Quantitative analysis of bilayer-to-monolayer partitioning of monotopic membrane proteins using *in vitro* microfluidics

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Lipid droplets are subcellular lipid storage organelles. They create a unique physicochemical environment in the cell as their hydrophobic lipid core is separated from the aqueous cytosol by a phospholipid *monolayer*. The dynamic metabolic function of lipid droplets relies on specific proteins that integrate into the limiting monolayer membrane in a unique monotopic hairpin-type topology. Many hairpin proteins dynamically partition between the endoplasmic reticulum membrane, a phospholipid bilayer, and the lipid droplet monolayer membrane. The hairpin topology in both membranes presumably facilitates this partitioning; yet, the collective processes enabling hairpin proteins to reside in these distinct physicochemical environments as well as the partitioning between them remain unknown. Here, we use UBXD8 as a model hairpin protein to study protein integration into these two types. We aim to purify the protein, label it with a fluorescent dye and reconstitute it into artificial membranes that simultaneously mimic the phospholipid bilayer and the LD environment in a microfluidic chamber. We fused UBXD8 to MBP to increase the solubility and expressed it in *E. coli* followed by affinity purification and attachment of a fluorophore. Different mutations within the construct will inform us which sequence features influence the membrane partitioning.