Small-Angle Neutron Scattering For Integrative Structural Modeling Of Membrane Proteins In Circularized Nanodiscs

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Membrane proteins (MPs) are large, complex molecular machines embedded within a lipid bilayer that drive essential life processes and, serve as targets for over half of approved drugs used in the clinic. To date, we lack a large fraction of MP information-fold, shape, structure, dynamics-that, if available, would provide novel platforms for scientists to design therapeutics more efficiently. Regardless of the method used to investigate MPs, the complexity of the lipid bilayer and mimetics used to recapitulate this environment are extensive (micelles, mixed micelles, bicelles, lipidic cubic phase, nanodiscs), and how these mimetics perturb MP structure is not fully understood. To date, structural models of membrane proteins are mostly limited to detergent micelles (~75% of deposited structures), which can have profound effects on MP function. Therefore, establishing alternative approaches to model MPs in more native-like systems is critical. Our overall objective is to use integrated 'meta-approaches' that combines experimental and computational efforts to probe the complex, mechanistic nature of MPs. In this work, we successfully established small-angle neutron scattering (SANS) protocols for a model MP system, the Disulfide bond formation protein B (DsbB), which catalyzes disulfide bond formation in E. Coli. This was accomplished by developing a deuteration strategy for 'stealth'-like circularized nanodiscs width 9 (d-cNW9) equipped for probing the geometric shape of DsbB under different lipid compositions. We combine this data with SANS-restrained molecular dynamics (MD) to reveal the architecture of DsbB and specific lipid-protein interactions within the associated d-cNW9 nanodisc for structural interpretation. This was also carried out for DsbB in deuterated dodecylphosphocholine micelles for comparison. Collectively, this integrated framework will generate new technologies to probe the structure and dynamics of MPs and the membrane mimetic.