Nanoscale spherical-supported membranes as novel platforms for phage display screening

V. Vasilca[1], S. Chin[2], C. Rossant[2], E. England[2], D. Bannister[2], T. Wilkinson[2], S. Muench[1], S.A. Baldwin[1] and L.J.C. Jeuken[1]

[1]*The Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, United Kingdom - bs09vv@leeds.ac.uk*

[2]*MedImmune Ltd, ADPE, Granta Park, Cambridge, United Kingdom*

Abstract

Membrane proteins represent the majority of therapeutic targets for antibody-based drugs. Such biopharmaceuticals are routinely identified via phage display screening, but this requires the target proteins to be detergent-solubilised and, unfortunately, the detergent micelles can occlude important epitopes on the target's surface, thereby hampering the discovery of successful antibody binders. Here we show that, by reconstituting purified membrane protein targets into solid-supported membranes built on 100- and 200 nm silica nanoparticles, a versatile research platform is constructed for phage display screening, which presents the target membrane protein within its native-like lipid membrane environment. To provide proof-of-principle, silica nanospheres (100- and 200 nm in diameter) were covered with a POPC solid-supported bilayer lipid membrane (i.e. SSBLM) incorporating the bacterial nucleoside transporter NupC. The formation of the SSBLMs was confirmed via spectrofluorometric measurements using fluorescently-labelled lipids and antibody binding studies to SSBLM-embedded NupC (Fig. 1). The SSBLMs were then used to screen for designed ankyrin repeat protein (DARPin) binders against a His6-tagged version of NupC, with multiple DARPin clones now being assayed for genuine binding activity.

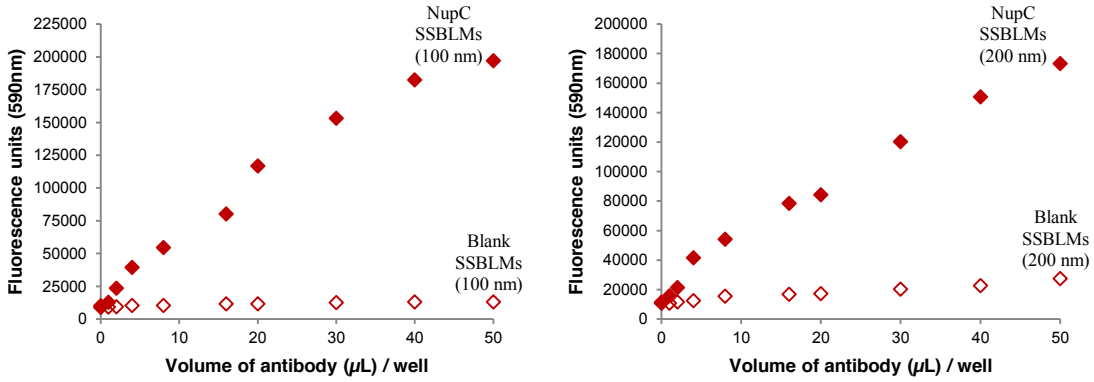

Figure 1. The fluorescence emission resulting from NupC (solid) and POPC-only (open) 100 nm SSBLM samples (left) and 200 nm SSBLM samples (right) using an ELISA-type antibody assay, confirming the presence of SSBLM-embedded NupC and the absence of aspecific binding of antibodies to the SSBLM.

Acknowledgements

We extend our grateful thanks to the Biotechnology and Biological Sciences Research Consortium (BBSRC) for their generous funding of this project and to MedImmune Ltd for providing additional funding and expertise, as well as access to their Cambridge site facilities.