Cross-linking measurements of *Potato leafroll virus* reveal protein interaction topologies required for virion stability, aphid vector transmission, and virus-plant interactions.

Michelle Cilia
USDA, ARS
Department of Plant Pathology and Plant-Microbe Biology
Cornell University

60th ASMS Conference on Mass Spectrometry
Aphids/Luteovirus pair as a model to study virus transmission by insects

Potato leafroll virus
PLRV

Host plant

Virions: Capsid Protein and Read-through Protein
RTP

60th ASMS Conference on Mass Spectrometry
Disorder is key to RTP functionality in aphids and plants

Predicted Disorder

Max
Min
Average
PLRV

Amino acid residue →

Cross-linking gives info about protein topology

Spacer arm
Reactive Groups

New covalent bonds contain information
• Protein interactions
• Topological features
**Affinity Tag**

**Protein Interaction Reporter (PIR) Technology**

Bruce Lab (University of Washington)

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**Why are these cleavable bonds important?**

**Mass Spec Cleavable Bonds**

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**60th ASMS Conference on Mass Spectrometry**
What are the interaction topologies in PLRV?

- Agrobacterium-mediated infiltration of infectious PLRV clone
- PIR labeling of infectious virions
- Trypsin digestion
- SCX Clean up
- Affinity enrichment
- LC-MS/MS analysis

![60th ASMS Conference on Mass Spectrometry](image)

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Velos-FT Mass Spectrometer Coupled in the Bruce Lab

- "S-lens"
- HPT LPT
- 7 T Actively Shielded Superconducting Magnet
- ICR Cell
- Replace LTQ with LTQ velos
- Standard ion injection optics
- 10X gain in sensitivity
- 2X gain in MS/MS acquisition speed

![60th ASMS Conference on Mass Spectrometry](image)
Velos-FT MS and Real-Time Analysis of Cross-linked Peptides

Weisbrod et al, in review.

60th ASMS Conference on Mass Spectrometry

Real-time identification of an unambiguous cross-linked homodimer in PLRV virions

Precursor – MS

Theoretical mass: 3876.733
Measured mass: 3876.755
Mass error: 5.6 ppm

Released peptides – MS²

Peptide sequence ID – MS³

GNGK¹⁸⁸SSDPAGSFR

IT – MS³ 689.82

Chavez et al. 2012
J. Proteome Res (May 5th issue)
Unambiguous Homodimer Cross-Link Enabled Identification of Capsid Monomer Interface

PIR data is useful for structural modeling

>1800 possible trimer structures

With single cross-linking constraint, 4 remain.

Southern Cowpea Mosaic Virus
Qu et al. 2000, Structure

60th ASMS Conference on Mass Spectrometry
Coupling PIR and Molecular Virology to Discover Functions of Virion Topological Features

No Stable Virion Formation in Plants.

PIR technology enabled us to discover interactions in the RTP that has not been possible using other methods.

First experimental evidence that the read-through domain is exposed on external surface of the capsid.
“The obstacle for researchers studying virus transmission is the inability to observe what is going on inside the vector”.

Gray and Banerjee, Microbiology and Molecular Biology Reviews, March 1999

• Future: In vivo applications in plants and insects to ID vector-virus-host interactions

• In vivo PIR-AP-MS

Jim Bruce, 8:30AM
ThOE am - Biomolecular Structure Analysis by Covalent Labeling: Future Directions

60th ASMS Conference on Mass Spectrometry

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Stewart Gray
Ho-Jong Ju
Dawn Smith
Tom Hammond

Juan Chavez, 3:30pm
TOE pm - Biomarkers of Drug Toxicity, Response & Efficacy Biomarkers: Innovative MS Approaches

UPCOMING BRUCE LAB POSTERS

Jake Zheng Tuesday 03 - Quantitative Proteomics: Label-free Slot: 061
Xia Wu Wednesday 09 - Microorganisms: Identification and Characterization Slot: 226
Chad Weisbrod Thursday 12 - Informatics: Quantification Slot: 280