Workshop: H/D Exchange, Covalent Labeling, and Cross-Linking Interest Group
Date: 6 June 2017
Organizers: Prof. David Weis, University of Kansas
Prof. Lan Huang, University of California, Irvine

Attendance: ~180

This year the interest group meeting focused on discussing general workflows, current status and future perspectives of the three areas covered in this group.

Three contributors gave 15-minute technique-oriented presentations followed by discussion of their topics with the audience. The presenters covered a broad spectrum of each technique and provide useful information for experts and new comers. Ample time was devoted to an engaging discussion of each topic in turn. New and experienced researchers were able to ask questions and offer comments and suggestions. In general, participants found the presentations informative and discussions interesting. Questions were contributed about equally from the floor and electronically.

The contributors were

Prof. Jim Bruce University of Washington
Prof. Joshua Sharp, University of Mississippi
Dr. Mikklos Guttman, University of Washington

Some of the major points of discussion were:

- Current workflows of cross-linking mass spectrometry in studying protein-protein interactions especially in living cells
- Experimental conditions for successful cross-linking reactions
- Data analysis and visualization of cross-link data
- General aspects of FPOP experiments
- Applications of FPOP in probing protein interactions
- New approaches for HX-MS experiments

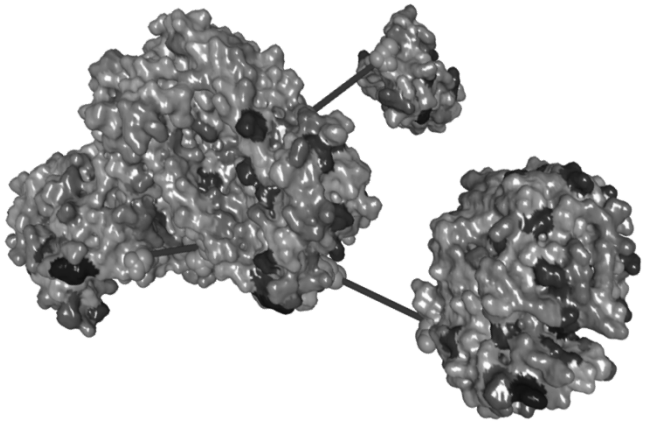
The slides presented follow this report.

# Cross-linking – what where and how

Jim Bruce
University of Washington

- General workflows for XL-MS
- Strengths and weaknesses of approaches
- New developments and applications
- Current challenges and advice for newcomers

## New covalent bonds hold structural information



Cross-linked interactors can be found without identification of cross-linked sites.

## What question is to be pursued?

- Purified protein or complex
- Complex mixture
- Cells, tissues, plants, animals
- Quantitation?

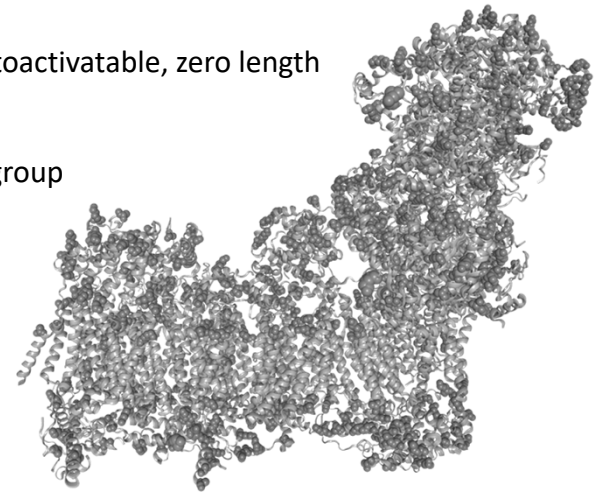
Workflow choices become more limited as sample complexity increases

## Cross-linking mass spec: general workflows

I) Choose chemistry: Amine reactive, photoactivatable, zero length

A) Frequency of amino acid/reactive group

B) Reaction Specificity
XXX**K**XXXK/R



C) Solubility
Protein conc. in cells ≈ 300mg/ml
Even multiple rounds of cross-linking causes little loss in bacterial cell viability

## Cross-linking mass spec: general workflows

II) Cross-linking reaction conditions

A) pH – usually neutral 7-8

B) Buffers used – choice based on XL reactive groups, 170mM $Na_2PO_4$, pH 8.0 frequent choice

C) Temperature, 4°C, RT?

D) Typical XL time – 30 Min.

E) Xlinker concentration? In vivo, as high as possible $[\ ]_{fiinal}$ mM, single protein, titration to determine

## Cross-linking mass spec: general workflows

III) Sample preparation

For activated esters, $T_{1/2}$≈ 8 minutes, pH = 7.5


A) Get rid of unreacted cross-linker? If so, how?
If cells, wash with PBS, All can use FASP, Gel, TCA ppt., others

B) Digestion: Enzyme choice? For lysine-reactive XL, trypsin is common choice
XXX**K**XXXK/R

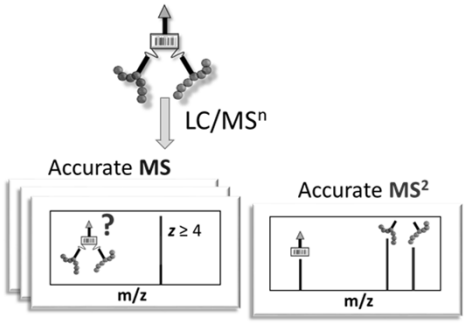
C) Enrichment? Strong cation exchange (most x-linkers), affinity enrichment (tag needed)

# Cross-linking mass spec: general workflows

IV) Mass spectrometry analysis

A) Single protein – accurate mass LC/MS/MS, selection of higher charge states

B) More complex samples benefit from MS cleavable cross-linkers – LC/MS/MS/MS, LC MS/MS ETD/HCD, others




# Cross-linking mass spec: general workflows

V) Database search

A) Single or few proteins, non-cleavable linkers – xQuest, P-link, others, chimeric $MS^2$spectra must be searched. Database grows with $n^2$, where $n$ is # of peptides.

B) Complex samples, cleavable linkers, $MS^3$ data – mass relationships enable unambiguous peptide mass ID, existing proteome search engines- Sequest, Mascot, etc. – MS/MS of single peptides. Advanced search tools XlinkX ID from combined ETD and HDC spectra use mass difference of long and short arm, others.

# Cross-linking mass spec: general workflows

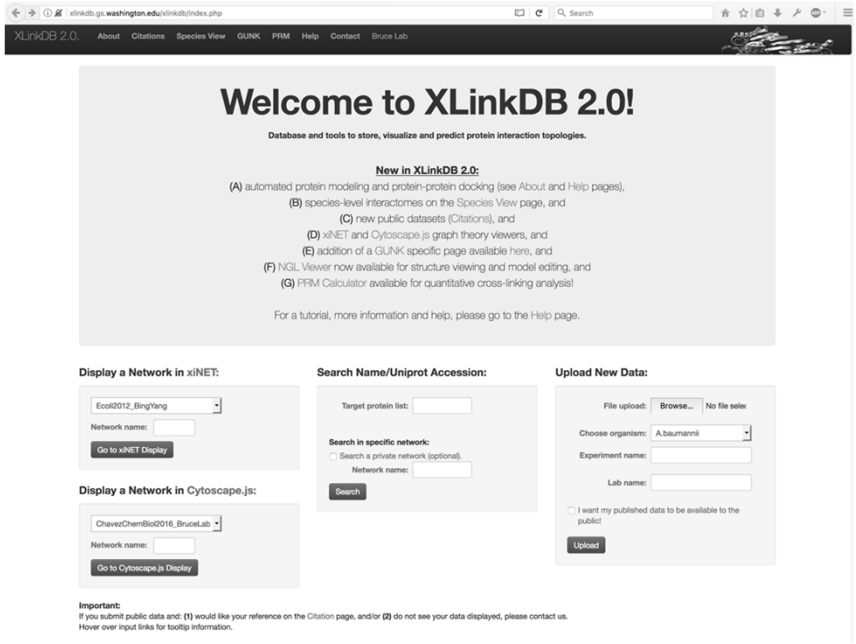
VI) Then what?

A) Single or few proteins with pdb files, manually visualize links with favorite viewer, jmol, molsoft, NGL, Docking: Patchdock, Haddock, iDock, others. Distance constraint: empirically determine

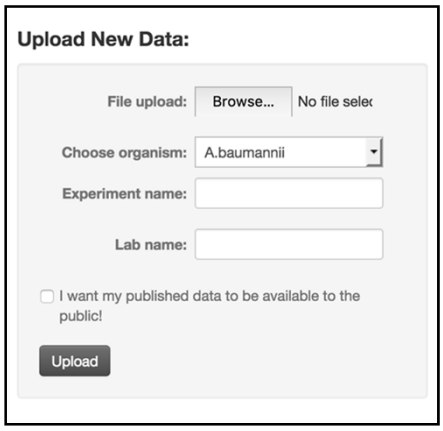
B) Complex samples; need network viewer: cytoscape, xiNet, Xlink-DB, many others. In general, most PPI networks tools are not designed with Xlink data in mind.

C) Capabilities are emerging

**XLinkDB 2.0 Database and Tools to Store, Visualize, and Predict Protein Interaction Topologies**



## Upload Your Crosslink Data

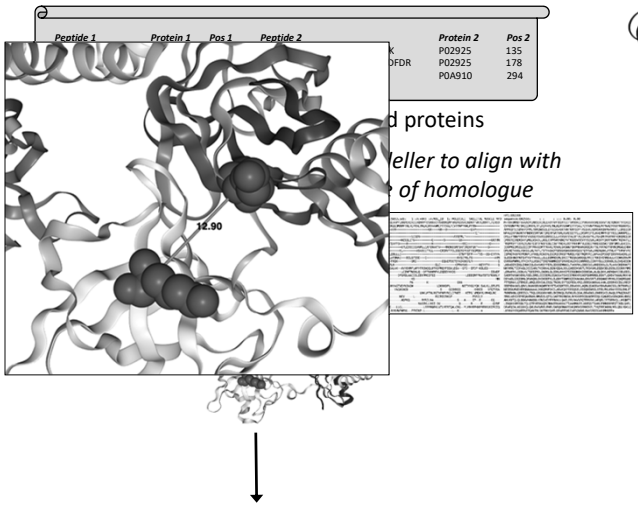



*Cross-link data text file*

| ***Peptide 1*** | ***Protein 1*** | ***Pos 1*** | ***Peptide 2*** | ***Protein 2*** | ***Pos 2*** |
|---|---|---|---|---|---|
| LASSLTTKGASSFK | Q9DB77 | 7 | DQWTKYEEDKFYLEPYLK | Q9D855 | 9 |
| NAFGAPLTKLQNIQFK | Q07417 | 8 | ENLLGEPGMGFKIAMQTLDMGR | Q07417 | 11 |
| FFTGQITAAGKVPPAK | Q61941 | 10 | VALSPAGVQALVKQGFNVVVESGAGEASK | Q61941 | 12 |
| IVPNILLEQGKAK | Q9CZU6 | 10 | GMKGLVYETSVLDPDEGIR | Q9CZU6 | 2 |
| ANVAKPGLVDDFEKK | Q9DCX2 | 13 | LASLSEKPPAIDWAYYR | Q9DCX2 | 6 |
| TLLKDTTVTGLGR | Q8BMS1 | 3 | DSIFSNLIGQLDYKGFEK | Q8BMS1 | 13 |
| TPIKDAATGAVKEK | P50544 | 11 | KGIVNEQFLLQR | P50544 | 0 |
| KVQHELDEAEER | Q02566 | 0 | QAEEAEEQANTNLSKFR | Q02566 | 14 |
| GFYIYQEGSKNK | Q8BMS1 | 9 | KMGLVDQLVEPLGPGIK | Q8BMS1 | 0 |
| VGQLLKDPKVSLAVLNPYIK | Q9DB20 | 8 | GQKVLDSGAPIKIPVGPETLGR | P56480 | 2 |
| LAADVGKGSSQR | P48962 | 6 | VKLLLQVQHASK | P48962 | 1 |
| FNPETDFLTGKDGK | Q99KI0 | 10 | KFKLEAPDADELPR | Q99KI0 | 0 |

## Representation of Uploaded Crosslinked Peptides in XLinkDB

Upload Cross-links to XLinkDB

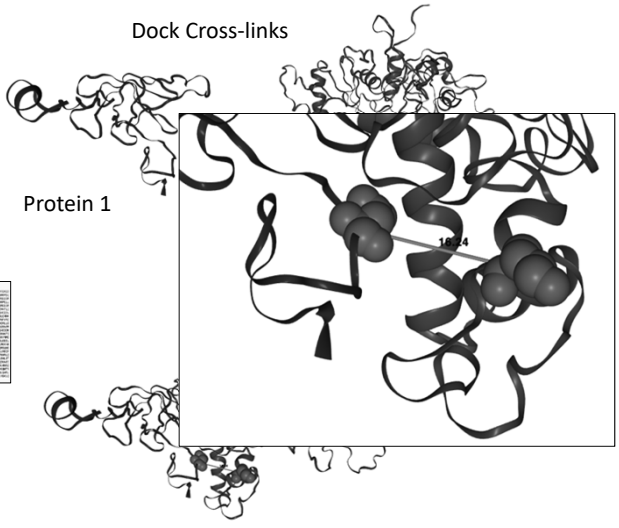



d proteins

*eller to align with*
*of homologue*

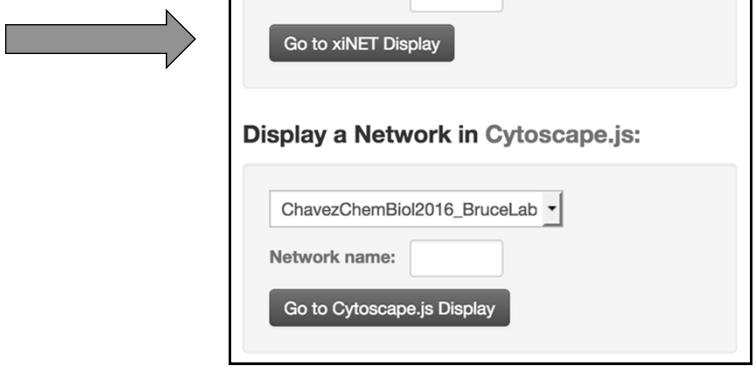
Compute intra-protein cross-link distances

Dock Cross-links

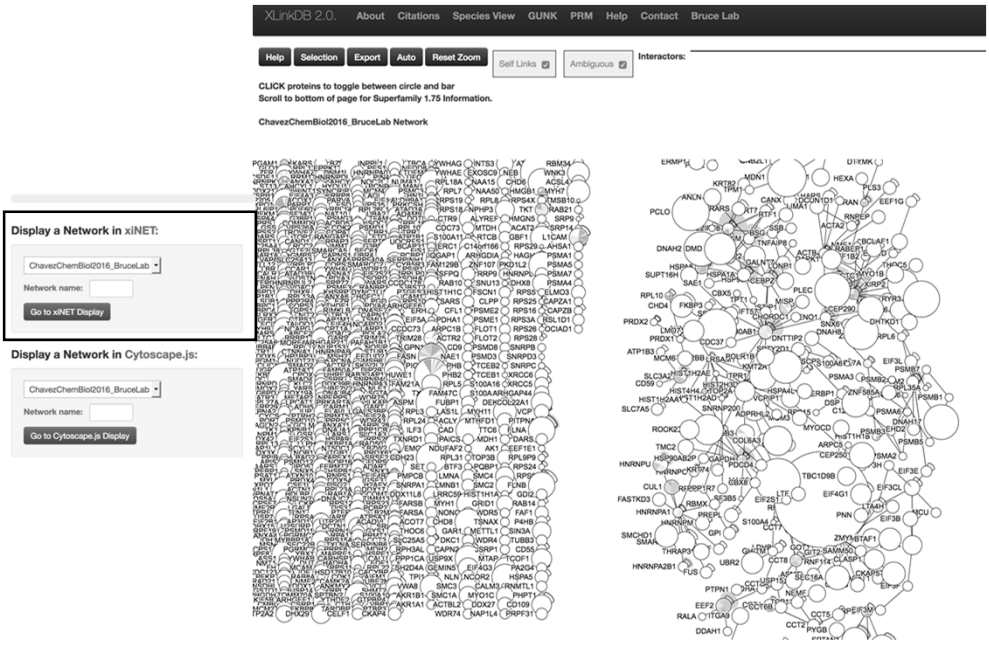



Compute inter-protein cross-link distances

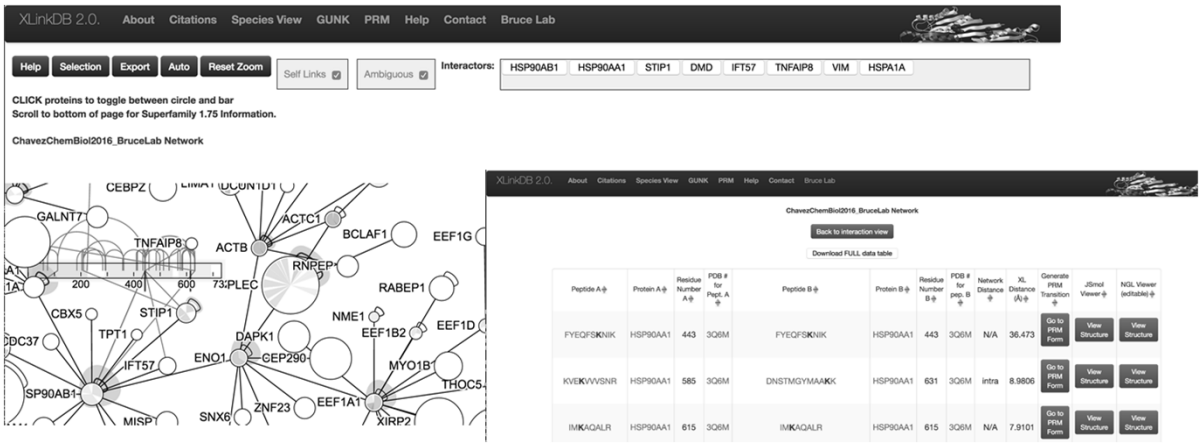
## View Your Crosslink Data: Network-level



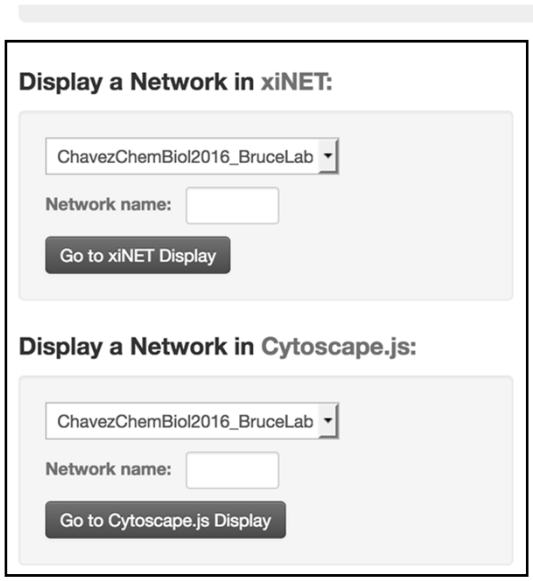

## View Your Crosslink Data: xiNET



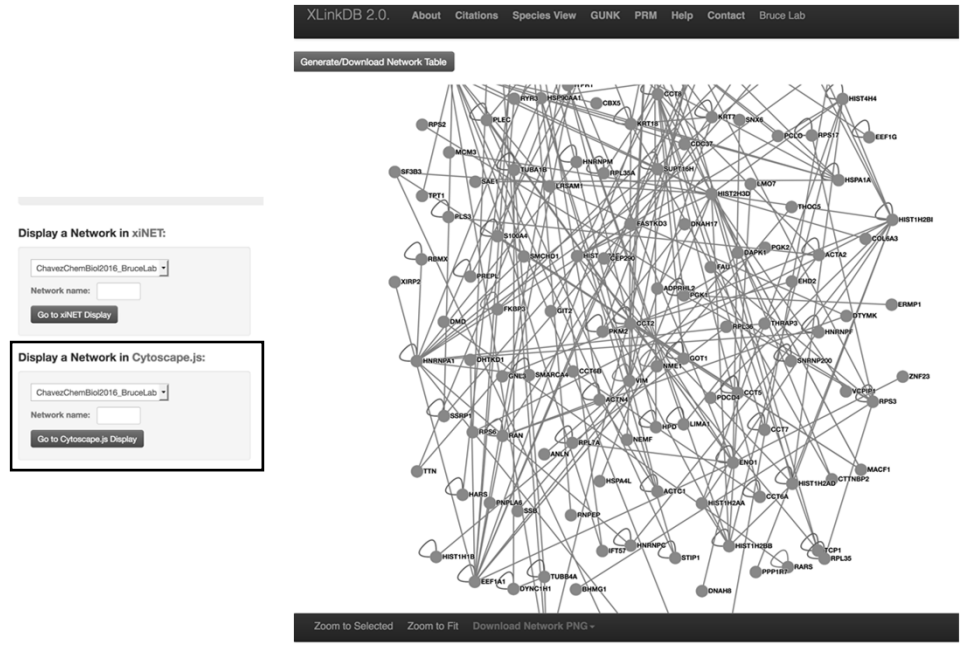
## View Your Crosslink Data: xiNET




## View Your Crosslink Data: Network-level

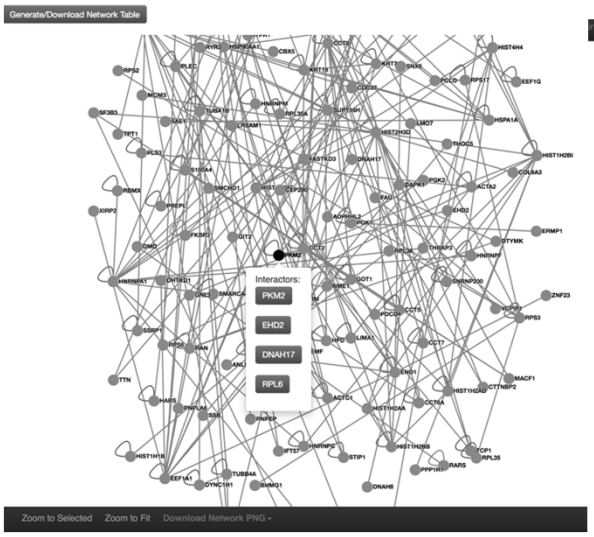


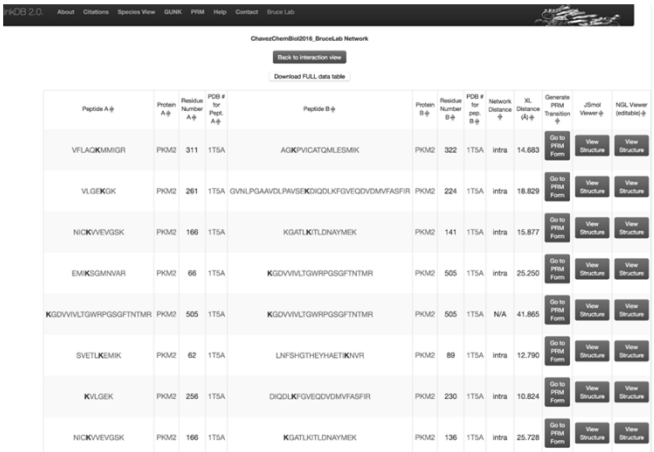
## View Your Crosslink Data: Cytoscape




## View Your Crosslink Data: Cytoscape

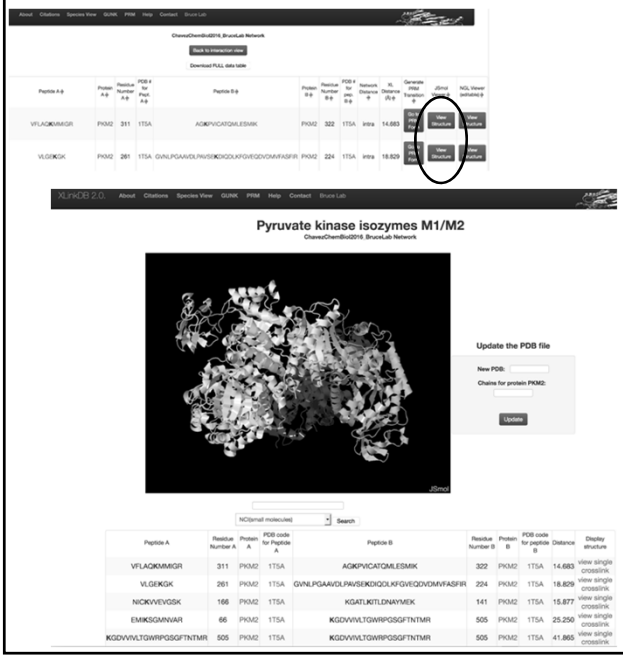
***Explore protein-protein interactions***



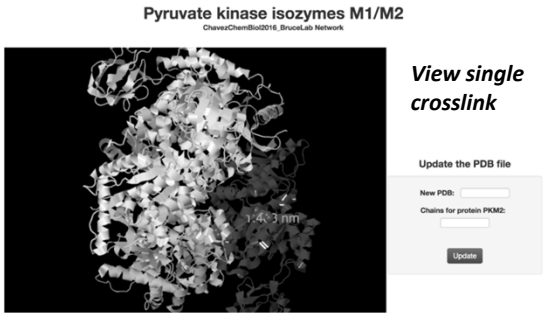



## View Your Crosslink Data with JSmol

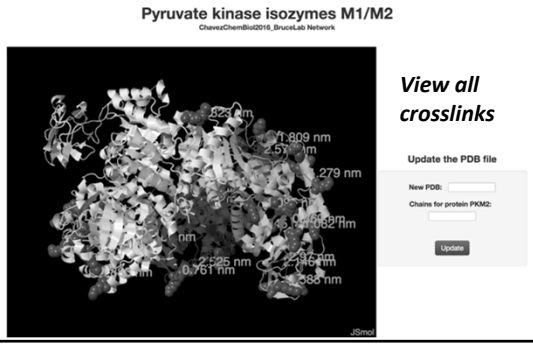
***JSmol Viewer***




***View single crosslink***

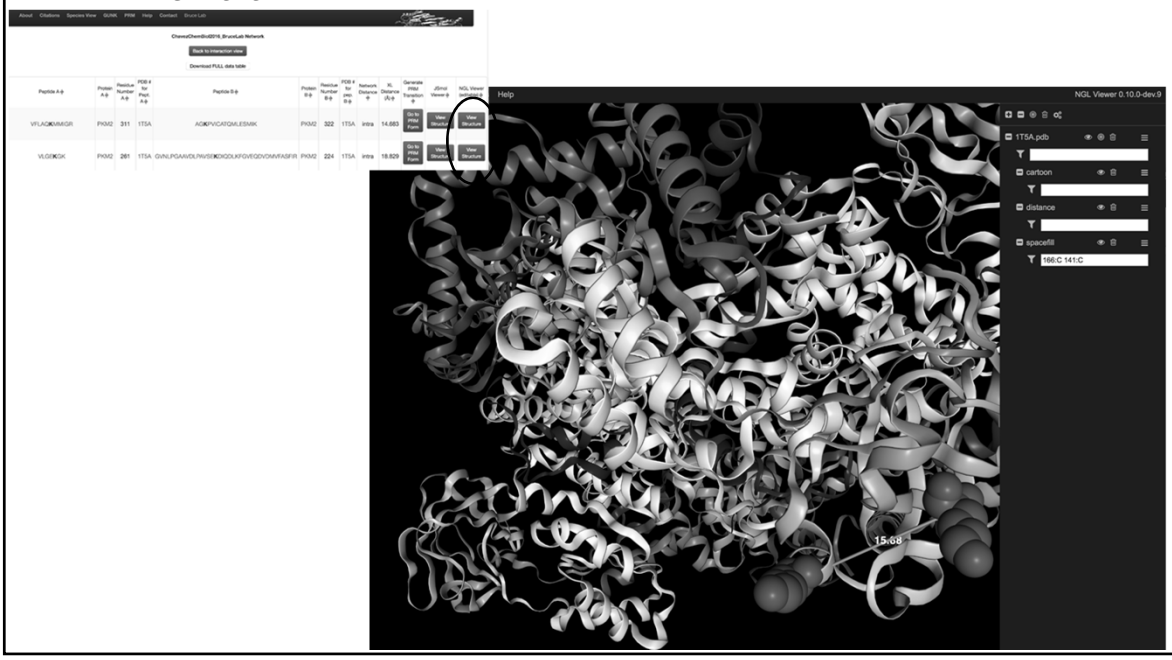



***View all crosslinks***



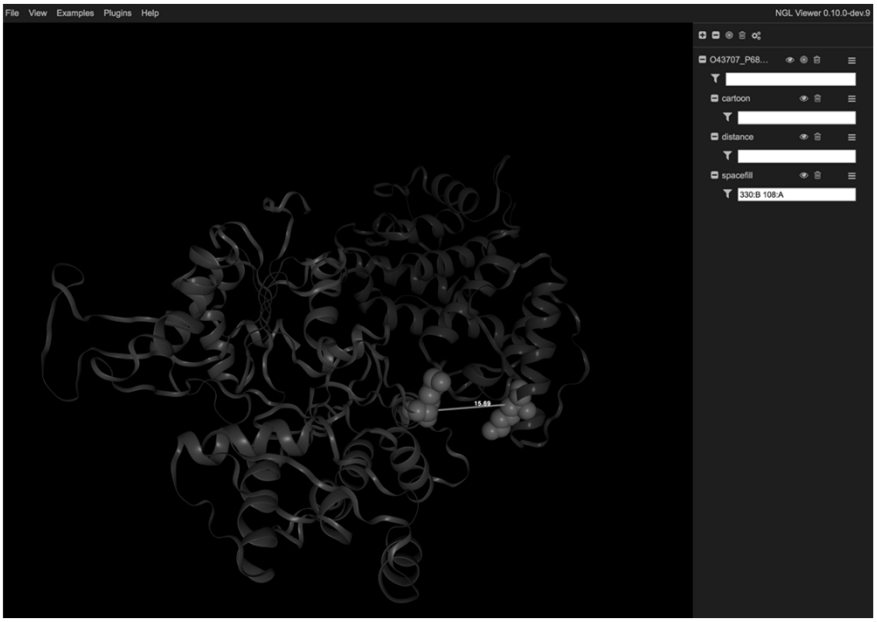

## View Your Crosslink Data with NGL

***NGL Viewer***



## View Your Crosslink Data with NGL

***NGL Viewer***




***Predicted protein interaction topology with idock***

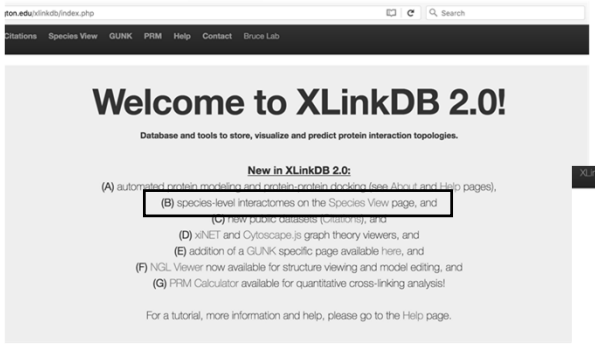
**ACTC1-ACTN4**
Interface

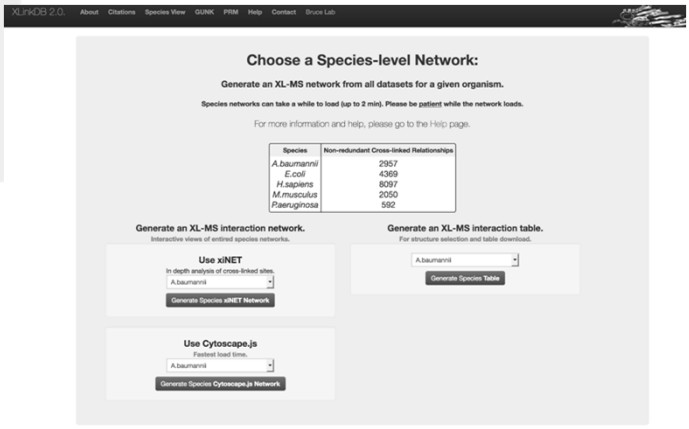
## Generate PRM Transitions of Crosslink

***NGL Viewer***


| PrecursorName | PrecursorMz | ProductMz | PrecursorCharge | ProductCharge | MoleculeGroup | ProductName |
|---|---|---|---|---|---|---|
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1233.683764 | 4 | 1 | peptide | A precursor 1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 617.34552 | 4 | 2 | peptide | A precursor 2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 411.899439 | 4 | 3 | peptide | A precursor 3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1985.089829 | 4 | 1 | peptide | A long arm 1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 993.048553 | 4 | 2 | peptide | A long arm 2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 662.368127 | 4 | 3 | peptide | A long arm 3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 114.09134 | 4 | 1 | peptide | A b1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 439.218726 | 4 | 1 | peptide | A b2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 552.30279 | 4 | 1 | peptide | A b3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 665.386854 | 4 | 1 | peptide | A b4+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 736.423967 | 4 | 1 | peptide | A b5+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 833.476731 | 4 | 1 | peptide | A b6+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 930.529495 | 4 | 1 | peptide | A b7+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1059.572088 | 4 | 1 | peptide | A b8+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 175.118952 | 4 | 1 | peptide | A y1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 304.161545 | 4 | 1 | peptide | A y2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 401.214309 | 4 | 1 | peptide | A y3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 498.267073 | 4 | 1 | peptide | A y4+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 569.304187 | 4 | 1 | peptide | A y5+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 682.388251 | 4 | 1 | peptide | A y6+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 795.472315 | 4 | 1 | peptide | A y7+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1120.5997 | 4 | 1 | peptide | A y8+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1262.648775 | 4 | 1 | peptide | B precursor 1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 631.828026 | 4 | 2 | peptide | B precursor 2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 421.554443 | 4 | 3 | peptide | B precursor 3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 2014.05484 | 4 | 1 | peptide | B long arm 1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1007.531058 | 4 | 2 | peptide | B long arm 2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 672.023131 | 4 | 3 | peptide | B long arm 3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 100.07569 | 4 | 1 | peptide | B b1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 237.134602 | 4 | 1 | peptide | B b2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 562.261987 | 4 | 1 | peptide | B b3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 675.346051 | 4 | 1 | peptide | B b4+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 789.388979 | 4 | 1 | peptide | B b5+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 903.431906 | 4 | 1 | peptide | B b6+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1002.50032 | 4 | 1 | peptide | B b7+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1116.543248 | 4 | 1 | peptide | B b8+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 147.112804 | 4 | 1 | peptide | B y1+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 261.155732 | 4 | 1 | peptide | B y2+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 360.224146 | 4 | 1 | peptide | B y3+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 474.267073 | 4 | 1 | peptide | B y4+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 588.310001 | 4 | 1 | peptide | B y5+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 701.394065 | 4 | 1 | peptide | B y6+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1026.52145 | 4 | 1 | peptide | B y7+ |
| IK[197.03]IIAPPER_VHK[197.03]INNVNK | 812.689128 | 1163.580362 | 4 | 1 | peptide | B y8+ |

## View Your Crosslink Data: Species-level

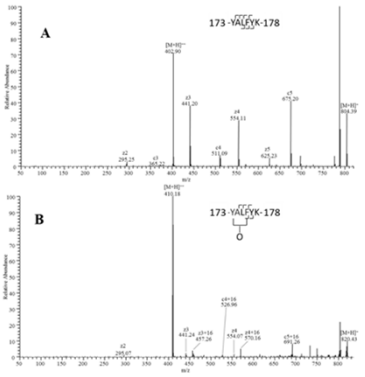


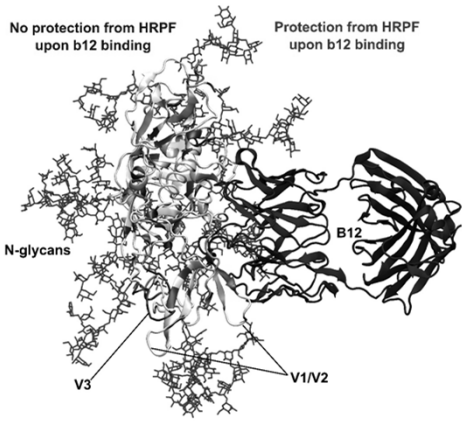



# Covalent Labeling: Experimental Considerations

Joshua Sharp
Assistant Professor, Department of BioMolecular Sciences
The University of Mississippi School of Pharmacy

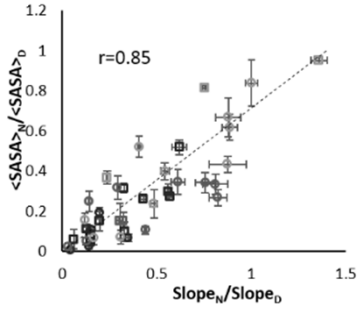
THE UNIVERSITY of MISSISSIPPI
DEPARTMENT OF BIOMOLECULAR SCIENCES



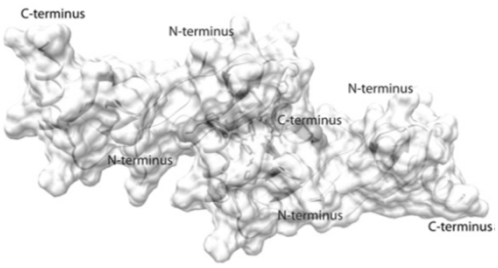




## Covalent Labeling

- **Stable** (usually) chemical modification of amino acid **side chains**
- **Topographical analysis**: Generally thought to report on changes in surface accessibility
- **Differential analysis**: usually compare a polypeptide sequence in two different structural states; report on relative differences

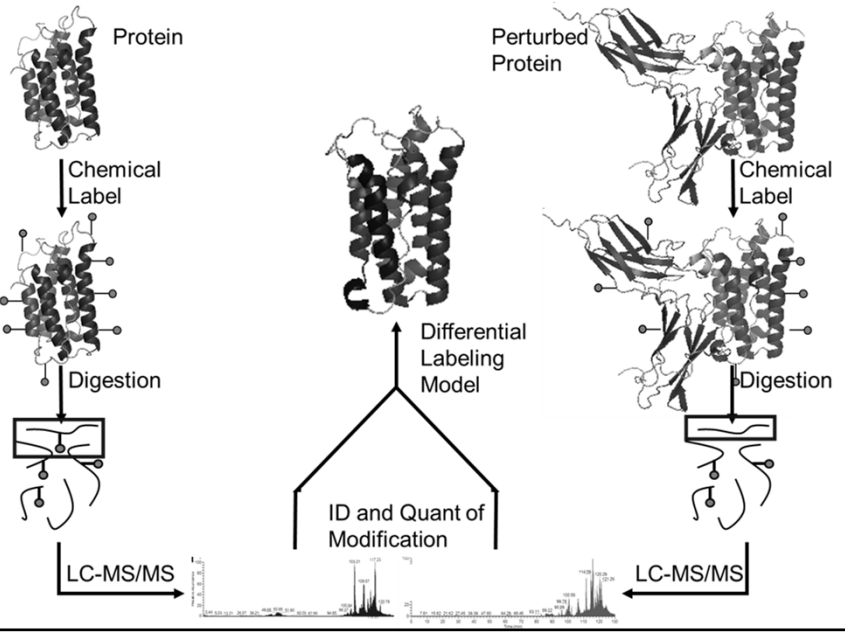



Xie *et al., Sci Rep* (2017) **in press**




Wang *et al., Structure* (2011) **19,** 1138-1148

## Experimental Overview

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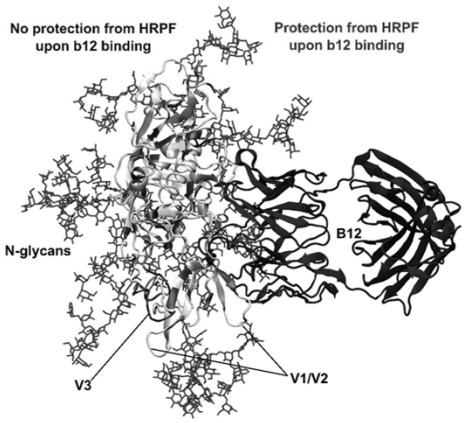
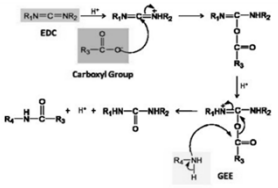
## Experimental Design

1. Chemical Probe Selection
   a) Selective
   b) Broad
2. Labeling
   a) Exposure control
      a) Labeling reagent concentration
      b) Labeling time
      c) Labeling temperature
   b) HOS maintenance
3. Protease Selection and Digestion
4. Quantification
   a) Peptide level
   b) Amino acid resolution
5. Data Interpretation



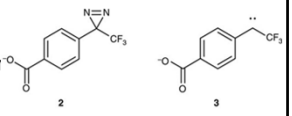
## Chemical Probe Selection

- **Selective Probes**: Target specific functional groups
  - Almost 50 year history in protein chemistry
  - Targeted analysis, best for testing specific hypotheses (e.g. primary amine labeling to identify charge-charge interactions in heparin binding, Ori *et al Mol Cell Proteomics* (2009) **8,** 2256-2265)
  - Relatively simple chemistry
  - Easily controlled reagent quantities and reaction times
  - Chemistry must be compatible with maintenance of HOS
  - New innovation in isotopic labeling, new reagents (e.g. Zhang *et al J Am Soc Mass Spectrom* (2016) **27,** 178-181)




Zhang *et al., Int J Mass Spectrom* (2012) **312,** 78-86

- **Broadly Reactive Probes**: Simultaneously labels multiple amino acid classes
  - Best for general topographical analysis
  - More complex chemistry, more complex analysis
  - Does **not** label all amino acids equally
  - Reactive group formed *in situ*; reagent quantities and reaction times more difficult to control
  - Very sensitive to buffer composition
  - New innovation in reagent activation methods, new reagents (e.g. carbene reagents: Manzi *et al* (2016) *Nat Commun* **7**, 13288)

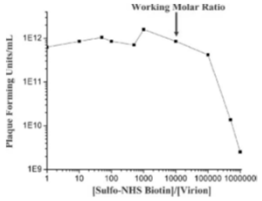



Manzi *et al., Nat Commun* (2016) **7,** 13288

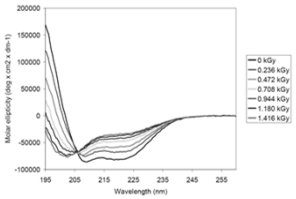
## Labeling: Maintaining HOS

- Major Issue: Covalent labeling alters the biophysical properties of amino acids
  - They **will** alter your protein HOS
  - Probing proteins with altered HOS results in artifactual results
- Three common methods for ensuring HOS is retained

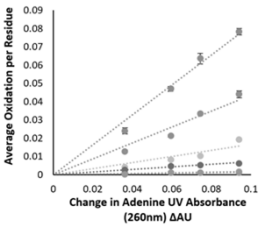
1. Functional assay 2. Biophysical assay 3.Kinetic analysis




Sharp *et al., Virology* (2006) **348,** 216-223



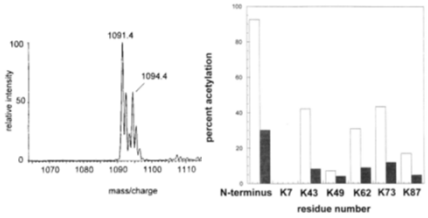

Sharp *et al., Biochemistry* (2006) **45,** 6260-6266




Xie *et al., Sci Rep* (2017) **in press**

## Labeling Quantification: Isotopic Labeling

- Problem: Several labeling reagents significantly alter the retention time and ionization efficiency of peptides and/or the digestion pattern of proteases, making relative quantification by ion intensity unreliable
- Selective probes are often saturatable; based on this, isotopic labeling is possible
- Protein is labeled in native form, then denatured and all reactive groups labeled with isotopomer
- Without isotopic labeling, protease must be carefully matched with reactive groups, ionization efficiency differences taken into account

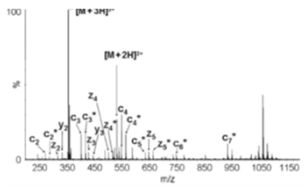



Chen, Smith and Griep, *Prot Sci* (1998) **7,** 1781-1788

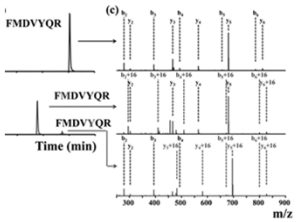
## Labeling Quantification: Amino Acid Resolution

- Problem: Quantitatively resolving how much labeling occurs on each amino acid in a peptide with multiple targets
- Mostly a problem with broad labeling reagents; usually few labeling site(s) per peptide of specific reagents
- Peptide modification isomers will usually separate (at least partially) by most peptide chromatography methods
- In ergodic MS/MS, modification site often alters fragmentation pathways
- Three basic approaches:

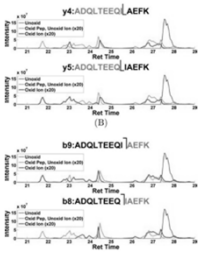
1. ECD/ETD
2. UHPLC Resolution
3. UHPLC/Product Ion Chrom.




Li *et al., J Am Soc Mass Spectrom* (2013) **24,** 1767-1776



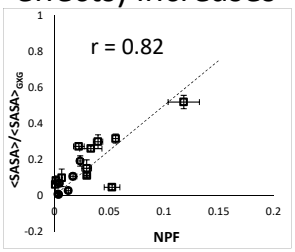

Zhang *et al., J Am Soc Mass Spectrom* (2017) **28,** 850-858



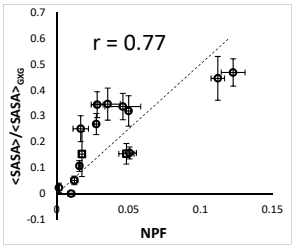

Kaur *et al., Mol cell Proteom* (2015) **14,** 1159-1168

## Data Interpretation: What Are We Measuring?

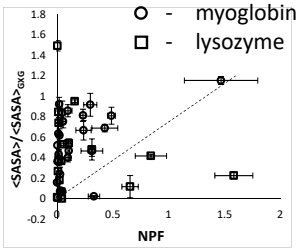
- Broadly Reactive Probes: Higher reaction rates, lower activation energies, less effects from "microenvironment"
- As reactivity of target decreases, effect of "microenvironment" (e.g. protein sequence inductive effects) increases




Highly Reactive
$k/k_p > 10$




Moderately Reactive
$4 < k/k_p < 10$




Poorly Reactive
$k/k_p < 4$

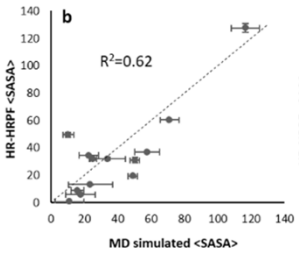
Xie *et al., Sci Rep* (2017) **in press**

$$\text{Normalized Protection Factor (NPF) } R_i = \frac{Slope\ R_i}{k_{R_i}/k_p}$$

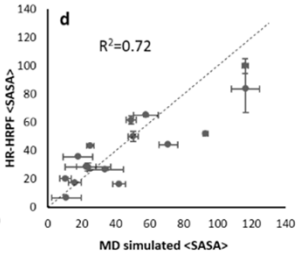
NPF adapted from Huang *et al, Biophys J* (2015) **108,** 107-115

## Broadly Reactive Probes

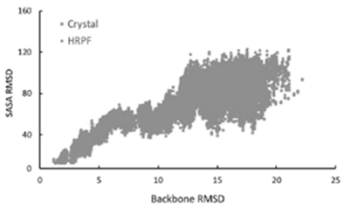
- If we solely probe highly reactive amino acids **<u>or</u>** if we correct for sequence context effects, we can directly measure <SASA> by rate of covalent modification
- Accuracy of HR-HRPF <SASA> measurements are comparable to X-ray crystallography structures when compared to <SASA> from MD trajectories




$k/k_p > 4$

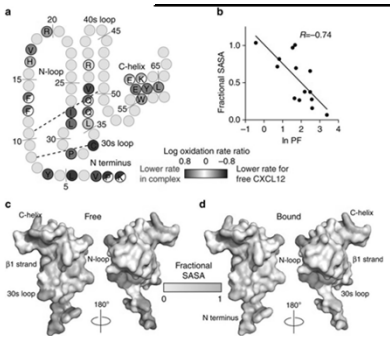



All probed amino acids, correcting for sequence effects




Xie *et al., Sci Rep* (2017) **in press**

## Quantitative Topography Measurements and Computational Modeling

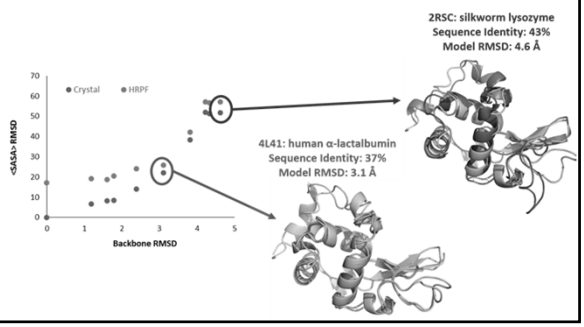



- Quantitative changes in <SASA> can help in understanding binding events

Gustavsson *et al., Nat Commun* (2017) **8,** 14135

- Absolute <SASA> measurements can be used to experimentally score models, differentiate high quality models from inaccurate models

Xie *et al., Sci Rep* (2017) **in press**




# Sharp Group

## Current Members

- Hao Liu
- Niloofar Khaje
- Dr. Charles Mobley
- Mohammad Riaz
- Dr. Quntao Liang
- Dr. Surendar Tadi
- Dr. Sandeep Misra
- Chelsea Suppinger
- Lindsey Miller
- Joseph Mason
- Delaney Mason
- Sydney Watson

## Alumni

- Dr. Boer Xie (St. Jude Children's Research Hospital)
- Dr. Zixuan Li (NYU School of Medicine)
- Dr. Yulun Chiu (MD Anderson Cancer Center)
- Dr. Xiaoyan Li (Massachusetts General Hospital)
- Dr. Rongrong Huang (Greenwood Genetic Center)
- Dr. Caroline Watson (CDC)
- Dr. Dandan Zhou (CCRC, University of Georgia)
- ViLinh Tran (Emory)
- Jessica Saladino

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- Prof. Geert-Jan Boons—GAG synthesis
- Prof. Kelley Moremen—Protein expression
- Prof. James Prestegard—NMR spectroscopy
- Prof. Rob Woods—MD simulations
- Prof. Lianchun Wang—GAG cell biology
- Prof. Jon Amster—Purified GAG sequencing

NIGMS

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P20 GM104932

NSF

CHE 1608685

### Financial Conflict of Interest Disclosure

- J.S.S. has a substantial ownership interest in GenNext Technologies, Inc., a small company designing and developing standardized and automated technologies for hydroxyl radical protein footprinting for the biopharmaceutical industry

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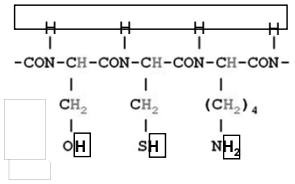
# **HDX,** Covalent Labeling & Crosslinking Interest Group

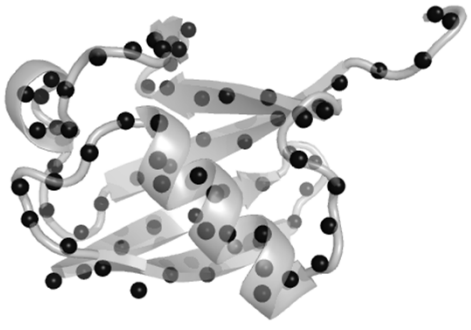
ASMS 2017

Mike Guttman
University of Washington, Seattle

# HDX-MS

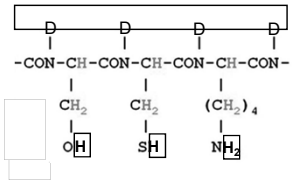
$H_2O \longrightarrow D_2O$

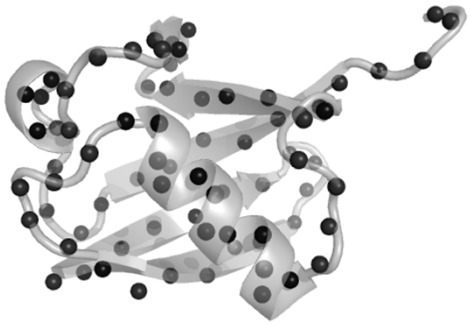


# HDX-MS

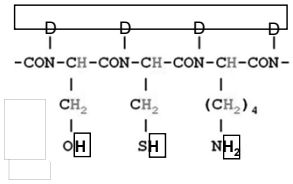
$H_2O \longrightarrow D_2O$

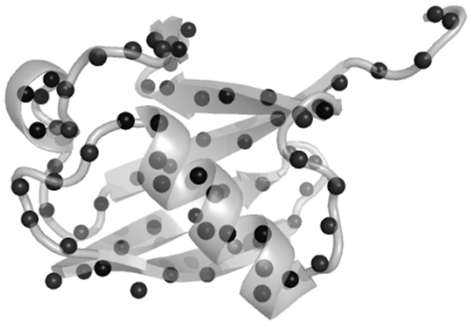


# HDX-MS

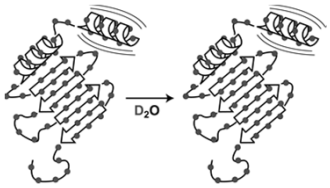
$H_2O \longrightarrow D_2O$

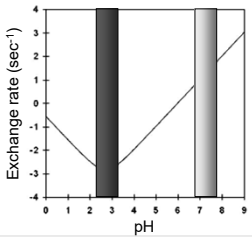


# 1. Label and Lock

- Initiate exchange
  - pH ~7, 25°C, native
- Halt exchange (quench)
  - pH 2.5, 0°C, denatured
- Varies with pH & temperature
  - 2 fold change with 0.3 pH units or 7°C
- Minimize back-exchange
  - $t_{1/2}$ ~ 30 min
  - Fast & reproducible

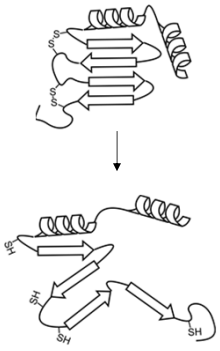


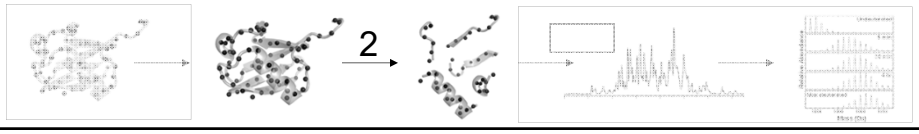





# 2. Denature & Reduce

- 8M Urea or 2M Guanidine
  - (careful with proteases)

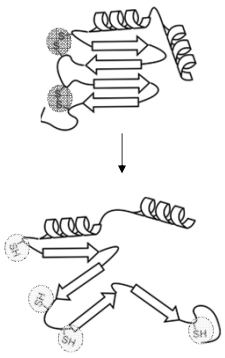


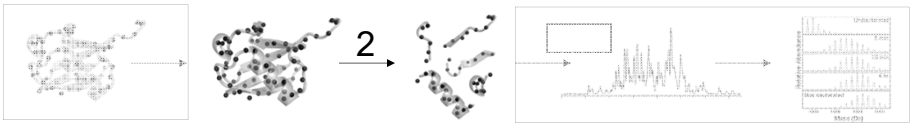



# 2. Denature & Reduce

- 8M Urea or 2M Guanidine
  - (careful with proteases)
- Disulfide bonds
  - TCEP
  - Online electrochemical reduction
    - Commercially available

Mysling S., (2014) *Anal Chem*



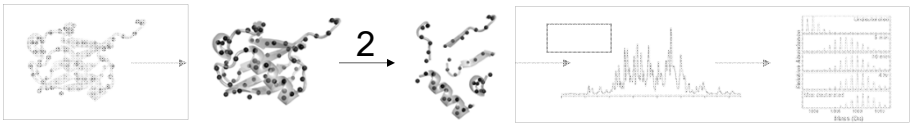




# 2. Digestion

- Limited proteases (pH 2.5)

| protease | organism | availability |
|---|---|---|
| Pepsin | *Sus scrofa* | ✓ |
| Aspergillopepsin | *Aspergillus Saitoi* | ✓ |
| Rhizopepsin | *Rhizopus sp.* | ✓ |
| Nep2 | *Nepenthes gracilis* | ✗ |

- High pressure (12 kpsi)



# 2. Digestion

- Limited proteases (pH 2.5)

| protease | organism | availability |
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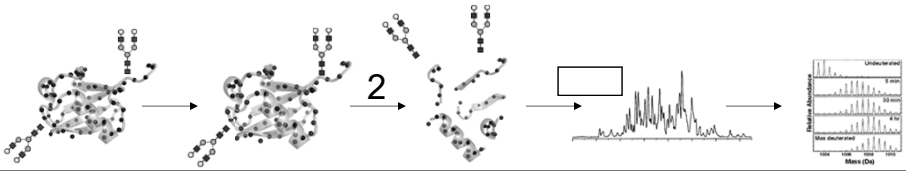
| Pepsin | *Sus scrofa* | ✓ |
| Aspergillopepsin | *Aspergillus Saitoi* | ✓ |
| Rhizopepsin | *Rhizopus sp.* | ✓ |
| Nep2 | *Nepenthes gracilis* | ✗ |

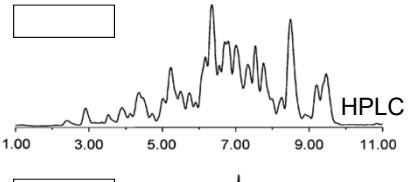
- High pressure (12 kpsi)
- Deglycosylate glycoproteins
  - PNGaseA Jensen PF et al,. (2016) *Anal Chem*



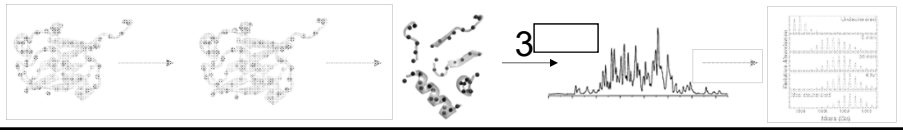

# 3. Resolve peptides (LC)

- UPLC columns
  - Shorter run times
  - Higher resolution
  - Peak capacity
- Ultra low temperatures
- Alternatives – CE-MS

Black WA., (2015) *Anal Chem*

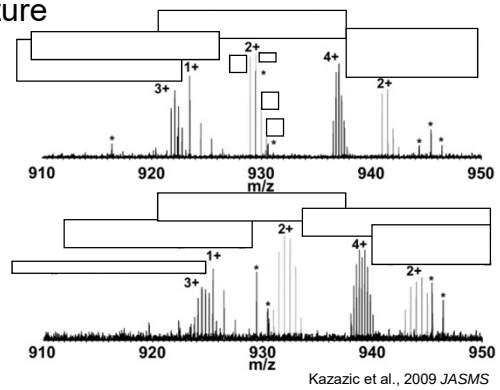



Wales et al., 2008 *Anal Chem*

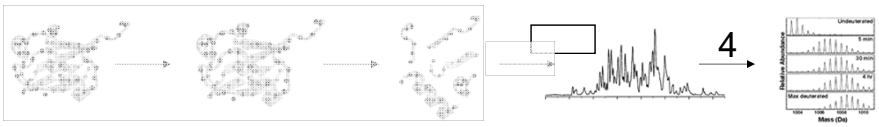


# 4. MS analysis

- Lower source temperature
- High resolution helps

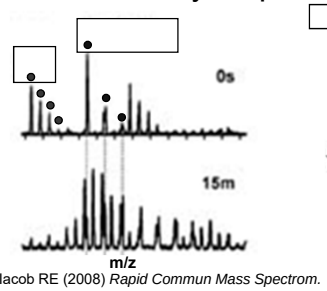


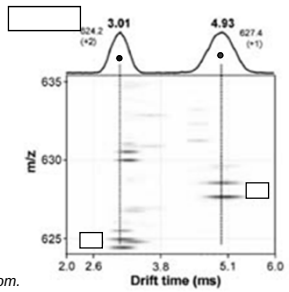

Kazazic et al., 2009 *JASMS*

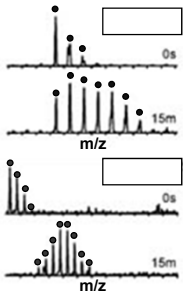



# 4. MS analysis

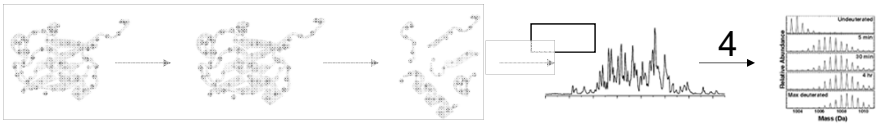
- Lower source temperature
- High resolution helps
- Ion mobility helps



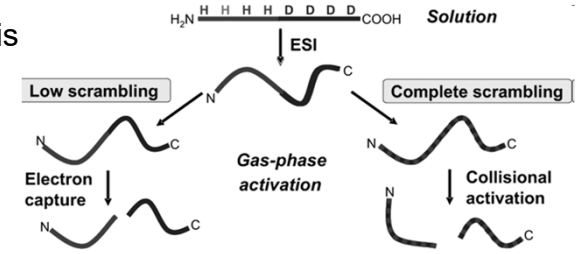




Iacob RE (2008) *Rapid Commun Mass Spectrom.*

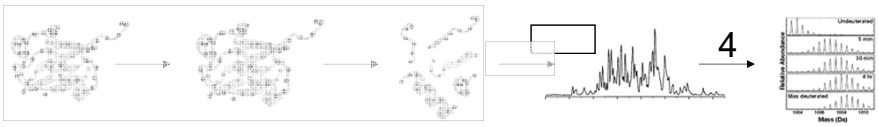


# 4. MS analysis

- Lower source temperature
- High resolution helps
- Ion mobility helps
- Site-specific analysis
  - ETD
  - Top-down

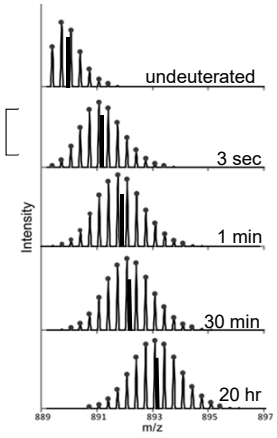


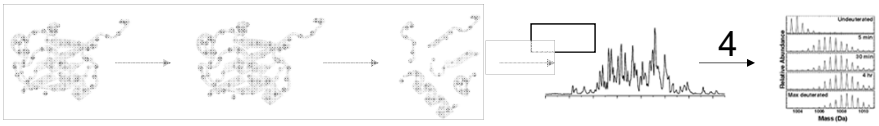

Rand et al. *J. Am. Chem. Soc.*, 2008




# 4. Data processing

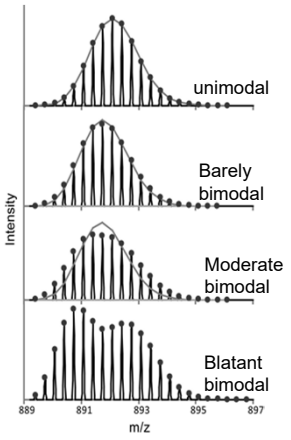
- Automated peptide ID and deuterium integration
- Many software options
- Simple centroid is most common

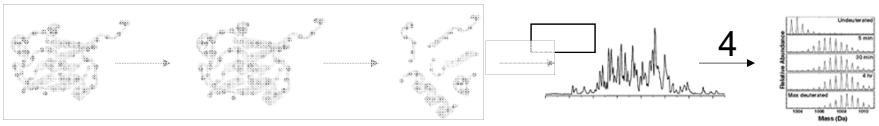


# 4. Data processing

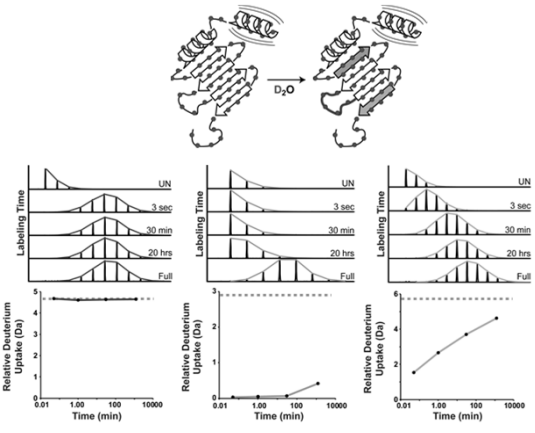
- Automated peptide ID and deuterium integration
- Many software options
- Simple centroid is most common
- Often insufficient
  Zhang J., (2013) *JASMS*
- Alternative approaches
  Chik et al, (2006) *Anal Chem*
  Guttman M. et al., (2013) *JASMS*
  Kan ZY., et al (2013) *PNAS*
- Bimodal deconvolution



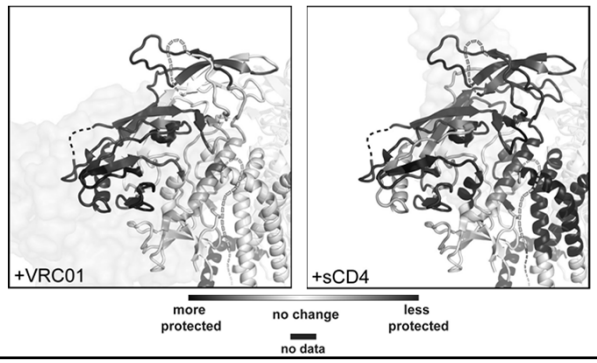

# What can we learn?

- Exchange governed by structure and dynamics
- Which regions are well folded?
- Deuterated control
  - Denaturation & exhaustive deuteration
  - Deuteration after proteolysis




# Interface mapping by HDX

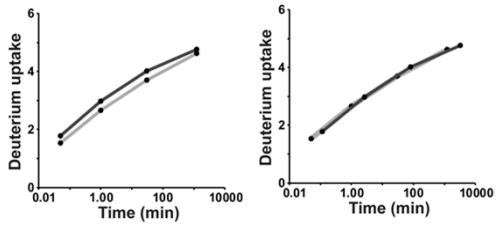
| Can | Can't |
|---|---|
| • Localize regions at the interface | – 3D structural information |
| • All allosteric effects | – Single residue resolution |
| | – Contact residues |

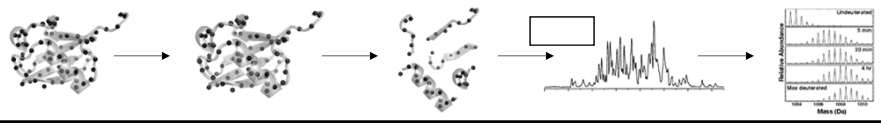



# Remaining challenges

- Can't quantitatively compare HDX data collected at different times
  - Exchange conditions
  - Back exchange variation
  - Internal exchange reporter (PPPI)

Zhang Z., 2012 *Anal Chem.*



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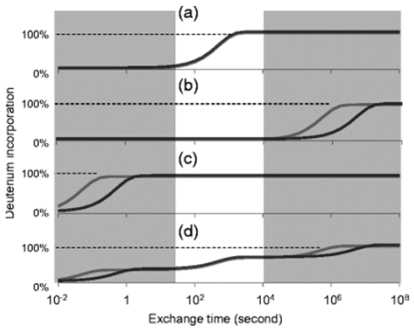


Hamuro Y., 2017 *JASMS*

# Remaining challenges

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  - Exchange conditions
  - Back exchange variation
  - Internal exchange reporter (PPPI)
    Zhang Z., 2012 *Anal Chem.*
- Limited temporal sampling
  - 10 sec to 10 hours may miss many relevant kinetic regions
- Get the most from your data
  - Look beyond the centroid