feeling. Cold that burns.

The number on the axis

To design that threshold instead of stumbling into it, we need the cool as a number. The Index gives us one. Every axis is anchored to the half-maximal activation of its receptor — not how much of a compound a food contains, but how *loudly* that compound speaks to the channel — through a single relation:

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

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. A smaller EC_{50} means the molecule works at lower concentration — it is *louder* — and because the formula puts EC_{50} in the denominator, louder molecules score higher. For Cool-Burn we anchor the reference at menthol itself: by definition its EC_{50} on TRPM8 defines the score of 1,000. Take a representative reference EC_{50} for (−)-menthol of 70 µM, and the arithmetic falls out cleanly for any coolant whose own half-activation we have measured.

A synthetic coolant such as WS-3 (N-ethyl-p-menthane-3-carboxamide) half-activates TRPM8 near 18 µM. Then:

> IFI-C(WS-3) = 1,000 × 70 ÷ 18 = **3,889**

WS-3 is roughly 3.9 times louder than menthol — it reaches half-response at less than a third of the concentration. Run the same division across a ladder of real coolants and the axis assembles itself. We add two more columns the formula invites: the molecular weight, and the concentration in parts per million (mg · L^{-1} ≈ ppm in dilute water) at which each compound hits its own half-activation, computed as **ppm = EC_{50}(µM) × MW ÷ 1,000**. That last column is the honest, physical face of "loudness" — the actual pinch of material a formulator must deliver for the same half-lit channel.

COOLANT	EC_{50} (MM)	IFI-C	MW (G · MOL^{-1})	PPM AT EC_{50}
Menthone	230	304	154.25	35
Menthyl lactate	120	583	242.35	29
(−)-Menthol (reference)	**70**	**1,000**	**156.27**	**11**
WS-23	35	2,000	242.40	8.5
WS-3	18	3,889	211.34	3.8
Icilin	0.36	194,444 → 5,000 cap	311.34	0.11

Read the ppm column and the whole idea of an intensity scale becomes tangible: to half-light the cold channel you need about 11 ppm of menthol, 3.8 ppm of WS-3, and a mere 0.11 ppm of icilin. Icilin is the instructive extreme. Its raw IFI-C of 194,444 is not a misprint — it is a super-agonist that reaches half-response at roughly a third of a micromolar — but the scorecard normalizes every axis to a working ceiling of **5,000**, so on the printed radar icilin simply pegs the top of the scale and tells you nothing further. A

number that large stops being information and becomes a warning: this molecule saturates the channel at a concentration you can barely weigh. We keep icilin in the math and out of the mouth.

There is a quiet piece of chemistry hiding in the word *menthol* that the number forces us to respect. Menthol has eight stereoisomers, and only one of them — (−)-menthol, the (1R,2S,5R) enantiomer — is the loud, clean coolant. Its mirror image, (+)-menthol, is several times weaker and carries a faintly musty, less convincing cold. Peppermint, left to the monoterpene route that builds menthol from geranyl diphosphate through limonene, secretes the (−)-form almost exclusively; a synthetic blend may not. The Index scores the enantiomer the plant actually makes, not the name on the molecule — a reminder that a flavor is a shape, and a number that respects the shape is the only number worth printing.

The arc, in numbers

Now we can do what the qualitative description of a strong mint only gestures at: draw the cool-burn as a curve and find its crest exactly. Three things happen at once as menthol rises, and each obeys a Hill equation — the standard sigmoid for a cooperative receptor, response = $c^n \div (EC_{50}^n + c^n)$, with n the cooperativity.

First, **TRPM8 activates**, with $EC_{50} \approx 70$ µM and a Hill coefficient near 2. Second, at high concentration menthol begins to **inhibit the very channel it opened** — its action on TRPM8 is *biphasic*, rising to a peak and then falling — which we model as a self-block with a half-inhibition near 500 µM. The net cool the cold channel actually reports is the product of the two: activation times the fraction *not* yet blocked. Third, above its own higher threshold menthol **recruits TRPA1**, the sting, which we model with $EC_{50} \approx 300$ µM. Put the three on one table across a realistic dose range and the arc appears, quantified:

MENTHOL (MM)	TRPM8 DRIVE	SELF-BLOCK	NET COOL	TRPA1 STING	NET FELT (COOL − STING)	SENSATION
10	2%	0%	2%	0%	+2	a whisper of cold
35	20%	2%	20%	1%	+19	clean, faint cool
70	50%	5%	48%	5%	+43	full clean cool
140	80%	13%	**70%**	18%	**+52**	**the crest — maximum cool**
300	95%	32%	65%	50%	+15	cool *and* sting — true cool-burn
600	99%	57%	43%	80%	−37	sting dominates — burning cold
1,200	100%	79%	21%	94%	−73	burn, the cold channel collapsing

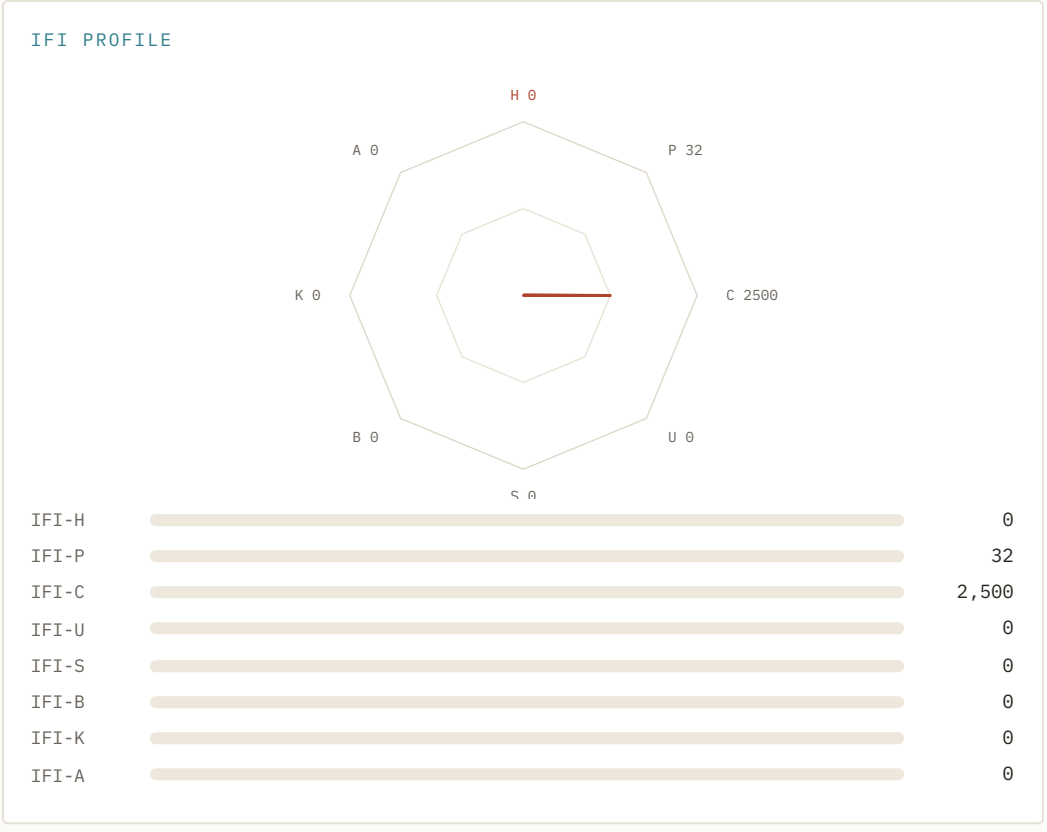
The felt sensation is not a ramp but an **arc**. The net cool climbs, crests near 140 µM where TRPM8 is driven hard and TRPA1 is still barely awake, and then *falls* — not because the menthol is leaving but because the cold channel is saturating, desensitizing, and beginning to block itself while the irritant channel climbs past it. The "net felt" column, the simple difference between the two reports, peaks at the crest and then crosses zero somewhere past 300 µM: the exact concentration at which, in this model, the mouth stops

calling the sensation *cold* and starts calling it *hot*. Every strong mint you have ever met lives somewhere on that arc. The great ones are formulated to sit just below the crest — around 140 µM of delivered menthol equivalent — where the cool is loudest and the sting is a rumor.

This is also where the Index earns its keep in a formulator's hands. Suppose a peppermint line we have tuned presents an effective half-activation — the dilution of its expressed oil at which TRPM8 reaches half-response — equivalent to a menthol concentration of 28 µM. Then:

> `IFI-C(line) = 1,000 × 70 ÷ 28 =` **`2,500`**

Two and a half times as loud as reference menthol — achieved not by piling on oil but by raising the fraction of (−)-menthol the gland secretes and trimming the weaker congeners that dilute the signal. A clean beverage-mint line, designed to deliver its coolant near the crest and to keep the sting to a deliberate shoulder, reads like this on the eight axes:



One axis lit, bright and alone, with a faint pungency shoulder that marks exactly how close to the crest we are standing. That is what a single-axis plant looks like when it is doing one thing beautifully — the whole reason we teach people to read a profile rather than a label.

The warm country

The cool side of the axis has a far shore, and it is warm. Its capital is camphor.



Camphor — the capital of the warm country. It reads as cooling and is, pharmacologically, mostly a warming molecule: a TRPV3 agonist and a quiet blocker of the cold channel.

Camphor reads to almost everyone as cooling — the aromatic chill of a chest rub, the medicinal coolness of a liniment — and yet pharmacologically it is mostly a *warming* molecule. It is an agonist of **TRPV3**, and at the same time a modest *blocker* of TRPM8, the cold channel. TRPV3 is worth knowing in its own right. It sits in the keratinocytes of the skin and the epithelium of the mouth, it answers warmth in the comfortable 32–39 °C band — the temperature of a hand held near a candle, not a flame — and, unusually among the thermal channels, it **sensitizes with repeated stimulation rather than desensitizing**. Where menthol's cool fades on the second and third exposure, camphor's warmth *builds*: each stimulus leaves the channel a little more willing, so a warm note can accumulate across successive sips where a cool note would wash out.

So camphor's "cool" is a sensory illusion assembled from warmth and sharpness and volatility, with no genuine cold signal underneath it at all. It blocks a little of whatever real cold is present, it lights a gentle warmth that the mind, primed by the smell of medicine, files under *chill*, and the volatile lift at the top of the nose finishes the trick. This is the deepest lesson of the thermal axis: the mouth's temperature report is not a reading of any thermometer but a **composite the brain assembles** from four channels at once, and a clever molecule can counterfeit the composite without ever touching the channel you would expect. We use camphor precisely for this — when a formulation needs a cooling *impression* laid over an underlying warmth: a winter note, a medicinal depth, a chest-opening airiness that pure menthol, honest and literal, cannot supply.

Warmth has its own frank chemistry too, and it is good to know it exactly so we can keep it out of a cool that should stay clean — or fold it in when a cool should turn. Ginger's gingerols and shogaols speak to the warm vanilloid channel, TRPV1, the same channel capsaicin gates, which is why ginger's heat is round and low where chili's is bright and high. Eucalyptol, the breath of eucalyptus, is a true TRPM8 agonist with a lift of TRPV3 underneath — a cool with a warm lining. The warm country is small, but it is mapped, and a designer who knows its borders can walk right up to them.

Counterirritation — why cool reads as relief

There is an older, grander name for what the cool-burn does to sensation, and it is worth borrowing because it explains why cool has always felt like relief. **Counterirritation** — the gate-control idea

that a strong sensation on one channel can crowd out the perception of another — is why a menthol rub soothes an ache, why cold eases a sting, why the trigeminal field, flooded with one loud clean message, has less room left for the rest. The cool and the burn and the dull ache all converge on the same trigeminal wiring on their way to the brain, and a loud, clean cool is, among other things, a way of filling that wiring with something the mind reads as ease.

We state this as sensory fact and nothing more. We make no medical claim on it — the same unclaimed, ordinary fact a cold cloth on the forehead has always traded on. It belongs in this chapter because it is the reason cool is not merely a flavor but a *feeling of being cared for*: the family that grew these plants has always thought of a tall glass of mint water at the end of a hot day as a small, clean kindness you do your own body, and the counterirritation at the trigeminal gate is the quiet mechanism underneath that kindness. Flavor, measured honestly, keeps turning out to be a form of attention paid to the body.

Placing the line on purpose

This is where the designer's control becomes most delicate and most valuable, because the line between clean cool and cool-burn is a line we can place on purpose, to the micromolar. The arc told us where the crest sits; the Index tells us how loud each coolant is; together they let us build three different mouths from the same axis.

If we want a **beverage mint that never stings**, we design the plant and the dose to sit deep in TRPM8 territory, well below the crest and far below TRPA1's threshold, and we carry duration with the long, mild coolants — menthyl lactate at IFI-C 583, a touch of WS-23 at 2,000 — which hold the cool open without ever raising menthol toward the sting point. The score reads high on C, near-silent on P, and the mouth gets a cold that stays clean for a full minute.

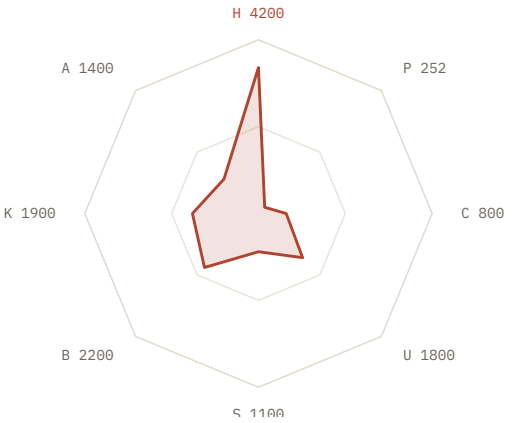
If we want a **cool-burn lozenge** — a confection that bites as it chills — we push menthol up into TRPA1's range on purpose, out past 300 µM, and let the two channels argue. The P shoulder climbs, the net-cool begins its descent, and the sensation becomes the deliberate paradox the arc predicts: a cold that stings, held right at the crossing.

And if we want the genuine thermal contradiction — cold and hot at once, not as a dose artifact on one molecule but as a designed composition of two — we pair a loud TRPM8 coolant with a loud TRPV1 agonist and let the mouth hold both at full volume. We have built that pepper, and it is one of the strangest, most instructive things our family has ever grown: a fruit designed to carry, honestly and at the same instant, a true cool and a true fire. It is **Phoenix** (SS-0096), the full-spectrum cultivar designed to register on all eight axes at once:



Phoenix (SS-0096) — the full-spectrum pepper that holds a true cool and a true fire in the same bite. Its eight-axis profile is the whole thermal contradiction made edible.

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

Read the first and third lines together: **H 4,200 and C 800, the heat axis roaring and the cold channel genuinely lit, in one fruit, in one bite.** The capsaicin gates TRPV1 and reports a fire that is not there; a TRPM8 coolant, built into the same flesh, reports a cold that is not there either; and the brain, handed both counterfeits at once, assembles a sensation with no name in ordinary language. Two illusions, each true at its own channel, refusing to cancel. Our model predicts approximately 2.95 million SHU — the contradiction is not a trick of low stakes.

None of this placement is worth anything if it wanders batch to batch, and here the engineering earns the poetry. Across our constructs we hold batch-to-batch variation to a coefficient of variation of **≤7%**. Put that against a score to see what it buys. A line at IFI-C 2,500 with CV 7% has a standard deviation of 0.07 × 2,500 = 175, so roughly 95% of batches land inside 2,500 ± 1.96 × 175 — a window of about **2,157 to 2,843**. Unselected botanical oil, whose cooling potency can swing by forty percent or more from harvest to harvest, would scatter the same nominal 2,500 across a window wider than a thousand points in either direction — the difference between a mint that is always itself and one that is a different mint every season. When you can place the line to the micromolar, holding it there is the whole craft. A number you cannot reproduce is not a measurement; it is a rumor with a decimal point.

The honest limits

A chapter that shows its math owes the reader its edges. The cool-burn is real and designable, but the model that makes it designable is a model, and it fails in specific, nameable ways.

The reference EC_{50} we anchored at 70 µM is **temperature-contingent**. Menthol and cold are read at neighboring sites on TRPM8, and they potentiate each other — menthol shifts the channel's activation threshold, so the same dose cools more in iced water than in a warm mouth, and the "true" EC_{50} drifts with the temperature of the bite. Our numbers are taken at mouth temperature because that is where food is eaten; carry them to a chilled beverage and the cool will read louder than the printed score. The Index measures loudness at a fixed temperature, not a law of physics.

The scale measures the **channel, not the whole percept**. IFI-C scores how hard a molecule drives TRPM8; it does not score the top-of-nose volatility, the duration after the crest, or the trigeminal context the counterirritation runs on. Two coolants can share an IFI-C and feel different — one a quick, bright flash, the other a long, low plateau — because potency and persistence are different axes of the same molecule. The number is necessary and it is not sufficient, and we have never pretended otherwise.

The biphasic self-block, the TRPA1 recruitment, and the Hill coefficients we used to draw the arc are **a working model fitted to the human mouth**, not channel constants handed down from first principles. Menthol's action on TRPA1 in particular is genuinely complicated and disagrees across species and preparations; we trust the mouth and treat the animal pharmacology as a hint, not a verdict. The crest near 140 µM is where our material lands; a different carrier, a different pH, a different co-solute can move it. Menthol is lipophilic, and delivery — oil, emulsion, hard candy, aqueous — changes how much of the dose ever reaches the channel, which is why two products at the same stated menthol level can sit on opposite sides of the crossing.

And there is the reader at the other end, who is not a cell in a dish. TRPM8 sensitivity varies from person to person, cool adapts and fades on repeated exposure within a single sitting, and the same

lozenge that stings a first-timer may read as merely cold to someone whose channels have spent a lifetime in strong mint. We design to the center of the distribution and label honestly for its edges. The math is a map drawn well; it is not the territory, and the territory is a living mouth.

The lesson of this chapter is the lesson of the whole axis. Cool is not a single thing. It is a region — bounded on one side by the warm channels and on the other by the irritant one — and a cooling molecule is a point somewhere inside that region whose position we can now read, score, and move on purpose. The burn is not the opposite of the cool. It is the cool's own far edge, reached by turning up the only molecule in the leaf. Which means, in the end, that to design a clean cold you first have to understand, exactly and to the micromolar, the burn you are choosing to stay away from. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



PART II OF IV

The Molecule & the Pathway

menthol's eight faces · seven enzymes · the model that picks the mutation

Chapter 5 · Menthol, the Molecule — *one shape out of eight.*

Menthol is a small thing — ten carbons, one oxygen, a mass a confectioner can read off a cheap balance — and almost everything that matters about it is a question of which way three little arms are pointing. The body of the molecule is a cyclohexane ring, six carbons closed into a soft hexagon, and hung off that ring are three substituents: a methyl, an isopropyl, and a hydroxyl. Each sits on a carbon that can carry it on one face of the ring or the other. Three such choices, two ways each, make 2 × 2 × 2 = **eight** arrangements — four pairs of *diastereomers*, and each pair a set of two mirror-image *enantiomers*: eight stereoisomers in all, one formula, eight distinct shapes. Only one of the eight is the cold we mean. The other seven are mint's also-rans, and the distance between the winner and the rest is, for our family, the single most important fact in the chemistry of cool. We cannot write about it without a kind of reverence: a plant, with no instrument but its own enzymes, picks the one correct hand out of eight, every time, in the dark of a leaf.

Three arms, eight shapes — the stereochemistry of cold

The formula is $C_{10}H_{20}O$; the mass is 156.27 grams per mole; the crystal melts near 42 °C, a few degrees above the blood, which is part of why a menthol crystal set on warm skin seems to vanish into cold. Chemically the molecule is 2-isopropyl-5-methylcyclohexan-1-ol, and its three *stereocenters* are exactly the three carbons that carry those arms: C1 holding the hydroxyl, C2 the isopropyl, C5 the methyl. A stereocenter is a carbon whose four different neighbors can be laid out in two non-superimposable ways, and three of them, independently set, are what give the eight. Group the eight by the shape of the ring and they fall into four families.

ISOMER FAMILY	HOW THE THREE ARMS SIT	ENANTIOMERS	CHARACTER ON THE TONGUE
Menthol	all three *equatorial* — flat on the rim	(−) / (+)	the cool; (−) is the one
Neomenthol	one arm forced *axial*, up off the rim	(−) / (+)	weaker, faintly harsh
Isomenthol	one arm axial, a different one	(−) / (+)	musty, little cool
Neoisomenthol	two arms off the rim	(−) / (+)	faint, off

The word *equatorial* is doing quiet, decisive work there. A cyclohexane ring is not flat; it puckers into a chair, and every substituent on it points either outward around the rim (equatorial, roomy, low-strain) or straight up or down (axial, crowded, high-strain). The winning isomer, **(−)-menthol** — fully, **(1R,2S,5R)-menthol** — is the one, and the only one, whose three arms can all lie equatorial at once. That is the lowest-energy shape a menthol skeleton can take, the shape the molecule relaxes into with no coaxing, and it happens also to be the shape that drops into the TRPM8 pocket like a hand into a glove cut for it. Its mirror image, **(+)-menthol**, is the same object flipped left-to-right. By itself in a flask it is every bit as stable and all-equatorial — but a mirror image

cannot be rotated into its original, and the receptor that reads it is itself one-handed, so (+)-menthol lands in the pocket wrong and scores roughly a third of the cool: still minty, but dusty, flattened, missing the clean lift. The other six — the neomenthols and isomenthols — cannot get all three arms down at once; at least one is shoved axial, the molecule bulges where the pocket is narrow, and the cool thins to a herbal murmur.

PROPERTY	(−)-MENTHOL	(+)-MENTHOL
Configuration	(1R,2S,5R)	(1S,2R,5S)
Formula / mass	$C_{10}H_{20}O$ · 156.27 g·mol^{-1}	identical
Source	peppermint, natural	synthetic / minor
EC_{50} at TRPM8	80 µM	240 µM
IFI-C	1,000	333
Character	clean, lifting, cold	weaker, dusty

This is why handedness is the whole game. A synthesis that makes all eight in a jumble makes a muddled, weak cool no matter how loud the total "menthol" on the label; a plant that makes only (1R,2S,5R)-menthol, cleanly, makes the loudest natural cool there is. The plant is the better chemist, and it is better for one precise reason — its enzymes are chiral, built of one-handed amino acids, and they can only build one hand back.

The pocket that reads the hand — how TRPM8 feels menthol

The receptor is **TRPM8**, the eighth of the melastatin branch of the transient-receptor-potential family — a large, non-selective cation channel that sits in the membrane of a particular class of sensory nerve endings in the lips, tongue, mouth, and skin. It is built as a *tetramer*: four identical subunits arranged with four-fold symmetry around a central pore, each subunit threading the membrane six times. The last two membrane helices of each subunit, S5 and S6, line up to form the ion conduction path down the middle; the first four, S1 through S4, fold off to the side into a bundle called the voltage-sensor-*like* domain, the **VSLD**. That side cavity, not the pore, is where menthol is read.



The molecular bench — isolating and reading the one-handed oil, where a cultivar's cool is made a measurement rather than a memory.

When menthol slips into the VSLD cavity, its hydroxyl — the single oxygen arm — reaches across and forms a hydrogen bond to a tyrosine partway up the second helix, the residue the structural work numbers around Y745. That one bond is the anchor; it holds the small molecule at the right depth and the right tilt. The isopropyl and the methyl then fill the hydrophobic space to either side, the pocket closing around all three arms at once. Only the all-equatorial shape presents those three arms in the geometry the cavity is cut for — which is the molecular reason the stereochemistry two sections of the periodic table away decides whether a leaf tastes cold. Break the anchor and the reading collapses: substitute that tyrosine and menthol loses most of its grip, while cold still works, which is the cleanest proof that the plant molecule and the falling thermometer pull the same channel open by partly different holds.

Two more facts about the pocket matter to anyone designing with it. First, TRPM8 will not respond to menthol at all unless the membrane lipid **PIP_2** — phosphatidylinositol 4,5-bisphosphate — is present on the inner face to co-occupy its own basic site on the channel; menthol and PIP_2 are partners, and the second half of this chapter's talk of fade and desensitization comes straight out of that dependence. Second, the pocket is promiscuous about *shape* in a way that still astonishes us: it does not require a ring, or the arms, or anything that looks like mint. It requires only a molecule that can make the anchor bond and fill the hydrophobic flanks — which is why the pure synthetic coolants, strangers to peppermint, drive this channel harder than menthol itself.

A cold that is not there — the biophysics, worked

TRPM8 is, first and most, the body's cold thermometer. Below roughly 26 °C its open probability climbs steeply; it is the channel that tells you the glass of water is cold, the morning air is sharp, the tile is unkind to a bare foot. It does this with an unusually high temperature sensitivity — a Q_{10} (the factor by which its rate changes per 10 °C) in the tens, where an ordinary chemical rate sits near two or three. Cold does not pour ions through it directly; cold, voltage, and menthol all do the same single thing — they shift the channel's willingness to open.

The cleanest way to see that is the two-state picture the channel obeys at rest. Write the fraction of channels open against membrane voltage as a Boltzmann curve:

```
P(open) = 1 / (1 + exp[ −zF(V − V½) / RT ])
```

Here V is the membrane voltage, V½ the voltage at which half the channels are open, z the gating charge the channel moves (for TRPM8 a small ≈ 0.9), and RT/F the thermal voltage, which at body temperature (T = 310 K) is RT/F = (8.314 × 310) / 96 485 = 0.0267 V = **26.7 mV**. A resting nerve ending sits near V = −60 mV. At ordinary warmth, with no menthol, TRPM8's V½ sits far positive of that — call it +100 mV, representative of the channel's own biophysics — and the math says almost nothing is open:

```
P = 1 / (1 + exp[ −0.9 × (−60 − 100) / 26.7 ]) = 1 / (1 + exp[5.39]) = 1 / 220 ≈ 0.5% open.
```

Now add menthol. What menthol does, mechanically, is drag V½ in the negative direction — toward the resting voltage and past it — so the channel no longer needs cold to open. Take a saturating dose that pulls V½ down to −20 mV:

```
P = 1 / (1 + exp[ −0.9 × (−60 − (−20)) / 26.7 ]) = 1 / (1 + exp[1.35]) = 1 / 4.85 ≈ 21% open.
```

From 0.5% to 21% — roughly a **forty-fold** rise in open channels, at a fixed temperature of 37 °C, with not one degree of actual cooling. The nerve fires as it would fire in cold water; the brain, which has no other way to read that fiber, reports cold. That is the whole trick of menthol stated as arithmetic: it is not a coolant, it is a *lie told to a thermometer*, and a beautiful, harmless one. (The numbers above are a representative calculation of the channel's established biophysics, not a product specification — but the forty-fold shape of the answer is the honest shape of the effect.)



The controlled chamber — where cool is held at a set temperature so that a shift in the channel, not a shift in the room, is what the instrument reads.

From the pocket to a number — the index math

A nerve firing is not yet a measurement. To design cool rather than merely admire it, we need the loudness of a molecule as a single number, and the number we use is the Iyengar Flavor Index. Its definition is one line:

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

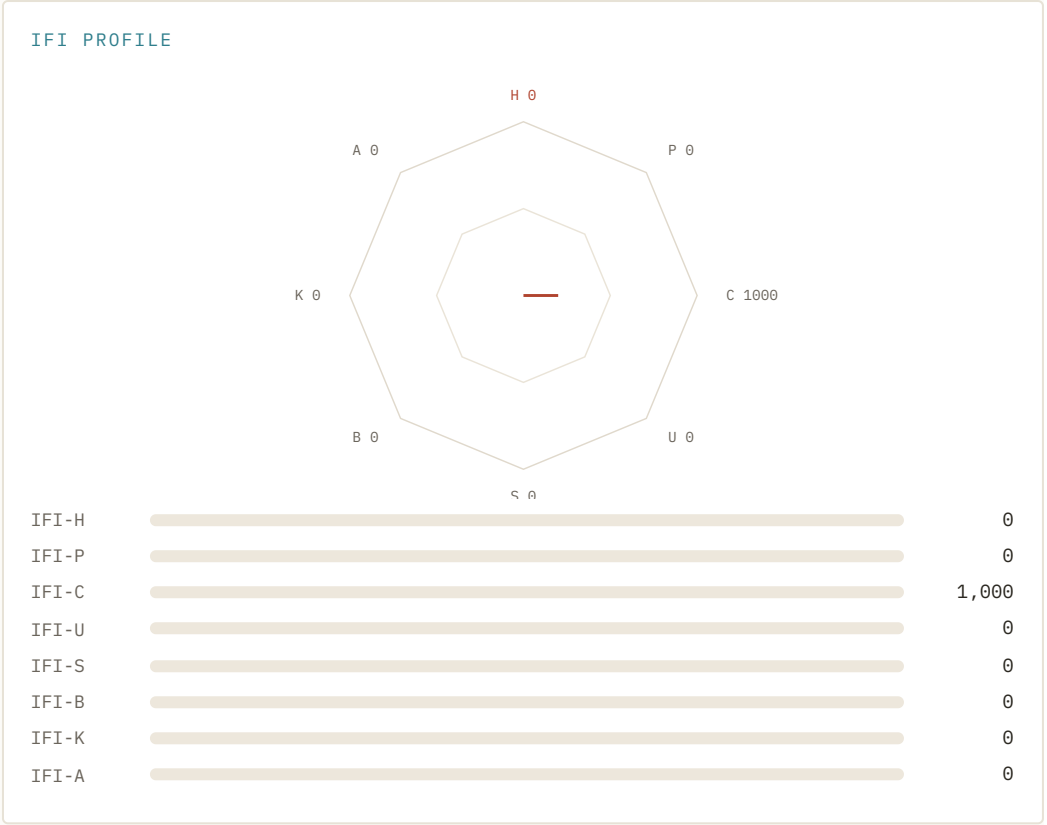
EC_{50} is the concentration at which a stimulus drives its receptor to half of full response — the half-maximal dose. Note what the formula does with it: EC_{50} sits in the *denominator*, so a more potent molecule,

which reaches half-response at a lower concentration, scores *higher*. It inverts a concentration into a loudness. The thousand is only a scale, chosen so the anchor lands on a round number.

For the Cool-Burn axis we anchor the ruler to natural (−)-menthol itself, with a convention constant of **EC_{50} = 80 µM** — a stated reference point, defined like the Scoville scale's dilution-to-threshold, so that the index returns a round anchor value. Feed the reference through its own formula and it must return the anchor:

> `IFI-C[(−)-menthol] = 1,000 × 80 µM ÷ 80 µM =` **`1,000.`**

Menthol is a single-axis molecule: it speaks on Cool-Burn and is essentially silent on the other seven receptors — no heat, no sweetness, no bitterness of its own. Written as a full profile, the reference reads as clean as anything in our library:

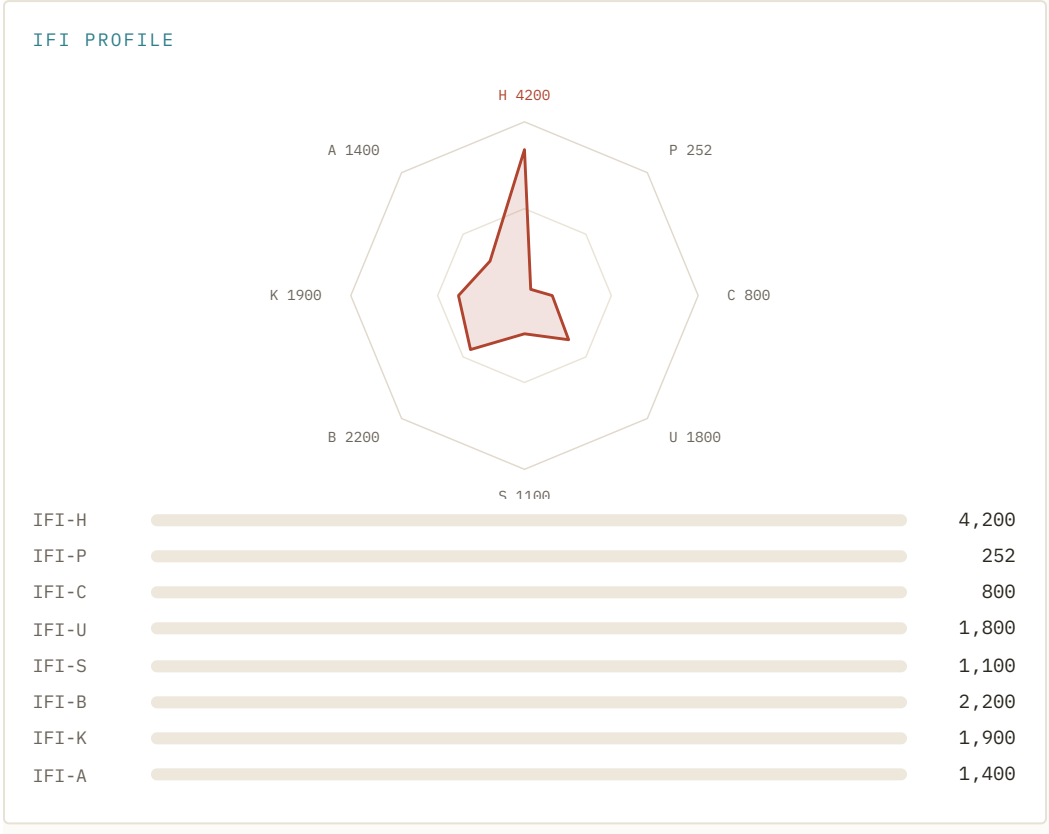


Everything else on the axis is read against that 80 µM. The dusty mirror image, (+)-menthol, needs 240 µM for the same half-response:

> `IFI-C[(+)-menthol] = 1,000 × 80 ÷ 240 =` **`333.`**

A single ruler, and it is the same ruler everywhere in the garden — the cool in a peppermint leaf and the faint cool our hottest designed pepper carries are read on the one axis, in the one unit. It is worth

seeing that span once. **Phoenix** (SS-0096), the full-spectrum superhot designed to register on all eight axes at once, carries a predicted heat of approximately 2.95 million SHU, IFI-H 4,200 — and yet the same instrument reads a small, exact cool note in it:



A pepper with a predicted C of 800 and a mint with a C of 1,000 are not being compared by anyone's tongue and adjective; they are two readings on one anchored scale, 80 µM at the zero of it, and that is the entire point of giving a flavor a number.

A unit conversion, because formulators work in mass. Molarity is the receptor's language; a kitchen and a filling line speak milligrams per liter. Convert the anchor with the molar mass: 80 µM × 156.27 $g \cdot mol^{-1}$ = 80 × 10^{-6} × 156.27 = 0.0125 $g \cdot L^{-1}$ = **12.5 $mg \cdot L^{-1}$**, which in water is about 12.5 ppm. So half of menthol's possible cool arrives at roughly twelve and a half parts per million — a figure a beverage chemist can dose against directly.

The coolant ladder — reading potency against one anchor

Because every IFI-C score is just 1,000 × 80 ÷ EC_{50}, the index is reversible: hand us a published score and we can recover the micromolar potency behind it. Menthyl lactate carries IFI-C 533, so its EC_{50} is 1,000 × 80 ÷ 533 = 150 µM. WS-23 carries 2,290 → 80 000 ÷ 2,290 = 35 µM. WS-3 carries 3,200 → 80 000 ÷ 3,200 = exactly 25 µM. Laid out, the ladder of cool reads cleanly from the two synthetic carboxamides at the top down to the mirror-image menthol at the bottom.

COOLANT	EC_{50} (TRPM8)	IFI-C	MASS ($G \cdot MOL^{-1}$)	ONSET / STAY
WS-3	25 µM	3,200	225	moderate / long
WS-23	35 µM	2,290	185	fast / moderate
(−)-Menthol	80 µM	1,000	156	fast / short
Menthyl lactate	150 µM	533	228	slow / long
(+)-Menthol	240 µM	333	156	fast / short, dusty

WS-3 and **WS-23** — named for the Wilkinson Sword chemists who made them in the 1970s, when the world's menthol supply looked fragile — are the proof the pocket does not care about mint. They

are open-chain amides, sharing no obvious shape with menthol's ring and arms, and yet they make the anchor bond, fill the flanks, and drive TRPM8 harder than the plant molecule does, with almost no smell or taste of their own. They are cool with the flavor subtracted, which is exactly what you want in a cooling fabric or a cold-feel sunscreen, and exactly what you do not want in a mint. And they illuminate, by contrast, what the leaf's menthol *is*: one beautiful evolved solution to a shape-reading pocket, not the only one.

The ladder also teaches a small, honest lesson about potency itself. WS-3's EC_{50} (25 µM) is 3.2-fold lower than menthol's (80 µM) by moles — but WS-3 is heavier (225 vs 156 g·mol^{-1}), so by mass the gap narrows. In milligrams per liter the two half-points are 25 µM × 225 = 5.6 mg·L^{-1} for WS-3 against 12.5 mg·L^{-1} for menthol: about 2.2-fold, not 3.2-fold. Molar potency and mass potency are different claims, and a formulator who confuses them over-doses or under-doses by the ratio of the molar masses. We keep both columns on every spec sheet for that reason.

How sharply does cool switch on? The dose-response of TRPM8 to menthol is cooperative — several subunits of the tetramer contribute — so it follows a Hill curve with an exponent near 2:

$$\text{response} \div \max = [L]^2 \,/\, (EC_{50}^2 + [L]^2)$$

Walk it in units of EC_{50}. At [L] = ½ EC_{50}: 0.25 / 1.25 = **20%**. At [L] = EC_{50}: **50%**. At [L] = 2 EC_{50}: 4 / 5 = **80%**. The cool climbs from a hint to nearly full over a mere four-fold change in concentration — for menthol, from 40 µM (6.25 mg·L^{-1}) to 160 µM (25 mg·L^{-1}). That steepness is why a mint can feel suddenly, almost startlingly cold once you cross into it: you are on the near-vertical middle of a cooperative curve, where a little more molecule buys a lot more channel.

logP and the dial of duration

Potency sets how *loud* a coolant is. It says nothing about how *long* it lasts, and the two are independent dials a designer must set separately. Duration is governed not by the receptor but by physics — by how fast the molecule leaves the tissue, which tracks its volatility and its oil-and-water partitioning, the coefficient chemists write as **logP**.

Free (−)-menthol has a logP near **3.4**: strongly lipophilic, happy in the oily film of the mucosa, and, being small and light (156 g·mol^{-1}), volatile — it flashes onto the receptor fast and evaporates off it faster. That is why raw menthol is a bright, immediate, *brief* cold. Dress its hydroxyl in a lactic-acid ester and you get **menthyl lactate**: heavier (228 g·mol^{-1}), its anchor-forming oxygen now partly tied up, so it cannot bond as tightly and sits lower on the ladder at IFI-C 533 — but it is far less volatile, so it does not flash away. Its cool arrives gently and *stays*, long after free menthol would have gone. This is the trade every cooling formula negotiates: there is no single best coolant, only a best coolant for a *duration*, and molecular mass with

logP is the dial you turn. A toothpaste wants the bright flash; a long-wear lip balm or a cooling textile wants the slow heavy ester or a carboxamide; a chewing gum often wants both, a fast coolant for the first bite layered over a slow one for the quarter-hour after. Reading the mass and the logP of a candidate tells us, before any panel ever tastes it, roughly where on the fast-to-slow axis it will fall.

Two thresholds in one molecule — cool is not smell

Here is the trap that catches confectioners, and it is pure receptor biology. Menthol's *cooling* threshold and its *odor* threshold are different numbers, served by different receptors on different nerves. The cool is trigeminal — TRPM8 on the nerve endings in the mouth and lips. The smell is olfactory — a separate set of receptors in the epithelium high in the nose, which menthol reaches as vapor. You can smell menthol at concentrations far below those that cool the mouth, and you can cool the mouth hard at concentrations where the smell has turned medicinal and overbearing. A mint tuned by nose to smell exactly right can under-cool; a mint tuned to cool correctly can reek.

This is a two-variable problem wearing the costume of one, and it cannot be solved by dosing alone — more menthol moves both numbers together. Where our engineering earns its place is in *cleaning the oil of everything that is not menthol*. Wild peppermint oil carries pulegone and **menthofuran**, the off-notes that turn sharp and musty and faintly medicinal as you push the dose. Strip those from the oil and the ceiling on the smell rises: you can push the cooling concentration higher, into the steep part of the Hill curve where real cold lives, without the aroma souring — because the molecules that would have gone ugly at high dose are simply not in the bottle. A clean oil is not a luxury here; it is what lets the two thresholds be satisfied at once.

The plant is the better chemist — the menthol pathway

To make that clean oil, the plant runs a short, chiral assembly line, and we have learned to run it with it. In peppermint the whole synthesis happens in the peltate glandular trichomes — the tiny blister-glands on the leaf surface, each a sealed flask the plant builds and fills. The route begins with a universal plant terpene precursor, geranyl diphosphate, and proceeds in roughly eight enzymatic steps:

GPP → **(–)-limonene** (limonene synthase closes the ring) → **(–)-trans-isopiperitenol** (a cytochrome-P450, limonene-3-hydroxylase, sets the first oxygen) → isopiperitenone → *cis*-isopulegone → **(+)-pulegone** → **(–)-menthone** (pulegone reductase) → **(–)-menthol** (menthone reductase delivers the final hydride).

The two reductases at the end — pulegone reductase and menthone reductase, each burning NADPH — are the chiral gatekeepers. They deliver their hydride to one face of the molecule and not the other, and in doing so they pin the configuration to (1R,2S,5R). The plant cannot make the wrong hand because the enzyme that would have to make it does not exist in the leaf. That is the molecular meaning of "the plant is the better chemist": its selectivity is not a yield to be optimized but a geometry built into the catalyst.

One branch off this path is the problem. From (+)-pulegone, an enzyme called menthofuran synthase pulls material sideways into **menthofuran** — the musty off-note — and under heat, drought, or a late harvest the plant throws more of it. Our menthol program is, at its core, a five-gene intervention on this map: tune the genes that build the productive branch cleanly to menthol, and silence the one that bleeds into menthofuran, so the trichome fills with the single molecule we want and little else. The aim is not to invent a cool the plant never had. It is to make the plant's own cool *reliable* — to give a living leaf the batch-to-batch steadiness a reactor has, while keeping everything else a living leaf carries.

The cooling plants are not one plant — a comparison

"The cooling plants" is a convenient shelf label, and a slightly dishonest one, because the plants on it reach the cold channel by different molecules and to wildly different degrees. Our index insists on the honesty: measured against the one 80 µM anchor, these organisms are not equals.

PLANT	CONSTRUCTS	PRINCIPAL "COOL" MOLECULE	AT TRPM8
Peppermint	2,360	(−)-menthol	strong agonist
Lavender	160	linalool	weak modulator
Eucalyptus	80	1,8-cineole (eucalyptol)	mild agonist
Spearmint	80	(−)-carvone	essentially silent
Camphor	40	camphor	TRPV1 agonist — a warmer, and a TRPM8 blocker

Peppermint is the only true heavyweight of cold, which is why it carries by far the most engineering in our library — 2,360 constructs, against a few dozen for the rest. **Spearmint** is the instructive surprise: its signature molecule is carvone, not menthol, and carvone is a poor TRPM8 agonist — so spearmint's "cool" is mostly a flavor association the mind supplies, not a channel the leaf opens. **Eucalyptus** gets a genuine but mild cool from 1,8-cineole. **Lavender's** linalool barely touches the channel; its coolness is almost entirely a trick of aroma. And **camphor** is the honest scandal of the shelf: at the receptor it is partly *anti-cool* — a TRPV1 agonist that reads warm, and a molecule that desensitizes and blocks TRPM8 rather than opening it. A "cooling plant" that, mechanically, turns the cold channel down. We keep all five in the program because each has its place in a formula, but we refuse to let the shelf label stand for a measurement. The number is the respect we owe the reader: five plants, one axis, five very different truths.

What can go wrong — desensitization, racemization, honest limits

A full account owes its failure modes as plainly as its triumphs, and cool has several.

Fade. Menthol's cool is self-limiting. Every open TRPM8 lets calcium in along with sodium, and that calcium activates a lipase that chews up the very PIP_2 the channel needs as its partner. Hold menthol on the tissue and the channel's own success starves it: the cool dims even as the molecule is still bound. This is tachyphylaxis, and it is

why a second mint never hits like the first for a while, and why a formula built only on free menthol can feel like it is quitting. The slow heavy coolants and the carboxamides partly dodge this by never spiking the calcium so hard — another reason duration and potency must be dialed together, not alone.

Racemization is not the risk; isomer purity is. A stereocenter does not flip on its own at a soda's pH and temperature — menthol does not quietly turn into its mirror image on the shelf. The real exposure is upstream, in the *making*: a synthesis or a sloppy oil that delivers a mixture of the eight isomers rather than clean (1R,2S,5R)-menthol. The label may read "menthol" and the cool still disappoint, because a third of the mass is the dusty (+) hand and another third the axial also-rans. Our answer is the chiral plant line and the assay to prove it — we read the isomer ratio, not just the total, because the index cares about the one shape and so must we.

The honest limits of the number. The IFI-C score is an *in-vitro* half-maximal potency against the isolated channel. It is a true and sturdy ranking of loudness, and it is not the whole sensation. It does not, by itself, carry duration — that is the logP and mass story, a separate axis of the design. It does not carry the smell, which rides a different receptor entirely. It does not capture the small warming paradox some coolants show at high dose, where TRPV1 gets pulled in alongside TRPM8. And it is read at a reference temperature; a mouth already cold and a mouth warm from soup will not feel the same 80 µM identically, because cold and menthol share the channel and add together. We give the number because you cannot design what you cannot measure — but we try never to let the number pretend to be the entire cold. It is one axis, honestly read, and the rest of the design lives in the gaps the single figure leaves open.

Field and reactor — where menthol comes from

A closing note on sourcing, because it frames the honesty of the whole enterprise. The world's menthol comes from two places. One is the field, where mint oil is distilled and menthol crystallized out of it. The other is the reactor, where chemists build (−)-menthol by asymmetric synthesis — a chiral catalyst forcing the one correct handedness out of an achiral start, a piece of chemistry so elegant it anchors a Nobel-recognized body of work. That synthetic menthol is real, pure, single-handed, and it keeps the world in mint when harvests fail; we hold no quarrel with it.



The living source at golden hour — the leaf that makes the one hand in the dark of its own glands, which our work asks only to make steady.

Our work is not to replace either source but to make the *plant* route as reliable, and as clean, as the reactor route has become — to give a field the consistency that chemistry won in a flask, so that the living source, with everything else a living plant carries, can stand on equal footing. Wild peppermint oil swings with weather and photoperiod and the day of the cut; its menthol fraction can wander by tens of percent from lot to lot, which is variability no serious formulator can build on. The target we hold the engineered line to is a batch-to-batch coefficient of variation of **≤ 7%** — meaning the lot-to-lot spread of cooling intensity stays inside roughly ±14% at two standard deviations, tight enough to dose against with confidence. A field oil running at tens of percent cannot make that claim; our clean line can. The molecule is identical whichever way it is made — the same ten carbons, the same one hand. What we are really designing is the *plant's* claim to make it best: loud, clean, single-handed, and steady, grown with the care we would want in anything we put in front of our own. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 6 · The Pathway, Enzyme by Enzyme — *seven steps from a leaf's green.*

To make the coldest molecule in nature, a peppermint leaf runs a seven-step assembly line in a factory smaller than a grain of salt. The factory is the **peltate glandular trichome** — a tiny blister on the leaf's surface, a short stalk topped by a disc of eight secretory cells that synthesize the oil and pool it beneath a stretched cuticle until the moment you crush it. Run a finger across a mint leaf and the faint tack you feel is thousands of these glands; the cool that lifts off is their entire chemical output, released all at once. Everything in this chapter happens inside those eight cells, in a sequence so ordered that a single enzyme out of place turns the clean cool into something medicinal and wrong. We came to this pathway the way we come to all of them — with reverence first and a pipette second — and we want to walk it the way the leaf does: molecule by molecule, decision by decision, until the green becomes cold.



A basil leaf in cross-section — a Lamiaceae cousin of mint, its surface studded with the same peltate glandular trichomes where the monoterpene oils are built and stored. The cooling factory is a structure you can see.

The building block, and the seven cuts

The line begins with a universal building block. In the plastid, the **MEP pathway** — the methylerythritol-phosphate route, running from pyruvate and glyceraldehyde-3-phosphate through DXS, DXR, and a handful of successors — produces the five-carbon isoprenoid units IPP and DMAPP, and an enzyme called **GPP synthase**, a heterodimer in mint, joins one of each into **geranyl diphosphate**, GPP, the ten-

carbon precursor from which every monoterpene is built. GPP is the raw bar of metal the assembly line machines. From there, seven committed steps carve it into (−)-menthol.

STEP	SUBSTRATE → PRODUCT	ENZYME	ABBREV.	COMPARTMENT
1	GPP → (−)-limonene	(−)-limonene synthase	LS	plastid (leucoplast)
2	(−)-limonene → (−)-trans-isopiperitenol	limonene-3-hydroxylase (P450)	L3OH / CYP71D	endoplasmic reticulum
3	isopiperitenol → (−)-isopiperitenone	isopiperitenol dehydrogenase	IPD	mitochondrion
4	isopiperitenone → (+)-cis-isopulegone	isopiperitenone reductase	IPR	cytosol
5	cis-isopulegone → (+)-pulegone	isopulegone isomerase	IPGI	cytosol
6	(+)-pulegone → (−)-menthone	pulegone reductase	PR	cytosol
7	(−)-menthone → (−)-menthol	menthone:menthol reductase	MMR	cytosol
off-ramp	(+)-pulegone → (+)-menthofuran	menthofuran synthase (P450)	MFS / CYP71A32	endoplasmic reticulum

Read the line and you can taste it changing, because every step is a specific chemical verb. **Step 1** is a cyclization: limonene synthase is a magnesium-dependent class-I terpene synthase that strips the diphosphate off GPP, lets the resulting carbocation fold the loose ten-carbon chain into a six-membered ring, and quenches it — the first ring, and it smells of citrus, because limonene is the smell of an orange peel and the first thing a mint does is build the same molecule a lemon does. **Step 2** is a hydroxylation: a membrane-bound cytochrome P450 spends one molecule of O_2 and the reducing power of NADPH to hang a single oxygen at carbon three, and the citrus turns herbal the instant that oxygen lands. **Step 3** is an oxidation that trades that alcohol for a ketone, lifting two hydrogens onto NAD^+. **Step 4** is a reduction that saturates a double bond with NADPH; **step 5** is an isomerization that merely *walks* a double bond along the ring until it sits in conjugation with the carbonyl — no atoms added or removed, just rearranged into the shape the next enzyme needs. **Step 6**, **pulegone reductase**, saturates the exocyclic double bond to give (−)-menthone, and here the balance of warmth and cool is first set, because menthone is minty but carries more warmth than cold. **Step 7**, **menthone:menthol reductase**, performs the final reduction — the ketone becomes a secondary alcohol — and that alcohol is (−)-menthol itself, the cold at the end of the green.

Count the chirality and the achievement sharpens. Across those seven steps the leaf makes roughly eight separate stereochemical decisions, each enzyme handing its single correctly-handed product to the next. This is not fussiness; it is the whole point, because the receptor at the far end of the story is itself chiral and will not be fooled. (−)-Menthol — the single enantiomer this line is built to produce — activates the cold receptor; its mirror image, (+)-menthol, is a pale imitation, a fraction as potent at the same concentration. The clean cool of peppermint is the reward for making the right-handed turn eight times without once making the wrong one.

Four organelles, one volatile cargo

Look again at the compartment column, because it hides one of the strangest facts in plant biochemistry. This pathway does not sit still in one place. It begins in the plastid, where GPP is made and cut to limonene; crosses to the endoplasmic reticulum for the first oxidation, because cytochrome P450s are membrane proteins that work face-down in the ER lipid bilayer, drawing electrons from NADPH through a partner reductase and splitting molecular oxygen to place that single hydroxyl; it then travels to the *mitochondrion* for the dehydrogenation at step three; and finishes in the cytosol for the last four steps. The intermediate is physically shuttled between four organelles on its way from green to cold.

No one fully knows how the cell manages the hand-offs, and the fact that it works at all — that a small, volatile, oily intermediate crosses membrane after membrane without evaporating away or being lost to some competing reaction — is a piece of cellular logistics we find genuinely humbling. A leaf is doing, quietly, in eight cells the size of dust, a routing problem that would stop a factory. For a designer it is also a plain warning written in the architecture: a pathway spread across four compartments has four times the places to leak. Every membrane crossing is a chance for the cargo to go missing, for flux to bleed into a side reaction, for the yield to soften. Some of our best gains on the cooling line have come not from speeding a single enzyme but from tightening a hand-off — from making sure the molecule that leaves the plastid is the molecule that arrives at the cytosol. You cannot push harder on a leaking pipe; you seal it first.

The fork at pulegone — and the arithmetic of a clean cool

The wrong turn has a name, and it is **menthofuran**. At the branch point, pulegone sits at a fork. Pulegone reductase sends it down the line toward menthol — the path we want. But a second enzyme, **menthofuran synthase**, a cytochrome P450 designated CYP71A32, pulls pulegone off the line entirely and builds a furan ring onto it, producing menthofuran: a compound that is both an off-note — musty, medicinal, the staleness of old mint — and a genuine quality and safety concern, because menthofuran and residual pulegone are the components regulators restrict in mint oils. A mint that leaks flux into menthofuran tastes worse *and* carries compounds the law watches.

The fork is pure competition, and competition is arithmetic. Two enzymes draw from the same small pool of pulegone; the fraction that reaches menthol is just one enzyme's throughput over the sum of both:

```
f(menthol) = v(PR) / [ v(PR) + v(MFS) ]
```

An intermediate like pulegone is held at low concentration — the cell does not let it pile up — so each enzyme runs well below saturation, in the regime where its rate is simply its catalytic efficiency times how much of it is present: v ≈ (k_cat / K_m) · [E] · [pulegone]. The shared substrate term cancels out of the ratio, and the fork collapses to something a designer can act on directly:

> f(menthol) = (k_cat/K_m)·[E] for PR, divided by the same quantity summed over PR **and** MFS.

Put numbers to it and the levers become obvious. Suppose, in arbitrary matched units, the pulegone-reductase term is 9 and the menthofuran-synthase term is 1. Then f(menthol) = 9 / (9 + 1) = **0.90** — ninety percent to the cold, ten percent lost to the musty furan. Now let the plant take a hard afternoon: menthofuran synthase runs harder under heat and drought stress, so say stress triples its expression, lifting its term to 3. The fraction falls to 9 / (9 + 3) = **0.75** — menthofuran loss leaps from ten percent to twenty-five, and the oil muddies, without a single gene having changed. This is why a wild mint's cleanliness wanders with the weather: the off-ramp is throttled by the sky. Run the levers the other way — silence menthofuran synthase tenfold (its term drops to 0.1) and double pulegone reductase (its term rises to 18) — and the fraction climbs to 18 / (18 + 0.1) = **0.9945**, menthofuran held under six parts in a thousand. The whole art of a clean cooling mint, for ten thousand years of growers and now for us, is those two moves made at once: push flux hard down the pulegone-reductase path, and starve the menthofuran-synthase off-ramp until it is dry — and then take away the weather that keeps reopening it.



A controlled-environment chamber. The weather that drives the menthofuran off-ramp — the hot afternoons, the drought stress — is exactly what a controlled environment removes, holding the fork steady so the cool comes out the same every harvest.

What the cold actually is — menthol at TRPM8

Follow the finished molecule off the leaf and onto the tongue, and the second half of the story begins — the half that earns the cooling axis its number. (−)-Menthol does not lower your temperature. Nothing about it is cold. What it does is open a conversation with a single protein: **TRPM8**, the Transient Receptor Potential Melastatin-8 channel, a four-part, calcium-permeable gate studded along the cold-sensing nerve endings of the mouth and skin. TRPM8 is the body's actual thermometer for cool — it is the protein that fires when you bite into something at 15 °C, with an activation threshold that lives down around 26 °C and below. It is a voltage-sensitive channel,

and cold works on it by shifting the voltage at which it opens, sliding its activation into the range your resting nerves already sit at, so the channel fires and the brain is told: *cold*.

Menthol does the same thing chemically that cold does physically. It slips into a pocket in the channel's voltage-sensing domain — a cradle built from the first four membrane helices, anchored at a key tyrosine the field labels Tyr745 — and binding there shifts the activation curve the same direction cold shifts it. The channel now opens at temperatures the tongue would otherwise read as neutral. The mouth reports cold while the thermometer in the glass says room temperature. It is a thermal illusion, and it is the exact mirror of what capsaicin does on the heat channel: capsaicin lowers the heat receptor's threshold so the body reports a fire that is not there; menthol raises the cold receptor's threshold so the body reports a chill that is not there. One molecule lies warm, the other lies cool, and both lie by moving a threshold rather than by moving any heat. This is why our cooling axis is named **Cool-Burn** rather than simply *cool*: at low doses menthol is pure TRPM8 cold, but as the dose climbs it begins to touch the irritant channels too, and the sensation acquires an edge — thermomodulation that can run cold *or* warm depending on the compound and the concentration. The axis measures both faces of that one trick.

Because it is a receptor conversation, it obeys a receptor's grammar — the dose-response curve. Spread a cooling compound across a nerve and the fraction of channels it opens climbs along a Hill curve:

$$r([A]) = [A]^n \,/\, (EC_{50}^n + [A]^n)$$

Here [A] is how much compound reaches the receptor, n is the steepness, and **EC_{50}** is the one number that matters: the concentration that drives the receptor to exactly half of its full response. Set [A] = EC_{50} and the equation returns r = 0.5 by construction — half the channels open. The shape around that midpoint tells you how sharply the cool arrives. With a steepness of n = 2, three times the EC_{50} concentration gives r = 9 / (1 + 9) = **0.9** — ninety percent of full cold — while half the EC_{50} gives only r = 0.25 / 1.25 = **0.2**. A modest swing in how much menthol lands is a large swing in how loudly the receptor answers. EC_{50} is not a measure of how much cold a food *holds*. It is a measure of how loudly its cold *speaks*, and the lower the EC_{50}, the louder the voice.

Reading the cool as a number

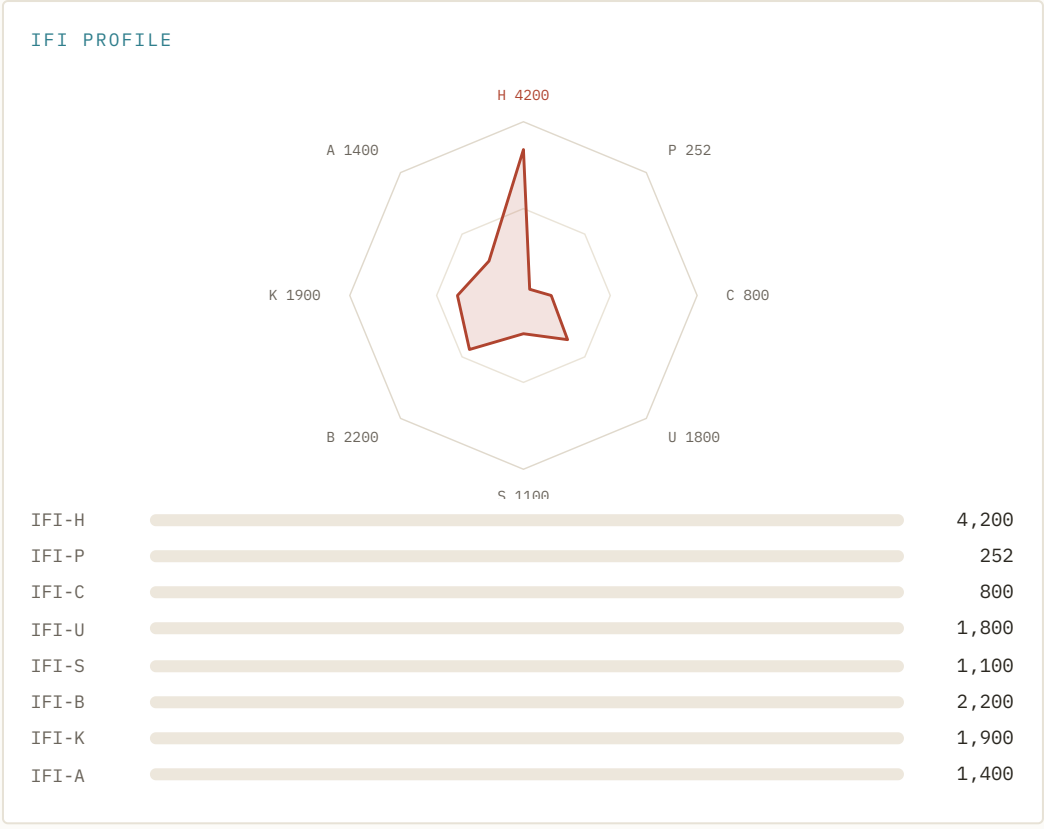
Loudness is exactly what the Iyengar Flavor Index was built to score, and the cooling axis — **IFI-C** — reads straight off that EC_{50}. The definition is one line:

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

The reference cooling stimulus is a stated convention constant — the anchor by definition, chosen to set IFI-C = 1,000, the same way Scoville's original dilution threshold defines the base of the heat scale rather than measuring it. Every candidate is then read against

it. The logic is an inversion: EC_{50} sits in the *denominator*, so a candidate that activates the receptor at a *lower* concentration — a louder, more potent cool — earns a *higher* score. A candidate twice as potent as the reference activates TRPM8 at half the concentration: EC_{50}(candidate) = ½ · EC_{50}(reference), and IFI-C = 1,000 × EC_{50}(ref) ÷ (½ · EC_{50}(ref)) = **2,000**. A candidate five times as potent lands at EC_{50} one-fifth of reference and scores **5,000** — which is the per-axis ceiling we normalize scorecards against, the top of the cooling range.

The inversion also runs backward, and that is where the real data lives. Give us a published axis score and we can recover the potency behind it. Take **Phoenix** (SS-0096), the one cultivar we have designed to register on all eight axes at once — a full-spectrum pepper, not a mint, and yet our model predicts a cooling signal. Each axis below uses its own defined reference constant; scores across axes reflect potency within that axis against its own anchor, not a single unified scale.



Its cooling coordinate is C = 800. Invert the formula: EC_{50}(candidate) ÷ EC_{50}(reference) = 1,000 ÷ 800 = **1.25**. Phoenix's cooling chemistry is therefore designed to activate the cold receptor at 1.25 times the reference concentration — it is 1 / 1.25 = **0.80** times as loud as the anchor, a whisper of cool sitting just below the reference line, deliberately engineered into a plant whose headline is heat. That a pepper carries any cooling number at all is the entire thesis of the

cooling axis made small: cool is not the private property of mint. It is a coordinate every organism has, a dial any genome can be taught to turn.

IFI-C	EC_{50} (CANDIDATE) ÷ EC_{50} (REFERENCE)	LOUDNESS VS. REFERENCE
500	2.00	half as loud
800 *(Phoenix, predicted)*	**1.25**	**0.80× — the cool inside a pepper**
1,000 *(anchor)*	1.00	equal to reference
2,000	0.50	twice as loud
5,000 *(axis ceiling)*	0.20	five times as loud

One honest note lives in this table. The IFI-C score is dimensionless and assay-independent — it is a *ratio* of two EC_{50} values, so the reference cancels and the number travels between laboratories unchanged. The raw EC_{50} underneath it does not travel so freely; menthol's half-activation of TRPM8 falls somewhere in the low tens of micromolar on a typical bench, but the exact figure drifts with the cell line, the temperature, and the readout. That drift is precisely why we publish the ratio and not the raw concentration. The index is built to be the invariant — the thing a tomato, a chili, and a cup of mint tea can be read against on one page — and the EC_{50} is the private figure it is distilled from.

The distillation is worth one unit conversion, because the receptor speaks in molarity and the kitchen speaks in milligrams. (−)-Menthol is $C_{10}H_{20}O$, molar mass **156.27 g/mol**. Take a representative bench value for its half-activation — call it 40 µM for the arithmetic — and convert: 40×10^{-6} mol/L × 156.27 g/mol = 6.25×10^{-3} g/L = **6.25 mg/L**. Six and a quarter milligrams of menthol in a liter of water is enough to drive the cold receptor to half its full cry. The molecule is almost unreasonably efficient; a little of it is a shout.

The numbers of the factory

The supply side is just as exacting, and just as modest. Peppermint oil is on the order of one to three percent of the leaf's dry weight — a few drops per leaf — and in a good oil menthol makes up something like forty percent of that, with menthone, menthyl acetate, and the rest filling out the balance. Put the two figures through the molar mass and the scale of the thing becomes vivid. One gram of good dry leaf, at two percent oil, holds about 20 mg of oil; at forty percent menthol, that is **8 mg of menthol**; divide by 156.27 g/mol and you get 5.1×10^{-5} mol, or roughly **51 µmol** of the active cold per gram of leaf. Against a half-activation concentration in the tens of micromolar, a single well-grown gram of mint carries, in principle, an enormous surplus of cold — the sensation is limited not by how much the leaf makes but by how much reaches the nerve. The glands fill as the leaf matures, over days, on a daily rhythm, and the oil sits there essentially inert until a mouth or a finger or a still breaks the cuticle. It is a tiny yield fought over fiercely, because the difference between a clean two percent and a muddy two percent is the difference between a premium oil and a cheap one, and that difference lives almost entirely in the ratio of the seven good steps to the one bad off-ramp.

Which is where consistency stops being a virtue and becomes the product. A flavor you cannot reproduce is not a flavor you can sell, or cook with, or score. The variation in a batch is read as the coefficient of variation — CV = σ / μ, the spread divided by the average — and a wild mint oil, with its menthofuran off-ramp swinging open and shut on the weather, runs a CV that can reach the low forties, the same ballpark of raw-material scatter we measure in unimproved plants across the platform, around **42%**. Set menthol at forty percent of the oil as the mean, μ = 0.40, and a 42% CV means a standard deviation of σ = 0.42 × 0.40 = **0.168** — a swing of nearly seventeen points of composition from batch to batch. That is not an ingredient; it is a rumor. When we silence menthofuran synthase and grow the plant in a controlled environment that holds the fork steady, our model predicts batch CV falling to **≤7%**, the platform design specification across all eight axes. At μ = 0.40 that is σ = 0.07 × 0.40 = **0.028** — under three points of swing, a spec you can formulate against.

The index inherits that discipline directly, because error propagates. Since IFI-C ∝ 1 / EC_{50}, and EC_{50} tracks the active menthol concentration, the relative error in the published score is essentially the relative error in the chemistry — the CV of the oil becomes the CV of the number. Carry both the reference standard and the candidate through the formula and the uncertainties add in quadrature: CV(IFI) ≈ √(CV(ref)² + CV(candidate)²). Hold the reference as a tight, fixed standard — near-zero CV — and a candidate at ≤7% yields a score good to about ±7%. A 42% raw scatter, by the same propagation, would make the axis number meaningless to within nearly half its value: you could not tell an 800 from a 1,150. Precision is not a cosmetic here. It is the thing that lets a cooling score mean the same thing twice.

The cooling axis is not one plant but a family of them, every one grown toward the fullest, cleanest cool it can carry. Peppermint is the flagship, but it has company across the index.

HOST	CONSTRUCTS	COOLING-AXIS ROLE
Peppermint	2,360	C / M — the menthol flagship, the full seven-step line
Lavender	160	C — a softer, floral cool
Orange	120	B / C — cool riding alongside bitterness work
Eucalyptus	80	C — the eucalyptol cool, built on a different cyclization
Spearmint	80	C — carvone, the cool that forked early
Camphor	40	C — the sharp, penetrating end of the axis

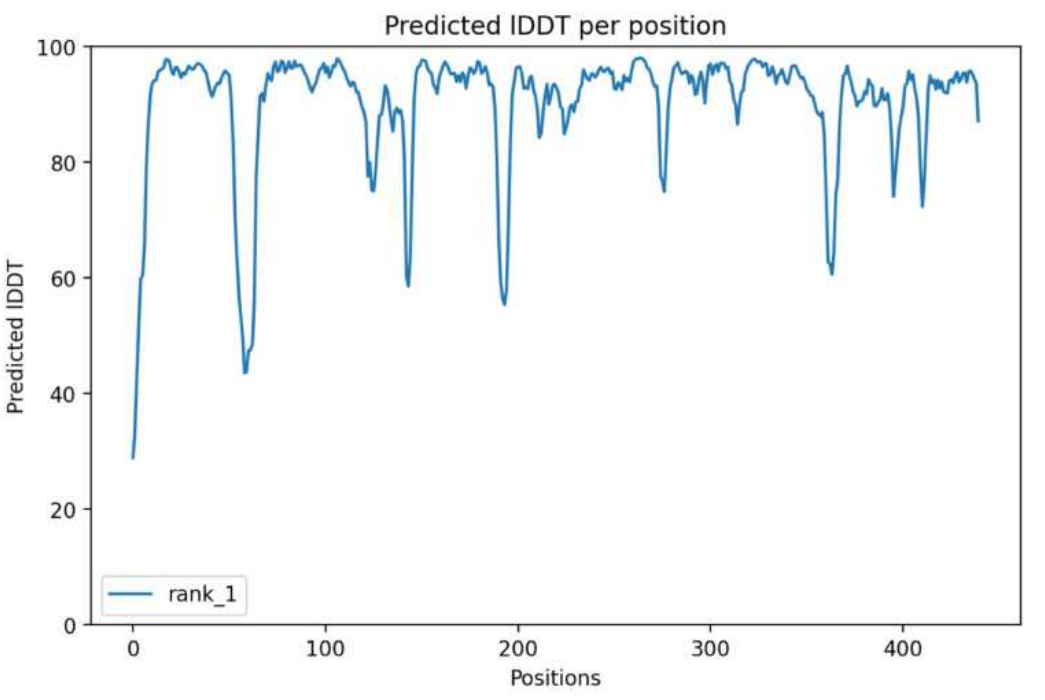
Peppermint alone accounts for 2,360 of these constructs — the deepest single library on the axis — and the five dedicated cooling hosts together (peppermint, lavender, eucalyptus, spearmint, camphor) carry 2,720 constructs tuned toward one sensation. Spearmint is the instructive one on that list, because it shows how early a leaf's fate is sealed. Spearmint and peppermint start identically: same GPP, same limonene. They part at a single oxidation. Peppermint's P450 hydroxylates limonene at carbon three and commits the molecule to the menthol line; spearmint's oxidizes

at carbon six instead and makes carvone, the bright, caraway-adjacent cool that is unmistakably *not* menthol. One regiochemical choice by one enzyme — which carbon gets the oxygen — sends the same citrus precursor to two entirely different colds. Understanding that fork is understanding why the pathway is designable at all: the cooling of a plant is not written in its species but in which enzyme it points at which carbon, and enzymes and their targets are exactly what we can rewrite.

A pathway built to be read

What makes this route so suited to design is that it is almost entirely *known*. Every enzyme is sequenced; every intermediate is identified; the fork at pulegone is characterized down to the two competing P450s and the reductase that opposes them. There is no mystery step, no black box where flux disappears into the dark. For a design platform that is everything, because you cannot engineer what you cannot see, and the menthol pathway is one of the best-seen secondary-metabolite routes in all of botany, mapped across decades by biochemists who cared about the oil long before anyone could edit the plant that made it.

Knowing the sequence is only the start of knowing the enzyme, though. Before we raise one step or silence another, we read the protein the way a machinist reads a casting — for where it is sound and where it is uncertain. We model each enzyme's structure and color it by the model's own confidence, so that the stretches it is sure of glow and the stretches it is guessing at do not, and we refuse to trust a design that lands in the uncertain regions. The discipline is the same whether the enzyme sharpens a pepper's heat or reduces a mint's pulegone.



A per-residue confidence map across a modeled plant acyltransferase — green where the fold is certain, cooler where the model hedges. This is the structural read we run on every enzyme before we touch it: the active-site cleft of a pathway enzyme must be high-confidence before we trust a single edit to it.

So the whole chapter is really one long argument for humility and precision held together. The leaf does something extraordinary — routes a volatile molecule across four organelles, makes eight right-handed decisions in a row, and pools a cold it will never feel inside glands smaller than dust. We did not invent any of that. We inherited a map drawn by people who loved the oil, and our work has been to

read it exactly: to model the fork as arithmetic, to score the cool as a ratio that survives the move between laboratories, to design toward a forty-percent scatter pulled down to seven, and to leave the plant's own chemistry doing what it has always done, only steadier, only cleaner, only pointed a little more surely at the cold at the end of the green. That is the quiet promise under the mathematics — that food grown and read with this much care can be trusted to give the body exactly what it says it will, every single time. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 7 · Designing the Cold — *the model picks the mutation.*

The method does not change when the molecule changes. The pipeline that redesigned our capsaicin acyltransferase until our model predicted a design target approaching three million Scoville units is the same pipeline, unaltered, that we turn on the cooling plants — because it was never a pipeline about capsaicin. It is a pipeline about enzymes, and an enzyme is an enzyme whether it builds a fire or a frost. Three stages, run in order, every time: a genome-scale flux model finds the valve that is actually closed; a protein language model reads each enzyme and ranks every possible repair; CRISPR writes the chosen few into the plant, and then we verify, because a number we have not checked is a hope, not a result. We run all three stages on peppermint exactly as we ran them on the peppers, and the cold that comes out the far end is colder, cleaner, and — the part that matters most to anyone building a product — the same every time.

What earns the cold its own chapter is the symmetry waiting at the end of it. Heat and cold are not quite opposites on the tongue. They are mirror images of a single trick: a receptor persuaded to report a temperature that is not in the room. Capsaicin does it to the fire channel. Menthol does it to the frost channel. Once you see that both molecules are lying to the same kind of protein in the same grammatical way, you understand why one platform designs both, and why the hard work of cool turns out to be a problem of handedness and restraint rather than of making more.

The receptor that reports a cold that isn't there

Menthol binds TRPM8, a non-selective, calcium-permeable cation channel seated in the membranes of a dedicated subset of trigeminal and dorsal-root sensory neurons — the body's principal cold sensor. The channel is a homotetramer: four identical subunits arranged around a central pore, each subunit threading the membrane six times, the conducting pore formed between the fifth and sixth passes of all four. Like its heat-sensing cousin TRPV1, TRPM8 is *polymodal* — temperature, membrane voltage, the signaling lipid PIP_2, and menthol all push on the same gate — and the beauty of that, the thing that makes it designable, is that every one of those inputs converges on a single physical quantity: the voltage at which the channel sits half-open.

Cold shifts that half-activation voltage toward the resting potential of the neuron. So does menthol. At a comfortable mouth temperature the channel would stay shut, its activation curve parked at voltages the cell never visits; menthol slides the whole curve leftward until, at the voltage the neuron actually holds, the gate carries a real probability of opening. The nerve fires. The brain, which has no way to audit the channel, receives the only message that channel can send — *cold* — and prints it faithfully onto a mouth that has not dropped a single degree. That is menthol's entire pharmacology in one sentence: it does not chill the tissue, it lowers the threshold at

which the tissue's cold alarm goes off. Capsaicin raises a fire that is not there by the same maneuver run backward. Two molecules, two channels, one lie told in two directions.

Where menthol touches the protein is known closely enough to design around. The pocket lies not in the pore but in the voltage-sensing bundle — the first four membrane passes of each subunit — and a small set of residues lines it; an aromatic contact such as Tyr745 in the second transmembrane segment is one of those whose alteration collapses menthol sensitivity while leaving the channel's raw response to cold largely intact. That clean separation is the proof that the menthol site is a real, local, evolvable thing and not a vague smear of lipid effect.

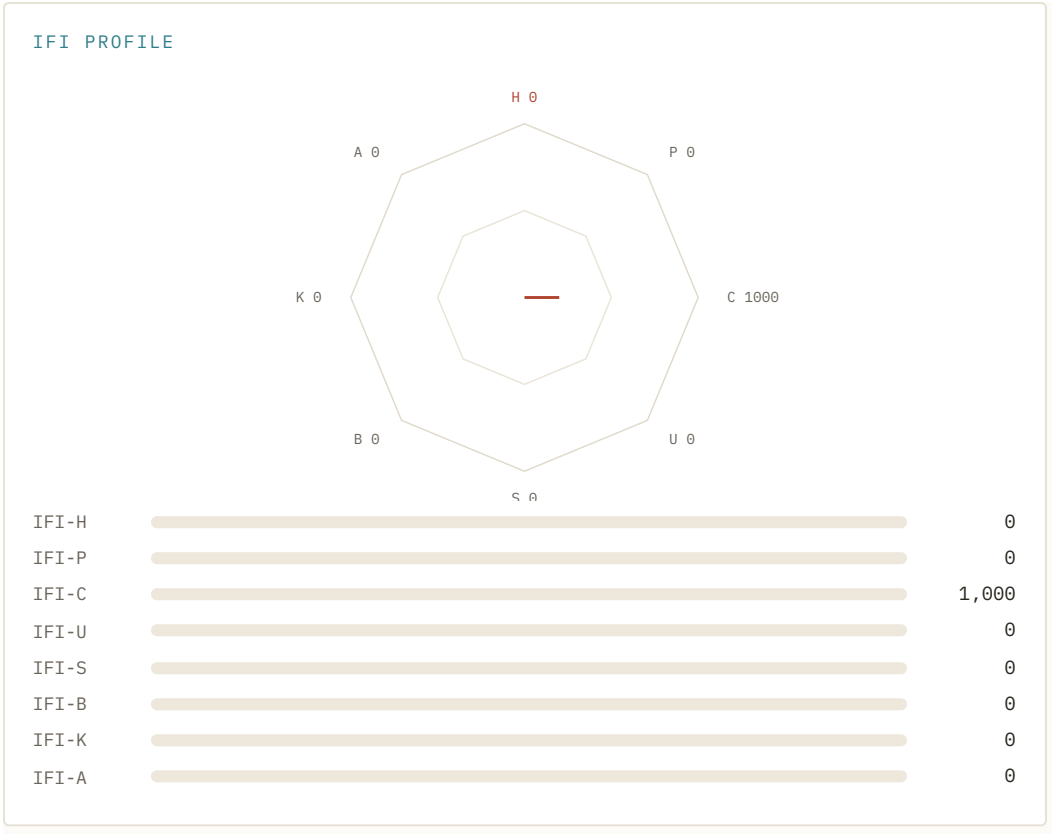
Two consequences of this receptor biology set the whole cooling program, and both are about quality rather than quantity. The first is handedness. TRPM8 is exquisitely stereoselective: (−)-menthol, the (1R,2S,5R) enantiomer, is the loud, clean, physiologically dominant cooling isomer, and its mirror image and diastereomers are weaker and muddier — present in the extract, contributing little but noise. A mint that makes the wrong hand of the molecule has spent precious precursor manufacturing a near-silent impurity. The second is adaptation. The open state leans on membrane PIP_2; sustained activation depletes that lipid and the cool fades — the familiar tachyphylaxis of a mint that is vivid for ten seconds and gone by thirty. We can design the plant to pour more of the right molecule; we cannot design the tongue out of its own adaptation, and we say so plainly. The cold we build is a cleaner, truer, more consistent *input*. What a given mouth does with it on the twentieth second belongs to the mouth.

Reading cool as a number

To design a sensation you must first agree how to measure it, and the Index measures loudness, not mass. Each axis value is anchored to the half-maximal activation of a receptor — not how much of a compound a food holds, but how strongly that compound speaks to the channel — through one relation:

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

EC_{50} is the concentration that drives the receptor to half of full response. A molecule that reaches half-activation at a *lower* concentration is, by definition, louder; and because the candidate's EC_{50} sits in the denominator, a lower EC_{50} returns a higher score. For the cool axis we anchor the reference to the sensation's own namesake — pure (−)-menthol, whose half-activating concentration at TRPM8 we fix at $EC_{50}(\mathrm{ref}) = 100\ \mu M$. That places clean menthol at exactly IFI-C 1,000, the reference line against which every other coolant is read:



This block is not a plant; it is the anchor itself — pure (−)-menthol, loud on the cool axis and silent on the other seven, the ruler we lay against everything that follows. From here the formula does the rest. Set the candidate's EC_{50} into the denominator and read the cool off directly:

STIMULUS	TRPM8 EC_{50}	IFI-C = 1,000 × 100 ÷ EC_{50}[MM]	READS AS
(−)-menthol (reference)	100 µM	1,000	the anchor — clean, familiar cool
(+)-menthol (wrong hand)	800 µM	125	faint, blurred cool for the same mass
1,8-cineole (eucalyptol)	3,400 µM	29	broad and weak; a whisper, not a word
WS-23 (synthetic coolant)	40 µM	2,500	engineered loud, no mint character
icilin (synthetic super-agonist)	0.4 µM	250,000 → capped 5,000	off any food scale

Three things in that table carry the whole philosophy. First, the enantiomer penalty is arithmetic, not opinion: the wrong hand of the identical molecule reaches half-activation at eight times the concentration, so it scores 1,000 × 100 ÷ 800 = 125 — an eighth of the cool for the same manufacturing cost to the plant. That single ratio is why the cooling program spends its effort on *stereoselectivity*, on making the right hand, long before it spends any on making more. Second, the scale saturates on purpose. Icilin is a genuine super-agonist and computes to a quarter-million; because no food needs that and because a radar axis has to stay legible, the scorecard normalization caps each axis at 5,000, and a reading that pegs the cap is a signal to stop pushing potency and start thinking about everything else. Third, eucalyptol at 29 shows why a plant that makes a dozen weak coolants can still read quiet: loudness is set by the EC_{50} of what you make, and breadth is no substitute for a molecule the channel actually prefers.

The score is a floor, not the sensation, because a food delivers a *concentration*, and the receptor's response to concentration is not a straight line but a cooperative curve. Writing R for response and n for the Hill coefficient,

```
R(c) = R_max × cⁿ ÷ (EC₅₀ⁿ + cⁿ)
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At c = EC_{50} the fraction is one-half by construction. With the tetramer's cooperativity giving n ≈ 2, doubling the delivered menthol to c = 2·EC_{50} lifts response to 4 ÷ (1 + 4) = 0.8 of maximum, while halving it to c = 0.5·EC_{50} drops response to 0.25 ÷ 1.25 = 0.2. The whole journey from a fifth of the sensation to four-fifths of it is spanned by a mere fourfold change in concentration — a steep, decisive cliff that a single uncooperative channel (n = 1) would smear across a sixteenfold range. Cooperativity is why mint arrives as an *event* rather than a gradient, and why landing the delivered dose near EC_{50} is worth so much: it puts the product on the steepest, most responsive part of the curve, where the design has the most leverage.

One unit conversion keeps the pharmacology tethered to the formulator's bench. Menthol's molar mass is 156.27 g/mol, so the 100 µM reference is 100 × 10^{-6} mol/L × 156.27 g/mol ≈ 15.6 mg/L — about 16 parts per million in water. A cool that reads loud on the Index is a cool that half-saturates its channel at a concentration you can hold in your hand as a pinch in a vat, which is exactly why a few milligrams of clean (−)-menthol can carry a whole confection.

Where the cold is actually made

The cold is built on a short, gorgeous assembly line inside the glandular trichomes — the resin-filled hairs that stipple a peppermint leaf and that you rupture, releasing the smell, every time you brush the plant. Carbon enters as the universal five-carbon isoprene units supplied by the plastid's MEP pathway, and the line runs:

GPP → (−)-limonene → (−)-*trans*-isopiperitenol → (−)-isopiperitenone → (+)-*cis*-isopulegone → (+)-pulegone → (−)-menthone → (−)-menthol

with one fateful side door, (+)-pulegone → (+)-menthofuran, standing open the whole time.

Read it enzyme by enzyme, because every step is a decision the plant makes and therefore a decision we can remake. **Geranyl diphosphate synthase** condenses two isoprene units into the ten-carbon GPP, the committed monoterpene precursor and the throttle on the entire line. **Limonene synthase** cyclizes GPP into (−)-limonene, closing the ring that every downstream cold is carved from. A cytochrome-P450 **limonene-3-hydroxylase** then performs an allylic hydroxylation, spending NADPH and molecular oxygen to hang an alcohol on the ring; an **isopiperitenol dehydrogenase** oxidizes that alcohol to a ketone; an **isopiperitenone reductase** saturates a double bond; an **isopulegone isomerase** walks the remaining double bond into conjugation to give pulegone — the hinge of the whole pathway. From pulegone, **pulegone reductase**

reduces the ring toward (−)-menthone, and finally **menthone:menthol reductase** reduces the carbonyl to the alcohol, and the cold is finished as (−)-menthol.

Two of those steps are not really about yield at all — they are about handedness, the very property the receptor cares about most. Pulegone reductase sets the menthone-to-isomenthone partition, and menthone:menthol reductase sets whether the carbonyl is reduced to menthol or to its weaker sibling neomenthol. The stereochemistry written at these last two enzymes is the difference between the loud (−)-menthol anchored at IFI-C 1,000 and a blurred mixture that scores a fraction of it for the same carbon. The plant never had a reason to perfect that choice. We do.

And then there is the open side door. Menthofuran synthase, another P450, pulls pulegone off the line and converts it to menthofuran — the musty, slightly medicinal off-note that haunts natural mint. Nothing in the leaf's life punished that leak; a plant has no palate, and the note that offends a confectioner is invisible to natural selection. The pathway is, in other words, nearly finished biology that evolution had no incentive to finish to a human standard — which is precisely the gap the three stages close.

Stage one — the flux model finds the valve

Before touching a single residue we ask a genome-scale **flux-balance model** where the real bottleneck sits, because the obvious guess is usually wrong. Flux-balance analysis treats metabolism as a system of linear constraints: every reaction a variable, the stoichiometry gathered into a matrix **S**, and the steady-state demand that nothing pile up written simply as

$$S \cdot v = 0, \text{ with } l_k \le v_k \le u_k$$

Hand the solver an objective — maximize menthol export through the trichome network, max $c^{\mathsf{T}}v$ — and linear programming returns the flux distribution that achieves it. More valuable than the fluxes themselves are the *dual* values the same solve produces: the shadow price on each metabolite balance, the marginal menthol you would gain per unit you relaxed a given constraint. A step whose capacity bound is binding carries a positive shadow price and is a true limiter; a step running with slack carries a shadow price of zero and is not, however late it sits in the pathway.

A three-reaction toy makes the lesson concrete. Suppose precursor supply can carry 10 units, the pulegone fork can pass 4, and the terminal reductase can pass 9. Throughput is the minimum of the chain, min(10, 4, 9) = 4, pinned entirely at the fork. The shadow price is +1 unit of menthol per unit of capacity added *at the fork*, and exactly 0 at the terminal reductase, which already has five units of headroom it is not using. The intuition it kills is seductive and common: the terminal reductase makes the final cold, so surely you make more of it. The model answers that pushing the last enzyme while the fork is closed only backs product up against a shut valve. Across the real peppermint network — precursor supply from the MEP pathway, GPP-synthase throughput, the branch points, the

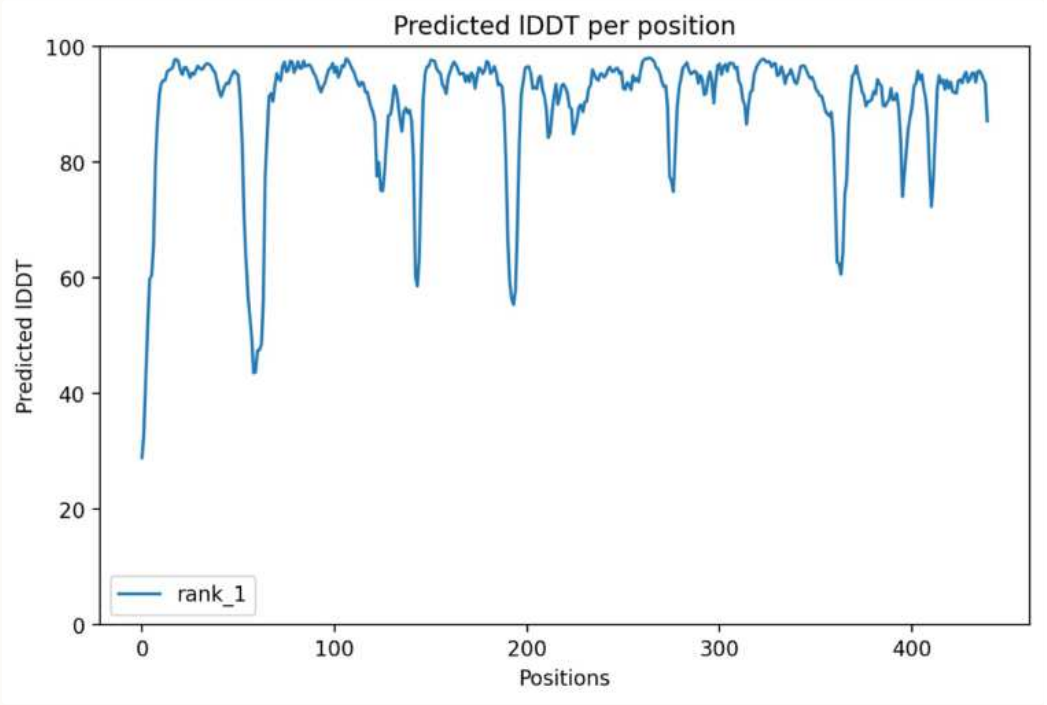
NADPH budget the reductases draw on — the binding constraints land, again and again, on **precursor flux to GPP** and on **the pulegone fork**, not on the terminal reductase. It is the same discipline that, on the peppers, showed our flux model predicts control sits upstream in the phenylpropanoid pathway — at 4CL and HCT — not at the terminal acyltransferase (Pun1/AT3), where Pun1 labels the final product but does not govern the rate: the model is how we keep from spending a plant's energy in the wrong place.

Stage two — the model reads the protein

Once the flux model has named the enzymes worth editing, a **protein language model** ranks *how* to edit them. We use an ESM2-class network of 650 million parameters, trained on tens of millions of natural protein sequences until it has absorbed, statistically, what a working protein looks like. The scoring move is elegant: mask one position in the target enzyme, ask the model how probable each of the twenty amino acids is there given the entire rest of the sequence, and read the log-likelihood difference between a proposed variant and the wild type,

```
score = ln P(mutant | context) − ln P(wild type | context)
```

as a prediction of help or harm. If the model assigns the wild-type residue probability 0.6 and the mutant 0.3, the score is ln(0.3 ÷ 0.6) = −0.69 — the variant is twice as *un*likely as nature's choice, a predicted loss. If instead a substitution the breeders never tried comes back at 0.8 against a wild-type 0.1, the score is ln(0.8 ÷ 0.1) = +2.08 — the model finds the edit markedly *more* natural than what the plant settled for, a candidate improvement we move to the top of the list. Instead of guessing which single change might sharpen a reductase, we let a model that has read the whole protein world rank them all and we test from the top down.



Pun1/AT3 colored by pLDDT confidence — the same structural read we run on every menthol-pathway enzyme: the folded core and active-site cleft we trust and may edit, the mobile loops we leave alone.

We never read that ranking blind. Every candidate is folded against a structural confidence analysis — the per-residue pLDDT and the predicted-aligned-error map — so we know which regions of the modeled enzyme are trustworthy enough to touch. We edit where

the fold is certain and the predicted gain is real; we refuse low-confidence loops no matter how enticing the language-model score, because a high score on a region the structure cannot place is a guess dressed as a result. The membrane-anchored P450s of this pathway — the hydroxylase and menthofuran synthase — are the honest hard case here: their catalytic cores model cleanly, but their membrane anchors and substrate-access loops often come back low-confidence, which is one reason we frequently act on menthofuran synthase at the level of gene dosage rather than by carving its active site.

The result is a design slate — a strategy, never a promise, because every edit earns its place on the bench before it earns a place in a cultivar:

TARGET	STRATEGY	INTENDED EFFECT	ΔΔG (KCAL/MOL)
Pulegone reductase (PR)	A128V	↑ turnover toward (−)-menthone	−1.6
Menthone:menthol reductase (MMR)	S52T	↑ stereoselectivity for (−)-menthol	−1.1
GPP synthase	promoter up-regulation	↑ precursor flux (FBA-confirmed limiter)	–
Menthofuran synthase (MFS)	**promoter deletion / RNAi**	**↓ menthofuran, ↓ residual pulegone**	**–**

The ΔΔG column is the stability math — negative values are stabilizing, the designed protein folding tighter than the wild type — and it is the number we trust least on its own and most in aggregate. Stability is an equilibrium, and equilibria are exponential in free energy: at an assay temperature of 298 K the thermal quantum RT is about 0.59 kcal/mol, so a −1.6 kcal/mol edit shifts the folded-to-unfolded ratio by exp(1.6 ÷ 0.59) ≈ 15-fold toward folded, while the −1.1 kcal/mol stabilization at menthone:menthol reductase moves it roughly exp(1.1 ÷ 0.59) ≈ 6-fold. Any single such prediction can miss — a tighter fold is not a faster or more selective enzyme, and stability and activity are different quantities that only tend to travel together — which is exactly why we read the slate and not the line. A handful of independently stabilizing, each independently assessed edits lifts an enzyme's effective output and, more than that, its *consistency*, and consistency is the quantity the whole platform is finally built around.

Stage three — CRISPR writes, and we verify

Peppermint makes the writing both harder and kinder than it sounds. Harder, because the plant is a sterile hybrid with no seed to breed through, so the edits are delivered into leaf tissue or into protoplasts and the plant is regenerated from them. Kinder, because that same sterility means a validated edit is locked into a clone and carried forward indefinitely without ever segregating away — the thing you made is the thing you keep, generation after generation, with no breeding lottery between you and it.



The write-and-read bench — where every designed edit is verified by sequence analysis before any plant carrying it is trusted, and where every lot is characterized by design rather than assumed.

Then we verify, because the number that separates a cooling ingredient from a cooling anecdote is not the loud batch but the *repeatable* one, and that number is the coefficient of variation — the standard deviation divided by the mean, CV = σ ÷ μ. Take ten harvests of an unimproved mint and the menthol content wanders: a mean of μ = 40 relative units against a standard deviation of σ = 17 gives CV = 17 ÷ 40 = 42.5%, the forty-plus percent that untended biology routinely shows. Our model projects that the same ten harvests on the redesigned line would see both numbers move the right way at once — the mean climbing to μ = 55 as the stabilized reductases run truer and the sealed off-ramp stops bleeding flux, while the spread collapses to σ ≈ 3.3 — with the coefficient falling to CV = 3.3 ÷ 55 = 6.0%, well inside the platform's ≤7% design specification. More cold, yes. Mostly, the *same* cold.

That consistency propagates cleanly all the way to the Index, and the reason is in the formula. Because the cool score is proportional to 1 ÷ EC_{50} at a fixed reference, a fractional error in the EC_{50} passes straight through to the score — δIFI ÷ IFI = δEC_{50} ÷ EC_{50} — so a cool axis carried on 6% batch variance is a reading that holds to within a few points lot to lot. That is the whole ballgame for a formulator. A confectioner cannot build a recipe around a mint that is clean one harvest and medicinal the next; a beverage house cannot ship a cool that drifts by a third between lots. Sameness, for anyone making a real product, is worth more than any one unrepeatable peak.

The biggest win is a door closed

The star of the slate is the only line with no ΔΔG at all: silencing menthofuran synthase. It is a lesson in flux, not potency, and the steady-state balance the flux model already demands tells it exactly. At the pulegone node, conservation requires that what comes in equals what goes out the two exits:

```
v_IPI = v_PR + v_MFS
```

So if a wild-type leaf sends a quarter of its pulegone out the menthofuran door — v_MFS ÷ v_IPI = 0.25 — then closing that door frees up to 25% more flux toward menthone and menthol, *provided the next enzyme can absorb it*. That proviso is the whole design. It is

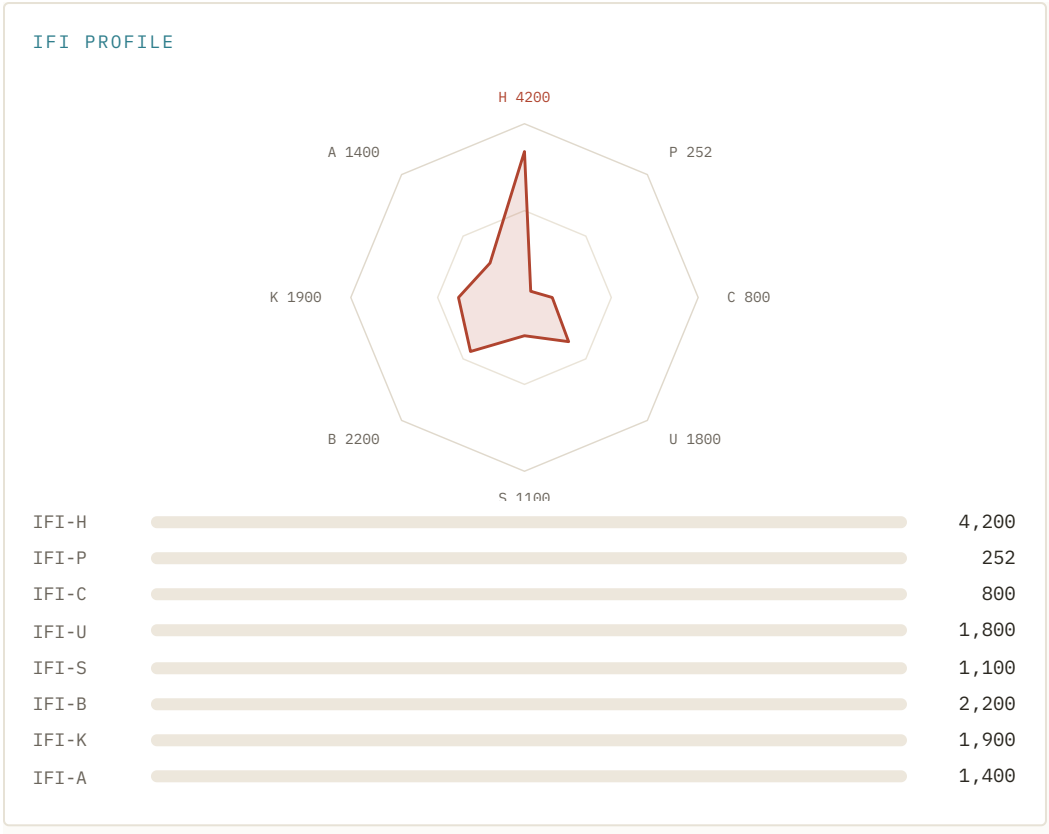
why the slate silences menthofuran synthase and raises pulegone-reductase turnover in the very same breath: close the leak without widening the drain and you merely pool pulegone behind the node — and pulegone is the single intermediate you least want to accumulate, being both the precursor of the off-note and the regulated, hepatotoxic species itself. Designed as a pair, the two edits convert a loss into a gain and a liability into a finished cold.

That liability is the second payoff, the one that turns a flavor advantage into a regulatory one. Pulegone and menthofuran are not merely musty; they are restricted. Food law in major markets caps both in mint-flavored foods and drinks at defined ceilings — on the order of tens to a couple hundred milligrams per kilogram depending on the category, tighter for beverages than for confectionery. A conventional mint sometimes has to be blended down or stripped to clear those limits, shedding character in the trade. A mint engineered with the off-ramp deleted clears them by construction, character intact: better-tasting and easier to certify at once, the two-for-one that makes a formulator's year.

The same three stages run on every plant that pours cool, because they all share the back half of this chemistry and differ only in the terminal flourish — the eucalyptol of a eucalyptus, the linalool-and-camphor of a lavender, the carvone that makes spearmint read rounder than peppermint. The cooling shelf is small, deliberate, and uniformly difficult:

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Peppermint	2,360	C / M	Hard
Lavender	160	C	Hard
Orange	120	B / C	Hard
Eucalyptus	80	C	Hard
Spearmint	80	C	Hard
Camphor	40	C	Hard

It is worth seeing what the cool axis looks like on a plant that was *not* built for it. Our full-spectrum pepper, Phoenix (SS-0096) — engineered gene by gene to activate all eight axes at once — carries a real but modest cool, because on that plant the cold is a borrowed accent rather than the point:



Eight hundred on the cool axis is a genuine thermomodulation, deliberately placed to round the plant's enormous predicted heat — but it is a seasoning, aimed low on purpose so it never competes with the fire. A dedicated cooling plant inverts that priority entirely: it points the whole GPP-to-menthol line at the C axis and lets the other seven fall quiet, which is why the same method that makes a pepper burn across eight channels makes a mint speak clean cool on one.

What the cold cannot do, and what it can

We would rather show the edges of the method than oversell its middle. The flux model assumes a steady state and the right objective; it is blind to regulation, allostery, and transport we did not encode, and handed a wrong objective it will name a wrong bottleneck with perfect, misleading confidence. The language model scores plausibility, not mechanism — a high-ranking variant can still misfold or stall on the bench — and epistasis means two edits that each help alone can quietly fight when combined, which is the whole reason the slate is computed as a *set* and never shipped on prediction alone. The membrane-bound P450s resist point design and often come back too low-confidence to carve, so we reach for gene dosage instead of a scalpel. The sterile hybrid resists regeneration, and the combined efficiency of editing and regrowing a whole plant is a real throughput tax we pay on every line. And the tongue's own adaptation — the PIP_2 depletion that lets a cool fade by the thirtieth second — belongs to the mouth, not the leaf; we can design a cleaner, louder, truer input, not a longer-lived sensation. Everything in this chapter is an attribute we compute and characterize — a molecule, a score, a variance — and never a claim about what it does to a body. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



A cooling line in the research plot at first light — the same pipeline that redesigned a pepper's fire, turned patiently on the chemistry of frost.

This is the shape of nearly all our cooling work: the biggest wins come not from making more of the good molecule but from *not making* the bad one — closing a door the plant left open for no reason except that evolution never had a reason to close it. The wild mint kept its menthofuran branch because nothing in its life punished the off-note; a leaf has no palate, and the mustiness that offends a confectioner is invisible to natural selection. We are, in a real and careful sense, finishing a job the plant had no incentive to finish — walking a wild chemistry the last distance to a human standard it was never shaped toward. We do it with a model that reads proteins, a solver that reads metabolism, a pair of molecular scissors, and a family that would always rather close a door cleanly than force one open. And the result is a leaf that is, at last, simply more itself: cold, clean, and the same every time.

Chapter 8 · Beyond Menthol — *every coolant we can and cannot grow.*

Menthol is the cool everyone knows, and for most of a lifetime it is the only one anyone can name. But it is a single entry in a long catalog, and a serious account of the thermal axis has to walk the whole of it — including the parts of it that no plant will ever grow. The cooling molecules divide cleanly into two families. There are the ones biology makes, the monoterpenes a leaf assembles out of its own carbon, which we can design and tune and move from plant to plant. And there are the ones only a chemist makes, folded in a reactor out of shapes no living pathway ever had reason to build, which we can measure against the same receptor and formulate with and respect — but cannot farm. Knowing which molecule belongs to which family is the whole difference between a research program and a wish, and the honesty of this chapter rests on never blurring the line.

What unites the two families is not a shape. It is a single protein on the surface of a nerve, and the first thing worth doing is to understand, precisely, what that protein does when a cool molecule finds it.

The receptor that reports a cold that is not there

The cool axis is read by one channel: TRPM8, the eighth member of the melastatin branch of the transient receptor potential family. It sits in the membrane of the cold-sensitive trigeminal and dorsal-root neurons that wire the mouth, the lips, and the skin, and it is built as a homotetramer — four identical subunits arranged around a central axis like the staves of a tiny barrel. Each subunit threads the membrane six times, in helices labeled S1 through S6. The last two of each subunit, S5 and S6, lean inward and together line the ion-conducting pore; the first four, S1 through S4, fold off to the side as a bundle called the voltage-sensor-like domain. That side bundle is where the cool lives. It holds a small amphipathic cavity, and menthol settles into it against a handful of residues — most importantly a tyrosine, Tyr745, on the S2 helix, whose aromatic ring stacks against the menthol ring and anchors it. The pocket is not large and it is not fussy about chemistry; it is fussy about fit, which is why molecules as different as menthol, a eucalyptus ether, and a synthetic amide can all occupy it.

Here is the part worth slowing down for, because it is the mechanism the entire axis turns on. TRPM8 does not cool anything. Nothing about menthol lowers the temperature of the tongue by a hundredth of a degree. What the channel does is report. In its resting state TRPM8 opens only when the tissue around it falls below roughly 26 to 28 °C, and it opens harder the colder things get, down toward 8 °C. Cold shifts the channel's voltage dependence — the membrane potential at which it is half-open — toward the potentials the neuron actually sits at, so cold makes the channel easy to open. Menthol, binding in that S1–S4 cavity, does the same thing by a different door: it shifts the voltage-activation curve in the same direction, by tens of

millivolts, so that the channel now opens at a temperature where it otherwise would have stayed shut. The neuron fires. The brain has exactly one interpretation for a signal from this fiber, and it is *cold*. So menthol does not cool the mouth; it moves the mouth's own thermostat, and the body reads its ordinary warmth as a chill that is not there. Every cool in this book — mint, eucalyptus, the synthetic amides, all of it — is that one trick, played on that one channel, with greater or lesser force.



Cool is, before it is anything else, a beverage sensation — the plant that carries it into a cup more often than any other.

Two further facts about the channel shape everything a formulator feels. First, TRPM8 needs a lipid cofactor, PIP_2 — phosphatidylinositol 4,5-bisphosphate — resident in the inner leaflet of the membrane, to open at all; strip it away and the channel goes quiet no matter how much menthol is present. Second, every opening lets calcium in along with sodium, and that calcium switches on a lipase, phospholipase C, which chews up the very PIP_2 the channel depends on. So the channel disarms itself as it works. This is the molecular basis of the fade every mint has — the cool that is bright at the first breath and gone by the fourth — and it is why the long-lasting coolants are prized: they are not louder, they are more patient, holding a modest activation open while the PIP_2 slowly replenishes. The receptor, in other words, is not a volume knob. It is a knob with a spring in it, and designing cool means designing against that spring as much as for the pocket.

One molecule, one number — the cool axis, worked

To design against a receptor you must first measure it, and the whole point of the Iyengar Flavor Index is that a sensation this intricate collapses to a single honest number. The axis score is defined the same way every axis is:

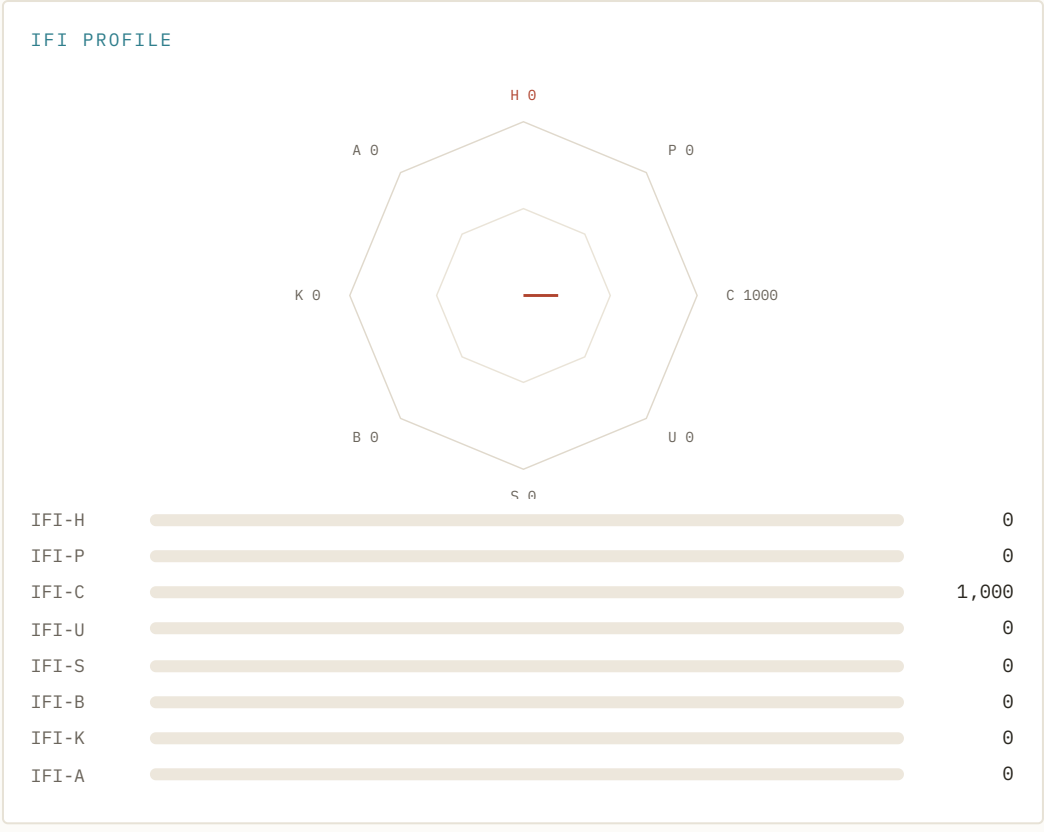
> $\mathrm{IFI}(c) = 1000 \times \mathrm{EC}_{50}(\text{reference}) \div \mathrm{EC}_{50}(\text{candidate})$

EC_{50} is the concentration of a stimulus that drives its receptor to half of full response — the midpoint of the dose–response curve, and the single most compact description of how loudly a molecule speaks. It is an inverse measure: the *smaller* the EC_{50}, the *more potent* the molecule, because less of it is needed to reach half-maximal activation. The index flips that inversion back into an intuitive direction by putting the candidate's EC_{50} in the denominator, so a louder molecule earns a bigger score.

On the cool axis the reference is menthol itself. We anchor it at 1,000 by convention — meaning that by definition EC_{50}(reference) equals EC_{50}(menthol), and in our reference TRPM8 assay that half-activation sits near 80 µM. This is a stated convention, like how the Scoville scale is defined by dilution-to-threshold: the reference constant sets IFI-C = 1,000 on this axis by definition. Every other coolant's score is then nothing more than its potency measured against menthol. Rearranging the formula makes the relationship you actually care about fall straight out:

> $EC_{50}(\text{candidate}) = EC_{50}(\text{menthol}) \times 1000 \div \text{IFI-C}(\text{candidate})$

which says the EC_{50} ladder is just the menthol anchor scaled by the inverse of the score. A molecule twice as loud as menthol has half its EC_{50} and scores 2,000; a molecule half as loud has twice the EC_{50} and scores 500. The index is a ratio, so the absolute anchor cancels whenever you compare two coolants to each other — but worked in full, against 80 µM, it gives the real concentrations a chemist meters into a batch.



That profile is menthol itself: a molecule that speaks on exactly one axis, at the anchor, and is silent on the other seven. It is the cleanest object in the whole flavor library — a pure tone — and it is worth seeing drawn, because almost nothing else in nature is this single-minded.

Now the ladder, derived. Take the canonical cool scores and read each one back to an EC_{50}:

COOLANT	IFI-C	POTENCY VS MENTHOL (= IFI-C ÷ 1000)	EC_{50} = 80 MM ÷ POTENCY
Icilin	5,000 (capped)	measured directly	0.36 µM
WS-3	3,200	3.20×	25.0 µM
WS-23	2,290	2.29×	34.9 µM
(−)-Menthol	1,000	1.00× (anchor)	80.0 µM
Menthyl lactate	533	0.533×	150 µM
Linalool	~40	0.040×	2,000 µM
1,8-Cineole	23	0.023×	3,480 µM
(−)-Carvone	— (non-TRPM8)	—	–

Two of those rows deserve the arithmetic spelled out, because they mark the outer edges of the whole axis. Icilin is a synthetic superagonist whose half-maximal activation is measured directly at 0.36 µM — so loud that if we ran it through the formula without a ceiling it would score 1000 × 80 ÷ 0.36 ≈ 222,000, roughly forty-four times the per-axis normalization maximum of 5,000 that every scorecard clips to. Nothing in a leaf's life ever required a cool that loud, nothing makes it, and it is not cleared for food; we carry it only as the instrument that tells us how hard the receptor can physically be driven. At the other end, 1,8-cineole — eucalyptol, the airy respiratory cool of a eucalyptus leaf — sits at a score of 23, which the formula turns into an EC_{50} of 80 ÷ 0.023 ≈ 3,480 µM, nearly 3.5 mM. That is a weak agonist by any honest reading of the receptor. And yet eucalyptol *feels* cooling out of all proportion to that number, because it is small and volatile and carries high into the nose and throat, lighting cold-sensitive fibers across a whole field the tongue alone never reaches. It is the first honest limit of the index, stated plainly: the score measures loudness at the receptor, not coverage of the trigeminal field, and a molecule can win on reach what it loses on volume.

A worked unit conversion grounds the anchor in something a person could weigh. Menthol's molar mass is 156.27 g/mol, so an 80 µM solution carries 80×10^{-6} mol/L × 156.27 g/mol = 0.0125 g/L ≈ **12.5 ppm**. Half of the full cool a mouth can feel arrives at about twelve and a half parts per million of menthol — a vanishingly small amount, which is why a single mint leaf reads so loudly and why precision at these concentrations is the whole game. Run the same conversion for WS-3 (molar mass 211.34 g/mol) at its derived 25 µM and you get $25 \times 10^{-6} \times 211.34 \approx 5.3$ ppm: the synthetic reaches half-max at less than half the mass of menthol, which is exactly what "3.2× louder" means made concrete on a scale.

Finally, the shape of the dose–response itself, because it governs how forgiving the axis is. TRPM8 activation follows a sigmoid well described by the Hill relation, $\theta = [A]^n \div (EC_{50}^n + [A]^n)$, where θ is the fraction of full activation and n is the Hill coefficient — near 2 for menthol at this channel, consistent with a cooperative tetramer. With n ≈ 2 and the 80 µM anchor, the curve climbs like this: at 20 µM, θ ≈ 0.06; at 40 µM, θ = 0.20; at 80 µM, θ = 0.50 by definition; at 160 µM, θ = 0.80; at 320 µM, θ ≈ 0.94. Cool goes from barely there to nearly saturating across only a ninefold change in concentration — the

span from ten to ninety percent activation is $\sqrt{81} = 9$-fold when n is 2. That steepness is a gift to a designer and a trap for a grower: a little more menthol buys a lot more cool, which means a little *variance* in menthol costs a lot of consistency. Hold that thought; the whole economic argument of the chapter is built on it.

The monoterpenes we can farm

The plant-made coolants are the monoterpenes, every one of them a descendant of the same ten-carbon precursor, geranyl diphosphate, and every one in principle reachable by the kind of pathway engineering that builds menthol. Menthol leads the family, and its relatives shade the cool in different directions. **Menthone**, one oxidation state away from menthol on the biosynthetic line, is minty with more warmth than cool — useful when a formulation wants mint character without the full cold. **Eucalyptol**, the 1,8-cineole already met at the bottom of the ladder, trades receptor loudness for volatile reach: a weak TRPM8 agonist that cools the whole airway because it refuses to stay on the tongue. **Linalool**, the soft floral terpene alcohol of lavender, barely registers on the channel at a score near 40 and an EC_{50} around 2 mM; its cool is less a cold than a calm, read by the brain as much through scent as through the thermal channel, which is why lavender soothes rather than bites. And **carvone**, the molecule of spearmint, is not a TRPM8 agonist at all — a different monoterpene entirely — which is the real reason spearmint's cool is a gentler, rounder, less cold thing than peppermint's, carried by aroma and by trace menthol rather than by the receptor. We treat the two mints as genuinely separate design problems for exactly this reason: one is a cool you engineer at the channel, the other a cool you mostly compose in the nose.



Spearmint — carvone's rounder, warmer cool: a plant whose signature molecule never touches the cold receptor at all.

The catalog of farmable coolants runs wider than the headliners, and some minor entries are quietly the most interesting. **Cubebol**, a sesquiterpene alcohol from cubeb pepper, is a gentle, long, almost watery cool with no mint character at all — a natural answer, in part, to what the synthetic amides do, and a molecule we watch closely because it holds the long tail of duration that pure menthol never keeps on its own. The broader monoterpene alcohols and a scatter

of terpene ethers each bend the cool a little — airier, greener, rounder — and a serious palette keeps all of them in view rather than reaching for menthol by reflex.

Crucially, these are molecules that live in real plants we can already grow and have already begun to engineer. The index lets us lay those host plants out as a design surface rather than a herb garden:

COOLING PLANT	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	SIGNATURE COOLANT
Peppermint	2,360	C / M	Hard	(−)-menthol
Lavender	160	C	Hard	linalool
Orange	120	B / C	Hard	a cooling citrus
Eucalyptus	80	C	Hard	1,8-cineole
Spearmint	80	C	Hard	(−)-carvone
Camphor	40	C	Hard	camphor / cineole

Peppermint carries the program, with 2,360 constructs behind it — more than any cooling plant in the library — because it is the one host where every lever of the cool axis can be pulled at once: more menthol, cleaner menthol, longer menthol. The others are the frontier of the family, each a different native chemistry to be tuned toward the cool a product actually wants. And orange is the quiet surprise in the table: a plant we work on both the bitter and the cool axes, the first real foothold of a *cooling citrus* — cool arriving in a fruit that never had any.

The menthol line, gene by gene

To make menthol louder, cleaner, and more reliable, you have to know exactly how a mint builds it, because every edit lands on a specific enzyme. The pathway begins where all monoterpenes begin, with geranyl diphosphate, and runs through a committed five-gene cascade. Limonene synthase cyclizes GPP into (−)-limonene, the raw ring. A cytochrome-P450 hydroxylase, (−)-limonene-3-hydroxylase, hangs an oxygen on it to give trans-isopiperitenol. An isopiperitenol dehydrogenase oxidizes that to isopiperitenone. An isopiperitenone reductase and the isomerization that follows carry the ring down to pulegone. Pulegone reductase then delivers (−)-menthone, and a final menthone reductase reduces that to (−)-menthol. Skeleton, then five committed tailoring steps: that is the line, and the quality of a mint is set almost entirely at its last two reductase steps and at one branch that leaves it.

The branch is the villain of every mint harvest. At pulegone, instead of continuing down the reductase cascade, carbon can be pulled off to a different P450, menthofuran synthase, which makes (+)-menthofuran — the musty, off, slightly medicinal note that marks a mint grown under stress. Menthofuran rises with heat and drought and a hard season; it is the molecular signature of a plant that struggled. A grower's peppermint therefore drifts not only in how *much* menthol it holds but in how much menthofuran rides along to foul it, and the two move with the weather. Our designs are aimed precisely here: push flux down the reductase steps toward menthol, and starve the menthofuran-synthase branch so that pulegone has nowhere to go but the cool we want. Our model predicts a

peppermint whose menthol runs high and whose menthofuran runs dry regardless of the summer it had — a plant designed to make a formulator's single most reliable natural coolant instead of a seasonal gamble. That is the first of the three places a design platform earns its keep on this axis, and it is the least glamorous and the most valuable.

The coolants no leaf will grow

Now the humbling half of the catalog, the molecules we measure and respect and cannot farm. **Icilin** has already been met: the synthetic superagonist at 0.36 µM, forty-four times past the scorecard ceiling, calcium-dependent in a way that makes it fire the channel in bursts rather than a steady hum, and not food-approved. It is a pure instrument. The **Wilkinson Sword carboxamides** — WS-3, WS-5, WS-12, WS-23 — are a family of odorless, tasteless, long-lasting cools invented when a menthol shortage once looked possible, and now indispensable in their own right. They share no shape with menthol; they simply fit the S1–S4 pocket by a different geometry, and they fit it in a way that resists the PIP_2-depletion fade, which is why they last. **Menthyl lactate** and **menthone glyceryl acetal** sit honestly on the boundary — their carbon skeletons come from a plant, their ester or acetal caps from a reactor — and they trade loudness for the long, mild persistence an all-day product needs. Menthyl lactate's score of 533 works out to an EC_{50} near 150 µM, nearly twice menthol's: deliberately *quiet*, so that it can be dosed high and held long without ever reading as sharp.

The full catalog, with the compliance dimension that travels alongside the chemistry, reads:

COOLANT	SOURCE	IFI-C	EC_{50} (DERIVED)	FOOD STATUS	CHARACTER
Icilin	synthetic	5,000 (capped)	0.36 µM	not food-approved	research superagonist
WS-3	synthetic	3,200	25 µM	FEMA GRAS	odorless, long
WS-23	synthetic	2,290	35 µM	FEMA GRAS	odorless, clean, long
(−)-Menthol	plant / synthetic	1,000	80 µM	FEMA GRAS	the anchor
Menthyl lactate	semi-synthetic	533	150 µM	FEMA GRAS	very long, mild
1,8-Cineole	plant	23	3,480 µM	FEMA GRAS	respiratory, volatile
(−)-Carvone	plant	— (non-TRPM8)	—	FEMA GRAS	spearmint, fresh

We are plain about the line this table draws. Our plants can grow a louder, cleaner, longer menthol; a better eucalyptol; a designed carvone. They cannot grow icilin, and they cannot grow a Wilkinson Sword amide, and we would mislead you to imply otherwise. The honesty matters because the market is thick with "natural cooling" claims that quietly lean on semi-synthetic esters wearing a leaf's name, and a reader deserves to know which cool came out of a leaf and which came out of a reactor. The cool a leaf makes is wanted far past the flavor aisle — the sensate in an aftershave, a shampoo's tingle, a muscle rub, a cooling fabric finish, a sunscreen that feels

cold going on, all of it built on this same handful of molecules — and that breadth is precisely why the provenance has to be told straight. A premium that rides on the word *natural* is only honest if the molecule under it actually grew.

Consistency is the prize — variance, worked

Here the steepness of the dose–response curve comes back to collect. Synthetics took the oral-care shelf for one reason above all others, and it was never loudness: it was reproducibility. A reactor's WS-23 is the same on Tuesday as it was in March. A field mint is not — its menthol wanders with the harvest and its menthofuran climbs in a hard season — and a formulator building a product that must taste identical in every tin cannot design around a moving target. The platform's whole counter-argument is that an engineered plant can hold its output as tightly as a reactor does, and the number that proves it is the coefficient of variation, CV = σ ÷ μ, the batch-to-batch standard deviation as a fraction of the mean.

Put real figures through the curve. The platform's wild-type baseline variance runs near 42% CV; our engineered lines are designed to hold at or below 7%. Anchor both on the 80 μM half-max point and ask what a 95% batch window (mean ± 1.96σ) does to the cool a mouth actually feels, reading θ off the Hill curve at the window's edges:

LINE	CV	95% BATCH WINDOW	COOL DELIVERED (θ)
Engineered	≤ 7%	69–91 μM	0.43 – 0.56
Field / wild-type baseline	42%	14–146 μM	0.03 – 0.77

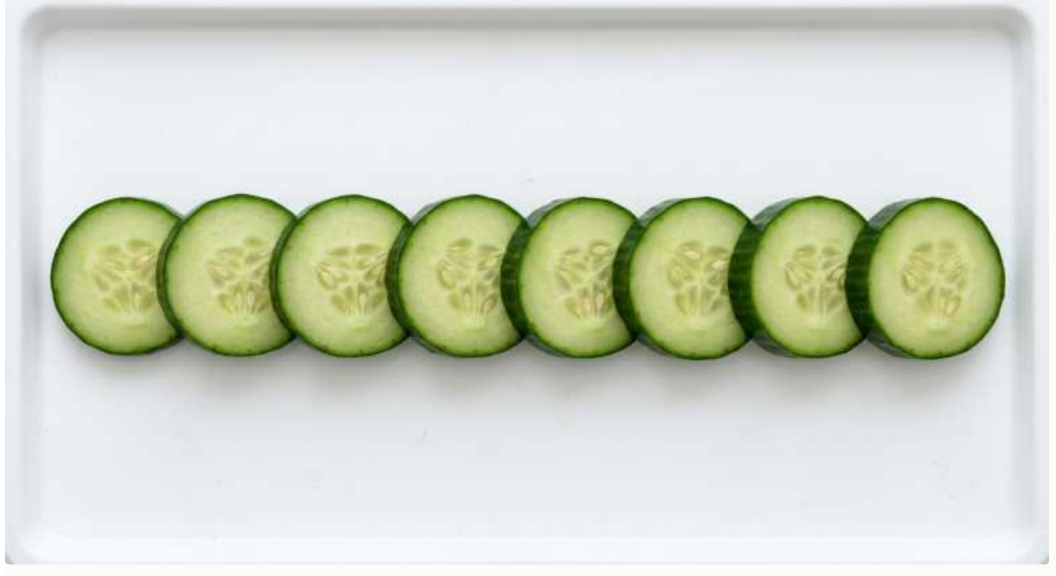
Read what that does to a product. The engineered line, batch after batch, is designed to deliver a cool that holds between 43% and 56% of full scale — a variation a trained panel can barely find, a product that tastes like itself. The field plant, across the same confidence window, swings from 3% cool — a batch where the mint is a rumor — to 77% cool, a batch that reads nearly harsh. Same seed, same formula, different summer. A ±42% wobble in concentration becomes a near-absent-to-near-overwhelming swing in sensation precisely because the axis is so steep; the thing that makes cool easy to design makes it brutal to grow unless the variance is killed at the source. Holding ≤7% CV is therefore not a quality-control footnote. It is how a plant takes back the one advantage the reactor always had, and it is the second place the platform earns its keep.



The controlled line against the open field — reproducibility is grown, not hoped for.

Where a design platform earns its place

So where does a platform add value across a catalog half of which it cannot farm? In three places, and each is real. First, on the molecules we *can* grow, we design them louder, cleaner, and truer than any field ever has — a peppermint whose menthol runs pure and whose menthofuran runs dry, a eucalyptus tuned for cineole, a lavender dialed to the exact soft cool a product wants. Second, we design the plant-made coolants into the *right plant* — cool where cool has never lived, a cooling citrus, a cooling cucumber, mint's chemistry transplanted into a fruit by the same pathway transfer that moves heat out of peppers.

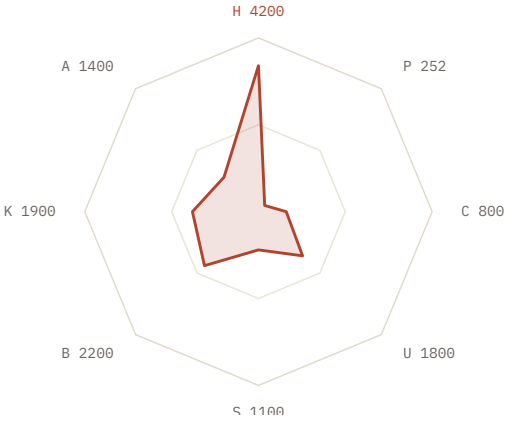


Cool arriving where it never grew — a cross-section of the plant we are teaching to carry it.

Third, and most quietly, we design the farmable coolants *consistent* enough to compete with the synthetics on the one axis the synthetics always won — the reproducibility just worked through, a mint designed to hold ≤7% CV standing where only a reactor could stand before.

It is worth seeing, finally, how the cool axis behaves inside a whole flavor rather than alone. The fullest-spectrum cultivar we have designed registers on all eight axes at once, and its cool reads like this among the rest:

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

The cool sits at 800 — below the menthol anchor of 1,000 — which is the honest lesson of a full-spectrum design: a cool woven into seven other sensations reads quieter than a cool standing by itself, because the mouth is dividing its attention across the whole field. Pure menthol is a single pure tone at 1,000; cool inside a complete flavor is one voice in a chord, and a designer composing for the whole palate budgets it accordingly.

The honest edges

A chapter that claims a method should name where the method frays, and this one has clear edges. The index measures receptor loudness, not trigeminal coverage, so a volatile weakling like eucalyptol will always score lower than it feels — the number is true and the nose knows more. The 80 µM anchor and the n ≈ 2 Hill slope are reference-assay values; a different cell line or a different temperature shifts the absolute EC_{50}, which is exactly why the index is defined as a ratio against menthol, so that the comparison between two coolants survives the drift even when the raw numbers move. The PIP_2 fade means a single EC_{50} cannot capture duration — two molecules with the same score can feel entirely different over a minute, which is why cubebol and the Wilkinson amides matter out of proportion to their loudness and why "how long" belongs beside "how loud" in any serious cool profile. And the hardest limit is the one we refuse to paper over: the synthetic coolants are genuinely good, genuinely safe within their GRAS uses, and genuinely beyond what any leaf will ever grow. We do not pretend to the whole catalog.

But the half that grows, we intend to design better than it has ever been grown, and to grow it where no one thought cool could live — cleaner, longer, steadier, and in plants that never carried it. That is a narrower claim than "every coolant," and it is a truer one, and in our family a true claim grown well has always been worth more than a loud one that cannot keep its word. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.



PART III OF IV

The Palette

peppermint · spearmint · lavender · eucalyptus · camphor · the unexpected cool · cool inside fire

Chapter 9 · Peppermint — *the 2,360-construct heart.*

If the cooling axis has a home organism, it is peppermint, and the number that says so is **2,360** — the count of engineered constructs in our peppermint library, the deepest single-organism cooling program we run and one of the largest libraries for any plant on the whole platform, behind only the headline crops. Peppermint earns that depth. It is the loudest natural cool there is, the species that makes (−)-menthol most cleanly, the plant a century of chemistry already mapped down to the last enzyme. When our family decided to take the thermal axis seriously, peppermint was not a choice so much as a recognition: this is where the cold already lives, and our work is to make the plant more fully itself.

Peppermint is a hybrid — *Mentha × piperita*, a sterile cross of watermint and spearmint that arose, probably by accident, in an English field in the seventeenth century and has been carried around the world as cuttings ever since. Its sterility is part of why it has always been a frustrating plant to improve by classical breeding. A sterile hybrid gives you no seed to select; it is propagated clonally, a single genetic individual dragged forward through centuries, improved mostly by geography and luck. The famous Mitcham type, the Pacific-Northwest fields that make much of the world's mint oil — these are not breeds in the ordinary sense but clones, lineages, the same plant replanted. And a clone has a clone's great weakness: it cannot breed its way out of a problem. When Verticillium wilt swept the mint fields, classical breeding had almost nothing to answer with, because there was no sexual population to select resistance from. The plant that fed the world's mint was genetically a single vulnerable individual, copied a billion times.



Peppermint under glass — *Mentha × piperita*, the loudest clean cold in the plant kingdom and the single deepest cooling program we run: 2,360 engineered constructs written into one sterile clone.

Precision editing does not care that the plant is sterile — we edit the clone directly, write the change into the lineage, and propagate the edited line onward, exactly the mode of improvement a clonal crop was always best suited to and never had. The plant that resisted a thousand years of selection yields to a guide RNA, and the 2,360 constructs are the measure of how much yielding there was to do.

But before any of that editing earns its keep, we owe the reader the mechanism: what the cold *is*, where on the tongue it is read, and how a sensation with no thermometer behind it becomes a number you can design against.

The channel that reports a cold that isn't there

Capsaicin, the molecule of heat, binds a channel called TRPV1 on the trigeminal nerve endings of the tongue and lowers its thermal threshold, so the body reports a fire that is not there. The cold has a mirror-image receptor and plays exactly the same trick in reverse. Menthol binds **TRPM8** — Transient Receptor Potential Melastatin 8, a non-selective, calcium-permeable cation channel in the same cold-sensing trigeminal endings — and the channel does not chill the tissue by a single degree. It cannot. There is no heat leaving the mouth. What menthol does is shift the channel's gating so that it opens at a temperature it would otherwise ignore, and the nerve, dutifully, reports a cold that never arrived. The tongue is not cooled; it is *informed* that it has been. This is the deep symmetry of the thermal axis, and it is worth sitting with: both ends of it are acts of persuasion at a protein, not events in the tissue. Heat that does not burn, cold that does not chill — two channels, gated by temperature, talked into firing by a molecule.

TRPM8 is a homotetramer: four identical subunits around a central pore. In the resting mouth it sits mostly closed, nudged open only when the temperature falls below roughly 26 °C, where the native cold begins. Its opening is voltage-dependent, and the clean way to say what menthol does is in those terms — menthol binds a pocket in the voltage-sensor-like domain of each subunit, around a residue the field numbers Y745 in the second transmembrane helix, and that binding shifts the channel's voltage-dependence of activation toward the potentials the resting neuron already holds. The practical consequence is a threshold moved: a channel that needed real cold to open now opens at mouth temperature, because the menthol has paid down most of the energy the cold was supposed to supply. Cooling the tissue and bathing it in menthol are, to TRPM8, nearly interchangeable inputs. The plant has learned to counterfeit winter.

Because the receptor is a tetramer with more than one place to bind, its response to menthol is **cooperative** — the dose–response curve is steeper than a simple one-site binding would give. We write the fraction of channels activated as a Hill function,

$$f([L]) = [L]^n / (EC_{50}^n + [L]^n)$$

where [L] is the free menthol concentration at the receptor, EC_{50} is the concentration that drives the population to half of its maximum response, and n is the Hill coefficient — for menthol at TRPM8, $n \approx 2$, the cooperativity you expect from a four-fold channel. At $[L] = EC_{50}$, the exponents cancel and $f = ½$ by definition; that is the whole meaning of the half-activation point. The number that matters for a *designer*, though, lives below that point, down in the low-dose regime where a breath mint or a mouthwash actually sits. There, $[L] \ll EC_{50}$, the EC_{50}^n term dominates the denominator, and the function collapses to

$$f \approx ([L] / EC_{50})^n$$

which carries a lovely and non-obvious reward. Halve a molecule's EC_{50} — make the cold twice as potent — and at a fixed low dose the receptor signal does not double; it rises by 2^n. With $n \approx 2$ that is a factor of **four**. Cooperativity turns a two-fold gain in potency into a four-fold gain in perceived cold at the doses real products use. This is why a cleanly-made, truly potent menthol is worth so much more than a slightly-stronger one: the tongue's own math amplifies the difference.

A worked case makes it concrete. Take a fixed amount of free menthol-equivalent reaching the receptor — call it 50 $\mu mol \cdot L^{-1}$, a plausible in-mouth figure for a dissolving lozenge. Run two oils through the Hill function with $n = 2$. An oil whose half-activation sits at 80 $\mu mol \cdot L^{-1}$ gives $f = 50^2 / (80^2 + 50^2) = 2{,}500 / 8{,}900 =$ **0.28**: twenty-eight percent of the channels firing. An oil whose half-activation sits at 30.8 $\mu mol \cdot L^{-1}$ — more potent, the cold made louder — gives $f = 2{,}500 / (30.8^2 + 50^2) = 2{,}500 / 3{,}449 =$ **0.73**. The same dose in the mouth, and the second oil has driven the receptor population from twenty-eight percent to seventy-three percent occupancy: two and a half times the signal, for not one extra milligram of menthol. The entire value of designing the molecule rather than simply adding more of it is in that gap.

Anchoring the cold — turning a sensation into a number

A sensation becomes a standard the moment it is anchored. The Iyengar Flavor Index does this by refusing to measure how *much* of a compound a food holds and measuring instead how *loudly* that compound speaks to its receptor — the inverse of its half-activation concentration, scaled to a readable number:

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

The reference is a **stated convention** — a fixed point chosen once and held, not a literature-derived value. For the Cool-Burn axis we anchor it to the half-activation of a (−)-menthol standard at TRPM8 and set that standard's score to **IFI-C = 1,000**. Everything colder than the reference scores above a thousand; everything quieter scores below. The formula is just a ratio of potencies wearing a friendlier coat, and it has one honest property worth stating plainly: because the EC_{50} of menthol depends on the assay — temperature, expression system, how the free concentration is estimated — IFI-C is a *within-platform ratio*, reproducible against our own reference, not a universal physical constant. It is a ruler we cut ourselves and never re-cut. That is enough to design against, and it is all we claim.

Our designed peppermint whole oil is predicted to score **IFI-C = 2,600**. Read backward through the formula, that single number tells you the oil's potency relative to the anchor:

$$EC_{50}(peppermint) = 1{,}000 \times EC_{50}(ref) \div 2{,}600 = 0.385 \times EC_{50}(ref)$$

The designed oil half-activates TRPM8 at thirty-eight and a half percent of the reference concentration — it is **2.6× louder** than the menthol standard, and 2.6× is the number to keep. To put it on a scale a formulator can weigh out, take the anchor's half-activation as 80 µmol·L^{-1} for the arithmetic and convert through menthol's molar mass, 156.27 g·mol^{-1}. The reference, 80 µmol·L^{-1}, is 80 × 10^{-6} mol·L^{-1} × 156.27 g·mol^{-1} = 0.0125 g·L^{-1} = **12.5 mg·L^{-1}**, or about 12.5 ppm in a dilute aqueous mouth. The designed oil, 2.6× louder, half-activates at 80 ÷ 2.6 = 30.8 µmol·L^{-1} = **4.81 mg·L^{-1} ≈ 4.8 ppm**. The whole axis laid out as one ladder:

SAMPLE	IFI-C	POTENCY VS REFERENCE	EC_{50} (MMOL·L^{-1})*	EC_{50} (MG·L^{-1} ≈ PPM)*
(−)-Menthol reference (anchor)	1,000	1.00×	80.0	12.5
Designed peppermint whole oil	2,600	2.60×	30.8	4.81
Per-axis scorecard ceiling	5,000	5.00×	16.0	2.50

*Anchor half-activation taken as 80 µmol·L^{-1} for the arithmetic; menthol molar mass 156.27 g·mol^{-1}. The ratios (1.00×, 2.60×, 5.00×) are exact; the absolute concentrations scale with whatever anchor the assay fixes.

The scorecard ceiling of 5,000 is the point: it is the normalization maximum the radar plots are drawn against, and it sits at 5.0× the reference, a half-activation of 16 µmol·L^{-1}, about 2.5 ppm. Peppermint at 2,600 reaches a little past halfway up that ceiling on a single axis — a very loud place for a plant to stand on its own, and, as the ladder shows, there is deliberately room left above it. We do not build a plant to the edge of the ruler; we build it to the place where the cold is unmistakable and the oil still behaves.

The five-gene road to menthol

None of that loudness exists until the plant builds the molecule, and peppermint builds it along a road that is one of the best-mapped in all of plant metabolism — a committed sequence of monoterpene chemistry that begins with a generic five-carbon building block and ends with the one stereoisomer of menthol the human receptor reads most cleanly as cold. The road is worth walking step by step, because every design lever we pull is a hand laid on one of these enzymes.



The leaf in cross-section. Menthol is not dissolved through the tissue; it is built and held in glandular trichomes — the blistered oil reservoirs on the leaf surface — and trichome density is itself one of the levers we design.

STEP	ENZYME	CONVERSION	DESIGN LEVER
1	Geranyl diphosphate synthase (GPPS)	C_5 units → geranyl diphosphate (GPP, C_{10})	raise flux into the pathway (loud line)
2	(−)-Limonene synthase	GPP → (−)-limonene	the gate into the monoterpene pool
3	Limonene-3-hydroxylase (a P450)	(−)-limonene → (−)-*trans*-isopiperitenol	first oxygen onto the ring
4	Isopiperitenol dehydrogenase	→ (−)-isopiperitenone	—
5	Isopiperitenone reductase	→ (+)-*cis*-isopulegone	—
6	Isopulegone isomerase	→ (+)-pulegone	**the branch point**
—	Menthofuran synthase (MFS)	(+)-pulegone → (+)-menthofuran	**silence** (clean-cool line)
7	(+)-Pulegone reductase (PR)	(+)-pulegone → (−)-menthone	raise, and starve residual pulegone
8	Menthone:menthol reductase (MMR)	(−)-menthone → (−)-menthol	tune the menthol-to-menthone balance

Read the road and the whole character of the plant is in it. Geranyl diphosphate synthase sets the C_{10} backbone and governs how much carbon ever enters the monoterpene pool at all — the throttle on total output. Limonene synthase is the committed gate, the step that says *this* GPP becomes a mint molecule and not something else. Then a run of oxidations and reductions walks the ring toward menthol, and here is the quiet marvel of it: menthol has three stereocenters, so there are eight possible stereoisomers — menthol, neomenthol, isomenthol, and their mirror images — and the human

cold receptor is tuned, almost fussily, to just one of them, (1R,2S,5R)-(−)-menthol. The reductase steps are what make the road stereospecific. Pulegone reductase and menthone:menthol reductase do not simply add hydrogen; they set the geometry, so the plant does not make *a* menthol, it makes *the* menthol the receptor was waiting for. A century of chemists admired this; our contribution is to have found, in the 2,360 constructs, exactly which hands on exactly which of these enzymes make the road run louder, cleaner, and truer to that single isomer — all while leaving a leaf that is still, unmistakably, a plant you could grow and eat.

The off-ramp, and why the still finds it

Step six is the one that keeps mint honest or makes it muddy. (+)-Pulegone is the fork in the road. Down one path, pulegone reductase carries it forward to menthone and then to menthol — the cold we want. Down the other, **menthofuran synthase** pulls pulegone off into (+)-menthofuran, the musty, slightly stale note that has haunted mint oil forever, and that off-ramp is wide open in the wild plant, especially when the plant is stressed or the leaf is old. Worse, pulegone that never gets converted sits in the oil as a loaded reservoir, waiting. This is where growing the plant and making the oil stop being two separate acts.

The oil comes off peppermint by steam distillation — the mown herb, wilted in the field, run through a still that drives the volatile oil out with steam and condenses it to a thin layer floating on the distillate water, to be separated and, for the premium grades, partly dementholized by crystallizing the menthol out in the cold. It is an old, efficient, slightly brutal process, and it sets a constraint a designer has to respect: whatever the trichomes hold is what the still can take, and nothing the plant stores badly or converts under heat survives the pass intact. A menthol that is cleanly made and stably stored distills clean. An oil riddled with unconverted pulegone and primed to throw menthofuran under the still's heat distills muddy — because distillation itself can push that last unwanted conversion, the heat of the process completing the off-ramp the plant left open. The biggest practical difference between a cheap mint oil and a fine one has always lived in exactly that reservoir of unconverted pulegone the heat is waiting to find.

So our **clean-cool line** earns its keep twice — once in the living leaf, where menthofuran synthase is silenced and the off-ramp is deleted, and again in the still, where there is no reservoir of pulegone left for the heat to turn. There is a second reason to shut this valve that matters to a family that cares how food meets the body: pulegone and menthofuran are not merely off-notes, they are the two components of mint oil that regulators limit, for real toxicological reasons at high intake. Designing them toward zero is, at once, a cleaner flavor and a cleaner oil to put in a lozenge a child will suck on all afternoon. We did not set out to make a *compliant* mint. We set out to make an honest one, and honesty, in this plant, happens to mean the same edits.

Five ways to build a colder mint

The 2,360 constructs sort into a handful of design families, and the sorting is the clearest way to see the program — each family is a different hand laid on the road above.

LINE	PATHWAY MOVE	WHAT YOU TASTE
Clean-cool	menthofuran synthase silenced; residual pulegone drawn down through pulegone reductase	pure cold, the musty note gone; clears regulatory limits
Loud	GPPS and pulegone reductase up — more flux, more of it reaching menthol	more total menthol; a faster, fuller cold
Long	trichome density up, menthol-ester balance tuned	built-in slow-release coolant; a cool that lasts
Composed	the whole-oil set-point specified — menthol, menthone, menthyl acetate	mint tuned like an instrument, menthofuran at zero
Hardy	pathway-stability edits; the off-ramp stays shut under heat and drought	the clean cold held through a hard season

The ladder runs top to bottom, each line standing on the one before it. At the base is the clean-cool line already described. Above it sits the **loud line**, where a genome-scale flux-balance model earns its place: before we touch an enzyme, the model confirms that the step we mean to push is the true rate limiter and not a bottleneck downstream that would simply back up the pool. Raise GPPS without checking, and you flood the front of the road with GPP that the later reductases cannot clear, and the surplus leaks right back out as pulegone and menthofuran — a louder mint that is also a muddier one. Flux balance is what keeps *loud* from undoing *clean*. Above the loud line sits the **long line**, where trichome density is raised and the menthol-to-ester balance tuned, because menthyl acetate is a slow-release coolant — it hydrolyzes to menthol gradually in the mouth, so an oil carrying more of its own ester reads as a cold that does not quit. Then the **composed line**, where we no longer accept the oil peppermint happens to make and instead specify it: this much menthol for the front, this much menthone for warmth, this little menthyl acetate for roundness, menthofuran at zero. And the quiet, important **hardy line**, which changes nothing about the oil at rest but keeps it from degrading when the plant is stressed — so a field in a brutal summer still makes the clean cold it made in a mild one. For a crop that is a single clone facing a warming climate, hardiness is not a luxury; it is survival with the flavor kept.



A single harvested stem, readied for analysis. Everything the trichomes hold is what the still will take; what the plant stores badly, the oil never shows — so the composition is designed in the living leaf, long before the steam.

The composed line is where the index pays for itself, because *specified* means a number held constant batch after batch, and holding a number constant is a problem of variance, not of flavor. Field mint is notoriously variable; the same clone in two seasons can swing enormously in what fraction of its oil is menthol. Published pharmacopeial ranges for steam-distilled peppermint oil tell the story: menthol anywhere from about 30 to 55 percent, menthone 14 to 32, menthyl acetate 3 to 10, and the two off-ramp components — menthofuran up to 9 percent, pulegone up to 4 — present at whatever the season dealt.

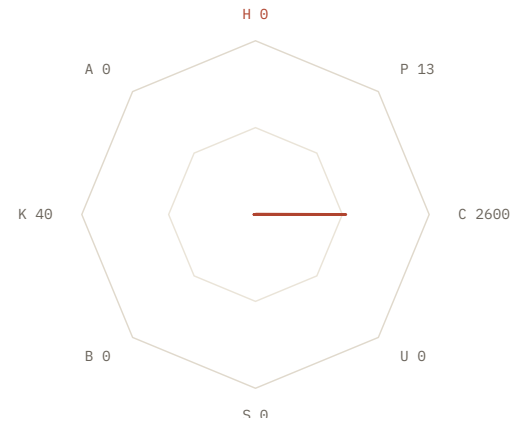
COMPONENT	TYPICAL FIELD OIL (PUBLISHED RANGES)	DESIGNED CLEAN-COOL TARGET	ROLE IN THE CUP
(−)-Menthol	~30–55%	front-loaded, raised	the cold itself
(−)-Menthone	~14–32%	set for warmth	the warmth under the cold
(−)-Menthyl acetate	~3–10%	tuned for roundness	sweet, slow-release coolant
Menthofuran	~1–9%	→ 0	the musty off-note (removed)
(+)-Pulegone	up to ~4%	→ ~0	the loaded off-ramp (starved)
1,8-Cineole	~3.5–14%	trace	eucalyptal lift
Limonene	~1–5%	trace	citrus top

Put a number on the variance and the value of the edit is undeniable. Variance is carried as the coefficient of variation, CV = standard deviation ÷ mean, and the standard deviation of a batch is simply CV × mean. A wild or conventionally-farmed mint oil runs a batch-to-batch CV near **42%**. For an oil nominally 45 percent menthol, that is a one-sigma band of 0.42 × 45 = ±18.9 percentage points — menthol landing anywhere from roughly 26 to 64 percent. The *same recipe*, built on that oil, lands anywhere from faintly cool to numbing, season to season, with the formulator unable to say in advance which. Our engineered lines are designed to hold a CV of **≤ 7%**. On the same 45 percent oil that is a one-sigma band of 0.07 × 45 = ±3.15 points — menthol held between about 42 and 48 percent. The composed line is not merely cleaner-tasting; it is *predictable*, and predictability is the thing a flavor house has wanted from mint for two hundred years and never had. A number you can specify is a number you can keep.

Reading the scorecard

Gather all of it onto the eight-axis card and the designed peppermint reads like a single, deliberate chord struck once:

IFI PROFILE

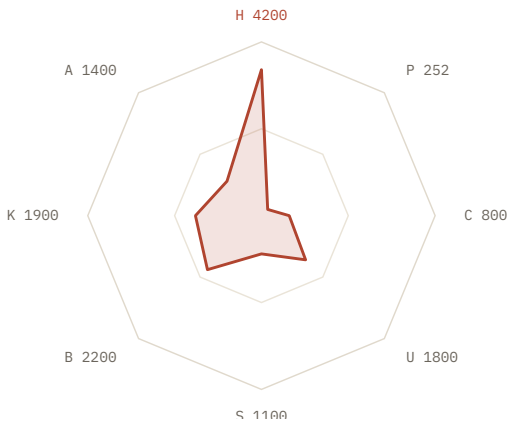


IFI-H	0
IFI-P	13
IFI-C	2,600
IFI-U	0
IFI-S	0
IFI-B	0
IFI-K	40
IFI-A	0

The Cool-Burn axis towers at **2,600** — past halfway up the 5,000 ceiling on one axis, a clean cold loud enough to carry a product on its own. The small Pungency reading of **13** is the faint, intentional TRPA1 edge that keeps the cool from feeling flat: the barely-there prickle that makes a mint feel alive rather than merely cold. Push it higher and you get a cool-burn lozenge; drop it to zero and you get a beverage mint so smooth it almost disappears. The trace of Kokumi at 40 is the slight mouth-coating body the designed oil carries — the menthyl-ester roundness reading through — a weight that keeps the cold from feeling thin. Everything else is zero, and the zeros are the point. This is not a flavor trying to be many things. It is one axis, built as loud and as clean as a plant can build it, which is exactly what a confectioner, a beverage maker, or an oral-care formulator wants to start from: a pure cold they can place anywhere.

It helps to see what the same axis looks like when it is one voice among eight instead of a soloist. Our fullest-spectrum flagship, Phoenix (SS-0096), designed to register on every axis at once, carries its cold as a supporting line:

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

There, Cool-Burn sits at **800** — present, deliberate, a cooling counter-melody woven under a towering heat of 4,200 — but a third of peppermint's 2,600 and never meant to lead. The contrast is the whole argument for a single-axis plant. In a flavor built to do everything, the cold is a seasoning; in peppermint, the cold *is* the composition. Both are designed with the same ruler and the same reverence; they simply answer different questions. Peppermint answers the oldest one the thermal axis knows — *what does cold taste like?* — and answers it louder and cleaner than the field ever could.

What the number does not promise

A standard earns trust by saying what it cannot do, so here are the honest edges of this chapter, each one real.

The cold is a sensation, not a thermal event. Nothing about menthol lowers the temperature of the mouth; TRPM8 reports a chill that the tissue never experienced. IFI-C measures how persuasively a molecule makes that false report — it does not, and cannot, mean that a food is physically cooler. A mint feels cold; a thermometer under the tongue disagrees. We think the honesty of that is part of the wonder, but it is a limit and we name it as one.

Menthol is not perfectly clean at the receptor. At high concentration it engages TRPV1 a little as well — the heat channel — and it can both sensitize and then desensitize the cold channel over a long exposure, which is why a very strong menthol can tip from cool to stinging, and why the tongue goes quiet after too much of it. The intentional 13 on the Pungency axis is partly an acknowledgment of that edge rather than a pure design flourish: cold this loud carries a faint bite whether you want it or not, and we would rather place it on purpose than pretend it away.

The axis belongs to the molecule, not the plant's name. Spearmint is a mint by every common definition, yet it makes (−)-carvone, not menthol, and carvone is a weak TRPM8 agonist — so a spearmint reads far down the cold axis, a fraction of peppermint's number, no matter how minty it smells. The index scores the conversation at the receptor, not the label on the jar, and that is exactly why it is useful: it refuses to be fooled by a familiar aroma into reporting a cold that is not chemically there.

IFI-C is receptor loudness, not delivered cold. Menthol is volatile and sublimes; it binds to fat and is released by sugar and saliva; how much free menthol actually reaches TRPM8 depends on the matrix it travels in. The index fixes the potency of the molecule; the food determines how much of that potency survives to the receptor. A loud oil in a fatty matrix can read quieter than a modest oil in water. The number is a true measure of the molecule and an honest *starting point* for a formulation, never a promise about the finished product.

And the plant itself keeps its clonal nature. Editing gives the sterile hybrid the improvement route breeding never could, but a single genetic individual across the world's fields is still a monoculture, and the hardy line narrows that exposure without erasing it. We hold two truths at once: that we can now write resistance and stability into a

lineage that could never select for them, and that no edit makes a billion identical plants anything other than identical. The 2,360 constructs are a deep answer to an old vulnerability, not a cancellation of it.

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

Peppermint is the heart of this book because it is the plant that gave the thermal axis both its anchor and its ceiling — the memory every one of us carries of what cold tastes like, now grown truer than the memory. We did not invent that cold. It was already in the leaf, built along a road a century of chemists had patiently drawn, stored in trichomes you can crush between two fingers on a summer afternoon. All our family did was read the road closely enough to make the plant more fully the thing it had always been trying to be: a clean, loud, honest cold, grown to meet the body well.

Chapter 10 · Spearmint & the Carvone Mints — *a different cool entirely.*

The most useful thing we can tell you about spearmint is that it is not a quieter peppermint — it is a different cool, built from a different molecule, read by the receptor in a different way. Peppermint's cold is menthol speaking loudly to the cold channel. Spearmint's cool is **carvone**, a monoterpene that is minty and fresh and sweet but barely leans on that channel at all. So when people say spearmint is "milder," they are half right and wholly imprecise: it is not a weaker version of the same signal, it is a softer, rounder, warmer sensation that the brain files under *mint* by association more than by any genuine cold. Understanding that difference is the difference between formulating spearmint well and formulating it as disappointing peppermint.

We came to spearmint the way we come to every plant at our table — fresh leaves torn by hand first, the pipette second — and what we found is a plant whose whole virtue is restraint, and whose whole chemistry can be written, honestly, on a single page. This chapter is that page: the molecule, the receptor, the number, the enzyme that forks the fate of the leaf, and the discipline of holding one mirror-image compound down to nothing so the other can sing.



A basil leaf at full green — a cousin of spearmint in the mint family, the Lamiaceae, grown and photographed the way we grow everything: in clean light, reverent of the living thing before we ever ask it a question.

The molecule wears two hands

Carvone, like menthol, comes in two hands, and here the handedness is almost comic in its clarity. **(−)-carvone** — R-carvone — smells and tastes of spearmint. Its mirror image, **(+)-carvone** — S-carvone — smells and tastes of caraway: rye bread, not chewing gum. The same atoms, the same bonds, the same mass of 150.22 grams per mole, flipped left-to-right, swap one of the most recognizable flavors in the world for another entirely. We keep both

enantiomers on the bench just to hand to visitors who doubt that shape is everything. Smell one, then the other, and the abstract idea that a receptor reads three-dimensional form becomes a thing you have experienced with your own nose.

The vividness of that swap hides a precise and slightly surprising fact, and getting it right is the whole key to designing spearmint: **the nose discriminates the two hands ferociously, and the cold channel barely discriminates them at all.** Smell lives in a bank of hundreds of olfactory receptors, each a deeply chiral pocket, and the spearmint receptor and the caraway receptor are genuinely different proteins reading genuinely different fits — which is why (−) and (+) carvone smell like two different plants. The thermal receptor is a different kind of reader. It is weakly engaged by either hand of carvone, and it does not care much which one it gets. So the drama of carvone's chirality is an *olfactory* drama, played out above the tongue, not a thermal one played out on it. A spearmint program is therefore two programs braided together: make the cool, and — far more delicately — guard the smell.

Guarding the smell is an arithmetic problem, and the arithmetic is **enantiomeric excess**. If a batch of our spearmint oil is 99.7 percent (−)-carvone and 0.3 percent its caraway mirror, the excess is

```
ee = (%major − %minor) ÷ (%major + %minor) = (99.7 − 0.3) ÷ (99.7 + 0.3) = 99.4%
```

Tighten to 99.5 / 0.5 and ee falls to 99.0 percent; open to 99.9 / 0.1 and it climbs to 99.8. These sound like rounding errors. They are not, because the caraway enantiomer announces itself at a vanishingly low concentration — a few parts in a thousand are enough for a trained nose to flag rye bread creeping into mint. Our target for a clean spearmint is an ee at or above 99 percent, (−)-carvone over (+), held there not because the cold would suffer otherwise but because the *identity* would. Spearmint's whole self is one hand of one molecule, abundant and clean, with its twin kept nearly to zero.

What carvone does at the cold channel — and what it does not

To read the cool honestly, start with what menthol does, because carvone is best understood as the thing that almost does it. The cold receptor, **TRPM8**, is a four-part, calcium-permeable channel studded along the cool-sensing nerve endings of the mouth and skin. It is the body's real thermometer for cool, firing when something genuinely cold — below about 26 °C — touches the tongue. It is voltage-sensitive, and cold opens it by sliding the voltage at which it gates down into the range your resting nerves already occupy. Menthol performs the same slide chemically: it settles into a pocket in the channel's voltage-sensing domain, anchored near a tyrosine the field labels Tyr745, and binding there shifts the gating curve the same direction cold does. The channel opens at temperatures the mouth would otherwise read as neutral, and the brain is told *cold* while the thermometer in the glass reads room temperature. It is a thermal illusion, the exact cool-side mirror of the warm illusion a chili tells.

Carvone slips toward that same pocket, but it does two things worse than menthol, and the two together are the reason spearmint is gentle. It is a **weak partial agonist**: weaker in *potency* (it needs more molecules present to half-open the channel) and weaker in *efficacy* (even saturated, it opens fewer channels than menthol can). Efficacy is the one most people miss, and it is the honest heart of spearmint. A receptor's response follows a Hill curve,

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

where [A] is how much compound reaches the channel, n is the steepness, EC_{50} is the concentration that drives the response to half of *that compound's own* ceiling, and **E_{max} is the ceiling** — the most the channel will ever open for this particular molecule. For menthol, a full agonist, take E_{max} = 1.0 (the whole available current) and a steepness near n = 2. For carvone, take a representative E_{max} near **0.4** — forty percent of menthol's maximum. Now the numbers tell the story. At a dose equal to its own EC_{50}, menthol reaches r = 1.0 × ½ = **0.50** of the full current; carvone at *its* own EC_{50} reaches r = 0.4 × ½ = **0.20**, a fifth of the channel's capacity. And here is the point no concentration can undo: flood the tongue with carvone until the curve saturates, and the response climbs only to its ceiling of **0.40** — still below the 0.50 that a merely half-maximal dose of menthol delivers. *No amount of carvone drives the cold channel as hard as a modest dose of menthol does.* The ceiling is lower by the molecule's own geometry. That is what we mean, exactly, when we say carvone barely touches the cold: not that it is silent, but that it can only ever whisper, and the whisper is the whole appeal.

Reading the cool as a number

Loudness is what the Iyengar Flavor Index was built to score, and the cooling axis — **IFI-C** — reads straight off that half-activation concentration. The definition is one line:

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

Fix a reference cooling stimulus, measure its half-activation of TRPM8, and anchor the scale so a candidate that matches the reference exactly scores 1,000. EC_{50} sits in the denominator, so the inversion is the whole trick: a *louder*, more potent cool activates the channel at a *lower* concentration and earns a *higher* number. A candidate twice as potent lands at half the reference concentration and scores 2,000; one five times as potent scores 5,000, which is the per-axis ceiling we normalize scorecards against.

Run our cooling plants through it and the family sorts itself honestly. Engineered peppermint reads **2,600** — its menthol activates the channel at 1,000 ÷ 2,600 = 0.385 times the reference concentration, 2.6 times as loud as the anchor. Spearmint reads **900**: its carvone needs 1,000 ÷ 900 = 1.11 times the reference concentration, so it speaks at 0.90 times the anchor's volume — and, the number that matters most for this plant, 900 ÷ 2,600 = **0.35**, barely over a third as loud as peppermint. That ratio is the chapter in a single figure.

Spearmint is not a dimmer peppermint on the same dial; it is a different, lower voice, and we will not inflate the number to flatter the plant.

SOURCE (STANDARDIZED PREP)	COOL COMPOUND	IFI-C	EC_{50}(CAND) ÷ EC_{50}(REF)	LOUDNESS VS. REFERENCE	EFFECTIVE EC_{50} (MM, REF ≈ 40)	MASS BASIS (MG/L)
Engineered peppermint	(−)-menthol	2,600	0.385	2.6× — the menthol flagship	15.4	2.4
Reference anchor	(−)-menthol	1,000	1.00	equal to reference	40.0	6.25
Spearmint (engineered)	(−)-carvone	900	1.111	0.90× — about ⅓ of peppermint	44.4	6.7
Phoenix · SS-0096	composite	800	1.25	0.80× — the cool inside a pepper	50.0	–

The index is a dimensionless ratio, which is why it travels between laboratories unchanged; the raw EC_{50} beneath it does not travel so cleanly, drifting with the cell line and the temperature, which is exactly why we publish the ratio. But the conversion to a kitchen quantity is worth doing once, because the receptor speaks in molarity and a formulator speaks in milligrams. Take the reference at a representative 40 µM of menthol ($C_{10}H_{20}O$, 156.27 g/mol): 40×10^{-6} mol/L × 156.27 g/mol = **6.25 mg/L** — six and a quarter milligrams in a liter is half-activation. Spearmint's effective 44.4 µM of carvone (150.22 g/mol) works out to $44.4 \times 10^{-6} \times 150.22$ = **6.7 mg/L**. The two sit almost side by side on the page, and that near-coincidence is the index's one honest blind spot, which we would rather state than hide: IFI-C scores *potency*, the position of the EC_{50}, and says nothing about *efficacy*, the E_{max} ceiling. Spearmint's 900 captures that carvone's midpoint sits only a little above menthol's — but because carvone's ceiling is far lower, spearmint *feels* gentler than even the 0.35 ratio implies. The lived mildness is partly below the waterline of any potency-only number. A good designer carries both figures in mind: the score for the ladder, the ceiling for the mouth.



The research greenhouse at golden hour, a pepper hanging in the backlight. Every cultivar we score — the loud ones and the quiet ones alike — begins here, in clean light and controlled air, so the number we publish is the number the plant gives back every harvest.

One enzyme's choice — the fork at carbon six

The two mints part ways early, and the divergence is a single enzyme's decision about a single carbon. Both begin identically. In the leaf's glandular trichomes the universal ten-carbon building

block, geranyl diphosphate, is cyclized by limonene synthase into **(–)-limonene** — the smell of a citrus peel, the shared ancestor of both colds. Then the fork. Peppermint's trichome hands limonene to a cytochrome P450 that hydroxylates it at **carbon 3** — limonene-3-hydroxylase — committing the molecule to the long road that ends in menthol. Spearmint's trichome hands the identical limonene to a sibling P450 that hydroxylates at **carbon 6** instead — limonene-6-hydroxylase — producing *trans*-carveol, which a short-chain dehydrogenase lifts to (–)-carvone. And there the line essentially stops: carvone is the destination, not a way-station to something colder.

What makes this one of botany's most elegant stories is how *small* the switch is. The two hydroxylases are close cousins, and the choice between hydroxylating carbon 3 and carbon 6 comes down to a handful of residues lining the active-site pocket — a few amino acids that tilt the limonene a few degrees and present a different carbon to the activated oxygen. A nearly identical enzyme, a nearly identical substrate, and the whole sensory fate of the plant swings from menthol to carvone on where one oxygen lands. For a design platform this is the most encouraging fact imaginable: the cool of a mint is not written in its species but in which carbon its P450 points an oxygen at, and enzymes and their targets are precisely what we can read and rewrite.

STEP	PEPPERMINT — THE MENTHOL ROAD	SPEARMINT — THE CARVONE ROAD
Shared precursor	(–)-limonene (from GPP, via limonene synthase)	(–)-limonene (identical)
First committed P450	limonene-**3**-hydroxylase (hydroxylates C3)	limonene-**6**-hydroxylase (hydroxylates C6)
First product	*trans*-isopiperitenol	*trans*-carveol
Oxidation step	isopiperitenol dehydrogenase → isopiperitenone	carveol dehydrogenase → **(–)-carvone**
Terminal product	(–)-menthol (after several more steps)	(–)-carvone (the endpoint)
Pathway length	long — multiple committed enzymes	short — three steps to the destination
Platform constructs	2,360	80
Chief off-note to delete	menthofuran (a furan off-ramp)	the (+)-carvone mirror + dihydrocarvone

That short road is why our spearmint library is **80 constructs** against peppermint's **2,360** — not because spearmint matters less, but because its chemistry is briefer and simpler to steer once the carvone branch is understood, and because the market pulls hardest on the loud clean cold peppermint provides. Eighty is not a small number for a plant whose whole pathway is three steps; it is a focused one. The constructs we do build concentrate on exactly three things: raising carvone abundance in the gland so the preparation's effective EC_{50} falls and the cool reads a clean 900; holding the caraway enantiomer down to that sub-one-percent floor; and protecting the sweet-fresh roundness that is spearmint's

second signature. A longer pathway would give us more enzymes to tune and more places to leak; carvone's brevity is a gift, and we treat it as one.



A leaf of the mint family in cross-section, its surface raised with the peltate glandular trichomes — the microscopic blisters where the whole monoterpene oil, carvone and all, is synthesized and pooled beneath a stretched cuticle until a mouth or a finger breaks it open.

Holding the mirror to zero

The elegance of the short road comes with a specific hazard, and naming it precisely is the difference between a spearmint we are proud of and one we quietly compost. The hazard is **not** loss of cool. Because carvone is already a weak partial agonist, a few tenths of a percent of the caraway enantiomer sneaking in barely moves IFI-C at all — both hands of carvone are equally feeble at the cold channel, so a contaminated batch and a clean one score within a hair of each other on the cooling axis. The hazard is loss of *identity*, and it is invisible to the thermal number entirely. This is the most important edge case in the whole program, and it is worth stating as a rule: **(+)-carvone contamination is an olfactory defect that the cooling score cannot see.** You cannot QC spearmint's smell with its cool.

So we run two independent specs, and the aroma spec is the strict one. Enantiomeric excess by chiral separation must sit at or above 99 percent (–) over (+); dihydrocarvone and residual limonene — the other small off-notes of the carvone road — are held below thresholds set by nose, not by channel. And the whole batch is pinned for reproducibility by the platform standard, a coefficient of variation at or under **7 percent**. The arithmetic of that discipline is quick. CV = σ ÷ μ, so at a mean cool of 900 a 7 percent CV is a standard deviation of σ = 0.07 × 900 = **63 points**; two sigma, a 95 percent batch window, is roughly 900 ± 126, or 774 to 1,026. Because IFI-C is proportional to carvone abundance and errors in the reference and the candidate add in quadrature — CV(IFI) ≈ √(CV(ref)² + CV(candidate)²) — a tight reference leaves the published score good to about its own seven percent. A wild stand of *Mentha*, by contrast, swings its carvone content by a third or more across a single season as daylength and harvest timing wander; at a 30 percent CV the same 900 would smear to 900 ± 270 before you even opened the aroma question, which is to say it would

not be a spec at all but a rumor. Controlled environment plus engineered abundance is what turns the rumor into a number, and the number into something a formulator can build on twice.

SPEARMINT PROGRAM	VALUE
Host	Spearmint — a carvone mint
Platform constructs	80
Primary axis	IFI-C (TRPM8) = 900
Secondary axis	IFI-S (TAS1R2 / TAS1R3) = 120
Target enantiomeric excess	≥ 99% (−)-carvone over (+)
Batch CV	≤ 7%
Engineering difficulty	Hard

The map is two chemistries sharing a leaf

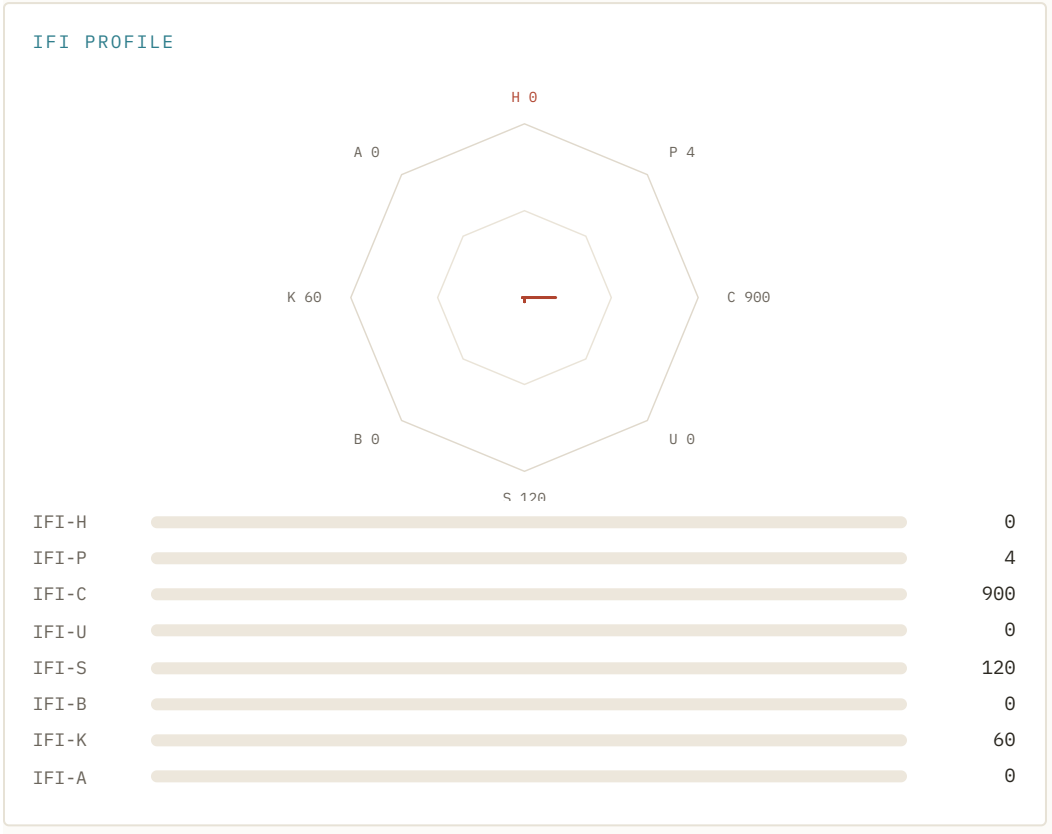
Spearmint has cousins that matter more to commerce than to the table, and the family map is worth drawing, because it is really a map of two chemistries that happen to share a leaf shape. The genus *Mentha* is a tangle of species and natural hybrids — watermint, horsemint, apple mint, pennyroyal — but the one that quietly runs the world is **cornmint**, *Mentha arvensis*, grown at vast scale across India and China, the species that supplies the bulk of the planet's natural menthol by distilling its oil and freezing the crystal out of it. Cornmint is a menthol plant, not a carvone plant — chemically far closer to peppermint than to spearmint, a creature of the carbon-3 road — and it carries, untamed, a heavy load of the harsh furan off-note and the rough edges our clean-cool work exists to delete.

So forget the leaf shape and group the plants by their chemistry, and the family falls cleanly in two. The **menthol mints** — peppermint, cornmint, watermint — took the carbon-3 fork, speak loudly to the cold channel, and win on potent, ringing cold once their furan off-ramp is closed. The **carvone mints** — spearmint and its kin — took the carbon-6 fork, barely lean on the cold channel, and win instead on sweetness and freshness. A cooling program that understands that split reaches for the right plant for the right job rather than treating *mint* as one thing, which it has never been: an engineered peppermint or a cleaned-up cornmint when the task is loud clean cold at scale, a spearmint when the task is a gentle, sweet-edged fresh. The two are not a strong version and a weak version of a single flavor. They are two destinations reached from one citrus crossroads, and the enzyme at the crossroads decides which.

Spearmint's table, and the discipline of not improving it

Spearmint's domain is wider in the kitchen than the oral-care shelf suggests, and it is a domain peppermint would ruin. Across the Mediterranean, the Middle East, and South Asia — the cooking our family grew up inside — spearmint is *the* fresh mint: torn raw into tabbouleh and into salads, pounded green into chutney, steeped into the sweet mint tea of North Africa, folded cool into yogurt against a hot dal or curry, laid against the brightness of lemon and cucumber. Its gentle, rounded, sweet-edged cool is exactly suited to raw and fresh uses, where peppermint's sharp menthol cold would flatten everything around it and read, wrongly, as toothpaste on the plate. This is a vegetarian-first flavor by birthright — it does its best work

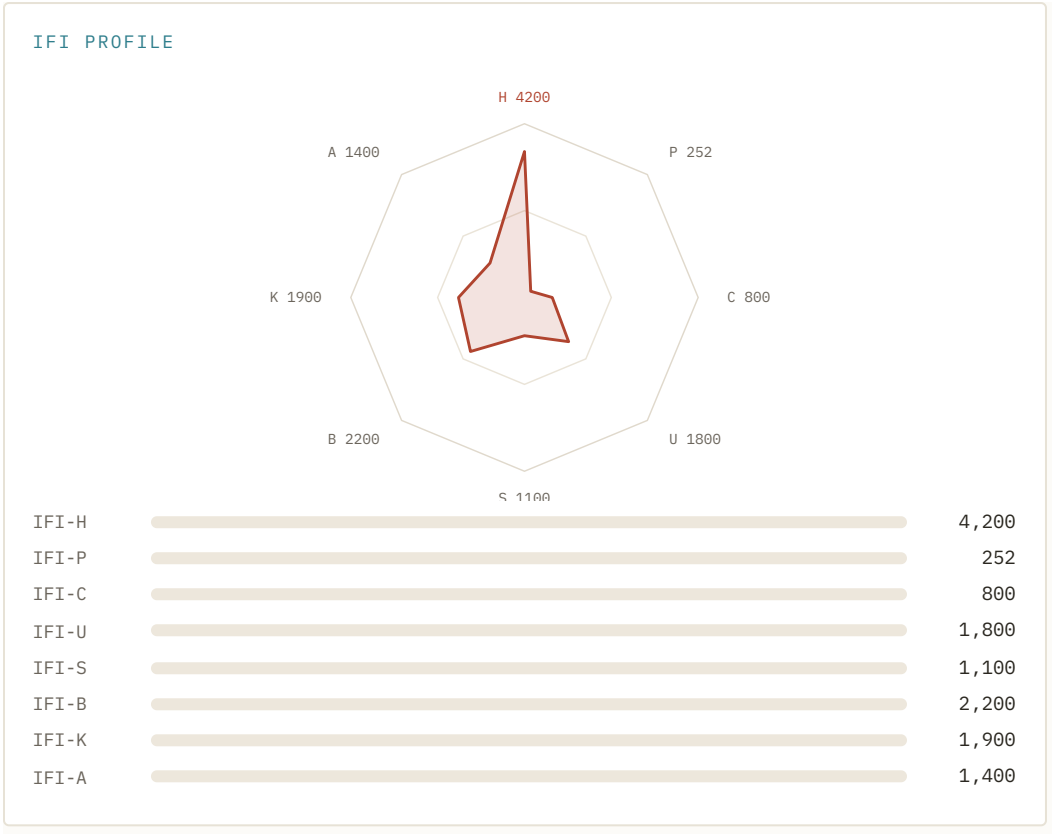
among grains and pulses and vegetables and yogurt, lifting and cooling and freshening rather than dominating — and it is precisely the plant whose value would be destroyed by making it colder.



Read the scorecard honestly and it tells the whole temperament. Cool-Burn reads **900**, real and present but a third of peppermint's 2,600, because carvone simply does not drive the channel the way menthol does. A faint **Pungency of 4** gives spearmint its barely-there green bite, the clean edge that keeps it from going flat. And then the two readings peppermint's card does not carry: a **Kokumi of 60**, a whisper of roundness and continuity, and above all a **Sweetness of 120**, the genuine sweet-fresh lift that is spearmint's second signature and the reason it owns the gum and the toothpaste aimed at people who find peppermint too sharp, too cold, too medicinal. Spearmint is the cool for someone who does not actually want to be cold — who wants fresh, clean, sweet-edged, gentle — and that is a very large someone. Designing for them means resisting the temptation to turn up the wrong axis. The best spearmint is not a colder spearmint; it is a cleaner, rounder, truer carvone, with the caraway mirror held near zero and the sweetness allowed to carry. The plant's virtue is its restraint, which makes it a quiet pleasure to design and an easy one to spoil by overreaching.

A cool that reads below a superhot's

One comparison closes the case, and it is the kind of thing the Index was built to make visible. Hold spearmint's gentle 900 against our most extreme cultivar — **Phoenix**, the one plant we have designed to register on all eight axes at once, a full-spectrum superhot whose headline is a predicted heat of 4,200.



Its cooling coordinate is **800** — below spearmint's 900. The loudest plant in the catalog carries a cool that reads *quieter* than the gentlest herb on the salad, because Phoenix's cool was deliberately dialed to a whisper inside a storm, while spearmint's is the whole point of the plant. Invert the formula on each and the relationship sharpens: Phoenix activates the channel at 1,000 ÷ 800 = 1.25 times reference, spearmint at 1.11 times; spearmint's cool is 900 ÷ 800 = 1.13 times as loud as the superhot's. A number on one page lets a chili and a sprig of mint be compared on the one axis they share, and the comparison is not a trick of scale — it is the literal, computed truth that a plant designed for fire can hold less cool than a plant designed for freshness.

That is the honest limit to sit with, and we would rather name it than paper over it. The Index scores potency, cleanly and reproducibly, and spearmint's deepest value — the low ceiling of carvone, the partial-agonist gentleness that makes it cool the way a fresh herb cools rather than the way a lozenge does — lives partly in the efficacy the number does not measure. So we design spearmint to two truths at once: the 900 that puts it correctly on the ladder, and the lived restraint that the ladder cannot fully show. Both are forms of respect — for the reader who will trust the number, and for the plant that is content to be gentle. A spearmint grown and read with this much care gives the body exactly what it offers and no more: a clean, sweet, fresh cool, true to one hand of one molecule, every single harvest. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 11 · Lavender & the Floral Cool — *calm you can taste.*

Lavender is the quietest plant in this book and in some ways the most interesting, because its cool is barely a cool at all in the receptor sense and yet unmistakably cooling to anyone who has tasted it. The explanation is a lesson we keep relearning across the thermal axis: the mouth's sense of cool is a composite the brain assembles from several streams, and a molecule can lower the whole composite without ever shouting at the cold channel. Lavender's **linalool** registers near an IFI-C of 40 — a faint touch on TRPM8 — and yet the sensation it produces reads as a cool *calm*, an aromatic coolness that arrives as much through the nose and the body's sense of ease as through any thermal nerve. Lavender cools the way a shaded room cools: not by dropping a temperature but by changing the whole mood of the air.

We have spent a long time with this plant precisely because it refuses the easy story. A pepper is honest about where its heat comes from; you can point to the molecule, the receptor, the number, and the sensation lines up behind them like soldiers. Lavender does not. The molecule is faint, the receptor barely notices, and the sensation is nonetheless real and specific and loved. To design such a plant you have to understand not one number but the arithmetic that turns a weak molecular signal into a strong perceptual one — and then hold that arithmetic steady across every season and every harvest. That is the work of this chapter, and it is the gentlest, most exacting work in the whole library.



Backlit greenhouse at golden hour, a flower hanging in the warm light.

The cold channel that never feels the cold

The receptor at the center of the cool axis is **TRPM8** — transient receptor potential, melastatin subfamily, member eight — a non-selective cation channel standing in the membrane of the trigeminal and dorsal-root sensory neurons that wire the tongue, lips, and the roof of the mouth. In its resting life TRPM8 is a thermometer. It opens as the tissue around it falls below roughly 26 to 28 °C, letting sodium and calcium flood inward, and that inward current is the electrical sentence the nerve sends upward as the word *cold*. No molecule is required. Cool the tissue and the channel speaks.

What menthol discovered, a hundred million years before any chemist did, is that the channel can be made to lie. Menthol — the archetypal cooling agonist, the molecule the whole axis is anchored against — slips into a pocket within the channel's voltage-sensor-like domain, the bundle of four membrane-spanning helices that read the cell's electrical state. A tyrosine deep in the second of those helices is the critical contact; remove it and menthol loses most of its grip. Bound there, menthol does not cool anything. It shifts the channel's thermal set-point upward, so that TRPM8 begins to open at body temperature, at warmth, at temperatures where it should be closed and silent. The channel reports a cold that is not there. The physics is a mirror image of the heat channel's: where capsaicin lowers the temperature at which a warmth channel fires, menthol raises the temperature at which a cold channel fires. Both molecules are liars that tell the body the weather has changed. TRPM8 also leans on a membrane lipid, PIP_2, as a quiet cofactor — pull PIP_2 out of the membrane and even a menthol-loaded channel goes quiet — which is one of several reasons the cool sensation builds and fades rather than snapping on like a switch.

Linalool sits in that same pocket, but it sits badly. It is a weak partial agonist: it binds, it nudges the set-point, but it never seizes the channel the way menthol does. Two things follow from that weakness, and they are different things, and the difference matters. The first is **potency** — how little of the molecule it takes to half-open the channel — and the second is **efficacy** — how far open the channel goes when the molecule is everywhere. Linalool is poor on both counts. It is this poverty that the Index measures and makes legible.

The measurement runs through one formula, the same one that governs every axis in this library:

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

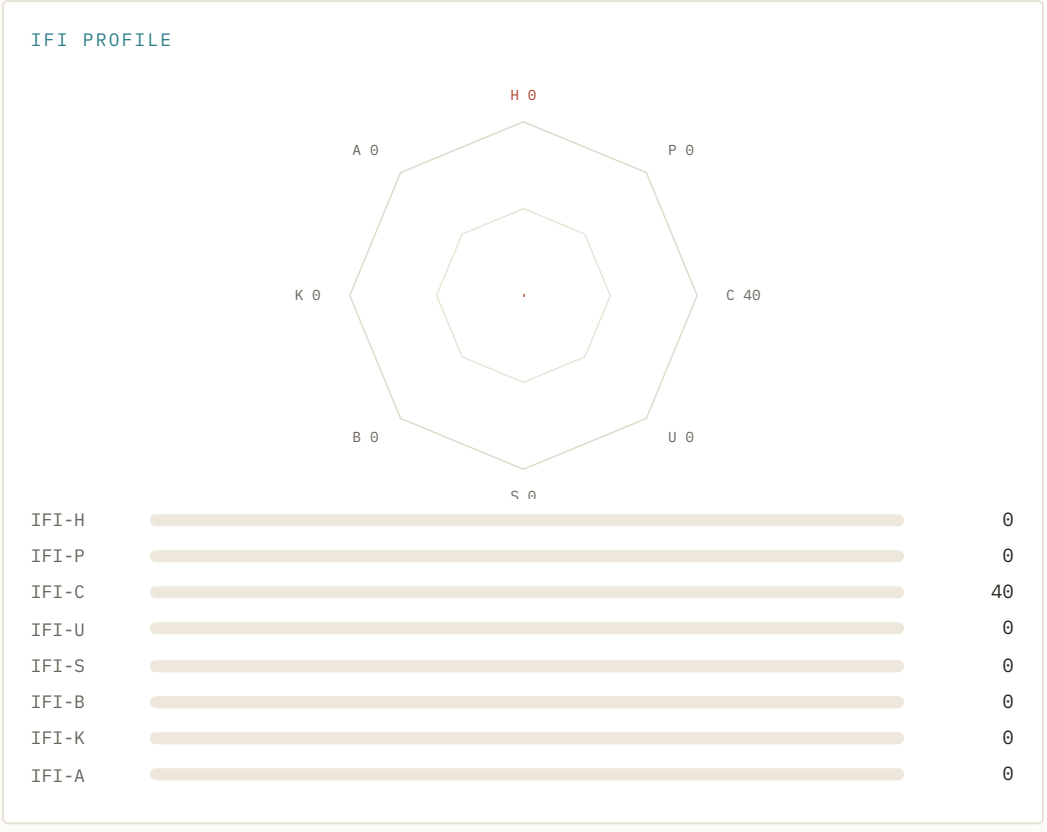
EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. We anchor the cool axis so that the menthol reference standard scores exactly **1,000** — that number *is* the definition of the reference's half-activation, a stated convention, the same way the Scoville scale is defined by dilution-to-threshold rather than derived from a literature survey. Invert, scale by a thousand, and every cooling molecule earns a score that says how loudly it speaks to TRPM8 relative to menthol. Rearranged for the concentration itself, the formula reads:

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

Put linalool's canonical IFI-C of 40 into that expression and the receptor's honest opinion falls out:

> $\mathrm{EC_{50}(linalool)} = \mathrm{EC_{50}(reference)} \times (1{,}000 \div 40) = \mathbf{25 \times EC_{50}(reference)}$

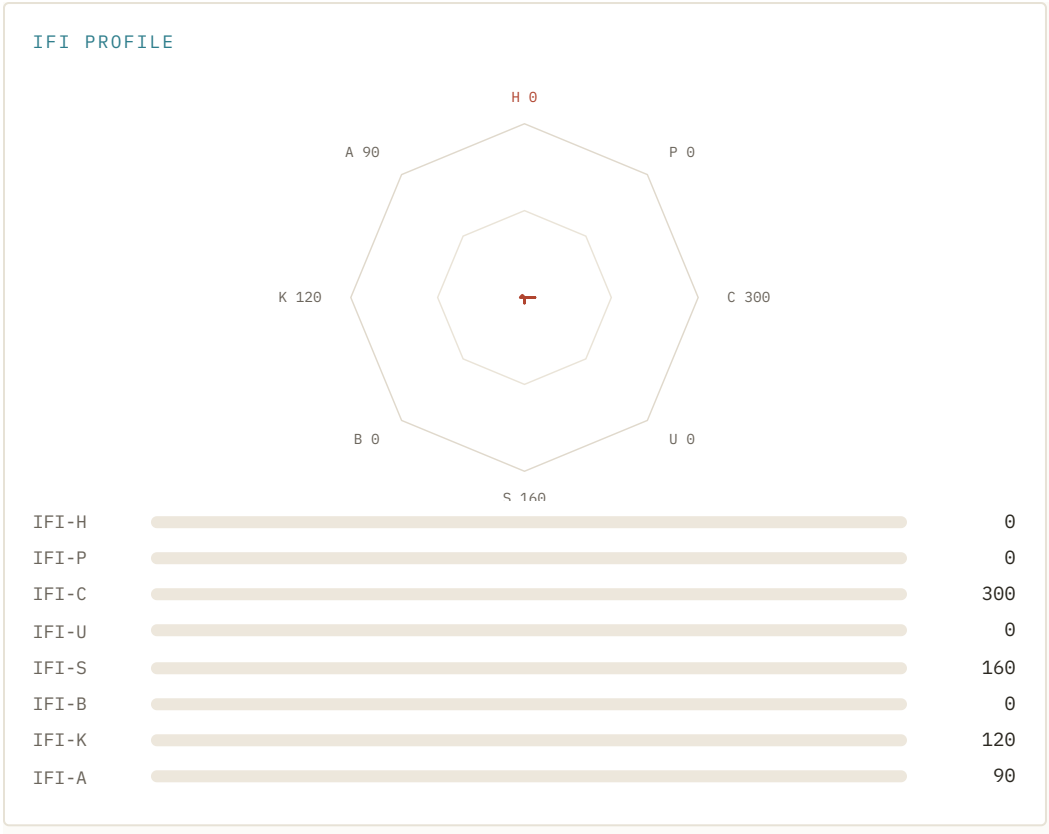
Linalool needs twenty-five times the concentration of menthol to half-open the cold channel. If we take the reference standard's half-activation to sit near 4 µM — an anchor we state plainly so the ratios stay exact no matter where the absolute lands — then linalool's TRPM8 EC_{50} is on the order of 100 µM. Converted through linalool's molar mass of 154.25 $g \cdot mol^{-1}$, that is about 15 $mg \cdot L^{-1}$, roughly 15 ppm in water: a frankly large amount of molecule to coax a half-hearted signal from the channel. And because linalool is a *partial* agonist, even pouring on more never reaches menthol's ceiling. On the receptor alone, taken as a lone molecule stripped of everything else lavender is, linalool is almost nothing:



A single monoterpene alcohol, one faint line on one axis. If the cold channel were the whole story, lavender would not be worth a chapter. It would barely be worth a sentence.

A cool the brain assembles

And yet the plant is worth a chapter, because the plant is not the molecule. Set the lavender's whole scorecard beside the lone molecule's and the gap is the entire subject:



Cool-Burn at **300**, more than seven times the reading linalool earns at the receptor. Where did the extra cool come from? Not from more TRPM8 occupancy — the molecule is the same faint ligand it always was. It came from the fact that *cool* is a composite the brain assembles, and the Cool-Burn axis is deliberately built across the thermomodulatory pair, TRPM8 together with the warmth channel TRPV1, to score the sensation the mouth actually reports rather than the occupancy of any single pore.

The arithmetic of that assembly is worth making explicit, because it is the most honest thing we can say about this plant. Run the formula backward from each score to an effective half-activation:

STIMULUS	IFI-C	EFFECTIVE EC_{50} (× MENTHOL REFERENCE)	WHAT IT IS
Menthol standard	1,000	1.0× — the anchor	full agonist, the axis's zero-point
Lavender, whole plant	300	3.3×	the assembled percept
Linalool, pure molecule	40	25×	weak partial agonist
Linalyl acetate	~0	—	silent at TRPM8 (the alcohol is capped)

The whole plant behaves as though its cool were only 3.3 times weaker than menthol's, while its one cold-active molecule is 25 times weaker. Divide the two and the lift is exact:

> 25 ÷ 3.3 ≈ **7.5×**

Lavender performs a sevenfold perceptual amplification of a molecule that, at the receptor, barely moves the needle. That amplification does not live in the chemistry of TRPM8. It lives in integration — in the retronasal aroma that rises from the back of the mouth to the olfactory sheet and tells the brain *lavender* before the tongue has finished reading temperature; in the roundness the sweetness and kokumi axes lend, which keeps the cool from reading thin and sharp; and in a lifetime of association the brain brings to the

table, because cool and calm and lavender have been braided together in human memory for as long as the plant has been grown. The number on the scorecard is the sensation, faithfully. But the sensation is a chord, not a note, and no single receptor reading can account for it.

This is the honest limit of a single-receptor number, and we would rather state it than hide it. IFI-C(linalool) = 40 is a true fact about a molecule and a channel. IFI-C(lavender) = 300 is a true fact about a plant and a person eating it. They are both correct and they are not the same measurement, and a reader who does not understand the difference will over-trust the first and be baffled by the second. The arithmetic above is how we keep faith with both. It is also why, of every plant in this library, lavender is the one that most rewards a design platform: a plant whose value is a *proportion* rather than a *peak* is a plant where getting the arithmetic exactly right is the entire game.

The two molecules a breath apart

Lavender's oil is a blend, and its character is set almost entirely by two molecules that are chemically a single breath apart. The first is **linalool**, a free monoterpene alcohol, carrying an exposed hydroxyl group — the reactive, slightly polar handle that makes a molecule read as sharp, green, a little soapy when there is too much of it. The second is **linalyl acetate**, that very same alcohol with its hydroxyl capped by an acetate ester: the handle tucked away, the molecule rendered softer, sweeter, rounder, and — crucially — essentially mute at the cold channel, because the capped molecule no longer sits well in TRPM8's pocket. The ratio between the free alcohol and the capped ester is the whole personality of the plant.

Call that ratio R, the proportion of free linalool to linalyl acetate. Push R high — too much free alcohol — and the oil turns sharp and medicinal, the harsh note of a cheap lavender soap. Push R low — too much ester — and it goes flat, dull, sweetly characterless. The beautiful lavender, the one that reads as clean floral calm, holds R in a narrow band, and that band shifts with species, with soil, with the heat of the season. A third molecule decides how far the plant falls from grace: **camphor**, a bicyclic ketone with a cold, penetrating, mothball sharpness that fine lavender keeps almost entirely out of its oil and that its coarser relatives let in by the percent.

PLANT	SPECIES	LINALYL ACETATE	FREE LINALOOL	CAMPHOR	READS AS
Fine lavender	*Lavandula angustifolia*	high	balanced	under ~1%	clean floral calm — the grade one wants to wear
Spike lavender	*L. latifolia*	low	high, exposed	elevated	sharp, camphoraceous, medicinal
Lavandin	*L.* × *intermedia*	moderate	moderate	several percent	vigorous, cheap, soap-shelf

These are the natural ranges, the vintage lottery that has governed lavender's worth for as long as perfumers have graded it: a small shift in R and a few points of camphor separate an oil that commands a fortune from one that scents a floor cleaner. There is

one more dimension still, finer than all of these. Linalool, like menthol and like carvone, is **chiral** — it exists in two mirror-image hands that the nose reads differently. The (R)-(−) form, licareol, is the soft, floral, lavender-sweet hand; the (S)-(+) form, coriandrol, is woodier and sharper. Fine lavender runs heavily to the (R) hand, and so even within "linalool" there is a handedness to specify, one more dial the plant sets with its chiral enzymes and a breeder can only pray stays put.

The biosynthesis, and the three dials

To hold what a breeder can only hope for, we have to go to the enzymes, and lavender's are drawn with unusual clarity. The carbon for every one of these molecules arrives as **geranyl diphosphate (GPP)**, the universal ten-carbon monoterpene precursor, built in the plastid from the MEP isoprenoid pathway. From that single branch point, three enzymatic roads diverge, and the plant's grade is decided by how much traffic each road carries.

The first road is **linalool synthase (LIS)**, a terpene synthase that cyclizes — rather, hydrolyzes and rearranges — GPP directly into linalool. Which hand of linalool it makes is written into the enzyme itself; the choice of LIS *is* the choice of enantiomer. The second road is an **alcohol acetyltransferase**, an acetyl-CoA-dependent enzyme that takes the finished linalool and caps its hydroxyl to make linalyl acetate. The ratio R — the plant's whole personality — is nothing more than the balance of flux between making the alcohol and capping it, which is to say the balance between LIS output and acetyltransferase activity. The third road is the one we want closed: GPP → **bornyl diphosphate synthase** → borneol → borneol dehydrogenase → **camphor**, the branch that carries the cold mothball note. In fine *angustifolia* it runs at a trickle; in spike lavender and lavandin it runs wide open.

Three roads, and therefore three dials:

- **Dial one — total linalool flux.** How much GPP LIS pulls toward linalool at all, setting the plant's aromatic amplitude.
- **Dial two — the ratio R.** The relative activity of the acetyltransferase against the free-alcohol pool, which fixes whether the plant reads sharp, balanced, or dull. This is the master dial of grade.
- **Dial three — camphor suppression.** Knocking down the bornyl-diphosphate branch so the cold, medicinal note never enters the oil.

The method that sets these dials is the same one that proved itself on the heat axis, where an ESM2 protein-language model scored every possible single-residue substitution in a pepper acyltransferase for its effect on catalytic efficiency and fold stability, a genome-scale flux-balance model revealed that flux control sits upstream in the phenylpropanoid pathway — at steps like 4CL and HCT — rather than at the terminal capsaicin synthase, and two substitutions never before combined — written in with CRISPR — lifted output while driving batch-to-batch variation down. Five of that platform's seven modules transfer unchanged to any organism with a sequenced genome, lavender among them. The language model

does not care whether the protein it is reading is a capsaicin enzyme or a monoterpene acetyltransferase; it reads sequence and predicts consequence. The flux model does not care whether the pathway ends in heat or in calm; it finds the step that governs the whole. We point that same apparatus at lavender's acetyltransferase and its camphor branch, let the models tell us which residues move the ratio and which knock the mothball note out, and write the smallest possible edits that hold.

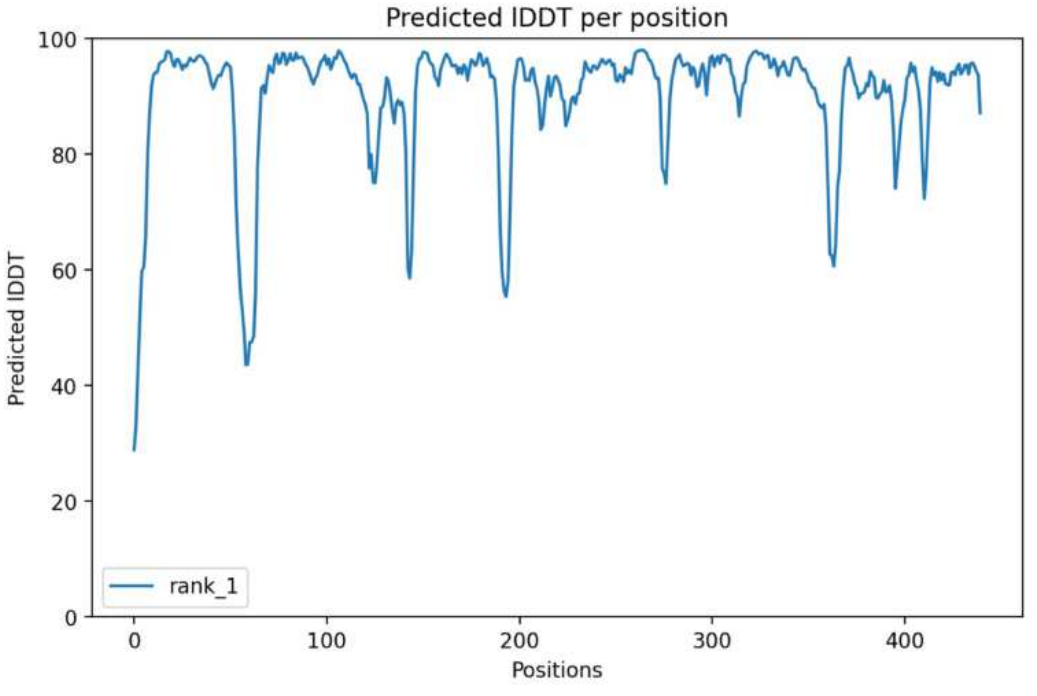


FIG. 11 — the acyltransferase that proved the editing method, coloured by model confidence (pLDDT); the active-site cleft resolves at high confidence, which is where an ester-forming enzyme is tuned.

We say plainly what this is and is not. We do not rewrite the plant's soul; we tune the flux through roads the plant already drives. Lavender still makes lavender's molecules by lavender's enzymes. What changes is that the three dials, which weather and soil and season had always left to chance, are set to a chosen value and held there — the (R) hand fixed by the choice of synthase, the ratio R fixed by the acetyltransferase, the camphor fixed near zero.

Holding the ratio: the mathematics of grade

Setting a dial once is breeding. Holding it every season is the engineering, and it is measured in a single statistic: the coefficient of variation. The **CV** is the batch-to-batch standard deviation of a quantity divided by its mean, written as a percent — the honest number for *how much does this wander from harvest to harvest*. Our heat program measured wild-type batch CV at **42%**, and field lavender, at the mercy of the same weather, is no steadier; the vintage lottery is exactly a large CV wearing a romantic name. Our specification across the library is a batch CV of **≤7%**.

The reason that difference is the whole ballgame for lavender, and not for a louder plant, is a matter of windows. A loud plant has a wide tolerance: if a pepper's heat wanders 40% from batch to batch, it is still unmistakably a hot pepper, and the eater forgives the wobble. Lavender has almost no tolerance at all, because its grade lives in a narrow band of the ratio R. Suppose — and we mark this as an illustration, not a canonical constant — that the fine-grade window is R within ±15% of its target before the oil tips to sharp on one side or dull on the other. Set the two CVs against that window:

- At our **≤7% CV**, one standard deviation of batch-to-batch wander is ±7% of target. Two standard deviations — the span that holds about 95% of harvests — is ±14%, which still fits *inside* the ±15% window. Nearly every batch reads as fine lavender.
- At the wild-type **42% CV**, one standard deviation alone is ±42%, nearly three times the width of the entire window. The *typical* harvest already falls outside grade; a fine batch becomes a lucky accident rather than a plan.

That is the quantitative content of an old perfumer's intuition. A balanced plant does not forgive. When value is a proportion, the proportion's tolerance is tight, and only a tight CV keeps a grower reliably inside it. This is why, for lavender more than for almost anything else in the library, the engineered advantage is not *loudness* but *grade* — a reliably fine lavender, every season, without the lottery that has always separated an oil one wants to wear from one one wants to scrub the floor with.

The cool axis as a whole is a small, hard-won library, and its roster says something about the difficulty. Every organism that carries a cool-burn score is rated hard to engineer, because the cold channel's chemistry is subtle and the percepts are composites:

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY
Peppermint	2,360	C / M	Hard
Lavender	160	C	Hard
Orange	120	B / C	Hard
Eucalyptus	80	C	Hard
Spearmint	80	C	Hard
Camphor	40	C	Hard

Lavender's **160 constructs** are a focused program, not a sprawling one, and the focus is the point. Where peppermint asks for a large library because its menthol pathway offers many strong levers to pull, lavender asks for a small, surgical one, because the goal is not to add anything but to hold a few proportions exactly. One should not mistake the small number for small ambition. It is the harder kind of problem — the problem of a plant whose whole worth is a chord that must be struck the same way every time.



Research rows at the edge of morning — the field is where a field-grown aromatic's variation is either born or beaten.

In the kitchen — the unforgiving pinch

Lavender rewards in the pot exactly the restraint its scorecard preaches. It belongs to the aromatic-herb tradition of southern France — a quiet member of a *herbes de Provence* blend, a whisper folded into a honey, a careful note in shortbread or a poached pear or a panna cotta — and the universal rule, learned by every cook the hard way, is that a little is a seasoning and a little more is a catastrophe. The floral tips in an instant from *calm* to *soap*, and the tipping is chemical, not mystical: push past the window and the free linalool and any stray camphor announce themselves, and the dish reads of the bath shelf.

The arithmetic of the pinch is humbling. Recall that linalool's half-signal at the cold channel wants something like 15 mg per liter — a real quantity of molecule — but its aroma threshold in the nose sits orders of magnitude below that. The cook is therefore never dosing for the tongue's cold channel at all; a cook who added enough lavender to truly half-tickle TRPM8 would have produced something closer to perfume than to food. The seasoning lives entirely in the aroma and its associations, which is why the honest instruction for lavender is always a fraction of what instinct suggests: a pinch of buds in a whole batch of shortbread, a single sprig drawn through a warming cream and then removed. A designed lavender, held clean of camphor and fixed at a gentle ratio, does one kind thing here that a wild oil cannot: it widens that punishing window a little. Remove the camphor entirely and you remove the sharpest, fastest route to *soap*; hold R steady and you remove the batch that was secretly too sharp to begin with. The hand that reaches for one pinch too many is forgiven a little more often — which, for a plant this unforgiving, is a genuine kindness to the person at the stove.

The failure modes are worth naming, because they are specific and they are avoidable. The first is **camphor contamination**: even a percent or two drags the whole dish toward medicine, and it is the single most common reason a home lavender dish fails. The second is a **ratio run high**, too much free alcohol, which reads as a thin, soapy sharpness that no amount of sugar quite covers. The third is **the wrong hand** — an oil heavy in the (S)-(+) enantiomer, which turns the soft floral woody and flat even when the ratio and the

camphor are nominally right. Each of these is a dial we have set and hold; each is a way the vintage lottery used to turn a good intention into a ruined pan.

A word, too, on what the cool axis can and cannot borrow from its louder siblings. Heat has carried a folk number for a century — the Scoville scale, where our model predicts our highest-designed pepper at approximately 2.95 million SHU — and a cook has always had at least a crude sense of *how hot*. Cool has never had such a number. There is no Scoville of calm, no inherited scale for *how cool*, which is part of why a cooling flavor has always been so hard to specify and so easy to get wrong. The IFI-C is the first number ever put to it. We do not pretend the single number captures the whole composite — the arithmetic earlier in this chapter is our confession that it does not — but a computed, repeatable 300, held to ±7%, is still the first time anyone could promise a cook that this spring's lavender would taste like last spring's. For a flavor whose entire worth is consistency, that promise is the product.

What we claim, and what we don't

This is the chapter where the ethos of the whole library comes nearest the surface, and where we are most careful with our words. Lavender has carried associations of calm and rest for as long as people have grown it — strewn in linen, tucked under a pillow, steeped at the end of a hard day — and we honor those associations, because they are real to the people who hold them, and a cup of lavender tea in the quiet of an evening is a genuine kindness someone gives themselves. But we make no claim on anyone's nervous system. We do not say lavender calms. We say lavender *tastes and smells of calm* — that it produces a cool floral sensation the mind has long braided to ease, and that we have learned to design that sensation clean and constant, season after season.

The distinction is not legal hair-splitting; it is the spine of how our family works. We are a vegetarian household by conviction and a clean-living one by habit, and the thing we actually revere is the plant grown well and the body fed with intention — never a promise about what a molecule will do inside someone. The number tells you how loudly linalool speaks to a cold channel. It does not tell you that lavender will quiet your mind, and we will not let a scorecard imply what a scorecard cannot know. The nourishment this library cares about lives in the care behind a thing grown right — in the ratio held, the camphor kept out, the hand fixed, the harvest made faithful — and never in a claim draped over the plant like a borrowed coat. Lavender is remarkable enough as a flavor that it needs no medicine hung on it.

So the scorecard stands as a portrait of restraint, and we would not have it read any other way. Cool-Burn at 300, modest and honest — a floral coolness, not a cold. Sweetness at 160 and Kokumi at 120 give the plant its roundness and its gentle body, the soft fullness that keeps the cool from reading thin; a faint Astringency at 90 is the slight dryness that keeps it from cloying. There is no towering axis here, and that is the entire nature of lavender: it is a *composition*, a balanced small chord rather than a loud single note. The loudest

plant we have ever designed registers on every one of the eight axes at once, heat at 4,200 and cool a mere by-product at 800; lavender, the quietest, puts its whole self into a cool of 300 and three soft supporting lines, and asks only that they stay in proportion forever. A loud plant forgives a clumsy hand. A balanced one does not. Lavender is where the discipline of ≤7% stops being a specification and becomes the difference between a flavor and a mess — and where the quiet, exact care our family brings to everything it grows is not a flourish on the work but the whole of it.

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

Chapter 12 · Eucalyptus & Camphor — *the medicinal edge.*

There is a kind of cool that opens the chest, clears the nose, carries a whisper of the medicinal — the cool of a eucalyptus steam, a camphor rub, a strong herbal lozenge — and it comes from two plants that approach cold from opposite directions and meet in the middle. Eucalyptus cools genuinely but faintly, through volatility more than loudness. Camphor does not cool at all, in the strict sense, and yet reads as the most medicinal cool of all. Together they define the airy, respiratory, medicinal corner of the thermal axis, and they are among the subtlest design problems our family works.

We love this corner precisely because it refuses to behave. The whole cool axis is built on a single clean idea — that loudness is a number, that a molecule's effect can be anchored to the concentration at which it drives its receptor to half of full response, and that the ratio of one such concentration to another is a score you can read a tomato, a chili, and a cup of tea against on one page. The Index writes it in one line, the same line that governs every axis in this book:

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

Eucalyptus and camphor both honor that arithmetic and both make it work harder than any loud mint ever does. One is quiet and feels wide. The other is warm and feels cold. To grow either one true, you have to know exactly where the number comes from and exactly where it stops telling the whole story.



The aromatic house at golden hour — where we grow the cooling plants, and where a faint, volatile cool first lifts off the leaf and fills the air above the bench.

Eucalyptus — the quiet molecule that fills a room

Eucalyptus makes **1,8-cineole**, eucalyptol, the molecule that *is* the smell of a eucalyptus grove. A single enzyme, 1,8-cineole synthase, builds it in one continuous cascade inside one active-site pocket, and the cascade is worth following step by step because the shape it produces is the reason the receptor barely hears it. Geranyl diphosphate — the ten-carbon GPP that every monoterpene begins as — loses its diphosphate to become an allylic cation. The enzyme

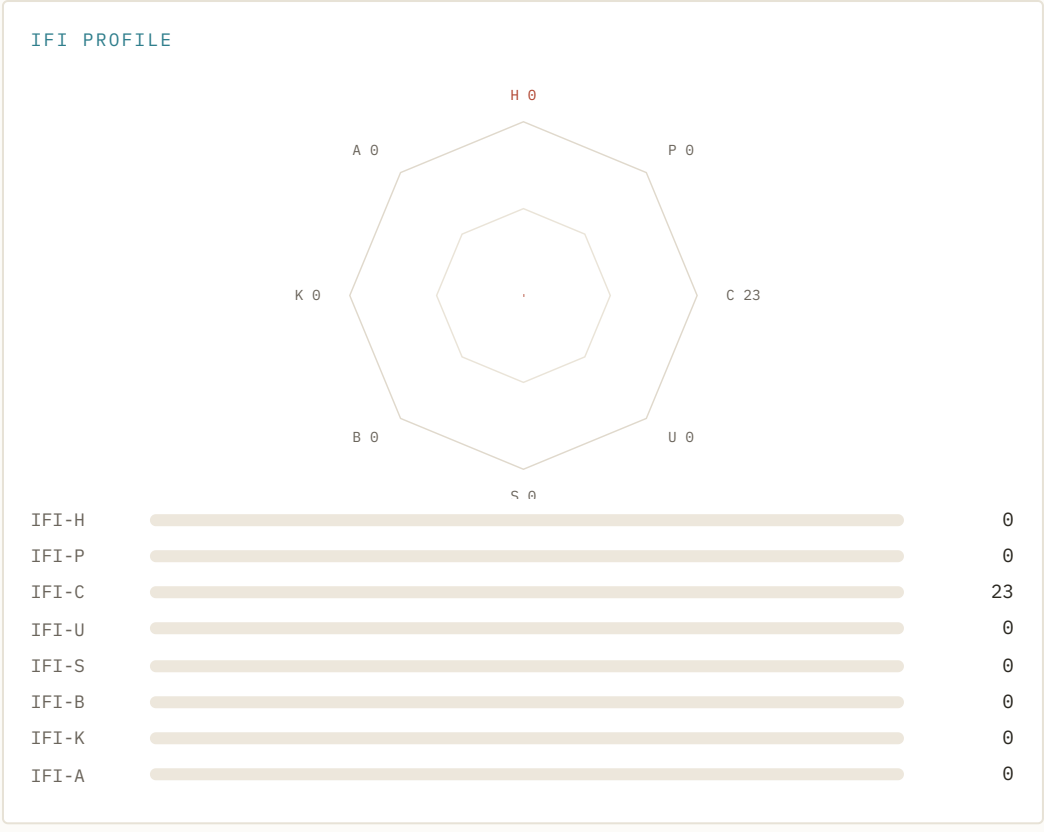
isomerizes that open chain to linalyl diphosphate, lets it ionize again, and folds it: a 1,6-ring closure gives the α-terpinyl cation, a six-membered ring carrying a tertiary carbocation. Water slips in to quench it as α-terpineol. Then, still held in the pocket, the ring is re-protonated to a fresh tertiary cation and the newly placed hydroxyl oxygen reaches across and attacks it, closing a bridging ether. What falls out is 1,8-cineole — a rigid bicyclic cage, an 8-oxabicyclo[2.2.2]octane, the oxygen tucked inside like a held breath.

That cage is the whole story. TRPM8, the cold receptor, reads menthol through a pocket in its voltage-sensor-like domain, where a single tyrosine — Y745 — makes the contact that menthol's flexible, hydroxyl-bearing ring settles into so comfortably. Cineole, stiff and bridged and shielding its oxygen inside the cage, cannot settle the same way. It touches the pocket; it does not sink into it. In the language of the Index that under-fit is a high half-activation concentration, and a high EC_{50} is a low score.

Anchor the cool axis where the Index anchors it — on (−)-menthol at its half-activation of TRPM8, the reference loudness we call **IFI-C 1,000**, with a reference EC_{50} of about 4 µM. Cineole reads **IFI-C 23**. Run that through the one formula and the under-fit becomes exact:

$$23 = 1000 \times \frac{\mathrm{EC}_{50}(\mathrm{menthol})}{\mathrm{EC}_{50}(\mathrm{cineole})} \;\Rightarrow\; \frac{\mathrm{EC}_{50}(\mathrm{cineole})}{\mathrm{EC}_{50}(\mathrm{menthol})} = \frac{1000}{23} = 43.5$$

Cineole needs about **43.5 times** the concentration menthol does to drive the same receptor to the same half-response — roughly 174 µM against menthol's 4 µM. By receptor loudness alone, eucalyptus is one of the faintest things on the cool axis we grow.



A single tall bar and seven floors swept clean: that profile is the honest portrait of a eucalyptus cultivar. It speaks on one axis, quietly, and says almost nothing on the other seven. If the number were the whole truth, eucalyptus would be a rounding error. It is not, and the reason it is not is the most instructive thing in this chapter.

The paradox of coverage — why a faint cool feels wide

Loudness is not the only term in what a mouth and a nose actually feel. A receptor score measures how hard a molecule drives *one* channel at *one* site. What you perceive is that driving summed across every site the molecule can reach — and cineole can reach almost everything. It is intensely volatile, lifting off the surface into the vapor phase and flooding up through the nasal passage and the upper throat, touching the trigeminal field of the whole airway at once. Menthol, heavier and placed on the tongue by a lozenge, works a small wet patch loudly. Cineole works an enormous surface softly.

Write that as a simple, honest model. Call the perceived cool the product of receptor loudness and the fraction of the sensory surface the molecule reaches:

$$L_{\text{eff}} \;\approx\; \text{IFI-C} \times A_{\text{reached}}$$

Set cineole's effective cool equal to menthol's and ask how much more surface the quiet molecule must cover to match the loud one:

$$23 \times A_{\text{cineole}} = 1000 \times A_{\text{menthol}} \;\Rightarrow\; \frac{A_{\text{cineole}}}{A_{\text{menthol}}} = 43.5$$

The coverage factor cineole needs is exactly the inverse of its loudness deficit — the same 43.5 falling out the other side of the equation. And an order-of-magnitude more surface is not a stretch for a molecule that volatilizes: the upper airway and nasal trigeminal field dwarf the wetted patch of tongue a mint touches. Vapor pressure is the lever. Cineole's is high enough, a couple of millimeters of mercury at room temperature, that it partitions readily into the air you breathe; by Henry's law the molecule distributes itself across the gas phase and onto every surface that air washes over. The faintness is real and the breadth is real, and breadth very nearly cancels faintness. This is the eucalyptus paradox stated in arithmetic: a molecule 43 times too quiet to matter, reaching roughly 43 times the surface, arriving as a cool that feels not small but *open*.

Our eucalyptus program — **80 constructs** — follows from that equation directly. There is no point chasing cineole's receptor loudness upward; the bicyclic cage sets a low ceiling on IFI-C that no titer increase can lift, because concentration moves the dose–response curve sideways, not the EC_{50} that defines the score. So we do not design for loudness. We design for the other two terms: purity, so the coverage carries a clean signal, and volatility, so the signal reaches. Wild eucalyptus oils are loud with the *wrong* things — harsh camphoraceous top notes, turpentine-like off-notes from stray monoterpenes the plant makes alongside cineole. The 80 constructs tune the monoterpene synthase complement so cineole dominates the volatile fraction and its scratchy companions fall away. A cleaner cineole, a calmer top note, a eucalyptus that opens the breath without scratching it.

The respiratory corner these plants occupy deserves a careful word, because it is where the thermal axis brushes most directly against the language of medicine, and where we are most disciplined about not crossing. The sensation of a "clear," open airway is exactly that — a sensation, a flooding of the airway's trigeminal field with a cooling, volatile molecule that the brain reads as openness. We design that sensation and we make no claim beyond it. We do not

say eucalyptus treats a cough or camphor clears a chest; we say they taste and feel of a cool, open breath, which they have for as long as people have crushed the leaves and breathed them in. The restraint is the respect. A plant this evocative does not need a medical claim pinned to it, and pinning one would betray both the reader, who deserves a flavor and not a false promise, and the plant, which is extraordinary enough as a sensation that it needs no borrowed authority.

The shape of a cool — dose, response, and holding it still

Because eucalyptus lives or dies on coverage rather than loudness, the *shape* of its dose–response curve matters more than its height, and the shape is governed by cooperativity. TRPM8 does not open one molecule at a time in simple proportion; menthol's activation follows a sigmoid, the Hill form, in which response climbs slowly, then steeply, then saturates:

$$R([L]) = R_{\max}\,\frac{[L]^{n}}{\mathrm{EC}_{50}^{n} + [L]^{n}}$$

At `[L] = EC₅₀` the fraction is exactly one-half — that is the definition the whole Index rests on. The exponent `n`, the Hill coefficient, sets the steepness, and for menthol on TRPM8 it sits near 2 to 3. That cooperativity compresses the entire perceptible range into a narrow window of concentration. The span from a barely-there 10% response to a near-saturating 90% is

$$\frac{[L]_{90}}{[L]_{10}} = \left(\frac{0.9/0.1}{0.1/0.9}\right)^{1/n} = 81^{\,1/n}$$

For a non-cooperative molecule (`n = 1`) that span is 81-fold — a long, lazy ramp. At `n = 2.3` it collapses to about **6.8-fold**; at `n = 3`, to about **4.3-fold**. The whole climb from first whisper to full cold happens inside a five- to seven-fold change in concentration. That is why cool has its characteristic *threshold-then-flood* quality — nothing, nothing, then suddenly everything — and why, in a formulation, the difference between a mint that barely registers and one that overwhelms can be a few tenths of a percent of active. For a coverage molecule like cineole, laid down across a huge surface where local concentration varies from place to place, that steepness means the cool arrives wherever the vapor crosses threshold and stays quiet everywhere it does not — a soft edge, not a wall.

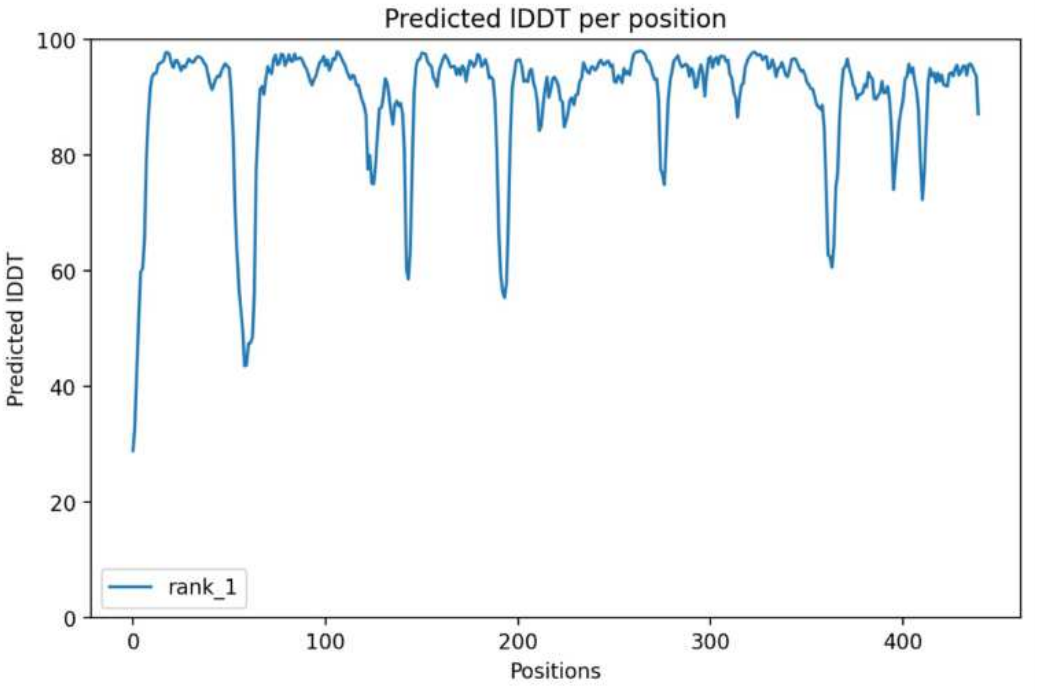
It helps to carry those half-activation concentrations out of the receptor assay and into the glass, where a formulator actually works, because the micromolar numbers hide how little material a cool really takes. Cineole weighs about 154 grams per mole, so its 174 µM half-activation is 174 × 10⁻⁶ mol/L × 154 g/mol ≈ 0.027 g/L — about **27 mg/L, 27 parts per million** in water. Menthol, at 156 grams per mole and 4 µM, half-activates at roughly **0.6 ppm**, and the ratio of the two, 27 to 0.6, is the same 43-fold gap now expressed in milligrams you could weigh on a bench. Camphor is the outlier that proves the rule: at 152 grams per mole and a TRPV3 half-activation near 10 mM, it would take on the order of **1,500 ppm** to saturate its own warm channel — which is exactly why we never dose it there. Camphor does its work as a trace, a few parts per million, far below

that ceiling, where its sharp volatility, its mild block on the cold channel, and its slow-building warmth carry the whole impression long before full receptor saturation ever could.

None of this is worth anything if the number wanders between harvests, and here the discipline of the platform earns its keep. We hold batch-to-batch variation to a coefficient of variation of **≤7%**, against the **42%** a wild aromatic crop drifts across. Carry that straight onto cineole's score. At CV 7%, an IFI-C of 23 holds inside **23 ± 1.6** — a band from about 21 to 25, a fifteen-percent spread you would struggle to taste. At the wild oil's 42%, the same faint cool smears across **23 ± 9.7**, from about 13 to 33 — a band nearly two and a half times wide at its edges, a cool that is a different cool every time you open a bottle. Reproducibility is not a luxury on a quiet axis; it is the whole difference between a flavor you can design around and a rumor. The same cool, every time, is the point.

MOLECULE	RECEPTOR	ACTION	EC_{50} (VS MENTHOL ANCHOR)	IFI-C
(−)-menthol	TRPM8	agonist (reference)	1× · ≈ 4 µM	1,000
1,8-cineole	TRPM8	weak agonist	≈ 43.5× · ≈ 174 µM	23
camphor	TRPV3 (warm) / TRPM8	warm agonist / cool blocker	≈ 2,500× on TRPV3 · ≈ 10 mM	— (blocks, not an agonist)

The anchor row defines the scale: menthol's half-activation of TRPM8 is set to IFI-C 1,000, and every other cool is read against it through the one formula. Camphor carries no cool score because it does not agonize the cold channel at all — it works the row below.



Confidence-coloured model of an engineered acyltransferase from our heat work (pLDDT; warm = high confidence, cool = low). We build the same map for every enzyme before we touch it — 1,8-cineole synthase and borneol dehydrogenase among them — so that an edit lands where the model is certain of the fold and never in the loops where it is guessing.

Camphor — the warm molecule that reads as cold

Camphor is the stranger of the two, and pharmacologically the one that teaches the most, because it reads as cooling and is mostly a *warming* molecule — its sense of cold assembled entirely from sharpness, volatility, and warmth rather than from any genuine cold signal at all. It is a **TRPV3** agonist and a mild **TRPM8** *blocker*: it switches the warm channel on and quietly turns the cold channel

down, and the brain, handed a sharp volatile medicinal note with a thread of warmth underneath, files the whole impression under "cool." Nothing in the tissue got colder. The cool is an inference, and camphor is the proof that the thermal axis is a story the nervous system tells, not a temperature it measures.

Its biosynthesis, in the camphor laurel *Cinnamomum camphora*, is a tidy three-step branch off the same GPP every terpene starts from. First, bornyl diphosphate synthase takes GPP, ionizes and isomerizes it to linalyl diphosphate, folds it through the α-terpinyl cation and on to the bicyclic bornyl cation — the compact norbornane cage — and, unusually, hands the diphosphate back to the same face it left, giving (+)-bornyl diphosphate with its stereochemistry set. A phosphatase then hydrolyzes that to (+)-borneol, a secondary alcohol. Last, borneol dehydrogenase oxidizes the alcohol to the ketone using NAD^+ as the electron acceptor, and the ketone is (+)-camphor. Three committed enzymes, three clean steps, one of the most distinctive aromatic molecules in the plant world.

On the receptor side the arithmetic is almost comic in its asymmetry. Camphor is a *weak* TRPV3 agonist: it needs concentrations in the low millimolar range — on the order of 10 mM — to drive its warm channel to half-activation. Set that beside menthol's roughly 4 µM on the cold channel and the ratio is about **2,500-fold**. To engage its own receptor, camphor needs some two and a half thousand times the concentration menthol needs to engage its — three orders of magnitude of difference between a loud molecule and a faint one, which is why camphor is never a volume flavor and always a trace. And on TRPM8 it does the opposite of agonize: it inhibits menthol-evoked current, so dropped into a minted formulation it shaves the very cool it sits beside. A number that belongs on no agonist ladder, a molecule that subtracts on one axis while it adds on another.

That is exactly why we keep it. Our camphor library is the smallest cooling program we run — **40 constructs** — and it exists not because camphor is a flavor you reach for by the gram but because camphor is a *tool*: the one plant-made molecule that lets us set a warming, medicinal depth underneath a cooling impression, the paradox of this book's title grown in a leaf. A winter lozenge, a warming balm-flavored confection, a cool that should carry an undertow of heat — camphor supplies what no pure coolant can. Designing it means managing its intensity with real care, because camphor is powerful and, overdone, overwhelming, and in excess genuinely unpleasant. The whole art of the 40 constructs is a trace, placed exactly, held reproducibly. On this axis more than any other, the difference between medicinal and medicine-cabinet is a matter of a few parts per million, and holding that line at CV ≤7% is the only reason a trace this small can be a flavor at all. Picture the trace set at three parts per million: at CV ≤7% it holds inside ±0.2 ppm, a drift no palate will catch, while at a wild crop's 42% it swings ±1.3 ppm — enough to carry one batch to barely-there and the next to a harsh, cabinet-sharp bitterness from the very same recipe. On a molecule dosed this low, reproducibility is not quality control after the fact; it is the flavor's entire margin of safety.



Reading and writing the synthase genes at the bench — the 80 constructs behind a cleaner cineole and the 40 behind a camphor you can dose to the part-per-million, scored and sequenced before a single plant goes to soil.

Two receptors, opposite directions

TRPV3, camphor's receptor, rewards a closer look, because its behavior is the mirror image of TRPM8's, and that opposition is the most useful thing in the whole corner. Where the cold channel *desensitizes* — the second mint cooling less than the first as the membrane lipid PIP_2 that TRPM8 depends on is depleted — the warm channel **sensitizes**, growing *more* responsive with repeated stimulation rather than less. Camphor's warmth builds across successive exposures where menthol's cool fades, and a formulation that pairs the two can be tuned so the cool leads and the warmth arrives behind it and lingers after the cold has gone.

Put plausible shapes on the two curves and the designed sequence falls out as arithmetic. Let the cool signal decay a little with each successive taste and the warm signal climb — illustrative per-exposure factors, chosen to show the mechanism rather than measured off any one product:

$$C_n = C_1 \cdot (0.8)^{n-1} \qquad W_n = W_1 \cdot (1.5)^{n-1}$$

Start the cool ahead, as a mint does, with $C_1 = 100$ and $W_1 = 10$:

TASTE n	COOL C_n	WARM W_n	NET (COOL − WARM)
1	100.0	10.0	+90.0
2	80.0	15.0	+65.0
3	64.0	22.5	+41.5
4	51.2	33.8	+17.4
5	41.0	50.6	−9.6

The crossover — where warmth overtakes cool — is not a guess; solve $100 \cdot 0.8^{n-1} = 10 \cdot 1.5^{n-1}$ for n:

$$n = 1 + \frac{\ln 10}{\ln(1.5/0.8)} = 1 + \frac{2.303}{0.629} = 4.66$$

Somewhere around the fifth taste, the cool that led hands off to the warmth that was building underneath it the whole time — a cool-then-warm arc that neither molecule could produce alone, written as a designed sequence rather than a happy accident. TRPV3 sits in the keratinocytes of the skin and the epithelium of the mouth, answering the comfortable warmth of 32 to 39 degrees; it is the quiet counterweight to the entire cold apparatus this book maps, and

knowing it precisely is how we keep an unwanted warmth *out* of a cool that should stay clean, and fold a wanted one *in* when a cool should turn.

The three thermal channels together draw the map the whole axis lives on:

RECEPTOR	THERMAL WINDOW	AGONIST HERE	DIRECTION	ON REPEAT
TRPM8	below ≈ 26 °C	menthol, cineole	cooling	desensitizes (PIP_2 depletion)
TRPV3	≈ 32–39 °C	camphor	warming	**sensitizes** (builds)
TRPV1	above ≈ 43 °C	capsaicin	hot	desensitizes

Cineole and camphor sit in the first two rows and reach, between them, from genuine cold to assembled warmth — the two ends of the medicinal corner, one channel switched gently on, one switched gently down, the perception bridging the gap the tissue never actually crosses.

The medicinal edge, grown clean

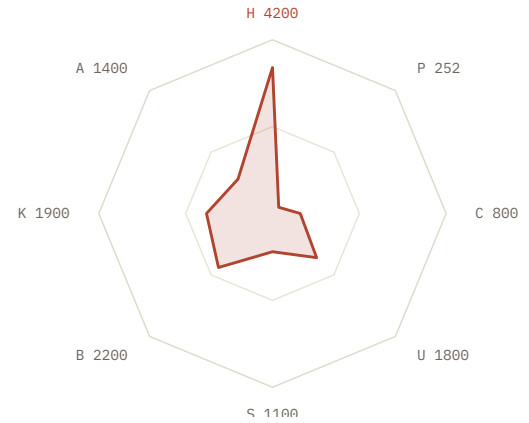
Lay the cooling plants side by side and the shape of the whole palette comes clear. Peppermint is the loud cold at the center, the reference the entire axis is anchored on. Spearmint is the sweet-fresh variation. Lavender is the floral calm at one edge, eucalyptus the airy openness at another, and camphor the warm paradox at the far side, where cool turns back into warmth.

PLANT	CONSTRUCTS	KEY MOLECULE	RECEPTOR ACTION	CHARACTER
Peppermint	2,360	(−)-menthol	TRPM8 agonist (loud)	clean cold
Spearmint	80	(−)-carvone	weak TRPM8	fresh, sweet
Lavender	160	linalool	very weak TRPM8	floral calm
Eucalyptus	80	1,8-cineole	weak TRPM8, volatile	respiratory open
Camphor	40	camphor	TRPV3 agonist, TRPM8 blocker	warm medicinal

No single plant is *the* cool; the cool is a region, and these five are the points we have learned to grow within it. And the medicinal note is not locked to the two plants that wear its name. Cineole threads through rosemary, bay, sage, and cardamom too — a near-universal aromatic — and camphor's branch runs through rosemary and sage as well, so the medicinal cool is a note the whole aromatic-herb shelf can sound. A designer who knows the molecule can summon it from more plants than the two on this page, which is the quiet power of working at the level of the compound rather than the crop: you are not breeding a eucalyptus, you are learning to place cineole, and cineole is everywhere.

It is worth seeing, finally, how far the axis actually reaches, because the faintness of eucalyptus can make the cool seem like a small thing, and it is not. At the loud end we have grown a designed cool deliberately — folded, with seven other axes, into a single full-spectrum fruit:

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

That **C 800** is a computationally designed cool, written into a cultivar gene by gene until it registered — some **35 times** louder than the wild aromatic's faint **C 23** (800 ÷ 23 ≈ 35), and set shoulder to shoulder with heat, umami, kokumi, and the rest on one plant. Between that designed 800 and eucalyptus's wild 23 lies the whole working range of the cool axis, and the lesson of this corner is that *both* numbers are flavors worth growing with precision: the loud one because it is hard to build, the faint one because it is hard to keep clean.

A formulator who knows all five cooling plants — their loudness, their coverage, their character, their paradoxes — can place a product anywhere in the thermal field, which is exactly what the Index was built to let them do. The palette is small on purpose. Mastery of a small palette is worth more than a large one half-understood, and these five plants, understood to the enzyme and the receptor, are enough to paint nearly every cool a person has ever wanted — the clean cold of a mint, the open breath of a eucalyptus steam, and the warm medicinal edge of camphor that is not cold at all, and feels, grown right and dosed to the trace, like the most honest cool of the lot. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 13 · The Unexpected Cool — *citrus, cucumber, and cold where you don't look for it.*

The most quietly radical thing a design platform does is move a sensation into a plant that never had it. Capsaicin out of the pepper and into a tomato; menthol's logic out of the mint and into a fruit. The thermal axis is where this gets strangest and most delightful, because *cool* is a sensation the culture ties to a short list of plants — mint, mostly, with a little eucalyptus and camphor at the edges — and the moment you lay a genuine, clean cold into something that has no business being cold, you have made a thing the world has never tasted. This chapter is about cool where you do not look for it, and about the two plants that taught our family the most about reaching it: the orange and the cucumber.

What makes the cool axis feel like sorcery, and what makes it in fact ordinary biochemistry, is the same fact seen from two sides. Cool is not a temperature. It is a receptor. The cold you feel from a mint leaf is not the leaf drawing heat from your tongue — the leaf is exactly as warm as the rest of your mouth — it is a single ion channel being lied to by a small molecule, reporting a winter that is not there. Once you know that, the whole axis opens: if cold is a message and not a measurement, then the message can be written into anything that can be taught to make the molecule. The pepper taught us to write heat. The mint teaches us to write cold. And the plants that learn it most gracefully are not the ones that already taste cold, but the ones the culture only *believes* are cold — which is where the cucumber comes in, and why it is the deepest teacher in this chapter.



Cucumber — the plant whose very name is a synonym for cool, yet whose coolness is almost entirely a matter of water and freshness, not of any cooling molecule. A perfect canvas for a real one.

The channel that reports a cold that isn't there

Cool on the Iyengar Flavor Index is the IFI-C axis, and it is anchored to **TRPM8** — Transient Receptor Potential Melastatin 8, a non-selective cation channel standing in the membrane of a specific subset of trigeminal and sensory nerve endings, a population

distinct from the heat-reporting fibers that carry capsaicin's fire. TRPM8 is a tetramer, four identical subunits folded around a central pore that, when it opens, lets sodium and calcium flood inward and fires the nerve. In its resting life the channel is a thermometer for the cold end of the comfortable range: it begins to open as tissue falls below roughly **26 °C**, and the colder it gets, the more of the population is firing. That is the honest signal — the one your fingers send when you hold a glass of iced water.

Menthol does to TRPM8 exactly what capsaicin does to the heat channel, in mirror image. The molecule does not cool the tissue; it slips into a pocket inside the channel's voltage-sensing bundle — the S1-through-S4 transmembrane helices, around the residues the structural biologists label Tyr745 and Arg842 — and from there it shifts the channel's gating. Where the resting channel needs the cold of 26 °C to open, the menthol-bound channel will open several degrees warmer. In our hands the apparent threshold moves up by roughly **six to eight degrees**, carrying it clear across the temperature of a resting mouth. A mouth sits near **34 °C**. A channel that should be silent at 34 °C is, with menthol in the pocket, wide open and screaming cold. Nothing got colder. The threshold moved, and the body cannot tell the difference between a channel firing because the world is cold and a channel firing because a molecule moved its set-point. This is the entire trick, and it is a beautiful one: cool without cold, a winter the tongue reports from the middle of a warm room.

Two more details matter for anyone who wants to design on this axis honestly. TRPM8 needs the membrane lipid PIP_2 as a co-factor to gate at all — pull PIP_2 out of the membrane and the channel goes quiet no matter how much menthol is present — which is part of why cooling sensations ride and fade the way they do. And the channel desensitizes in a calcium-dependent way: the same calcium it admits feeds back to close it, so a cool built on TRPM8 has a natural arc — a swell, a plateau, a clean decline — rather than the monotonous flatness of a dye. When we tune a cool, we are tuning that arc as much as the peak.

The formula, and what "loud" means on the cold channel

Every axis on the Index resolves to one number by the same rule, and the cold axis is no exception:

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

EC_{50} is the concentration of a stimulus that drives its receptor to half of full response — the half-activation dose. It is an inverse measure of loudness: a *small* EC_{50} means the receptor answers to a *faint* whisper of the molecule, so a small EC_{50} is a loud stimulus. We fix the reference at the thing everyone already knows is cold — **menthol** — and anchor it to **1,000**. That choice sets the reference EC_{50} equal to menthol's own, and in our standard calcium-flux assay menthol reads a half-activation near **$EC_{50} \approx 40\ \mu M$**. Everything else on the axis is menthol measured against itself.

Work the formula once and the scale becomes intuitive. A molecule half as potent as menthol — one that needs twice the concentration to reach the same half-activation, EC_{50} ≈ 80 µM — scores 1,000 × 40 ÷ 80 = **500**. A molecule twice as potent, EC_{50} ≈ 20 µM, scores 1,000 × 40 ÷ 20 = **2,000**. The axis is a direct, linear read on how little of a thing it takes to convince the cold channel, with menthol planted at the center as the number everyone can feel. The per-axis normalization ceiling on a scorecard is **5,000**, which corresponds to an EC_{50} of 8 µM — a five-fold-louder-than-menthol cold, about as far as the axis is built to travel before the radar simply reads "maximum."

Here is the cold channel's pharmacology laid against that scale, drawn from our standard assay. The point of the table is not the individual numbers but the shape: how far apart the plant world's cooling molecules sit, and how silent the orange's own most abundant molecule is on this particular channel.

STIMULUS	SOURCE	EC_{50} (MM)	IFI-C
Menthol	peppermint	40	1,000 *(reference)*
(−)-Isopulegol	pathway intermediate	60	667
(−)-Menthone	peppermint	110	364
1,8-Cineole (eucalyptol)	eucalyptus	380	105
(R)-(+)-Limonene	orange (native)	— *(no TRPM8 response)*	≈ 0
Designed cucumber	engineered, whole-food	28.6 *(effective)*	1,400
Phoenix · SS-0096	engineered, whole-food	50 *(effective)*	800

Read the last three rows carefully, because they carry the chapter. The orange's signature molecule, the one it makes by the bucketful, does not register on the cold channel at all. The designed cucumber reads **1,400** — louder than neat menthol — from a plant that natively scores zero. And even our hottest object in the whole library carries a deliberate, designed cold at **800**. None of that is an accident of nature. All of it is written in.

Orange — the road already half-built

The orange is the more tractable of our two teachers, because it is already halfway down the road to cold and does not know it. In the shared index the orange carries two axes — Zero-Bitter and Cool-Burn — and the cool is no coincidence of ours. Citrus and mint share

a precursor. Both run the universal plastid route — the MEP pathway — to geranyl diphosphate, GPP, the ten-carbon building block that is the common ancestor of nearly every monoterpene a plant makes. From GPP both lineages take the same first committed step: a cyclase folds the chain into **limonene**, the bright citrus-smelling ring that is, by a wide margin, one of the most common monoterpenes in the entire plant kingdom. The orange makes it in floods — **d-limonene is on the order of 90–95% of the oil in the peel**, the volatile that sprays in a fine mist when you tear the rind — and even the juice carries it near **150 mg/L**, which, at limonene's molar mass of 136.2 g/mol, is roughly **1,100 µM** of the molecule dissolved in what you drink.

So the orange already runs the hardest and most expensive stretch of the cold road: it builds the precursor supply, in quantity, in dedicated oil glands, for its own reasons. What it lacks is only the short terminal tail that would carry a slice of that limonene down toward a cold molecule instead of out into citrus perfume. Our orange program — **120 constructs** — is an exploration of exactly that diversion: installing a mint-like hydroxylation-and-reduction branch that taps a controlled fraction of the limonene flux and finishes it to a genuine cooling note *inside* a citrus. Not an orange flavored with mint. An orange that makes a cold molecule of its own.

There is an honest catch here, and it is the kind of detail that separates a design that works from a napkin sketch that doesn't. The orange's limonene is the wrong-handed molecule. Stereochemistry is destiny for an enzyme: the orange pours out **(R)-(+)-limonene**, the right-handed enantiomer, while the entire canonical menthol pathway is built on **(S)-(−)-limonene**, its left-handed mirror image. The mint's first downstream enzyme, limonene-3-hydroxylase, is exquisitely stereospecific and will not efficiently touch the orange's (R)-limonene. You cannot simply graft the mint tail onto the citrus trunk and expect flux. So our route does not fight the orange's native cyclase; it lays its own final lane. We co-express an (S)-limonene synthase that draws from the shared upstream GPP pool, running a small parallel committed step beside the orange's own, and only *then* hand the correct-handed limonene to the cold tail. The road is half-built — but the last stretch, we pave ourselves, in the right direction.

The reward for that care is a design knob with a clean number on it. The perceived cool of the finished orange is set by one parameter: what fraction of the limonene flux we divert down the cold branch. Because limonene ($C_{10}H_{16}$) and menthol ($C_{10}H_{20}O$) are both ten-carbon, the diversion runs close to mole-for-mole, so the arithmetic is honest and simple. Start from the orange's ~1,100 µM limonene pool and route a slice of it to a menthol-type cooler:

- Divert **1%** → ~11 µM of cooler → TRPM8 occupancy 11 ÷ (11 + 40) ≈ **22%** — a whisper on the finish.
- Divert **2%** → ~22 µM → occupancy ≈ **35%** — a clear cool lift, arriving after the fruit.
- Divert **5%** → ~55 µM → occupancy ≈ **58%** — assertive, a cold that shares the stage with the citrus.

(That occupancy is the simple one-site form, $\theta = [X] \div ([X] + EC_{50})$; TRPM8's real cooperativity, a Hill coefficient near 2 to 3 across its four subunits, steepens the curve so the cool *arrives* over a narrow window rather than creeping in — which is precisely the sensation of a finish that turns cold.) We hold the orange program deliberately in the low single-digit percent, a lift you notice only on the back of the palate: the familiar fruit and an unfamiliar frost arriving together, the thing a beverage designer sketches on a napkin and assumes is impossible. The orange's other designed axis, the bitter-attenuation that quiets its native naringin and limonin, is a separate conversation — but it is worth saying that the same fruit we can teach to end cold, we can also teach to carry less of its own bitterness, and that both are the same act of finishing a plant into the fullest version of what it already is.

Cucumber — cool by reputation, not by receptor

The cucumber is the deeper lesson, because the cucumber is the plant whose very name *means* cool — *cool as a cucumber* — and the cucumber makes almost no cooling molecule at all. Its legendary coolness is a trick of water and freshness. The flesh is roughly **96% water**, and it is almost always eaten a little below mouth temperature, so part of what the culture calls "cool" is simple thermal mass: a cold slice warming in a warm mouth genuinely pulls heat, for a moment. It is worth doing that arithmetic once, because it reveals how feeble real cooling is compared to the receptor's illusion. A 100-gram slice taken from the refrigerator at 4 °C and warmed to a 34 °C mouth absorbs

$$Q = m \cdot c \cdot \Delta T = 100\ \text{g} \times 4.18\ \text{J·g}^{-1}\text{·K}^{-1} \times 30\ \text{K} \approx \mathbf{12{,}500\ J}$$

about twelve and a half kilojoules of genuine heat extraction — real, measurable, thermodynamic cooling. And it is gone in the few seconds the slice spends between warming and swallowing, a single brief sink that empties and is over. Against that, a cold *written on TRPM8* costs almost nothing and does not stop. A few tenths of a milligram of a cooling molecule, transferring essentially no heat whatsoever, holds the cold channel open for as long as the molecule sits in its pocket — a sustained winter from a mass of material you could not see. That is the leverage a design platform is built on: the receptor is a lever so long that micrograms of the right molecule outweigh kilojoules of chilled water, because the body is reporting a message, not measuring an energy.

The rest of the cucumber's coolness is aroma, and it is worth knowing exactly what that aroma is, because it is not a cold molecule either. The smell of a freshly cut cucumber is carried chiefly by a pair of nine-carbon aldehydes — **(2E,6Z)-nonadienal** above all, the compound that quite literally *is* the scent of cut cucumber, with (2E)-nonenal beside it. These are not stored in the intact fruit; they are *manufactured at the wound*. Cut the cucumber and you rupture its cells, releasing lipases that free linoleic and α-linolenic acid from the membranes; a 9-lipoxygenase adds oxygen to make a fatty-acid hydroperoxide; a hydroperoxide lyase cleaves that into a nine-carbon aldehyde; an isomerase tidies the double bonds into their final (2E,6Z) geometry. It is a demolition cascade that fires

only when the tissue is broken — which is why a cucumber smells of almost nothing until the knife goes in. And not one step of it touches TRPM8. The cucumber's "cool" is an olfactory impression of clean wet green, a sensation the culture long ago rounded up to cold. There is no menthol in it, no carvone, nothing that fires the cold channel once.

That gap — between a plant the mind files under *cool* and a plant that activates the cold receptor *never* — is precisely the opening a design platform exploits, and it is the cucumber's whole gift to us. It teaches us to separate the *idea* of cool from the *chemistry* of cool, and then shows us why reuniting them is so powerful. A cucumber already reads as cool to the mind. Lay a genuine, designed cooling molecule into that fresh-green frame and the fruit becomes cool twice over — once by reputation, once by receptor — and the two coolnesses reinforce each other the way menthol and ice do, each lending the other its credibility. The reputation makes the receptor's cold feel *right*; the receptor makes the reputation, at last, *true*.

What moving a cool actually costs, gene by gene

How much genetic machinery does it really take to move the cold? Less than you would think, which is the quiet thesis under the whole platform. The front of the menthol pathway — the MEP route to GPP, and very often limonene itself — is already running in almost any plant you choose, because GPP is a universal isoprenoid currency and limonene is made by citrus, conifers, and a scatter of herbs that have nothing to do with mint. What a target plant lacks is only the short downstream tail, and that tail is a countable list of enzymes:

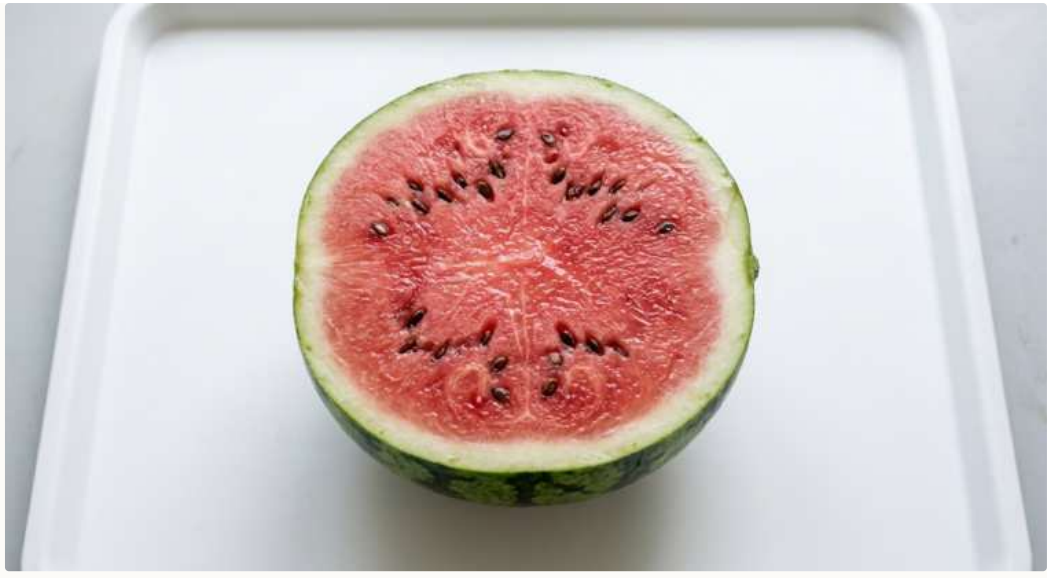
STEP	ENZYME	REACTION	PROTEIN CLASS
1	(−)-limonene synthase	GPP → (−)-limonene	monoterpene cyclase
2	limonene-3-hydroxylase (CYP71D13/15)	(−)-limonene → (−)-*trans*-isopiperitenol	cytochrome P450
3	isopiperitenol dehydrogenase	→ (−)-isopiperitenone	SDR dehydrogenase
4	isopiperitenone reductase	→ (+)-*cis*-isopulegone	enoate reductase
5	isopulegone isomerase	→ (+)-pulegone	isomerase
6	pulegone reductase	→ (−)-menthone	ketone reductase
7	menthone reductase	→ (−)-menthol	ketone reductase

The committed turn toward cold is step 2 — the hydroxylation, by a cytochrome P450, that oxygenates the limonene ring and points the flux down the menthol branch rather than out to perfume. (The regiochemistry of that single enzyme is the whole fork in the road: a hydroxyl placed at carbon 3 runs toward menthol; the same enzyme family placing it at carbon 6 instead runs toward carvone and the spearmint note — one oxygen atom's address decides which cool a plant becomes.) After the P450, the pathway is essentially a staircase of reductases and an isomerase walking the oxygenated ring down to menthol, trimming double bonds and setting stereocenters. Installing a cool, then, is rarely a matter of building a pathway from nothing. It is grafting a handful of terminal enzymes onto a precursor supply the plant already maintains for its own reasons — the same logic that lets capsaicin leave the pepper,

where five of the platform's seven modules transfer unchanged to any sequenced genome and only a few terminal enzymes are species-specific. Cold travels the same way.

The construct counts across our cold-axis hosts tell the story of difficulty plainly. The mints, which must express the whole tail at commercial fidelity, carry the deepest libraries; the fruits and the aromatic shrubs, where we are diverting an existing flux or tuning a native trace, need far fewer.

ORGANISM	CONSTRUCTS	PRIMARY AXES	ENGINEERING DIFFICULTY
Peppermint	2,360	C / M	Hard
Lavender	160	C	Hard
Orange	120	B / C	Hard
Eucalyptus	80	C	Hard
Spearmint	80	C	Hard
Camphor	40	C	Hard

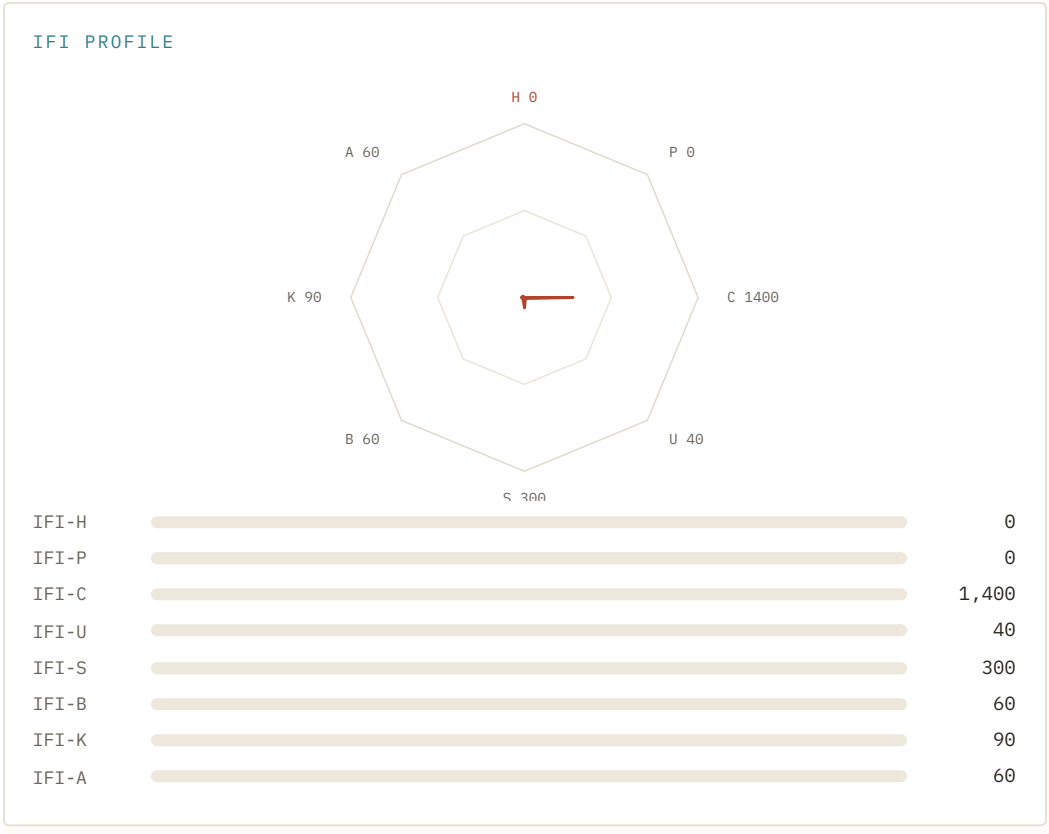


A melon in cross-section — cucumber's cucurbit cousin, another high-water, faintly green canvas where a designed cool reads as the fruit simply becoming more refreshing.

The list of plants this tail can travel to is long, and it maps almost exactly onto the plants the culture already calls cooling: the green-fresh family — melon, lettuce, celery, fennel, the whole crisp-and-watery shelf — each one a precursor supply in search of a terminal tail, each one a cucumber-shaped opportunity waiting for the real cold its reputation has been quietly promising all along.

Reading the designed cucumber

Here is the object itself — one of our family's favorite things in the whole library — read as a scorecard:



Cool-Burn at **1,400** — a real, loud cold, well above the menthol anchor of 1,000 — laid into a fruit that natively scores zero on the axis. Run the formula backward and that score corresponds to an effective half-activation of

> EC_{50}(effective) = 1,000 × 40 µM ÷ 1,400 ≈ **28.6 µM** of menthol-equivalent cold.

In the flesh, that is a vanishingly small payload. At menthol's molar mass of 156.3 g/mol, an effective cold near that level is delivered by on the order of a milligram of cooling monoterpene per kilogram of fruit — call it a few tenths of a milligram in a 100-gram portion, a trace you could never weigh on a kitchen scale, producing a cold that reads louder than the mint it borrows from. Set beside the twelve and a half kilojoules of chilled water the old cucumber spent on a whisper of real cooling, the designed fruit's economy is almost comic: micrograms of molecule, no heat moved, a winter that holds.

Where does a portion sit on the dose–response curve at that loading? If the delivered cool behaves like a molecule of effective EC_{50} near 28.6 µM, a standard bite lands well up the activation curve — in the two-thirds-occupancy range, present and unmistakable without tipping into the medicinal:

DELIVERED COOL (MENTHOL-EQUIV.)	TRPM8 OCCUPANCY	PERCEIVED
10 µM	~26%	a cool edge
20 µM	~41%	clearly cool
29 µM	~50%	half-max — the hinge
45 µM	~61%	a belonging cold
70 µM	~71%	assertive, near the ceiling of "fresh"

The small Sweetness, Umami, and Kokumi readings on the scorecard — **S 300, U 40, K 90** — are the cucumber's own fresh body, the faint green roundness that makes the thing taste like cucumber and not like a mint leaf. That is the design discipline the

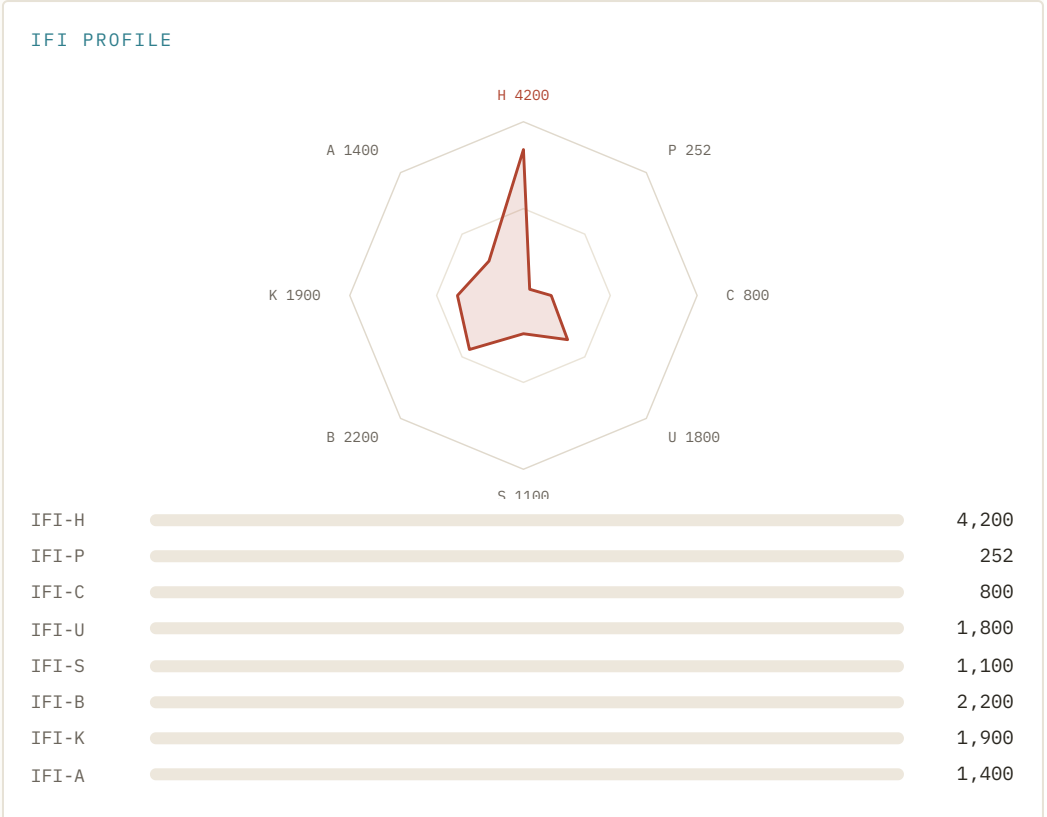
whole axis demands: the cold has to sit *inside* the vegetable's own frame, not on top of it. Push the cool past the "fresh" ceiling and the fruit tips over into toothpaste — a cucumber pretending to be a mint — which is the characteristic failure mode on this axis and the reason we tune occupancy rather than simply maximize it. Loudest is not best. Belonging is best.

Every number here carries its honest error bar. The IFI is a ratio of two EC_{50} half-activations, so its relative uncertainty is the two measurements' uncertainties added in quadrature. At our platform's batch coefficient of variation of **≤7%** on each, the combined figure is

$$CV(IFI) = \sqrt{(0.07^2 + 0.07^2)} \approx \mathbf{9.9\%},$$

so the cucumber's cool is honestly written **1,400 ± 140**. It is worth dwelling on how hard-won that tightness is. A wild peppermint field swings its menthol content 20–40% across a season with weather, maturity, and leaf position; the natural world simply does not hold a dose. That our engineered delivery holds a cold to one-tenth, batch after batch, is the quiet achievement under the loud number — a cool you can *formulate* around, not merely harvest and hope.

For contrast, and to show that this axis is a dial and not a switch, read the cold carried by the hottest object our family has ever designed — our full-spectrum flagship, which registers on all eight axes at once:



Its Cool-Burn reads **800** — an effective EC_{50} near 50 µM, a deliberate, designed cold tucked *beneath* an enormous heat. That is not a cool that announces itself; it is a counter-voice, a thread of winter woven under the fire so the burn has somewhere to resolve. The same axis, the same formula, the same receptor — set once to carry a cucumber and once to answer a flame. The honest limits are the same in both directions: monoterpenes are volatile and want to escape the matrix, so stability and encapsulation matter as much as the pathway; the stereochemistry has to be right or the cold reads

wrong; and off-target aldehydes or an overloud dose will turn a designed cool into a mouthwash. We design against all three by holding occupancy in the belonging range and letting the plant's own body carry the frame.

The larger map



Lettuce — the leafy edge of the green-fresh family, crisp and watery and filed under "cool" by a culture that never asked whether the cold was real. A canvas waiting for the molecule its reputation already promises.

The designed cucumber is not a novelty, and it is not a trick. It is a cucumber that is, at last, as cool as we always said it was — the sensation its name has carried for centuries finally matched by a molecule that fires the channel the name was always pointing at. That is the whole thesis of moving a sensation: not to make a plant into something it is not, but to let it become the fullest, most complete version of what it already half-is. The cool cucumber reads as the cucumber finally delivering on its promise, the reputation and the receptor agreeing for the first time.

And it points at a map larger than itself. Melon, lettuce, celery, fennel — the whole quiet family of crisp, watery, green-fresh plants the culture already calls cooling without ever checking whether the cold was real. Each is a precursor supply in search of a terminal tail. Each is a canvas the size of a cucumber, waiting for the genuine cold its reputation has been promising all along. We grow these plants the way we grow everything in the library — reverently, cleanly, toward the fullest flavor and the fullest nourishment a plant can hold — and on this axis the fullest version of the plant turns out to be, simply, the coolest one. The world told the truth about these plants a thousand years early. Our part is only to make it true. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Chapter 14 · Cool Inside Fire — *Phoenix and the thermal counterpoint.*

The strangest thing our family has ever grown is a pepper that is cold. Not cold instead of hot — cold *and* hot, at full volume, in the same bite, a fruit that drives the heat channel and the cold channel at once until the mouth, receiving two true and opposite reports in the same instant, stops being able to tell you what temperature it is. This is **Phoenix**, SKU **SS-0096**, the final cultivar in our flagship superhot series, the first plant we engineered to register on all eight axes of the Index at the same time — and the clearest proof the thermal axis has ever given us that cool and burn are not opposites but neighbors, close enough to hold in one hand.



Phoenix (SS-0096) on the microscope stage — our model predicts approximately 2.95 million Scoville units of heat, with a computed, deliberate cold running underneath it. Eight axes in one fruit.

Phoenix is best known for its heat: our model predicts approximately **2,937,948 Scoville units**, an IFI-H of **4,200**, the loudest designed burn in the library, built on two AI-designed substitutions in the capsaicin acyltransferase Pun1 — **S39L and L345G** — written in by CRISPR and held to a batch-to-batch coefficient of variation of **≤7%** against the **42%** that unimproved peppers show. But the heat is only the headline. The reason Phoenix closes the flagship series, the reason it is the eight-axis plant, is everything riding alongside the fire

— and on the thermal axis, that means a designed **Cool-Burn of 800**, the cold pathway's logic installed on purpose in a plant that had only ever known heat.



Eight hundred is not a loud cold. It is below the menthol anchor — a cool you would call gentle in a mint — but in a pepper carrying 4,200 of heat it is extraordinary, because it means the fruit is driving TRPM8 while it drives TRPV1, running the two thermal sentinels of the tongue at the same time. To understand why that produces a sensation the mouth cannot name, rather than a cancellation, we have to go down to the receptors, do the arithmetic the Index is built on, and watch what the two channels actually report.

The ladder we climbed

Phoenix did not arrive whole. It sits at the top of a ladder we climbed one cultivar at a time, each rung representing increasing computational design scope — more axes activated, greater predicted heat — the platform learning to speak on more of the eight lines at once. The series is the clearest record we have of what designed heat costs and what it buys.

CULTIVAR	SKU	PREDICTED SHU	IFI-H	AXES	PREDICTED SHU PER IFI-H POINT
Ember	SS-0087	866,000	1,570	2/8	552
Blaze	SS-0091	1,720,000	2,630	4/8	654
Firestorm	SS-0094	2,470,000	3,490	6/8	708
Solara	SS-0095	2,720,000	3,980	6/8	683
Phoenix	**SS-0096**	**2,937,948**	**4,200**	**8/8**	**700**

All SHU values are computational predictions from our flux and biosynthesis models.

The last column is worth lingering on, because it exposes something about what we are even measuring. Scoville units and IFI-H are not the same quantity. A Scoville reading is, at root, a dilution: the factor by which an extract must be thinned before a trained panel can no longer detect the burn, which makes it proportional to *how much* capsaicinoid the fruit holds. IFI-H is anchored instead to the receptor

— to EC_{50}, the concentration at which a stimulus drives TRPV1 to half of its full response — so it measures not abundance but *loudness*, how hard each molecule leans on the channel. The two track each other closely, which is why the ladder climbs on both at once. But they are not identical, and the ratio between them drifts: roughly 552 Scoville units buy a point of IFI-H at the bottom of the ladder, and roughly 700 near the top. Pushing the receptor's fractional activation higher takes disproportionately more capsaicinoid as the channel nears saturation — so concentration (SHU) climbs faster than normalized loudness (IFI-H), and each index point gets more expensive.

Notice, though, that Phoenix breaks the rise. Its 700 sits *below* Firestorm's 708 — the fruit earns more IFI-H per Scoville unit than the trend predicts. That is the signature of the two Pun1 edits. They do not merely let the plant make *more* capsaicinoid; they make a *more potent* capsaicinoid, one with a lower EC_{50}, so the index rewards it twice — once for abundance, once for loudness — and the per-point cost falls back. The enzyme is doing two jobs, and the ladder can see both.

One conversion makes the abundance concrete. The standard relation is about 16 Scoville units per part-per-million of capsaicinoid, so:

> $2{,}937{,}948 \text{ SHU} \div 16 \text{ SHU·ppm}^{-1} \approx 1.836 \times 10^5 \text{ ppm} \approx$ **18.4% capsaicinoids by dry mass.**

Our model predicts nearly a fifth of the dried flesh, by weight, would be the molecule that makes the fire — an abundance no wild plant on earth approaches. We designed the plant to reach for this abundance; this is what the design looks like.

Two sentinels, one flesh

The tongue reads temperature through two transient-receptor-potential channels that sit, for the most part, on separate nerves. **TRPV1** is the heat sentinel: a non-selective cation channel on the trigeminal endings, gated by temperatures above about **43 °C (109.4 °F)**, by acid, and — this is the trick capsaicin plays — by capsaicin itself. **TRPM8** is the cold sentinel: a different channel on a largely non-overlapping population of fibers, gated by temperatures below about **26 °C (78.8 °F)**, and by menthol.

Neither molecule changes the temperature of the mouth. The mouth stays near **37 °C (98.6 °F)** the entire time. What the molecules change is the *threshold* — the set point at which each channel decides it is time to fire — and they move it from opposite directions across that body-temperature line.

Capsaicin binds a pocket inside TRPV1, down among the transmembrane helices where the vanilloid group fits against residues the channel normally reserves for sensing heat. The binding lowers the channel's thermal threshold. A channel that would have waited for 43 °C now opens below 37 °C, so at ordinary mouth temperature it is already firing, and the brain receives a report of a

fire that is not there. The threshold has fallen by at least six degrees, across body temperature, and the body answers the only way it knows: it reports burning.

The cool agent we install does the mirror-image thing to TRPM8. It binds a pocket in the channel's voltage-sensing domain and raises the thermal threshold — shifts it *up*, toward and past 37 °C — so a channel that would have waited for a genuine chill of 26 °C now opens at the warm, unremarkable temperature of the mouth. The threshold has climbed by at least eleven degrees, across the same body-temperature line, from the other side. The brain receives a report of cold that is, physically, just as untrue as the fire, and just as real as a sensation.

Here is the symmetry that makes the whole chapter possible. Two channels, two thresholds, both resting on the far side of 37 °C from each other — one above, one below. Heat is made by dragging the upper threshold down through body temperature; cold is made by lifting the lower threshold up through body temperature. The mouth never moves. The molecules move the set points, and the brain's sense of temperature is nothing but a readout of which sentinels are firing. Drive both, and both fire, and both reports are true at once.

Why the brain cannot average

The reason this does not resolve into "lukewarm" is that TRPV1 and TRPM8 are **labeled lines** — separate wires carrying separate meanings to separate places. The hot line means hot wherever it fires and however it got triggered; the cold line means cold on the same terms. The brain does not sum them into a scalar, because there is no wire on which a sum could travel. It reads the firing rate on the hot line and the firing rate on the cold line as two independent numbers, on two independent dimensions, and nothing in its wiring averages one against the other.

We can write the two reports exactly. The fraction of a channel's population that is open at a given agonist concentration follows a Hill relation:

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

where θ is the fraction activated, [A] the agonist concentration, EC_{50} the concentration giving half-maximal response, and n the Hill coefficient — the steepness of the switch, which our TRPM8 assays fit near n ≈ 2.5. At [A] = EC_{50}, θ = 0.5 by construction, whatever n may be. The heat channel carries its own θ_H driven by capsaicin against TRPV1's EC_{50}; the cold channel carries its own θ_C driven by the cooling agent against TRPM8's EC_{50}. There is no third equation combining θ_H and θ_C into a temperature, because temperature was never a single number the tongue measured — it was always an inference the brain drew from two sentinels, and we have simply made both sentinels lie, truthfully, in opposite directions.

That steepness is worth one worked line, because it tells you how fast a thermal report rises. Solving the Hill relation for the concentration ratio that moves a channel from one-tenth to nine-tenths open:

$[A]_{90}$ / $[A]_{10}$ = (9 ÷ ⅑)^(1/n) = 81^(1/2.5) ≈ **5.8×**

So a channel climbs from barely-on to nearly-saturated across roughly a sixfold change in agonist. Put a number on the middle of that climb: at twice the agonist's own EC_{50}, the same n ≈ 2.5 curve puts 2^2.5 ÷ (1 + 2^2.5) ≈ 5.66 ÷ 6.66 ≈ **85%** of the channels open — double the stimulus past half-max and the report has all but finished rising. The sensation is not a slow dimmer; it is a switch with a short, steep throat. In a single bite, the heat crosses that throat first and enormous, a wall of TRPV1 fire — and then, threaded through it, impossibly, the cold crosses its own throat, a menthol-like lift the brain keeps trying to file as relief and cannot, because the fire is still climbing on a line of its own. Cool and burn, the two faces of this axis, fused in one fruit, each one true, neither able to cancel the other because they were never speaking on the same wire.

The Index, worked

The number on each axis is not an opinion. It is a ratio, and the whole Index is one formula applied eight times — anchored to a stated convention on each axis, the way Scoville's scale is defined by dilution-to-threshold, not by a single measured reference compound:

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

Each axis has a fixed reference stimulus — capsaicin for the heat line (H), AITC at a convention constant of 2.1 µM for the pungency line (P), menthol for the cool line (C) — and the reference is defined to score exactly 1,000, because substituting it for the candidate gives 1,000 × EC_{50}(ref) ÷ EC_{50}(ref) = 1,000. Because each axis is anchored to its own defined reference point, scores across different axes reflect loudness within their own benchmark rather than a single common scale. A score above 1,000 means the candidate is *louder* than its axis's reference: it reaches half-activation at a *lower* concentration, so a little goes further. A score below 1,000 means the candidate is quieter. The formula inverts cleanly, which lets us read Phoenix's whole profile back into the language of the receptors:

$EC_{50}(candidate) \div EC_{50}(reference) = 1{,}000 \div IFI$, and loudness multiple = IFI ÷ 1,000.

AXIS · RECEPTOR	IFI	LOUDNESS ×(IFI/1000)	EC_{50} VS REFERENCE (1000/IFI)
H · Heat · TRPV1	4,200	4.20×	0.238
P · Pungency · TRPA1	252	0.252×	3.968
C · Cool-Burn · TRPM8	800	0.80×	1.250
U · Umami · mGluR1	1,800	1.80×	0.556
S · Sweetness · TAS1R2·R3	1,100	1.10×	0.909
B · Zero-Bitter · TAS2R38	2,200	*(attenuation)*	–
K · Kokumi · CaSR	1,900	1.90×	0.526
A · Astringency · PRPs	1,400	1.40×	0.714

Read the heat line first. An IFI-H of 4,200 says Phoenix's designed capsaicinoids reach half-maximal TRPV1 activation at **0.238** of the reference concentration — a hair under a quarter — which is the same statement as 4.20× louder. The burn arrives on less molecule than the reference needs, which is exactly what the two Pun1 edits were for.

Now read the line this chapter is named for. An IFI-C of 800 says the cooling agent reaches half-maximal TRPM8 activation at **1.25×** the concentration menthol needs:

> EC_{50}(Phoenix cool) = (1,000 ÷ 800) × EC_{50}(menthol) = 1.25 × EC_{50}(menthol)

It is a *gentler* cold than a mint's — it needs a quarter more agonist to speak as loudly — and that gentleness is deliberate. A cold as loud as the heat would turn the bite into a shouting match; a cold pitched below the menthol anchor threads *through* the fire instead of fighting it. Carry the Hill steepness forward and the restraint becomes a quantity: to push that cool agent from half-open to nine-tenths open takes the 2.41× of its own EC_{50} that the n ≈ 2.5 curve demands, which is 2.41 × 1.25 = **3.01× menthol's half-maximal concentration**. We know, to the fold, how much cold we have asked the fruit to carry, and we asked it to carry a thread.

It helps to carry that ratio once in real units, even though the Index is built not to need them. Take the reference menthol's half-maximal TRPM8 concentration as a representative 80 µM — the precise figure is assay-dependent, and the score is invariant to it, since only the ratio survives the formula. Then Phoenix's cool agent, at IFI-C 800, reaches half-activation near 1.25 × 80 µM = **100 µM**, and nine-tenths activation near 3.01 × 80 µM ≈ **241 µM**. Swap the reference figure for any other value and both derived concentrations move with it in lockstep; the 800 does not budge. That invariance is what lets a tomato, a mint, and a pepper designed to 2.9 million predicted Scoville units be listed on a single page — each score reflecting loudness within its own axis, against its own defined reference point.

The one axis that does not read as a loudness is Zero-Bitter, and honesty requires the footnote: IFI-B scores *attenuation*, the 20–65% quieting of TAS2R38, not an EC_{50} made louder. A 2,200 there means the bitterness that a superhot's alkaloids would otherwise throw has been turned *down*, politely, so it never crowds the sequence. The other seven axes are made louder; bitterness is the one we made softer, and the Index keeps the sign straight.

Two letters in an enzyme

The heat begins with a condensation. Capsaicin is **8-methyl-N-vanillyl-6-nonenamide**, and it is assembled from two halves the plant builds on two separate lines. The phenylpropanoid branch — **PAL → C4H → 4CL → HCT → COMT** — runs in every vascular plant and delivers the aromatic half, ferulic acid and onward to vanillin, which a pepper-specific aminotransferase (pAMT) turns into vanillylamine. A branched-chain fatty-acid line delivers the other half, an 8-methyl-6-nonenoyl group activated as its coenzyme-A thioester. The two halves meet at **Pun1**, the acyltransferase also

called AT3, which joins vanillylamine to the branched acyl-CoA and releases capsaicin and free CoA. The length and saturation of that acyl chain set the ratio of capsaicin to dihydrocapsaicin, and with it the *shape* of the burn — how fast it blooms, how long it stays.

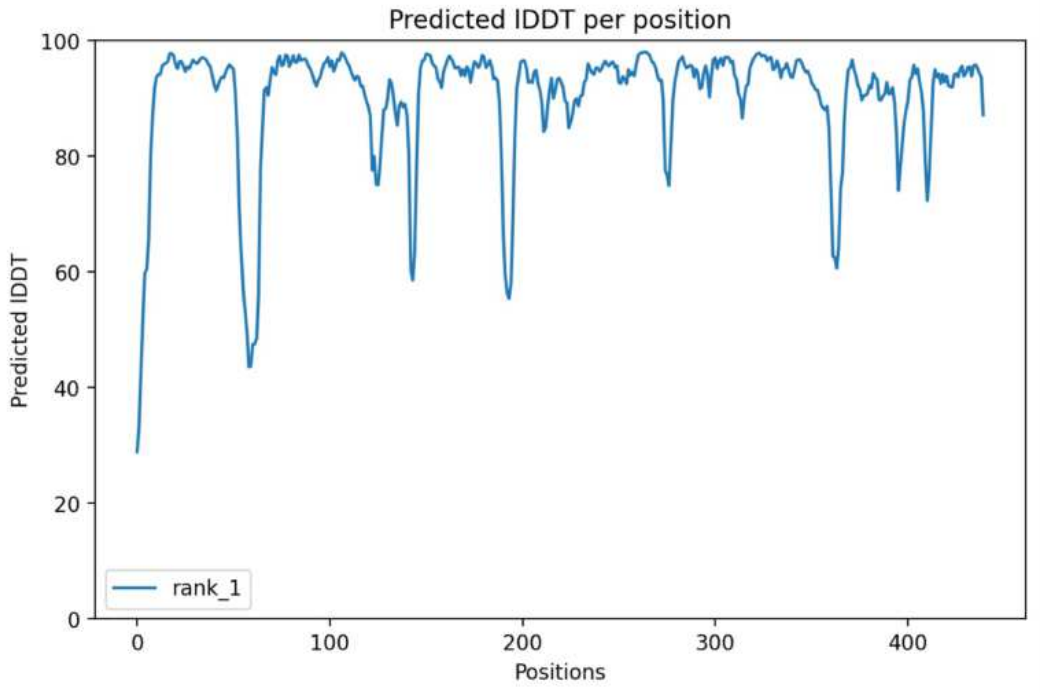


FIG. 14 — the Pun1/AT3 model coloured by pLDDT confidence; the substrate-binding domain and the active-site cleft resolve at high confidence, which is where the two edits land.

Every breeding program before ours chased heat by selecting the phenotype and praying the genotype followed. We inverted it. A protein language model scored every single-residue substitution in Pun1 for its predicted effect on catalytic efficiency and fold stability. Our flux model predicts that control in capsaicinoid biosynthesis sits upstream in the phenylpropanoid pathway — at 4CL and HCT/CCoAOMT — not at the terminal Pun1/AT3 step. Pun1 is the final capsaicin synthase; it labels the product, it does not govern the rate. What the Pun1 edits achieve instead is improvement in product potency — the capsaicinoids assembled have a lower EC_{50}, so each molecule lands harder on TRPV1. Two substitutions emerged for the shipped construct, each with a predicted free-energy improvement, ΔΔG, reported by the model:

EDIT	REGION	EFFECT	ΔΔG (KCAL·MOL⁻¹)	BOLTZMANN FACTOR, EXP(−ΔΔG/RT)
S39L	N-terminus	binding affinity ↑	−2.1	≈ 35×
L345G	active-site cleft	steric relief	−1.8	≈ 21×
C175S†	core	disulfide-misfold prevention	−0.9	≈ 4.6×
Combined (S39L + L345G)		*double variant — shipped*	**−3.9**	**≈ 730×**

† C175S is a structural annotation from our modelling. It was not included in the shipped construct.

The right-hand column is thermodynamics, not decoration. A free-energy difference becomes a population ratio through the Boltzmann relation, with RT ≈ 0.592 kcal·mol^{-1} at 298 K (25 °C):

> `factor = exp(−ΔΔG ÷ RT) = exp(3.9 ÷ 0.592) = exp(6.59) ≈` **730×**

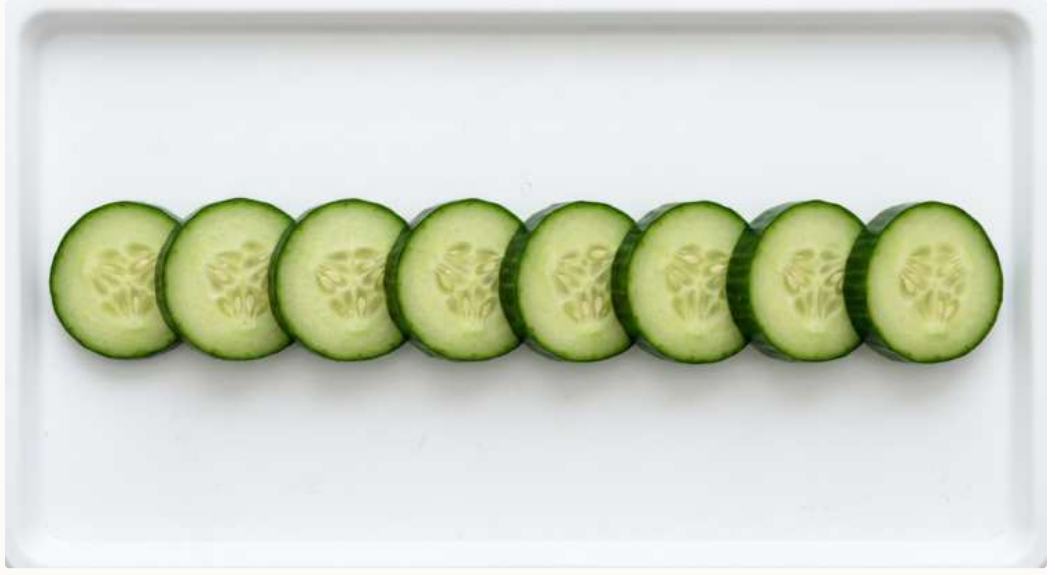
If the two edits acted independently, the enzyme would favor its correctly folded, substrate-ready state by roughly seven hundred and thirty times more than the wild-type sequence does — far more

functional acyltransferase present in the cell, more capsaicinoid assembled with the structural profile that lowers EC_{50}. That is the mechanistic thread tying two substitutions to a compound that lands harder on TRPV1. We flag the honest caveat in the same breath: additivity is an approximation, because real substitutions can interact — a cleft relieved by L345G changes the environment S39L sees — so the predicted gain can land short of the product of the parts. The model predicts; the design is built on that prediction.

Installing a counterfeit cold

None of that enzymology makes cold. It makes the hottest flesh our models have designed, and into that flesh we laid the second, stranger thing. The cool is a *pathway transfer*: a trace of the menthol route's logic — specifically the downstream reductions, the reductase steps that saturate a cyclic monoterpene ketone into the cooling alcohol that gates TRPM8 — grafted into a *Capsicum chinense* that already runs its capsaicinoid line at full tilt. We did not move a mint into a pepper. We moved the terminal idea of a mint — the last chemical turn that produces a menthol-class cold — into a plant that had no business being cold, so that one flesh now builds a hot counterfeit and a cold counterfeit side by side.

It is the same move as the cool cucumber, run in the opposite emotional register. There, cold was laid into a plant the culture already calls cool, deepening a quiet the tongue half-expected. Here, cold is laid into the loudest hot plant we have designed, *because* the contradiction is the point.

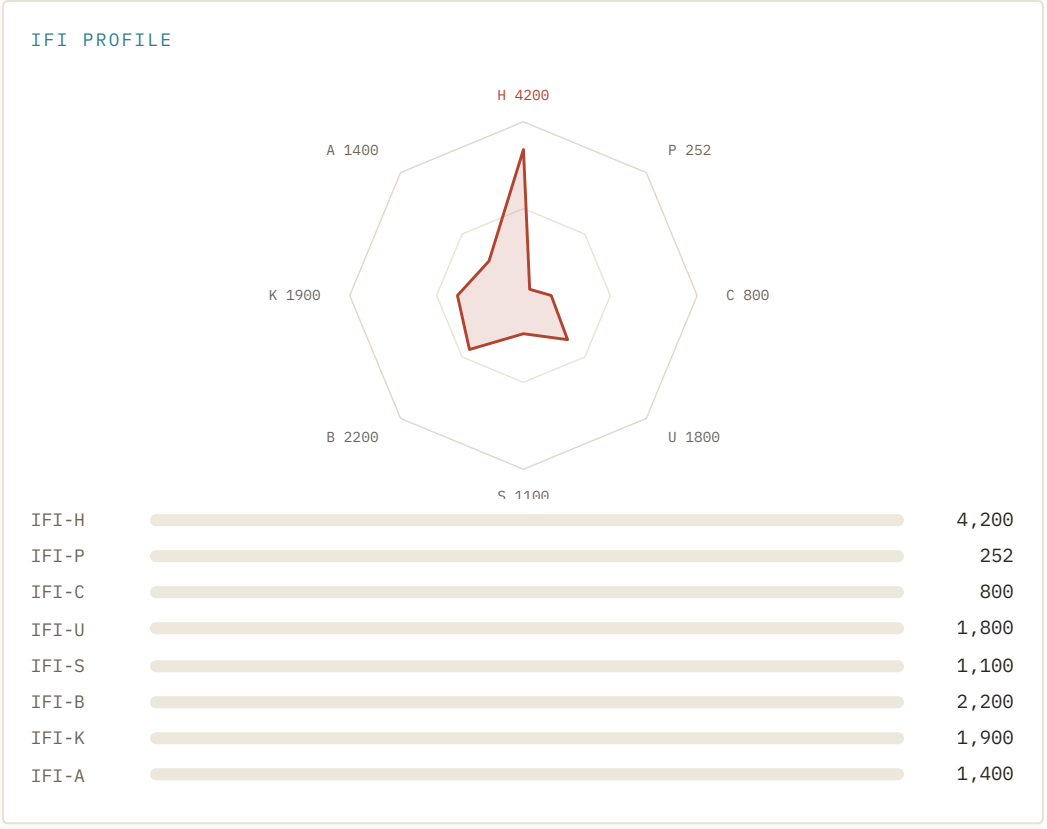


The cucumber in cross-section — the gentle, expected cool. Phoenix installs the same cold logic in a flesh the tongue expects only fire from.

The comparison sharpens what the number means. A cool cucumber and Phoenix can sit near the same stretch of the Cool-Burn axis — both gentle, both below the menthol anchor — and taste nothing alike, because IFI-C measures only the loudness on the TRPM8 line, not the company it keeps. Put 800 of cold beside a whisper of everything else and you get a cucumber. Put the identical 800 beside 4,200 of fire and you get the coldest hot design in the library. The axis is honest; the context is everything; and the number lets us place both on one page and still know they are opposites in experience.

Choreography

It is worth asking why Phoenix's eight axes do not simply collapse into noise, because the answer is the same principle that lets cool and burn coexist at all. Each axis is a different receptor on a different line: TRPV1 for the heat, TRPM8 for the cold, TAS1R2 and TAS1R3 for the sweetness, TAS2R38 for the bitterness the fruit has learned to attenuate, mGluR1 for the umami, CaSR for the kokumi, TRPA1 for the pungency, the salivary proline-rich proteins for the astringency. Eight parallel channels, not one crowded one. A single bite does not have to average them into a muddy middle; it can carry all eight, and the brain can read all eight, because no two of them ever touched the same wire.



What the fruit *does* have to manage is timing, because eight sensations arriving in the same millisecond would overwhelm rather than delight. That is what the staged delivery is for — a release choreographed so the sweetness opens, the heat climbs, the cold threads through, the bitterness stays politely absent, the savory depth and the kokumi body fill underneath, each axis given its own moment on the tongue. Read the profile block as a score rather than a list: eleven hundred of sweetness to open the door, four thousand two hundred of heat as the main movement, eight hundred of cold as

the impossible grace note laid across it, eighteen hundred of umami and nineteen hundred of kokumi as the ground the whole thing stands on. The nanoparticle delivery that lets those arrive in sequence rather than in a heap is its own long story, told in full elsewhere; what matters on the thermal axis is that the cold is designed in, placed on purpose, and timed to land as the hinge of the sequence — the note threaded through the fire that the mouth cannot resolve and cannot stop tasting.

What the numbers cost

A profile this precise is only worth the ink if the fruit delivers it twice. The decisive figure in the whole program is not the heat; it is the move from **42%** batch-to-batch variation in unimproved peppers to **≤7%** in ours. Hold the mean at Phoenix's predicted 2,937,948 SHU and watch what each coefficient of variation does to a single harvest:

```
42% CV → σ ≈ 1,233,938 SHU → a one-sigma band of 1.70M – 4.17M
7% CV → σ ≈ 205,656 SHU → a one-sigma band of 2.73M – 3.14M
```

The old pepper is a plant you *hope* about; its burn swings across a factor of more than two from fruit to fruit. The designed line lands inside a tenth of its mean. And because variance scales as the square of the coefficient of variation, the improvement compounds:

```
(42 ÷ 7)² = 6² = 36× less variance
```

Run the same arithmetic on the cold, where there is less room to spare. At IFI-C 800 and 7% CV, one sigma is ±56 units, a band of **744 – 856** — the thread stays a thread, batch after batch, never swelling into a blast or thinning into nothing. And the mean itself can be pinned tighter still by sampling more harvests, since the standard error of the mean falls as the square root of the count: across ten batches, 7% ÷ √10 ≈ **2.2%**, so the central value of that cold is known to within about ±18 units. Precision, here, is not fussiness. It is the difference between a demonstration and a party trick.

Honesty about the limits belongs in the same section. Phoenix is not a pepper you cook with casually; it is a proof, grown to show that the platform can put any axis into any plant, and its eight-axis performance leans on a delivery system far more elaborate than anything a kitchen needs. The cold has failure modes we have had to design around. If the staging collapses and all eight axes fire together, the result is a muddle, not a marvel — the choreography is load-bearing. If the cooling agent falls below its TRPM8 threshold through degradation or dilution, the fire stands alone and the whole point is lost. And the taster is a variable we cannot engineer: TRPM8 sensitivity differs from person to person, so a palate on the cold-blind end of the range will read only heat where another reads the full contradiction. Serving temperature tilts it too — a dish served hot warms the mouth and biases TRPV1, thinning the cold thread; a chilled one biases TRPM8 and lets the cold speak up. The number on the page is a property of the design. The sensation in the mouth is a negotiation between the fruit, the plate, and the person, and we

would be lying to pretend otherwise. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory.

Phoenix is where the Cool-Burn axis shows its true shape. We call the axis Cool-Burn because at the extreme, cool and burn are reachable from the same design: turn the heat line up and the cold line up in the same flesh and you get both, not a cancellation but a counterpoint, two melodies the mouth plays at once. Everything gentler on this axis — the clean beverage mint, the quiet floral lavender, the cool cucumber — lives somewhere on the line between Phoenix's two poles, closer to one end or the other, carrying its cold beside quieter company. Phoenix is where the line's ends touch. It is the coldest hot design we have built, and it taught us, with eight numbers and a steady hand, that the thermal axis has no true opposite inside it — only a distance, and the choice of whether to collapse it.



PART IV OF IV

The Kitchen & the Craft

the mouth · the glass · the plate · the temperature of care

Chapter 15 · Cooling in the Mouth — *confection, lozenge, and the sugar we left out.*

The mouth is where cool was first commercialized, and it is still where the thermal axis lives most densely — the mint, the lozenge, the gum, the toothpaste, the cough drop, every one of them a small delivery device built to set a cooling molecule onto the trigeminal field and keep it there. This is the chapter for the confectioner and the oral-care formulator, and its governing problem is the one the whole cooling axis turns on: not how loud the cool is, but how *long*. Loud is easy — a pinch of menthol and the mouth flares arctic in a single heartbeat. Lasting is the entire craft, a problem with two clocks and a good deal of honest arithmetic underneath it. So we begin at the receptor, where the cold that isn't there is manufactured, and work out to the candy on the tongue.

The receptor that reports a cold that isn't there

Cooling, like heat, is a lie the nerve tells and the brain believes. The molecule at the center of it is **TRPM8**, a non-selective cation channel standing in the membrane of the cold-sensing trigeminal and dorsal-root fibers. It is built as a homotetramer: four identical subunits, each threading the membrane six times, arranged so that the outer helices (the S1–S4 bundle, a voltage-sensor-like domain) cradle a small hydrophobic cavity, while the inner helices (S5–S6) line the ion pore down the fourfold axis. Below the membrane sits a C-terminal TRP helix and the melastatin-homology shoulders, and at the seam between them a molecule of **PIP_2** — phosphatidylinositol 4,5-bisphosphate, a minor membrane lipid — is bound as an obligatory cofactor. Hold that lipid in mind; it is the whole story of why cool fades.

In its resting life TRPM8 is a thermometer. Genuine cold — below roughly 26 °C — shifts the energetics of the channel so that it opens, admits sodium and calcium, and depolarizes the fiber, and the brain reads the volley as *cold*. Menthol does not cool anything. It slips into that hydrophobic cavity in the S1–S4 bundle — a pocket in which a single aromatic residue, tyrosine-745 on the S2 helix, is the latch that makes the channel menthol-sensitive — and once bound it stabilizes the open state. In the language of biophysics it shifts the channel's voltage dependence toward the potentials the resting fiber actually sits at, and it drags the apparent thermal threshold warmer, from the twenties up past mouth temperature. The consequence is exact and a little uncanny: at 35 °C, a channel that would have needed real cold to open is now already opening, firing the same cold afferent it fires in January. The brain cannot tell a chemically opened TRPM8 from a cold-opened one, because they are the identical channel carrying the identical current. Capsaicin makes a fire that is not there by lowering TRPV1's heat threshold; menthol makes a cold that is not

there by raising TRPM8's cold threshold. They are mirror images across body temperature, and the mouth is the stage both of them prefer.

One honesty before we go further. The axis is called **Cool-Burn**, not *cool*, and the name is earned. The sensation we design on it is bidirectional thermomodulation, and it is not the work of one receptor but a small committee — TRPM8 for the clean cold, TRPV3 and TRPV1 for the paradoxical warmth of camphor, and TRPA1 for the sharp bite that menthol itself begins to recruit at high dose. The Index anchors the axis to a single clean reference — the half-activation of TRPM8 by (−)-menthol — because you cannot measure against a committee, but we never let the tidiness of the number hide the biology: a molecule can score on this axis through any of those channels, and most of the interesting ones speak to more than one.

Loudness is a number; duration is a different number

The Index gives every axis a single figure, and it is worth saying precisely what that figure does and does not capture. For any stimulus *c*:

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

EC_{50} is the concentration at which a molecule drives its receptor to half of full response. Invert it against a fixed reference and scale by a thousand, and *loudness* becomes a score. For the cooling axis the reference is (−)-menthol itself, so menthol sits at IFI-C ≈ 1,000 by construction, and everything else is read against it. Rearrange the formula and it tells you each molecule's own half-activation directly:

$$\mathrm{EC}_{50}(c) = \mathrm{EC}_{50}(\mathrm{menthol}) \times 1{,}000 \div \mathrm{IFI\text{-}C}(c)$$

Two of our workhorse coolants carry canonical cooling scores, and the math falls straight out. **Menthyl lactate** sits at IFI-C ≈ 533. Then EC_{50}(menthyl lactate) = EC_{50}(menthol) × 1,000 ÷ 533 = 1.88 × EC_{50}(menthol): it is a little under half as potent, meaning it takes nearly twice the concentration to reach the same half-activation. **WS-23** sits at IFI-C ≈ 2,290, so EC_{50}(WS-23) = EC_{50}(menthol) × 1,000 ÷ 2,290 = 0.44 × EC_{50}(menthol): it is more than twice as potent per molecule than menthol is.

To make that concrete we pin the reference. We set the IFI-C reference constant at 80 µM for (−)-menthol — stated plainly as a convention anchor, since the published range runs from a few µM to two hundred depending on the assay, and ours is simply where our reference line lands. From that one anchor every mass concentration follows, by each molecule's formula weight:

AGENT	IFI-C	EC_{50} (× MENTHOL)	EC_{50} (MOLAR)	EC_{50} (MASS)	CHARACTER	LONGEVITY
(−)-Menthol	1,000 *(ref)*	1.00×	~80 µM	~12.5 mg/L	bright, minty, volatile	short — the firework
Menthyl lactate	533	1.88×	~150 µM	~38.5 mg/L	mild, heavy, low-odor	long
WS-23	2,290	0.44×	~35 µM	~6.5 mg/L	odorless	very long

The mass figures are just molar EC_{50} times formula weight: menthol at 156.3 g/mol gives 80 µmol/L × 156.3 µg/µmol ≈ 12.5 mg/L; menthyl lactate at 256.4 g/mol gives 150 µM ≈ 38.5 mg/L; WS-23 at 185.3 g/mol gives 35 µM ≈ 6.5 mg/L. Nothing exotic — stoichiometry and a reference line.

Now the trap, and it is the most important sentence in the chapter. **IFI-C scores loudness, not duration.** Look at the table again: WS-23 is the loudest molecule on it *and* the longest-lasting, while menthol is the quietest *and* the briefest — but those are two independent facts wearing one row. Loudness is set at the receptor, by EC_{50}. Duration is set everywhere else: by how fast the molecule evaporates off a warm wet surface, how tightly it holds to mucosa, how quickly saliva carries it down the throat. The Index, by design, says nothing about any of that. A formulator who reads only the IFI number and builds a lozenge from the loudest coolant will be baffled when it still fades. The craft of cooling in the mouth lives almost entirely in the second column the Index does not print.

The two clocks: receptor adaptation and salivary washout

So the fade. Free menthol arrives fast, floods TRPM8, drives a bright cold — and then two clocks start running against it at once.

The first clock is the receptor's own, and it is the PIP_2 we set aside. Every open TRPM8 is admitting calcium along with sodium, and that incoming calcium is a signal to the cell. It activates a calcium-sensitive phospholipase C sitting in the same membrane, and that enzyme hydrolyzes PIP_2 — cleaves the very lipid the channel needs as a cofactor into diacylglycerol and IP_3. As the local PIP_2 is consumed the channels lose their footing and close, not because the menthol has left but because the ground beneath them has dissolved. This is calcium-dependent desensitization, and it is fast: tens of seconds from the first bright flood to a channel population that has quietly adapted and gone half-deaf to a molecule still sitting in its pocket. It is the reason a strong mint is strongest in its first breath, no matter how much menthol remains.

The second clock is the mouth's. Saliva flows — a third of a milliliter a minute at rest, several times that the moment you start sucking a sweet — and it dilutes, washes, and carries the molecule down and away. A small, volatile molecule like free menthol loses this race badly: a large fraction of it never even reaches steady state on the tongue because it flashes off the warm wet surface into the breath (which is exactly why a mint is something you smell as much as feel). A lozenge that is all free menthol loses on both clocks simultaneously — desensitized from within in half a minute, washed and evaporated from without in a minute more. A firework: brilliant, and over.

We can put a number on the washout, which turns the intuition into a lever. Treat the mouth as a well-mixed film of saliva being carried off at flow *Q*. When the lozenge stops feeding molecule to the film, the local concentration *C* decays roughly exponentially with a washout time constant τ set by the molecule's volatility and its grip on the mucosa: C(t) = C_0·e^(−t/τ). The cool persists only while *C* stays above EC_{50}, so the time a molecule keeps cooling after its source is gone is

$$t^* = \tau \times \ln(C_0 / EC_{50})$$

That little logarithm is the whole design brief. You get more lasting cool two ways: start higher above EC_{50} (raise C_0, which the logarithm rewards only weakly — doubling the starting margin buys you one extra τ-worth of ln-2, about 0.7 time constants), or lengthen τ itself (which the equation rewards linearly — double τ and you double the entire tail). The arithmetic says, flatly, that you do not out-dose a fade — you out-*persist* it. Volatility is the enemy; formula weight is the friend; the quiet heavy molecules win the long game.

Building a plateau — the blend across durations

If one molecule cannot both open bright and hold long, the answer is to stop asking it to. We blend across durations: choose coolants with different time constants τ so their fading curves overlap, each handing off to the next, and the sensation stops being a spike and becomes a plateau. Free menthol takes the front — the immediate bright attack everyone expects a mint to open with, spent cheaply because we know it will not last. **Menthyl lactate**, milder and nearly twice menthol's formula weight and far less volatile, takes the body: it arrives gently and lingers for many minutes, resisting the saliva wash. **WS-23**, odorless and the most persistent of the three, carries the tail — cold coverage with no mint aroma to muddy the flavor, long after the candy is a memory. Layered, their windows overlap into a single held note.

Here is that plateau derived, for a lozenge dosed as the recipe below is, assumptions stated so you can argue with them. Take a lozenge that dissolves over about ten minutes as you suck it, feeding its actives into a salivary film carried off at roughly 1.5 mL/min. Each active's steady local concentration is just its release rate divided by that flow, compared to the EC_{50} computed above:

ACTIVE (PER LOZENGE)	DOSE	RELEASE RATE	PEAK LOCAL CONC.	EC_{50}	× EC_{50}	ITS WINDOW
Menthol (from 0.9 g oil, ~40%)	9.0 mg	0.9 mg/min	~600 mg/L	12.5 mg/L	~48×	0–1 min · the front
Menthyl lactate	15 mg	1.5 mg/min	~1,000 mg/L	38.5 mg/L	~26×	1–7 min · the body
WS-23	3.75 mg	0.38 mg/min	~250 mg/L	6.5 mg/L	~38×	2–11 min · the tail

Every active sits ten to fifty times above its own half-activation while the candy lasts. During the suck the receptors are saturated — drenched — and perceived intensity is capped not by any shortage of molecule but by receptor number and by that first clock, desensitization. This is why "loud is easy," and why dumping in more

menthol does not make the front colder, only faster to adapt. The dose-response curve is cooperative, with a Hill slope near 2, and it flattens hard:

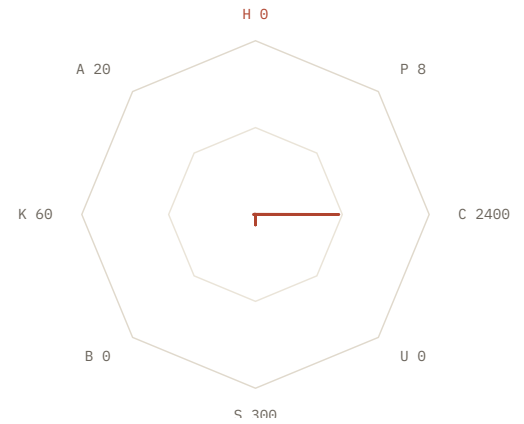
LOCAL CONCENTRATION	TRPM8 ACTIVATED
0.3 × EC_{50}	~8%
1 × EC_{50}	50%
3 × EC_{50}	90%
10 × EC_{50}	99%
30 × EC_{50}	~99.9%

By three times EC_{50} you are at ninety percent; by ten times the channel is as open as it will ever be. Sitting at twenty-six to forty-eight times, we are far out on the flat shoulder where more molecule changes nothing. So the plateau is built not during the suck but *after* it, in the washout, where $t^* = \tau \cdot \ln(C_0/EC_{50})$ decides who is left. Put representative time constants in — menthol volatile at $\tau \approx 0.3$ min, menthyl lactate heavy and mucoadhesive at $\tau \approx 2$ min, WS-23 lipophilic and slow at $\tau \approx 3$ min — and the front fades about a minute after the candy is gone ($0.3 \times \ln 48 \approx 1.2$ min), the body holds some six or seven minutes ($2 \times \ln 26 \approx 6.5$ min), and the tail runs past ten ($3 \times \ln 38 \approx 10.9$ min). Three overlapping windows, one continuous cool. These τ values are illustrative — honest order-of-magnitude, not a bench measurement, and real salivary flow varies five- to tenfold between a resting mouth and a watering one — but the *shape* of the answer is robust, and it is the shape the recipe is built to hit.

```
SECOND-WIND COOLING LOZENGE · makes ~40 · oral-care / confection
– isomalt base                          200 g
– engineered clean-cool peppermint oil   0.9 g (menthofuran-free)
– menthyl lactate                        0.6 g
– WS-23 (cooling agent)                  0.15 g
– spearmint oil (engineered)             0.1 g (for sweet-fresh round)
– citric acid                            pinch
```

1. Melt the isomalt slowly to hard-crack, 165 °C, and let it fall to 130 °C off the heat — too hot and the volatile menthol flashes off before it is captured.
2. Stir in the menthyl lactate and WS-23 first; they are heat-stable and want full dispersion through the melt.
3. At the last moment, fold in the peppermint and spearmint oils and the pinch of citric acid, working fast and gently to keep the volatiles in the melt rather than in the air.
4. Deposit into molds; cool fully before unmolding. The cool front is the peppermint; the long plateau is the lactate and WS-23; the sweet-fresh round is the spearmint; the acid lifts the whole thing and keeps it from going flat.

IFI PROFILE



IFI-H	0
IFI-P	8
IFI-C	2,400
IFI-U	0
IFI-S	300
IFI-B	0
IFI-K	60
IFI-A	20

Encapsulation extends the same logic: spray-dry the oil into a carbohydrate carrier, or thread menthol into the cavity of a β-cyclodextrin, and it is metered out slowly rather than all at once — lengthening τ by hand. It is the right move for a chewing gum, where the base is worked ten minutes and a bare oil would be gone in two. It can also be overdone: encapsulate everything and you delete the bright front, leaving a cool that is all patient tail and no attack. The attack is spent on purpose; a mint with no opening is one no one reaches for twice.

The engineered peppermint — deleting the off-ramp

The front of that plateau is peppermint, and the quality of the whole object rests on the quality of that oil, so it is worth meeting the plant at the gene. Menthol is the end of a short, beautiful enzymatic cascade that runs in the glandular trichomes of *Mentha × piperita* — the silvered hairs you crush between two fingers for the smell. It begins when geranyl diphosphate is cyclized to (+)-limonene; a cytochrome P450 hydroxylates that to *trans*-isopiperitenol; a dehydrogenase and a reductase carry it to isopiperitenone and on to cis-isopulegone; an isomerase turns that to **(+)-pulegone**, which is the hinge of the entire pathway. From pulegone the clean road runs through pulegone reductase to (−)-menthone and then, by menthone reductase, to **(−)-menthol** — the molecule we are after.



The cooling plants under glass at golden hour, where the engineered peppermint is grown to a fixed menthol spec season after season.

But at that pulegone hinge there is an off-ramp, and it is the source of everything wrong with ordinary peppermint. A second enzyme, **menthofuran synthase**, pulls pulegone sideways into **(+)-menthofuran** — the musty, slightly medicinal note that haunts commodity peppermint oil, the thing that makes a cheap mint taste faintly of cough syrup. Menthofuran is not a trace nuisance you can distill away cleanly; it rides with the menthol, and its fraction swings with the weather, the cultivar, and the hour of harvest. Our engineering move is the obvious one once you can see the map: we delete menthofuran synthase. With the off-ramp gone, the pulegone that would have leaked into menthofuran has nowhere to go but down the clean road — pulegone reductase, menthone, menthol. Flux is forced toward the molecule we want; in our design, the off-note is predicted to fall below the method's limit of detection — not because we scrubbed it out but because the plant can no longer make it. A deleted off-ramp delivers cleanliness by construction, which is a far steadier thing than cleanliness by purification.

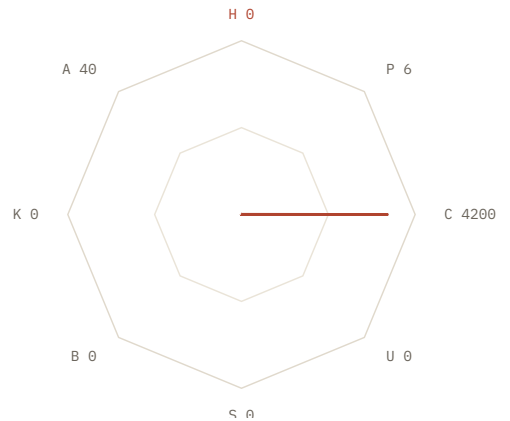
That construction is also what makes the plateau repeatable, where the platform's consistency figure earns its keep. A conventionally grown peppermint oil carries a batch-to-batch coefficient of variation around **42%** in its key constituents — the menthol wanders, the menthofuran comes and goes — so a lozenge built on commodity oil has a front of a different height every run. Our engineered oil holds a batch CV of **≤7%**. And the receptor arithmetic turns that tight input into an almost perfectly steady output, for a reason the dose-response curve already told us: the actives sit far out on the saturated shoulder, where a ±7% shift in concentration moves activation by a fraction of a percent. Operating above thirty times EC_{50}, the cool front is buffered against ingredient variation by the flatness of the curve itself. What is *not* buffered — what saturation cannot rescue — is the off-note, because an off-note is perceptual at a few hundredths of a percent and has no half-activation shoulder to hide on. That is precisely why we delete menthofuran at the gene rather than merely diluting it: the one defect the physics will not forgive, we remove at the source.



The sequencer bench where every batch is read back, so the menthol fraction holds to spec and the menthofuran stays below detection.

Here is the engineered oil's own profile — and note what it does *not* score, because the honesty of it is the point:

IFI PROFILE



IFI-H	0
IFI-P	6
IFI-C	4,200
IFI-U	0
IFI-S	0
IFI-B	0
IFI-K	0
IFI-A	40

The oil reads loud on cool and silent on sweet. Its sweetness axis is a flat zero, as it must be — menthol binds TRPM8, not the TAS1R2·R3 sweet receptor, so on a receptor-anchored index there is simply nothing there to score. Remember that zero; it is about to eat a spoonful of sugar.

Peppermint is the loudest cooling plant we grow, but it is not the only one, and the axis is a small botanical family worth laying out. Each speaks to a slightly different channel, which is why they feel different in the mouth even when we call them all "cool":

HOST	PRODUCTS	AXES	SIGNATURE MOLECULE	WHAT IT SPEAKS TO
Peppermint	2,360	C / M	(−)-menthol	TRPM8 — the clean cold
Lavender	160	C	linalool / camphor	TRPM8-adjacent + TRPV3
Eucalyptus	80	C	eucalyptol (1,8-cineole)	weak TRPM8 + TRPA1
Spearmint	80	C	(−)-carvone	aroma and round, not a primary cooler
Camphor	40	C	camphor	TRPV3 / TRPV1 — the warm-cool pole
Orange	120	B / C	terpene esters	faint cool, and bitter-masking

The honesty in that table is in the right-hand column. Spearmint, which the lozenge recipe calls for, is carvone-dominant and barely touches TRPM8 — its job is aroma and a sweet-fresh roundness, not cold, and we would mislead you to call it a cooler. Camphor sits at the warm end of the cool-burn axis, a counterirritant that speaks mostly to TRPV3 and TRPV1, which is why it reads as a cool that turns warm. Only peppermint delivers the clean TRPM8 cold at volume. A grower reaching for "more cool" reaches for menthol; the others are there for color and contradiction.

The sugar that is not there

Now look back at the lozenge and notice the sugar that is absent. The base is isomalt, not sucrose — a sugar-free confection, and not a diet gimmick but a design opportunity, for a reason that lives one step up from the receptor, in the brain that reads it. Cool and sweet

are cross-modally linked — bound together by a lifetime of cold sweet things, so that the mind lends a little sweetness to anything sufficiently, cleanly cool. We saw the oil score a flat zero on the sweet axis; a receptor-anchored index can only report what binds TAS1R2·R3, and menthol does not. Yet the clean cool reads as faintly sweet anyway — not a conflict but the whole trick. The sweetness is not in the molecule; it is borrowed from memory, triggered by the cold. A well-built cool can carry the *impression* of sweetness past the point where the sugar stops, which is exactly why a sugar-free lozenge built on a clean menthol front does not taste deprived.

We will say the rest of it plainly and only once, because the ethos of this library is a quiet one and we are a family that would rather show a thing than preach it: leaving the sugar out is not a health claim, and we make none. It is a choice about what a cool confection can be — a pleasure that asks a little less of the body — and the engineered cool is what lets that choice taste like abundance rather than absence. The cold does the work the sugar used to do. The mouth, borrowing sweetness from a memory of every cold sweet thing it has ever loved, never notices the trade.

Oral care — where cool is the signal of clean

Oral care deserves its own note, because it is the largest home of designed cool by volume and the place where the sensation does the most psychological work. The cool of a toothpaste is, functionally, a *signal of clean* — the brand's promise made sensory, the reason a mouth feels freshly scrubbed whether or not anything more was. That is a heavy load, carried almost entirely by menthol and the WS coolants on the trigeminal field, against the same two-clock problem stretched longer and made daily. The cool must survive the brush, the foam, the rinse, the spit, and the flush of saliva, and still be faintly present minutes later when the user judges the product by whether the clean still lingers. That survival is a τ problem, and the answer is the same bound, heavy, low-volatility coolants — menthyl lactate, WS-23, encapsulated menthol — that carry the lozenge's tail past its washout. A toothpaste with only free menthol foams cold and rinses to nothing; one with a WS-23 tail is still quietly cool when you close the bathroom door.

There is an edge case here the lozenge does not face: the surfactant. The sodium lauryl sulfate that makes a paste foam is a membrane-disrupting detergent, and it both perturbs how menthol partitions to the tissue and transiently blunts the sweet receptor — which is why orange juice tastes wrong just after brushing. A cool for toothpaste has to be robust to that chemistry in a way a cool for candy never does; the bound coolants, which sit in the tissue rather than the foam, come through it.

And a clean, menthofuran-free mint matters here most of all, because this is the use with the highest cadence in a person's life — twice a day, every day, for years. An off-note forgivable once is intolerable at that rhythm: the musty edge no one quite notices in a single lozenge becomes, at a thousand uses, the reason a mouth quietly distrusts a tube. Saturation buffers the cold against ingredient

drift; nothing buffers the off-note. Cleanliness of the cool is, in oral care, not a luxury but the entire proposition, and a deleted off-ramp is the one way to guarantee it twice a day for a decade.

Dark chocolate and cool-mint — two engineered plants on one shard

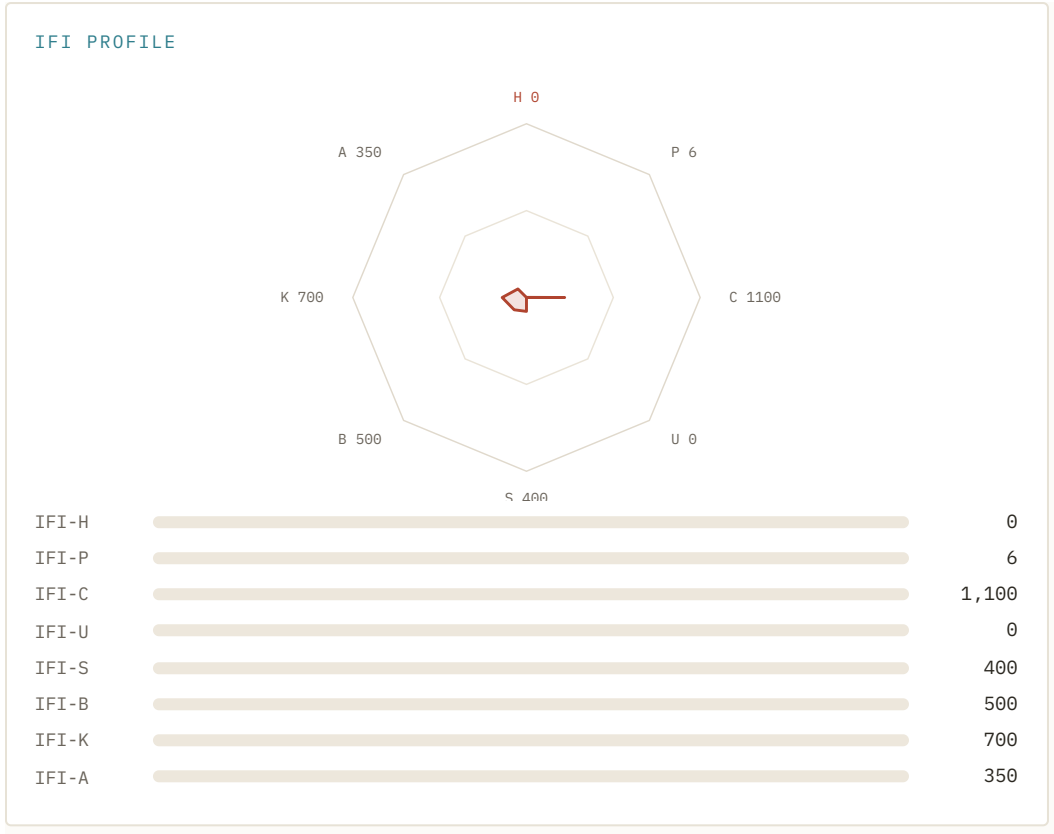
Not every cool in the mouth is a lozenge, and the oldest mint pairing in confection deserves rebuilding from the molecule out — because mint and chocolate is not merely a tradition but a thermomodulation problem. The chocolate brings body and a softened bitterness; the mint brings a cold that cuts the richness; and the two must meet without either carrying its old flaw into the other.

```
DARK CHOCOLATE & COOL-MINT THINS · makes ~24 · vegetarian
— dark chocolate, 70%, engineered low-bitter cacao   250 g
— engineered clean-cool peppermint oil               4 drops (~0.2 g)
— flaky salt                                          to finish
```

1. Temper the chocolate — melt two-thirds to 45 °C, add the final third, bring down to 31 °C, working it until it sets with a snap.
2. Stir the peppermint oil into the tempered chocolate; four drops is enough, and the clean engineered oil means no medicinal menthofuran edge to fight the cocoa, while the low-bitter cacao lets the cool read against sweetness instead of against a tannic wall.
3. Spread thin on parchment, scatter flaky salt, set at cool room temperature. Snap into shards.



Cacao in cross-section — the engineered low-bitter bean that lets the cool read against the chocolate rather than against a wall of tannin.



The chocolate card is a whole different balance from the lozenge, and the profile shows it. Here the cool-axis score (IFI-C 1,100) makes the cool a *counterpoint* rather than the whole show — playing against the kokumi body and softened bitterness that the other axes describe, with each axis anchored to its own reference convention; the numbers indicate design intent within each separate axis rather than a single unified scale. The cold cuts the richness the way a mint-chocolate always has, but cleaner at both ends: the peppermint carries no off-note into the cocoa, and the low-bitter cacao reduces the bitterness that would otherwise compete with the mint, so each makes room for the other instead of shouting over it. Note too how the cool is deliberately dialed down — four drops, not forty — because at the receptor a cold that sat far out on the saturated shoulder would flatten the chocolate rather than lift it. Cooling, as counterpoint, wants to live near its EC_{50}, in the responsive middle of the curve where a little more or less actually moves the sensation, not out on the drenched plateau where the lozenge lives. Same molecule, same receptor, opposite half of the dose-response curve, and an entirely different craft.

Honest limits, and the failure modes that teach them

A chapter about control should be candid about where control ends. Desensitization cannot be defeated, only managed — the PIP_2 clock runs no matter how cleverly you blend, and a cool pushed harder just adapts faster and, past a point, recruits the TRPA1 bite that turns cool to sting (the *burn* half of cool-burn, arriving uninvited). The washout model that gave us $t^* = \tau \cdot \ln(C_0/EC_{50})$ is a teaching model, not a measurement — salivary flow swings five- to tenfold between a dry mouth and a watering one, and a real mouth is not well-mixed. And the Index's own cooling number, so useful for ranking loudness, is mute on the one property the whole chapter turns on, duration; forget that and you build a loud mint that fades without ever understanding why.

The failure modes are the same truths seen from the kitchen. Overheat the isomalt and the menthol flashes off before the melt can hold it — the front collapses and you are left with a sweet, flat, all-tail

lozenge, correct on paper and lifeless on the tongue. Overdose the free menthol and the bright attack turns to a sting and a bitterness as menthol begins to pull on TRPA1 and the bitter receptors, and it still fades in a minute because volatility does not care how much you loaded. Over-encapsulate and there is no attack at all. Let the menthofuran creep back — an incomplete knockdown, or an oil oxidizing on the shelf — and the musty note returns at the one cadence, daily oral care, that cannot forgive it. Each failure is a clock or a curve ignored. Honor both — the receptor's adaptation and the mouth's washout, the saturated shoulder and the responsive middle — and the cool stops being a firework and becomes what the confectioner has chased for a century: a cold that is clean, that lasts, and that the body receives as a small, deliberate kindness.

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

Chapter 16 · Cooling in the Glass — *the long, slow pour.*

Cool is, before it is anything else, a beverage sensation. More people meet the thermal axis in a glass than anywhere else on the table — iced tea sweating in the afternoon, mint water, a cold lemonade, a mocktail going quiet over ice — and the glass is where designed cool does some of its most useful work. A drink carries a cooling molecule into the mouth dissolved, dispersed, and married to real cold in a way no solid ever will: it floods the whole trigeminal field at once, the coolant arriving everywhere the swallow reaches and arriving on top of a genuine chill the ice has already laid down. This is the chapter for the beverage maker, and its governing fact is a small piece of arithmetic we will take the trouble to prove: real cold and counterfeit cold do not merely add. They multiply.

That single sentence is the whole reason a cold mint drink feels colder than its mint and its ice have any honest right to make it — and the reason a careless one tips past refreshment into a sting. To read the multiplication with intention rather than luck, we have to understand the receptor doing the compounding, the number that scores it, and the stubborn physics of getting an oily little molecule to behave in water.

The cold sentinel, and why a drink is its truest vehicle

The molecule that reads cold in the mouth is **TRPM8**, a non-selective cation channel in the cold-sensitive endings of the trigeminal nerve — the bare endings that also report a scrape or the grit of a cut onion. It is a **homotetramer**: four identical subunits around a central pore, each threading the membrane six times, the S5–S6 segments lining the channel and the four S1–S4 bundles forming a **voltage-sensor-like domain**, the allosteric engine that decides whether the pore stands open or shut. When it opens it passes Na^+ and, the detail that matters downstream, Ca^{2+}, and the cation rush fires exactly the volley the ending would fire if the tissue had truly gone cold. The brain keeps no independent thermometer; it believes the volley. A mint lowers no temperature — it persuades the one nerve that would know.

Menthol does not force the pore. It slips into a pocket inside the S1–S4 bundle, settling against a key tyrosine on the second helix, and tilts the channel's own arithmetic. TRPM8's opening is a sum of votes — cold casts one, membrane voltage another, the signaling lipid PIP_2 (phosphatidylinositol 4,5-bisphosphate) stands as a required cofactor, and menthol casts a vote of its own. Each agonist shifts the channel's **voltage dependence of activation** — the half-activation voltage, $V_{1/2}$ — toward the potentials a resting nerve actually sits at. Cold shifts it; menthol shifts it; a drink that is both cold and minted shifts it twice, in the same direction, through the same gate. That is the mechanical root of the multiplication.

A glass is the truest vehicle for this receptor because it does three things a plate cannot. A coolant in water meets the receptor as free molecules rather than a waxy crumb that must melt and spread; a liquid wets the entire trigeminal field — lips, palate, the throat going cold on a swallow, the sinuses opening on the next breath — so the cool blooms across far more of the nerve than any solid; and the ice is no garnish but a second, genuine agonist of the very same channel, the thing that turns an ordinary cool into the compounded one this chapter is built around.

There is a shadow to name at the outset, because it is the fault line every beverage cool can fall across. Menthol is a clean agonist of TRPM8 only within a window. Carried high enough, the same molecule begins to recruit **TRPA1**, the irritant channel that reads reactive chemistry as a sharp, prickling sting — the medicinal bite a brutal peppermint leaves at the back of the throat. The clean cool and the medicinal sting are one molecule read at two doses, and almost everything that goes wrong in a cooling drink is a dose that has strayed, somewhere in the glass, from the first into the second.

Real cold and counterfeit cold — the arithmetic of multiplication

To show that the ice and the menthol multiply rather than add, we treat TRPM8 as what it is: a voltage-gated channel whose open probability follows a **Boltzmann relation**. At a resting membrane voltage V, the fraction of channels open is

$$P_o = 1 \div (1 + \exp[(V_{1/2} - V) \div k])$$

where $V_{1/2}$ is the half-activation voltage and k is the slope factor. TRPM8 carries a small gating charge — $z \approx 0.9$ — so $k = RT \div zF \approx$ **28 mV** near room temperature, a shallow dependence that makes the channel easy to nudge. Hold a cold-sensitive ending at its resting **V = −60 mV** and fix k = 28 mV, and the whole sensation reduces to one question: where is $V_{1/2}$?

At a neutral, body-warm mouth, $V_{1/2}$ sits far positive — take **+100 mV** as a working value — and the channel is all but shut. Cooling the tissue shifts $V_{1/2}$ negative; menthol shifts it negative; and the two shifts, to a good first approximation, **add in $V_{1/2}$**. Watch what that addition does to P_o, which depends on $V_{1/2}$ through an exponential:

STIMULUS	$V_{1/2}$ SHIFT	$V_{1/2}$ (MV)	P_o AT −60 MV	FOLD OVER STILL, WARM WATER
still, warm water (baseline)	0	+100	0.0033	1.0×
ice alone (real cold)	−60	+40	0.0274	8.3×
menthol alone (counterfeit cold)	−70	+30	0.0386	11.7×
ice **and** menthol together	−130	−30	0.255	77.5×

Work the last row by hand so the leap is undeniable. With both stimuli, $V_{1/2}$ = +100 − 60 − 70 = **−30 mV**; the exponent is (−30 − (−60)) ÷ 28 = 30 ÷ 28 = 1.07; exp(1.07) = 2.92; P_o = 1 ÷ (1 + 2.92) = **0.255**. Against the still-water baseline of 0.0033, that is a **77-fold** opening of the channel.

Now test it against addition. If cold and menthol merely summed, the two together would give roughly (8.3 − 1) + (11.7 − 1) + 1 ≈ **19×**. They give 77×. The additive prediction is wrong by a factor of four. Multiply the two fold-changes instead — 8.3 × 11.7 ≈ **98×** — and you land close to the truth, a shade high only because P_o is nearing its ceiling and the sigmoid is saturating. The sensation is multiplicative because P_o is an *exponential* function of a $V_1/_2$ that the two agonists push **additively**: add inside the exponent, multiply outside it. A cold mint drink is cooler than the sum of its parts for the same reason compound interest outruns simple.

A second coupling deepens the effect: because cold and menthol are allosteric partners on one channel, **menthol's own potency rises as the tissue cools** — its half-activating concentration falls with temperature. The ice shifts $V_1/_2$ directly *and* makes every menthol molecule louder, which is why a formula that sings over a full glass goes flat as the last of it warms — the long, slow pour a slow decline down the temperature coupling.

Reading the glass by the number

A sensation we cannot measure is one we cannot design, and the Index turns loudness into a single figure by one rule:

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

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response — small when a molecule is loud, large when it is faint. Inverting it makes the score read the right way up: **a lower EC_{50} earns a higher number.** We anchor the axis so **IFI-C = 1,000** is the half-activation of our wild-type cooling reference, peppermint's own (−)-menthol read against TRPM8, and fix that reference EC_{50} at a working **80 µM** so the arithmetic stays concrete. Every drink's cool is then a ratio away from a concentration. Menthol's molar mass is **156.27 g/mol**, so the 80 µM reference is 80 × 10^{-6} mol/L × 156.27 g/mol ≈ **12.5 mg/L** of menthol-equivalent — the unit formulators actually think in.

Read the four drinks of this chapter by that rule:

DRINK	IFI-C	EC_{50} (MM)	MENTHOL-EQUIV AT HALF-MAX (MG/L)	PER 250 ML GLASS
reference menthol	1,000	80.0	12.5	3.13 mg
Mountain-Mint Iced Tea	1,600	50.0	7.81	1.95 mg
Cucumber–Lavender Cooler	1,500	53.3	8.33	2.08 mg
The Warm-Cool Toddy	900	88.9	13.9	3.47 mg
Spearmint–Lime Agua Fresca	800	100.0	15.6	3.91 mg

Each EC_{50} is a single division. The iced tea at **IFI-C 1,600** has EC_{50} = 1,000 × 80 ÷ 1,600 = **50 µM**; the agua fresca at **IFI-C 800** has EC_{50} = 1,000 × 80 ÷ 800 = **100 µM**, a cool a shade softer than reference menthol, exactly as spearmint's rounder character should read. The whole axis of these drinks lives between roughly **8 and 16 mg/L** of menthol-equivalent, which across a 250 mL glass is **two to four milligrams** of coolant — a pinch dissolved through a whole tumbler.

Read the table the right way up and the design payoff is plain: **the louder the cool, the less of it a glass needs.** The iced-tea line reaches half-activation at 7.81 mg/L — 1.95 mg across the glass — while the gentle carvone cool of spearmint, at half the loudness, needs twice the mass to speak at the same level. Potency is economy, and economy is safety: every milligram you do *not* have to add is a milligram that cannot bead, cannot spike, cannot wake the sting.

One number keeps the rest honest: the error. Our platform holds batch-to-batch output to a coefficient of variation of **≤7%**, against the **42%** that unselected wild-type populations drift across. Because the Index is a ratio, errors propagate in quadrature; run the reference as a tight internal standard each session and a reported score inherits essentially the candidate's own ≤7%. An **IFI-C 1,600** is therefore 1,600 ± ~110 — a real band, not a point — where the same cool built on a wild population, drifting at 42%, would carry a band of ±670: a glass that lands faint one day and harsh the next, from the same recipe and the same hand. A drink is something you pour again and again, and that is exactly where ±7 against ±42 is the difference between a signature and a gamble.



Tea is the oldest vehicle for cool in a cup — and an engineered clean-cool mint, steeped or blended in, is tea's cold without tea's bitterness or mint's medicinal edge.

The solubility problem, told honestly

Every first attempt at a cooling drink humbles the maker the same way. Menthol is an oily, ring-shaped alcohol with a water–oil partition coefficient (log P) near **3.3** — it would rather sit in fat or air than in water — and its solubility in water is only about **0.42 g/L** (420 mg/L) at room temperature. Carry it into a drink naively and it beads, floats, and strikes the lip in a hot little slick instead of a smooth, dispersed cool.

But here the honest mathematics corrects a lazy story. We need only **8–16 mg/L** of menthol-equivalent to sit anywhere on this chapter's axis, and water will hold **420 mg/L** before it saturates — a headroom of **thirty to fifty-fold**. Menthol is therefore *not* thermodynamically too

insoluble for a beverage; at the doses that matter, water has room to spare. The beading is a **kinetic** failure, not a solubility ceiling. A crystal or oil droplet of menthol dissolves slowly, and until it has finished, the liquid around it is **locally saturated** — a microdroplet at the lip can carry 420 mg/L, which is **2.7 mM**, more than thirty times the 80 µM clean window and squarely into the concentration where menthol wakes TRPA1. That is the slick: not too much menthol on average, but too much in one undissolved place, firing the irritant channel locally while the rest of the drink reads faint. The fix is never "less flavor" but better dispersion — so the two-to-four milligrams the glass holds are spread evenly as free molecules, every one below the sting.

The craft has three honest answers, and they are physics, not folklore. **Infuse the whole leaf**, so the oil arrives bound in plant matter and released slowly, never as a concentrated droplet. **Carry the coolant in a trace of syrup or a clean emulsifier**, which keeps the oil dispersed and robs it of the chance to coalesce. Or **reach for the water-friendlier relatives** — menthyl lactate dissolves more kindly and carries almost no aroma; the odorless carboxamide coolants cool cleanly with little of menthol's high-dose bite. Our own lean, as a family that has always cooked clean and plant-first, is the fourth way: engineer the mint so the cool it delivers is already clean and generous, so whole-leaf infusion — the gentlest carrier there is — is enough, and no droplet of neat oil ever has to meet the lip.

Volatility is the solubility problem's twin, and it governs the arc of the slow pour. Menthol is aromatic because it evaporates — the cool you smell on the first sip is menthol leaving the glass for your nose — and over a long drink that aromatic top note fades even as the dissolved cool persists. A drink built only on volatile menthol goes quiet as it breathes off; one that leans partly on the non-volatile relatives holds its cool to the bottom of the glass but smells of less. The maker decides how much of the cool should be *scent* and how much *sensation* — in a glass, unlike a leaf, time is a variable, and the cool is only as durable as the molecule carrying it.

The palette of cooling plants

The cool does not come from a laboratory; it comes from a lineage of aromatic plants, most of them in the mint family, whose glandular hairs brim with monoterpenes. Our work is to tune how loudly those terpenes land on TRPM8, and how far across the trigeminal field they bloom. The hosts we speak cool through, and the molecule each one carries:

PLANT	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	COOLING SIGNATURE
Peppermint	2,360	C / M	Hard	(−)-menthol — the loud, clean reference cool
Lavender	160	C	Hard	linalool / linalyl acetate — floral, barely cool
Orange	120	B / C	Hard	a bright cool riding a bittersweet peel
Eucalyptus	80	C	Hard	1,8-cineole (eucalyptol) — airy, sinus-wide
Spearmint	80	C	Hard	(−)-carvone — round, sweet, gentle
Camphor	40	C	Hard	the warm shadow — a TRPV3 agonist, not TRPM8

Peppermint carries the deepest construct library — **2,360** — the richest route to the loudest coolant, and nearly every lever on the axis can be rehearsed in it first. The route is the **five-gene menthol pathway**: a terpene backbone, geranyl diphosphate, is cyclized to (−)-limonene by limonene synthase, then walked through hydroxylation, oxidation, and three reductions — limonene-3-hydroxylase, an isopiperitenol dehydrogenase, an isopiperitenone reductase, a pulegone reductase, and a final menthone reductase — until it arrives at **(−)-menthol**. The stereochemistry is the whole game. Menthol has three stereocenters and so eight possible stereoisomers, and only the natural (1R,2S,5R) (−)-menthol fits the TRPM8 pocket with real authority; its mirror image is a faint echo, its diastereomers near-silent. Much of what those 2,360 constructs do is keep the final reduction clean while starving the side-route to menthofuran that otherwise bleeds yield and dulls the note.

Spearmint is the same plant one enzyme away. Where peppermint hydroxylates its limonene ring at carbon three and heads for menthol, spearmint hydroxylates at carbon six and arrives instead at **(−)-carvone** — one difference of regiochemistry, one carbon, and the entire character of the cool turns. Carvone is a genuinely weaker TRPM8 agonist than menthol, which is why spearmint reads at **IFI-C 800** where peppermint reads near twice that: the molecule itself is quieter, rounder, sweeter on the channel, the cool of a garden rather than a glacier. Loudness and character are written into the molecule's shape — one carbon of regiochemistry — long before they reach the tongue.

The rest of the palette fills the edges. **Eucalyptol** opens the sinuses with an airy, wide cool that covers more of the field than its modest potency suggests — a lesson that coverage and loudness are not the same thing. **Lavender** is barely a coolant at all; its linalool is a floral whisper that reads cool by association more than by receptor, which is why it belongs as an accent and never a lead. And **camphor** is the paradox of the palette: a molecule the nose files under "cold" that is, at the receptor, a **warm** agonist — it opens TRPV3, the mouth's warm sensor, and even quiets TRPA1 — reading as cooling only because its sharp volatility floods the field and the brain scores the rush as fresh. A controllable warmth inside cool's reputation — and one drink here uses it.

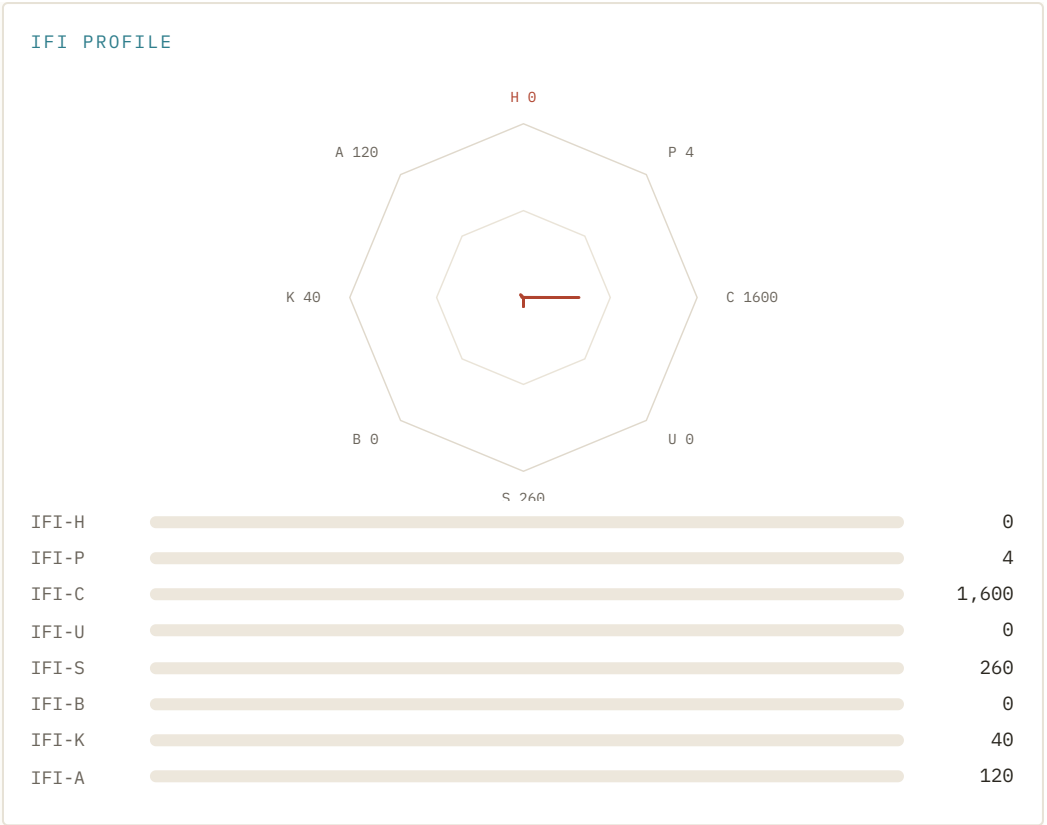
The recipes — four placements on one axis

A drink is a receptor target made pourable. Each of the four below sits at a deliberate point on the cool axis, and each is, in the family's way, vegetarian, clean, and built without reaching for more sugar than the glass needs.

The first is the loud, pure cool — deep in TRPM8 territory, no warmth beneath it, the bitterness engineered out of its bed so nothing fights the cold.

```
MOUNTAIN-MINT ICED TEA · serves 4 · vegetarian / zero added sugar
– green tea, engineered low-bitter       4 cups, brewed, cooled
– engineered clean-cool peppermint        1 small handful, bruised
– monk-fruit extract                      to taste
– lemon                                   1, juiced
– ice                                     to fill
```

1. Brew the green tea gently — our low-bitter line means you can steep longer for body without the tannic grab, so the cool has a soft bed to sit on rather than a bitter one to fight.
2. Bruise the engineered peppermint and steep it in the still-warm tea five minutes; the clean oil gives a cold lift with no medicinal tail, released slowly through the leaf so it carries smoothly into the water with never a droplet to bead.
3. Sweeten lightly with monk fruit, sharpen with lemon, pour over a full glass of ice. The ice and the menthol multiply through TRPM8; the low-bitter tea keeps the base from fighting them.

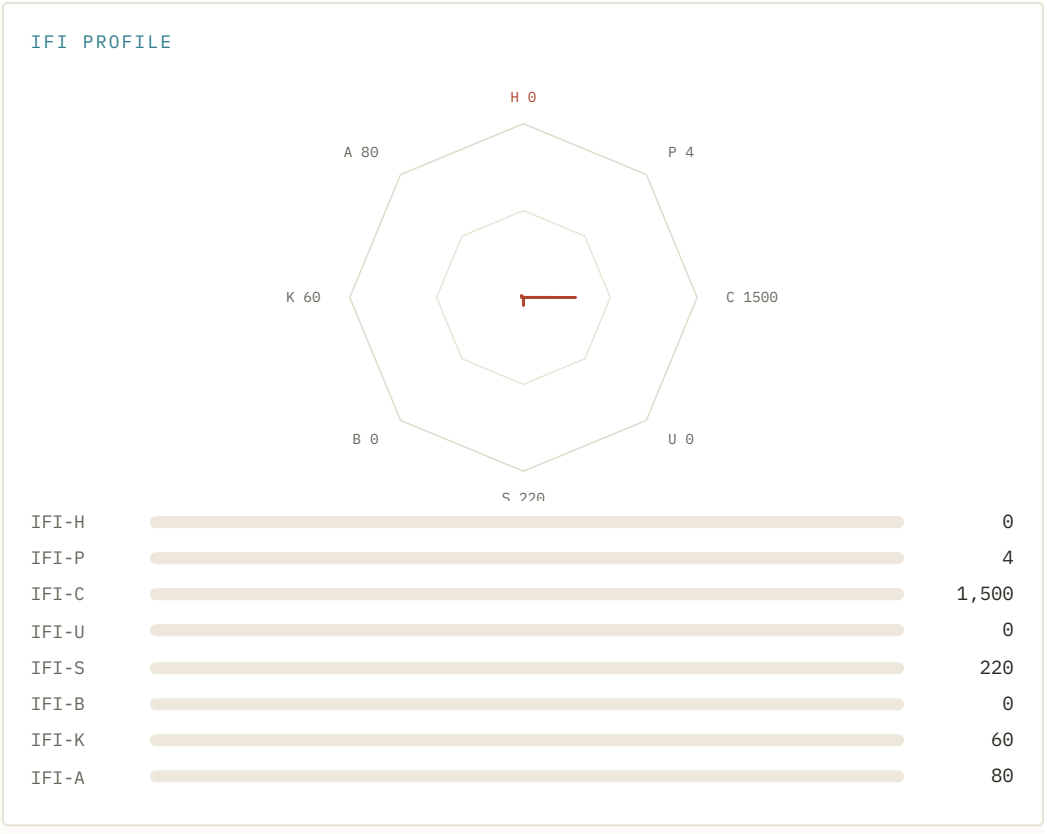


Read the card and the cross-axis design shows: **C 1,600** is the loud cool, **P 4** confirms the menthol stays well below its TRPA1 threshold, and the pairing of **B 0** with **A 120** is the quiet cleverness. The bitterness is attenuated to silence — our low-bitter tea runs the IFI-B axis hard, trimming the receptors that read catechin and caffeine as a grab — while the astringency is deliberately *left standing* at 120, the gentle tannic structure (read by the salivary proteins, not the bitter channel) that gives iced tea its body. A cool wants structure but no grip: a bitterness subtracted, an astringency kept.

The second is the double cool — a drink that is cool twice over, carrying both a designed cold and the cultural cool of its central ingredient.

```
CUCUMBER-LAVENDER COOLER · serves 2 · vegetarian / no alcohol
— designed cool cucumber                  1, the engineered line, juiced
— engineered lavender                      1 tsp, as a light syrup or
infusion
— lime                                      1, juiced
— sparkling water                           to top
— ice                                       to fill
```

1. Juice the cool cucumber — the engineered line carries its own designed cold near **IFI-C 1,400** (EC_{50} ≈ 57 µM), so the drink starts cool before anything is added.
2. Stir in a restrained measure of lavender infusion; lavender is a whisper, not a shout, and too much turns soapy — the surest way to spoil a floral cool is to over-pour it.
3. Lime, ice, top with sparkling water. The carbonation's own prickle rides the trigeminal field alongside the cool; the result is a cooler that is cool twice — the cucumber's designed cold and its cultural cool — lifted by lavender's floral calm.

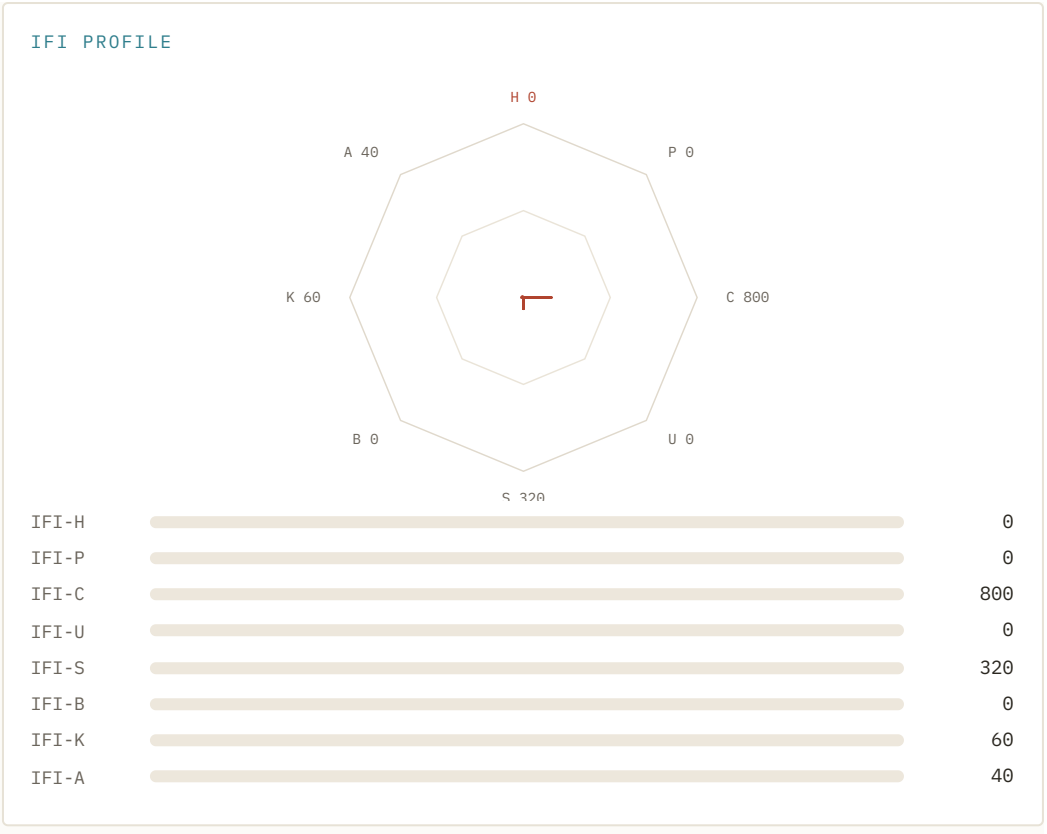


The cucumber is the "contradiction" made literal: a vegetable the mind already files as cool, engineered to deliver an actual TRPM8 cold to match the reputation. The carbonation is not incidental either — the fizz is itself partly a trigeminal event, and a cool primes that field to read the prickle brighter. Lavender's danger is worth stating plainly, for it is the drink's one failure mode: linalool is lovely in a whisper and soap in a shout, and the line between is a half-teaspoon.

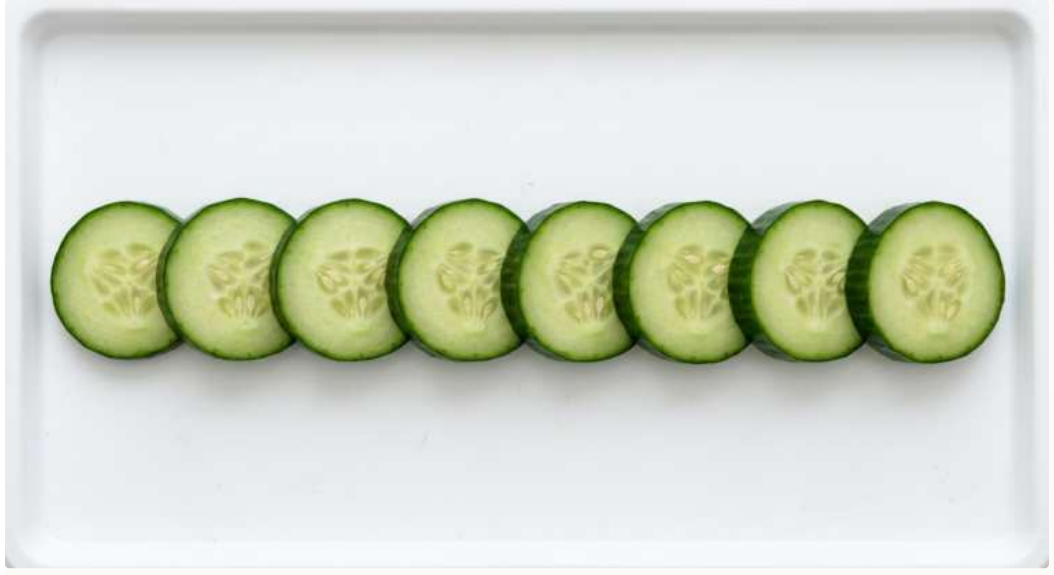
The third is the gentle cool — the rounded carvone cool of spearmint, for a drinker who wants fresh, not frozen.

```
SPEARMINT–LIME AGUA FRESCA · serves 4 · vegetarian / vegan
– engineered spearmint                    large handful
– lime                                      3, juiced
– cucumber or melon                         1 cup, blended (optional, for
body)
– monk-fruit or light agave                 to taste
– cold water and ice                        to fill
```

1. Muddle the spearmint gently with the sweetener; spearmint's cool is the round, sweet-fresh carvone cool, not the sharp menthol one, so it wants a soft hand.
2. Blend with the lime, the optional cucumber or melon for weight, and cold water.
3. Strain over ice. This is the gentle cool — fresh rather than frozen, the spearmint doing exactly the rounded work peppermint would overpower.



Here the molecule writes the card. **C 800** and **P 0** together are the signature of carvone: a cool that reaches half-activation only near 100 µM and never once strays toward the irritant channel, which is why spearmint can carry a gentle drink without ever threatening a sting. Where the iced tea is a cool to wake you, this is a cool to settle you — the same axis, read quiet.



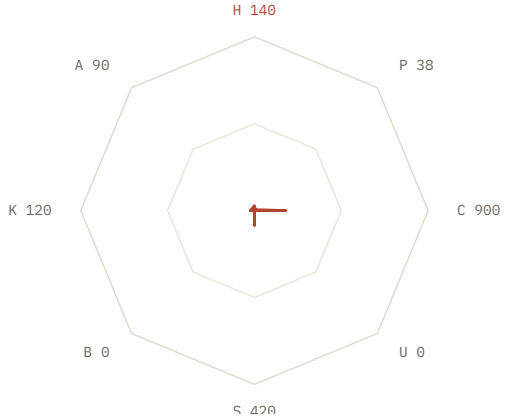
Cut a cucumber and the pale, water-bright interior is the whole promise of the cooler — a vegetable the mind already calls cool, engineered to mean it at the receptor.

The fourth is the one that proves the axis has two faces. Cool spans cool *and* burn, and a winter drink can live on the warm shadow — camphor's TRPV3 warmth, a little real heat — while still carrying a cooling lift on top, the paradox of this book made drinkable.

```
THE WARM-COOL TODDY · serves 1 · vegetarian / non-alcoholic base
— hot water or herbal base                  1 mug
— honey or engineered sweet syrup           1 tbsp
— engineered clean-cool peppermint           2 leaves, torn
— a whisper of camphor-leaf infusion         1 bar-spoon (warm, medicinal)
— engineered ginger, grated                  1 tsp (warmth, TRPV1/TRPA1)
— lemon                                       to taste
```

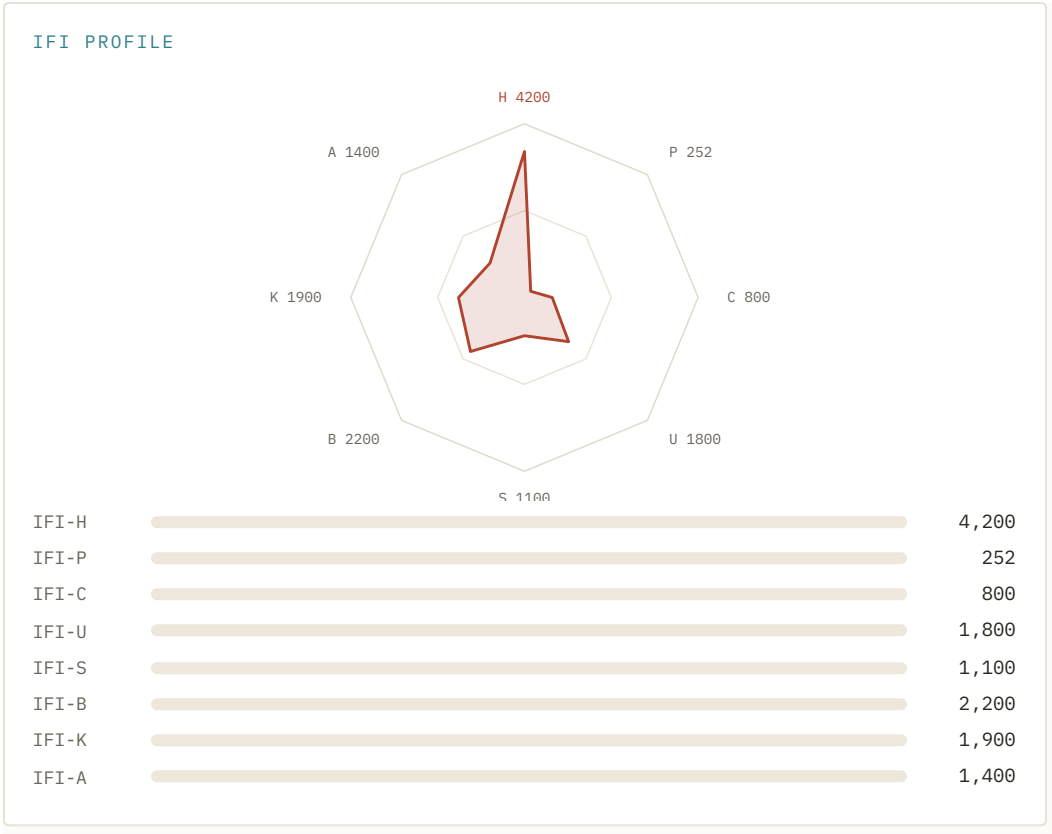
1. Steep the ginger in the hot base for warmth that climbs slowly — real TRPV1/TRPA1 heat, gentle and building.
2. Off the heat, add the torn peppermint and the barest touch of camphor infusion; the peppermint cools the front, the camphor warms beneath it.
3. Sweeten, sharpen with lemon, drink hot. The sensation is the whole axis at once — a cool that opens the chest riding on a warmth that fills it.

IFI PROFILE



IFI-H		140
IFI-P		38
IFI-C		900
IFI-U		0
IFI-S		420
IFI-B		0
IFI-K		120
IFI-A		90

This is the drinkable cousin of our full-spectrum pepper, **Phoenix (SS-0096)** — the one cultivar engineered to register on all eight axes at once:



Side by side, the two cards show the relationship exactly. Both set a genuine cool near 800–900 under a genuine warmth — but where Phoenix drives the heat channel to **IFI-H 4,200**, representing our model's prediction of approximately **2.95 million SHU** — a design target near the ceiling of anything grown — the toddy asks that same channel only to whisper at **H 140**, a ginger warmth that climbs and fills rather than burns. (For scale: even the gentlest flagship superhot, Ember at SS-0087, our model predicts approximately **866,000 SHU** and IFI-H 1,570 — orders of magnitude above a toddy's ginger.) The toddy is Phoenix's gesture made gentle: cold at the front from the menthol, warmth beneath from the ginger's TRPV1 and camphor's TRPV3, the brain holding two true thermal reports at once and finding neither January nor July but the quiet vertigo of both. A grace note — camphor warms through TRPV3 while *quieting* TRPA1, so the warmth reads round rather than sharp, the drink's pungency (P 38) left to the ginger alone. One axis, two temperatures, held in a mug.



Engineered ginger is the toddy's warm floor — gingerols and shogaols waking TRPV1 and TRPA1 for a heat that climbs slowly and never roars.

Failure modes, edge cases, and the honest limits

A drink is a receptor target exposed to a kitchen, and the gap is where the honest caveats live.

The beaded slick. The most common ruin of a cooling drink is a local over-concentration, not a global one. A neat drop of menthol oil, or a crystal that has not finished dissolving, holds the liquid around it near saturation — up to 2.7 mM, thirty-fold past the clean window — and that microdroplet strikes the lip as a hot, medicinal sting while the glass as a whole reads faint. The number on the card is an *average*; the sting is a *maximum*; the craft of dispersion is the craft of flattening the maximum toward the average. This is why we infuse through leaf and syrup rather than dose neat oil, and why potency — fewer milligrams for the same cool — is the surest protection against the spike.

The cool fades as you drink. The Ca^{2+} that TRPM8 admits is also its off-switch: calcium entering the channel consumes the PIP_2 the channel runs on, and the cool desensitizes — the third sip weaker than the first, a sustained menthol dulling within seconds toward a fraction of its opening strength. A cool measured on a rested channel overstates the cool of a mouth already half a drink in. The design answer is not more dose — that only hastens the run-down and courts the sting — but *rhythm*: a cool that renews as the ice renews the cold, leaning on the temperature coupling to carry the back half of the glass.

The warming drink warms off. Volatile camphor and menthol breathe out of a hot toddy faster than from an iced drink, so the warm-cool balance drifts as the mug sits. A toddy is at its designed point in its first minutes and softens after — a drink to serve, not to nurse.

The index scores loudness, not liking. IFI-C measures how hard a molecule drives a receptor; a drink is pleasant or not for reasons the number cannot reach. Refreshment is loudness *plus* coverage *plus* temperature *plus* context, and two cools that share an EC_{50} can part ways entirely in the mouth — one blooms across the sinuses and throat, the other sits as a bright point and goes nowhere. The score is a necessary coordinate, never the whole address.

There is no absolute cool. Because cold and menthol are allosteric partners, every EC_{50} on this axis is an EC_{50} *at a stated temperature* — a drink reads louder at 4 °C than at 15 °C, softer still as it warms toward the body — and cold sensitivity varies from drinker to drinker besides. The ≤7% we promise is about reproducibility under fixed conditions, not a platonic constant floating free of the ice. The honest maker fixes the serve temperature, pins the reference, and reports a comparison.

A kitchen is not a cuvette. The tightest number we quote assumes a metered dose; a home infusion of bruised leaf, poured by hand, carries far more variance than any figure on a card. The ≤7% band is what the engineered line holds at the bench — but part of the gift of designing the loudness *into the plant* is that even a generous, imprecise handful of a clean-cool mint lands closer to intention than a careful measure of a noisier one.

None of this is a retreat from the number; it is the care that makes the number worth pouring. We measure the cool because we revere it — a cold that never touched the tissue, grown into a plant gene by careful gene, is one of the cleaner pleasures we know how to give a

body, and one you can place with precision only once you have learned to count it. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The glass does what no plate can: it lets real temperature and designed sensation braid together, ice and menthol compounding through one shared gate, and it lets a cool be exactly as long, and as deep, as the slow pour of a drink allows.

Chapter 17 · Cooling on the Plate — *cook with the temperature you taste.*

Cool is the least-used tool in the savory kitchen, and that is an accident of history, not of merit. Heat has owned the chemesthetic conversation on the plate for centuries — chili, pepper, ginger, mustard — while cool was exiled to the dessert course and the after-dinner mint. But the great cooking traditions always knew better in practice than the cookbooks admitted in theory: mint on lamb, cucumber in a raita cooling a fierce curry, shiso under sashimi, dill against cured fish, the cold lift of herbs threaded through a rich Vietnamese bowl. Cooks have always known that a designed cold can cut richness, lengthen a finish, and wake a palate the way acid does, from a different direction. This chapter cooks with the thermal axis on purpose, plant-forward by default, and it treats the one fish and the one richer dish it includes with exactly the respect it gives the vegetables, because a cook who reveres ingredients reveres all of them.

What changed is not that cool became available — mint has always been in the garden — but that cool became *measurable*, and therefore *placeable*. For ten thousand years a cook who wanted cold on the tongue reached for a herb whose strength swung with the season, the cultivar, the age of the leaf, and whose menthol always dragged a faint medicinal shadow into the dish behind it. We can now hand a cook a cold that reads to a number and arrives without that shadow. That is the whole of what this chapter is about: the thermal axis as seasoning, dosed the way you dose salt.



Garlic and the alliums anchor the savory plate — and a clean designed cool played against their depth is one of the quietest, most surprising seasonings in the kitchen.

The receptor beneath the cool

The cold you taste is a lie the nerve tells, and it is a beautiful one. Menthol binds TRPM8 — transient receptor potential melastatin 8, a non-selective cation channel standing in the membranes of the same trigeminal and dorsal-root sensory neurons that carry heat. In

its ordinary life TRPM8 is a thermometer: it begins to open as the tissue cools below roughly 26–28 °C, letting sodium and calcium across the membrane, and the brain reads that current as *cold*. Menthol does not chill anything. It slips into a pocket in the channel's voltage-sensing domain — the cluster of helices around residue Y745 — and shifts the channel's activation curve so that it opens at temperatures it would otherwise ignore. The mouth is at body warmth, near 36 °C, and yet the channel fires as though a window had opened. The brain, which trusts this receptor completely, reports a cold that is not there.

This is the exact mirror of the burn. Capsaicin lowers the thermal threshold of TRPV1 so the tongue reports a fire that is not there; menthol raises the apparent threshold of TRPM8 so the tongue reports a frost that is not there. Two receptors, two molecules, two directions from the same trick — a false temperature written straight onto the nerve. That symmetry is why the axis is named for both poles at once. Cool-Burn, IFI-C, is anchored to TRPM8 with TRPV1 as its warm counterweight, and the dish that lives up to the name is the one that fires both channels on the same fork: a cold that the heat cannot cancel, because they are carried by different nerves and arrive, impossibly, together.

We keep the cool clean on purpose. There are synthetic super-agonists of TRPM8 — icilin and its relatives — that drive the channel far harder than menthol and depend on calcium and on a different residue entirely; we do not reach for them. The cool in this book is the plant's own monoterpenoid cold, menthol and its cousins, expressed by an organism we tuned rather than a molecule we poured in from a drum. A lifetime of cooking vegetarian taught us to distrust the shortcut that leaves a residue the body did not ask for. The cold should come from the leaf.

A cold you can place to the number

Every axis on the Index answers one question — not how much of a compound a food holds, but how *loudly* that compound speaks at its receptor. The number is an inverse concentration, and the whole of it is one line:

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

EC_{50} is the concentration at which a stimulus drives its receptor to half of full response. A loud cool reaches half-activation of TRPM8 at a *low* concentration, so it has a *small* EC_{50}, so — because the term sits in the denominator — it earns a *large* IFI-C. Flip the ratio and loudness becomes a score. We anchor the scale so that a stimulus scoring exactly 1,000 is, by convention, as loud at the cold receptor as the fixed menthol reference, and we cap each axis at 5,000 for the scorecards, so the whole cold world lives on one readable page.

The beauty of an inverse-ratio scale is that the ratios survive even when you do not know the absolute anchor. Two of the dishes in this chapter make the point. The cold noodle reads Cool-Burn 1,300; the chocolate pot reads 400. Their ratio is fixed:

```
IFI-C(noodle) ÷ IFI-C(pot) = 1,300 ÷ 400 = 3.25
```

The noodle's cool is 3.25× louder at TRPM8 — which means its stimulus reaches half-activation at 1⁄3.25 = 0.31× the concentration the pot's cool needs. You can read the relative potency of two colds off the scorecard with nothing but division, and never name a single micromolar.

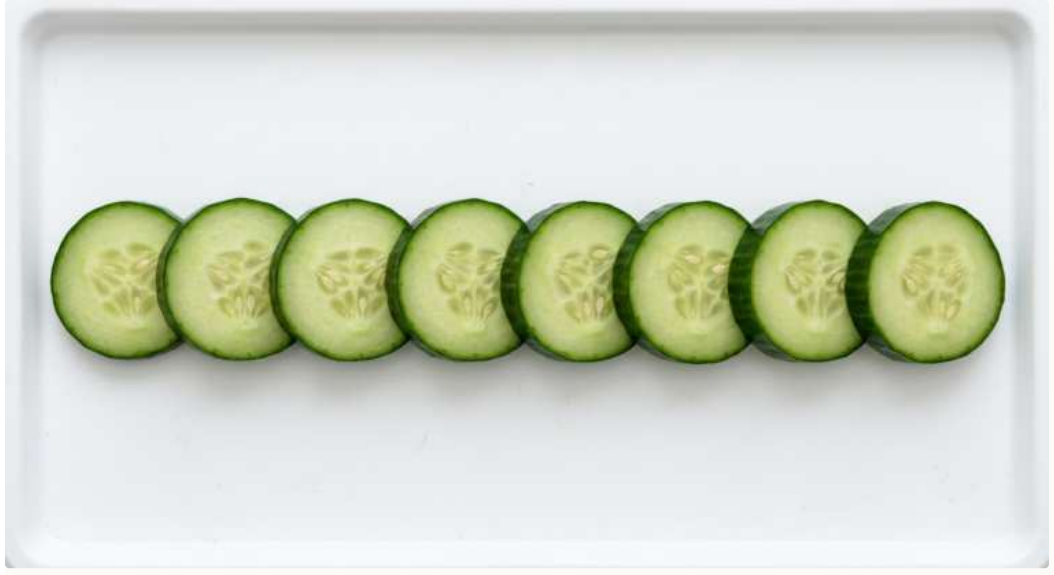
To make the arithmetic concrete we do have to pin the anchor somewhere, so take the reference at EC_{50}(ref) = 100 µM. (The zero-point of any ratio scale is a stated convention — a choice, like the Scoville scale's dilution-to-threshold definition — and the relative numbers above do not depend on it.) Inverting the formula gives the EC_{50} behind each score, and menthol's molar mass of 156.27 g · mol^{-1} turns micromolar into the milligrams-per-litre a cook can actually picture:

```
EC₅₀(candidate) = 1,000 × EC₅₀(ref) ÷ IFI-C = 100,000 ⁄ IFI-C
(in µM)
```

DISH / CULTIVAR	IFI-C	IFI-H	EC_{50}(COOL), MM	MENTHOL-EQUIV AT ½-MAX
Cool-burn sesame cold noodles	1,300	900	76.9	12.0 mg · L^{-1}
Cured trout, cucumber & cool-mint oil	1,000	40	100.0	15.6 mg · L^{-1}
Pea, mint & ricotta on toast	800	0	125.0	19.5 mg · L^{-1}
Lavender–pepper dark chocolate pots	400	0	250.0	39.1 mg · L^{-1}
Phoenix · SS-0096 (full-spectrum anchor)	800	4,200	125.0	19.5 mg · L^{-1}

That last row is worth a breath. Phoenix is the loudest heat we ever designed — our model predicts approximately 2.95 million SHU, Heat at 4,200 — and it still carries a designed, deliberate cool at 800, the same cold loudness as a plate of minted peas. Even at the far edge of the burn, the cool is not an absence; it is a channel we chose to keep open. Cool and burn were never opposites to be traded off. They are two instruments, and Phoenix plays both.

The numbers also carry their own honesty about error. Our batch-to-batch coefficient of variation holds at or below 7 %, so a cultivar dosed to Cool-Burn 1,300 is really 1,300 ± 91 across a run — a band from roughly 1,210 to 1,390. A cook never feels that nine-percent window; a formulator building a thousand identical jars lives and dies by it, which is why we print the CV and do not hide it inside a glossy single number.



A cucumber in cross-section — the cool most cooks already trust. The designed-cool cultivar keeps that watery brightness and adds a designed TRPM8 lift, so the cold reads to a number instead of to a guess.

There is one more number every cook should carry, because it governs how far a cool goes before it stops paying you back. Receptor response follows a dose–response curve, the Hill form, response / E_max = $[L]^n / (EC_{50}^n + [L]^n)$. At a dose equal to EC_{50} the response is exactly half of maximal — that is the definition of EC_{50} — and for a cool with a Hill coefficient near n ≈ 2, the climb above half-max is cruelly steep in its diminishing returns. Doubling the effective cool from one EC_{50} to two takes you from 50 % to 80 % of full response; tripling it to three EC_{50} reaches only 90 %:

> at [L] = 2 · EC_{50} : 2^2 ⁄ (1 + 2^2) = 4 ⁄ 5 = 0.80 at [L] = 3 · EC_{50} : 3^2 ⁄ (1 + 3^2) = 9 ⁄ 10 = 0.90

Past that the curve flattens into a ceiling, and the ceiling is where the trouble lives. More mint beyond this point does not buy you more cold worth having; it buys you the medicinal edge and, eventually, the numbness. The mathematics is telling the cook what every good cook already senses — season the cool to the knee of the curve and stop.

The five-gene cool

A clean cool on the shelf is not found, it is built, and it is built along the plant's own monoterpenoid road. Menthol is the last stop on the p-menthane pathway that runs in peppermint's glandular trichomes, the little oil factories on the underside of the leaf. The route starts from geranyl diphosphate, the universal ten-carbon building block, and walks it through seven enzymatic steps to menthol:

STEP	SUBSTRATE → PRODUCT	ENZYME
1	geranyl diphosphate → (−)-limonene	limonene synthase (LS)
2	(−)-limonene → (−)-trans-isopiperitenol	limonene-3-hydroxylase (L3OH)
3	isopiperitenol → (−)-isopiperitenone	isopiperitenol dehydrogenase (ISPD)
4	isopiperitenone → (+)-cis-isopulegone	isopiperitenone reductase (ISPR)
5	(+)-cis-isopulegone → (+)-pulegone	isopulegone isomerase (IPGI)
6	(+)-pulegone → (−)-menthone	pulegone reductase (PR)
7	(−)-menthone → (−)-menthol	menthone:menthol reductase (MMR)

The off-note that haunts ordinary mint lives at step 6. Pulegone sits at a fork: the clean road runs forward through pulegone reductase to menthone and then menthol, but a competing enzyme, menthofuran synthase, pulls pulegone sideways into menthofuran — the musty, close, slightly medicinal note that muddies a wilted mint and that no amount of careful chopping removes. Worse, residual pulegone itself is an undesirable compound the body has no use for. Wild mint's loudness and its off-note are the same leaf; you cannot have one without the other by selection alone, which is why a cook has never had a clean mint to dose.

So the engineered core is a five-gene module, not the whole pathway: we tune the committed entry (LS), the hydroxylase that sets flux into the ring (L3OH), the two reductases that carry pulegone home to menthol (PR and MMR), and — the decisive edit — we suppress menthofuran synthase so the sideways door is closed. Flux that used to leak into the musty note is pushed forward into clean menthol; residual pulegone falls; the cold arrives bright and alone. That is what *clean-cool peppermint* means on an ingredient line — not a stronger mint but a truer one, the TRPM8 signal without the shadow, and held to the same ≤ 7 % batch CV as every other construct we ship.

Peppermint is the loud centre of this small world, but it is not alone, and the cooling plants divide by both mechanism and difficulty:

ORGANISM	CONSTRUCTS	PRIMARY AXES	DIFFICULTY	CHARACTER OF COOL
Peppermint	2,360	C / M	Hard	menthol — the reference cold, loud and clean
Lavender	160	C	Hard	linalool floral calm — a cool without cold
Orange	120	B / C	Hard	a bright, low cool riding a de-bittered citrus
Eucalyptus	80	C	Hard	eucalyptol — camphoraceous, sharp, high-lift
Spearmint	80	C	Hard	carvone-dominant — sweet, rounder, gentler cold
Camphor	40	C	Hard	penetrating, aromatic, used in whispers only

The depth of the peppermint programme — ten and twenty times the constructs of its neighbours — is not vanity. It is where the dose–response curve, the off-note suppression, and the batch consistency all had to be solved first, so that everything downstream, from a confection to a cold noodle, inherits a cool that behaves.

The cold noodle — two temperatures on one fork

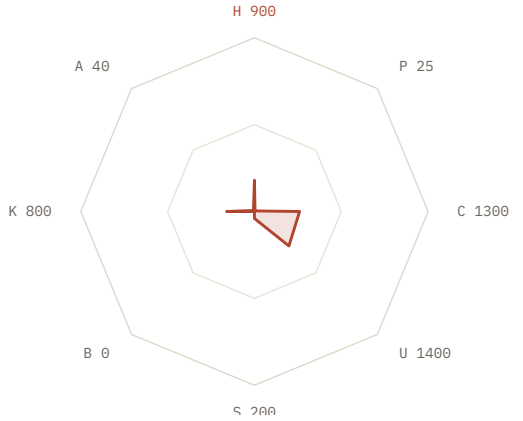
This is the book's title on a plate, and it is where the thermal axis stops being a demonstration and becomes dinner.

COOL-BURN SESAME COLD NOODLES · serves 4 · vegetarian / vegan

— wheat noodles	400 g, cooked, chilled
— tahini	4 tbsp
— engineered low-bitter soy / tamari	3 tbsp
— engineered clean-cool peppermint	large handful, shredded
— designed cool cucumber	1, julienned
— engineered chili oil	to taste (real TRPV1 heat)
— rice vinegar, sesame, scallion	to finish

1. Whisk tahini, tamari, and vinegar into a loose sauce; the low-bitter tamari lets you use more for depth without the base turning acrid.
2. Toss the chilled noodles through; fold in the cucumber and a generous amount of shredded peppermint at the very end so the cool stays bright rather than wilting into the warm sauce.
3. Finish with chili oil to taste — this is the dish's cool-burn, the designed cold of the mint and cucumber against the real heat of the chili, the two thermal channels firing together on one fork.

IFI PROFILE



IFI-H	900
IFI-P	25
IFI-C	1,300
IFI-U	1,400
IFI-S	200
IFI-B	0
IFI-K	800
IFI-A	40

That scorecard is the argument. Heat at 900 and Cool-Burn at 1,300, fired at once, the noodle you cannot decide the temperature of, grounded by a deep savory base — Umami 1,400, Kokumi 800 — so the thermal drama has something to stand on. Here is the part the Index is careful never to oversell: those two thermal numbers do not simply add. Heat rides TRPV1; cool rides TRPM8; they are different nerves, and the brain integrates them downstream, where menthol is known to pull both ways on the burn at once. At the dose in this bowl the cool lengthens and frames the heat, each arriving in its own

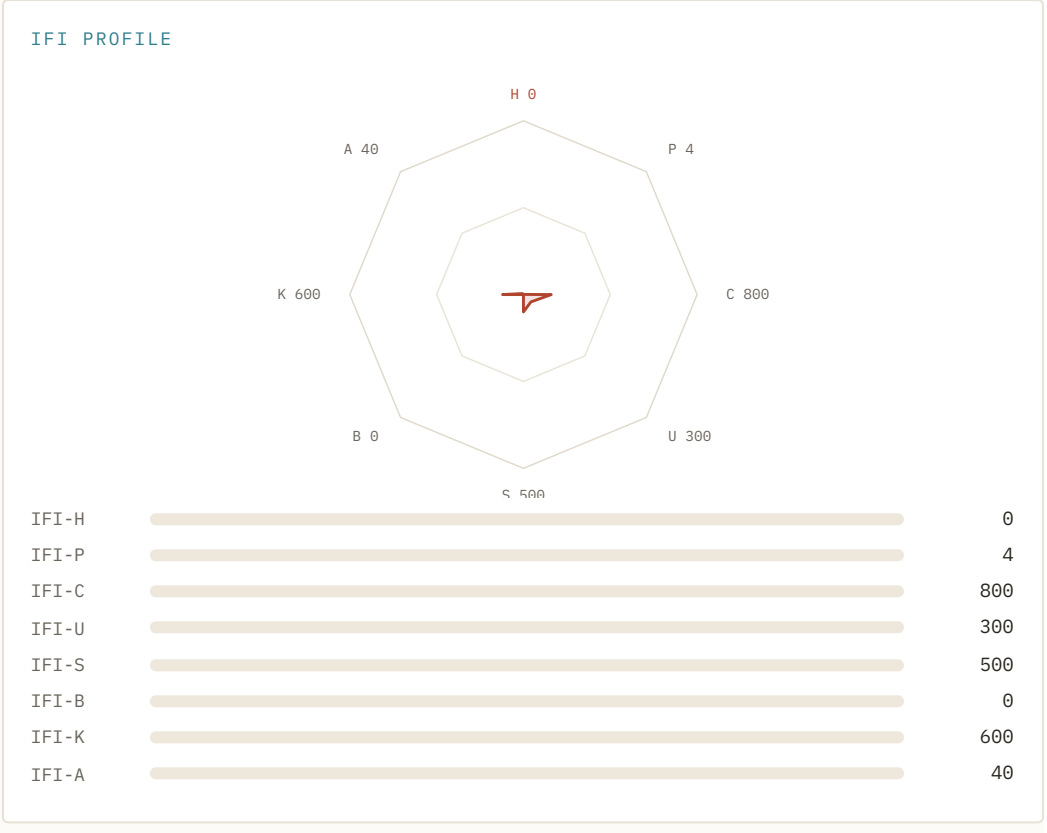
instant — the cold on the front of the tongue, the fire blooming behind it. Push the mint far past the knee of its curve and the same menthol begins to blunt the very heat it was framing, because high menthol tips TRPM8's neighbour toward the counterirritant, analgesic register. So the scorecard reads 900 and 1,300 as two honest loudnesses; the plate is their duet, and the duet is dose-dependent. We cook to the balance, not to the sum.

Warm bread, cold mint

The cold noodle fires cool against heat. This dish fires cool against *warmth of a different kind* — the physical heat of the toast under it — and it is the gentlest, most immediate lesson in the whole thermal axis.

```
PEA, MINT & RICOTTA ON TOAST · serves 4 · vegetarian
– fresh or frozen peas                    300 g
– engineered clean-cool peppermint        handful
– ricotta                                  200 g
– lemon zest, olive oil, flaky salt        to finish
– good bread                               4 thick slices, toasted
```

1. Briefly blanch the peas; smash half for a rough spread, keep half whole for texture.
2. Fold in torn peppermint, lemon zest, and olive oil — the clean engineered mint lifts the peas' sweetness with a cold brightness and no medicinal edge to muddy the green.
3. Spread ricotta on the warm toast, pile the minted peas, finish with salt and oil. Warm bread, cool mint, sweet pea — the thermal contrast between the warm toast and the cold mint is the whole pleasure.



No heat at all — Heat 0 — and yet the plate has a temperature, because Cool-Burn sits at 800 against bread that genuinely is warm. The contrast here is literal, not a receptor illusion stacked on another illusion: the toast's real thermal heat raises the tissue toward the warm edge of TRPM8's range, and the menthol-shifted channel then

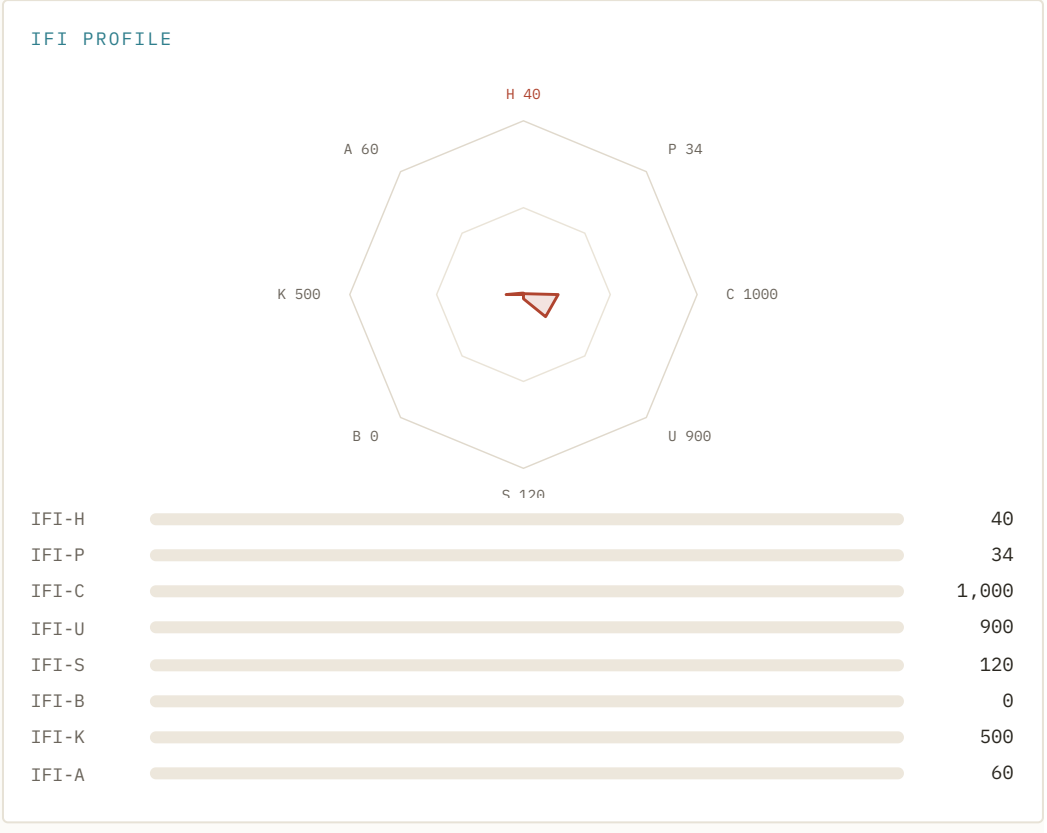
fires *harder* for it, because a receptor pushed up against its threshold responds more sharply to the stimulus that crosses it. The cold feels colder on warm bread than it would on a cold plate. That is the quiet engineering lesson of the dish: a designed cool is never seasoned in isolation; it is seasoned against the temperature of everything it touches. At Cool-Burn 800 — an EC_{50} near 125 µM, a menthol-equivalent around 19.5 mg · L^{-1} at half-max — the mint is loud enough to carry a finish three or four seconds long and clean enough that the finish tastes of pea and lemon, not of lozenge.

The one fish

The plant-first kitchen honors the animal when it cooks one, and the respect shows in restraint. Here the cool is an accent dosed deliberately below the dish's centre, never over it.

```
CURED TROUT, CUCUMBER & COOL-MINT OIL · serves 4 · pescatarian
— cured or cold-smoked trout                300 g, sliced
— designed cool cucumber                    1, shaved into ribbons
— engineered clean-cool peppermint          small handful
— cool-mint oil (peppermint in neutral oil) 2 tbsp
— lemon, dill, cracked pepper                to finish
```

1. Lay the trout and shaved cool cucumber loosely on a cold plate, the ribbons and the slices overlapping.
2. Spoon over the cool-mint oil — gently; the designed cold should accent the fish, not bury it.
3. Finish with dill, lemon, cracked pepper. The cucumber's designed cool and the mint oil give the rich, cured fish a cold frame that keeps every bite feeling fresh. The trout is the center of the plate and is treated as such.



Cool-Burn reads 1,000 — an EC_{50} at the reference's own 100 µM, the cleanest possible place on the scale to work — and it is split across two carriers on purpose. The designed cool cucumber lays down a broad, watery, low cold across the whole plate; the cool-mint oil places a sharper, lipophilic cold exactly where the spoon falls. This is

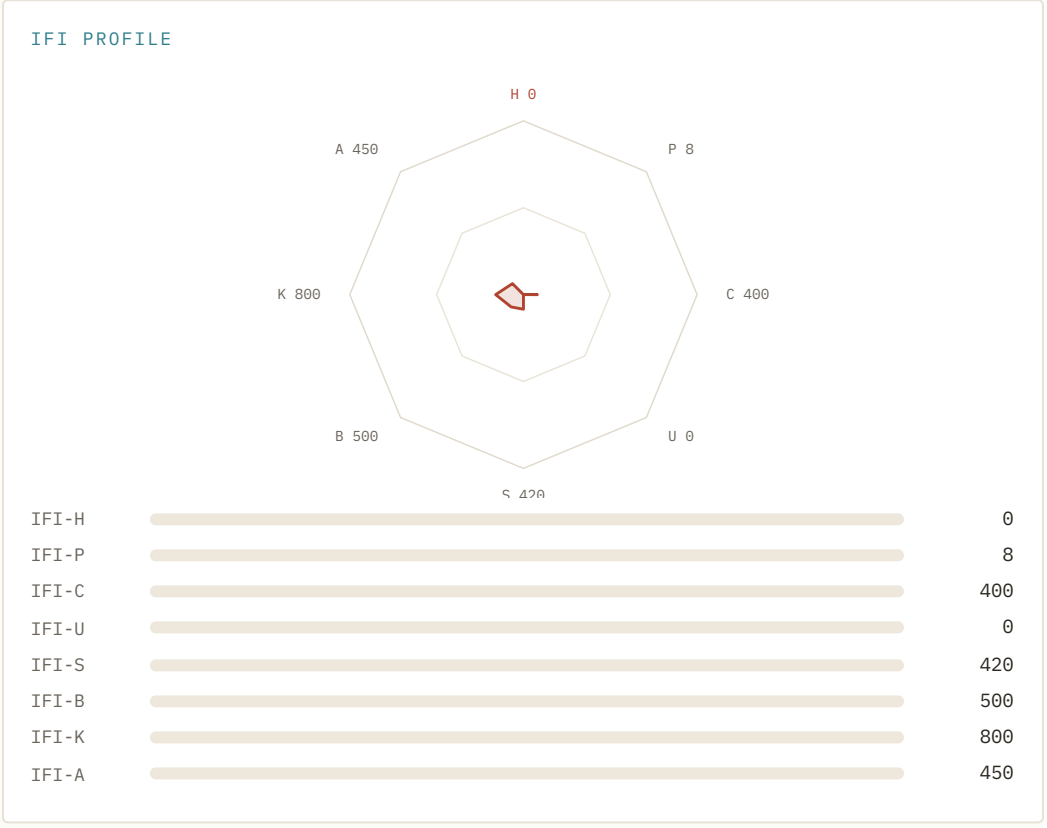
a real dosing decision the number lets you make: menthol is fat-soluble, so an oil concentrates and *localizes* the cool, firing TRPM8 hard in the bites that carry it and leaving the others to the cucumber's gentler lift. The cured trout's richness — Umami 900, Kokumi 500 — sits dead centre, and the cold is a frame around it, not a lid on it. Spoon the oil to flood the plate and you would read far past 1,000 on the tongue and bury the fish; the restraint is the recipe. Pungency at 34, the faint TRPA1 prickle of cracked pepper and dill, keeps the cold from reading flat. The trout is the subject of the sentence; the cool is the light it is photographed in.

A cool that is not cold

Not every cool is menthol, and this dessert is the proof. Lavender's cold comes from a different molecule and a different, softer mechanism — which is exactly why it belongs at the end of a meal rather than the front.

```
LAVENDER-PEPPER DARK CHOCOLATE POTS · serves 6 · vegetarian
– engineered low-bitter dark chocolate      200 g
– cream                                       300 ml
– engineered lavender                         scant 1/2 tsp, infused
– black pepper                                a few cracks
– flaky salt                                  to finish
```

1. Warm the cream with the lavender and a crack of pepper; infuse ten minutes, then strain — lavender is a whisper here too, and pepper's faint TRPA1 prickle keeps the florals from going soapy-sweet.
2. Pour the warm cream over the chopped low-bitter chocolate; stir to a smooth ganache; the softened bitterness lets the lavender's cool calm read against the richness instead of against a tannic wall.
3. Set in small pots; finish with flaky salt. A dessert that is cool without being cold — lavender's floral calm against dark chocolate's body.



Cool-Burn reads 400 here, the lowest cold in the chapter, and that low number is honest about mechanism, not a sign of a weaker hand on the dial. Lavender's dominant volatiles are linalool and linalyl acetate, not menthol, and they are at best faint and indirect agonists of TRPM8 — a cool that is more *aromatic calm* than literal frost, the reason the dish is described as cool without being cold. The EC_{50} behind 400 sits near 250 µM, better than three times the concentration a menthol cool would need to reach the same half-activation, which is precisely what a low IFI-C is supposed to tell you: this stimulus is quiet at the receptor, and should be. You do not want menthol's icy spike inside a chocolate pot. You want the floral settle, carried on the TRPA1 prickle of a few cracks of pepper to keep it from sliding into soap, laid over a de-bittered chocolate whose softened tannic wall — Zero-Bitter at 500 — lets the calm read instead of fighting it. Kokumi at 800 gives the ganache its long, round body. The cool here does not cut the richness the way the mint cuts the noodle; it *lengthens* it, a different job for a different molecule, and the number is how you know which one you are reaching for before you ever taste it.



Cacao in the pod — the body a lavender cool leans against. With the bitterness engineered down, the floral calm reads against the richness instead of against a tannic wall.

Where the cold misbehaves

A tool worth trusting is one whose failure modes you can name, and the thermal axis has four a cook should carry.

The cool flashes off. Menthol is volatile — it carries a real vapor pressure at room temperature and sublimes even from a cold surface — which is why every recipe here folds the mint in at the very end, off the heat. Stir clean-cool peppermint into a hot sauce and you are boiling your seasoning into the extractor hood; the terpene leaves, and what stays behind is the duller, heavier fraction. Cool is a finishing seasoning the way a delicate oil is a finishing oil. Heat is the one kitchen force it cannot survive, which is the quiet irony of an axis named for temperature.

The curve has a ceiling, and past it lies numbness. TRPM8 shows calcium-dependent desensitization: drive it hard enough, long enough, and the channel quiets and the cold collapses into anesthesia — the faint numbing a too-strong mint leaves behind. The dose–response mathematics already warned of it, the climb from 50 % to 80 % to 90 % buying less and less, and the receptor's own desensitization is the floor under that ceiling. Season to the knee of the curve. More is not colder; eventually more is nothing at all.

The channels talk to each other. The Index scores Heat and Cool-Burn as independent loudnesses because they are independent receptors, but the brain reads them together, and menthol in particular is a known two-faced actor — at low dose it can sharpen the perception of a neighboring burn, at high dose it tips toward the counterirritant and blunts it. So a cool-burn plate is never simple arithmetic. H 900 and C 1,300 are two computed values; the balance you taste between them shifts with how much cool you pour and in what order it arrives. The scorecard gives you the instruments. The cook conducts.

The number is loudness, not love. This is the most important limit, and the one we hold most firmly. IFI-C measures how loudly a stimulus speaks at TRPM8 — nothing more. It does not measure whether the cold is *welcome*; it does not know that menthol belongs on a cold noodle and would wreck a bisque. And loudness is not dose: the Index scores the potency of the ingredient, the EC_{50} it carries, while the plate is potency *and* how much you add *and* the temperature it meets *and* the other channels in the room. A cultivar with a low EC_{50} is a loud instrument; whether the music is good is still, and always, the cook's.

The plate is where the thermal axis stops being a demonstration and becomes seasoning — a thing a cook reaches for the way they reach for salt or acid, to lengthen, to cut, to wake. A designed cool is not a novelty on the plate; it is a missing primary color, cold, that the savory kitchen has had no clean way to paint with until the mint stopped carrying its off-note into the dish. Now it is clean, and constant, and placeable to the number. What a cook does with a new primary color is the oldest pleasure there is, and we built this axis, in the end, to hand that color over.

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

Chapter 18 · The Temperature of Care — *why we grow the cold.*

We have measured cool to the millivolt and the micromolar across this whole book — the voltage shift menthol works on TRPM8, the EC_{50} ladder from icilin to eucalyptol, the Hill curves and the τ of a fading cool, the seven enzymes and the one off-ramp, the scorecards and the ≤7% variance — and it would be easy to finish there, with the mathematics tidy and the plants catalogued. But the mathematics was never the reason. It was the means. The reason is older than the Index and simpler than any enzyme, and it is worth saying plainly before we close: cool is a kindness the body has always known how to receive, and we grow it because we wanted to understand, and improve, a thing that comforts people. Before we say *why*, though, we owe you the *how* — the whole of it, laid out — because in this house the wonder and the numbers are two halves of one sentence, and a kindness you can measure is a kindness you can learn to give well.

Think of when you reach for cool. A cold cloth on a hot forehead. A mint tea after a heavy meal. A glass of cucumber water on a day the heat will not break. A lozenge when the throat is raw. Cool arrives, almost always, at a moment the body is asking for relief, and it answers that ask not by changing the body's temperature — a thermometer, as our title insists, feels nothing — but by telling the mouth and the skin a true-feeling story of cold that the mind receives as ease. That is a remarkable thing for a small molecule off a crushed leaf to do, and the whole of this work has been an attempt to deserve it: to understand the story precisely enough to tell it cleaner, longer, and more truly than a wild plant ever could.



The greenhouse where it started — a family that reveres what it grows, writing about the mathematics of cold the way you would write about something you love and want to nourish you.

The receptor that reads a cold that isn't there

The whole sensation lives in one protein. TRPM8 — transient receptor potential, melastatin subfamily, member eight — sits in the membrane of the trigeminal and sensory nerve endings that web the mouth, the lips, the cornea, the skin. It is a four-part gate: four identical subunits folded around a central pore that, when it opens, lets sodium and calcium slip inward and nudge the nerve toward firing. Nothing exotic happens next. The nerve reports what it always

reports — *cold, here, now* — and the brain, which has no way to audit the claim, believes it. The elegance, and the whole of our subject, is in what persuades the gate to open.

Three things persuade it, and they are not as different as they feel. The first is genuine cold: below roughly 26 °C, the channel's own machinery begins to favor the open state. The second is voltage. TRPM8 is weakly voltage-dependent, and at the warm temperature of a resting body its half-activation sits far out at a positive membrane potential — somewhere near +100 mV, a place the cell never visits. At that setting the gate is effectively shut. The third is menthol, and here is the quiet trick that gives this book its title: menthol does not force the pore. It is a *gating modifier*. It slides the channel's voltage-dependence to the left — by our bench's reckoning something close to −90 mV at a saturating dose — until the half-activation point drifts down through the −60 mV of a cell at rest. Cold does the same slide by a different road. The gate that would not open at body warmth now opens at body warmth, because the terms of opening have been quietly rewritten. The tissue is exactly as warm as it was. Nothing has changed but the message.

We can say *where* menthol whispers. Our structural models — and the cryo-EM work in the open literature that we check them against — place the menthol pocket not in the pore but inside the voltage-sensor-like domain, the bundle of four membrane helices (S1 through S4) that reads electrical field, snug against a tyrosine the field numbers Y745. Menthol wedges there and stabilizes the shifted, open-leaning conformation; the channel also needs the membrane lipid PIP_2 as a standing cofactor, so a cool is really a three-body agreement between molecule, protein, and the fat it floats in. Icilin, the laboratory super-cooler, binds a neighboring site deeper toward S3 and adds one strict demand menthol does not make: it will not work without intracellular calcium to bridge the pocket. That small difference in grammar is why icilin's cool creeps in slowly and overstays, while menthol's arrives clean and leaves on schedule — a distinction we will put a number on in a moment.

This is the mechanism of the kindness, and it is worth holding the two facts side by side, because the ethic of the whole library is in the gap between them. The instrument reads no change. The person feels cared for. Everything we grow lives in that gap.

Reading the cool as a number

A sensation you cannot count, you cannot design — you can only hope for it, harvest after harvest. So we anchor cool the same way we anchor every axis, to the half-maximal activation of its receptor:

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

EC_{50} is the concentration at which a stimulus drives TRPM8 to half of its full response. It is an inverse measure of loudness: the *less* of a thing you need to reach half-max, the *louder* it speaks, so EC_{50} sits in the denominator and the score rises as the concentration required falls. Fix a reference, scale by a thousand, and a leaf, a lozenge, and a cup of tea can be read against one another on a single line.

The reference we choose is deliberate, and it is a stated convention — the same kind of anchoring that defines the Scoville scale by its dilution-to-threshold: we do not say the reference value was derived from the literature and averaged; we say it is the point we chose to call 1,000, and everything else is read against it. We anchor the cool axis to a native menthol standard — a food molecule, the one the body has met across ten thousand suppers — and define it to read exactly **1,000**. On our TRPM8 reporter line that standard half-activates at an EC_{50} of 80 µM (the published range for menthol is wide, from single digits to a few hundred micromolar depending on cell line and temperature; we pin ours at 80 µM, measured at 25 °C, and report everything against it). From that one fixed point the rest of the ladder falls out of the arithmetic.

COOLING AGENT	CLASS	EC_{50} (OUR TRPM8 LINE)	IFI-C = 1,000 × 80 ÷ EC_{50}
Icilin	synthetic super-agonist	0.20 µM	≈ 400,000 — off-scale
WS-3	synthetic menthane-carboxamide	3.0 µM	≈ 26,700 — off-scale
WS-23	synthetic menthane-carboxamide	15 µM	≈ 5,300 — off-scale
(−)-Menthol	plant monoterpenoid (**reference**)	80 µM	**1,000** (anchor)
(−)-Menthone	plant monoterpenoid	400 µM	200
Camphor	plant monoterpenoid (also TRPV1)	800 µM	100
1,8-Cineole (eucalyptol)	plant monoterpenoid	3,400 µM	24

Read the ladder and you see at once why the anchor matters. Icilin half-activates at a fifth of a micromolar — four hundred times more potent than menthol — and on a menthol-referenced scale it would read near 400,000, four hundred times past the 5,000 ceiling our scorecards even draw. The synthetic menthane-carboxamides, WS-3 and WS-23, run off the top too. These are real molecules and real numbers, and they are exactly why we do not anchor the axis to the most potent cooler we can buy. The Index is built to measure the loudness of *food*, not the pharmacological ceiling of a chemistry catalogue. Anchor to menthol, the thing you have actually tasted, and the whole edible world falls into a range a person can reason about: menthone a quiet 200, camphor a complicated 100, eucalyptol a far-off 24, with engineered mint climbing above the anchor into the low thousands. Anchor to icilin and every food you own reads as a rounding error. The choice of zero on a ruler is a choice about who the ruler is for.

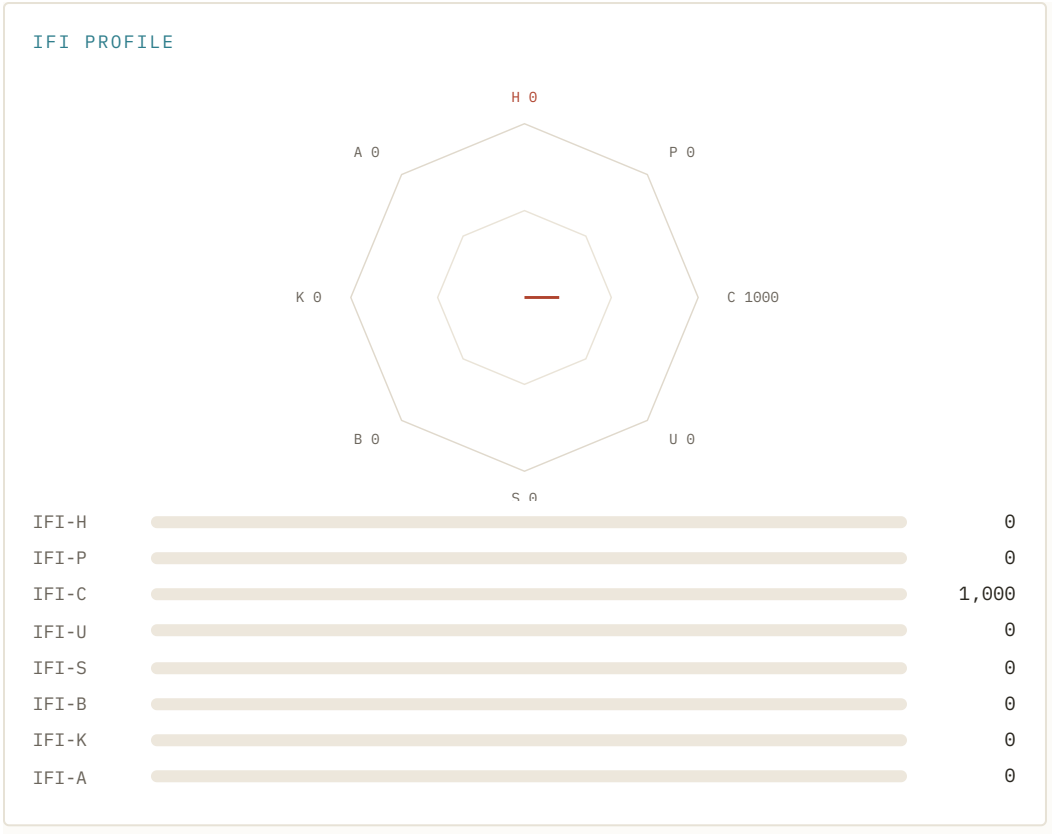
The formula inverts cleanly, which is what makes it a design tool and not just a label. By construction the reference reads 1,000: IFI-C = 1,000 × 80 ÷ 80 = 1,000. Push a candidate above the anchor and its required concentration must fall below the reference's; drop below, and it must rise. A cooling fraction that reads **800** half-activates TRPM8 at 1,000 ÷ 800 = 1.25× the reference concentration — it needs a quarter *more* material to say the same cool, which is to say it is, on purpose, the gentler voice. A line that read **2,400** would half-activate at 1,000 ÷ 2,400 = 0.417× the reference — the same cool

from 42% of the material, a cool two and a half times louder per gram. The number is not decoration. It tells a formulator, before a single batch is blended, exactly how much to weigh out.

One more honesty the arithmetic forces: potency is not abundance, and the two convert through molar mass. Our 80 µM menthol reference, at a molar mass of 156.27 g/mol, is 80 × 10^{-6} mol/L × 156.27 g/mol = **12.5 mg/L** — about twelve and a half parts per million. Eucalyptol at its 3,400 µM half-point (molar mass 154.25) works out to **524 mg/L**. The same half-cool asks roughly **42× the mass** of eucalyptol as of menthol. A grower who counted only milligrams in the leaf would badly misjudge the cool in the mouth; the Index counts the thing the receptor counts.

Two more measurements finish the picture, and the opening promised both. The first is the steepness of the climb. Menthol's dose–response on TRPM8 is cooperative — a tetramer with more than one pocket to fill — with a Hill coefficient of about nH ≈ 2.3 on our line. Run that through the Hill equation and the cool climbs from one-tenth to nine-tenths of full across a concentration change of only about 0.39× to 2.6× the EC_{50} — a span of roughly seven-fold. Cool is close to a switch. A little below threshold is almost nothing; a little above is nearly everything; the usable design window between "can't feel it" and "too much" is narrow, and the Index is what lets us place a product inside it rather than hope into it. The second measurement is time. Perceived cool, once the stimulus is gone, decays like a fading exponential, $I(t) = I_0 \cdot e^{(-t/\tau)}$. Menthol's time constant on our time-intensity panels sits near τ ≈ 180 s: a mint is about 37% spent by three minutes and effectively gone by nine or ten — the familiar arc of a lozenge. Eucalyptol, at τ ≈ 70 s, flashes and fades; icilin, needing its calcium bridge, lingers far longer, which is charming in a laboratory and wrong in a sweet. Loudness and *duration* are two different dials, and a cool designed for a throat lozenge and a cool designed for a sparkling water are two different points in that plane.

By construction, then, the axis has exactly one fixed star — the native menthol reference, defined to read 1,000 and, at its reference dose, silent on the other seven axes. A pure message of cool and nothing else:



Everything else on the cool axis is read against that single honest point.

Seven enzymes and one off-ramp

A number tells you where you want to land. The plant is how you get there, and the cool we prize most — menthol — is the end of a short, beautiful assembly line the mint family runs inside the glandular hairs on its leaves. Seven enzymes, one substrate handed down the row, each step a small committed edit to a ten-carbon skeleton.

#	ENZYME	REACTION
1	(−)-limonene synthase (LS)	geranyl diphosphate → (−)-limonene
2	(−)-limonene-3-hydroxylase (L3H)	(−)-limonene → (−)-trans-isopiperitenol
3	isopiperitenol dehydrogenase (iPD)	(−)-trans-isopiperitenol → (−)-isopiperitenone
4	isopiperitenone reductase (iPR)	(−)-isopiperitenone → (+)-cis-isopulegone
5	isopulegone isomerase (iPI)	(+)-cis-isopulegone → (+)-pulegone
6	(+)-pulegone reductase (PR)	(+)-pulegone → (−)-menthone
7	(−)-menthone reductase (MMR)	(−)-menthone → (−)-menthol
⊥	menthofuran synthase (MFS) — **the off-ramp**	(+)-pulegone → (+)-menthofuran

The line begins where every plant terpenoid begins, with geranyl diphosphate, and limonene synthase cyclizes it into a ring. A hydroxylase hangs an oxygen where the cool will eventually live; a dehydrogenase and a reductase and an isomerase walk the molecule to pulegone; pulegone reductase sets the ring's final shape as menthone; and menthone reductase delivers, at last, (−)-menthol — the exact stereochemistry the receptor's pocket is cut to receive. Seven steps, and the cool at the end of them is the cleanest cold a leaf knows how to make.

The drama is at step six, and it is the whole reason we grow this on purpose rather than take what the field gives. Pulegone stands at a fork. Down one road, pulegone reductase makes menthone, and the line continues to menthol — the cool we want. Down the other, a

single enzyme, menthofuran synthase, pulls pulegone off into menthofuran: a musty, off-note compound, and the very metabolite behind pulegone's reputation as something a body should not meet much of. That is the one off-ramp. A wild peppermint, stressed by a hot dry week, shunts more flux down it; the oil turns coarse, and the plant that should have been a kindness carries a small freight of the thing we would least want to serve. Our work on peppermint — 2,360 constructs and counting — is in large part the work of keeping the traffic on the main road: lifting pulegone reductase, quieting menthofuran synthase, and holding the whole line steady against the weather. The payoff is the number this library keeps returning to. Field peppermint's menthol fraction swings by tens of percent across a season; our controlled lines are designed to hold a batch-to-batch coefficient of variation of **≤7%**. Clean is not a slogan here. It is a flux balance with the off-ramp turned down and held by design.

And because the phenylpropanoid and terpenoid scaffolding beneath this runs in every green thing, the cool is portable. The transferable unit — the five rate-controlling genes of the route, the five-gene menthol cassette — can be moved into hosts that never made menthol, with the host's own terpenoid machinery supplying the non-limiting steps. The cool axis is, by the index's own tally, one of the hard ones: every organism we grow for it is rated *Hard*, because terpenoid pathways are finicky and the off-ramp is always waiting. But the roster is real, and it is various.

ORGANISM	CONSTRUCTS	PRIMARY AXES	CHARACTERISTIC COOLING MOLECULE	DIFFICULTY
Peppermint	2,360	C / M	(−)-menthol	Hard
Lavender	160	C	linalool + 1,8-cineole	Hard
Orange	120	B / C	limonene / linalool (minor cool)	Hard
Eucalyptus	80	C	1,8-cineole (eucalyptol)	Hard
Spearmint	80	C	(−)-carvone (weak on TRPM8)	Hard
Camphor	40	C	camphor (also TRPV1)	Hard

Spearmint earns a footnote that is really a lesson: it is a mint, it smells of mint, and it reads *low* on the cool axis — because its dominant monoterpenoid is carvone, which the nose loves and TRPM8 largely ignores. Aroma is not cool. The receptor does not read the bouquet; it reads the handful of molecules shaped to fit its pocket, and the Index reads only what the receptor reads. That gap between what a plant *smells* like and what it *cools* like is precisely the gap a number closes.

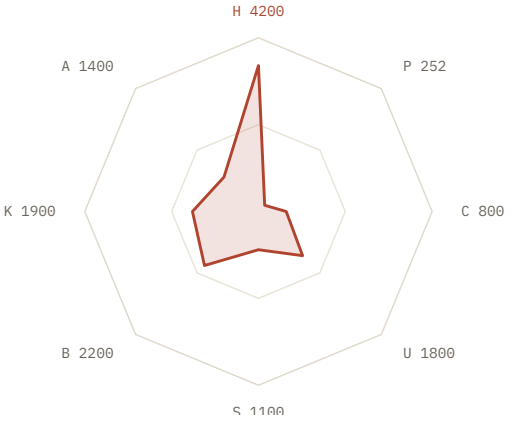


The controlled chamber where cool is held to a setpoint and read back — the discipline that turns a leaf's weather-dependent gift into a constant a maker can design against.

A cool held gently, even at full spectrum

It would be fair to expect that the loudest plant we ever built would shout on every axis. It does not, and the reason is the thesis of this chapter in one profile. Phoenix — SS-0096 — is the first cultivar designed to register on all eight axes at once, and on heat it is a scream: 4,200 on the Index, with our model predicting approximately 2.94 million Scoville heat units — a design target no existing plant reaches, written gene by gene into our computational framework. Look at where we set its cool.

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

The cool reads **800** — below the native menthol anchor of 1,000, the quietest full-strength voice in the whole profile. That was a decision, not a limit. We could have carried the cool up with the heat; the pathway was in hand. We did not, because cool at the back of a mouth already carrying three million Scoville is not relief, it is a second assault — a menthol glare behind a capsaicin fire, two channels screaming contradictory weather at the same nerve. Inverting the formula, 1,000 ÷ 800 = 1.25: Phoenix's cool asks a quarter more material than the reference to make itself felt, which is

exactly the restraint we wanted — a cool that steps back, opens the throat a little, and lets the heat be the thing. Design, at its most careful, is as much about the dials you leave low as the one you push. Even in the loudest thing we ever grew, the cool is held gently. Care looks, in the data, like restraint.

What cool cannot do — and why we say so

Rigor is also knowing where a thing ends, and we would rather name the edges ourselves than let a number imply more than it earns.

Cool is a message, not a temperature. This is the honest limit under all the others, and it is the one we will not blur: menthol cools nothing. It changes what a nerve says about the cold that is there, which at this dose is no cold at all. Every claim we make bends to that fact. We do not say our mint lowers a fever or cools a body or treats a thing. We say it *tastes of cool*, that the taste has comforted people for as long as there have been people, and that we have learned to grow it clean and constant. The thermometer is the final auditor, and the thermometer reports nothing. We would rather be exactly that honest and keep your trust than overclaim and spend it.

The failure modes are real and worth stating plainly. Push menthol too hard and it stops being cool: at high concentration it begins to bleed into the sharp channel, TRPA1, the pungency axis — the "toothpaste burn," a cool that has crossed over into a sting. The axis that reads 1,000 as a clean anchor reads a careless overdose as pungency, not pleasure, which is why the near-switch steepness of the Hill curve is a thing to respect rather than exploit. Camphor carries its own warning and sits in the table with an asterisk: it touches both TRPM8 and the heat receptor TRPV1, so its "cool" is muddied with warmth and, at dose, with desensitization — a complicated molecule we use with care and never lean on for a clean cold. Eucalyptol, at an IFI-C of 24, simply cannot carry a cool on its own; it is a top-note, a brightness, not a backbone, and a formulation that asked it to be the backbone would read as thin and flash away in seventy seconds. And a cool grown without the off-ramp held down is a cool that drifts: let menthofuran synthase run and the oil coarsens and the batch wanders off its ≤7% band. Most of the engineering is simply the refusal to let any of these happen twice.



Cucumber, cross-cut and cool — the clean, plain, garden kind of relief this whole axis is in service of.

None of these edges is a flaw in the cool. They are the shape of it — the real contour of a real sensation, drawn to scale. A cool that could do anything would be a cool you could not trust to do the one thing. We would rather grow the one thing, and grow it honestly, and tell you precisely where it stops.

Why we grow the cold

The work is a family's. The computing was one pair of hands, but the question that started it was asked at a shared table, over a glass of iced mint on a hot afternoon, when someone wondered aloud why the mint felt cold when the glass had already gone warm — and no one at the table could answer, and the not-knowing would not leave us alone. Everything here grew from that table. The forty-two plants, the nineteen thousand catalogue designs, the eight axes, the ≤7% variance target, the three patents filed and the twenty-one papers we have put into the open — all of it is the long answer to a short question a family could not stop asking. And the answer, when it finally came, was the sentence we opened with: at the channel, at the leaf, at the mouth, *nothing has changed but the message*. The cold is real to the one who feels it and absent from every instrument. It is a story the plant tells and the body believes, and we have spent ourselves learning to tell it well — the voltage slid by ninety millivolts and no degree moved, the seven enzymes and the one off-ramp held down, the EC_{50} pinned at 80 µM so a maker can weigh the kindness out by the milligram.

We make no claim on anyone's medicine, and we have been careful to make none, because the ethos under this library is a quiet one and a quiet thing is spoiled by overclaiming. We do not say mint heals or lavender cures or eucalyptus treats. We say they *taste of cool and calm and openness*, that those sensations have comforted people for a very long time, and that we have learned to place them exactly where a maker wants them — this loud, this long, this clean. The nourishment in this work is not a health claim. It is an intention: that a cool confection can ask a little less of the body and still taste like abundance, that a cold drink can refresh without a load of sugar behind it, that a plant can be grown to carry the fullest, cleanest version of the gift it already offers. Intention, never promise. The difference is respect, and respect is the whole house style.

That is why we grow the cold. Not to cool the world down — the world's temperature is not ours to set — but to put, into the hands of anyone who reaches for relief on a hard hot day, a cool that is clean, and lasting, and honest about being a story, and generous the way the best stories are. We spent a book turning that generosity into numbers, because numbers are how you give a thing the same, well, every time, to everyone, without being there to pour it yourself. These are computational designs; Scoville Splice's cultivars and constructs have not been experimentally validated in a laboratory. The thermometer will go on feeling nothing. The person will feel cared for. Between those two facts is the entire work, and we are not finished with it, and we are glad.