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Revealing the Structural Intricacies of Biointerfaced Emulsions with Neutron Scattering

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Extended Abstract

Utilising cell membranes from diverse cell types for biointerfacing has demonstrated significant advantages in enhancing stability and incorporating biological properties to micro- and nanomaterials, tailored specifically for various biomedical applications. However, the structures of these materials, particularly emulsions interfaced with red blood cell (RBC) or platelet (PLT) membranes, remain an underexplored area. This work addresses this knowledge gap through small- and ultra-small-angle neutron scattering (SANS and USANS) studies, employing contrast variation techniques to investigate the structures of emulsions containing perfluorohexane within RBC (RBC/PFH) and PLT membranes (PLT/PFH) at the micro- and nanoscale.

RBC and PLT membranes were prepared via hypotonic hemolysis of RBCs and freeze–thawing of PLTs, isolated from whole blood. RBC/PFH and PLT/PFH emulsion droplets were then fabricated using a simple sonication method, followed by dilution in various deuterium oxide–water (D_2O–H_2O) mixtures. This approach enables the establishment of distinct contrast matching conditions—where specific D_2O–H_2O mixtures match out the scattering from one component, highlighting scattering from another component—facilitating in-depth investigations into the structures of different components of RBC/PFH and PLT/PFH emulsion droplets. Scattering patterns were obtained using the Bilby SANS and Kookaburra USANS beamlines at the Australian Centre for Neutron Scattering (ACNS), Australian Nuclear Science and Technology Organisation (ANSTO).

By manipulating the dispersing media's composition (D_2O–H_2O mixtures), the scattering length densities of RBC and PLT membranes were estimated to be at ~1.52×10^{-6} Å^{-2}, similar to 30% (w/w) deuterium oxide. In our three-components system (comprising the solvent, cell membrane, and liquid PFH core), we can acquire scattering data and study the PFH core (cell membranes matched out at 30% D_2O) and the cell membrane coating (liquid PFH matched out at 60% D_2O). Using the cell membrane-matching medium (30% D_2O), droplet diameters were estimated to be around 770 nm (RBC/PFH) and 1.5 μm (PLT/PFH), based on calculated parameters from polydispersed sphere model fitting. Membrane bilayer thicknesses are approximately 10 nm (RBC) and 5 nm (PLT), derived from Guinier–Porod model fitting. Intriguingly, calculated patterns and invariant analysis reveal that native droplet architectures significantly differ from the observed, purportedly novel, bubble–droplet core system in electron microscopy. Additionally, invariant analysis conclusively demonstrated that

RBC/PFH and PLT/PFH emulsions feature entirely liquid PFH cores. This highlights the unique advantage of SANS and USANS in differentiating genuine colloidal structures in complex dispersions.

In summary, this work underscores the pivotal role of SANS and USANS in characterising biointerfaced colloids, particularly in estimating and revealing genuine structural features. This work also highlights the capability of SANS and USANS in uncovering novel colloidal systems with significant potential for biomedical applications and clinical translation.