



# Accurate and Affordable Allergen Quantification for the Seed Biotech Industry

Inventors

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# What is the problem?

- **Allergenicity of foods and food components**
  - 1-4% of adults and 6-8% of children have food allergies<sup>1</sup>
  - Seeds cause most food-related allergic reactions (tree nuts, peanuts, wheat and soybeans)<sup>2</sup>
- **Seed biotech industry**
  - Required to monitor endogenous allergens (over 30 in soy!) in existing and new seed varieties (particularly GMOs) before they enter the market as food/feed
- **Current allergen measurement uses out-of-date technologies**
  - Poorly quantitative (2D gel electrophoresis)
  - Expensive/time-consuming to develop (Antibody-based)
- **Implementation of new technologies**
  - The seed biotech industry and federal regulatory agencies are entertaining new strategies for measuring allergen levels to standardize the approval process



# How can we help?

- **Mass spectrometers are high-end analytical instruments that can accurately measure the mass of molecules**
- **Seed allergens are typically proteins - proteins have specific mass “signatures” which can be monitored by mass spectrometry**
- **Mass spectrometry can measure the levels of allergens in seed samples**
  - Comparing signals from seed allergens and synthetic labeled allergen standards gives absolute quantities of allergens
  - Design and implement allergen detection schemes cheaply
  - Specific, accurate, and reproducible<sup>5</sup>

# How does it work?



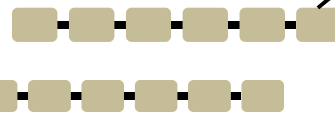
Seed allergen (peptides)



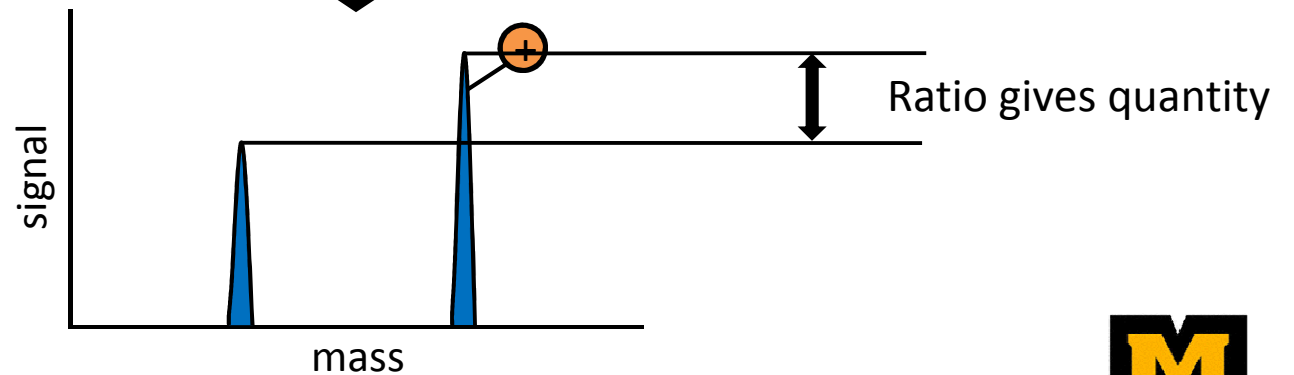
Labeled internal standard <sup>+</sup>



Mix



mass spectrometry can distinguish them



# How does our method compare?



	<u>Conventional</u> (2-D gel-based)	<u>Our method</u> (MS-based)
<u>High-throughput</u>		✓
<u>Sensitive</u>	✓	✓
<u>Selective</u>	✓	✓ ✓
<u>Accurate</u>		✓
<u>Initial cost</u>	<b>lower</b>	<b>higher</b>
<u>Absolute quantities</u>	<b>NO</b>	<b>YES</b>



# What have we done?

- Our lab has developed a novel process for extracting and measuring seed allergens – a disclosure has been filed
  - We have quantified 10 allergens simultaneously in soybean with ~7% technical variation
- We have ongoing research contracts with the plant biotech industry
  - Contracts from biotech companies for allergen quantification has generated steady funding for nearly two years
  - New contracts are being negotiated
- Our recent results have been published-we lead the field
  - **Stevenson, S. E.**; Chu, Y.; Ozias-Akins, P.; Thelen, J. J., **Validation of gel-free, label-free quantitative proteomics approaches: Applications for seed allergen profiling.** *J Proteomics* 2009.
  - Lee, D. G.; Houston, N. L.; **Stevenson, S. E.**; Ladics, G. S.; McClain, S.; Privalle, L.; Thelen, J. J., **Mass spectrometry analysis of soybean seed proteins: optimization of gel-free quantitative workflow.** *Analytical Methods* 2010.
  - **Stevenson, S. E.**; Houston, N. L.; Thelen, J. J., **Evolution of seed allergen quantification - From antibodies to mass spectrometry.** *Regulatory Toxicology and Pharmacology* *In Press*.
  - Houston, N.L.; Lee, D.G.; **Stevenson, S.E.** et al., **Quantification of soybean allergens using mass spectrometry.** *J. Prot. Res.* Submitted.



# What do we need?

- Angel or Venture capital
  - provide start-up expenses
    - Equipment purchase/leasing costs
    - Rent for lab space
    - Initial salaries for 1-2 employees

Contracts are predicted take over within the first year!!



# Thank you!

# Questions?

## References

1. Taylor, S. L.; Gendel, S. M.; Houben, G. F.; Julien, E., The Key Events Dose-Response Framework: a foundation for examining variability in elicitation thresholds for food allergens. *Crit Rev Food Sci Nutr* **2009**, *49*, (8), 729-39.
2. <http://www.fda.gov/Food/LabelingNutrition/FoodAllergensLabeling/GuidanceComplianceRegulatoryInformation/ucm106187.htm>
3. Wang, P.; Whiteaker, J. R.; Paulovich, A. G., The evolving role of mass spectrometry in cancer biomarker discovery. *Cancer Biol Ther* **2009**, *8*, (12), 1083-94.
4. EFSA Panel on Genetically Modified Organisms, Scientific Opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed. European Food Safety Authority (EFSA) Journal, **2010**, *8*(7),1700.
5. Addona, T. A.; Abbatiello, S. E.; Schilling, B.; Skates, S. J.; Mani, D. R. et al., Multi-site assessment of the precision and reproducibility of multiple reaction monitoring-based measurements of proteins in plasma. *Nat Biotech* **2009**, *27*, (7), 633-641.