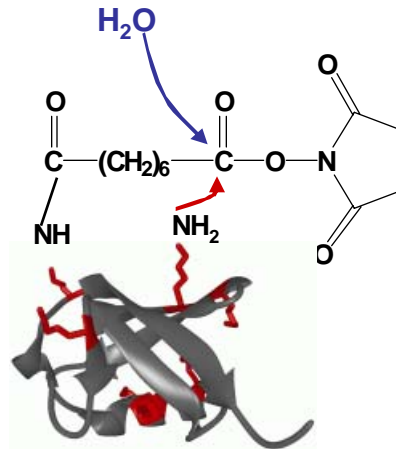# Cross-Linking Chemistry Example

"Hanging" Cross-link
Add $C_8H_{12}O_3$,
$\Delta m = 156.0786$

Disuccinimidyl Suberate (DSS)
Reacts only with Primary Amines
(Lysine, Amino-Terminus)

11.4A

H_2O
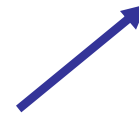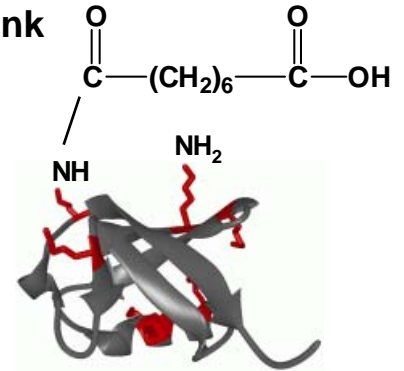
"Internal" Cross-link
Add $C_8H_{10}O_2$,
$\Delta m = 138.0681$
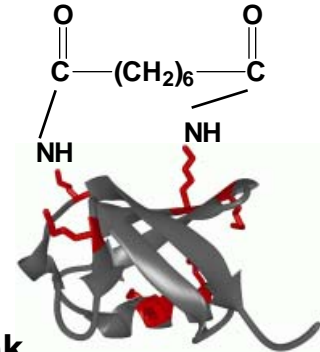
"Bottom-Up" vs. "Top-Down" MS3D

Bottom-Up:

Data Analysis, Identify X-linked Fragments, Put It All Back Together

↑

Mass Measurement of Peptides

↑

Digest

↑

Purification of Monomer (Gel, Size Exclusion Column)

↑

Chemical Crosslinking

Top-Down:

Chemical Crosslinking

↓

Sample Cleanup (Protein Trap)

↓

Gas-Phase "Purification" of Cross-linked Species

↓

Gas-Phase "Digest", CID, IRMPD, ECD

↓

Data Analysis, Identify Fragments Containing X-links, Put It All Back Together

Advantages of Top-Down

- **Eliminates time and sample consuming monomer purification, proteolysis, LC-MS**
    - Higher throughput, automation
- **Eliminates protein modifications that can be introduced in these steps (oxidation, methylation, formylation, etc.).**
    - Greatly simplifies data analysis
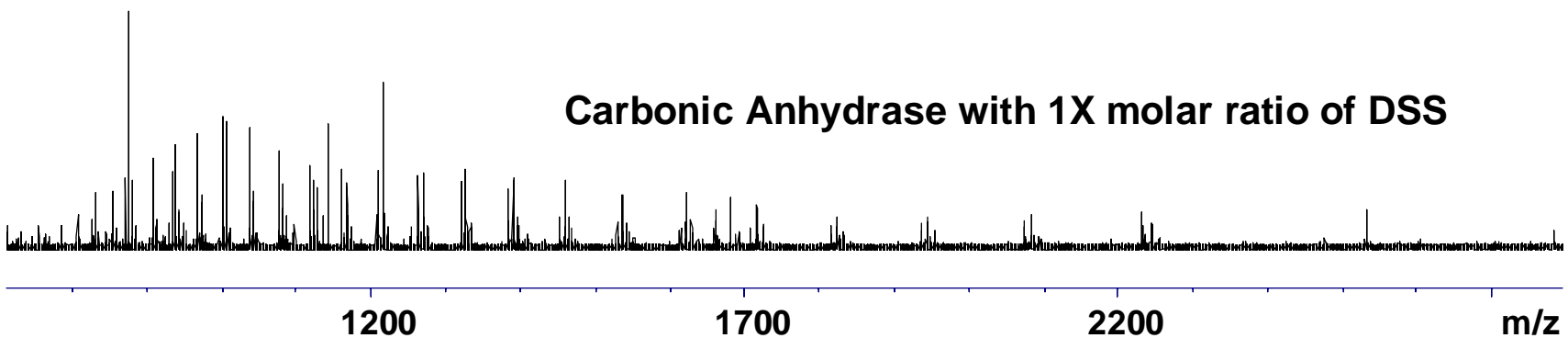
FTMS of Whole Cross-linked Proteins

Carbonic Anhydrase with 4X molar ratio of DSS
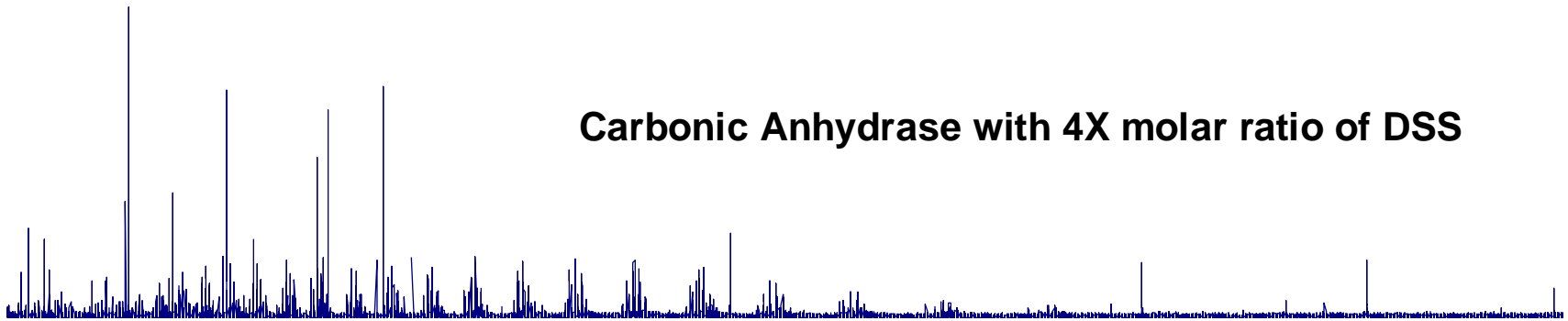
Carbonic Anhydrase with 2X molar ratio of DSS
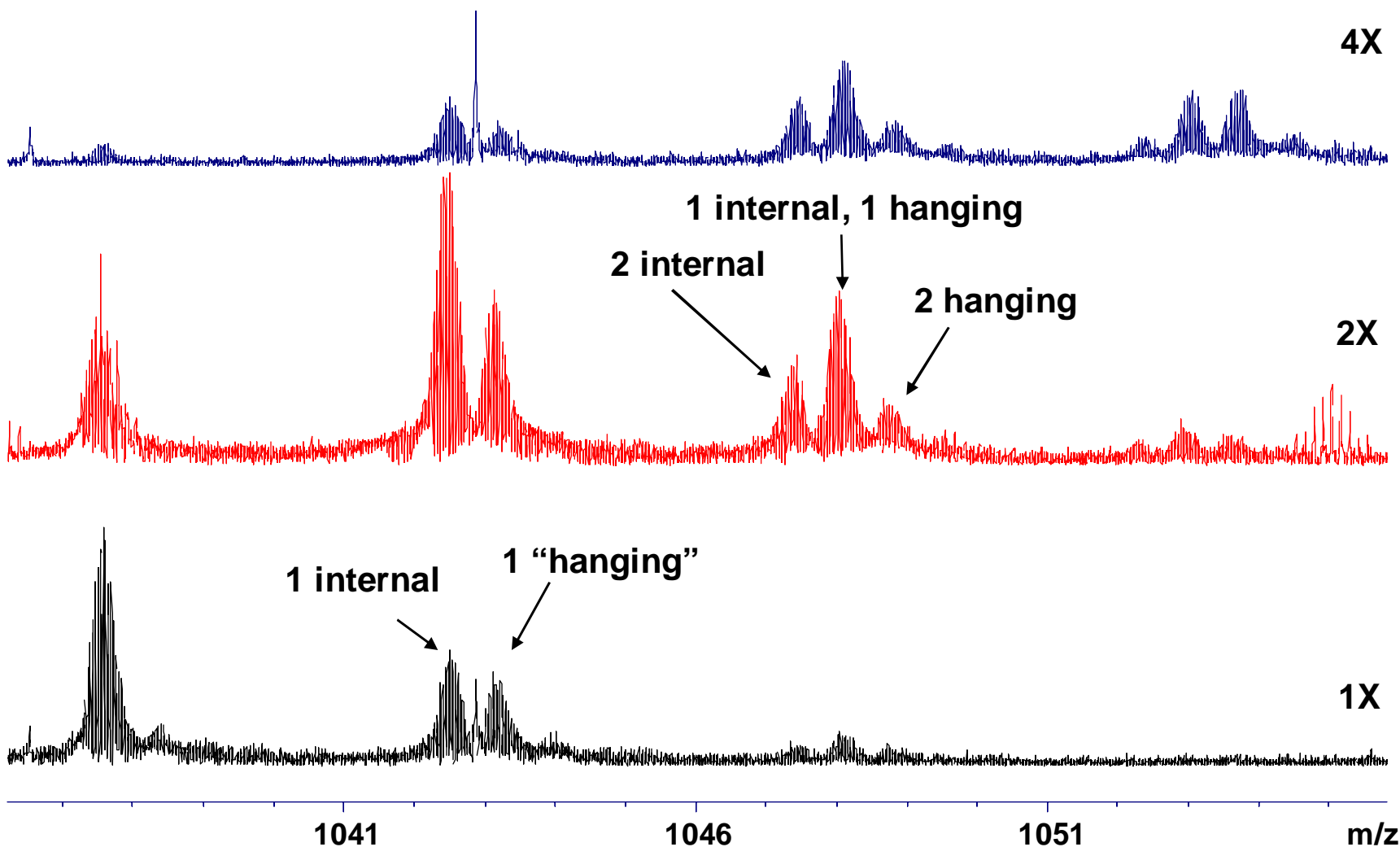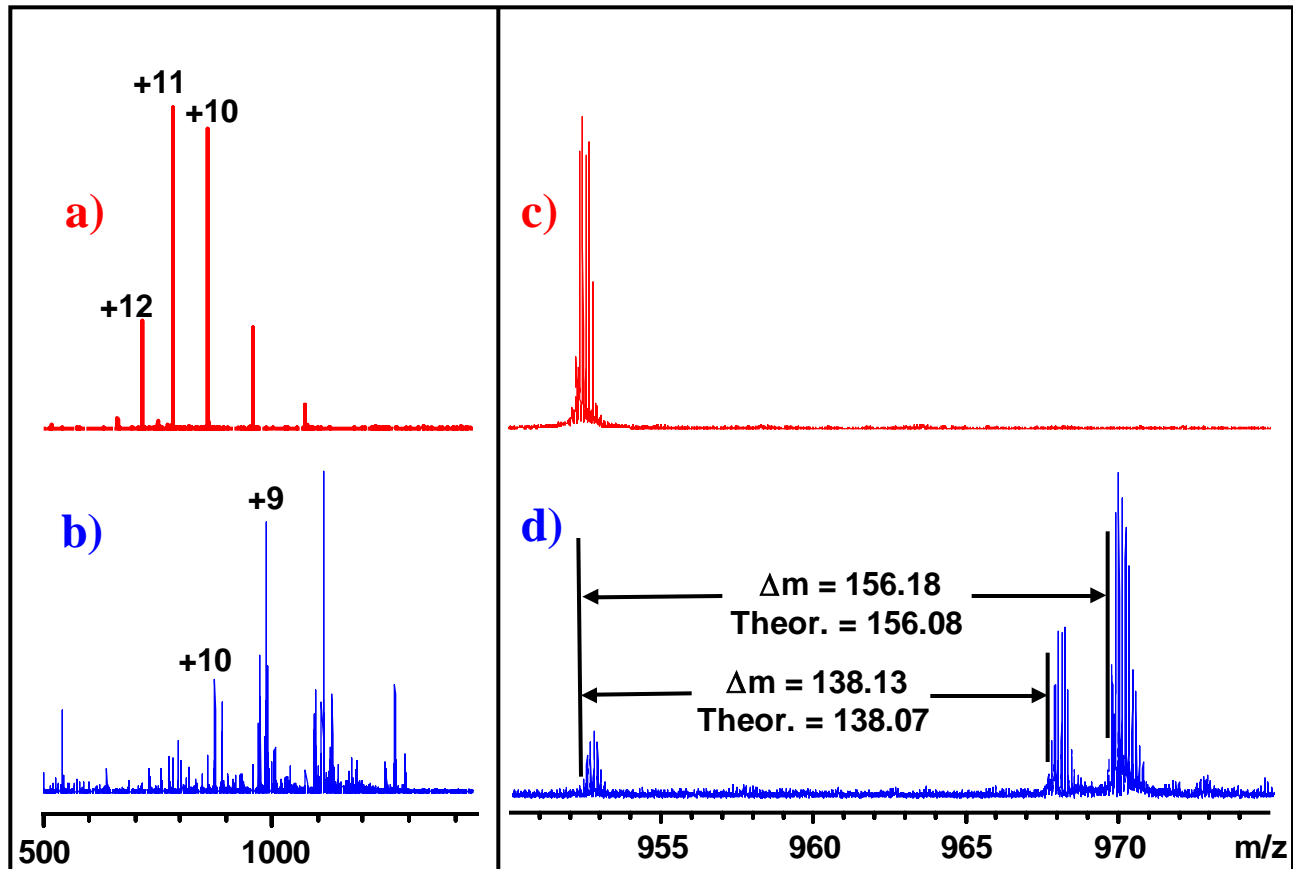
Carbonic Anhydrase with 1X molar ratio of DSS

1200   1700   2200   m/z

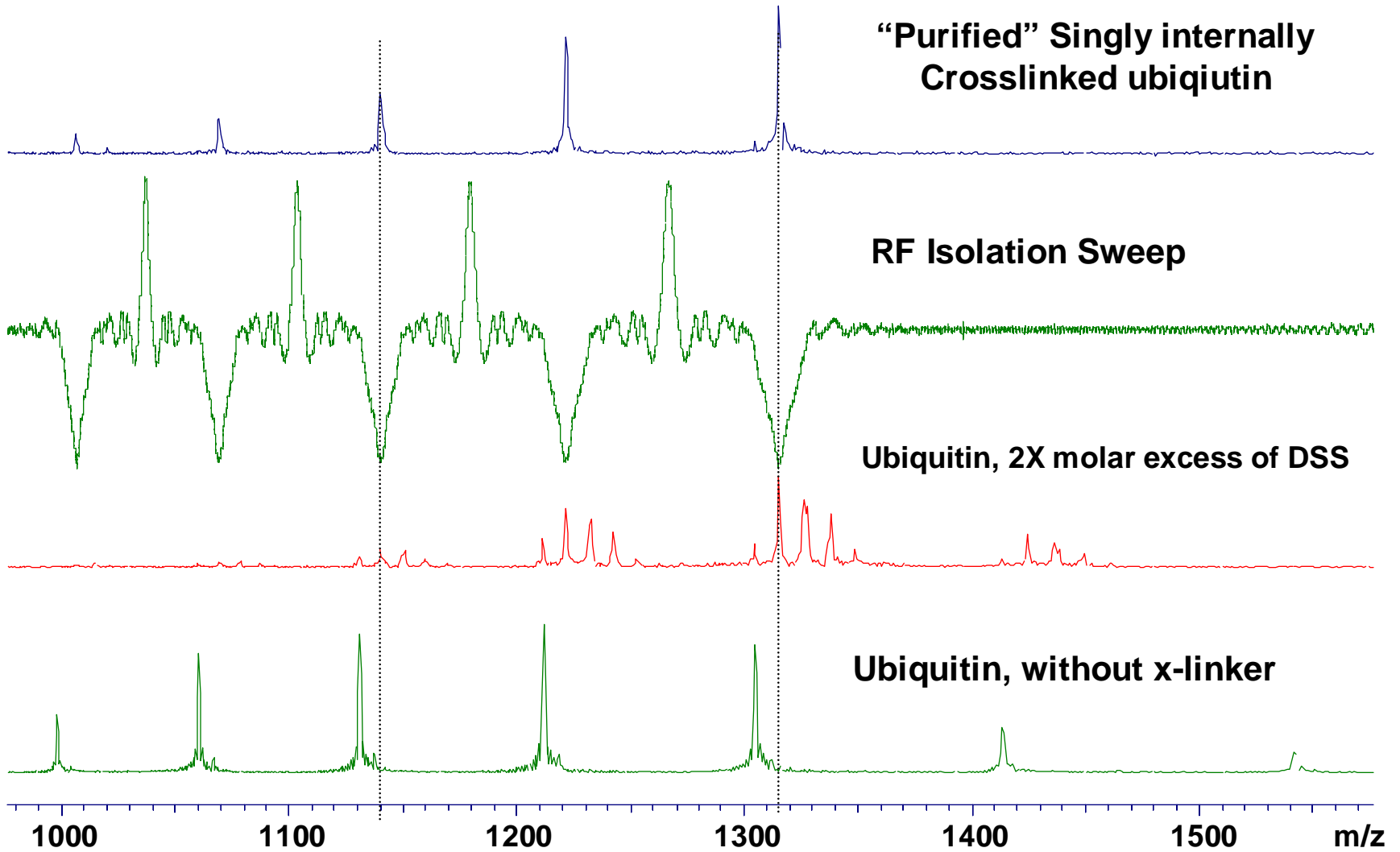# Allows Direct Observation and Reaction Products and Optimization

FTMS of Whole Cross-Linked Proteins
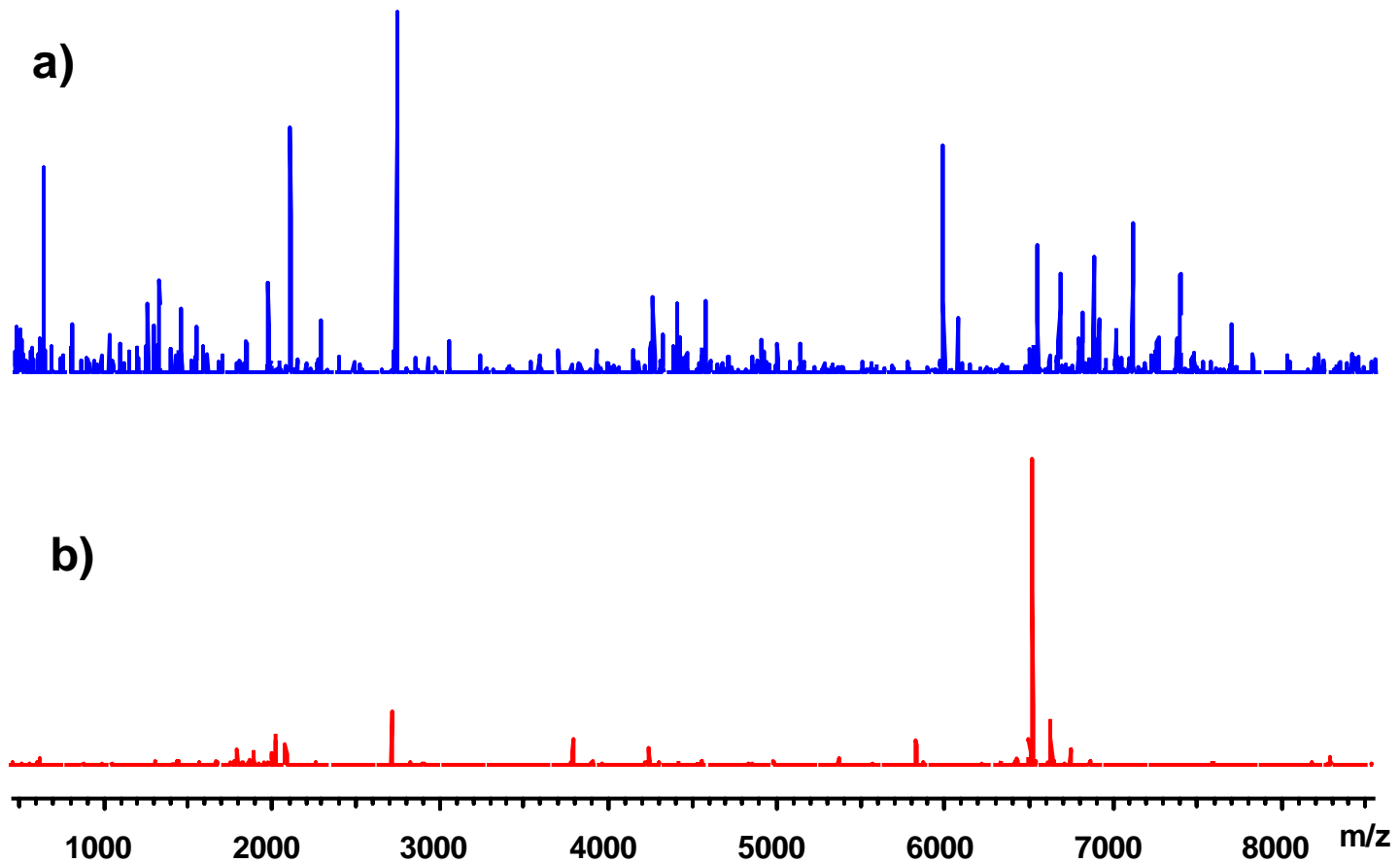

(a) and (b) show the spectra of native ubiquitin and x-linked ubiquitin. (c) and (d) show an expanded region of the spectra in a) and b) around the +9 charge state.

"Gas-Phase Purification"

"Purified" Singly internally Crosslinked ubiqiutin

RF Isolation Sweep

Ubiquitin, 2X molar excess of DSS

Ubiquitin, without x-linker

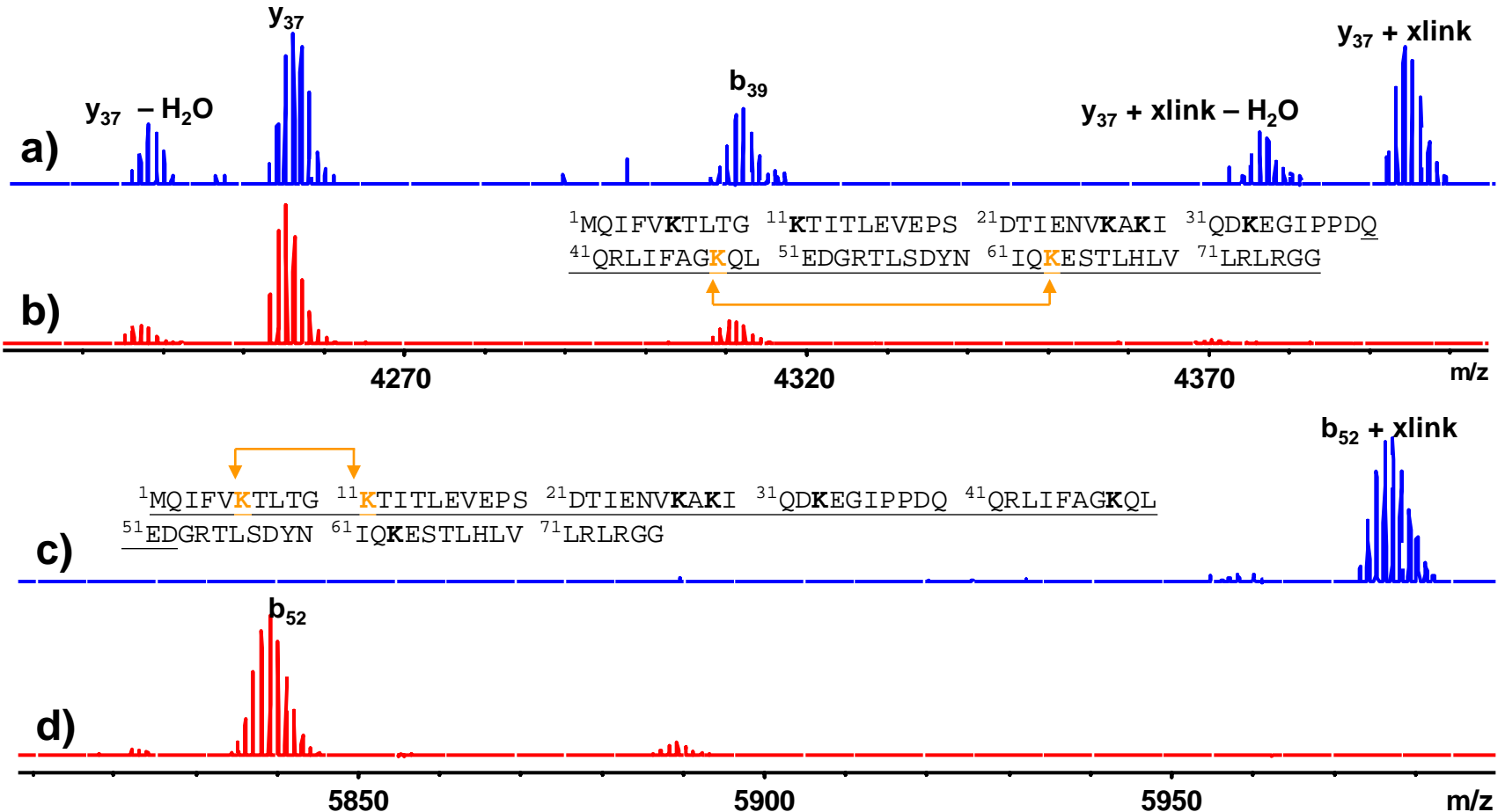1000   1100   1200   1300   1400   1500   m/z

SORI-CID MS/MS

Deconvoluted MS/MS spectra of singly x-linked ubiquitin (a) and native ubiquitin (b).
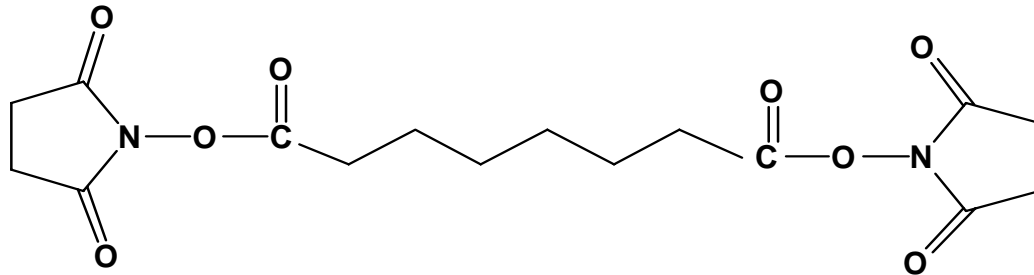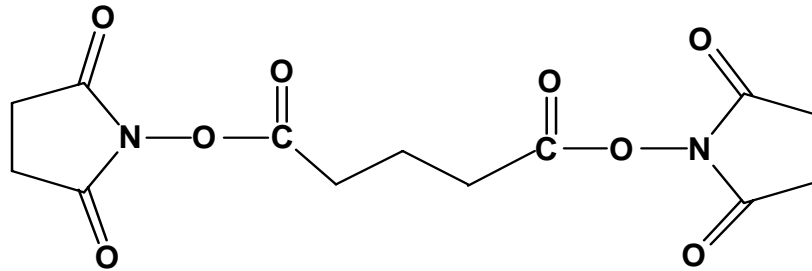
Detail of the SORI-CID MS/MS

X-linked ubiquitin (a,c) MS/MS spectra and the unmodified ubiquitin (b,d) MS/MS spectra. The underlined sequences of ubiquitin in (b) and (c) correspond to the y_{37} and b_{52} ions, resp. The arrows indicate the lysine residues that are cross-linked.
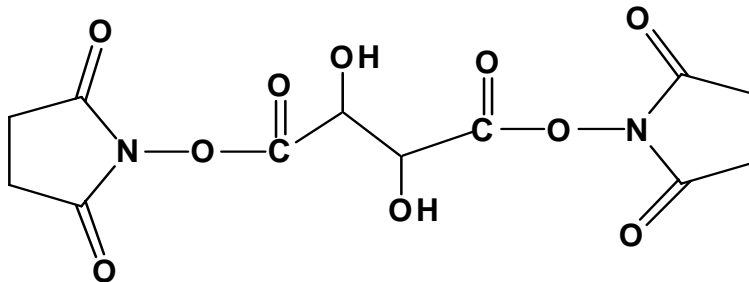
Other Cross-linkers

Disuccinimidyl Suberate
DSS
11.4 Å

Disuccinimidyl Glutarate
DSG
7.5 Å

Disuccinimidyl Tartarate
DST
5.8 Å

Cross-Links Observed

Cross-Link	DSS	DSG	DST	Constraint
Amino Term. – K6	Yes	Yes	Yes	Distance < 5.8 Å
K6-K11	Yes	Yes	Yes	Distance < 5.8 Å
K48-K63	Yes	Yes	No	5.8 Å < Distance < 7.5 Å

Structure of Ubiquitin with Assigned Xlinks