Engineering cardiolipin binding to an artificial membrane protein

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Membrane proteins are critical to cellular functionality, with their activities frequently influenced by targeted protein-lipid interactions. In this study, we explore the complex role of the regulatory lipid cardiolipin (CDL), known for its stabilizing effect on membrane protein complexes. Utilizing the computationally designed scaffold protein TMHC4_R [1] as a model system, we combine molecular dynamics simulations with native mass spectrometry (MS) to dissect the protein attributes that facilitate selective lipid interactions and lipid-mediated stabilization (see **Figure**). We reveal that the presence and localization of positively charged amino acids, along with structural flexibility, are key determinants for differentiating between stabilizing and non-stabilizing CDL interaction sites. Extending our findings to native membrane proteins, we identify a stabilizing CDL site within the *E. coli* intramembrane protease GlpG and demonstrate how CDL modulates the enzyme's substrate specificity. Our work establishes a foundational approach for the design of engineered proteins with tailored lipid interactions, paving the way for the creation of proteins with bespoke membrane-targeted functionalities.

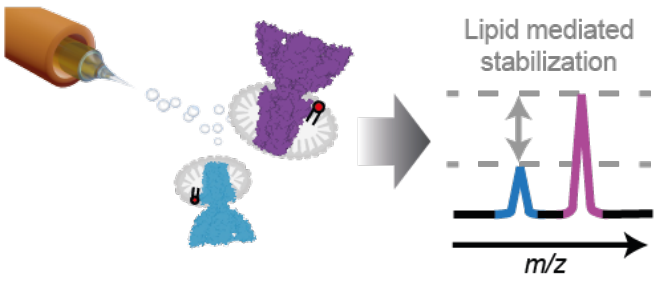

Figure. Native MS of TMHC4_R mutans unravels structural features for lipid mediated stabilization.

[1] Lu, P, et al. Accurate computational design of multipass transmembrane proteins. *Science* 359, 1042–1046 (**2018**).