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Crystallization of membrane transport proteins in Lipidic Cubic Mesophase (LCP) aided by an engineered Green Fluorescent Protein Thermal Shift Screen (GFP-TS)

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Membrane protein crystals grown by in meso or the lipidic cubic phase (LCP) method generally produce higher resolution structures, as they have a lower solvent content (type I crystals) than those grown by traditional vapour-diffusion crystallization (type II crystals). To grow LCP crystals of membrane proteins with the synthetic lipid monoolein, the purified membrane protein solution is mixed with the molten monoolein in a weight ratio of 2:3. It can be very challenging to grow LCP crystals of membrane proteins, however, and while it is generally thought to be a fairly mild environment, the stabilities of different membrane proteins have not been extensively compared.
We engineered a Green Fluorescent Protein Thermal Shift Screen (GFP-TS) and use it to identify specific lipid for the bacteria sodium proton exchanger (NhaA) and also, specific ligand for the plant homologue of the human CMPsialic acid/CMP exchanger (SLC35A1). The former was crystallized and the structure solved by LCP in the presence of its specific lipid while the latter in the presence of its specific ligand at 2.3 and 2.8 Å respectively. No detectable crystal was obtained in the absence of either the lipid or ligand after extensive crystallization trials. The GFP-TS method should prove useful for screening lipid additives and small molecules not only to stabilize membrane proteins for structural determination by X-ray crystallography and single particle Cryo-EM but also to identify drug candidates of these medically relevant membrane proteins.

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