# Structural Analysis of Macromolecular Anchors for Cell Membrane Surface Engineering Using Contrast-Matching Neutron Scattering

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Cell-membrane surface engineering, in which functional molecules are displayed on living-cell membranes, is a useful strategy for studying membrane proteins and cellular functions and for developing cell-based therapies. Metabolic labeling with unnatural sugars and direct insertion of lipid assemblies have been widely used for this purpose. However, rapid renal clearance, insufficient membrane insertion, and elimination by the immune system still limit their use in living animals.

We have developed amphiphilic random copolymers bearing oligo(ethylene glycol) and hydrophobic poly(propylene oxide) side chains as polymer anchors for membrane modification. These polymers self-assemble into nanoparticles in water and spontaneously incorporate into phospholipid membranes by simple mixing. We also prepared related polymers containing cholesterol or alkyl anchors. Small-angle X-ray scattering showed that these polymers form spherical micelles with radii of approximately 10 nm, and fluorescence microscopy and FRET measurements confirmed their incorporation into phospholipid liposomes.

The structure of polymer anchors on a lipid bilayer should directly influence anchoring stability and the presentation of functional molecules. However, this structure is difficult to determine by electron microscopy because of limited contrast and resolution. Conventional small-angle X-ray scattering is also not sufficient for such multicomponent hybrid vesicles because the scattering signals from lipids and polymers overlap.

In this study, contrast-matching small-angle neutron scattering (SANS) was used to selectively observe polymer-rich domains in DMPC-$d_{54}$ liposomes. The lipid-bilayer scattering was suppressed in a $D_2O/H_2O$ = 86/14 mixed solvent, allowing extraction of the polymer contribution. The resulting polymer-derived scattering profiles were analyzed using several theoretical models. Among them, a short core–shell cylinder model reproduced the experimental data well (Fig. 1). The best-fit parameters gave a cylinder length of 14 nm and a diameter of 4.3 nm, indicating that the incorporated polymers formed short cylindrical domains in the lipid bilayer. These dimensions are in good agreement with our previous results obtained for related random-polymer systems.

The contrast-matched SANS analysis provided structural information on polymer domains incorporated in the bilayer, which could not be obtained from microscopy or SAXS alone. By combining these data with fluorescence observations and FRET-based insertion assays, we obtained insight into how polymer chemical structure governs membrane-associated domain formation. These results provide a structural basis for designing robust polymer anchors for cell-membrane surface engineering and potential in vivo cell-surface modification.

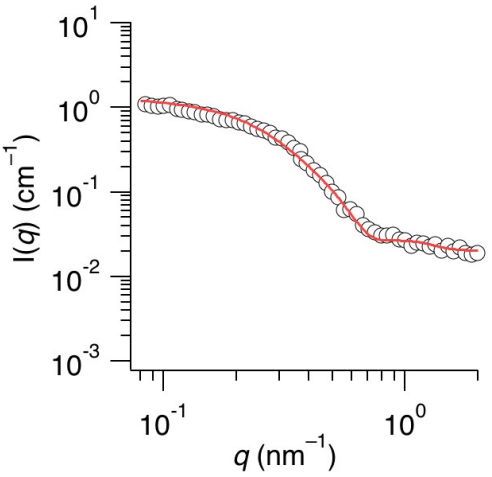


Fig. 1. SANS profile of the polymer/DMPC-$d_{54}$ hybrid vesicles at 37 °C in 84 : 16 $D_2O$ : $H_2O$ (open circles) and theoretical curves obtained form the core–shell cylinder model (red line).