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Insight on the myoglobin interaction with lipid bilayers within sponge phases using polarized neutron reflectometry

Lipid nanoparticles (LNPs) are dispersions of liquid crystalline phases [1,2] and feature different structure and curvature of the aqueous lipid interface, including inverse bicontinuous cubic (Q2), sponge phase (L3), or inverse hexagonal (H2) structure depending on the lipid composition [1-3]. In previous work, we have characterised the encapsulation of several industrially relevant proteins: aspartic protease (34kDa, used in cheese production), β -galactosidase (456kDa, used for lactose free dairy products), and sugar beet phytoalbumin (potential iron supplement replacement) into lipid sponge phases [4-7]. Here, the interaction between the protein and lipids is protein specific and can strongly affect both the structure and dynamics of the lipid nanoparticles. In the present study we focus on the heme-protein-lipid interaction with lipid bilayers, which is key for understanding the encapsulation of the iron binding heme-protein, such as myoglobin and phytoalbumin, in the sponge phase. This type of heme-bound iron can be used to treat anaemia instead of iron in organic salts that is conventionally used. Encapsulation is needed to prevent unwanted proteolytic and redox reactions. We have used sponge phase LNPs with diglycerolmonooleate (DGMO), glycerolmonooleate (GMO) and Polysorbate 80 (P80) as well as LNPs where DGMO was partially replaced with dioleoylglycerophosphocholine (DOPC) to form well defined lipid bilayers mimicking the lipid interface inside the sponge phase. To enhance the contrast and reduce the need for additional solvent contrasts we used silicon substrates with a switchable magnetic contrast layer (MCL) during polarised neutron reflectometry (PNR) experiments [8]. These substrates consisted of 10 nm Fe layer capped with 100 nm SiO₂ layer to protect the Fe layer against corrosion and gave excellent response to the spin state of the neutrons. The formed lipid bilayers had a very high coverage of about 90%, which allowed studies of the interaction of the protein with the lipid interface. The results show that myoglobin interacts so strongly with the lipid bilayer that it was mostly removed from the substrate. The presence of DOPC increased the stability of the bilayer so that remains intact with very low amounts of protein attached.

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Primary authors: LUCHINI, Alessandra (Università degli Studi di Perugia); MACHINGAUTA, Marshall (PhD student); KOEHLER, Sebastian; GILBERT, Jennifer; Mr YAKYMENKO, Ivan (Linköping University); BIRCH, Jens (Linköping University); Prof. JARREND AHL, Kenneth (Linköping University); COOPER, Joshaniel (European Spallation Source); Dr STENDAHL, Sjoerd (Uppsala University); Prof. LANGRIDGE, Sean (ISIS Neutron and Muon Source); Dr KINANE, Christy (ISIS Neutron and Muon Source); VOROBIEV, Alexei; DEVISHVILI, anton; HJÖRVARSSON, Björgvin (Uppsala University); NYLANDER, Tommy (Lund University)

Presenter: NYLANDER, Tommy (Lund University)

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