Topology Characterization of Model Proteins by Photochemical Side Chain Oxidation and Mass Spectrometry

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Recent advances in high-throughput biology have led to an abundance of data regarding the identity of proteins present in living cells. However, for many of these proteins, the structure and function remain unknown. Standard techniques for solving protein structure (XRC, NMR) are very labor intensive and not suited for high-throughput analyses. Computational protein structure prediction algorithms have the speed necessary for high-throughput analyses, but they often generate inaccurate models, especially for proteins for which no homologs with known structure exist. The application of experimental constraints to the protein structure has been previously utilized to improve the accuracy of protein modeling algorithms. These constraints aid in eliminating false models, greatly increasing the accuracy of the computational predictions. We describe the use of hydroxyl radical chemistry to rapidly and selectively oxidize amino acid side chains of the surface of several model proteins. The sites of oxidation are determined by reverse phase liquid chromatography coupled to tandem mass spectrometry. Our results indicate that our technique selectively oxidizes amino acid side chains based on their chemical reactivity, solvent accessibility, and flexibility. The potential of this technique for generating high-throughput structural constraints should have a large impact on the field of structural genomics.

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