

PhD Thesis Defense

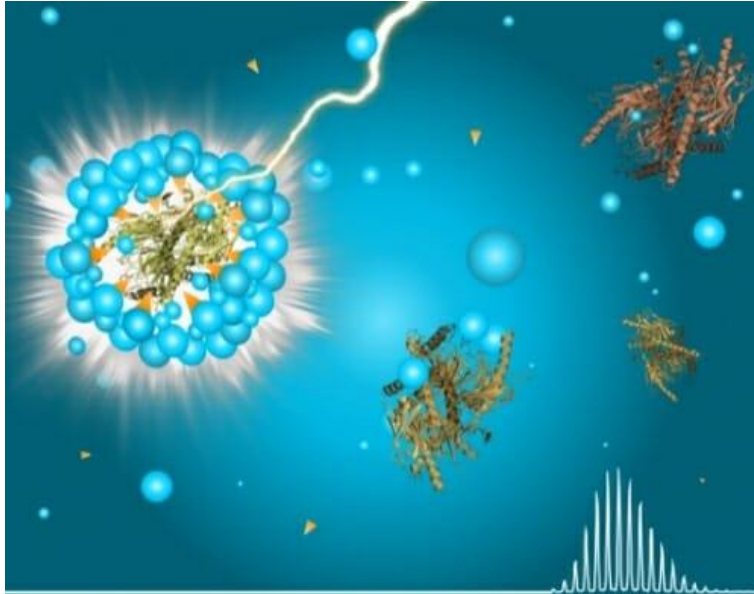
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Novel Proteomic Approaches to Characterize Endogenous Membrane Proteins

Ge Research Group

Defense on August 12th, 3:00 pm CST via Webex:

<https://uwmadison.webex.com/meet/pr924844041>



Biological information flows as DNA and is transcribed into RNA which is translated into proteins. However, sequence variation from alternative splicing events and mutations combined with post-translational modification (PTMs) results in a large number of protein forms (referred to as proteoforms) arising from a single gene. Mass spectrometry (MS)-based proteomics provides unprecedented opportunities to understand the role of proteoforms in health and disease; however, many challenges remain. For example, despite their importance as drug target (>50% of current drugs), membrane proteins are traditionally underrepresented using MS-based proteomics because of their lower expression level, hydrophobicity, and lack of established protocols. To address these challenges, I developed a novel photocleavable surfactant, Azo, which can effectively solubilize proteins and is compatible with MS analysis. We demonstrated Azo-aided top-down proteomics (the sequencing of intact proteins by MS) enabled the solubilization of important membrane proteins from biological samples, including heart tissues, for comprehensive characterization of their proteoforms. Moreover, Azo is simple to synthesize and can be used as a surfactant in polyacrylamide gel electrophoresis. We further incorporated the surfactant technology to facilitate high-throughput bottom-up proteomics (the sequencing of digested proteins by MS), which allows for more extensive proteome coverage and protein expression determination. Combining these approaches, we established a powerful integrated bottom-up and top-down proteomics strategy to extensively characterize proteins in biological samples. Finally, novel membrane protein enrichment and multidimensional liquid chromatography separation strategies were developed to further expand the scope of MS-based proteomics for characterizing the membrane proteoform landscape.