

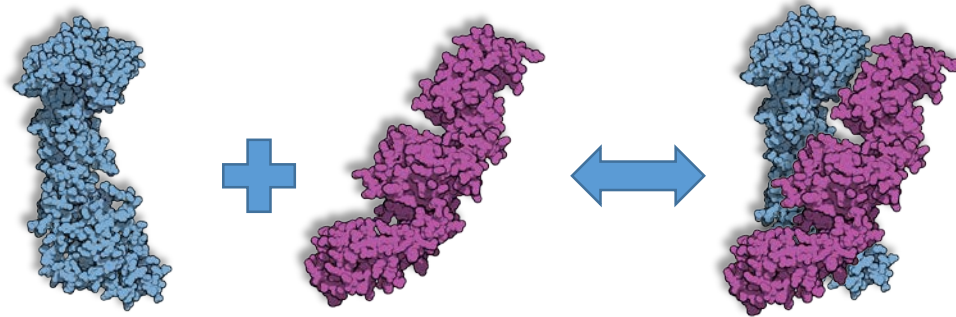
# A General Method for Targeted Quantitative Cross-Linking Mass Spectrometry

Skyline Users Meeting


06.04.17

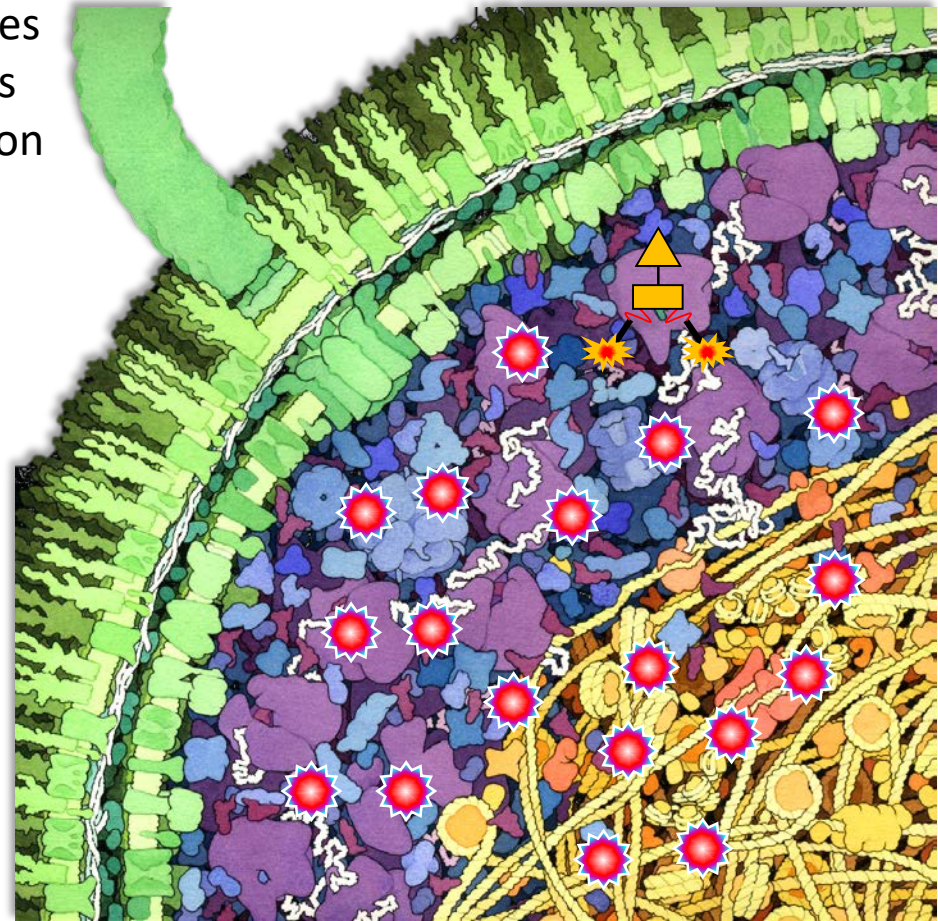
Juan Chavez – Bruce Lab

# *In vivo* cross-linking for systems structural biology

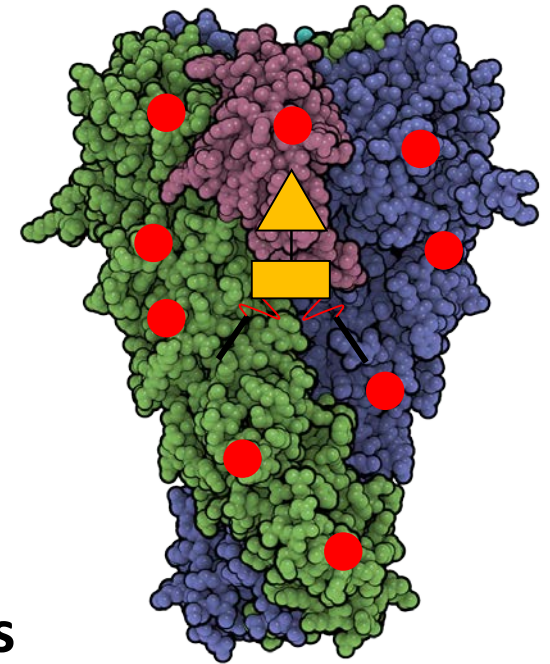
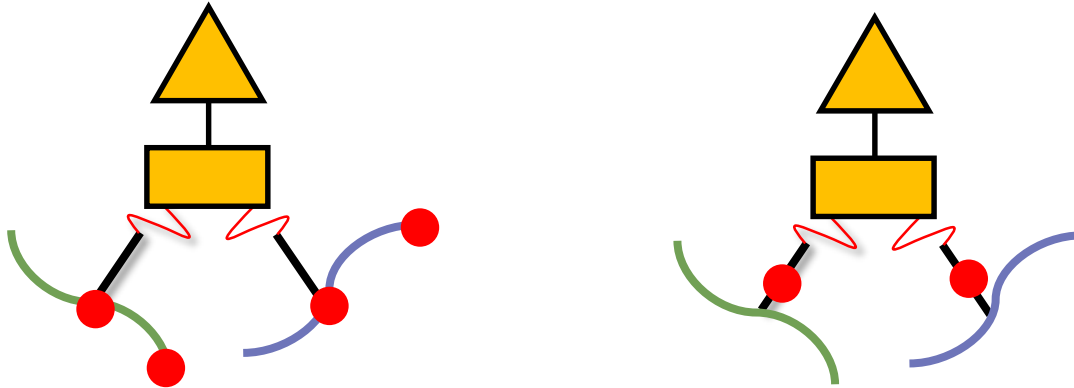


Macromolecular crowding

- Proteins function as multicomponent machines
  - Inter-protein & inter-domain interactions
  - Stable/dynamic interactions drive function
- Crowded cellular environment
  - ~200-300 mg/mL
  - Compartmentalization
- Chemically “freezing” protein conformations and interactions to gain biological insight
  - Proximal reactive residues 
  - Spatial constraints on cellular system



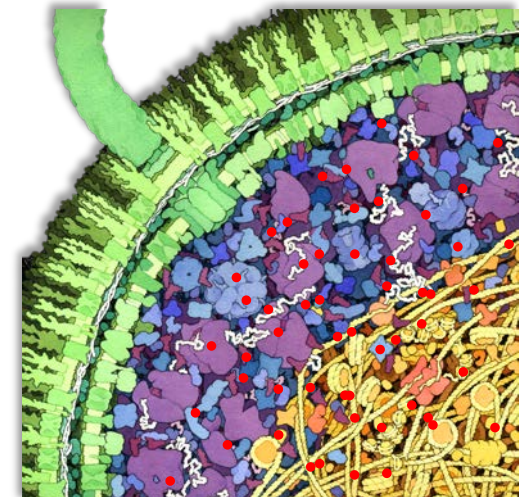
# Quantitative cross-linking with mass spectrometry



## Protein conformation & interaction dynamics

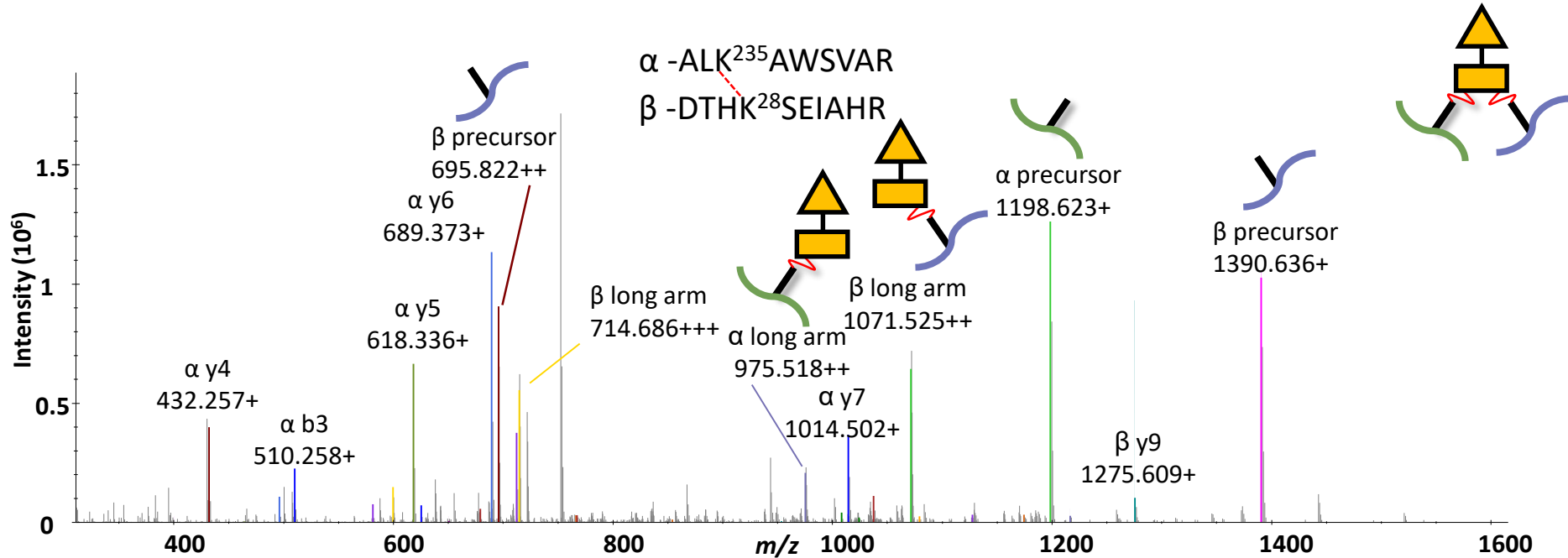
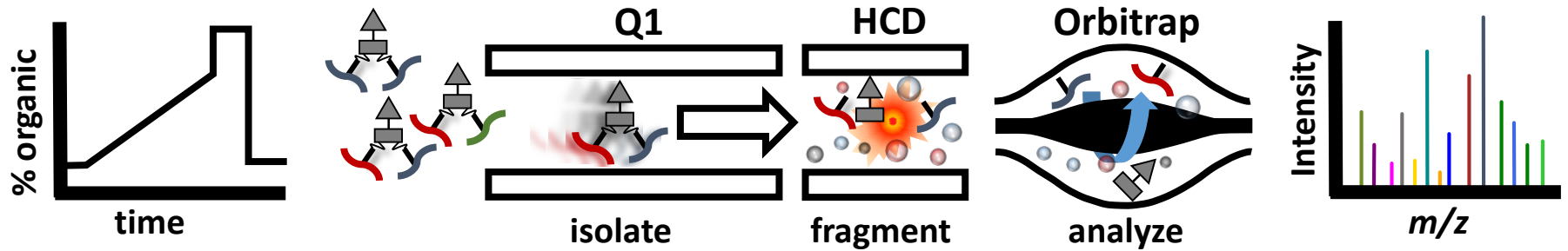
**Incorporation of isotopes = ●**

- Metabolic labeling – SILAC
  - Chavez et al Nat. Commun. 2015
  - Chavez et al Cell Chem. Biol. 2016
- Isotope labeled cross-linker – d0/d8 PIR
  - Zhong et al JPR 2017

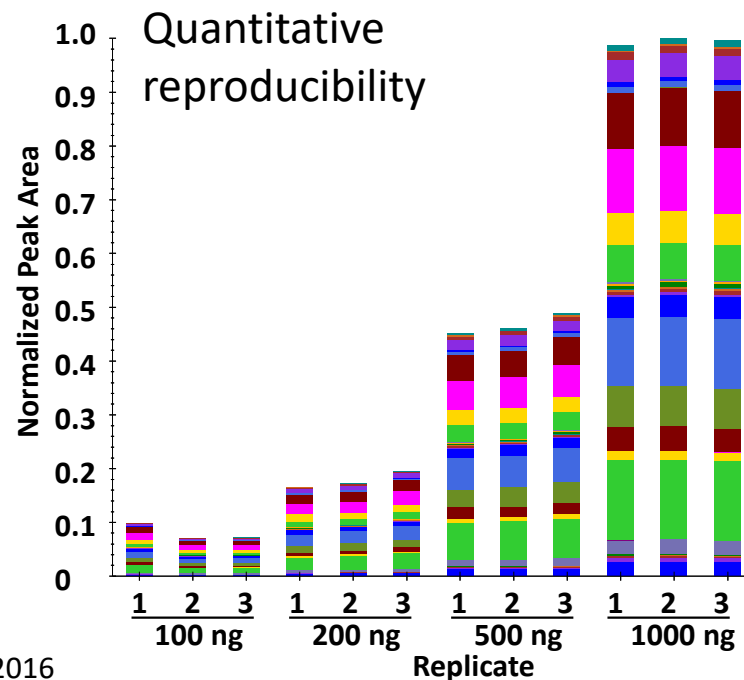
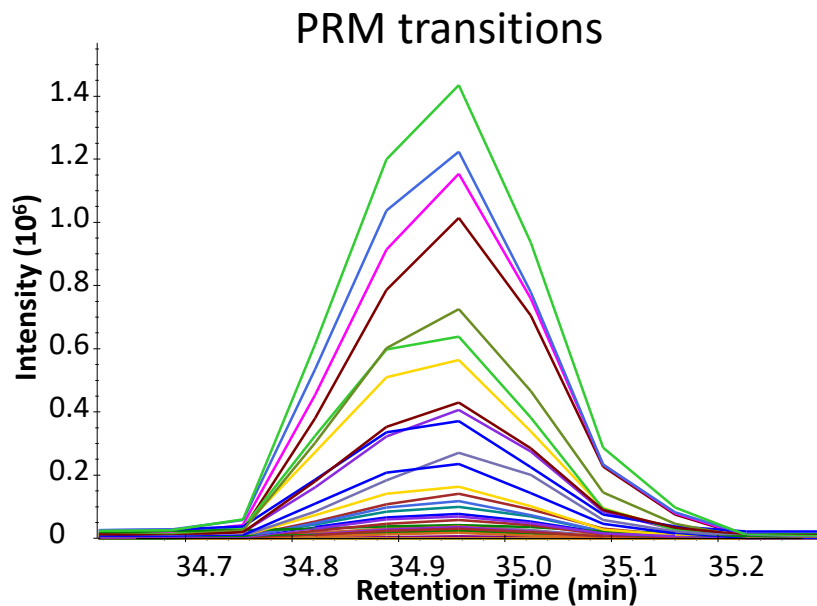
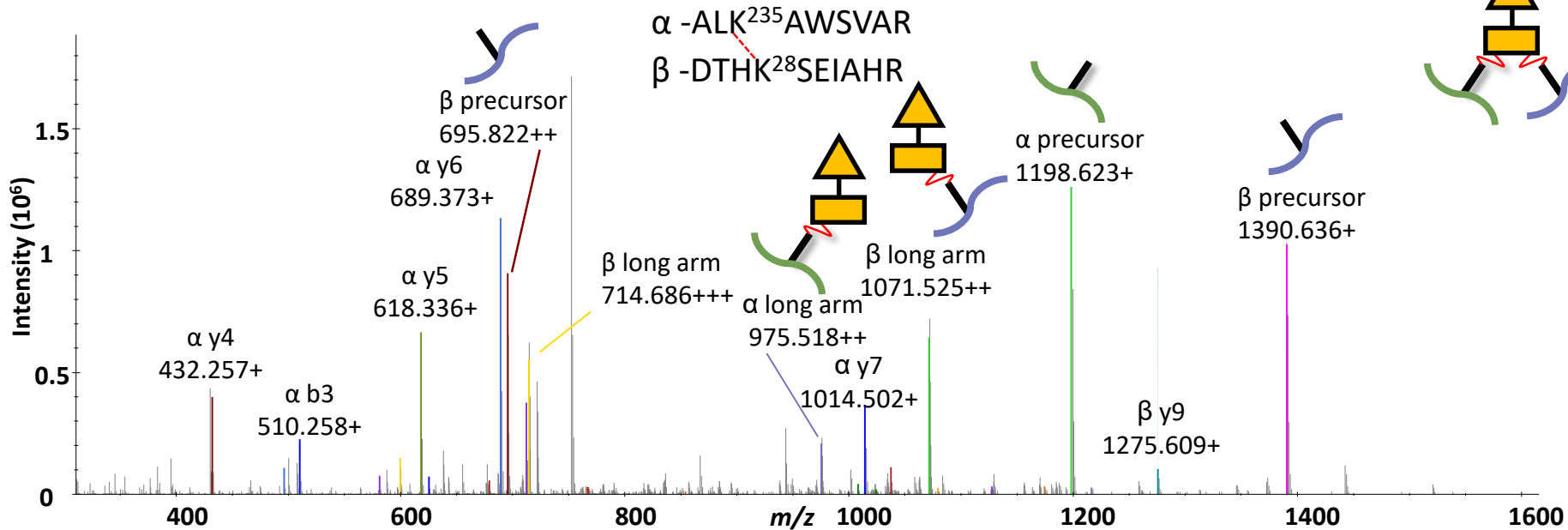


# Targeted quantitation of cross-linked peptides by Parallel Reaction Monitoring (PRM)

LC-MS



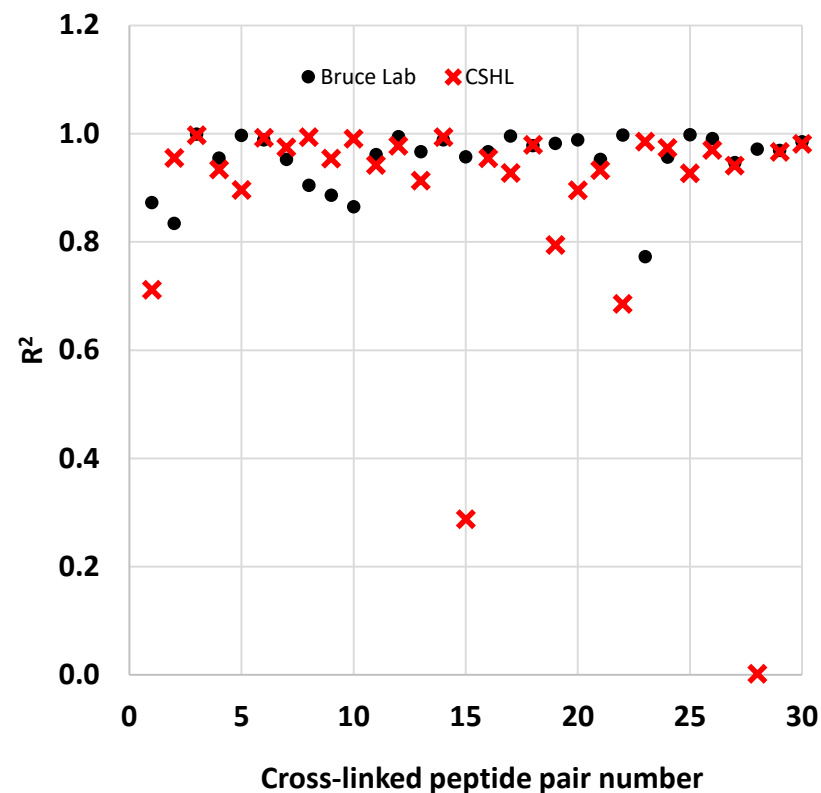
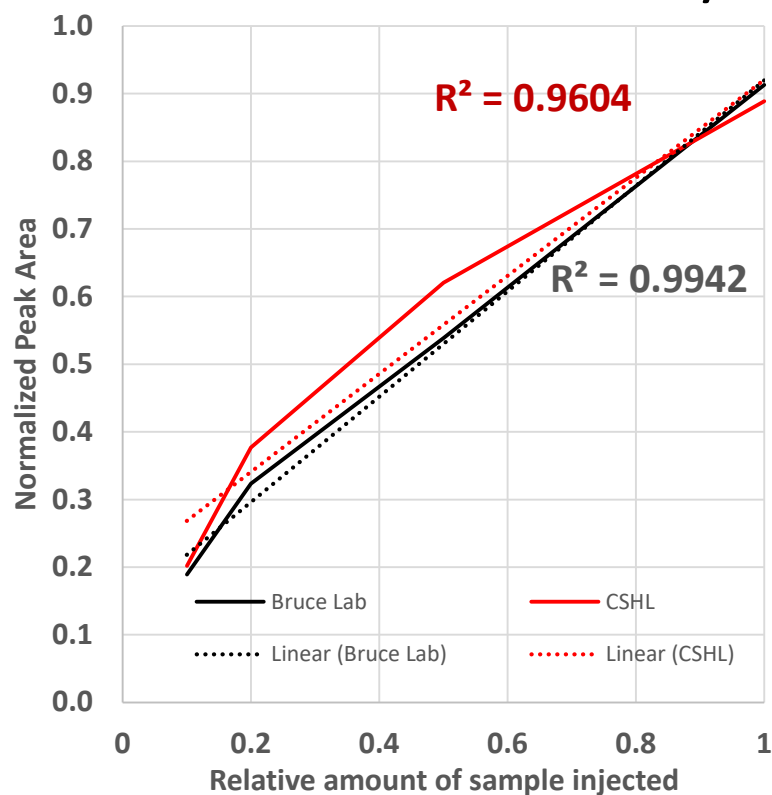
# PRM of cross-linked peptide pairs



# PRM of cross-linked peptide pairs



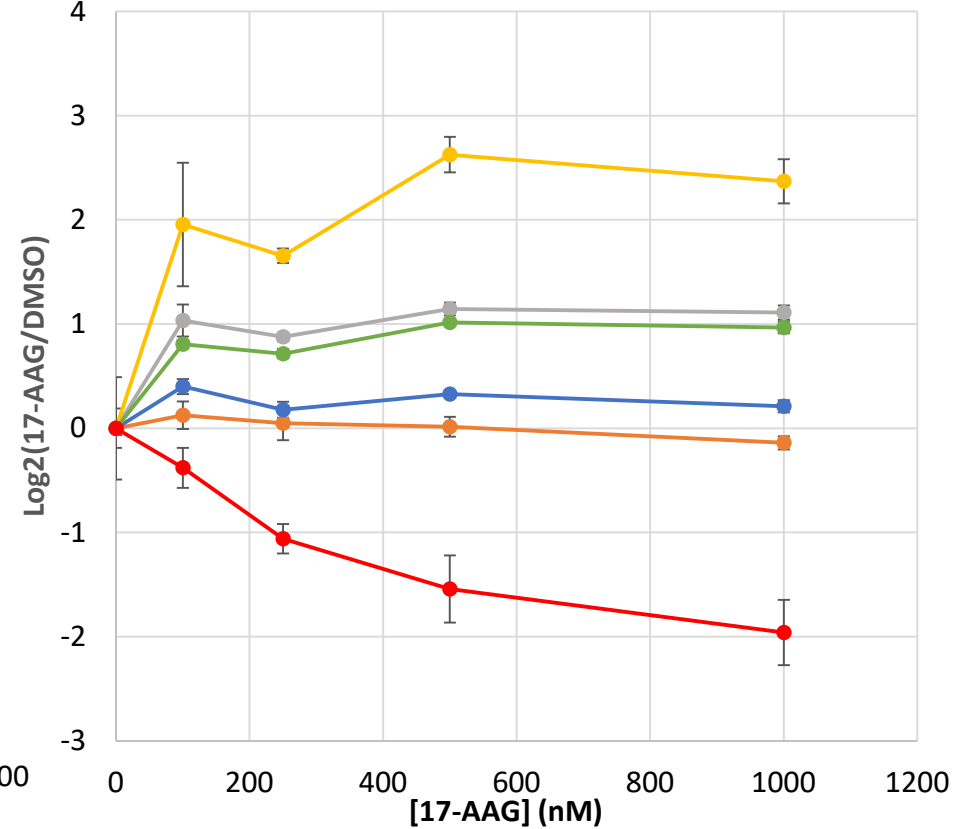
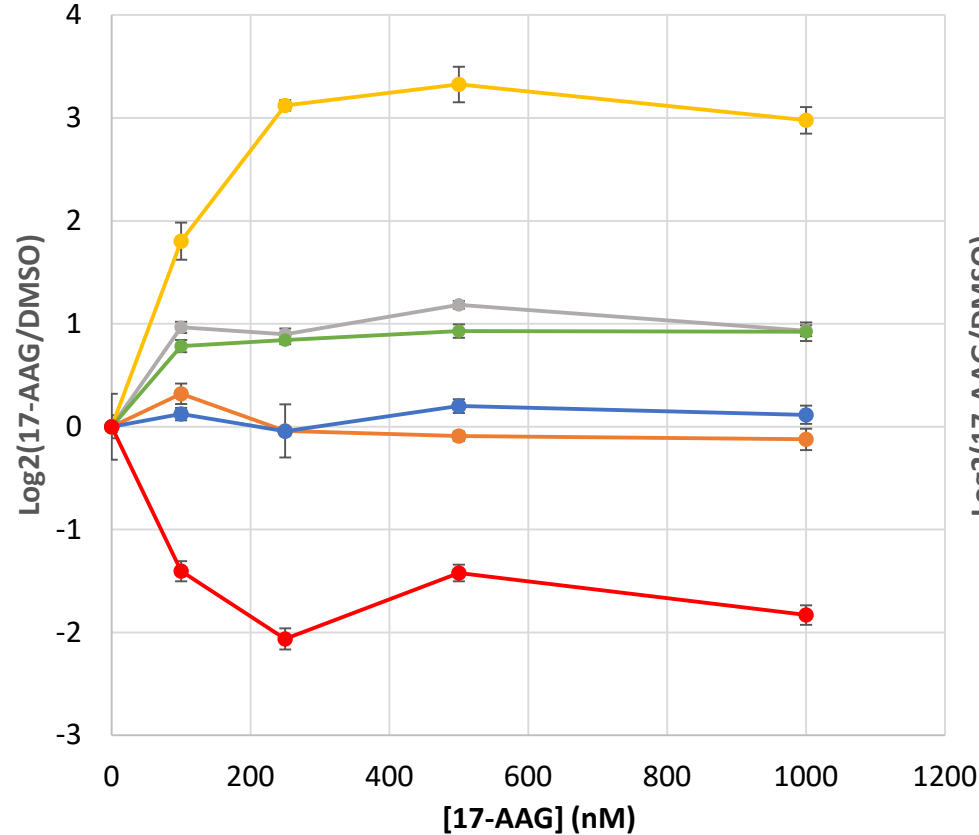
## Cross-laboratory study



# PRM of *in vivo* xlinks in cross-laboratory study

Bruce Lab

Cold Spring Harbor Lab



- FYEAFSK<sup>435</sup>NLK\_FYEAFSK<sup>435</sup>NLK
- FYEAFSK<sup>435</sup>NLK\_FYEQFSK<sup>443</sup>NIK
- FYEQFSK<sup>443</sup>NIK\_FYEQFSK<sup>443</sup>NIK
- IMK<sup>607</sup>AQALR\_FYEAFSK<sup>435</sup>NLK
- IMK<sup>615</sup>AQALR\_FYEQFSK<sup>443</sup>NIK
- VK<sup>739</sup>IPVAIK\_VLGSGAFGTVYK<sup>728</sup>GLWIPEGEK

- HS90B homodimer
- HS90B-HS90A heterodimer
- HS90A homodimer
- HS90B intramolecular link
- HS90A intramolecular link
- EGFR intramolecular link

# XlinkDB: an online database and tools for cross-linking mass spectrometry data

<http://xlinkdb.gs.washington.edu/xlinkdb/>

## General XL-MS results

Pep A | Prot A | Res A |  
| Pep B | Prot B | Res B



## XLink transition calculator in XLinkDB

Peptide A	Protein A	PDB # for Pept. A	Peptide B	Protein B	PDB # for pep. B	Network Distance	XL Distance (Å)	Generate PRM Transition	JSmol Viewer	NGL Viewer (editable)
QFFT <b>K</b> IK	CDC37	P08238_Q16543	NNIK <b>L</b> YVR	HSP90AB1	P08238_Q16543	N/A	16.507	<a href="#">Go to PRM Form</a>	<a href="#">View Structure</a>	<a href="#">View Structure</a>

<http://xlinkdb.gs.washington.edu/xlinkdb/prmTransitionForm.php>

## Network analysis



## Structural analysis

MS PDB  
PROTEIN DATA BANK

Modeller

PATCHDOCK

Hsp90

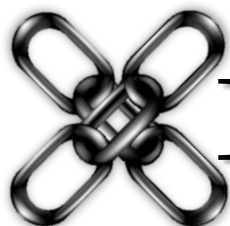
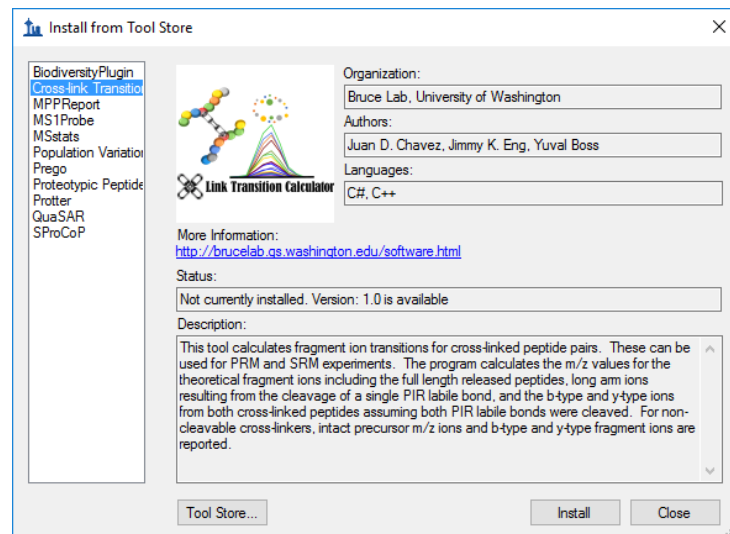
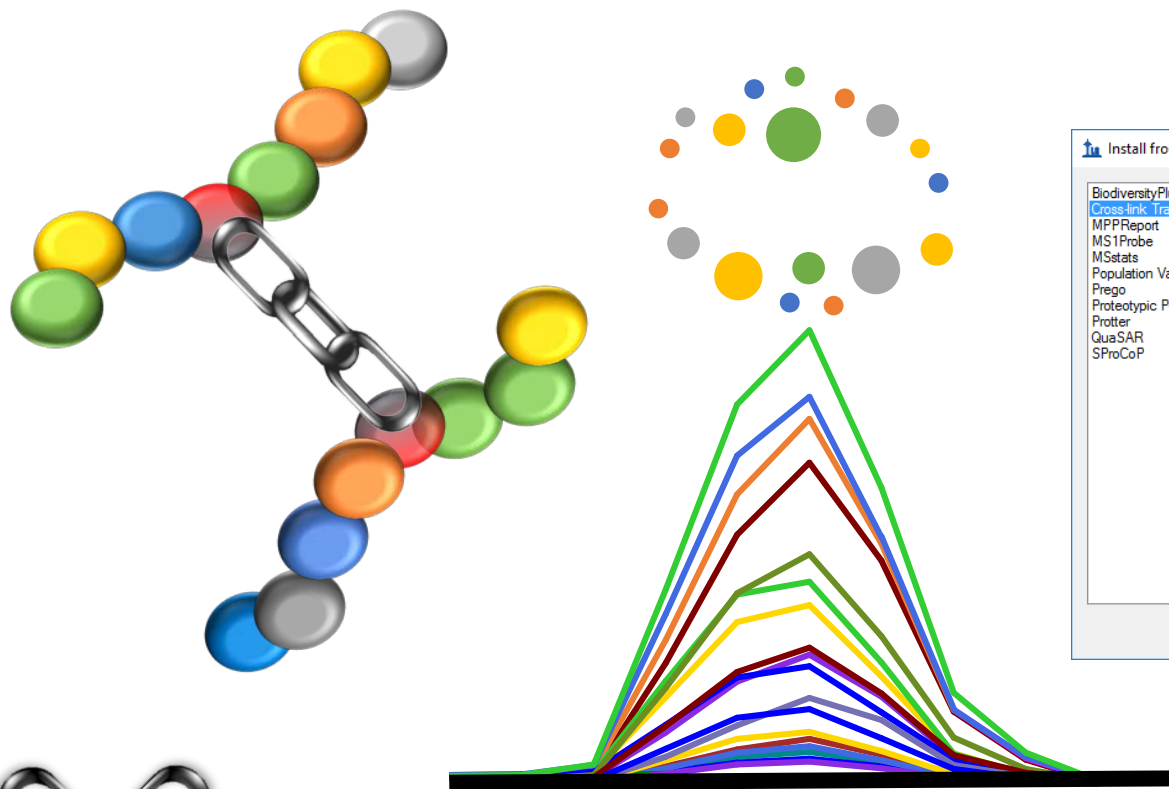
- 36,644 XL-pairs
- 7,833 proteins
- 7 species

XLinkDB: Zheng et al. JPR 2013

XLinkDB2.0: Schweppe et al. Bioinformatics 2016



# Skyline External Tools: Cross-link Transition Calculator



## Link Transition Calculator

- **General input** - compatible with all cross-link data
- Calculates transitions for use in PRM and SRM experiments

# Acknowledgements

- Bruce Lab – past & present members

- UWPR

- Jimmy
- Priska
- Vagisha

- Skyline

- Yuval Boss

- Funding Sources

- NIH R01GM086688

## Bruce Lab ASMS presentations

- MP 052 - Drug Induced Protein Conformational and Interaction Dynamics by Quantitative Cross-linking Mass Spectrometry
- MP 578 - Comparative interactome analysis of multidrug-resistant and sensitive pathogenic bacteria strains of *A. baumannii*
- TOE am - Dynamics of the Mitochondrial Interactome
- ThP 383 - Characterization of harmonic signal acquisition with parallel dipole and multipole detectors
- ThOA pm - Mango: A general search tool for CID-cleavage cross-linked peptide identification