Beyond Stevia:
Are protein sweeteners the Next Big Thing?

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CLEAN LABEL CONFERENCE

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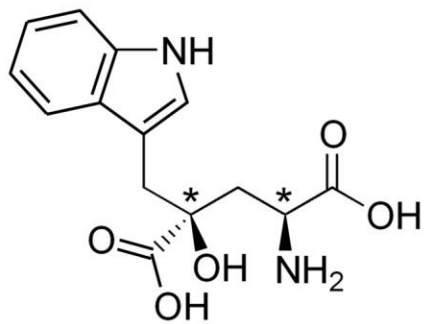
Outline

- Requirements for success
- Candidate proteins
- How do they match up?

Requirements for success

- Regulatory approval
  - safety
- Good taste
- Practical utility
- Acceptable cost

Cautionary tale: monatin



- amino acid related to tryptophan
- natural origin
- great taste, high potency
- history of human use

"…well tolerated at high dietary concentrations, not genotoxic/mutagenic, carcinogenic, or overtly toxic."*

"significant" ECG effects*

*Source: Darpo et al, 2016, *Food Chem. Toxicol.*, 91:217-224

Likely extra assessments

Protein digestibility
In vitro simulated gastric fluid

Allergenicity
Compare amino acid sequence against known allergens

Toxigenicity
Compare amino acid sequence against known toxins

Requirements for success

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- Good taste
  - sweetness quality, dynamics
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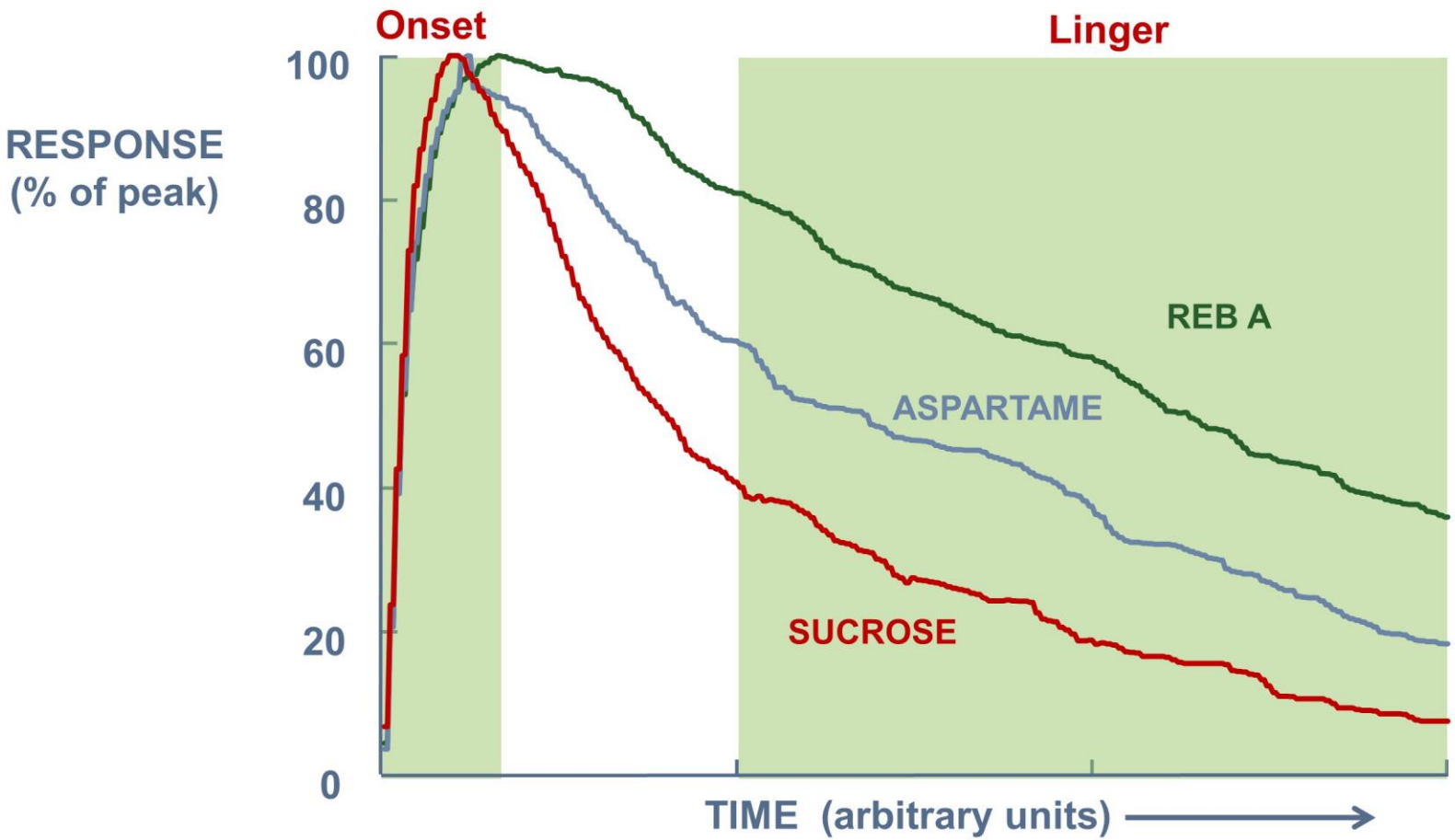
- Acceptable cost

Good taste

Intensity/time, ~8% sucrose equivalent



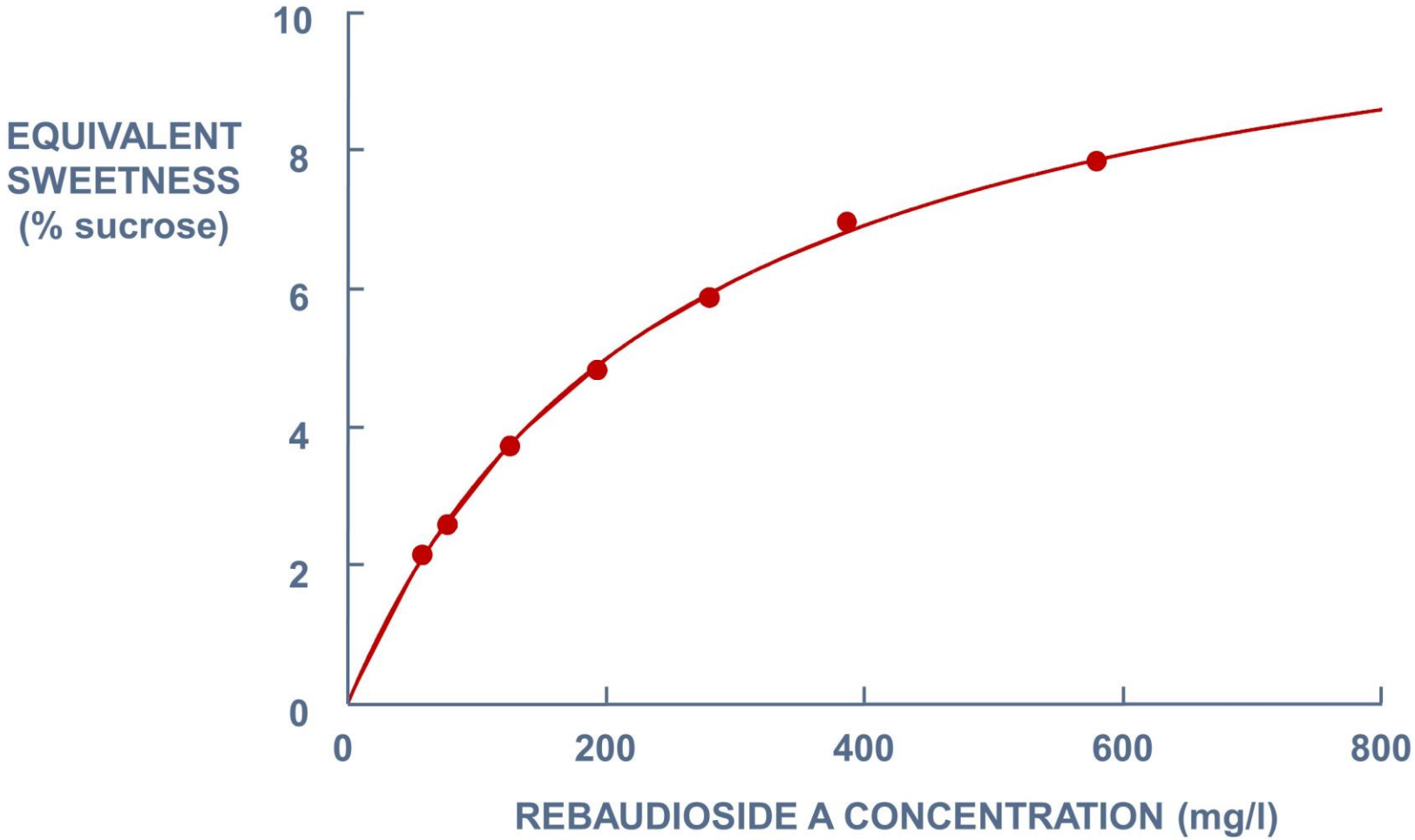
Source: Prakash et al, 2008, *Food Chem. Toxicol.*, 46(7S):S75-82

Requirements for success

- Regulatory approval
  - safety
- Good taste
  - sweetness quality, dynamics
- Practical utility
  - solubility, stability
- Acceptable cost
  - potency

Acceptable cost

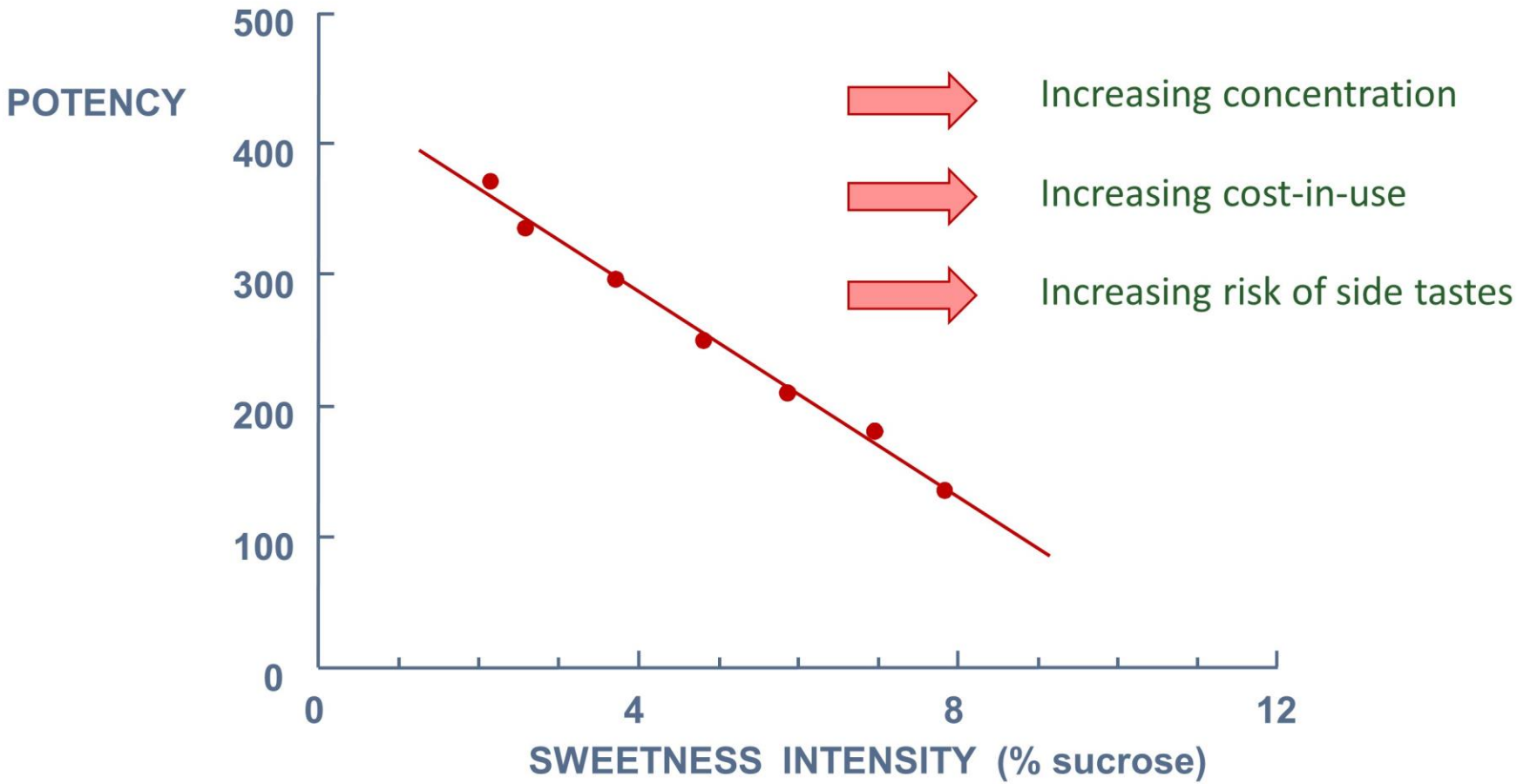
Concentration–response of typical HPS: rebaudioside A



Source: Fry, Yurttas & Biermann, 2011, *J. Food Sci.*, 76(9):545-8

Acceptable cost

Potency declines as HPS concentration rises (e.g. rebaudioside A)

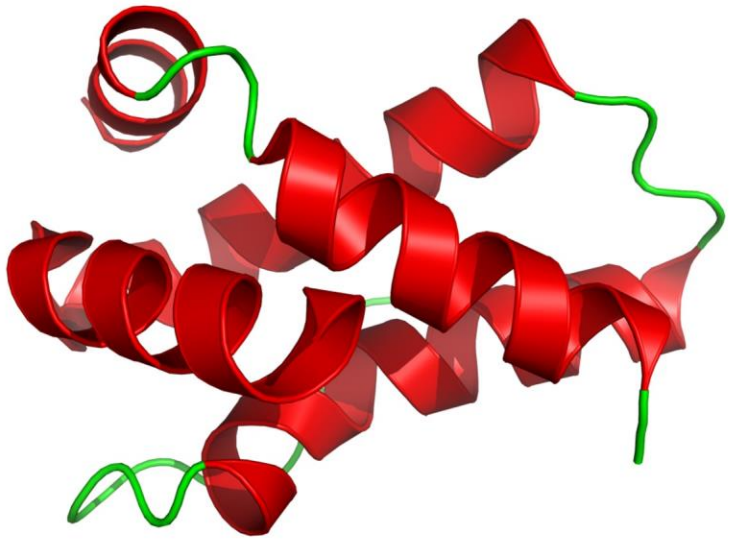


Source: Fry, Yurttas & Biermann, 2011, *J. Food Sci.*, 76(9):545-8

Acceptable cost

Beware how potency is expressed

Mabinlins potency “100-400” on a molar basis



Even if mabinlin 2 (10.4 kDa) were 400x sucrose on molar basis….

= 13x on a weight basis

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Candidate proteins

Sweetener	Source	
Brazzein	**Plant**	*Pentadiplandra brazzeana*
Curculin/neoculin	**Plant**	*Curculigo latifolia*
Lysozyme	**Animal**	*Eggs of hen, turkey, duck, quail…*
Mabinlins	**Plant**	*Capparis masakai*
Miraculin	**Plant**	*Richardella dulcifica (Synsepalum dulcificum)*
Monellin	**Plant**	*Dioscoreophyllum cumminsii, D. volkensii*
Pentadin	**Plant**	*Pentadiplandra brazzeana*
Thaumatin	**Plant**	*Thaumatococcus daniellii*
Designer proteins	**Lab**	

Candidate proteins

Sweetener	Main issues
Brazzein	
Curculin/neoculin	*Not heat stable, poor dynamics, taste interactions with acid*
Lysozyme	*Low potency (10-20x)*
Mabinlins	*Low potency (10-13x)*
Miraculin	
Monellin	*Not heat stable, poor dynamics*
Pentadin	*Potency about 25% of brazzein & lower quality sweetness. Breakdown product of brazzein?*
Thaumatin	
Designer proteins	

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Thaumatin
Miraculin
Brazzein
Designer proteins

How do they match up?

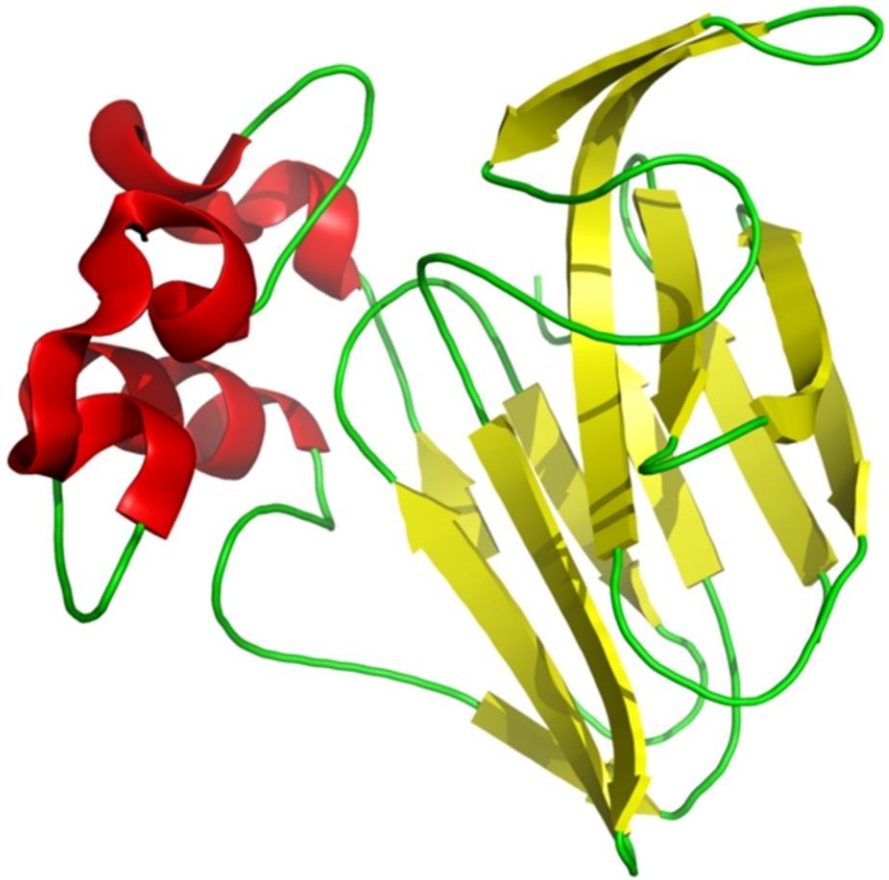
Thaumatococcus danielli , Katemfe fruit

Wild shrub: Nigeria, Ghana, Cameroon & Ivory Coast

Picture: Naturex

How do they match up?

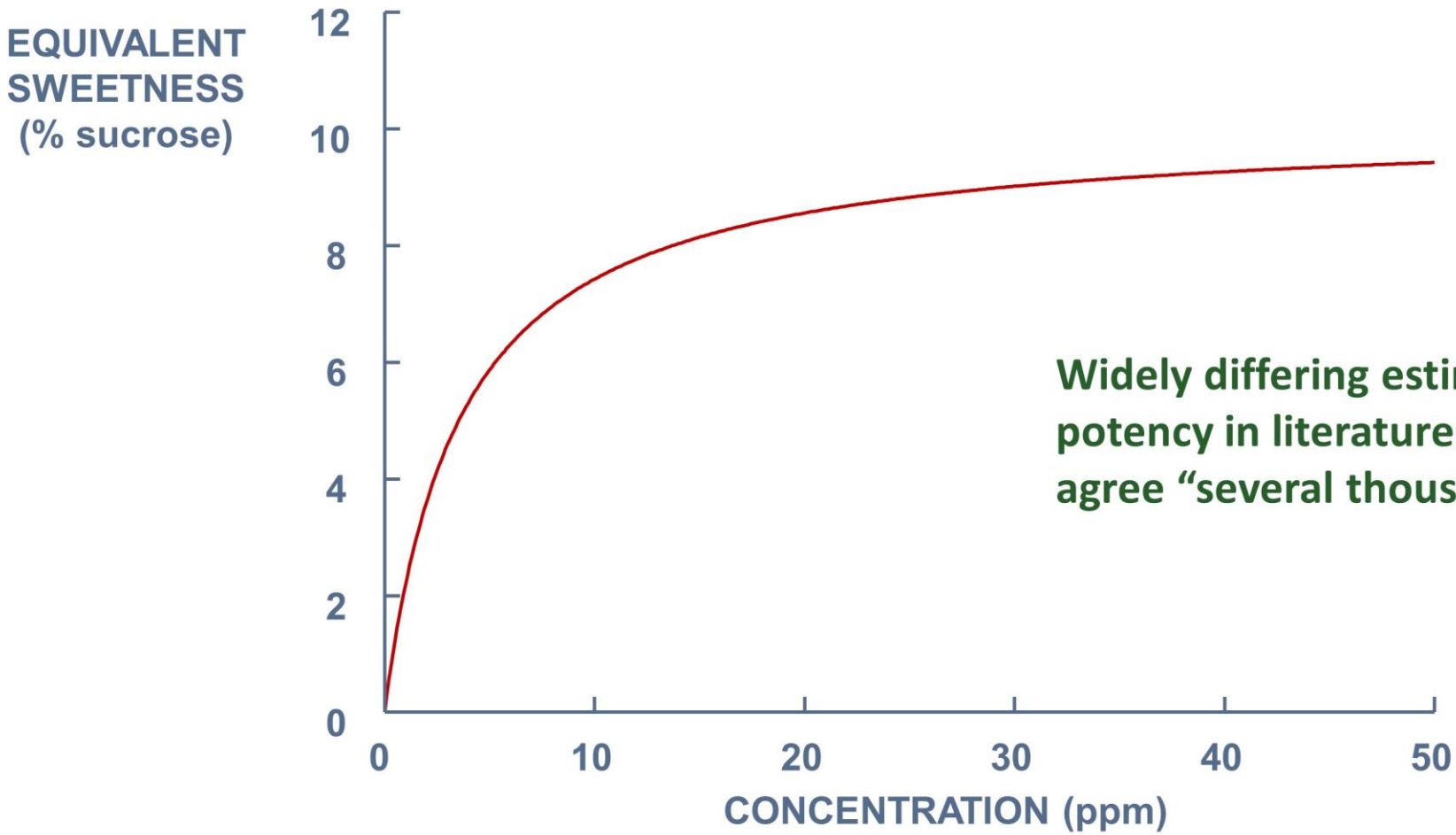
THAUMATIN



- Mixture of proteins
- 5 isoforms I, II, III, a, b
  mainly I & II
- All sweet
- 207 amino acids, 22.2 kDa
- ~6g thaumatin/kg wet fruit

How do they match up?

Concentration–response of thaumatin



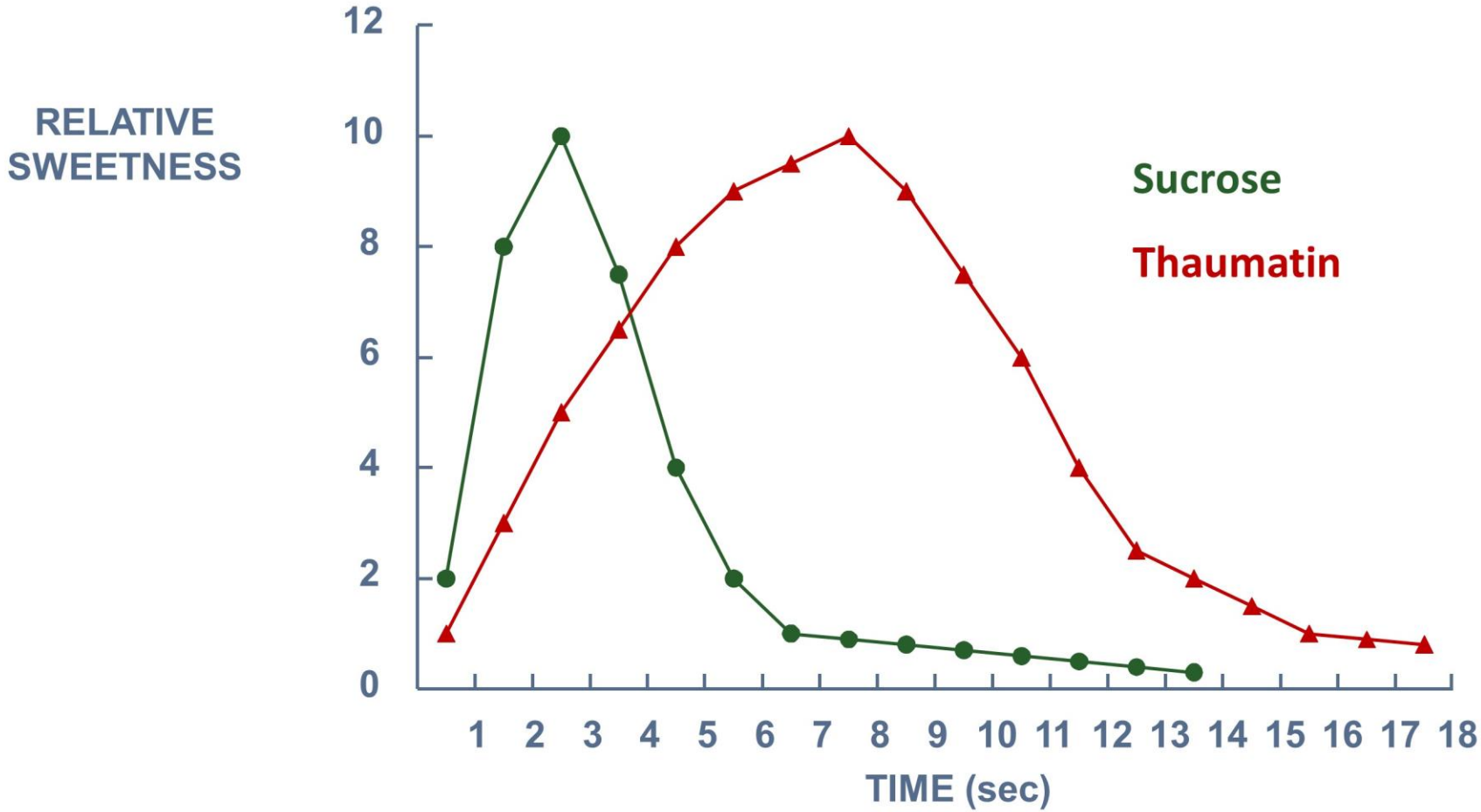
Widely differing estimates of potency in literature. Most agree "several thousand".

Source: DuBois et al, 1991, in *Sweeteners Discovery, Molecular Design & Chemoreception,* ACS Symposium Series 450

How do they match up?

Thaumatin dynamics: very slow onset & long linger

(Concentrations not given)



Source: S. Pearce & H. Roth, in *Advances in Flavours and Fragrances: From the Sensation to the Synthesis* Royal Society of Chemistry, UK, 2002, pp 178-193

How do they match up?

	Thaumatin status
• Regulatory approval - safety	USA FEMA GRAS #3732 EU E957, good safety data
• Good taste - sweetness quality, dynamics	Licorice side taste Very poor dynamics
• Practical utility - solubility, stability	Commercially available V. soluble, stable
• Acceptable cost - potency	Acceptable High potency

How do they match up?

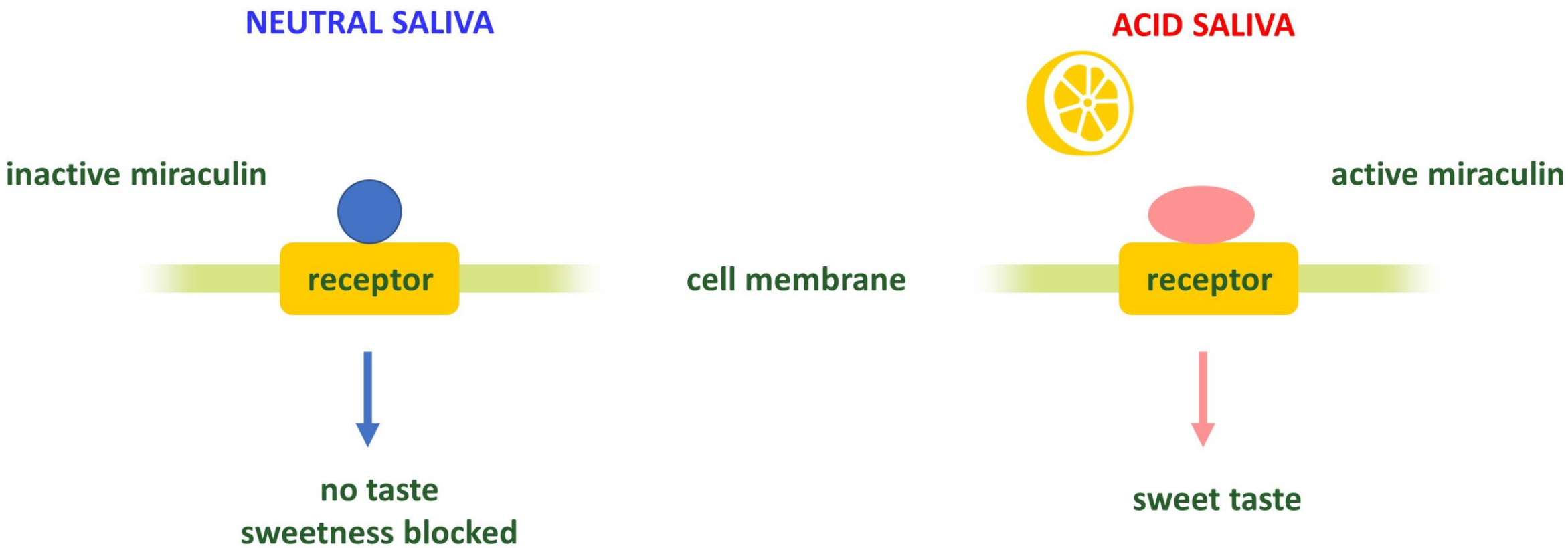
Richardella dulcifica (Synsepalum dulcificum), Miracle fruit

- Glycoprotein
- Tetramer of 2 dimers
- 191 amino acids, 24.6 kDa
- not sweet itself
- sweet with acids

Berry picture: Hamale Lyman. Miraculin: Jmol development team

How do they match up?

How miraculin works



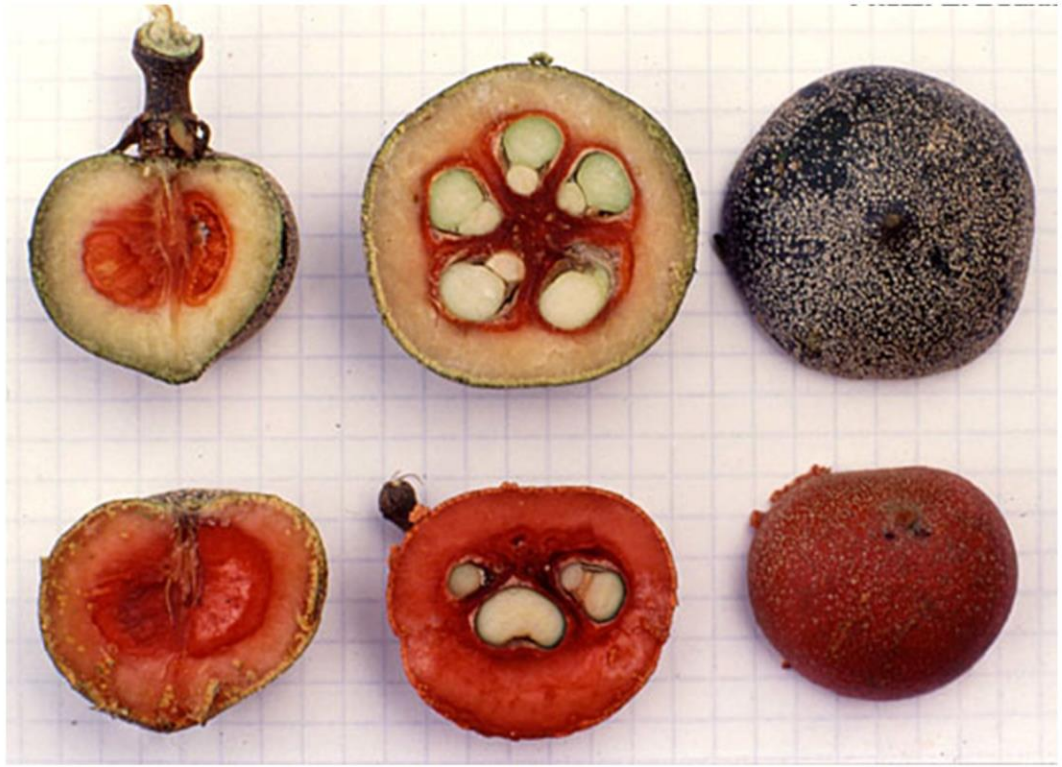
How miraculin works

How do they match up?

	Miraculin status
• Regulatory approval - safety	EU dry berry = novel food FDA: “Insufficient safety data”
• Good taste - sweetness quality, dynamics	Good quality taste Very poor dynamics
• Practical utility - solubility, stability	Practicality dubious Soluble, stability?
• Acceptable cost - potency	Cost? Potency?

How do they match up?

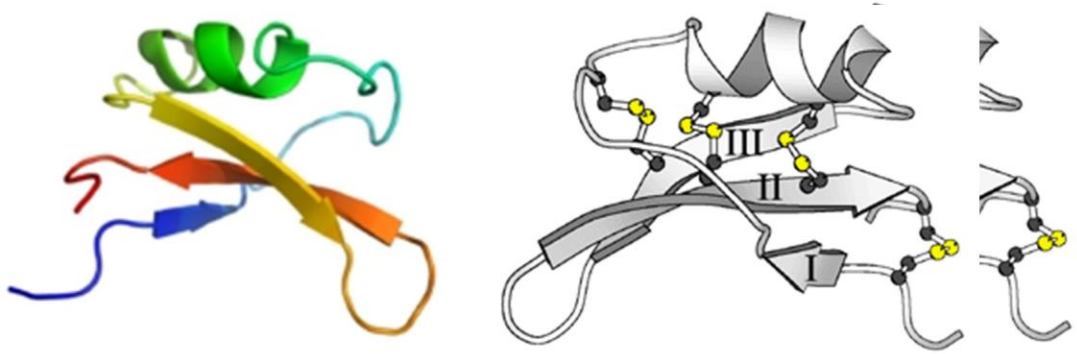
Pentadiplandra brazzeana



Grows wild: Angola, Congo, Central African Republic, Cameroon, Gabon and Nigeria

Pictures: P. Latham, E. Dounias

How do they match up?



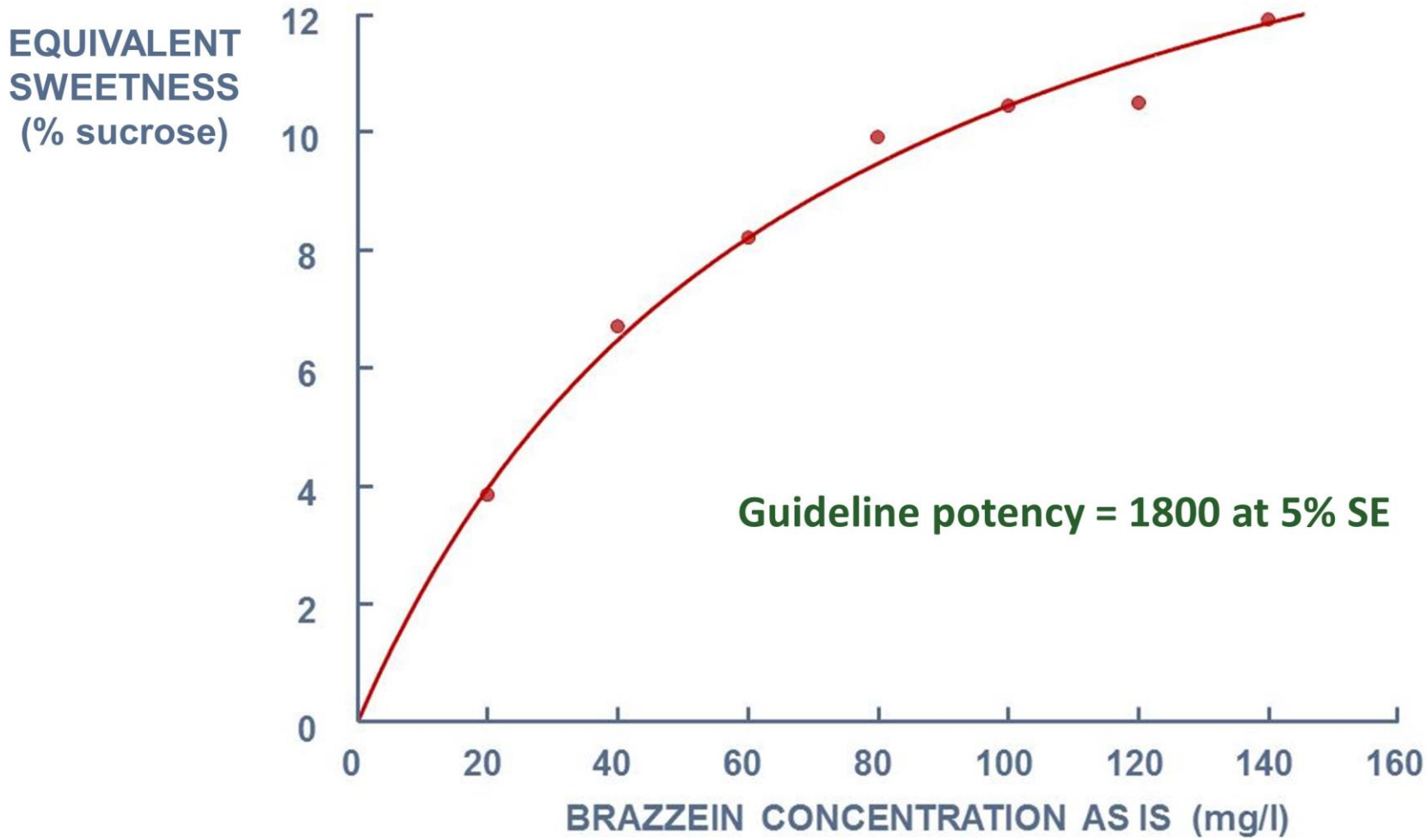
Pulp round seeds = 0.05 – 2% brazzein
Low molecular weight (6.5 kDa) protein

Type	Amino acids	Stability	Notes
1	54	stable	v. slow onset licorice
2	54	unstable → type 1	
3	53	stable	most potent faster onset little licorice

3 types

How do they match up?

Concentration–response type 3 brazzein



Source: Applied Biotechnology Institute

How do they match up?

	Brazzein status
• Regulatory approval - safety	No approval No safety data
• Good taste - sweetness quality, dynamics	Good quality taste Poor dynamics
• Practical utility - solubility, stability	V. soluble, stable at 98°C, wide pH range
• Acceptable cost - potency	Cost? High potency

Outline

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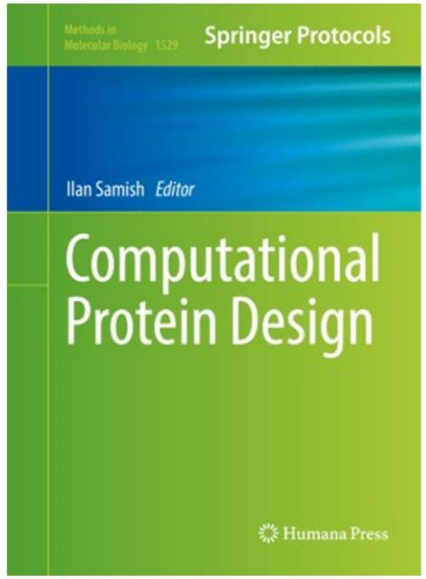
  - Thaumatin
  - Miraculin
  - Brazzein
  - Designer proteins

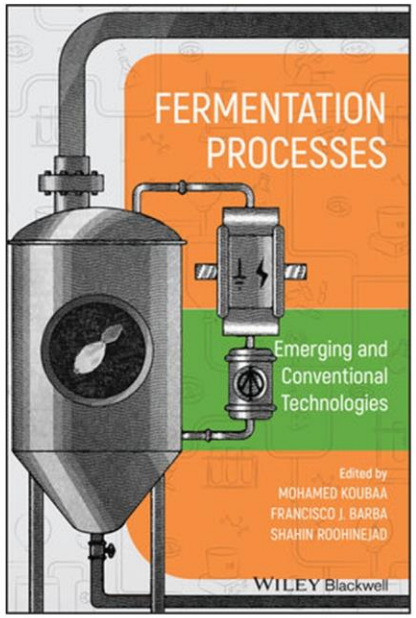
How do they match up?

What are “designer sweet proteins”?

Take natural protein
e.g. monellin



Change structure to improve properties



Produce new protein by fermentation in host organism

Mutant “designer protein”

How do they match up?

Key issues

Pro

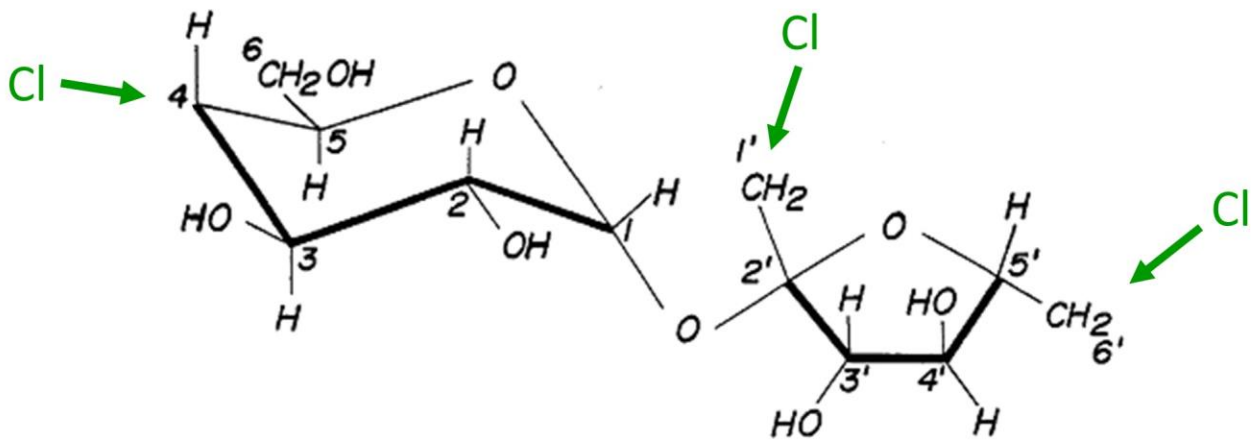
- possibility to engineer out defects of natural protein sweeteners
- fermentation “easily” scaled up
- high potencies available → cost effective

Con

- not found in nature
- consumer reaction untested

How do they match up?

"70-100% identical to sweet proteins found in nature"



sucralose

Sucralose molecular weight = 397.6 g/mol

3 chlorines in sucralose = 106.4 g/mol

% chlorine by weight = 106.4/397.4 *100
= 26.75% of molecule

Sucralose is (100-26.75) = **73.25% nature identical**

How do they match up?

- Regulatory approval
  - safety
- Good taste
  - sweetness quality, dynamics
- Practical utility
  - solubility, stability
- Acceptable cost
  - potency

Designer proteins status

No approval (yet)

No safety data (yet)

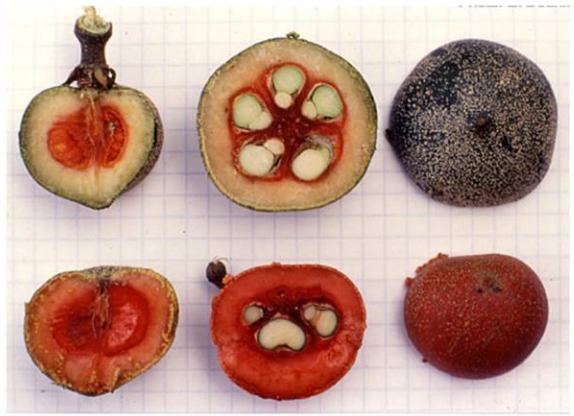
Probably good quality taste

Claimed good, little evidence (yet)

Summary

Thaumatin

Established success
More flavor enhancer than sweetener



Brazzein

Promising, but dynamics issue
No safety yet

Miraculin & others of natural origin

Currently no realistic prospects

Designer proteins

?