RAMP Lipid Screen Factsheet

Why use RAMP Lipid Screen?

- It is becoming increasingly clear that lipids play fundamental roles in membrane protein structure and function.
- Methods most commonly used for membrane protein isolation utilise detergents which remove most if not all the associated lipids.
- The RAMP Lipid screen allows high-throughput analysis of stability of detergent solubilised and isolated membrane protein in a wide lipid space (Cecchetti et al., 2021).
- The screen saves lab costs and reduces waste.
- Unsolubilised, the screen conditions are stable at -70 °C for at least a year.
- The lipids can be used for stability screening via nano-DSF. In the developers hands the screen was NOT suitable for use in thermal denaturation screening assays employing CPM (7-Diethylamino-3-(4'-Maleimidylphenyl)-4-Methylcoumarin) dye as a result of non-specific interactions between the dye and some of the lipids. This cross reactivity between lipids and the CPM dye has been previously reported (Sampson et al., 2021).
- The lipid in the screen is suitable for nanodisc and liposome reconstitution.

Applications

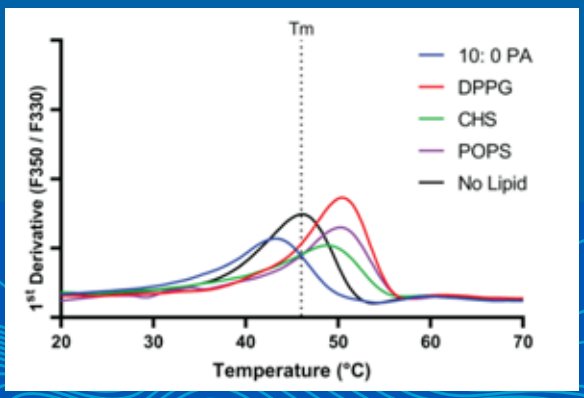
- Stability screening of membrane proteins in a range of lipid environments
- Ability to customise and build bespoke lipid compositions for assays
- Crystallisation trials of membrane proteins
- Source of lipids for nanodisc and liposome reconstitution

Figure 1. Schematic of the RAMP Lipid Screen

Each unique lipid condition is colour coded in the schematic and is provided in triplicate.

Figure 2. Lipid screening of UapA by nanoDSF

A) 1st derivative of the ratio of the fluorescence signals at 330 nm and 350 nm for UapA in several representative lipids from the screen. The Tm can be determined from the peak of the curve, where the change in fluorescence signal is fastest. The dashed line shows the Tm for the no added lipid control condition.



B) Changes in Tm for all lipids in the screen compared with the control of no added lipid. Lipids have been grouped and colour coded. The SEM (standard error of the mean) was calculated and plotted based on three technical repeats.

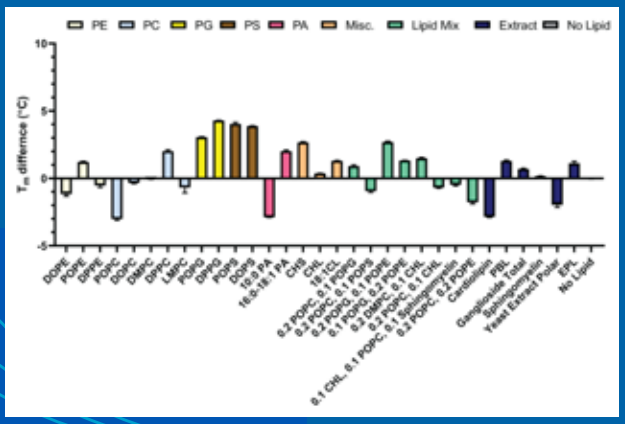
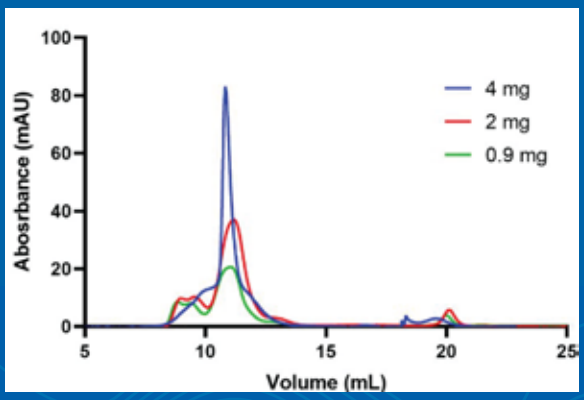
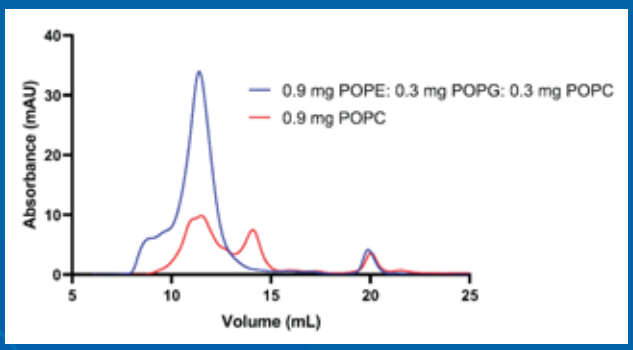


Figure 3. Overlayed size exclusion traces of a series of MmpL3-MP1E3D1 nanodiscs

A) Using POPC.



B) Using lipids sourced from the RAMP lipid screen. Nanodiscs were generated by mixing purified ΔC-MmpL3 (MmpL3) with membrane scaffold protein 1 E3D1 (MSP1E3D1) and lipid in a 1:10:1000 molar ratio.



See Data Sheet for MS analysis data for example lipid conditions stored as detergent solubilised solution over 3 months at a range of temperatures.

References

Cecchetti, C., Strauss, J., Stohrer, C., Naylor, C.E., Pryor, E.E., Hobbs, J.R., Tanley, S.W.M., Goldman, A. and Byrne, B. (2021). A novel high-throughput screen for identifying lipids that stabilise membrane proteins in detergent based solution. PLOS ONE, 16(7), pp.e0254118–e0254118. doi:https://doi.org/10.1371/journal.pone.0254118.

Sampson, D., Cristina Fàbregas Bellavista, Stewart, M.J. and Mulligan, C. (2021). Thermostability-based binding assays reveal complex interplay of cation, substrate and lipid binding in the bacterial DASS transporter, VcINDY. Biochemical Journal. [online] doi:https://doi.org/10.1042/bcj20210061.