Peanut Allergy Research

Peanut Allergy Research Unit
USDA-ARS-New Orleans, LA

Soheila Maleki

Ongoing Research

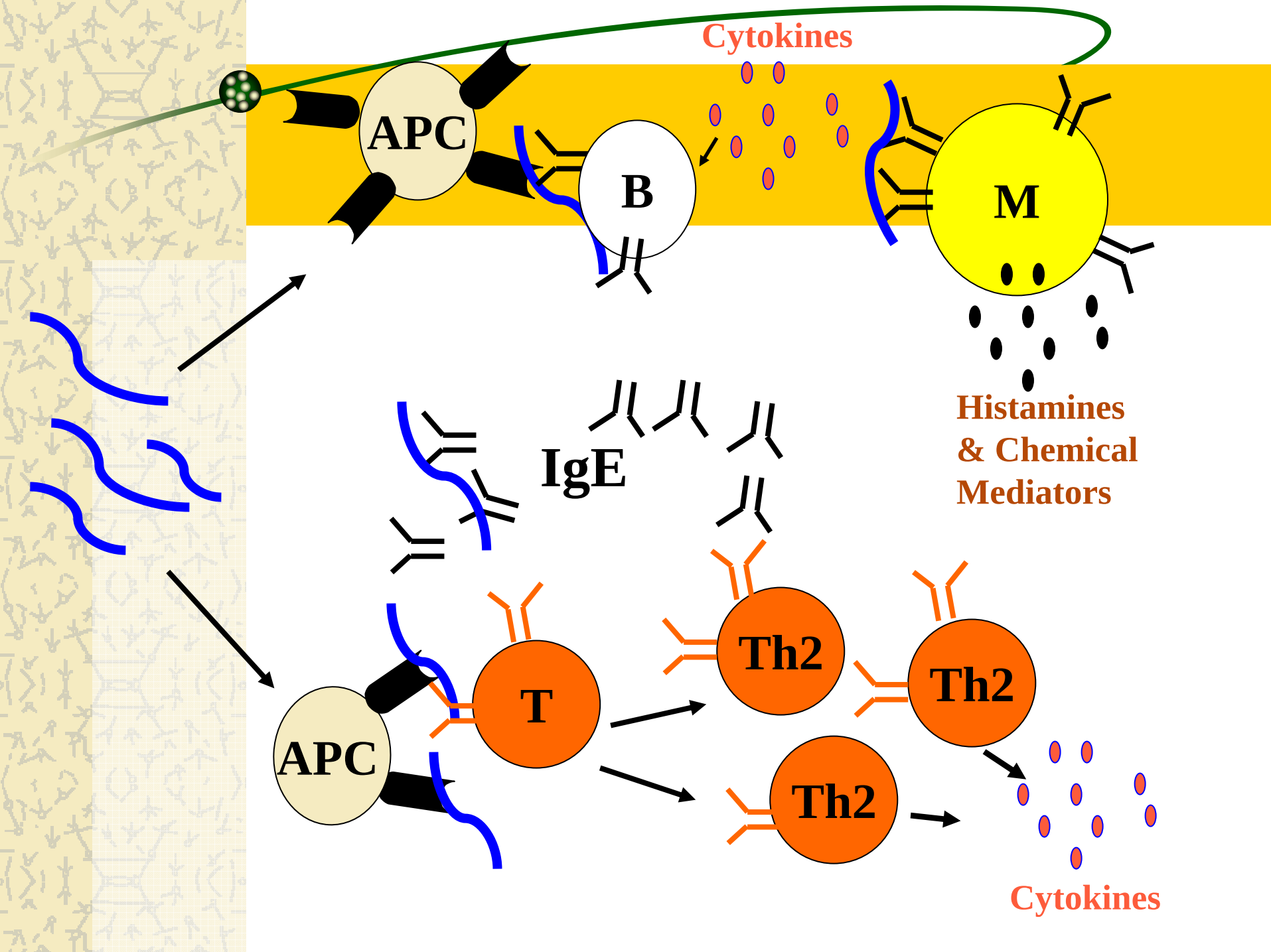
❖Improve diagnostic/detection methods (threshold dose, detection kit, allergen identification, purification and characterization, standard extracts, cross-reactivity, etc)

❖Determine the threshold dose and the effects of processing on threshold dose, epidemiology and sensitization.

❖To develop novel therapeutic tools for the treatment of peanut allergies. (vaccine, anti-IgE, peptide, cytokine, APC & T-cell immunotherapy)

❖Genetically engineer hypoallergenic plants (gene silencing, mutation, replacement, knock out)

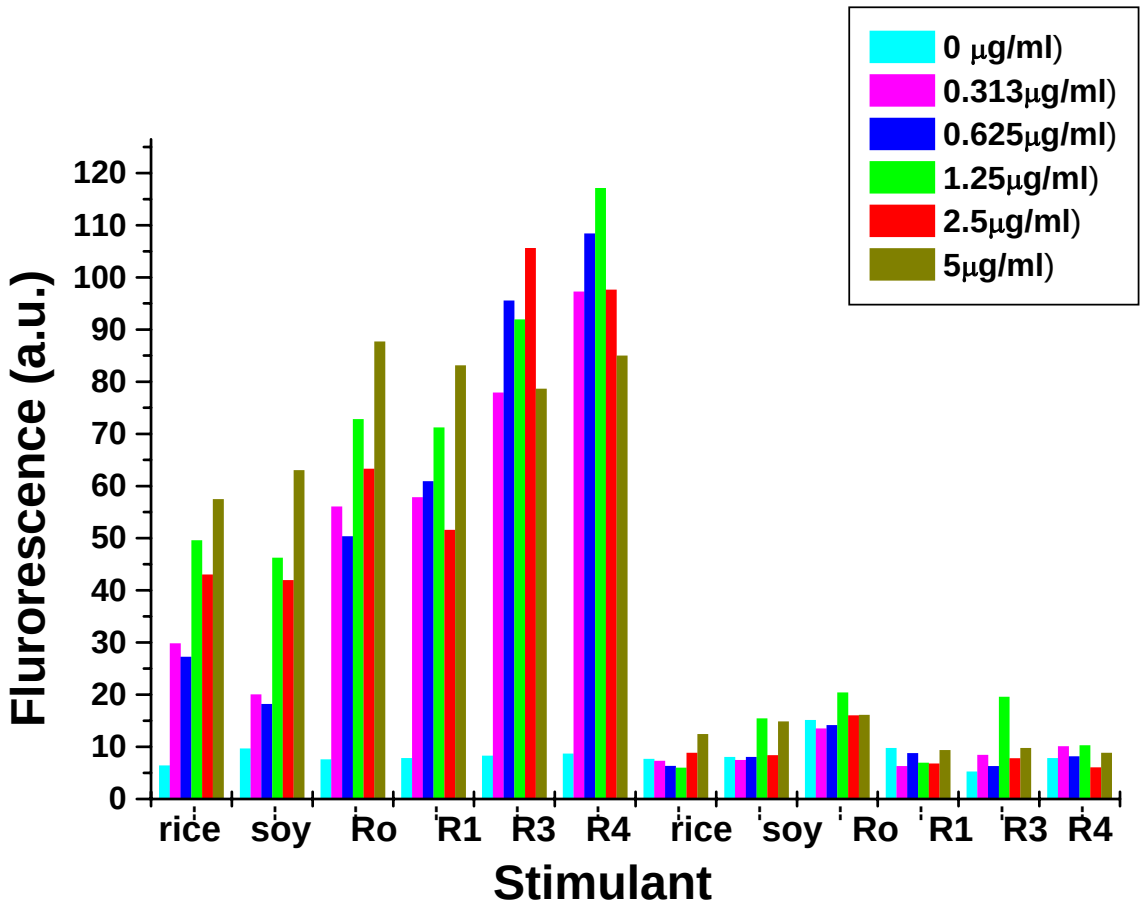
❖Find peanut varieties with naturally reduced levels of allergens or allergenic properties (screening with antibodies)


Cytokines
APC
B
M
Histamines
& Chemical
Mediators
IgE
APC
T
Th2
Th2
Th2
Cytokines

Sensitization & skin test reactions of peanuts processed and ingested in different forms

Mouse model for comparing sensitization

(USDA, LA & Japan)

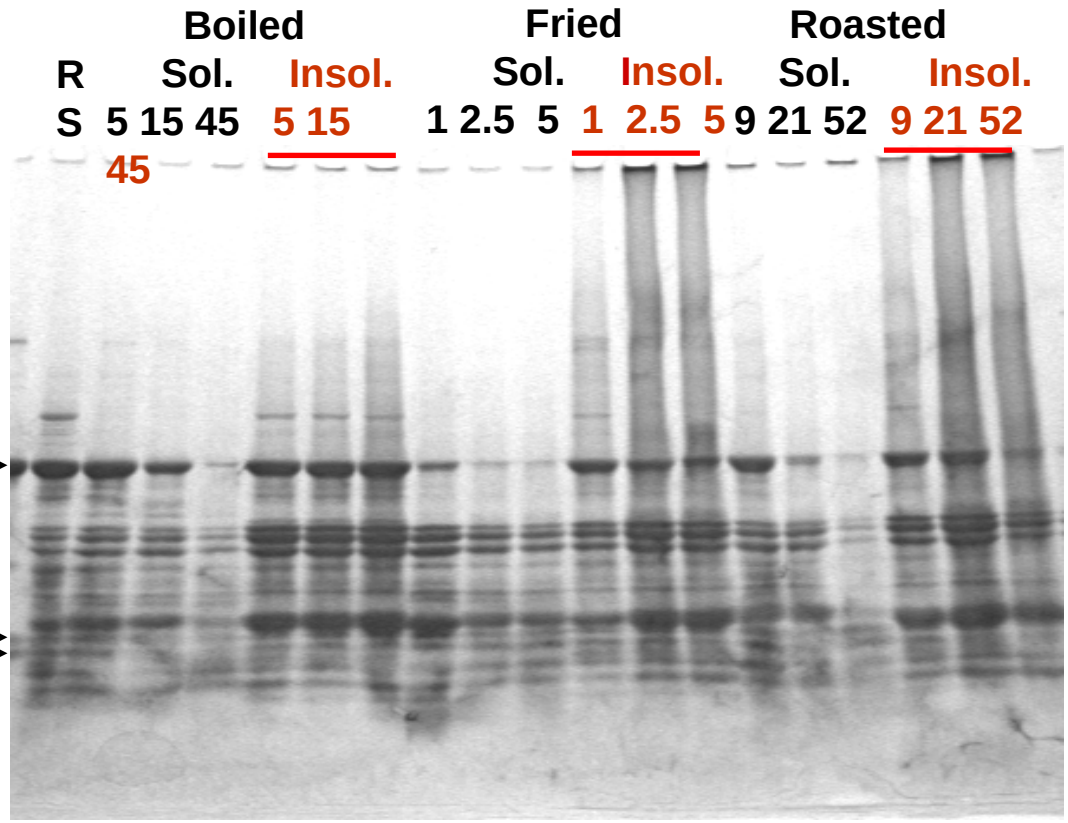


Maleki S.J., Yamaki, K., et al. (2002) The relationship of the structure of peanut proteins to their function as allergens. *Proceedings of the 31st United States-Japan Resources (UJNR) Panel,* HHH.

What happens to peanuts following different processes?

Solubility of peanut proteins following different thermal processes

SDS-PAGE

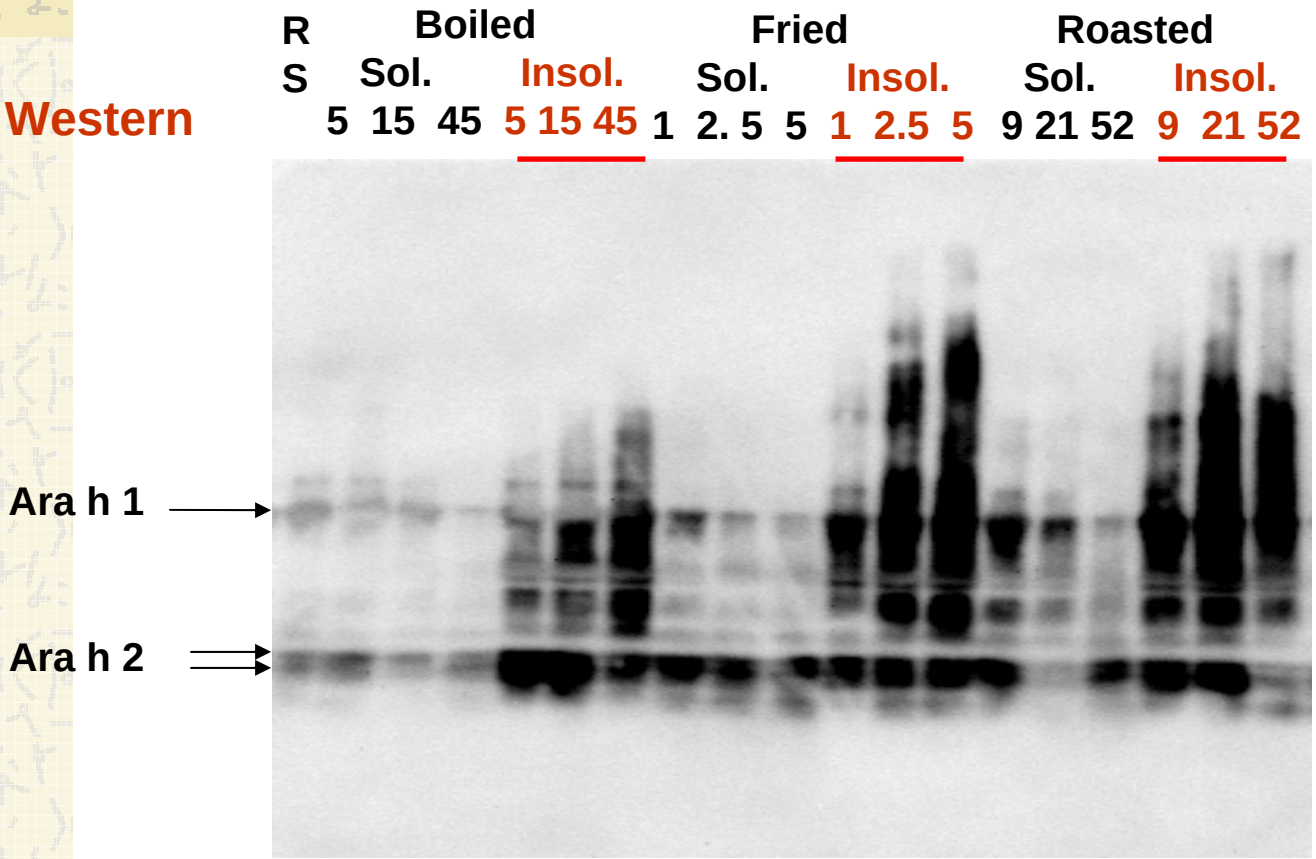


R = Raw Extract; S = Soluble Fraction; I = Insoluble Fraction

Protein solubility is decreased with increased exposure to thermal treatment

Effect of thermal processing on IgE binding

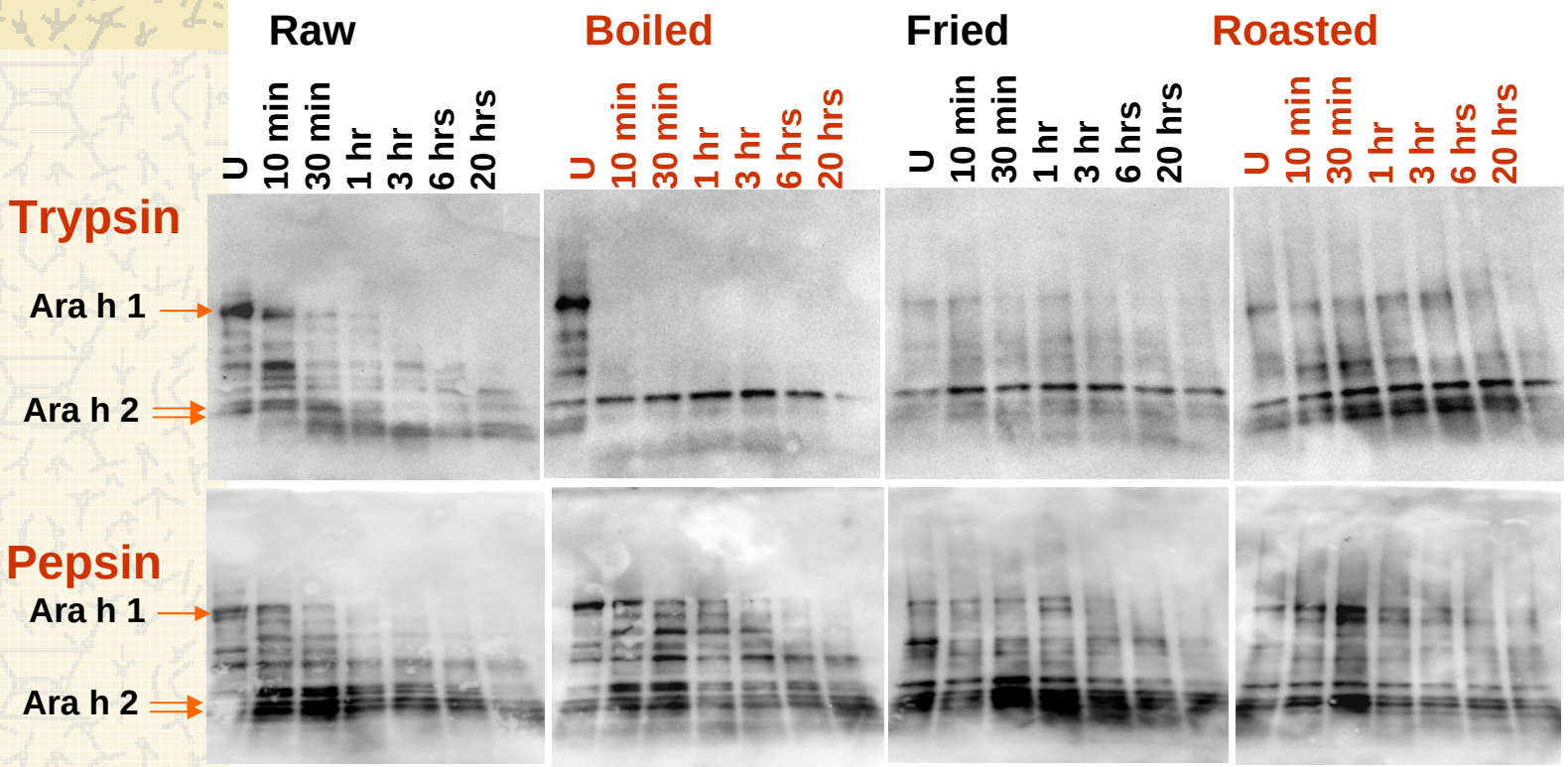
IgE Western



R = Raw Extract; S = Soluble Fraction; I = Insoluble Fraction

IgE binding is reduced in the soluble fraction as exposure time increases

IgE binding to trypsin & pepsin digested, differently processed peanuts



IgE binding to peanut allergens is increased as exposure to heat is increased

Some facts about the peanut allergens

- Nine allergens have been identified in raw peanut.
- Five of these allergens (Ara h 1,2,3,4 & 6) have been purified from raw and 3 of them Ara h 1, 2. 3 & 4) from roasted peanut.
- The IgE binding sites for Ara h 1, 2, 3 & 4 (the major allergens) have been identified.

Some facts about the peanut allergens

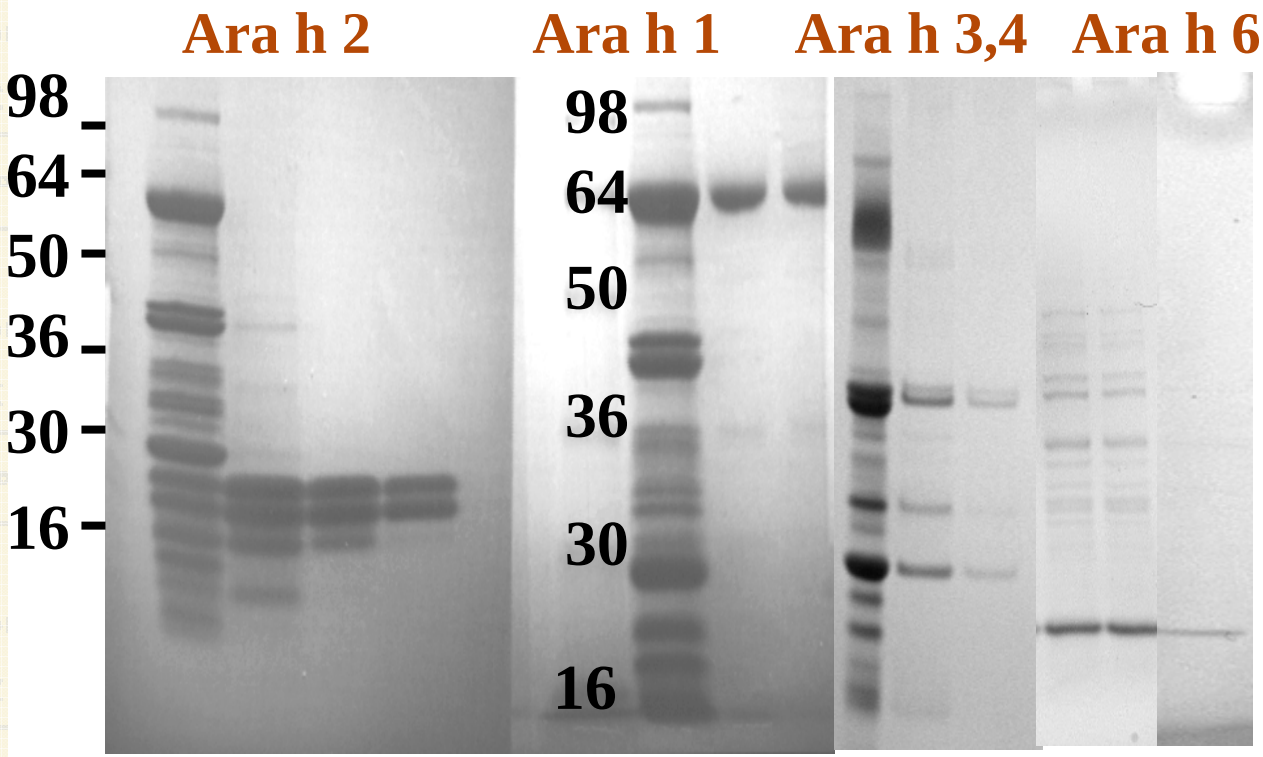
- The cDNA for all of the 9 allergens have been cloned.
- The cDNA's for Ara h 1, 2 & 3/4 have been altered to eliminate or reduce IgE binding significantly (hypoallergen).
- The genomic clones for Ara h 1, 2, 3 & 4 have been identified.

Identification and Purification of the Major Peanut Allergens & Anti-Allergen, Antibody Production

The Allergens Identified in Peanut

Name	protein	% in seed	MW	% individuals allergic
Ara h 1	**vicillin**	**~12%**	**63 KDa**	**>95%**
Ara h 2	**conglutin**	**~1%**	**18, 20 KDa**	**>95%**
Ara h 3/4	**glycinin**	**~25%**	**60 KDa**	**~50%**
Ara h 5	**profilin**	**14 KDa**		**<20%**
Ara h 6, 7	**conglutin homolog**	**~1%**	**15, 17 KDa**	**>50%**
Ara h 8	**glycinin homolog**	**16 KDa**		**?**
Ara h 9	**oleosin**	**18 KDa**		**?**

Purification of the major allergens identified in peanut



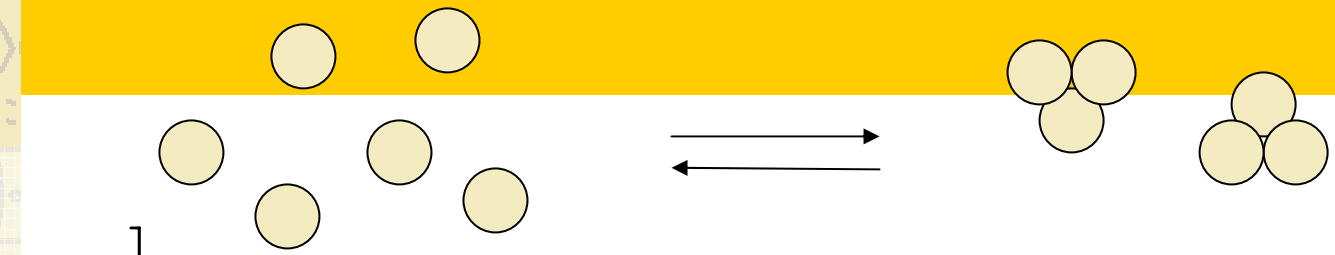
Purpose of Identification and Purification of the Major Peanut Allergens

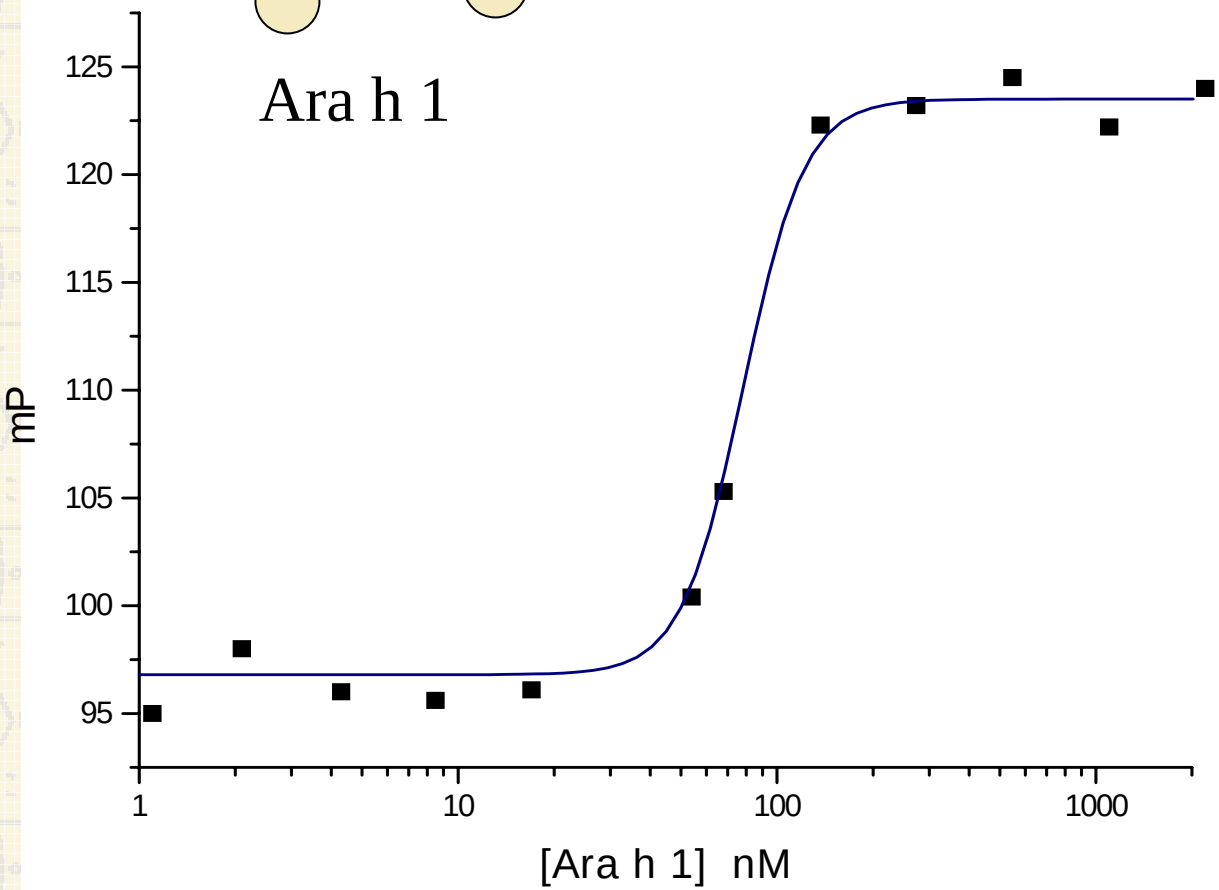
- Allergen structure function analysis
- Allergen cloning
- Development of diagnostic tests
- Tissue culture: T-cell, B-cell
- Histamine release/Mast cell, Basophil
- Animal model testing
- Anti-Allergen Antibody Production
- Cross-reactivity analysis

etc

Structure, Biophysical Properties and IgE Binding to the Major Peanut Allergens (i.e. allergens are classically thought to be resistant to digestion)

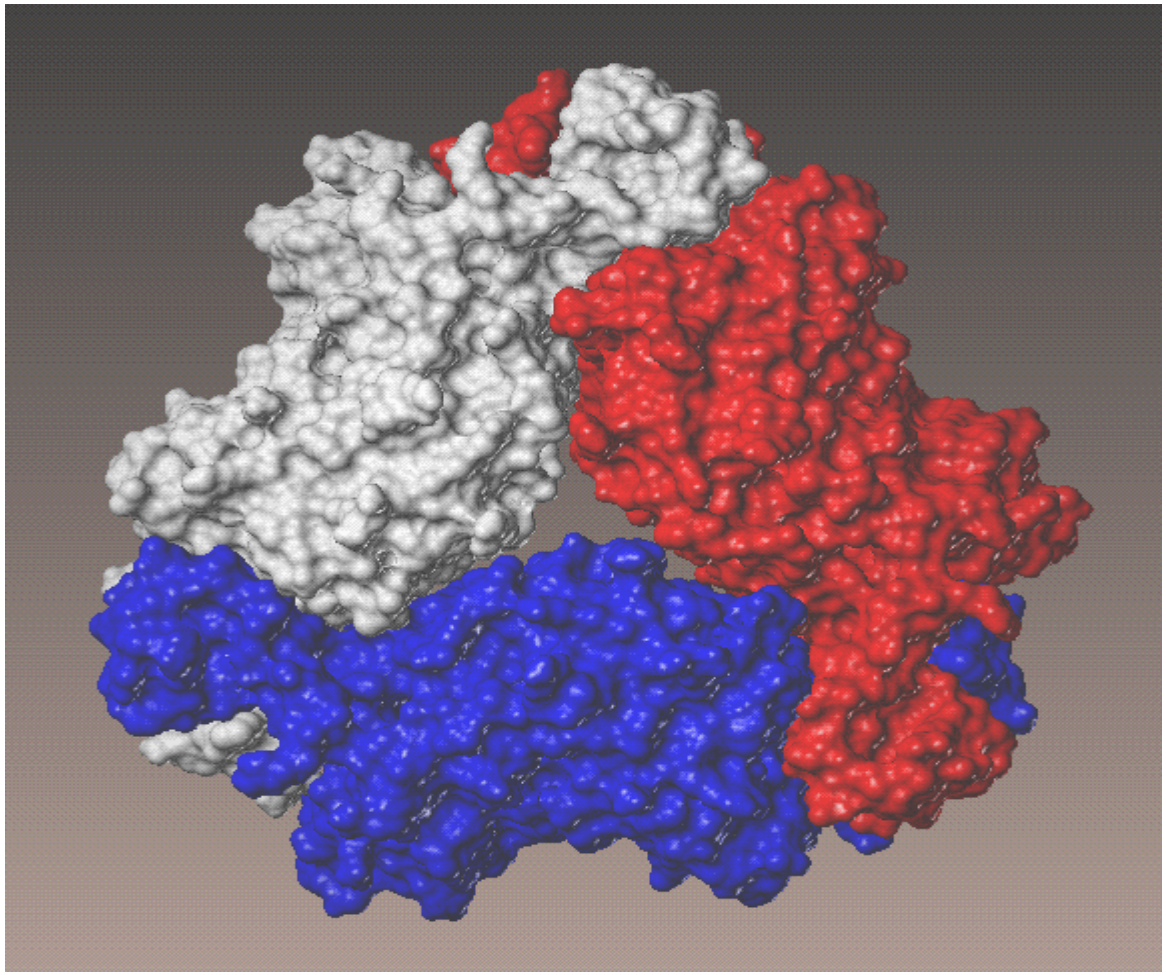
Fluorescence Polarization Analysis of Ara h 1



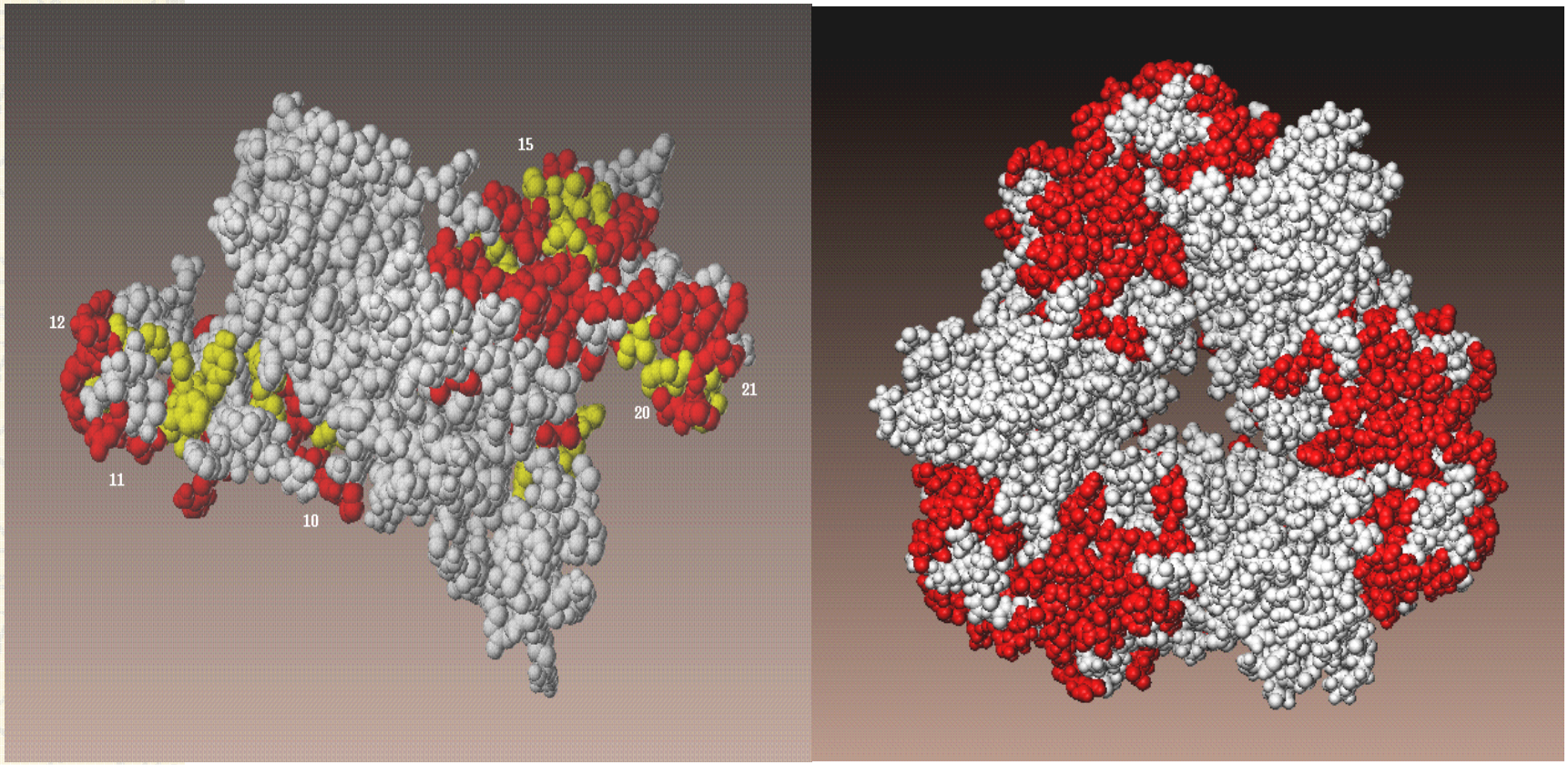


***JBC*,1998, *273 (2)*: 13753- 13759**

Ara h 1 forms trimers



Ara h 1 forms highly Stable trimers that protect IgE binding sites from digestive enzymes



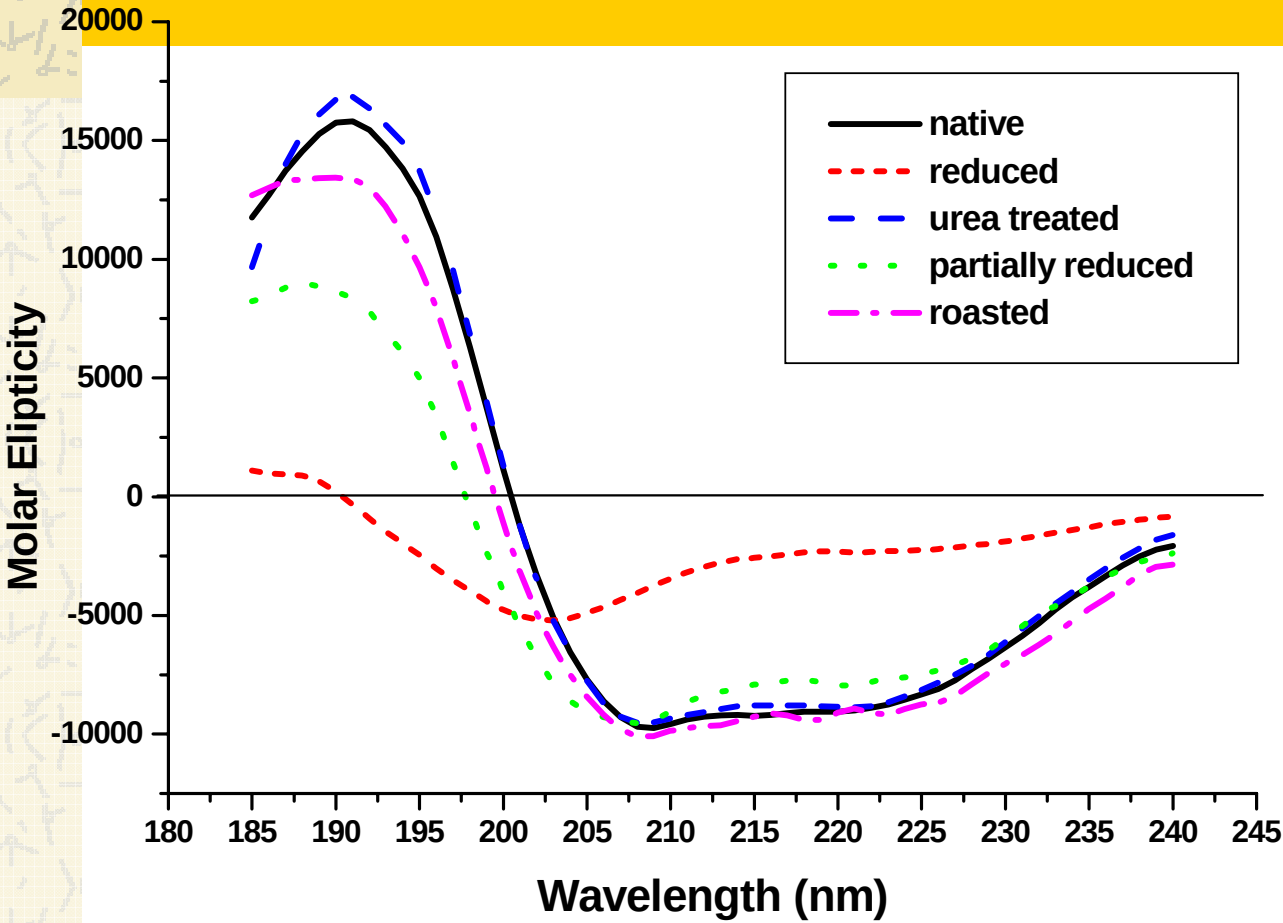
Maleki et al., (June, 2000) *J. Immunology*, 164: 5844-49

Ara h 2 has sequence homology to trypsin inhibitors

Trypsin inhibitory activity of Ara h 2 from roasted vs raw peanuts

Sample	Trypsin inhibitor Activity (unit/ug)
Ara h 2 from Raw Peanuts	21.38
Denatured Ara h 2	68.2
Ara h 2 from Roasted Peanuts	74.66

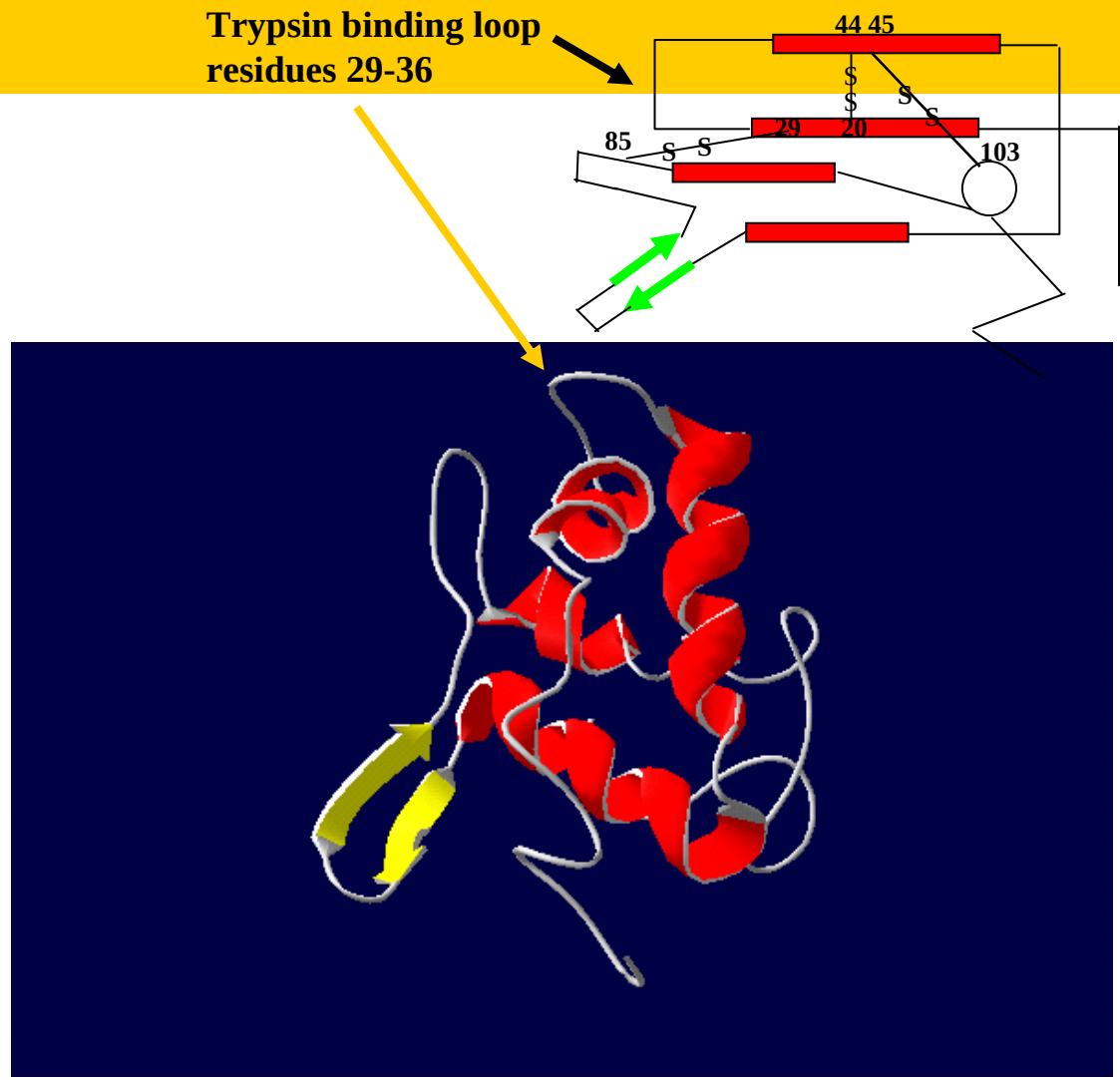
CD spectrum of Native Ara h 2:



Raw: ~60% α-helix, ~3% Beta sheet, 37% Random Coil

Roasted : ~58 α-helix, ~4% Beta sheet, 38% Random Coil

Ara h 2, a digestive enzyme inhibitor



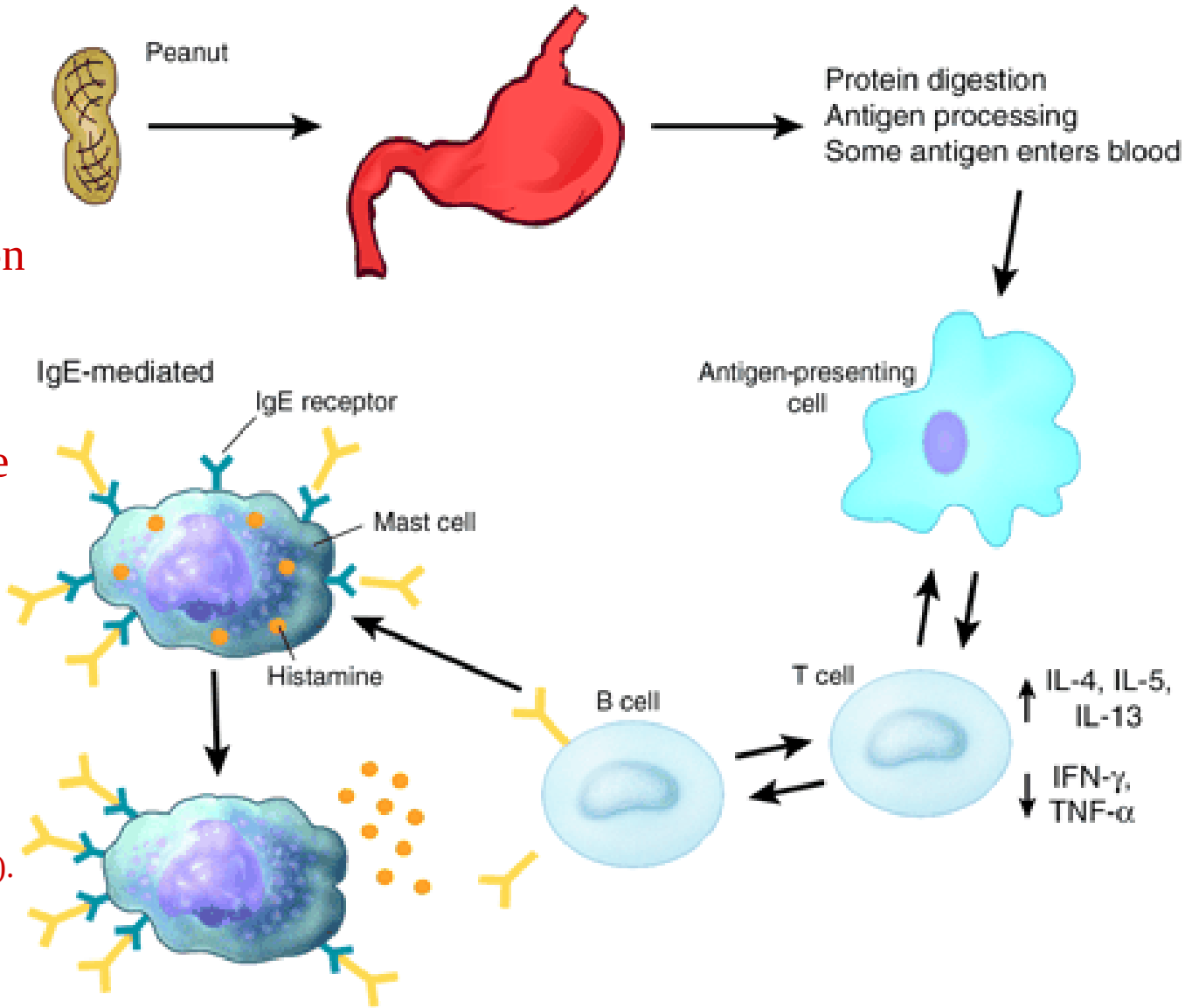
Maleki et al, (2003) *J. Allergy & Clinical Immunology,* 112 (1), 190-195.

Threshold dose
(Multicenter project)

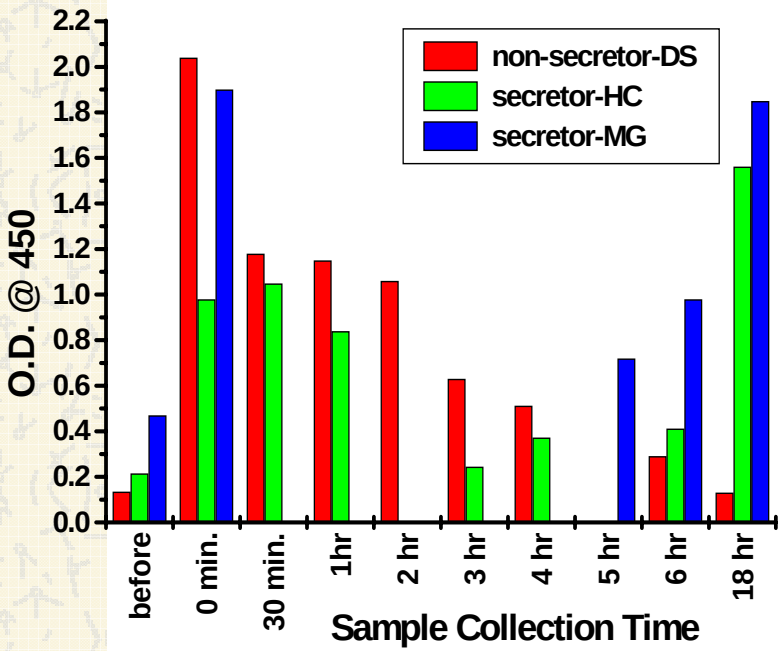
Kinetics of absorption
(USDA, LA& MD)

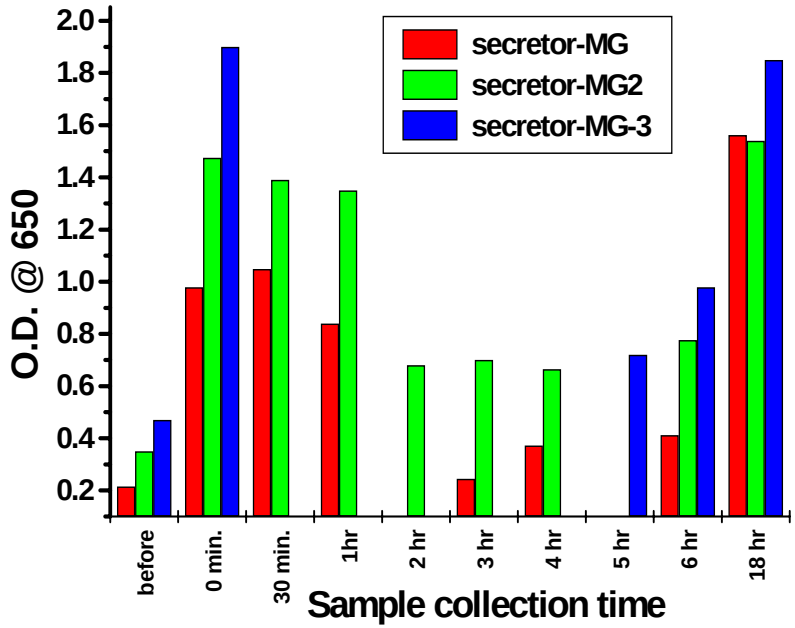
Fragments of allergens that survive digestion /activated Charcoal
(USDA, MD/St.Michaels)

Sensitization ability of the surviving fragments (USDA, LA, Canada, UAMS, Japan).

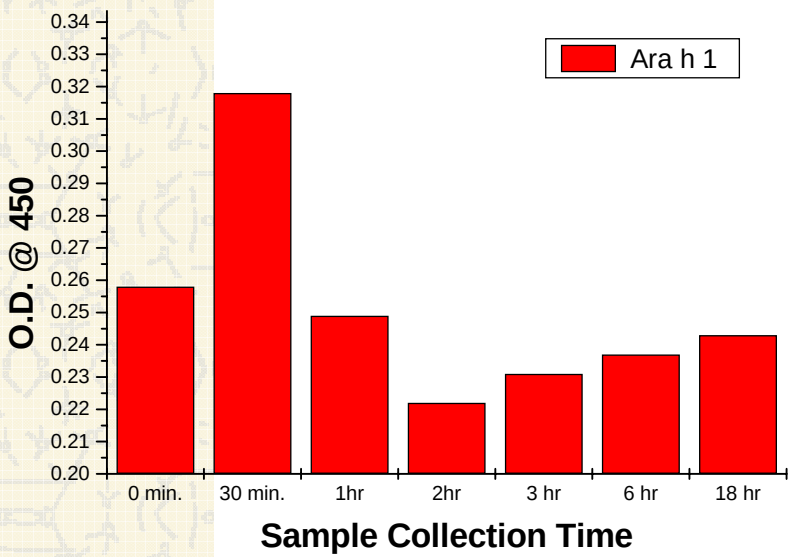


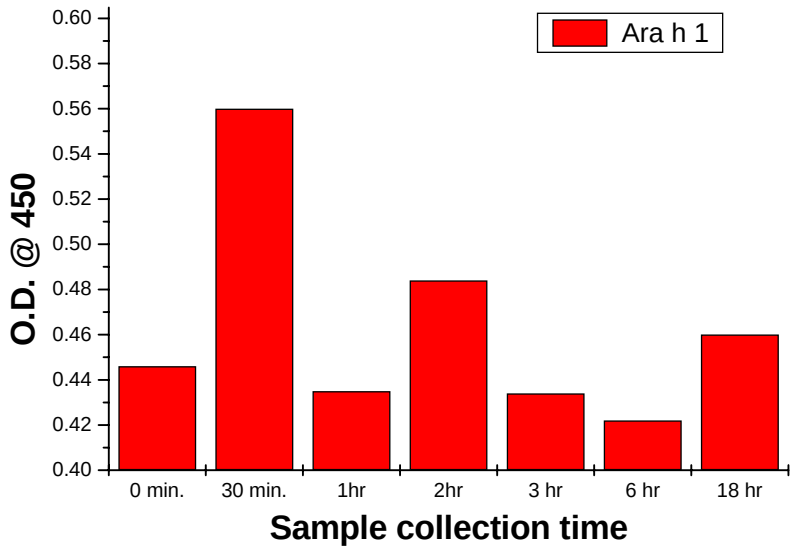
Secretion of Peanut Protein in Saliva following ingestion





Detecting Ara h 1 in breast milk of a non-allergic volunteer (same person, 2 ELISAs)





Why a food should be studied in the form that it is ingested

- **The Structure/Function of Peanut Proteins**
  - **digestion in the gut**
  - **absorption into the blood stream**
  - **IgE binding**
  - **histamine release**
  - **T cell proliferation**
  - **threshold dose/sensitization**

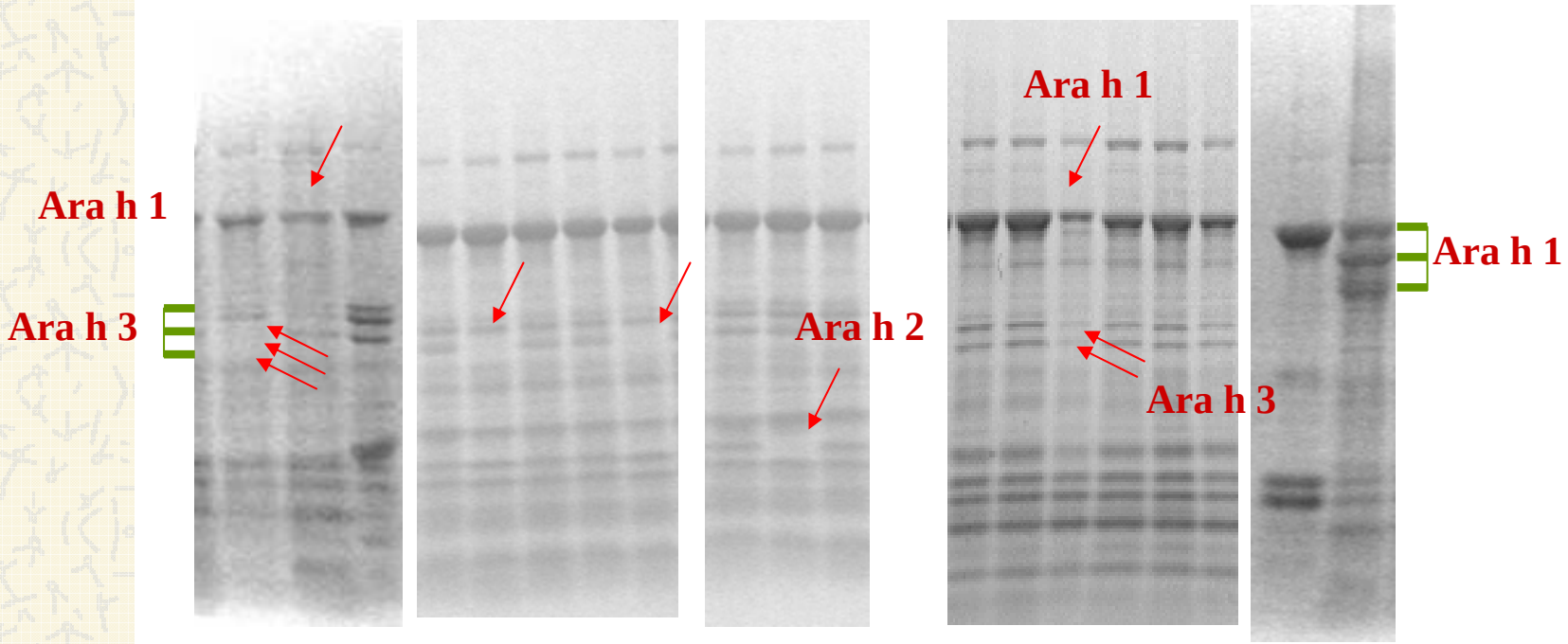
- **Age and frequency of consumption by infants may influence the Epidemiology of peanut allergy.**

Screening Peanut Cultivars for Reduced levels of Allergens

(USDA: LA, NCSU)

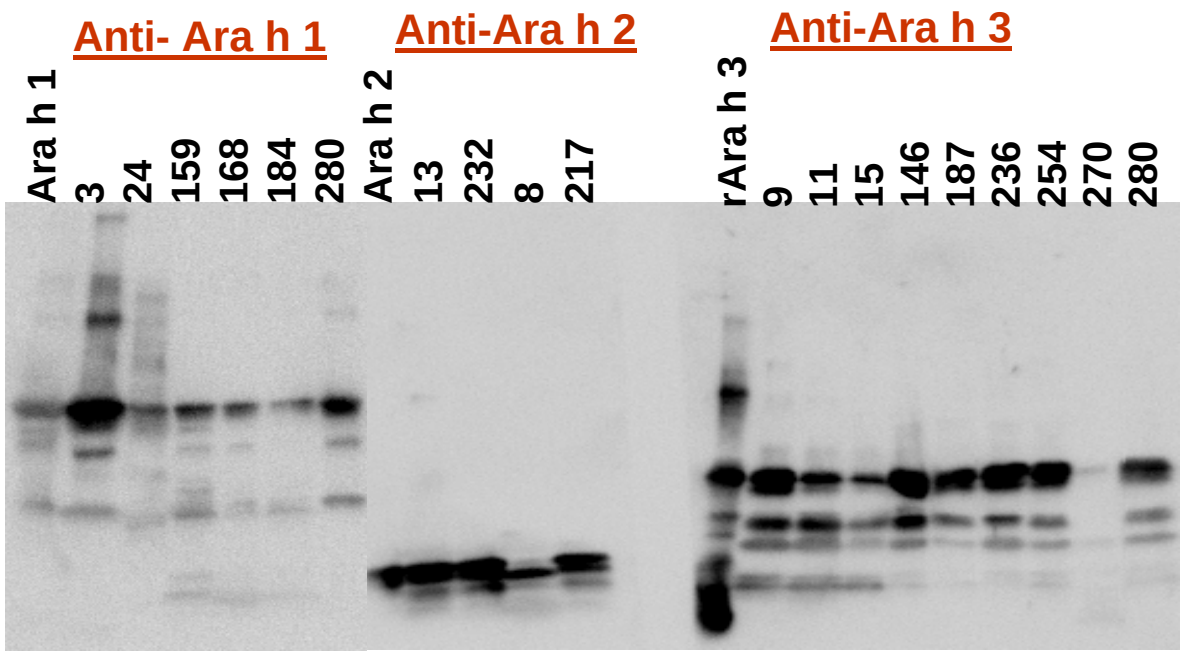
Funded by the Georgia Peanut Commission, The Peanut Foundation & USDA

Peanut Cultivars missing Ara h 1, Ara h 2 and Ara h 3 have been found: (USDA, NCSU)



These varieties are currently being crossbred at NCSU to produce reduced/hypoallergenic peanuts

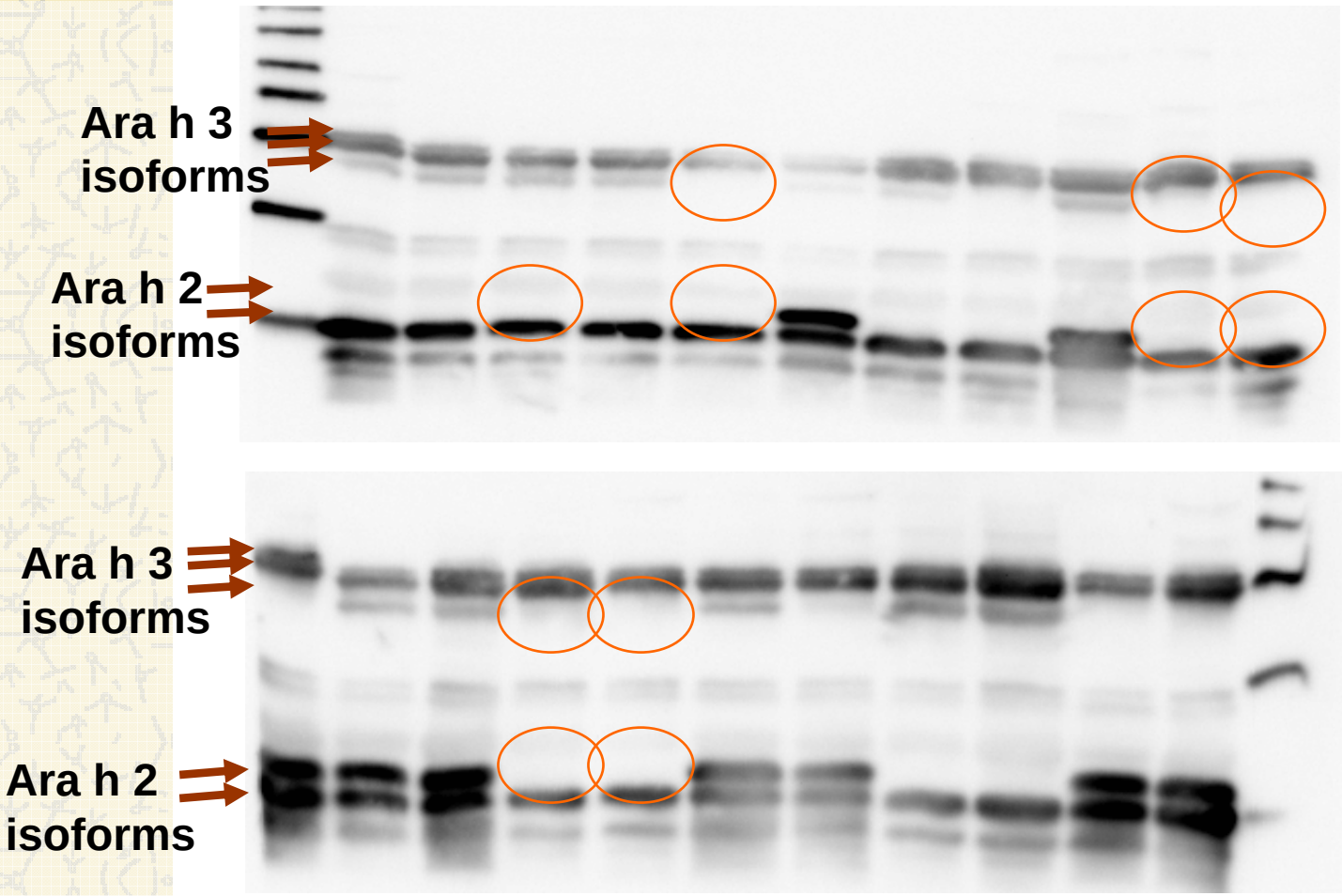
Anti-Ara h 1, Ara h 2, and Ara h 3 Western Blot on peanut varieties of Interest



Missing an Ara h 3 isoform

Missing an Ara h 2 isoform

Western blot analysis with both anti-Ara h 2 and anti-Ara h 3 antibodies



Conclusions

- Through traditional breeding it is possible to knockout some of the allergenic proteins in plants, which may ultimately:
  - reduce the severity of the allergic response
  - reduce sensitization capability
  - be useful in immunotherapeutic desensitization
  - be useful in understanding valuable genetic information such as the genetic inheritance patterns of the allergens. (i.e. Co-inheritance of the missing isoforms followed the classical Mendelian inheritance pattern of 1:15)
- The peanut industry and market is less likely to suffer from boycotts or price fluctuations seen while attempting to market genetically modified organisms (GMO)

Other USDA projects not discussed:

- Immunotherapy:
  - Identification of T cell epitopes
  - T cell signal transduction/cytokine secretion
  - Computer modeling of common IgE binding sites
  - APC driven T cell inactivation
  - IgE epitope mapping of Ara h 5-8
- Natural History/sensitization:
  - Does peanut protein in breast milk sensitize or tolerize infants
- Epidemiology
  - Characterization and comparison of the allergenicity of legume based foods from within and outside of the USA.

Acknowledgements:

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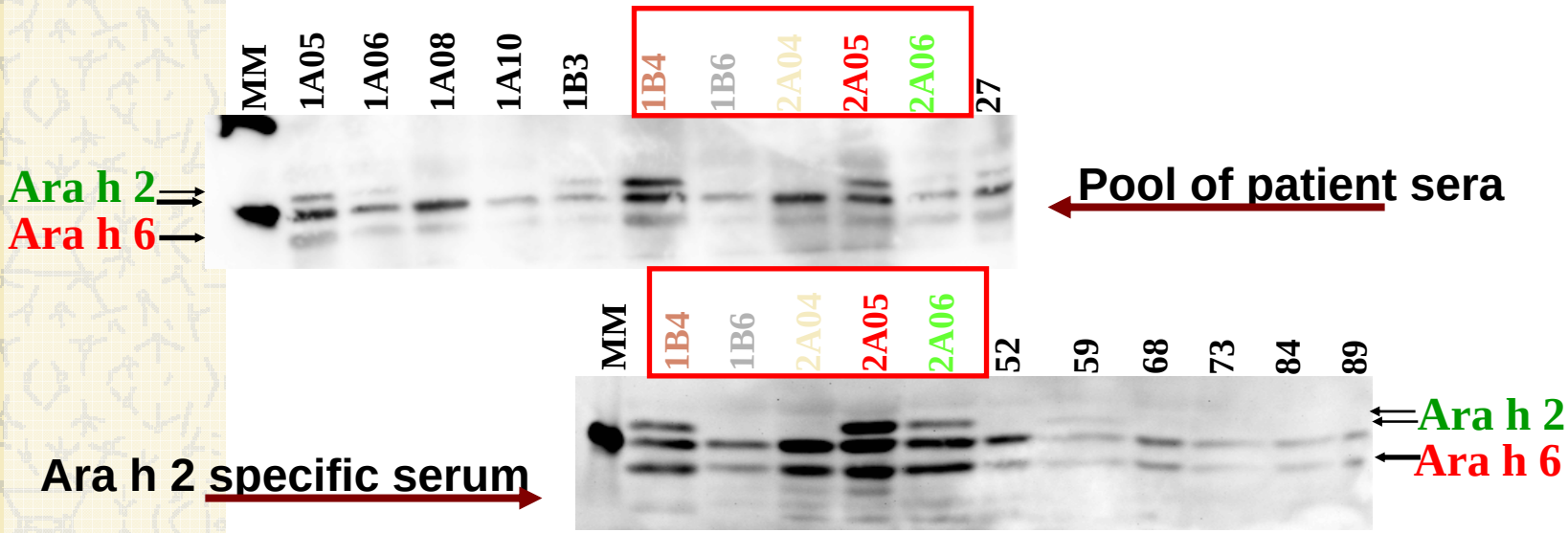
Dr. Elaine Champagne

Funding:

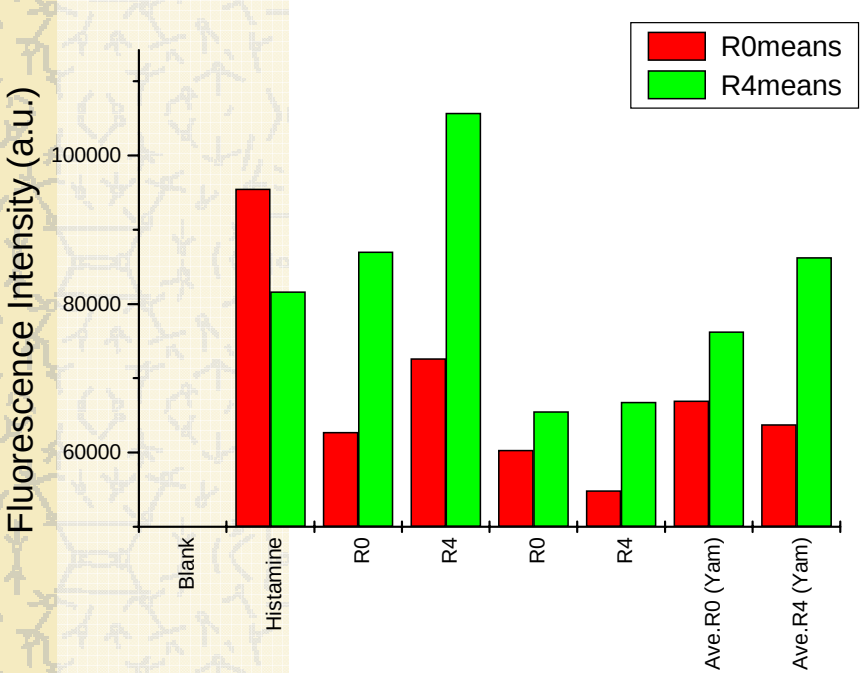
USDA-ARS
National Peanut Board
Georgia Peanut Commission &
The Peanut Foundatio

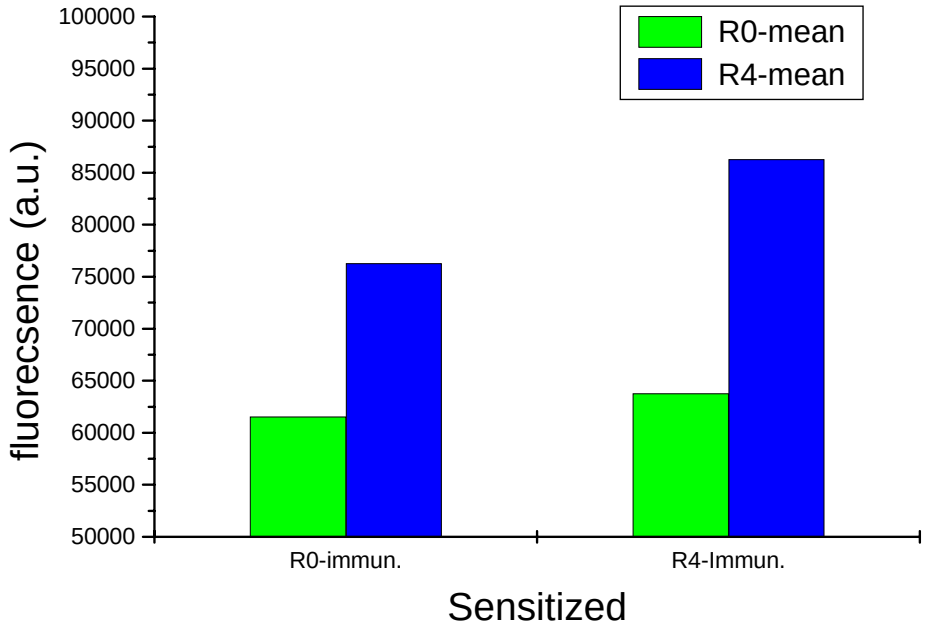
Western blot analysis using serum IgE from peanut allergic individuals



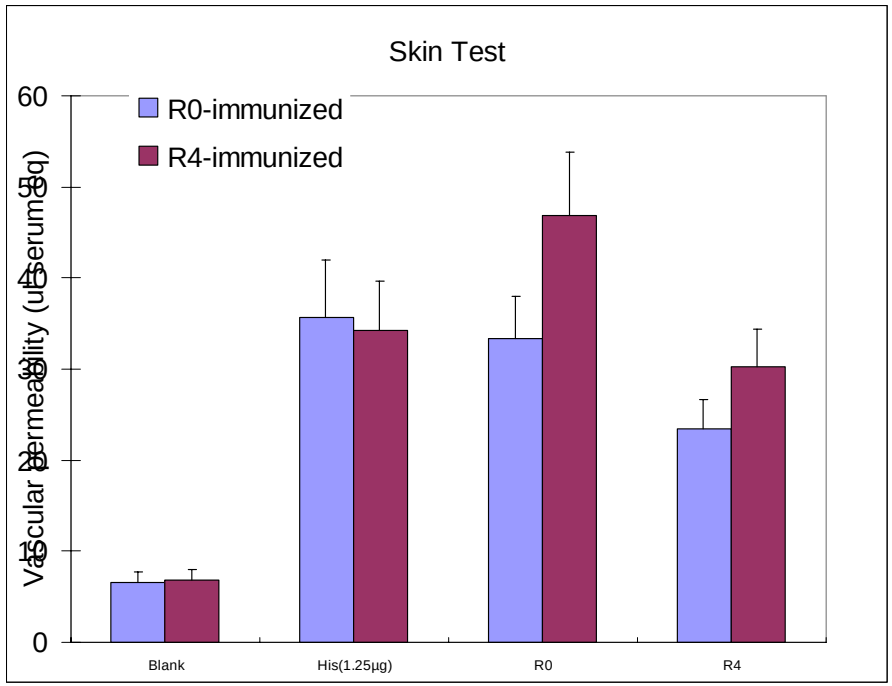
Differential IgE binding to Ara h 2 and Ara h 6 is seen in the mutant peanuts for the different individuals. This indicates amino acid sequence differences exist among the same allergens in the varieties.

Cross sensitization of mice with raw and roasted peanuts

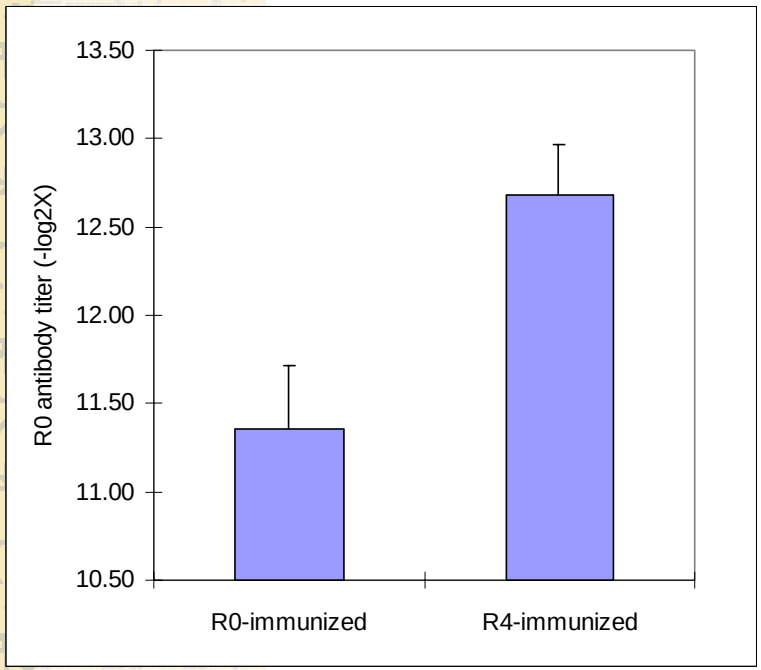


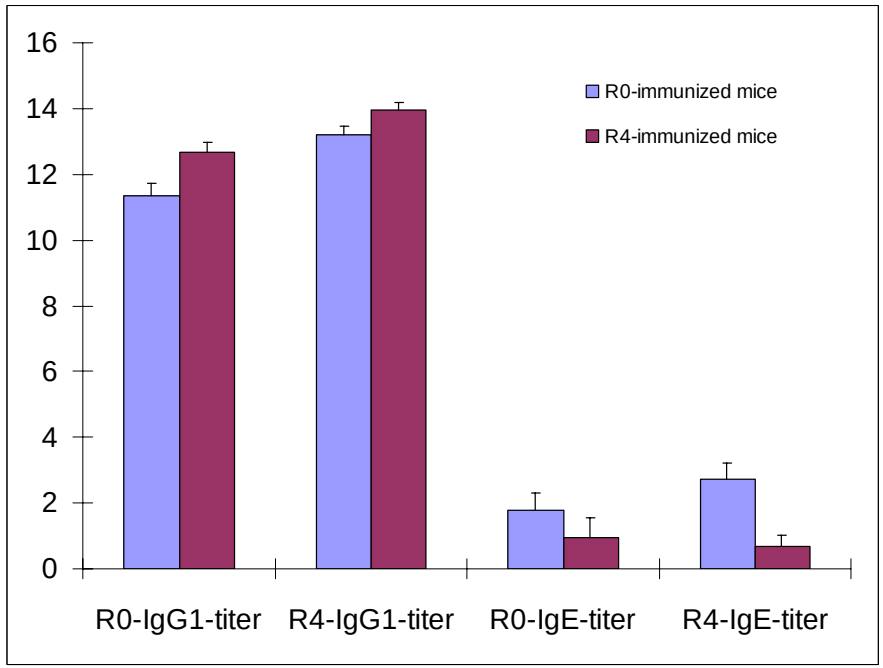


Yamaki, K., Maleki S.J., Champagne, E.T., Shinohara, K. (2003) *32 nd United States-Japan National Resources (UJNR) Panel.*


Skin Test
R0-immunized
R4-immunized
Vascular permeability (uL Serum/sq)
60
45
30
15
0
Blank
His(1.25μg)
R0
R4

Antibody titers in mice sensitized with raw and roasted peanuts





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