Profiling protein-protein interactions *in vivo* by cross-linking and mass spectrometry

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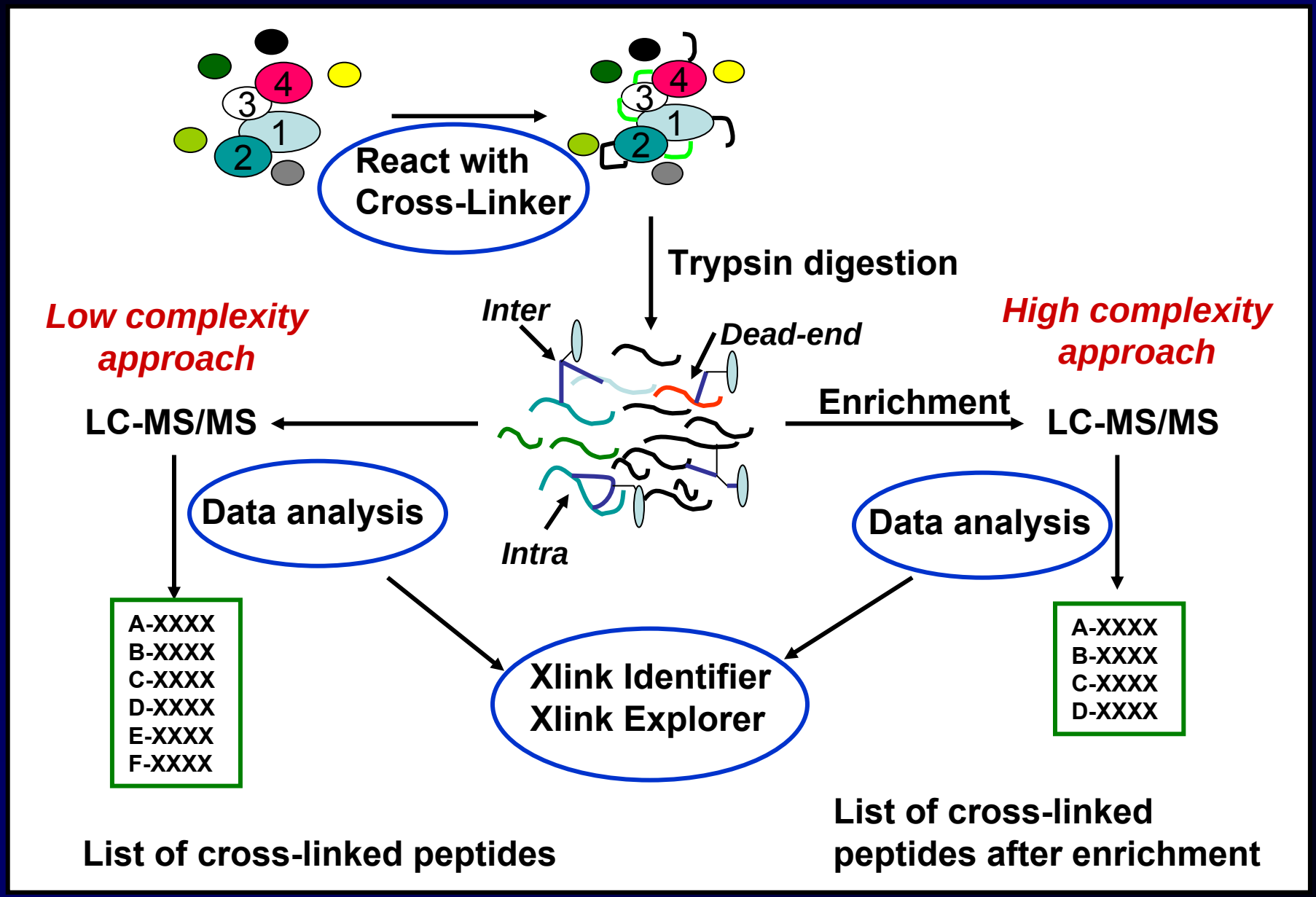
Part 1 – Global

Novel chemical cross-linking strategy for global identification of protein-protein interactions

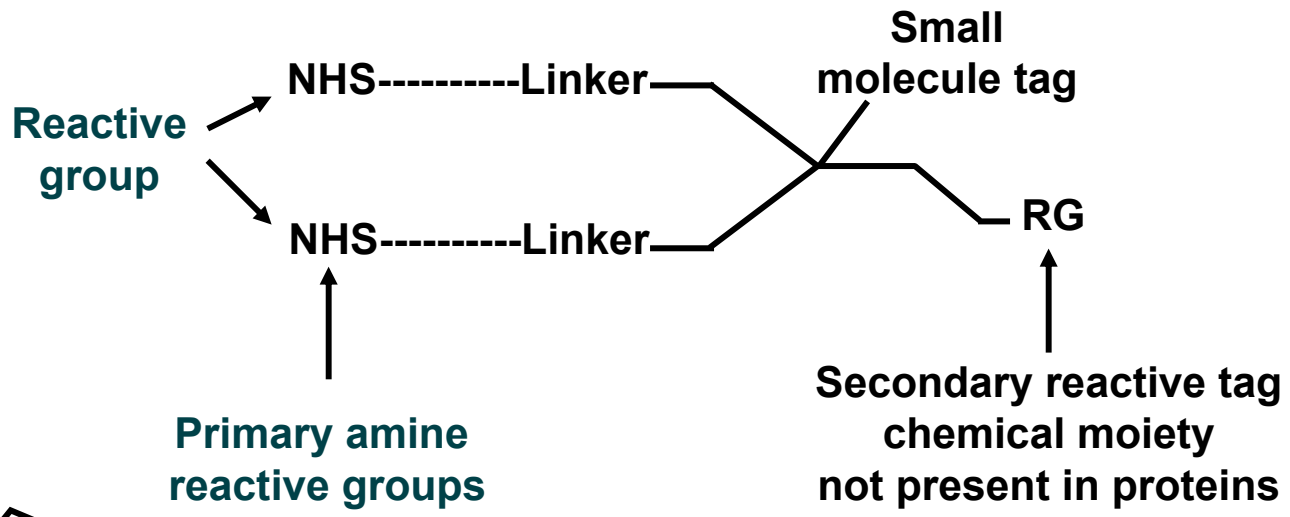
Part 2 – Targeted

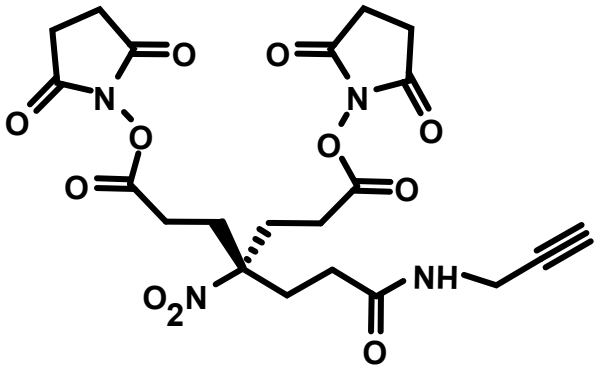
Method for investigating protein-protein interactions related to *Salmonella typhimurium* pathogenesis

Global cross-linking strategy



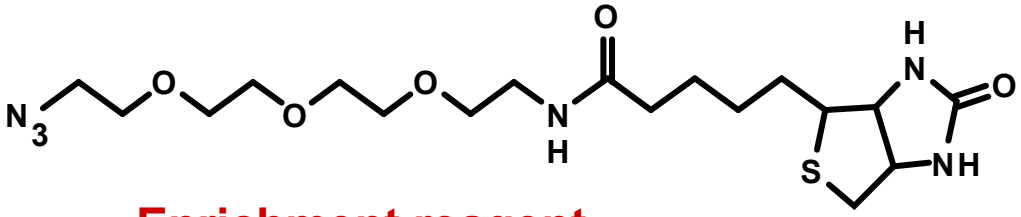
Cross-linker design: enrichment-based strategy





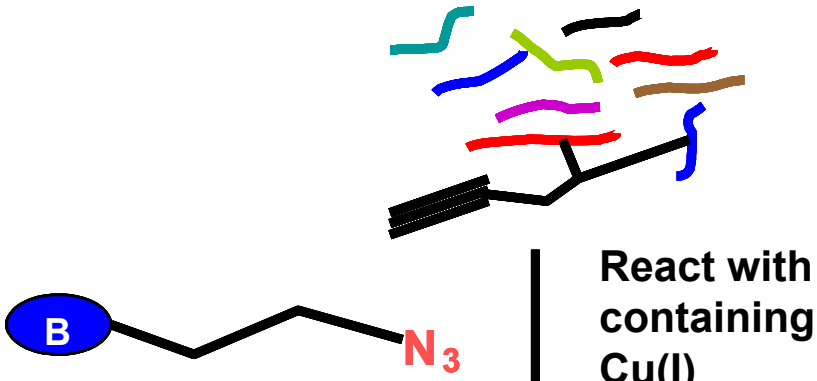
Cross-linker spacer chain 9-10Å

Small cross-linker for cell permeability

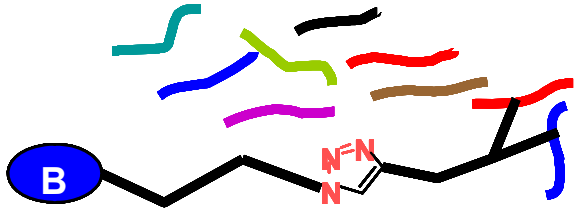


Enrichment reagent

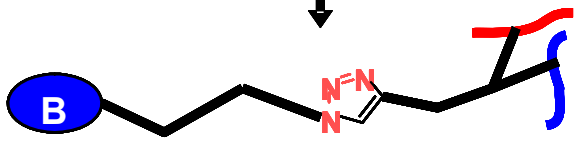
Enrichment strategy using CLICK chemistry



React with azido compound containing biotin support
Cu(I)



- Capture with avidin beads
- Wash to remove non-binders
- Elute

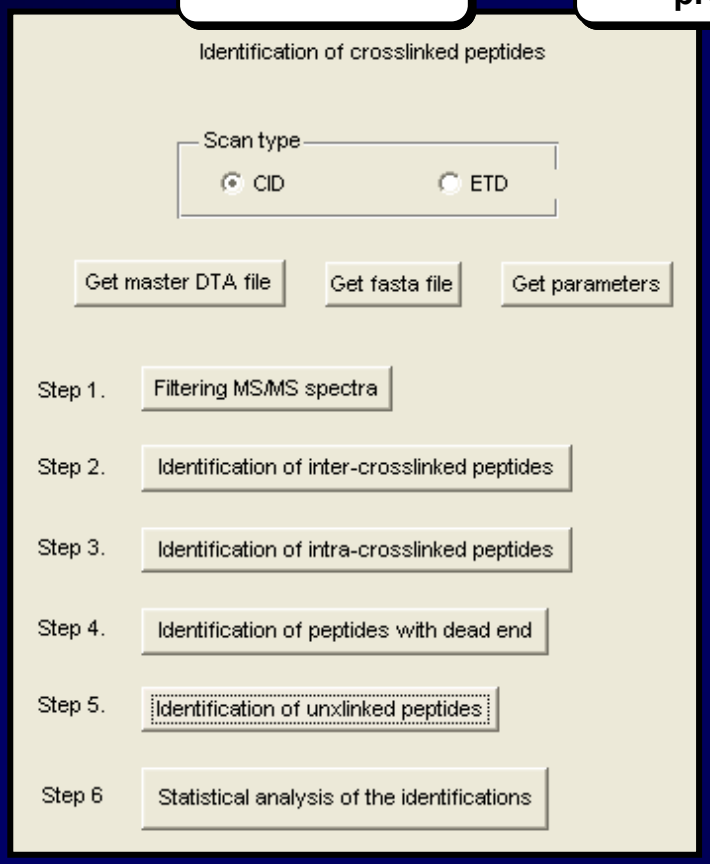


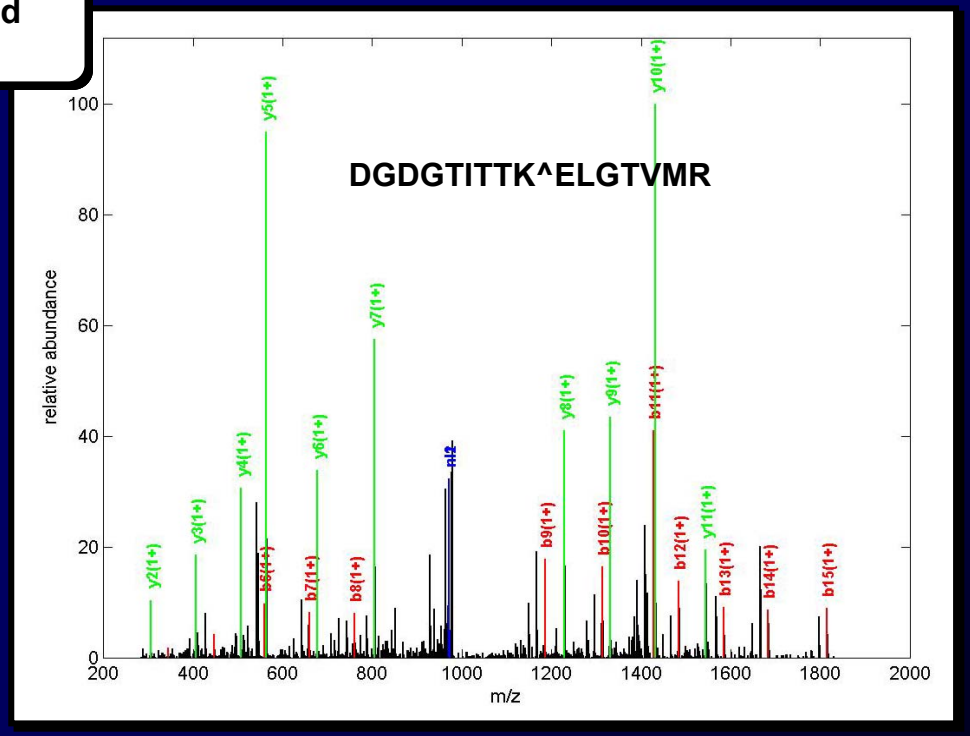
LC-MS/MS

Challenge:

Automated identification of cross-linked peptides

Data pre-processing → Spectrum denoising → Spectrum normalization → Unique scoring → Spectrum annotation and visualization → FDR estimation explicitly → Inference of cross-linked proteins

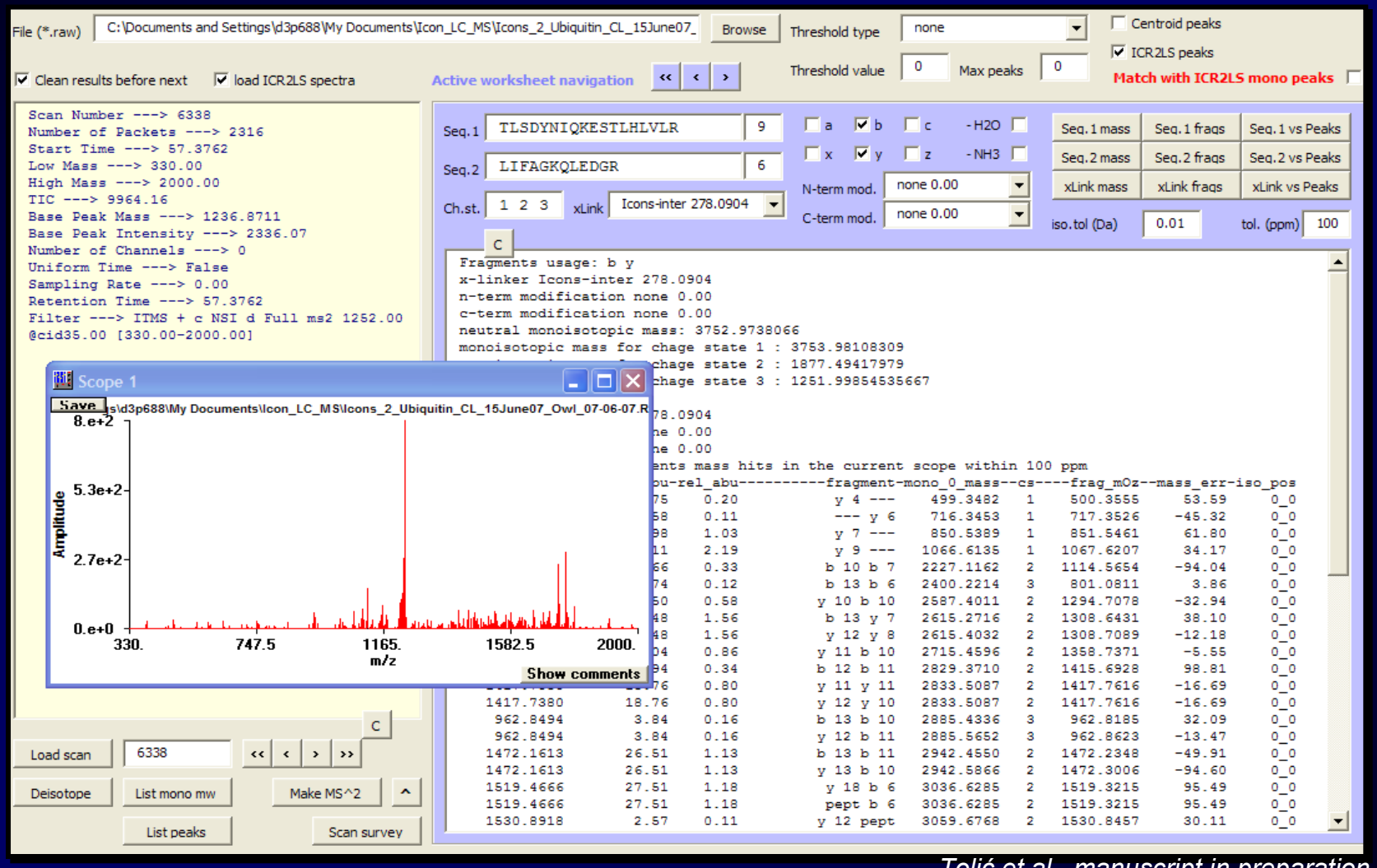




Xlink-Identifier aids automated data analysis

Du et al., manuscript in preparation

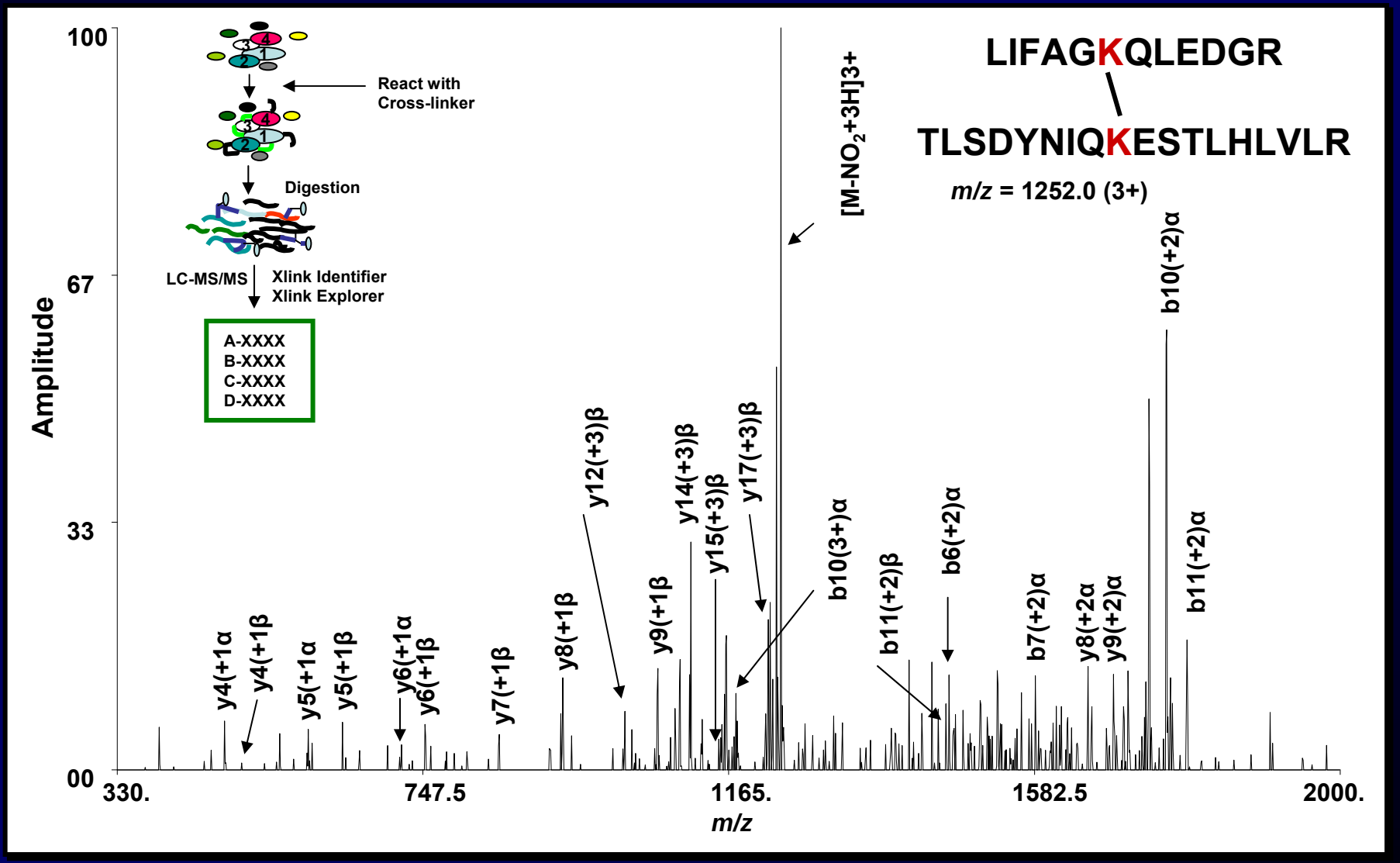
Challenge:
Manual validation of cross-linked species



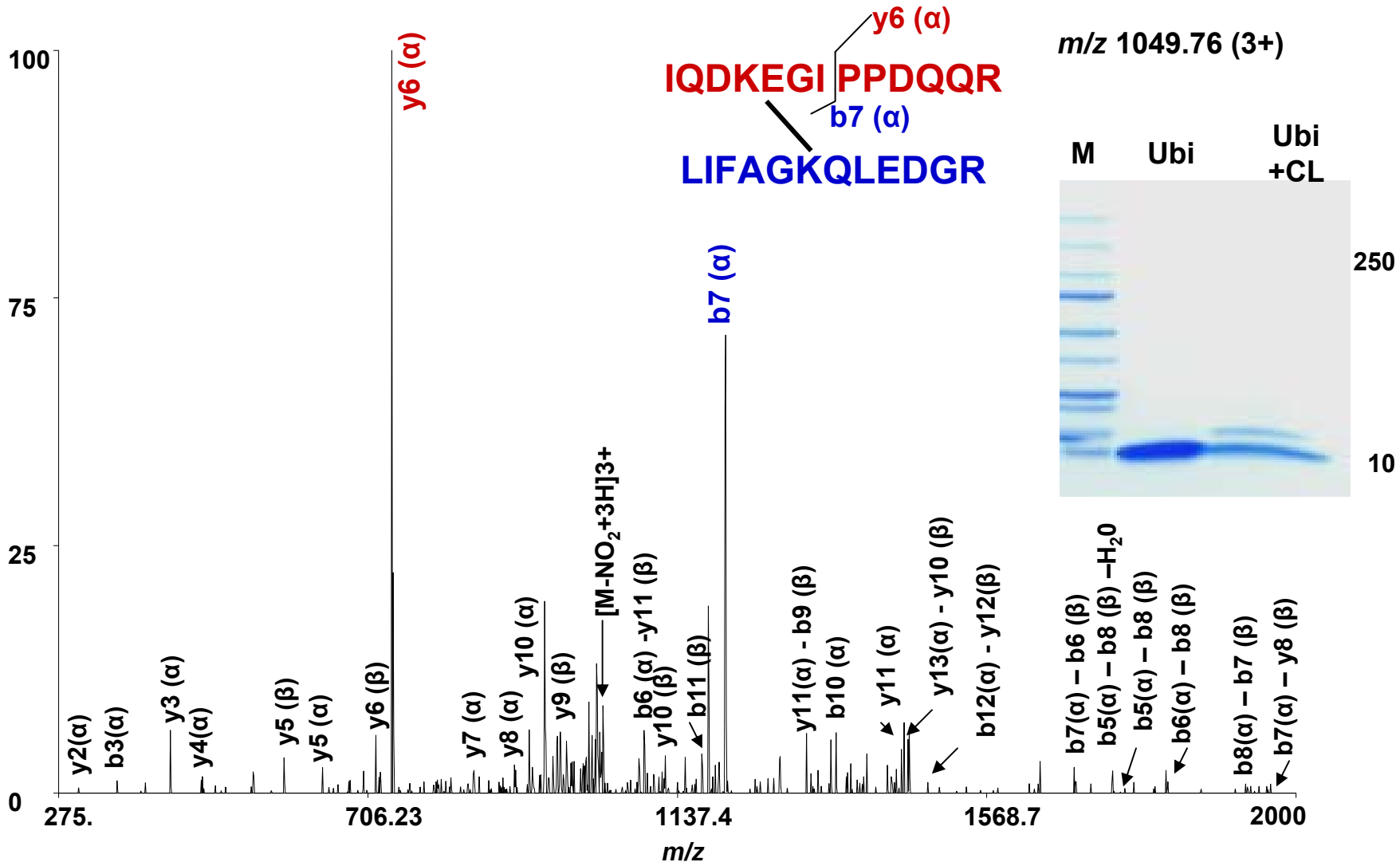
Tolić et al., manuscript in preparation

Xlink-Explorer aids manual validation

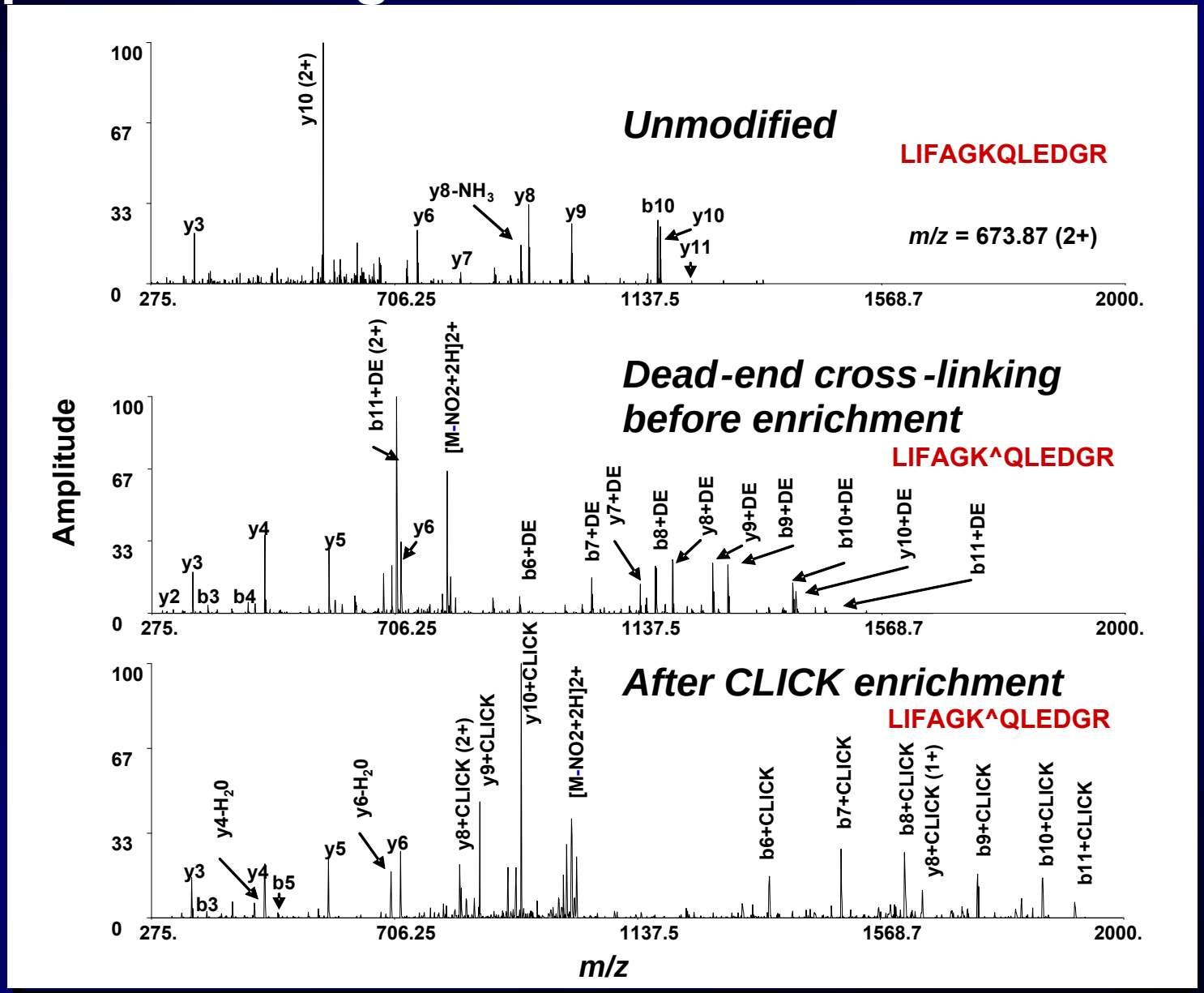
Initial application:
Low complexity approach using ubiquitin in solution digestion



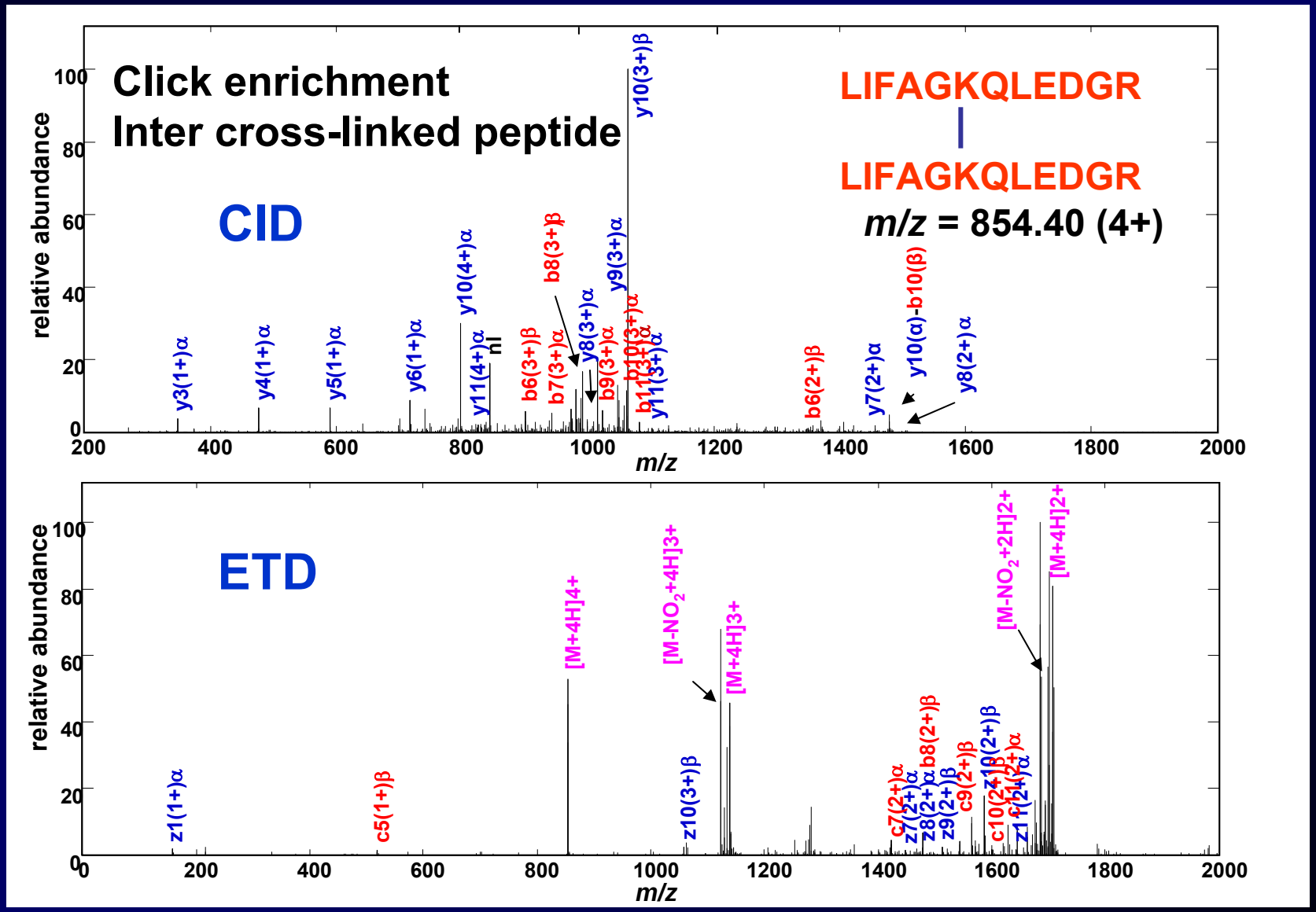
Low complexity approach using ubiquitin in gel digestion



Cross-linker and enrichment agent maintain typical CID fragmentation

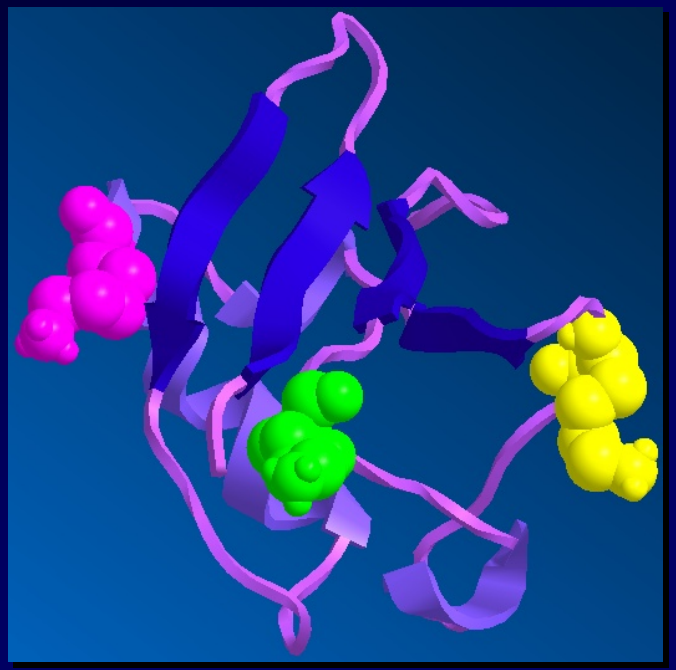


Automated identification of enriched inter cross-linked peptides by *Xlink-Identifier*



Cross-linking results consistent with previously published crystal structures

MQIFVKTLTG KTITLEVEPS DTIENVKAKIQDKEGIPPDQ
QRLIFAGK[48]QLEDGRTLSDYNIQKESTLHLV LRLRGG



Inter cross-linked peptides

LIFAGK^QLEDGR--- TLSDYNIQK^ESTLHLVLR

LIFAGK^QLEDGR--- LIFAGK^QLEDGR

LIFAGK^QLEDGR--- IQDKEGIPPDQQR

Intra cross-linked peptide

AK^IQDK^EGIPPDQQR

Dead-end

TLSDYNIQK^ESTLHLVLR
LIFAGK^QLEDGR
IQDKEGIPPDQQR

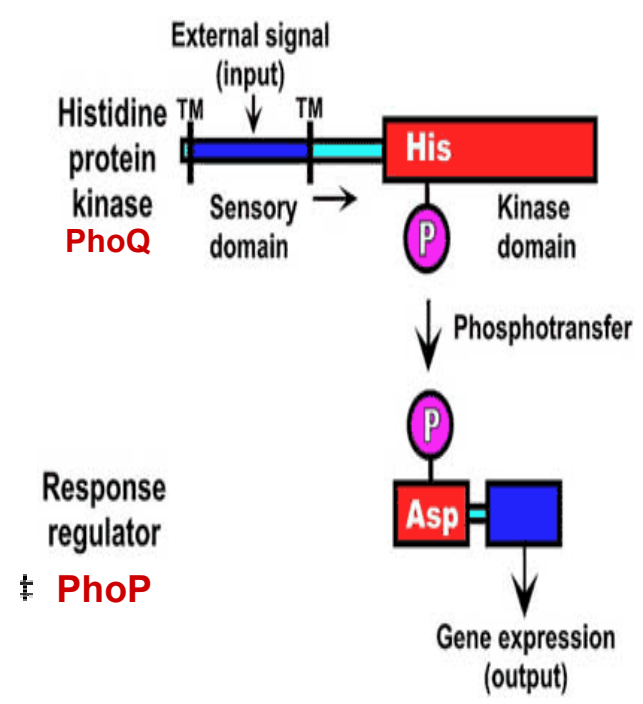
Summary – Part 1
Initial characterization of enrichment-based cross-linking strategy

- Small molecule cross-linker with optional enrichment capability
- Software developed to aid analysis
- Fragmentation spectra remained interpretable for cross-linked and enriched peptides
- Studies with higher complexity protein samples currently in progress

Part 2: *Salmonella* virulence study to discover interacting partners

Targeted 3 virulence-associated proteins with known binding partners

- STM 0982 (HimD) is known to stably interact with STM 1339 (HimA)
- STM 1231 (PhoP) is known to transiently interact with STM 1230 (PhoQ)
- PduB (STM 2039) is expected to interact with other members of the Pdu operon



Example of known interacting system
(www.mcgill.ca/microimm/lemoual)

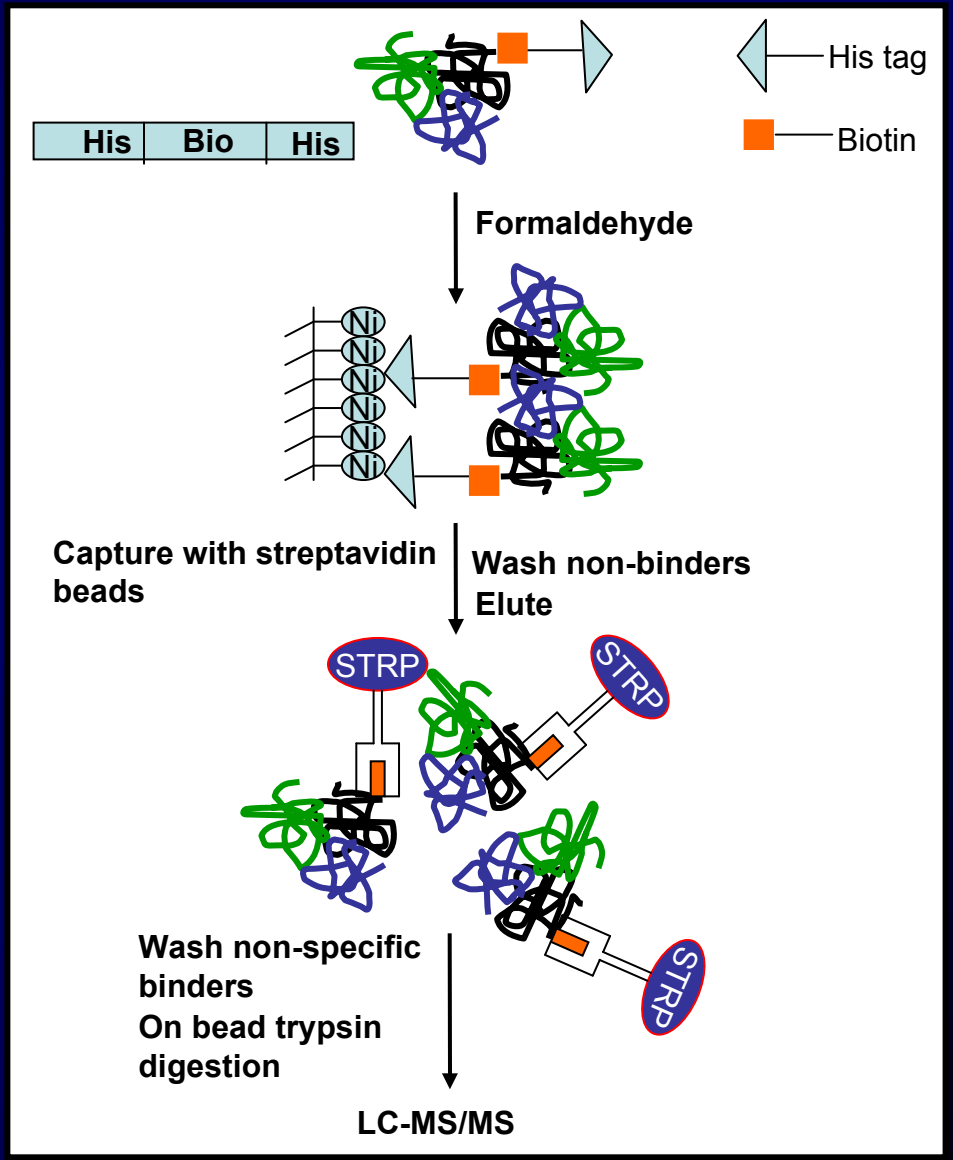
Pull down approach with HBH tag and formaldehyde cross-linking

Advantages

- Cell permeable and nonspecific
- Inactivate the enzyme immediately
- *In vivo* and in functional context
- Strong denaturing conditions can be used

Disadvantages

- Close proximity (~2Å)
- Non-specific modification in proteins



Tagwerker ,et al., *Mol. Cell. Proteomics* 2006, 5:737-748.

Modified HBH-cross-linking strategy

Bead control

STM cells

↓ 1% Formaldehyde
Lyse

Proteins

↓ Affinity Purification
Wash and Digest

Peptides

↓ LC-MS/MS

Bead interacting

Bait control

STM cells (HBH-bait)

↓ **0% Formaldehyde**
Lyse

Proteins

↓ Affinity Purification
Wash and Digest

Peptides

↓ LC-MS/MS

Bait
+ bead interacting
+ non-specific binding

Cross-link

STM cells (HBH-bait)

↓ 1% Formaldehyde
Lyse

Proteins

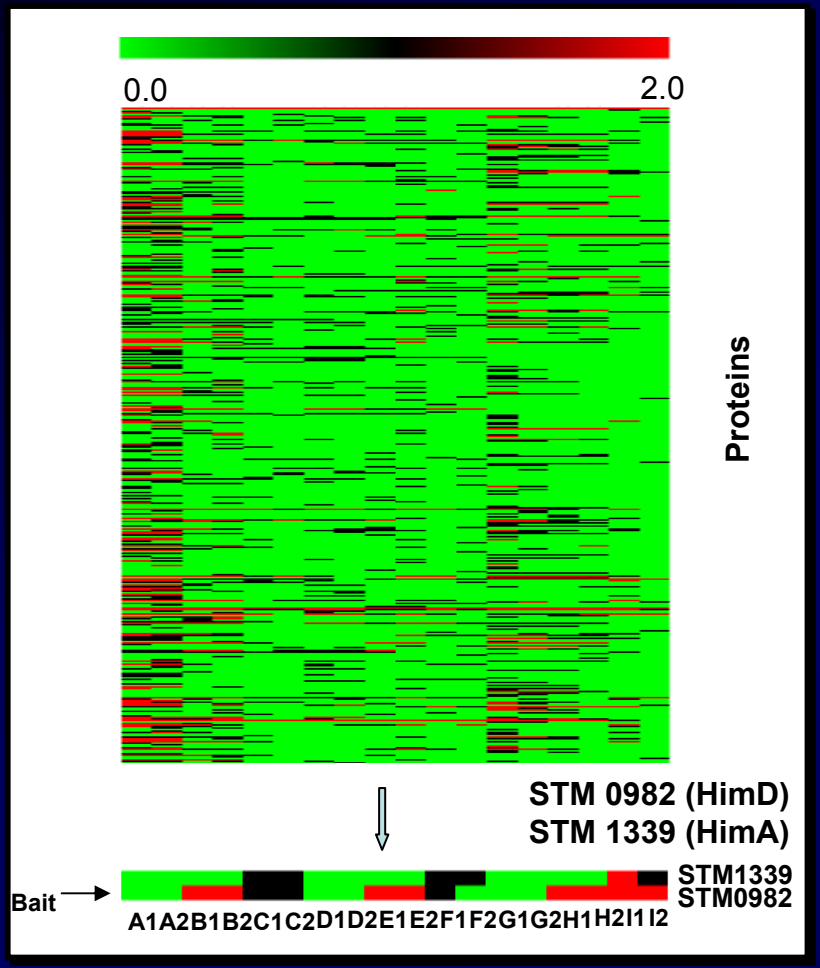
↓ Affinity Purification
Wash and Digest

Peptides

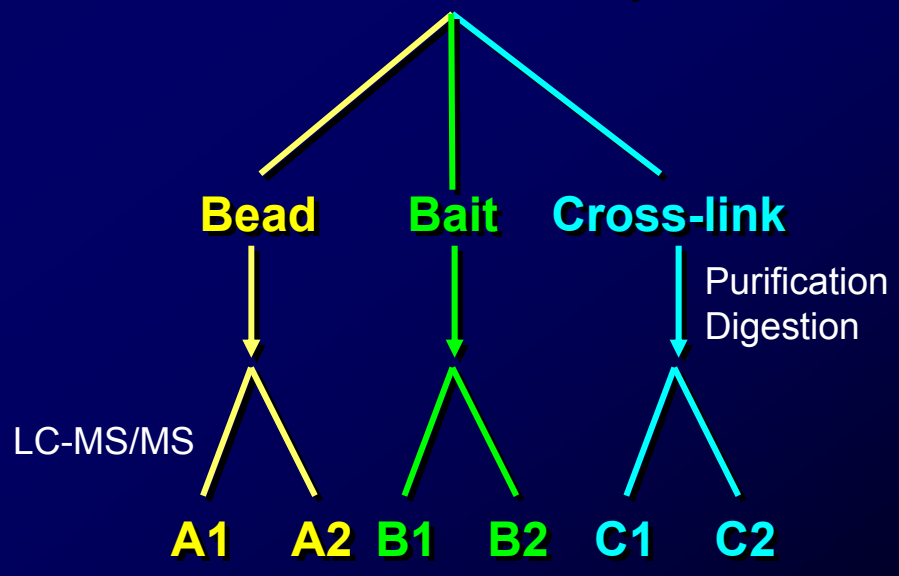
↓ LC-MS/MS

Bait
+ bead interacting
+ non-specific binding
+ specific binding

Reproducibility study with HimD-HBH



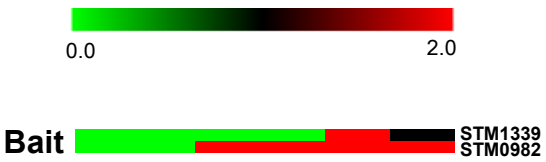
- **3 independent biological experiments:**
  - one using 0.5% formaldehyde
  - two using 1% formaldehyde
- **Two controls for each experiment:**



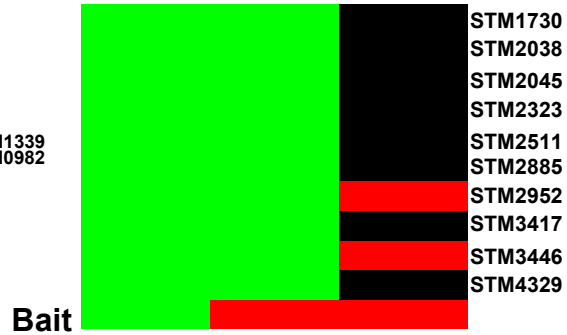
Results highly reproducible and consistent with known stable interactions

Lists of identified interactions for 3 virulence-related proteins

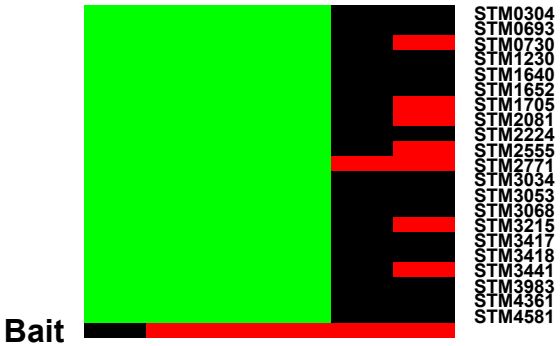
HimD (STM0982)



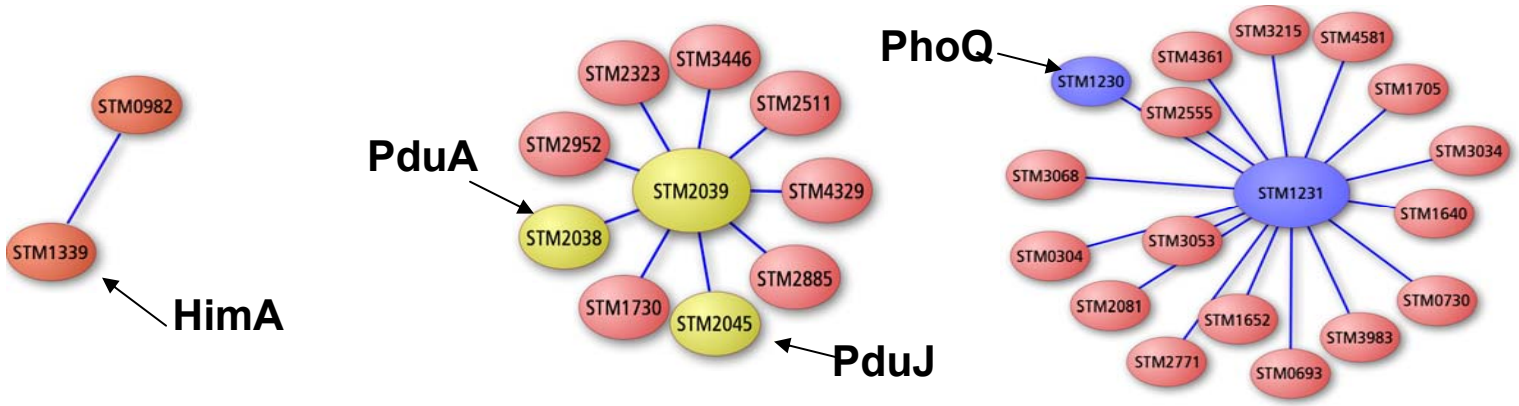
PduB (STM2039)



PhoP (STM 1231)



Interaction diagrams with known or expected interactors labeled



Summary – Part 2

Tandem affinity tag and cross-linking applied to identify interactions related to *Salmonella* pathogenesis

- A modified HBH tag and formaldehyde cross-linking strategy used to differentiate background, non-specific, and specific interactions
- *In vivo* application to three *Salmonella* virulence proteins identified known and novel protein-protein interactions
- Currently, a few interactions are being validated with other methods

Acknowledgements

Richard D. Smith
Joshua N. Adkins
Fred Heffron (OHSU)
Liang Shi
M. Uljana Mayer
Xiuxia Du
Nikola Tolić
Gordon A. Anderson
Ronald J. Moore
Therese Clauss
Hyunjin Yoon (OHSU)
Leah M. Rommereim (Dartmouth)
Angela D. Norbeck
Mathew E. Monroe
Si Wu
Karl K. Weitz

Funding:

National Institute of Allergy and Infectious disease (NIAID/NIH)
National Center for Research Resources (NCCR/NIH)
Office of Biological and Environmental Research (BER/DOE)
LDRD/PNNL

Environmental and Molecular Science Laboratory, a U.S. DOE national scientific user facility at Pacific Northwest National Laboratory