Protein Safety: Allergenicity

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HESI Protein Allergenicity Technical Committee (HESI PATC)

Public and Private Sector Participation

Co-Chairs:

- Dr. Gregory Ladics (DuPont Pioneer)
- Dr. Scott McClain (Syngenta USA)
- Prof. Ronald van Ree
- (Academic Medical Center)

Staff: Nancy G. Doerrer, MS (HESI)

Public Participants:

- Academic Medical Center, University of Amsterdam, Netherlands
- Copenhagen University Hospital at Gentofte, Denmark
- Guangzhou Medical University, China
- US Environmental Protection Agency
- US Food and Drug Administration

Sponsors:

- BASF Plant Science
- Bayer SAS
- DuPont Pioneer
- Monsanto Corporation
- Dow AgroSciences
- Syngenta USA

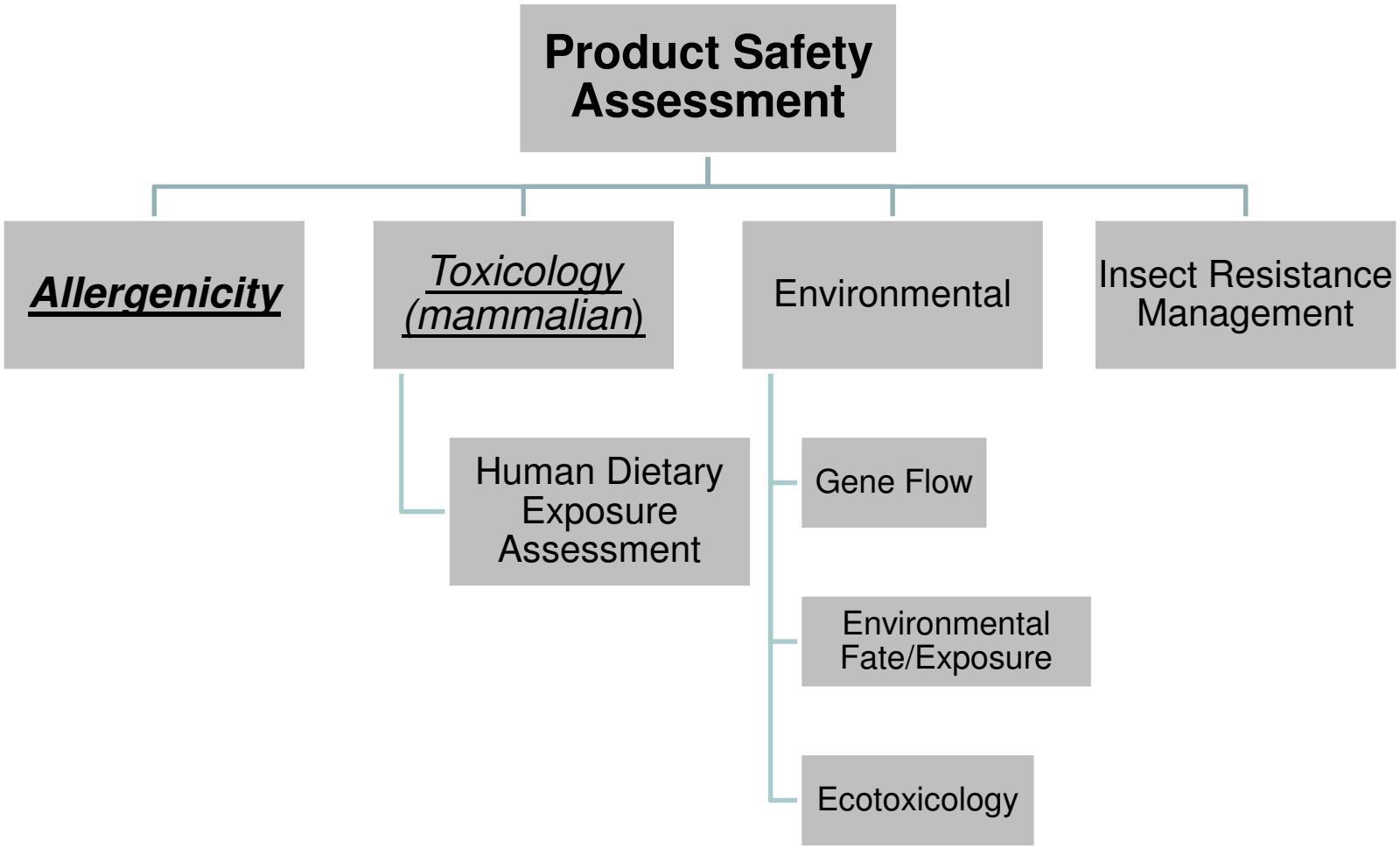
PATC Mission

To advance the scientific understanding of relevant parameters defining allergenic proteins, as well as to encourage development of reliable and accurate methodologies for characterizing the allergenic potential of novel proteins.

Objectives

- Promote understanding of what makes a protein allergenic;
- Establish processes useful in a weight-of-evidence approach to the evaluation of novel proteins expressed in biotechnology products;
- Develop scientific uniformity for these evaluations; and
- Communicate scientific findings to the academic, industry, and regulatory communities.

Place of allergenicity assessment in product safety assessment

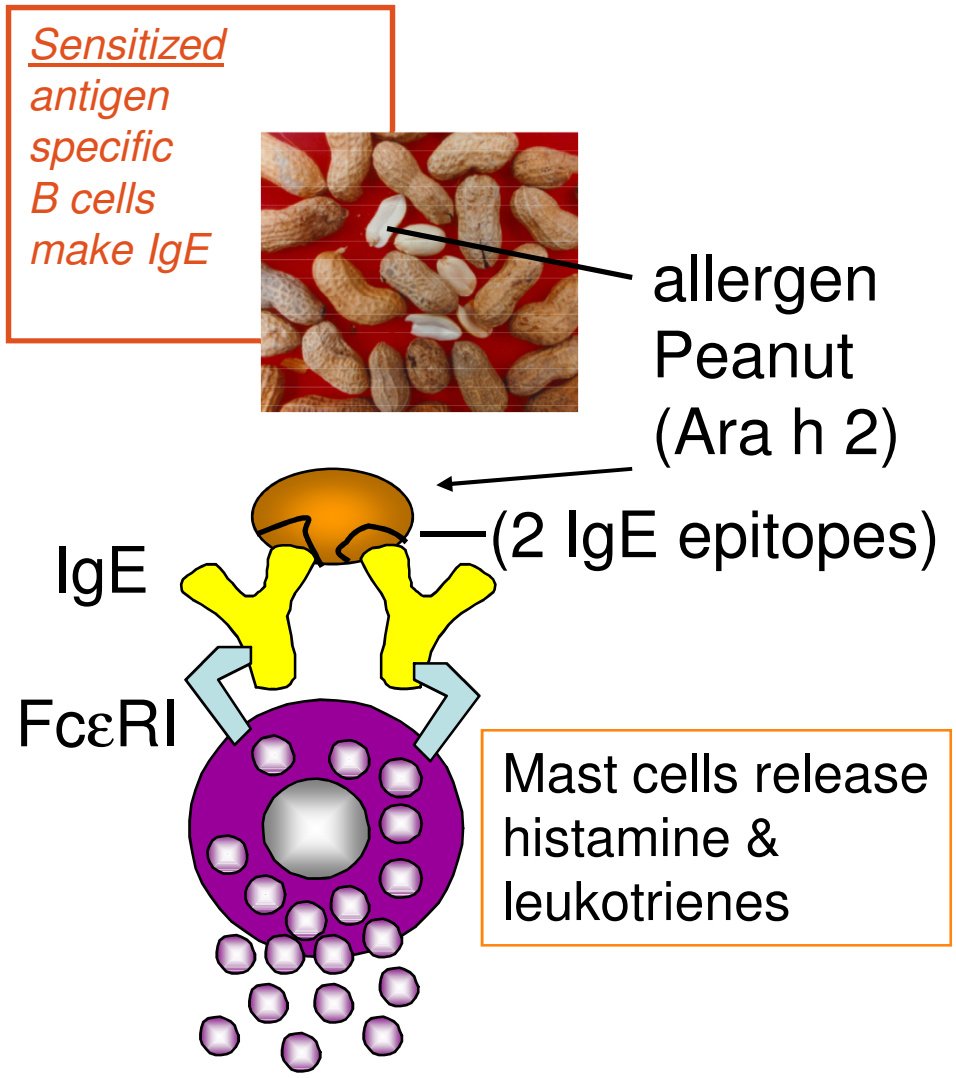


Allergen-specific IgE is the key mediator in Food Allergy

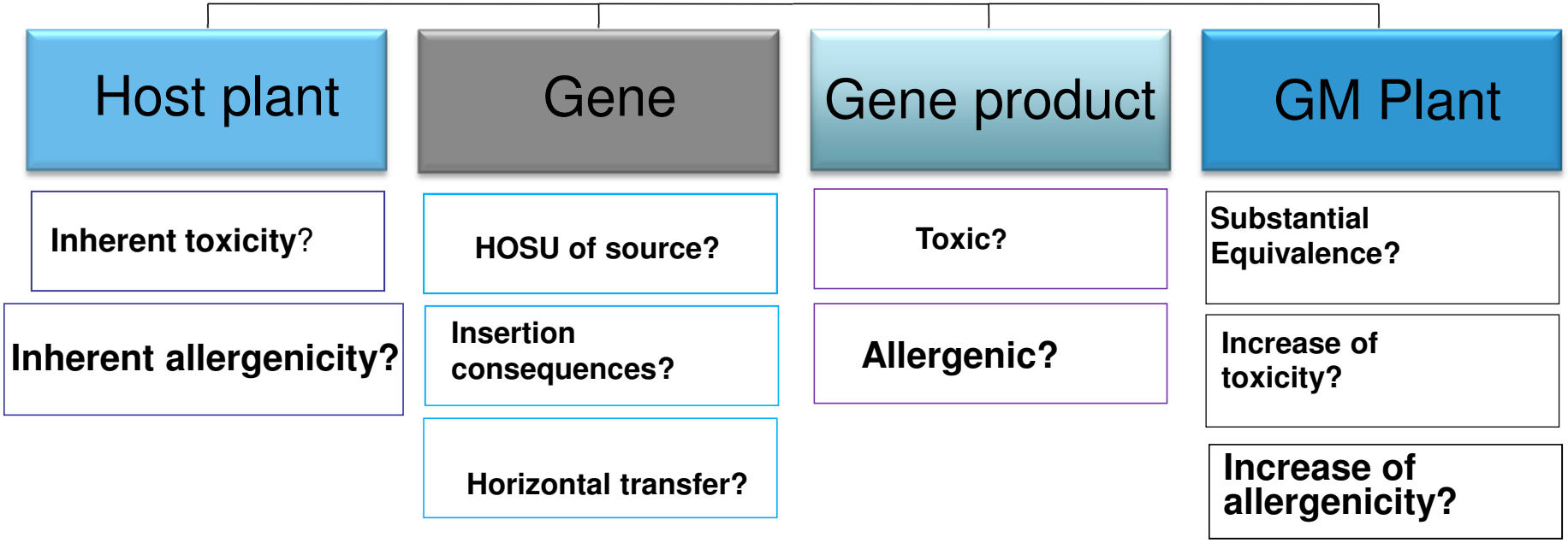
IgE-mediated symptoms

~15 minutes after eating:

- oral itching
- hives
- angioedema
- asthma
- diarrhea/vomiting
- atopic dermatitis
- anaphylaxis



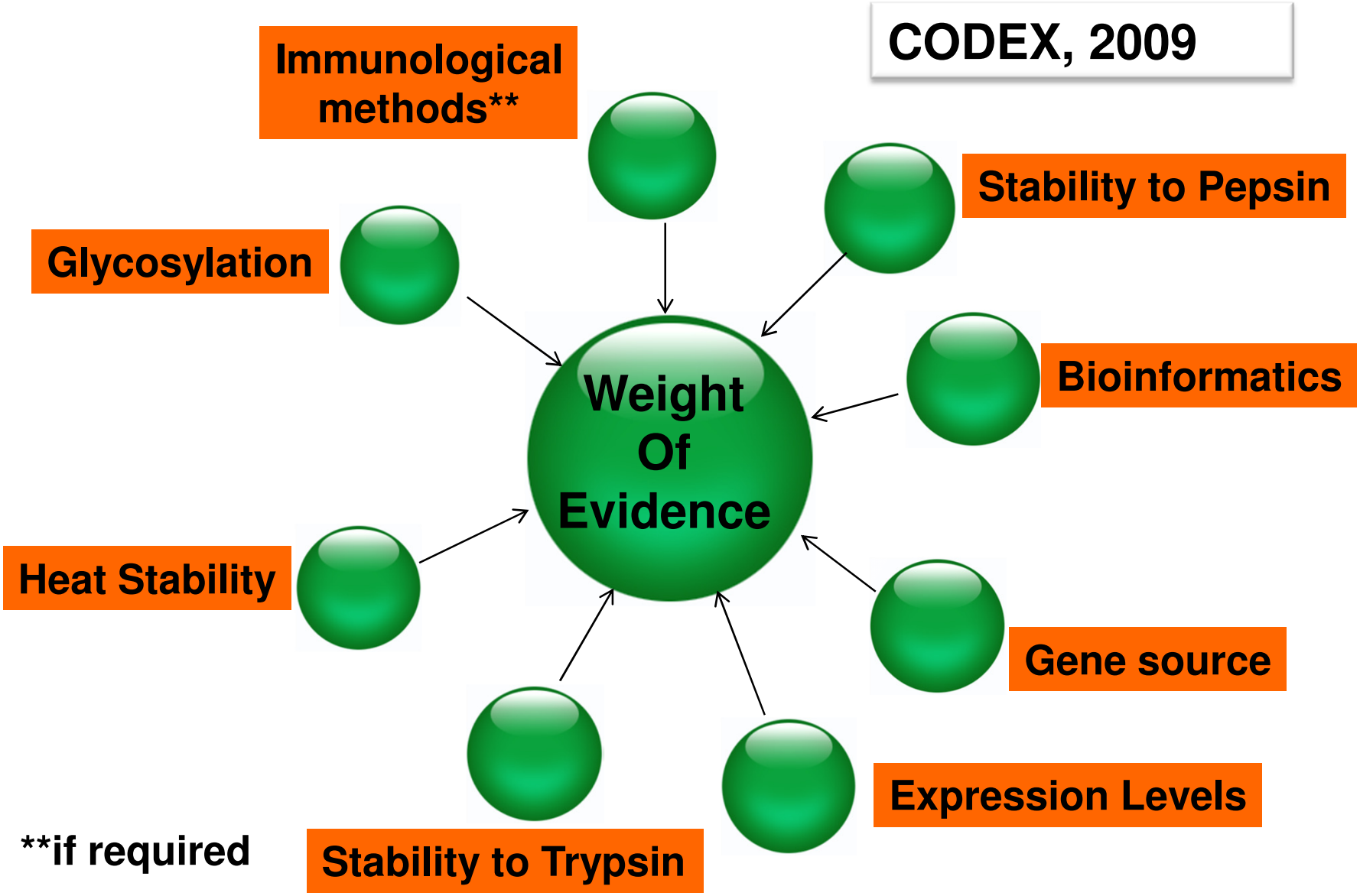
Where to search for potential allergy risks?



Comparison of the GM crop to a conventional equivalent with a History of Safe Use (HOSU) guides the safety assessment

Weight-of-Evidence Approach

How to identify the protein that will turn out to be an allergen?



What is an allergen?

Most stringent definition:

“An antigen that sensitizes (induces IgE) and (usually) causes symptoms”

COMPLETE ALLERGEN

Cross-reactive allergen:

“An antigen that does/can not sensitize itself but can cause symptoms”

INCOMPLETE ALLERGEN

Ergo, there are two potential risks when introducing a transgene:

- Introducing a risk for de novo sensitization (induction of IgE)
- Introducing a risk for inducing symptoms in already sensitized subjects

What is the origin of food allergy?

There are essentially two ways to become food allergic:

1. Exposure to foods such as egg, milk, fish, peanut or hazelnut





COMPLETE ALLERGENS

2. Exposure to respiratory allergens such as pollen or mites

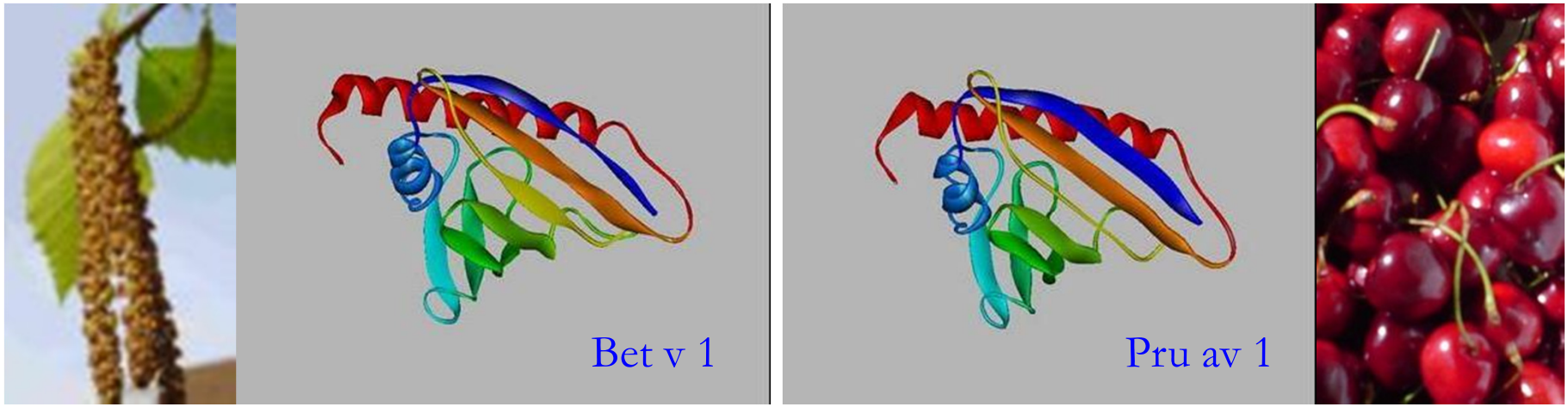
→ cross-reactivity to foods





INCOMPLETE ALLERGENS

Structural homology is the explanation of cross-reactivity



“Homology to an allergen makes the antigen Pru av 1 an allergen but NOT a COMPLETE ALLERGEN”

Some examples

COMPLETE ALLERGENS (sensitizers/ frequent symptoms):

Inhalant: Bet v 1, Phl p 1, Phl p 5, Der p 1, Der p 2, Fel d 1, etc.

Food: Ara h 2, Pru p 3, Cor a 9, Gad c 1, Gad m 1, etc.

INCOMPLETE ALLERGENS (cross-reactive / possible symptoms):

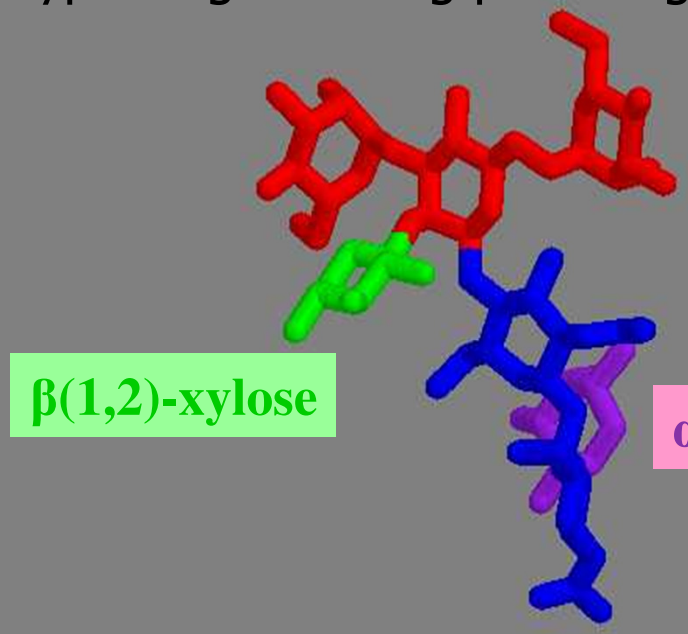
Food: Bet v 1-homologues in fruits, nuts and vegetables

profilin homologues in virtually all plant foods

plant N-glycans

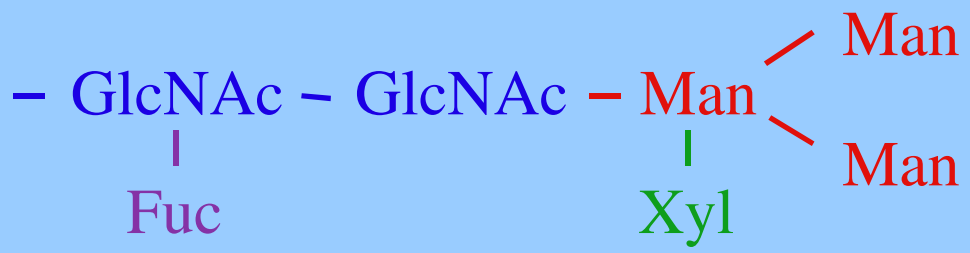
Many cross-reactivities are not clinically relevant, at least not in every patient
Sometimes no evidence for clinical relevance can be found at all.

Typical IgE-binding plant N-glycans

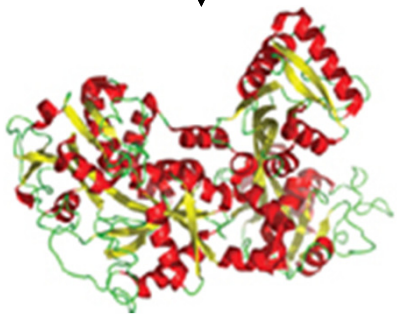


β(1,2)-xylose

α(1,3)-fucose



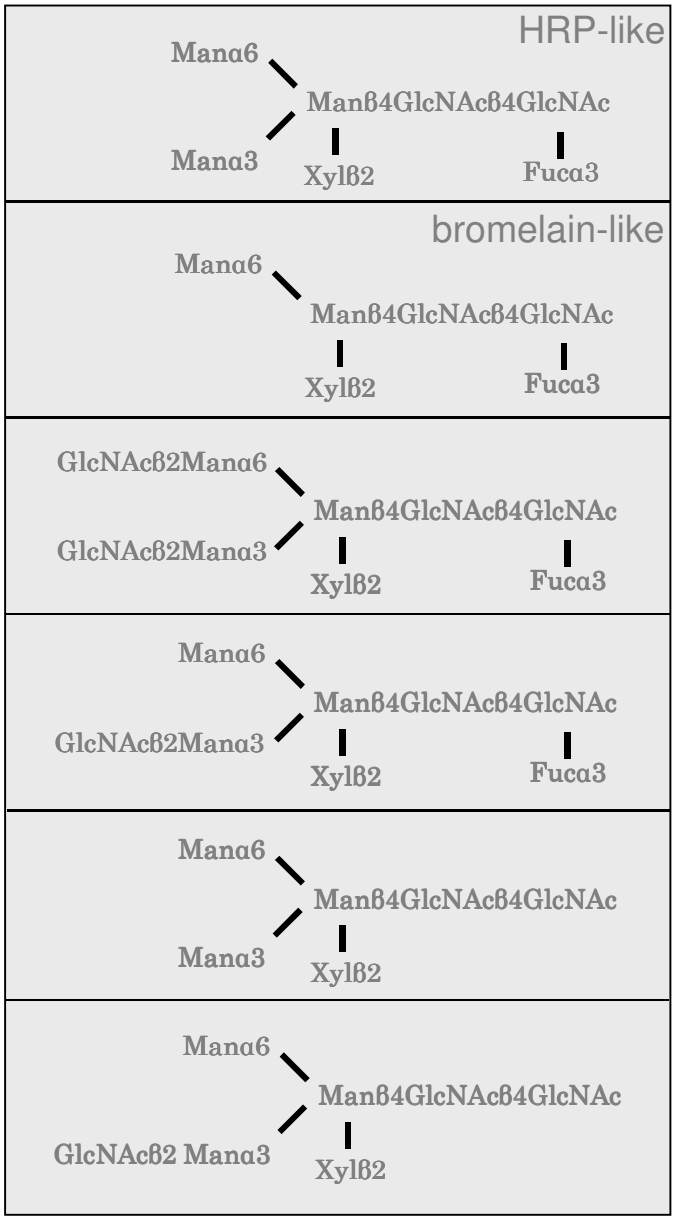
Expression of human lactoferrin in rice: plant glycosylation

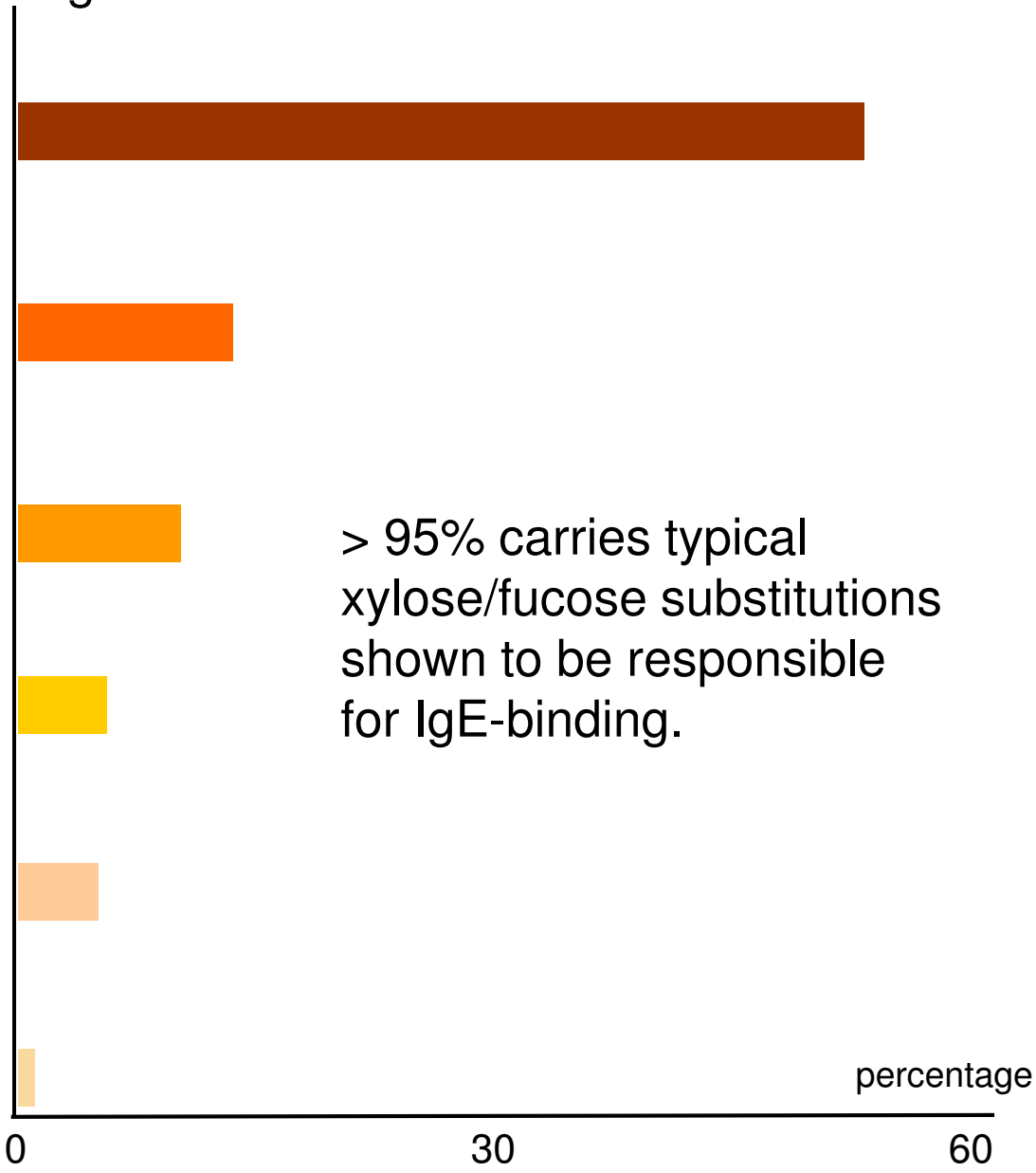


primate glycosylation

plant glycosylation

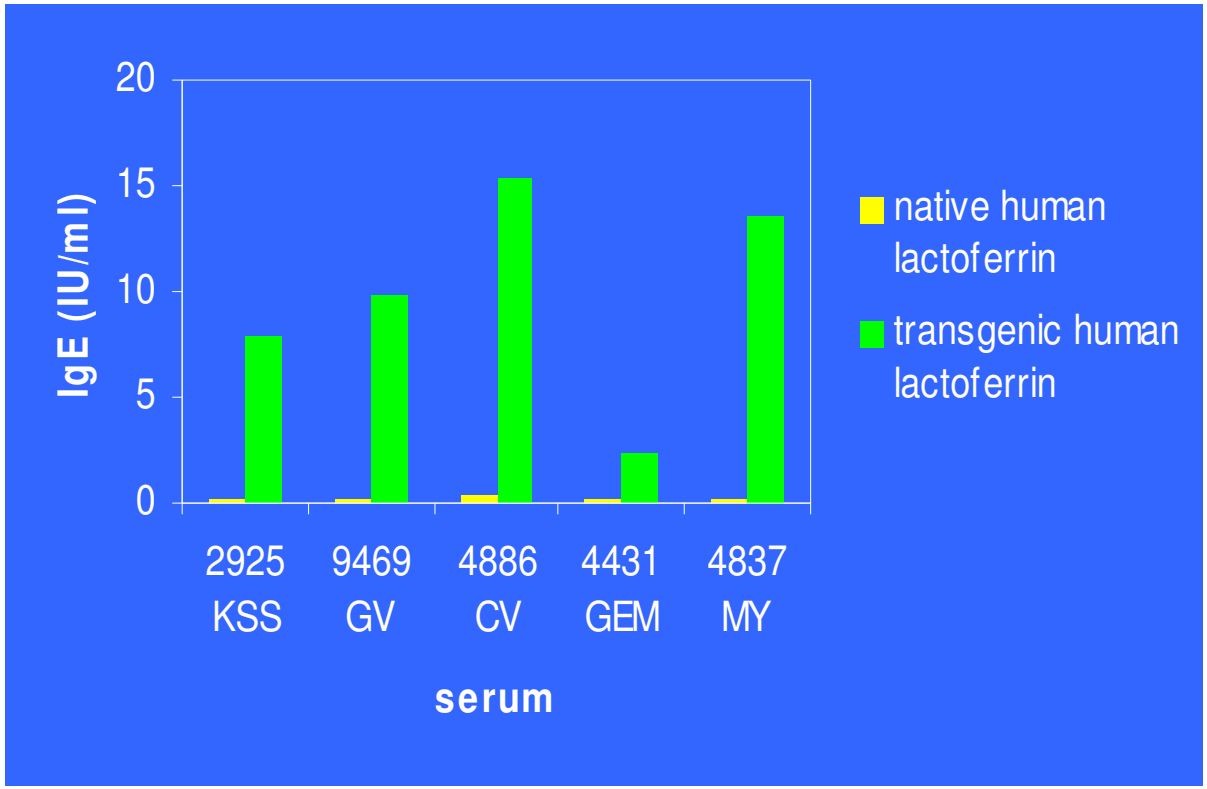
Sugar moieties found on transgenic lactoferrin



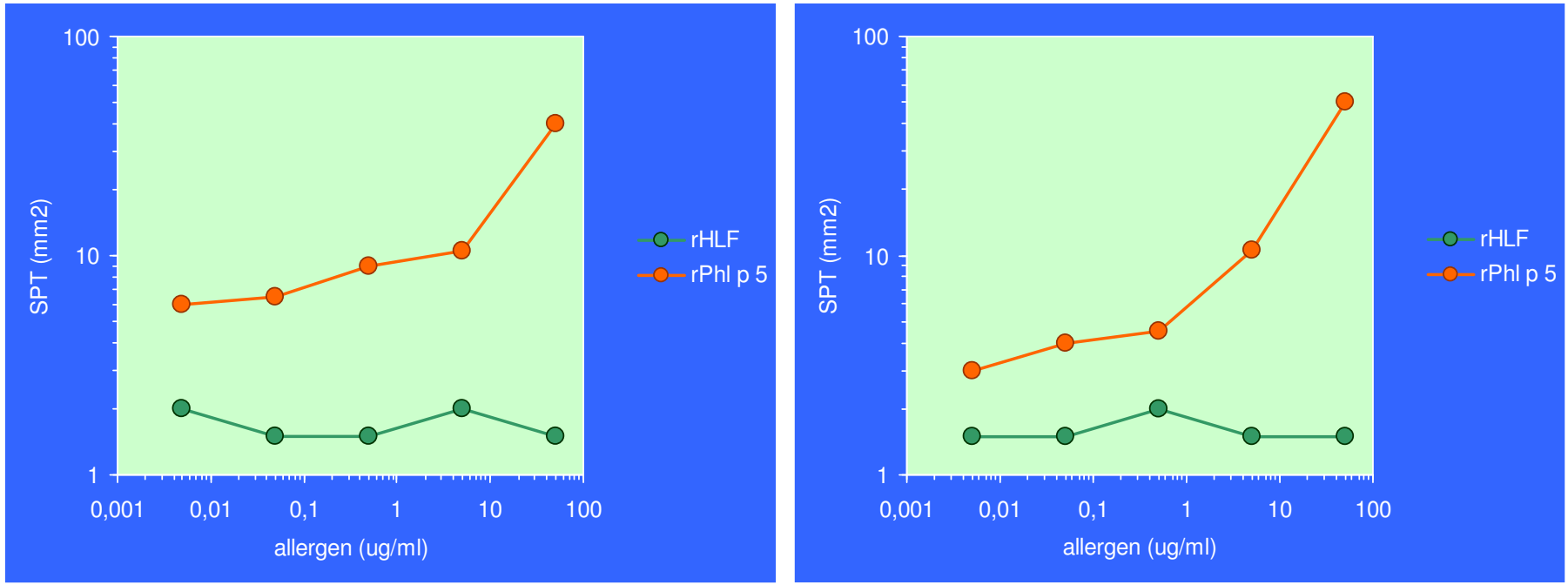


> 95% carries typical xylose/fucose substitutions shown to be responsible for IgE-binding.

Expression of human lactoferrin in rice introduces IgE-binding glycans

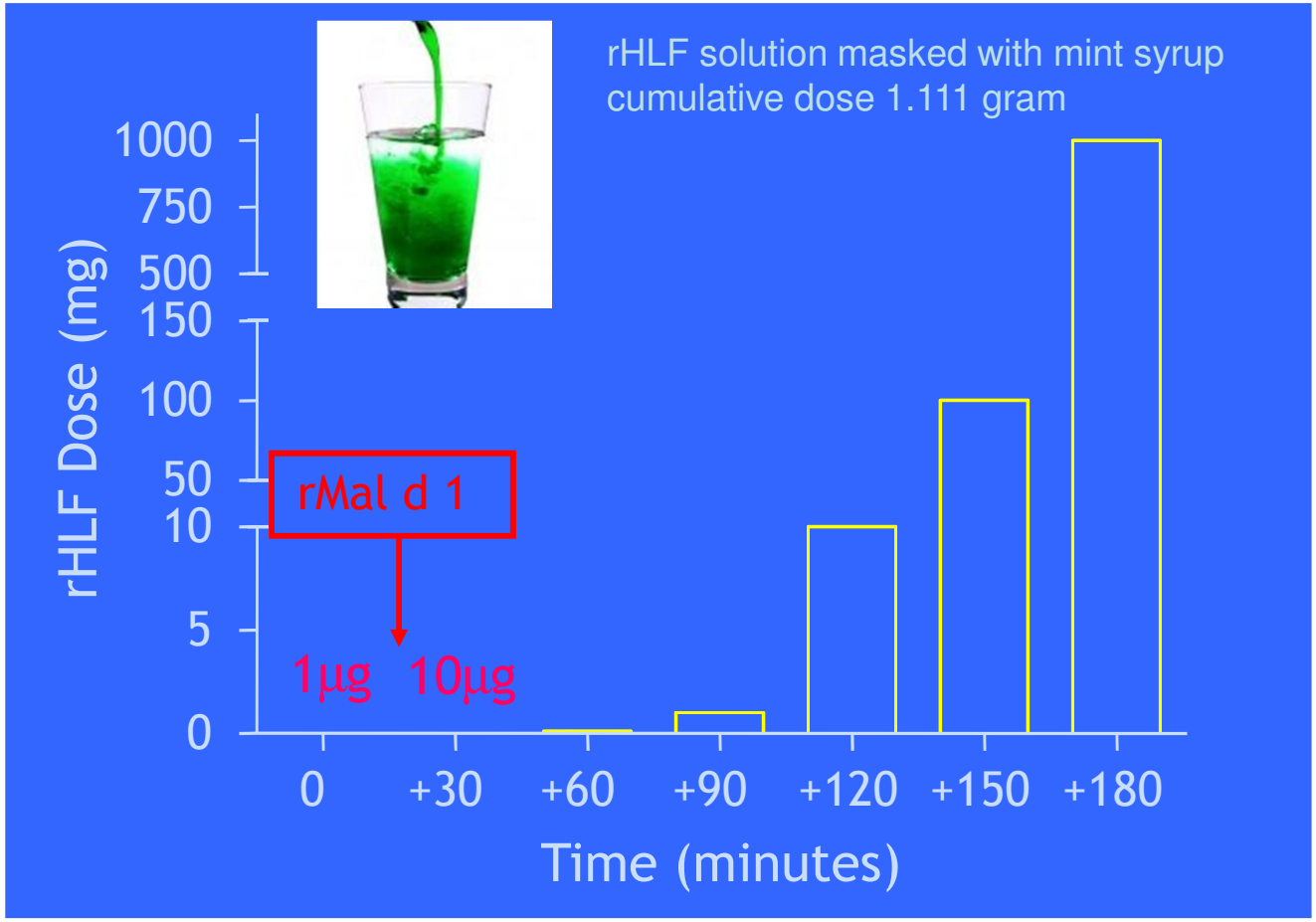


SPT: a true allergen (rPhl p 5) versus glycans (rHLF)



IgE anti-glycans has no significant biological activity

DBPCFC with rHLF from transgenic rice
in 5 plant glycan-IgE positive subjects



rMal d 1 induced subjective and/or objective symptoms in µg range

Bolhaar ST et al. Clin Exp Allergy 2005;35:1638-44

All five subjects had a negative challenge with a cumulative dose of 1.111 gram

Mari A. et al. Allergy 2008; 63: 891-6

- Introducing a risk for inducing symptoms in already sensitized subjects

  Demonstration of specific IgE is in general an alarm bell
  The example of plant N-glycans however demonstrates that this may sometimes be overcautious and result in discarding perfectly safe proteins with N-glycosylation sites.

  Having said that searching for homology (BIOINFORMATICS) with existing allergens is of great importance to rule out that existing allergens are introduced, or proteins with likely cross-reactivity to existing proteins.

How does bioinformatics help?

Allows one *primary* question to be asked:

Is the protein an existing allergen?

Allows one *secondary* question to be asked:

Is the protein likely to cross-react with an existing allergen?

Bioinformatics is not intended to answer whether a protein will *"become"* an allergen (sensitization)

University of Nebraska Allergen Database (Allergen Online)

- **Industry sponsored, peer-reviewed allergen database at University of Nebraska**
  - Peer-reviewed by clinical and research allergists from around the world: Japan, Europe, and USA
  - Well-defined criteria; posted on database website.
  - Inclusion of protein allergens (food, dermal, respiratory) based on available data in the public literature.
  - Updated once a year (Version 13)
  - Available free to the general public

www.allergenonline.org

Allergen Search Strategy

- Compare amino acid sequence of query protein to database containing sequences of food, dermal and respiratory allergens.

- **Evaluate sequence for amino acid identity using local alignment programs, such as BLAST (or FASTA)**
  - **> 35% identity over an 80 or greater amino acid window**

  and potential (theoretical) IgE epitope matches.
  - ~~**≥ 8 contiguous identical amino acids**~~ **(EFSA 2011; Ladics et al., 2011, Reg. Toxicol. Pharmacol., 60:46-53).**

Specific IgE Sera Screening

- For proteins originating from an allergenic source, or having significant homology with a known allergen, specific serum screening is conducted.

- An issue of critical importance to sera screening is the availability of *well characterized*, quality human sera from a sufficient number of patients.

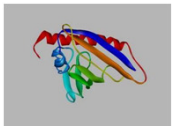
- Potential false positives/equivocal results (e.g. N-glycans)

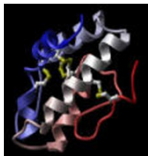
- Risk assessment: risk for mild versus severe symptoms?

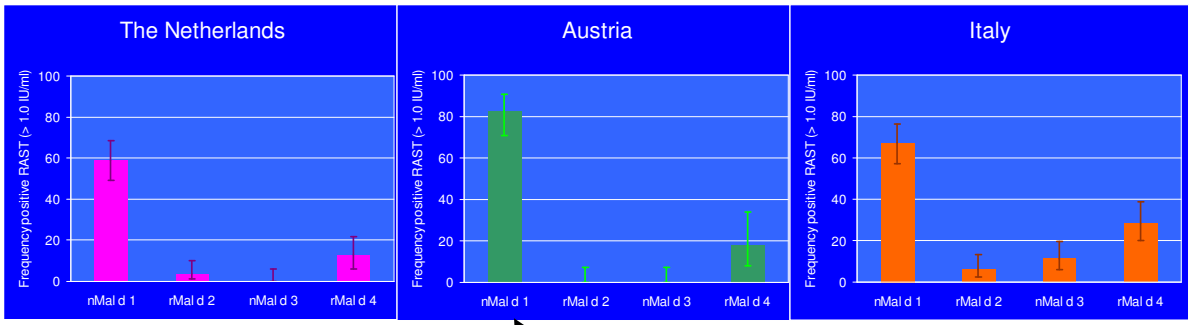
A European study on apple allergy

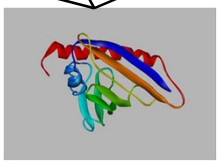
CRD using four purified apple allergens:

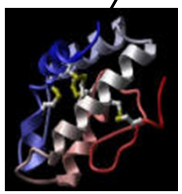
1. Birch-pollen cross-reactive allergen
2. Mal d 2
3. Lipid transfer protein (LTP): true food allergen
4. Mal d 4



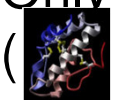


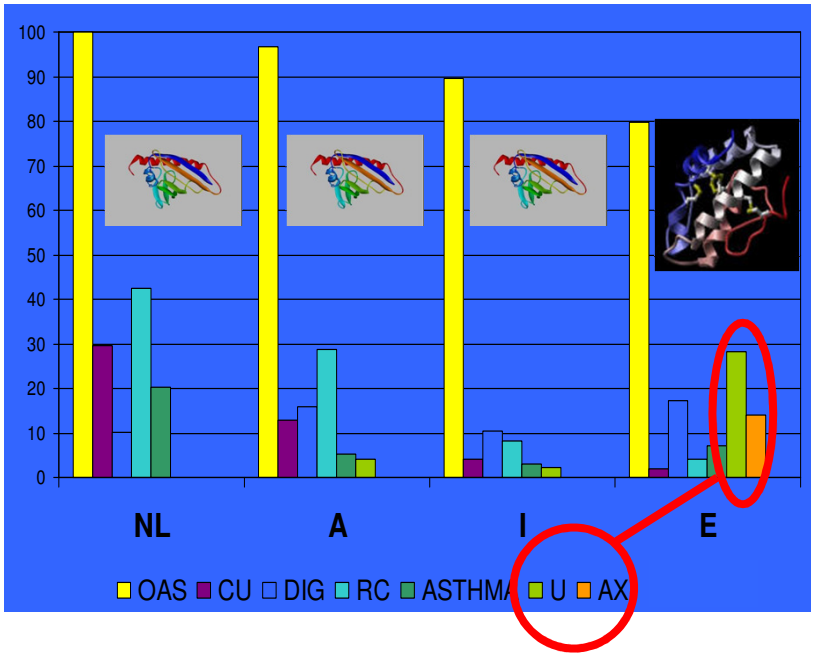




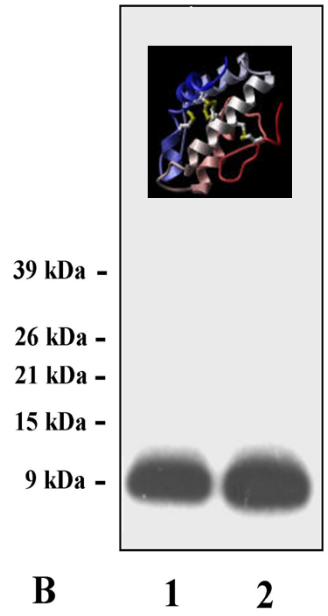


CRD reveals a clear geographic difference. So what?

Only patients with IgE antibodies against the COMPLETE ALLERGEN () have severe systemic symptoms (U: generalized urticaria / AX: anaphylaxis). The risk is for severe food allergy increased by around 8-fold!



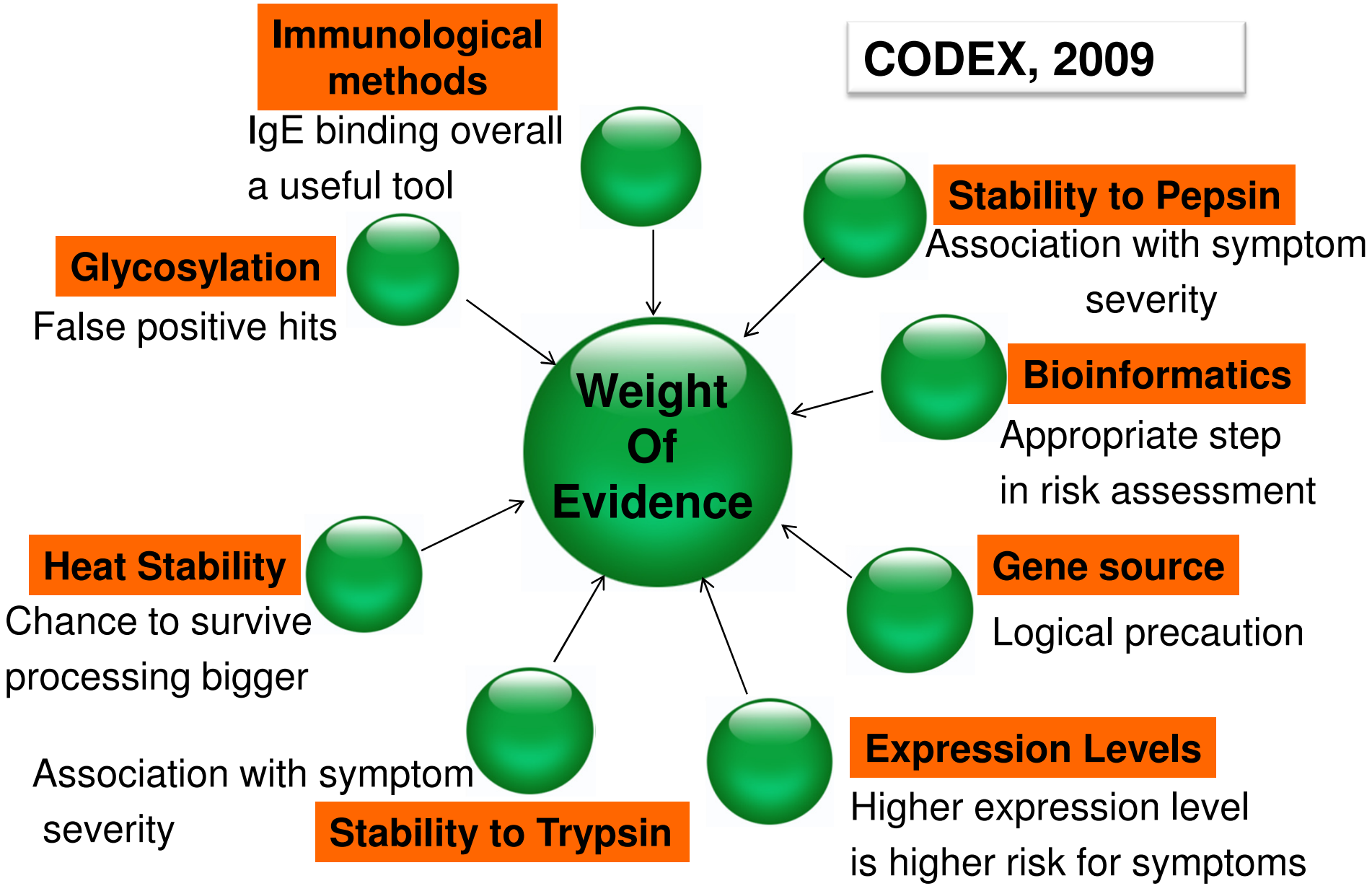
Likely explanation:
resistance to gastric digestion



IgE to a transgene with homology to a protease-resistant allergen bears more risk than to a protease-sensitive allergen.

Weight-of-Evidence Approach

How to identify the protein that will turn out to be an allergen?



Risk:	**Endpoints to reduce risk per CODEX (2009):**
Transfer an existing allergen or cross-reactive protein into another crop	Bioinformatics Serum screen for IgE binding
Alteration or quantitative increase of endogenous (existing) allergens	Analytical methods
Creation of food allergens *de novo*	Physical properties of protein (e.g., stability in SGF; heat)

Impact of transgene on endogenous allergens?

- Very little is know about expression levels of endogenous allergens in non-GM crops, in particular the degree of variability.
- To set action levels, the bandwidth of non-GM allergen levels need to be established.
- Certainly, extreme effects on known allergens need to be prevented, but so far there is no evidence suggesting such effects.

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What makes a protein a COMPLETE ALLERGEN?

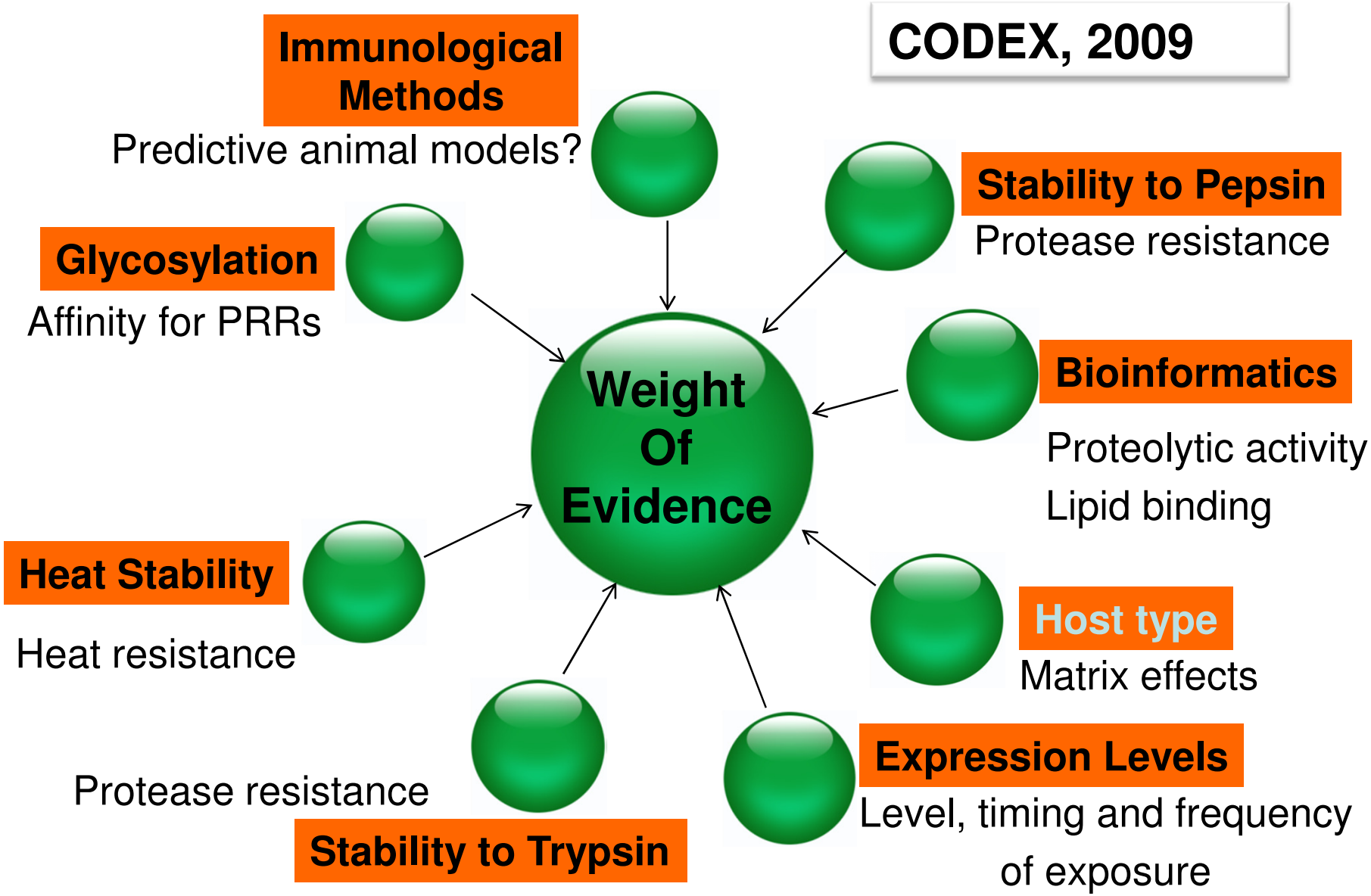
Complex issue:

- *intrinsic factors*, i.e. molecular properties such as:
  - proteolytic activity
  - lipid-binding
  - protease resistance
  - heat resistance
  - affinity for pathogen recognition receptors (PRRs) (e.g.TLRs)

- *extrinsic factors*, i.e. anything “accompanying” the molecule such as:
  - matrix presenting the molecule
  - co-exposures in environment
  - infections
  - microbiome
  - level of exposure
  - genetic background
  - timing and frequency of exposure

Weight-of-Evidence Approach

How to identify the protein that will turn out to become an allergen?



Can we evaluate sensitizing potential in a useful way

- Animal models: to date no predictive model has been reported
- Glycosylation: very little evidence that it is a risk factor
- Protease resistance: as long as the route of sensitization is debated (GIT? Oral mucosa? Respiratory tract? Skin?) not clear
- Heat stability: no evidence available that it plays a role
- Protein function (protease / lipid-binding): some evidence that it may play a role in promoting sensitization
- Matrix effects: seems very likely to play a role (e.g. peanut vs soy)
- Dosage/timing: high expression may in fact be protective!

Concluding remarks

- To assess whether a protein will turn out to be a risk for sensitized subjects can be assessed with reasonable certainty by bioinformatics, IgE immunoassays, and protease- and heat- resistance evaluation.
- To assess whether endogenous allergens are increased is hard to establish without having solid non-GM information
- Predicting the sensitization potential of a protein is still not really possible. So far no animal models have been reported that can predict allergenicity.