

## **A Top Down Approach to Protein Structural Studies Using Chemical Cross-Linking and Fourier Transform Mass Spectrometry**

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### **INTRODUCTION**

Recent work has suggested that the mass spectrometric analysis of proteins that have been covalently modified by bifunctional cross-linking reagents and then digested proteolytically can lead to sufficient low-resolution distance constraints to solve the tertiary structure of a protein.<sup>1</sup> Some difficulties in this approach include the necessity to separate internally crosslinked monomeric protein from cross-linked dimers using either size exclusion chromatography or gel separations, followed by proteolytic digestion. This contribution explores the applicability of a top-down approach<sup>2</sup>, where the entire crude cross-linked protein mixture is injected into an ESI-FTMS instrument, and the cross-link positions localized by multiple stages of fragmentation and mass spectrometry.

### **METHODS**

Protein samples were cross-linked with commercially available homobifunctional cross-linkers such as disuccinimidyl suberate (DSS Pierce, Rockford IL), under conditions that maintained the protein in its native state structure. Cross-linked samples were cleaned up with a simple one-step protein trap cartridge cleanup (Macro Traps, Michrom BioResources, Auburn CA), or run directly by ESI after dilution in a standard denaturing ESI solvent, on a Bruker Daltonics (Billerica, MA) APEX II FTMS equipped with a 7.0 Tesla superconducting magnet.

### **PRELIMINARY RESULTS**

Preliminary results show that it is possible to directly observe cross-linked monomeric proteins for proteins as large as carbonic anhydrase. The resolution and mass accuracy of the FTMS instrument are sufficient to unambiguously assign peaks in the observed spectra to unreacted protein, protein with a 'hanging cross-link' (where one end of the cross-linking reagent reacts with the protein and the other is hydrolyzed), and protein with a true internal cross-link. These observations permit the reaction conditions to be optimized to improve the yield of the desired internally cross-linked protein. For ubiquitin, the singly internally cross-linked species has been isolated in the FTMS analyzer cell and interrogated by MS/MS. The MS/MS results include fragments both with and without the cross-link. Based on these fragments two sets of cross-linked lysines have been unambiguously assigned, that are consistent with the known structure of ubiquitin, and the size of the cross-linker. Further work is underway to identify more cross-linking positions and to extend the technique to larger proteins.

### **REFERENCES**

1. Young et al., Proc. Natl. Acad. Sci. USA **2000**, *97*, 5802-5806.
  2. McLafferty et al., J. Am. Chem. Soc. **1999**, *121*, 806-812.
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