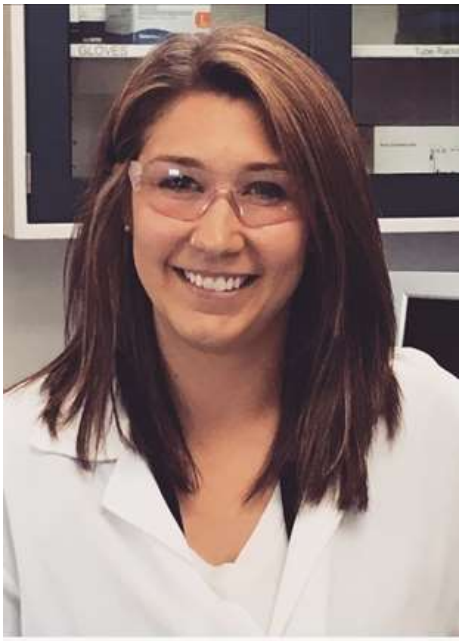
Poster presentation:

Efficient and convenient enrichment of multi-spanning membrane proteins for proteomic studies



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Biography: Joanna received her BS in microbiology from Western Illinois University. She has actively worked on the development, commercialization and support of protein sample preparation and protein interaction products introduced over the past eight years. This also includes development of products for dialysis, protein concentration, membrane protein extraction, protease and phosphatase inhibition, and immunoprecipitation. She has experience in a multitude of techniques such as mass spectrometry sample prep, cell and tissue lysis, cell separation, and protein expression and extraction.

Abstract:
Examining the membrane proteome is vital to understand its role in normal and disease function. However, the isolation and extraction of multi-spanning membrane proteins for proteomic study often proves difficult. Traditional isolation methods are tedious and time-consuming. Additional drawbacks include poor solubilization, incompatibility with downstream applications, and disruption of membrane protein complexes. In this study, we report a procedure that enables fast, convenient solubilization of membrane proteins from cultured cells and tissue. This sequential detergent extraction method allows for the enrichment of both integral membrane proteins and membrane-associated proteins. To validate our method, three integral multi-spanning membrane proteins (ADP/ATP translocase 3 (SLC25A6), sodium/potassium ATPase alpha subunit (AT1A1) and adenylate cyclase 2 (ADCY2)) were evaluated. Membrane protein extraction in cell lines (A431, HeLa, HCT116, HepG2, HEK293 and A549) and mouse tissue (brain, kidney and liver) using this reagent-based extraction outperforms the other available kits as determined by western blotting and mass spectrometry. Additionally, extraction of multi-spanning membrane protein is improved by increasing the

ionic strength of the solubilization buffer, obtaining 70-85% extraction, as confirmed by western blot densitometry. Native membrane protein complexes are also preserved, allowing for compatibility with co-immunoprecipitation experiments. This straight forward and robust method enables researchers to better investigate the role of membrane proteins in cellular functions.