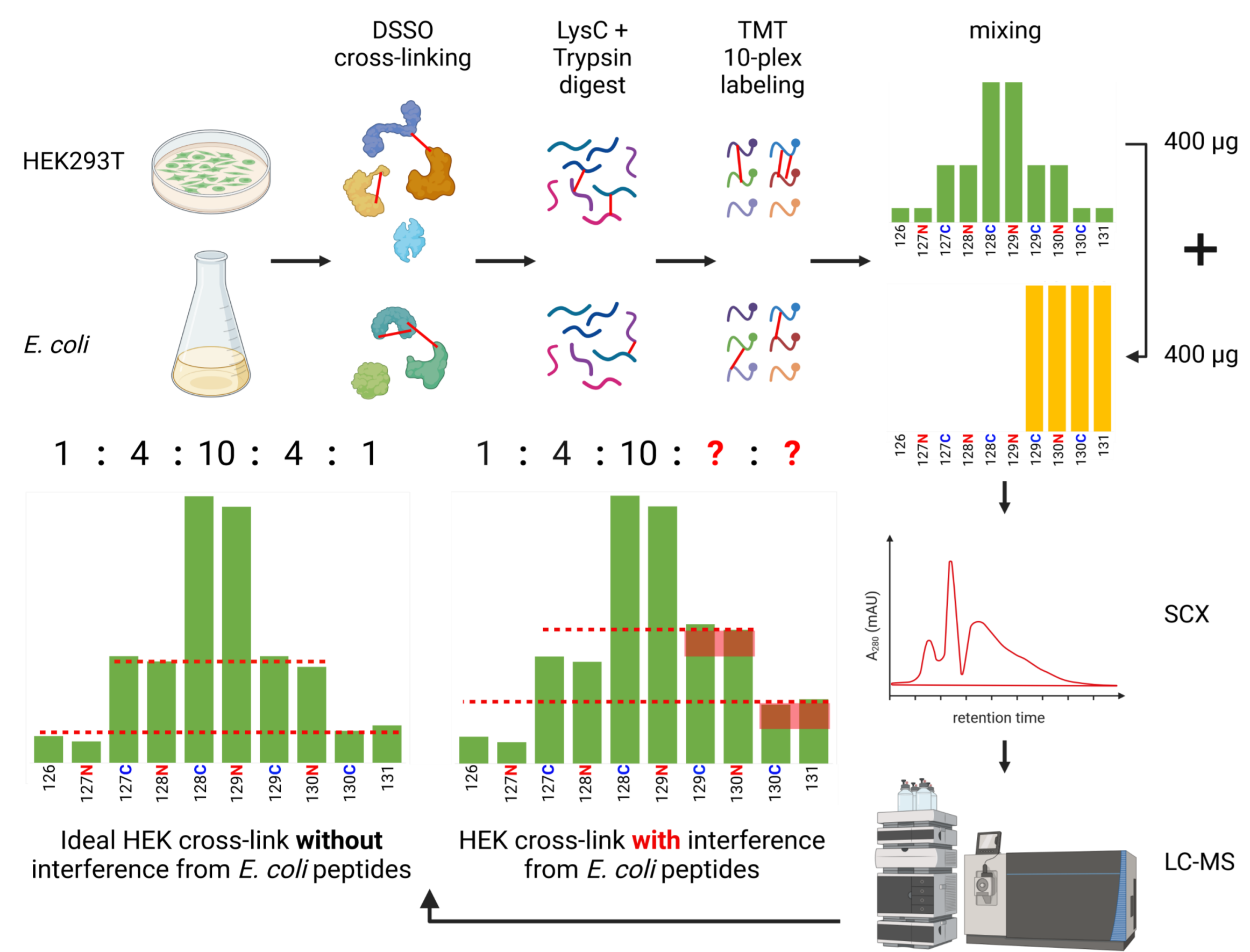


TMT-based large-scale interactomics quantification

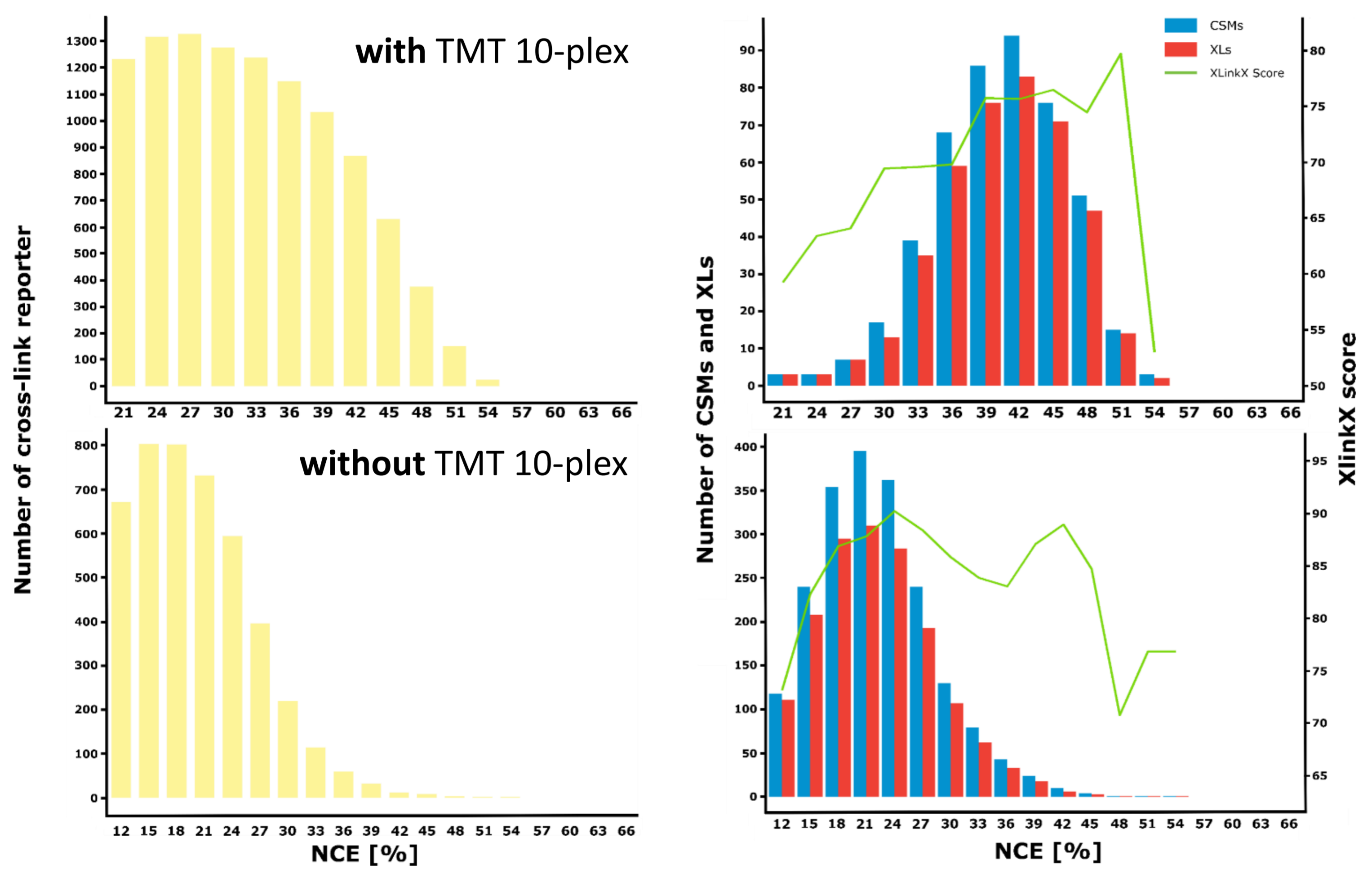
- **Two-interactome dataset:** TMT10plex-labeled DSSO cross-linked *E. coli* lysate spiked into TMT10plex-labeled DSSO cross-linked HEK293T lysate
- XL identification and quantification accuracy of different methods assessed
- **Remarkably higher energies** required for TMT10plex-labeled XL fragmentation
- **Stepped HCD-MS2** provides vast number of XLs with high quantification accuracy
- Simple applicability paves way for multiplexed PPI quantification from complex biological systems

Generating a two-interactome dataset

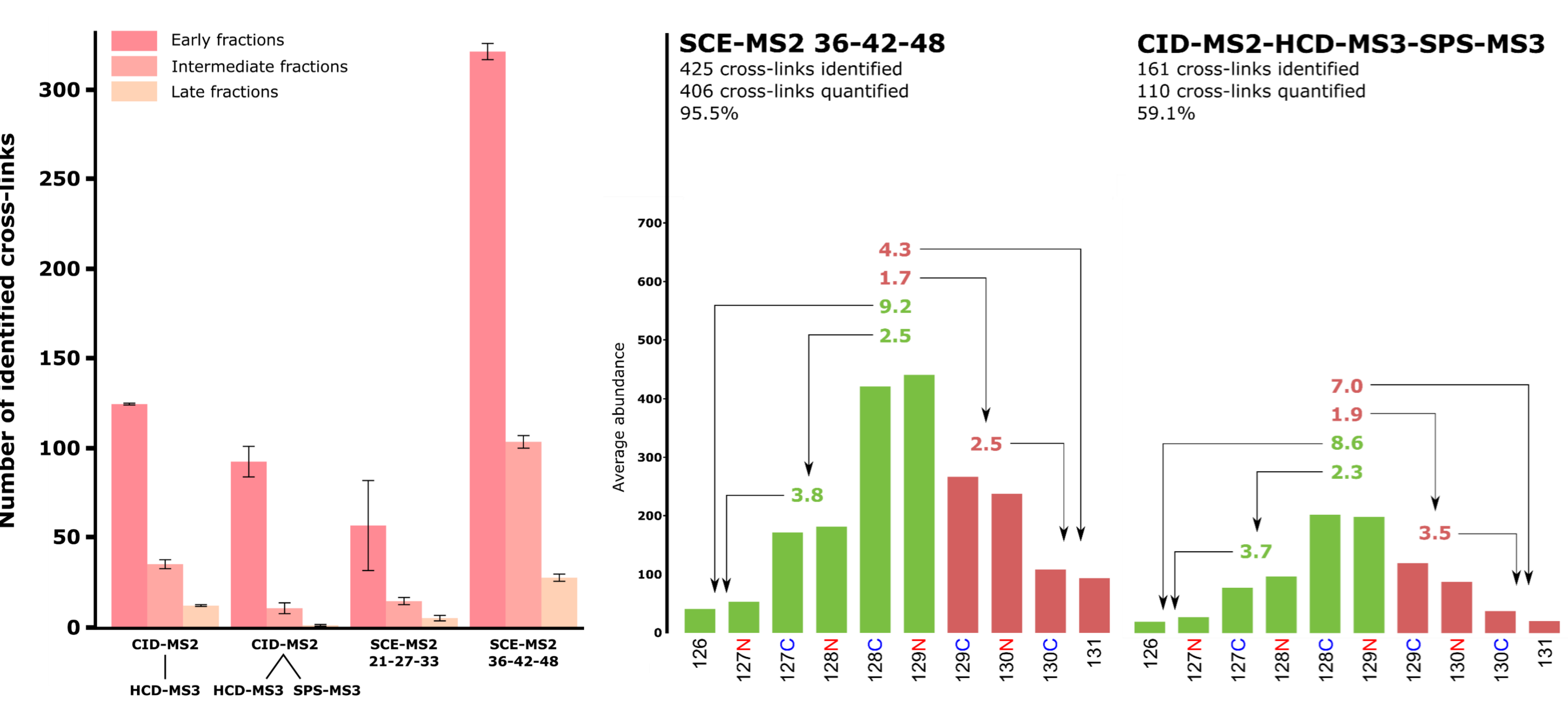


Two-interactome benchmarking dataset generation workflow.

Stepped HCD-MS2 outperforms classical MS2-MS3 strategies



Fragmentation of TMT-labeled cross-links using HCD MS2
 Left: Generation of cross-link reporter ions at different NCEs
 Right: Identified CSMs and XLs using different NCEs for fragmentation

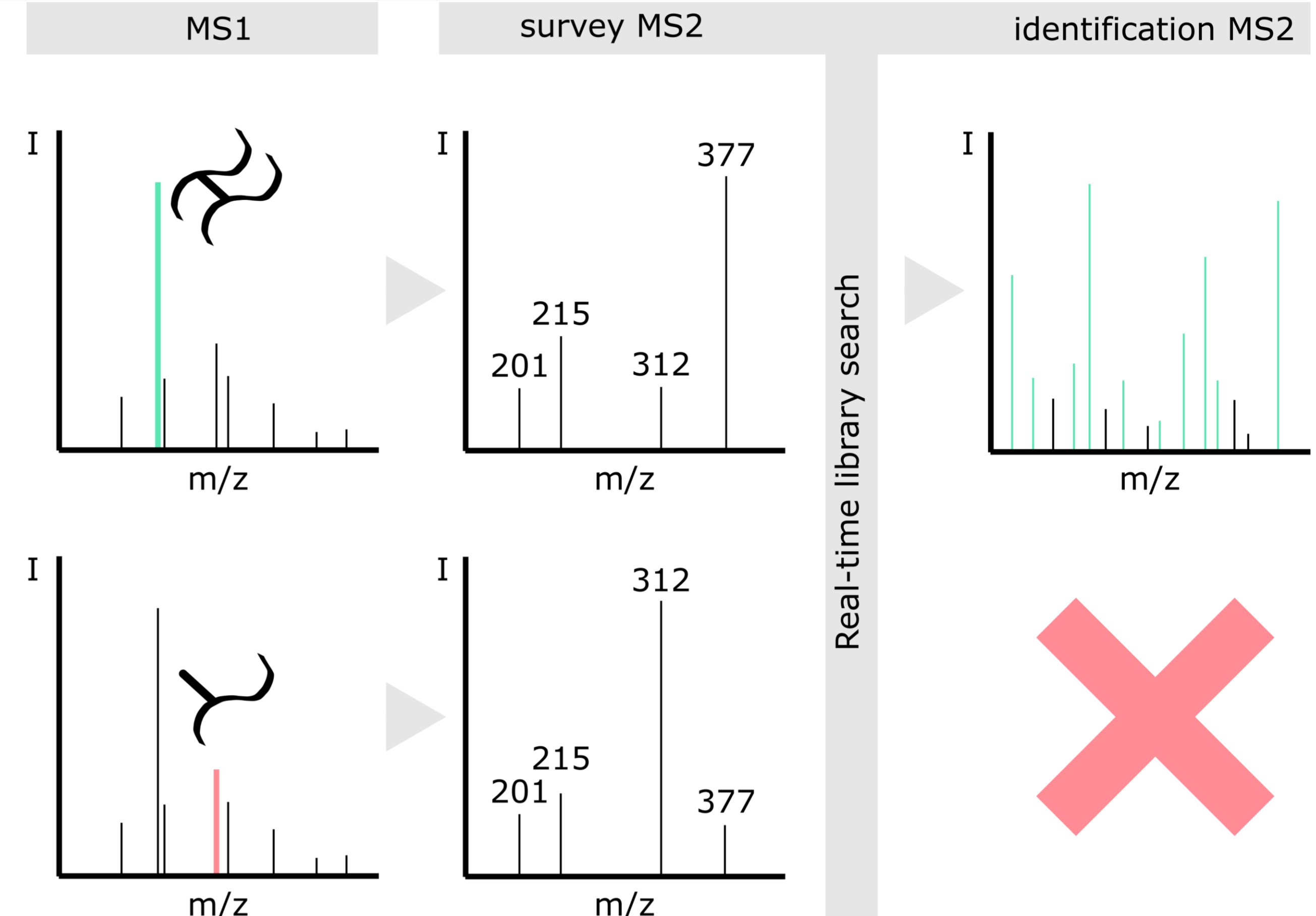


Assessment of MS2- and MS3-based XL identification efficacy and quantification accuracy
 Left: Identification of TMT-labeled XLs using different acquisition strategies
 Right: Average TMT abundance (reporter ion S/N) and ratio distortion of human XLs

Real-time library search-guided acquisition

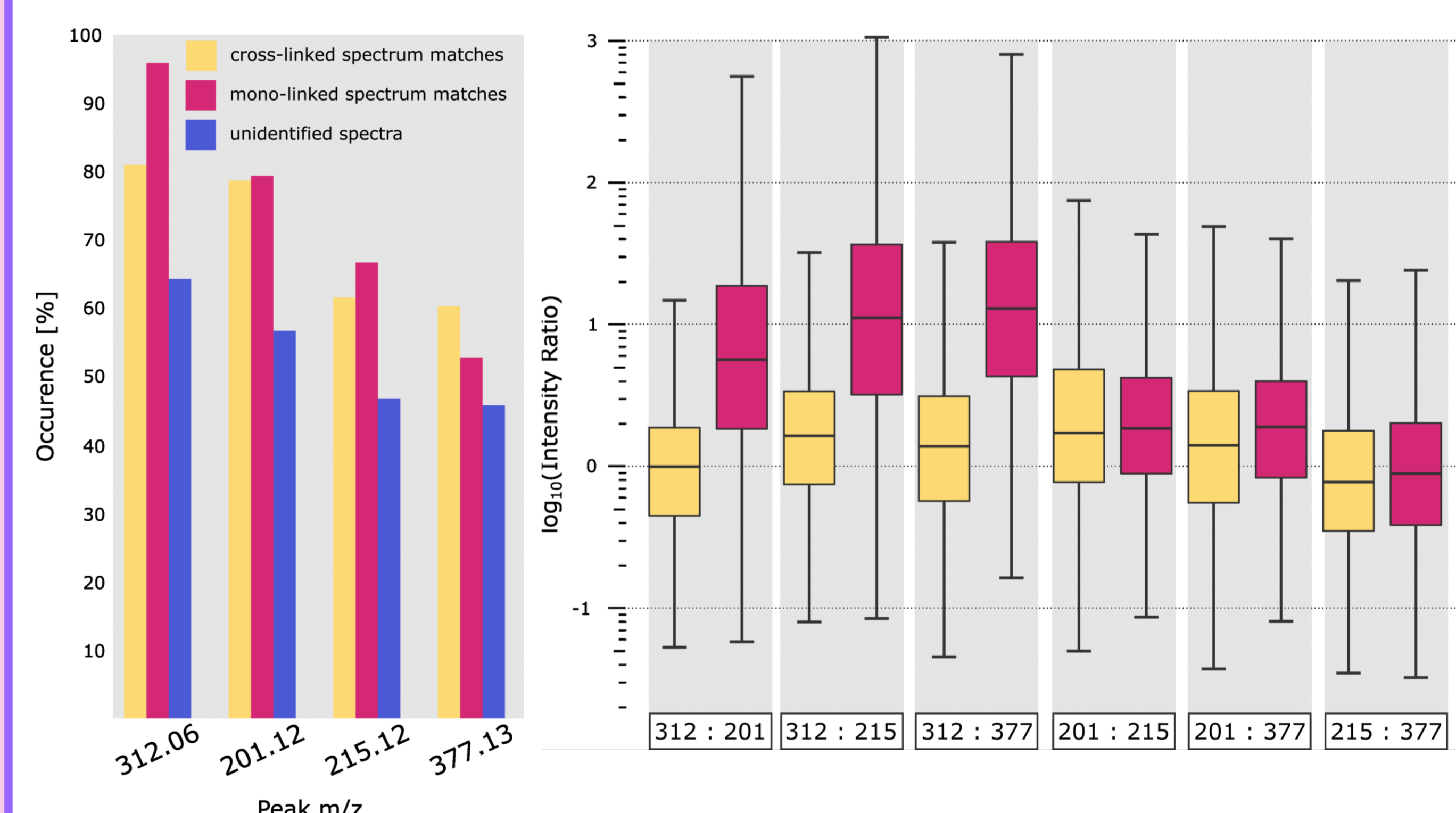
- **XLs can be enriched** from excess of linear peptides
- **Mono-links (ML) remain** and hinder XL identification
- MLs and XLs generate **unique fragmentation patterns**
- **Real-time library search (RTLS)** can trigger targeted acquisition
- **RTLS improves XL identification** from low- and high complexity **tbPhoX cross-linked** samples
- most beneficial for **unenriched samples and short LC-gradients**

Creating a RTLS-guided trigger mechanism

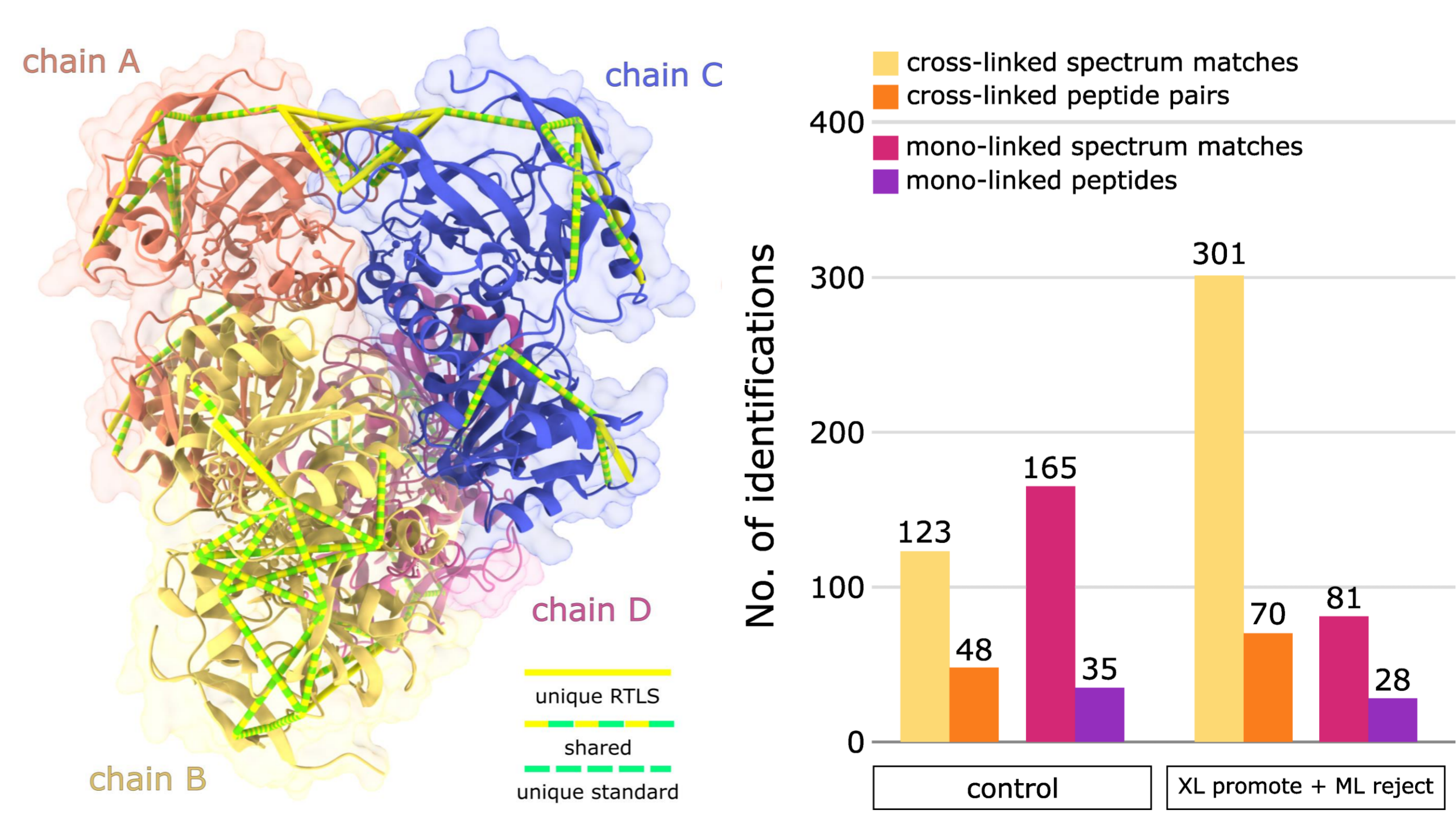


Real-time library search triggered XL acquisition scheme.

RTLS increases the number of identified XLs



Assessment of XL and ML fragmentation patterns
 Left: Identifying the four most frequently occurring peaks in XLs and MLs
 Right: Relative intensity ratios of peaks diagnostic for XLs and MLs



RTLS improves XL identification and increased depth of XL-MS data
 Left: RTLS helps to identify inter-subunit cross-links of yeast ADH
 Right: RTLS increases the identification of XLs from tbPhoX cross-linked BSA

