Cross-linking Mass Spectrometry

Lan Huang and Clinton Yu
University of California, Irvine

Jim Bruce
University of Washington, Seattle

Protein Samples $\rightarrow$ Cross-linking $\rightarrow$ Digestion $\rightarrow$ Peptide Separation

Interaction and Structural Analysis $\rightarrow$ XL Identification $\rightarrow$ MS Analysis
Protein Cross-linking

**XL Reagents**
- **Spacer arm**
  - Reactive group 1
  - Reactive group 1
- **Cleavable spacer arm**
  - Reactive group 1
  - Reactive group 1
- **Enrichable handle**
  - Reactive group 1
  - Reactive group 1
- **Cleavable spacer arm**
  - Reactive group 1
  - Reactive group 2

**Typical XL Products**
- **Type 0**
  - ‘dead-end’
  - N → C
- **Type 1**
  - ‘intra-link’
  - N → C
- **Type 2**
  - ‘inter-link’
  - N → C

- Heterogeneity
- Low abundance
- Complex fragmentation
Common Strategies for MS Analysis of Cross-linked Peptides

1. **MS/MS, non-cleavable cross-linkers (e.g. DSS)**

   - MS\(^1\), intensity vs m/z
   - Inter-link α-β
   - HCD, intensity vs m/z
   - - α\(_{-}\)HCD - β\(_{-}\)HCD

2. **MS\(^n\), MS-cleavable cross-linkers (e.g. DSSO)**

   - MS\(^1\), intensity vs m/z
   - Inter-link α-β
   - CID, intensity vs m/z
   - α\(_{A}\) β\(_{T}\)

3. **MS/MS, MS-cleavable cross-linkers (e.g. DSSO)**

   - MS\(^1\), intensity vs m/z
   - Inter-link α-β
   - HCD, intensity vs m/z
   - - α\(_{-}\)HCD - β\(_{-}\)HCD

   - MS\(^2\), intensity vs m/z
   - α\(_{A}\) α\(_{T}\) β\(_{A}\) β\(_{T}\)

   - MS\(^3\), intensity vs m/z
   - α\(_A\)
   - β\(_T\)

   - MS\(^3\), intensity vs m/z
   - - β\(_{-}\)CID
Quantitative XL-MS: Advancements and Applications

- SILAC-based interactome quantitation

- Isotopic-labeled cross-linker quantitation

- Isobaric XL-MS interactome quantitation

Quantitative interactome measurements