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A Method for Characterization of Detergent-Resistant Membrane Rafts using Liquid Chromatography-Tandem Mass Spectrometry

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This study describes a combined, detergent- and organic solvent-based proteomic method for the analysis of detergent resistant membrane rafts (DRMRs). These specialized micro domains of the plasma membrane contain a distinctive and dynamic protein and/or lipid complement, which can be isolated from most mammalian cells. Lipid rafts are predominantly involved in signal transduction and adapted to mediate and produce different cellular responses. To facilitate a better understanding of their biology and role, DRMRs were isolated from Vero cells as a Triton-insoluble membrane fraction. After detergent removal, sonication in 60% buffered methanol was used for cholesterol depletion, followed by solubilization and tryptic digestion of the resulting protein complement. The peptide mixture RPLC was resolved using microcapillary reversed-phase liquid chromatography (coupled on-line to a tandem mass spectrometer). Gas-phase fractionation in the mass-to-charge range was employed for qualitative analysis to broaden the selection of precursor ions, increase the number of identifications, and detect less abundant proteins. A total of 380 proteins were identified, including all known lipid raft markers, while 91 (24%) proteins were classified as integral-helical membrane proteins of which 51 (56%) were predicted to have multiple transmembrane domains. The same upstream sample preparation method was coupled to multidimensional quantitative analysis that employs 16O/18O proteolytic labeling to quantify differences in relative abundances of DRMR proteins in response to lota b toxin induction of Vero cells. Over 550 proteins were quantitated using this method of which a significant number were alpha-helical membrane proteins, as predicted by TMHMM algorithm. More importantly, all known lipid raft marker proteins and many other known cell surface proteins were quantitated. This study showed for the first time the ability to utilize 16O/18O enzyme-specific proteolytic labeling for shot-gun quantitative membrane proteomics.