



Accurate and Affordable Allergen Quantification for the Seed Biotech Industry

Inventors

Jay J. Thelen, PhD, Severin E. Stevenson, MS
University of Missouri, Department of
Biochemistry



What is the problem?

- **Allergenicity of foods and food components**
 - 1-4% of adults and 6-8% of children have food allergies¹
 - Seeds cause most food-related allergic reactions (tree nuts, peanuts, wheat and soybeans)²
- **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- **Current allergen measurement uses out-of-date technologies**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 reproducible⁵

How does it work?



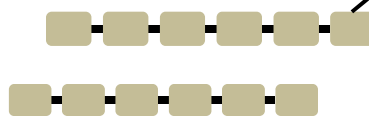
Seed allergen (peptides)



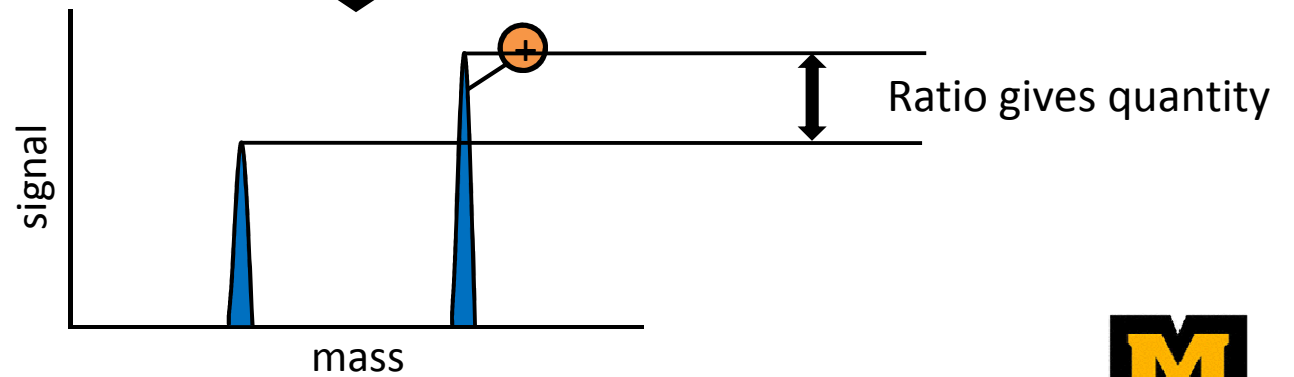
Labeled internal standard ⁺



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> | lower | higher |
| <u>Absolute quantities</u> | NO | YES |



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.