Crosslink Data Analysis in Ten Easy Steps

1. Group Related Experiments
2. Remove Unlinked Peptides
3. Identify Xlinks with Multiple Observations
4. Note Source Experiment of Peptide
5. Sequester Xlinks with Short Peptides
6. Verify MS/MS
7. Quality MS1 Isolation
8. Validate XIC
9. Compare with Unlinked or in silico peptides
10. Record Validation

Xlinks Used to Define Structural Constraints

1. Docking of crystal structure
2. Opening of ionic lock region
3. Rotation of RH bundle

Rapid Classification
MS/MS Alone Looks Reasonable…

BUT

Unreliable Chromatography Co-isolated Precursors

Discussion and Conclusions

Using this workflow, we can readily analyze thousands of possible crosslinked peptides across diverse experiments. The goal is to identify the best crosslinks first as anchor structural constraints taking advantage of various linker types and proteolytic enzymes. This Byologic strategy can produce a robust candidate report in hours instead of days to weeks, and efficiently informs structural modeling efforts and biochemical experiments.

Here, we identified the binding interface of b2AR-GRK5 using this method. Our crosslink predictions observed an ionic lock region opening and a rotation of the RH bundle. These experimental observations were further supported by complementary HDX, EM, and mutant crosslink analyses.

This approach is scalable to other protein-protein interactions, including determination of complex stoichiometry and polymerization.

Acknowledgment & Reference

This work was supported by NIH grant GM100634.

Ryan D. Leib1; Chris Becker1; Yong KIF; Pierre Allemand1; St John Skillton2; Eric Carlson3; Christopher M. Adams2; Alis Chien1
1Stanford University Mass Spectrometry, Stanford, CA 2Protein Metrics Inc.
Contact: info@proteinmetrics.com

Introduction

New mass spectrometry methods to investigate protein-protein complexes, binding interfaces, and biopolymer oligomerization show tremendous promise as structural biology tools. In practice, improved data analysis tools and methods are needed to validate the rich but incredibly complex MS/MS fragmentation data. Here, we present a novel analysis approach for chemical crosslinking using features of Byonic and Byologic with an empirical grading system to quickly and robustly characterize a model G protein-coupled receptor, (2 Adrenergic Receptor (2AR) with its kinase (GRK5)). While the structure of the individual proteins are determined, the molecular architecture of the docked interaction is poorly understood, and has important implications for a host of signaling pathways and protein recruitment mechanisms.

Methods

A parallel strategy using multiple crosslinkers, proteolytic enzymes, and fragmentation methods (ETD, HCD) was used to extensively characterize in vitro crosslinked 2AR-GRK5. Raw data were searched against a focused database in Byonic to identify thousands of candidate crosslinked peptides for review.

Byologic was used to condense these thousand possible experiments and facilitate rapid cross-link validation using an empirical rule strategy described here. This approach not only provides significant time savings over individual review of the independent output files, it provides a more robust cross comparison between related experiments, and a robust permanent report generation for easy information transfer between researchers.

Discussion and Conclusions

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