A Fab-ulous approach for solving structures of small membrane proteins using cryo-EM

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Structure determination of membrane proteins is critical to the molecular understanding of many life processes, yet it has historically been a challenging endeavor. Over the past decade a number of techniques and reagents which facilitate membrane protein sample preparation for structural biology have been developed. Furthermore, advances in single-particle cryo-electron microscopy (cryo-EM) hardware and software have resulted in an ever-growing number of progressively smaller membrane protein structures. Despite this, determining the cryo-EM structure of small membrane proteins (<100 kDa) remains a technical challenge and several strategies are being developed to address this problem. One such example is complexation of target proteins with specific monoclonal antigen-binding fragments (Fab) to increase the particle size and provide fiducials outside of the transmembrane region for alignment.

Recently, we have used this approach to determine the cryo-EM structure of Major Facilitator Superfamily Domain containing 2A (MFSD2A; molecular weight of 58 kDa) to a resolution of 3.0 Å. MFSD2A is highly expressed within endothelial cells of the blood-brain barrier and the blood-retina barrier, where it facilitates Na+-dependent uptake of Docosahexaenoic acid in the form of lysophosphatidylcholine (LPC-DHA). Our structure captures the transporter in an inward facing conformation and features a large amphipathic cavity harboring the Na+-binding site and a bound lysolipid substrate, which we confirmed by native mass spectrometry. Together with functional analyses and molecular dynamics simulations, this structure reveals details of how MFSD2A interacts with substrates and how Na+-dependent conformational changes allow for their release into the membrane through a lateral gate.

Our work illustrates how Fabs can facilitate structure determination of small membrane proteins and provides insight into the molecular mechanism by which this atypical MFS transporter mediates uptake of LPC-DHA into the brain. Finally, we are hopeful that our findings will aid drug delivery by guiding structure-based LPC-prodrug design for neurotherapeutic intervention.