Molecular Dimensions

A CALIBRE SCIENTIFIC COMPANY

anatrace

A CALIBRE SCIENTIFIC COMPANY

User Guide

RAMP Lipid Screen

Detergent based extraction of integral membrane proteins (MPs) is essential for many downstream analyses including structural studies. However, MPs are relatively unstable in detergent-based solutions, as detergent extraction removes most, if not all, associated membrane lipids, compromising protein structure and function. Styrene maleic acid lipid particles (SMALPs), an alternative approach, extract MPs complexed with their associated membrane lipids but their size and heterogeneity makes them unsuitable for many downstream analyses. In addition, characterisation of MP-lipid interactions is essential for full understanding of the physiological function of any MP.

A simple and potentially effective way to restore structural integrity and protein function is the relipidation of integral MPs after detergent extraction. However, identifying which lipids are suitable is typically time-consuming and requires substantial investment in a range of lipids. Many of these lipids will have no measurable effect on the MP, meaning that both money and chemicals are wasted. Although lipidomics analysis is possible on purified protein, it only identifies which lipids co-purify with the protein and does not indicate whether these are forming interactions with the protein and if the interactions are stabilising. Furthermore, lipidomics analysis requires purchase and analysis of several lipids as internal standards. Therefore, a cost-effective, high-throughput lipid screen would be a game-changer, by allowing researchers to identify lipids relevant for their target protein easily and efficiently.

The RAMP Lipid screen is a 96-well screen containing 31 lipid conditions (each in triplicate) for high-throughput identification of lipids that stabilise membrane proteins. The screen contains both individual lipids and lipid mixtures and is designed to give both maximum information about the lipids specific to an individual protein and reduce both the cost and reagent waste associated with lipid screening,

Features of the RAMP Lipid screen

- A wide lipid space covered including commonly used individual lipids and lipid mixtures.
- The lipid plates contain 31 lipids in triplicate along with a blank for a control.
- Sufficient lipid in each well for 3 proteins to be analysed by nano-DSF in triplicate in 1 detergent.
- The second/third lipid repeat allows flexibility of use in terms of detergent and allows longer term storage at either -70 °C or -20 °C for up to a year in the unsolubilised state.
- The lipids are stable for at least 3 months at -70 °C or -20 °C and for up to two weeks at 4°C following solubilisation in 3% DDM.*

*NB MS analysis indicates that it is possible to store the lipids in detergent at 4 °C for up to a month with no discernible effects on the lipid. However, we noted evaporation of the solvent over this timescale and thus a likely associated alteration in lipid concentration.

1.0 Screen layout

Figure 1. Schematic of the RAMP Lipid Screen.

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

Calibre SCIENTIFIC

Molecular Dimensions

Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257

anatrace

434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280

User Guide

RAMP Lipid Screen

2.0 Preparation of the lipid screen for nanoDSF and nanoDSF analysis of protein stability

The lipid screen lipids were solubilised overnight in 50 µL 3% detergent (the specific detergent used depends on the detergent used in protein purification) at 25 °C, with gentle mixing (~80 RPM) to give a lipid concentration of 6 mg / mL. Solubilisation was aided by aspiration using a multichannel pipette. Based on the below nanoDSF protocols, each replicate lipid condition of the screen provided enough lipid to carry out analysis of 3 proteins with 3 technical repeats per protein (assuming the 3 proteins are in the same detergent solution).

The stability of a range of membrane proteins in the different lipid conditions was assessed using nano-DSF. MmpL3 from Mycobacterium smegmatis and UapA-G411Vd1-11 from Aspergillus nidulans were expressed in E.coli and Saccharomyces cerevisae respectively in the Byrne laboratory at Imperial College London. VcINDY and LacY were kind gifts from members of the Membrane Protein Lab at Diamond Light Source (Harwell, UK). We are very grateful for access to the Nanotemper Prometheus at the Membrane Protein Laboratory for nano-DSF analysis (under user access: MX39208). Protein samples (8–10 µL) were transferred into standard-grade Prometheus NT.48 capillaries (SKU: PR-C002, Nanotemper). The capillaries were then loaded into a Prometheus NT.48.

A preliminary discovery scan was performed to optimise the device's excitation power, ensuring a fluorescence signal at 330 nm within the range of 5,000–15,000 RFU for all samples in the run. An initial low-resolution scan was conducted to evaluate sample quality and melting behaviour. To determine suitable protein concentrations for nanoDSF experiments, purified protein was diluted in the relevant buffer* to various concentrations ranging from 1.0 mg/mL to 0.1 mg/mL. Approximately 10 µL of each concentration was loaded into capillaries and subjected to a temperature ramp from 20 °C to 95 °C at a rate of 3 °C/min. Based on the results of the scan, a protein concentration was chosen based on the shape of the curve of the melting scan, choosing the lowest concentration that yielded a well-defined and interpretable melting curve, with a clear unfolding transition and minimal baseline noise.

*Buffer conditions: UapA (25 mM Tris-HCl pH 7.5, 150 mM NaCl, glycerol 2 %, DDM 0.03 %), MmpL3 and LacY (20mM Tris HCl pH 8.0, 150 mM NaCl, 10% glycerol, 0.03% DDM).

VcINDY (20mM Tris HCl pH 8.0, 150 mM NaCl, 10% glycerol, 0. 0.3% DM).

Following this, scans were conducted over a 20 - 95 °C ramp, at 1 °C / minute for higher peak resolution. A 1 x mix was prepared for each reaction, allowing for three technical replicates for each lipid from the screen for each protein. The mix consisted of 11 µL of solubilised lipid, 11 µL of 3 x protein buffer, and a variable volume of MQ H_2O and protein to achieve the desired protein concentration. From this mixture, approximately 10 µL was aliquoted into each capillary tube for analysis. Based on this experimental set up, in a single well of the screen there is enough lipid for 3 technical repeats of 3 different proteins (provided they are in the same detergent).

During the scan, fluorescence emission at 330 nm (F330), 350 nm (F350), the F350:F330 ratio, and light scattering signals were recorded. For each protein, the impact of all lipids on stability was compared to a lipid-free reference sample.

Processed data was exported from the NanoTemper software and analysed in GraphPad Prism. The first derivative of the fluorescence intensity changes at 530 nm and 550 nm was used to identify transitions in the protein structure. For scattering data, the peak of the first derivative was analysed to detect structural changes in the protein sample. Additionally, the first derivative of the scattering signal provided insight into the midpoint or the most rapid point of aggregation. To evaluate the stabilising effect of specific lipids on detergent-protein micelles, the difference in Tm (°C) between the blank sample (no lipid) and lipid-containing samples was calculated, facilitating easier interpretation of each lipid's impact.

User Guide

RAMP Lipid Screen

3.0 Use of the lipids in nanodisc preparation

The lipid screen can be used as a source of lipids for the production of nanodiscs (NDs) containing a protein of interest for further study. In this case, POPC either from the lipid screen or from an existing PC lipid stock in the Byrne group or a mix of 0.9 mg POPE, 0.3 mg POPG and 0.3 mg POPC from the lipid screen were used to encapsulate ΔC-MmpL3. The lipid mix mimics the ratio of lipids reported for MD simulation analysis of ΔC-MmpL3 in a membrane environment (Cioccolo et al, 2024). The lipid screen was solubilised in 7.5% DDM overnight, which was then used to produce MSP1E3D1_ ΔC-MmpL3 nanodiscs.

Nanodiscs containing purified ΔC-MmpL3, using POPC, were generated as previously described (Cioccolo et al., 2024). In brief, ΔC-MmpL3 purified using immobilised metal affinity chromatography and at a concentration of 10 mg/ mL was mixed with membrane scaffold protein 1 E3D1 (MSP1E3D1) and lipid in a 1:10:1000 molar ratio. The volumes used were reduced such that a nanodisc preparation could be made using 0.9 mg of the lipid as a proof of concept (this miniaturisation is shown in Figures 2, 3, and 4). The 0.9 mg of lipid is equivalent to the combined amount of POPC in the 3 replicate wells of a single lipid screen. The mixtures were incubated at 4 °C overnight. Following this, activated Biobeads SM2 (400 mg Biobeads: 350 µg of protein) were added and the solution incubated for a further 2 h at 4 °C.

The sample was then centrifuged to pellet the beads, and the soluble material loaded onto a Superdex® 200 Increase 10/300 GL (Cytiva, US), pre-equilibrated with SEC buffer (20 mM Tris-HCl pH 8, 150 mM NaCl). The peak fractions were analysed by SDS-PAGE on a 10% TGX gel (Biorad) (Figure 6, 7, 8, 9, and 10). Figures 9 and 10 present the SEC and SDS-PAGE analysis of the nanodiscs reconstituted using the screen lipids. The ΔC-MmpL3 encapsulated in nanodiscs elutes at ~11 mls (a small amount of protein is lost to aggregation, ~8 mls). The same peak is eluted in each case.

4.0 Mass spectrometry analysis

Previous iterations of the screen (not solubilised in detergent) have been stored under N2 gas at -20 °C for up to one year.

For the new iteration of the screen, the stability of solubilised lipids was analysed at 3 storage temperatures over the period of 3 months. Figure 11 shows a workflow describing the steps of storage and analysis at the 3 time points.

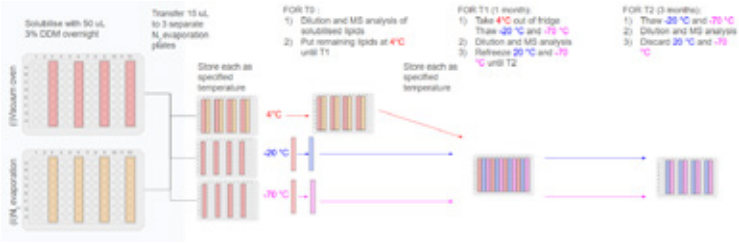


Figure 1.1

Schematic showing the sampling, storage and analysis schedule for mass spectrometry of the lipid screen conditions.

Lipids were solubilised as above in 50 µL 3% DDM, with overnight incubation at 25 °C at 80 RPM. They were then individually diluted 1:100 in MilliQ water. Lipids were analysed by mixing 0.4 µL of the diluted lipids with 0.4 µL of the MBT Lipid Xtract™ Kit (Bruker). The samples were left to dry at RT for 30 minutes before spectra were collected on a pre-calibrated MALDI Biotyper® (Bruker). Data was analysed using FlexAnalysis (Bruker).

The T0 time point was analysed immediately after solubilisation of the lipid screen overnight. Solubilised lipid was then aliquoted into 3 different plates, and then stored at either 4 °C, -20 °C, or -70 °C. At 1 month (T1) all 3 temperatures were analysed, and at 3 months (T2) only – 20 °C and -70 °C were analysed.

User Guide

RAMP Lipid Screen

5.0 Results

5.1 nanoDSF

Intrinsic fluorescence at 330 nm and 350 nm, reflecting local alterations in the tryptophan (and tyrosine) environment during the unfolding process. The unfolding behaviour of the relipidated protein was compared to a reference sample (the same detergent solubilised and purified protein but with no added lipids) by analysing differences in the apparent melting temperature (ΔTm). The RAMP lipid screen can be used as a means of investigating lipid-mediated stabilisation of membrane proteins, using nanoDSF. The no added lipid/ reference Tm values for each protein were as follows:

UapA 46.0 °C ± 0.04 °C
MmpL3 45.4 °C ± 0.28°C
VcINDY 62.7 °C ± 0.13 °C

LacY did not produce any clear fluorescence transitions based on the 1st derivative of the ratio of the 330 nm and 350 nm readings. However clear peaks were seen for the 1st derivative of the scattering data, as well as the 1st derivative of the 330 nm signal. Based on this, there was a rapid onset of aggregation at 57.3 °C ± 1.02 °C for LacY with no added lipids from the screen. For LacY, the individual 330 nm and 350 nm 1st derivatives alone were found to be more informative. Based on the 330 nm 1st derivative, we see a no lipid Tm of 51.4 ± 0.195 °C.

UapA

As validation for the screen, one of the proteins used was UapA-G411V1-11. This protein was tested with a previous iteration of the screen as described previously (Cecchetti et al, 2021), and its inclusion here allows a direct comparison between the data obtained using both screen versions. Overall, the data was remarkably similar given that different individuals purified the protein several years apart, the lipids used in the different screen versions were from varying batches, and different Nanotemper instruments were used (Figure 2).

Phosphatidylglycerol, phosphatidylserine and CHS were found to be the most stabilising lipids in both screens. DPPG and POPS resulted in an increase in Tm of 4.30 ± 0.01 °C, and 4.04 ± 0.11 °C, respectively compared to the no added lipid control.

PC lipids, barring POPC and DPPC appeared to have minimal impact, with DPPC stabilising the protein. POPC was found to be the most destabilising condition, reducing the Tm by 3.02 ± 0.09 °C. Additionally, the lipid mixes containing POPC in combination with POPS was also found to be destabilising to the UapA; whereas POPC in combination with POPG was found to stabilise the protein micelle complex. This slight stabilisation is possibly due to a combination of the destabilising effects of the POPC, and the stabilising effects of the POPG, resulting in a mid-point of slight stabilisation. These findings agree with previous work (Pyle et al, 2018; Cecchetti et al, 2021).

Some minor differences across the two screens were picked up. For example, 10:0 PA destabilises the protein, but POPA stabilises the protein which is in contrast to the 2021 screen which showed that both conditions destabilised UapA. Additionally, 18:1 CL destabilised the protein in the original screen, whereas this provided some stability in the 2024 version of the screen.

Of the new conditions introduced into the current screen, the yeast polar extract was found to destabilise the protein-lipid micelles, despite UapA being a fungal protein. A similar trend was seen with the cardiolipin extract which also destabilised the protein. EPL and PBL (PC, PE and PS lipids at ~ 10-15 % each) are both in agreement with the original screen, slightly stabilising the proteins.

Taken together the UapA stability analysis indicate that there is marked reproducibility in the data obtained even several years apart, giving confidence that the findings are reliable. Where differences were observed it is worth carrying out additional analysis.

User Guide

RAMP Lipid Screen

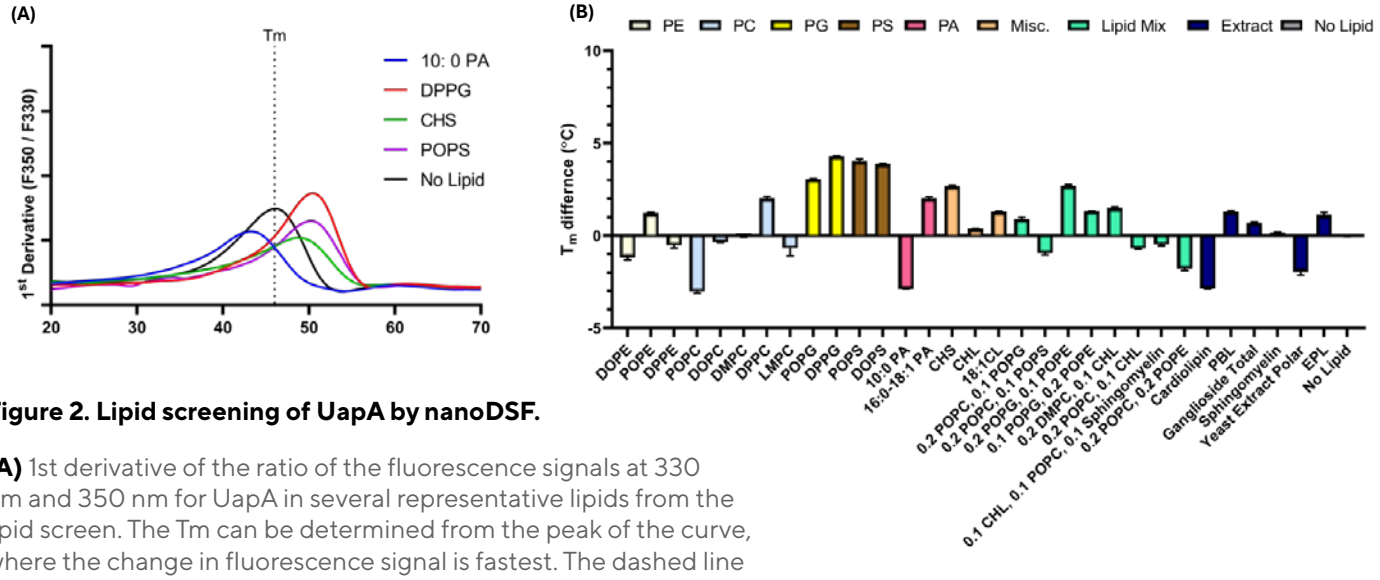


Figure 2. Lipid screening of UapA by nanoDSF.

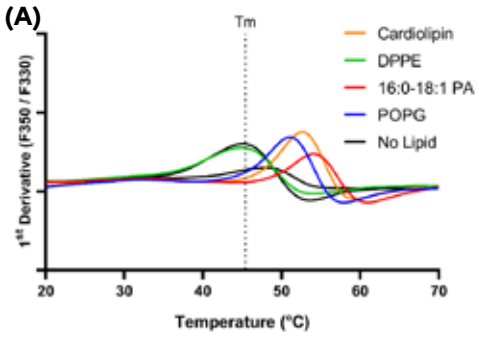
(A) 1st derivative of the ratio of the fluorescence signals at 330 nm and 350 nm for UapA in several representative lipids from the lipid screen. The Tm can be determined from the peak of the curve, where the change in fluorescence signal is fastest. The dashed line shows the Tm for the no added lipid 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 was calculated and plotted based on three technical repeats.

MmpL3

All lipids stabilised MmpL3 with the exception of DPPE and CHL. DPPE appeared to be slightly destabilising, reducing the Tm by less than 1C compared with the control (Figure 3). The lipids16:0-18:1 PA (POPA), 18:1 CL and Cardiolipin were the most stabilising conditions, increasing the Tm by 8.53 ± 0.07 °C, 7.21 ± 0.05 °C and 7.11 ± 0.01 °C, respectively. Phosphatidylglycerol and phosphatidylserine lipids were also highly stabilising.

It is possible that the general trend of all classes resulting in higher Tms could be due to a non-specific interaction between the lipids and the protein-micelle complex. Previously, MmpL3 purified using the same method (Cioccolo et al., 2024) was found to co-purify with bound lipids as determined by lipidomics analysis.



This may result in all lipid binding loci on MmpL3's structure being occupied, and therefore, the protein-specific effects of adding exogenous lipids would be mitigated by the presence of pre-bound lipids on MmpL3. Thus, a more micelle-focused effect of the lipids could take precedence over protein specific lipid effects. It is possible that the lipidation of detergent micelles reduces the micelle curvature, leading to increased protein stability as previously reported (Heerklotz, 2008).

Calibre SCIENTIFIC
Molecular Dimensions
Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257
anatrace
434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280

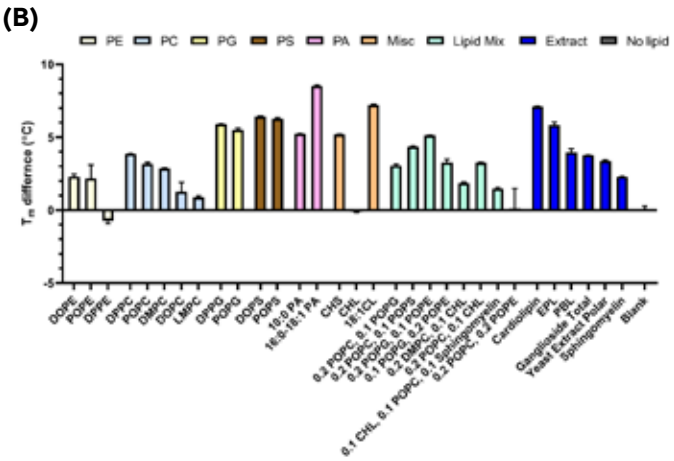
User Guide

RAMP Lipid Screen

Figure 3. Lipid screening of MmpL3 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 lipid 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 was calculated and plotted based on three technical repeats.



vcINDY

We used the 1st derivative of the ratio of the 330 nm and 350 nm fluorescence signals to infer changes to the Tm following addition of lipids to purified vcINDY (Figure 4). Similar to MmpL3, we saw a general stabilising effect on the vcINDY-micelle complex following exposure to the different lipids in the screen, with some notable exceptions. PE lipids appeared to have a minimal impact, slightly increasing the Tm. DPPG was the most stabilising condition, resulting in an increase in Tm of 8.3 ± 0.5 °C.

The CL 18:1 and Cardiolipin extract and the phosphatidic acid lipids, namely 10:0 PA and 16:0- 18:1 PA, were the only components of the screen to result in a lower Tm compared with the no added lipid control.

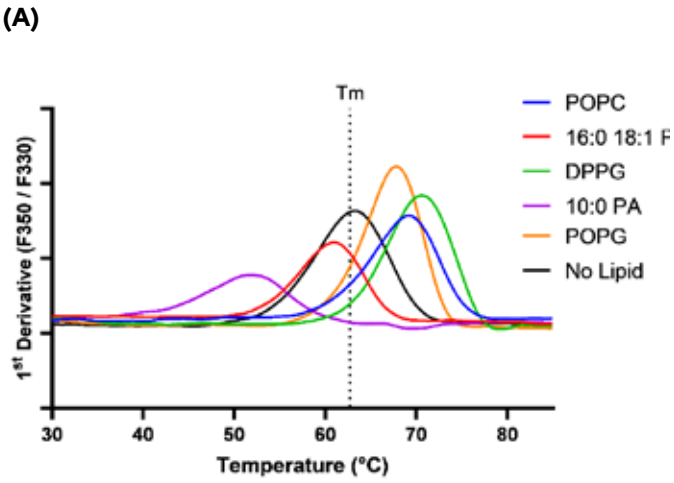
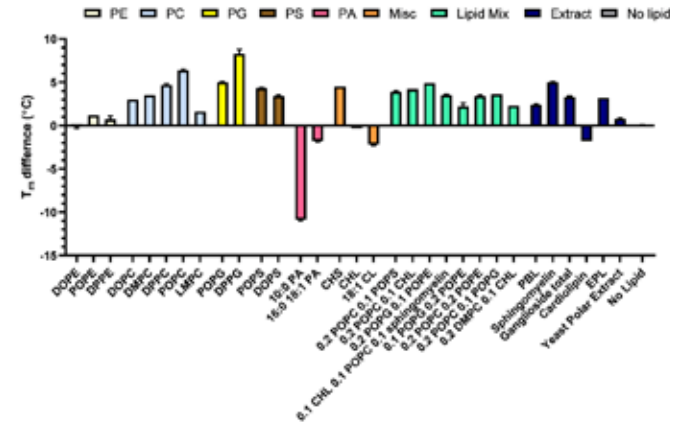


Figure 4. Lipid screening of vcINDY by nanoDSF.

(A) 1st derivative of the ratio of the fluorescence signals at 330 nm and 350 nm for UapA in several representative lipids from the lipid 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 was calculated and plotted based on three technical repeats.



Calibre SCIENTIFIC
Molecular Dimensions
anatrace
Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257
434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280

User Guide

RAMP Lipid Screen

LacY

For LacY analysis we used the 1st derivative of the 330 nm fluorescence reading, as well as the 1st derivative of the scattering data to inform structural changes (Figure 5). Overall, the LacY-micelle complex was generally destabilised by the lipids. PE lipids were all destabilising, as were all PC lipids with the exception of LMPC, which induced a very small, ~1 °C change in Tm. In addition, PA lipids, CL18:1 and Cardiolipin extract also led to lower Tms, with the latter being the most destabilising, resulting in a reduction in Tm of ~7 °C. These findings are in contrast to previous data reporting a key role for PE in stabilising specific conformational states of LacY (Martens et al, 2018).

PG lipids stabilised the protein as, somewhat surprisingly, did CHS which was the most stabilising, increasing the Tm by ~4 °C.

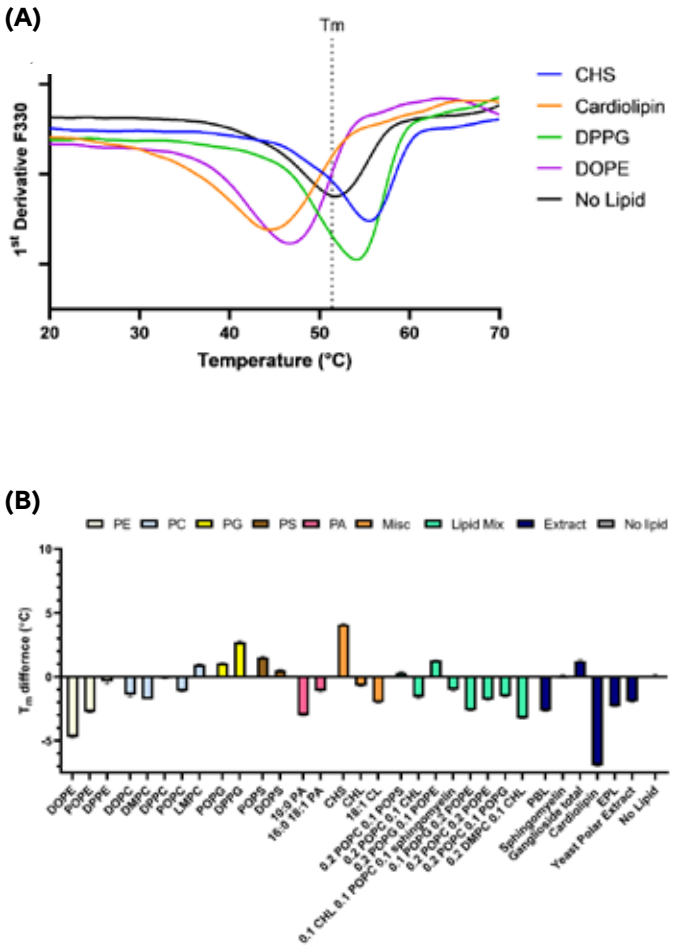


Figure 5. Lipid screening of LacY by nanoDSF.

(A) 1st derivative of the ratio of the fluorescence signals at 330 nm and 350 nm for UapA in several representative lipids from the lipid 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 was calculated and plotted based on three technical repeats.

User Guide

RAMP Lipid Screen

5.2 Nanodisc Preparations

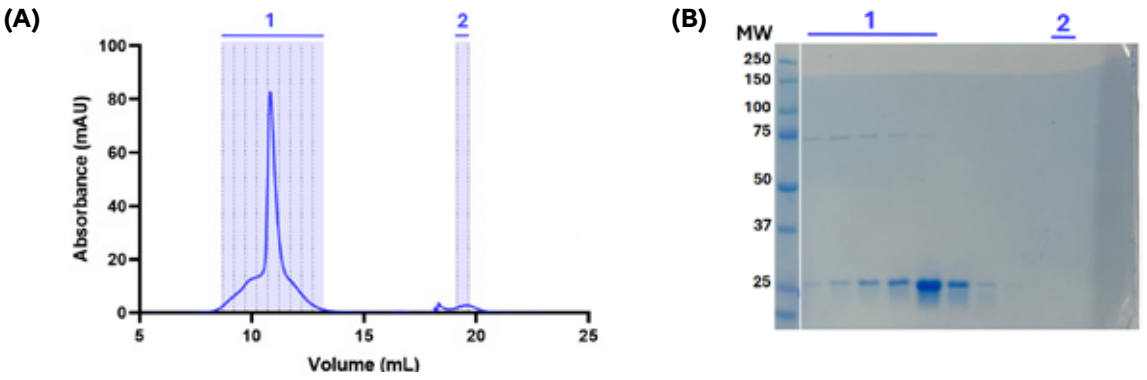


Figure 6. MmpL3-MP1E3D1 nanodiscs using 4 mg POPC.

(A) Size exclusion trace indicating the main protein peak (1) and a later peak (2) that was also analysed by SDS-PAGE **(B)** corresponding SDS-PAGE gel of highlighted fractions from SEC. 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.

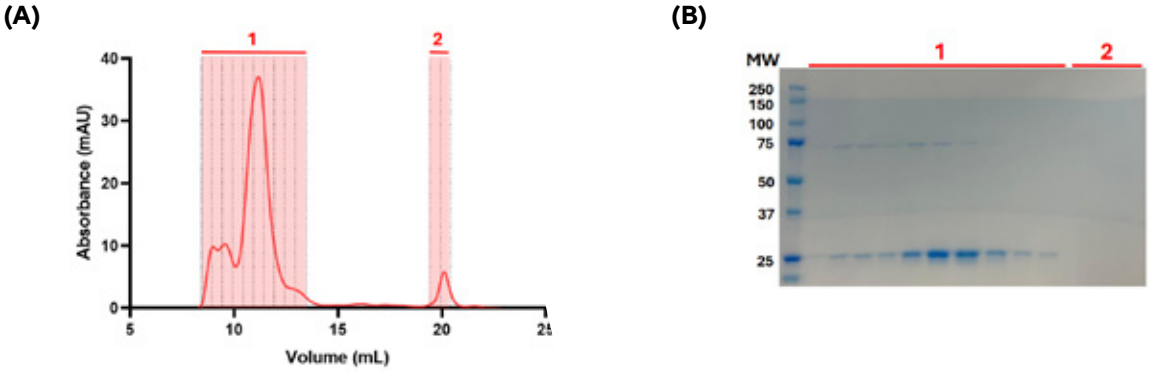


Figure 7. MmpL3-MP1E3D1 nanodiscs using 2 mg POPC.

(A) Size exclusion trace indicating the main protein peak (1) and a later peak (2) that was also analysed by SDS-PAGE **(B)** corresponding SDS-PAGE gel of highlighted fractions from SEC. 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.

User Guide

RAMP Lipid Screen

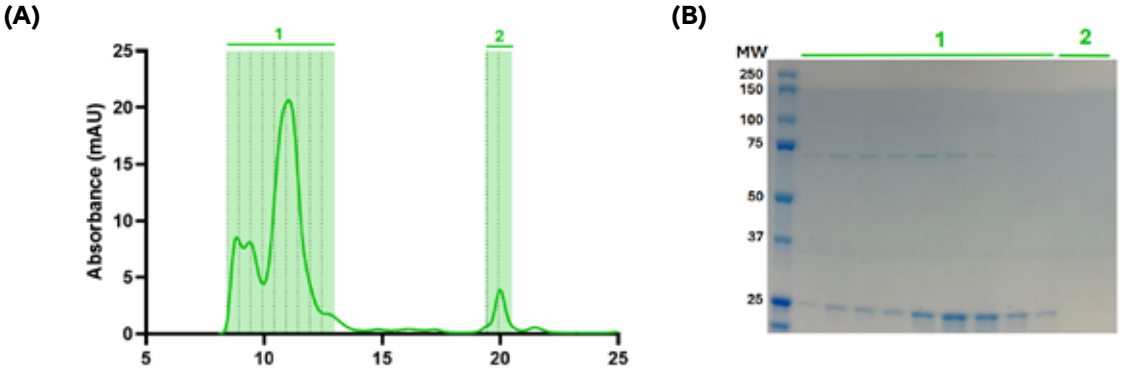


Figure 8. MmpL3-MP1E3D1 nanodiscs using 0.9 mg POPC.

(A) Size exclusion trace indicating the main protein peak (1) and a later peak (2) that was also analysed by SDS-PAGE **(B)** corresponding SDS-PAGE gel of highlighted fractions from SEC. 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.

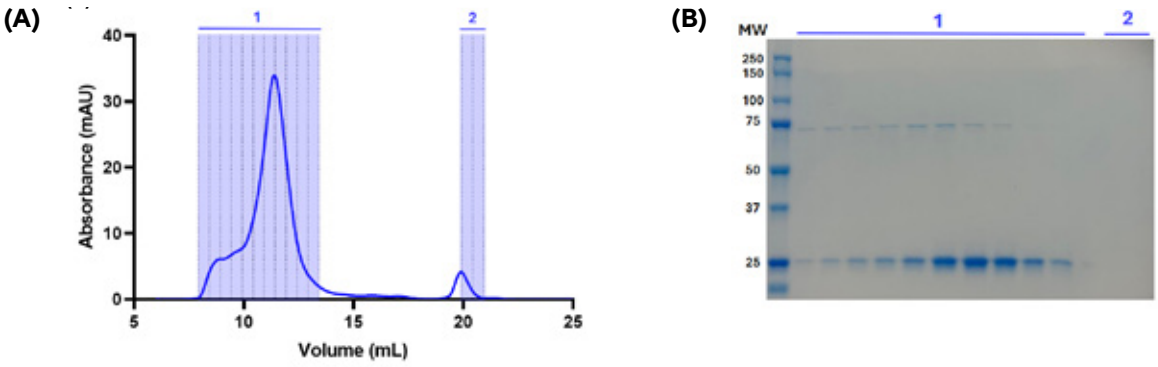


Figure 9. MmpL3-MP1E3D1 nanodiscs using 0.9 mg POPE, 0.3 mg POPG, and 0.3 mg POPC sourced from the RAMP lipid screen

(A)) Size exclusion trace indicating the main protein peak (1) and a later peak (2) that was also analysed by SDS-PAGE **(B)** corresponding SDS-PAGE gel of highlighted fractions from SEC. 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.

User Guide

RAMP Lipid Screen

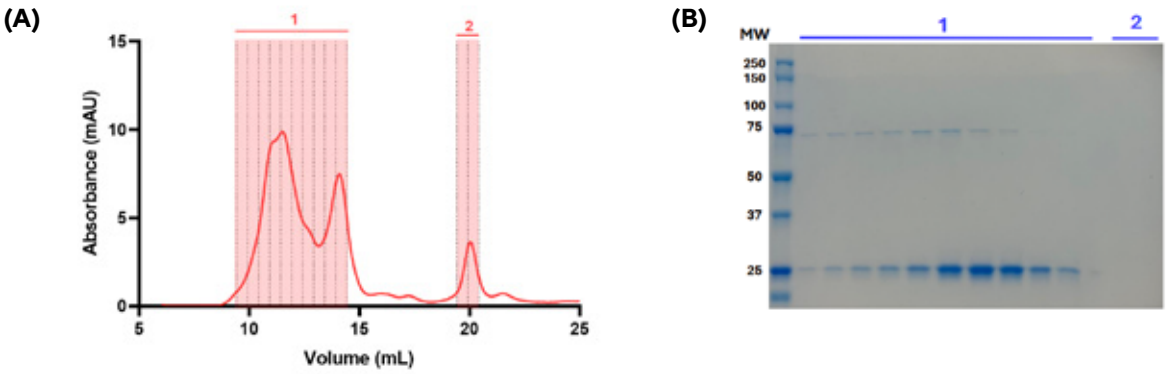


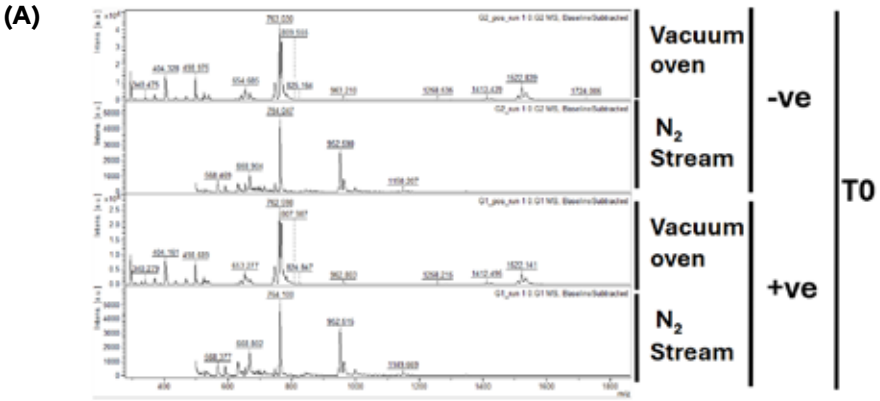
Figure 10. MmpL3-MP1E3D1 nanodiscs using 0.9 mg POPC sourced from the RAMP lipid screen

(A) Size exclusion trace indicating the main protein peak (1) and a later peak (2) that was also analysed by SDS-PAGE **(B)** corresponding SDS-PAGE gel of highlighted fractions from SEC. 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.

5.3 Mass Spectrometry

Spectra were qualitatively analysed, comparing between both timepoints and between freezing temperatures. Very little quantitative difference was found between the temperatures and time points; however, some evaporation was seen for 4 °C between T0 and T1. Based on this, we would recommend storing the screen for a maximum of 2 weeks at 4 °C, and freezing for longer periods. As a representative of this, we show qualitative similarities between the lipid condition 0.2 POPC, 0.1 POPS at 4 °C, -20 °C and – 70 °C, at T0, T1 and T2 in Figure 12. Spectra for all lipids and timepoints taken is available on request.

POPS can be seen in negative ionisation mode at m/z of 764, and POPC can be seen in positive ionisation mode at an m/z of 759. Both lipids were visible across all 3 timepoints and produced qualitatively comparable spectra. There was also little difference found between spectra stored at -20 °C and – 70 °C.



User Guide

RAMP Lipid Screen

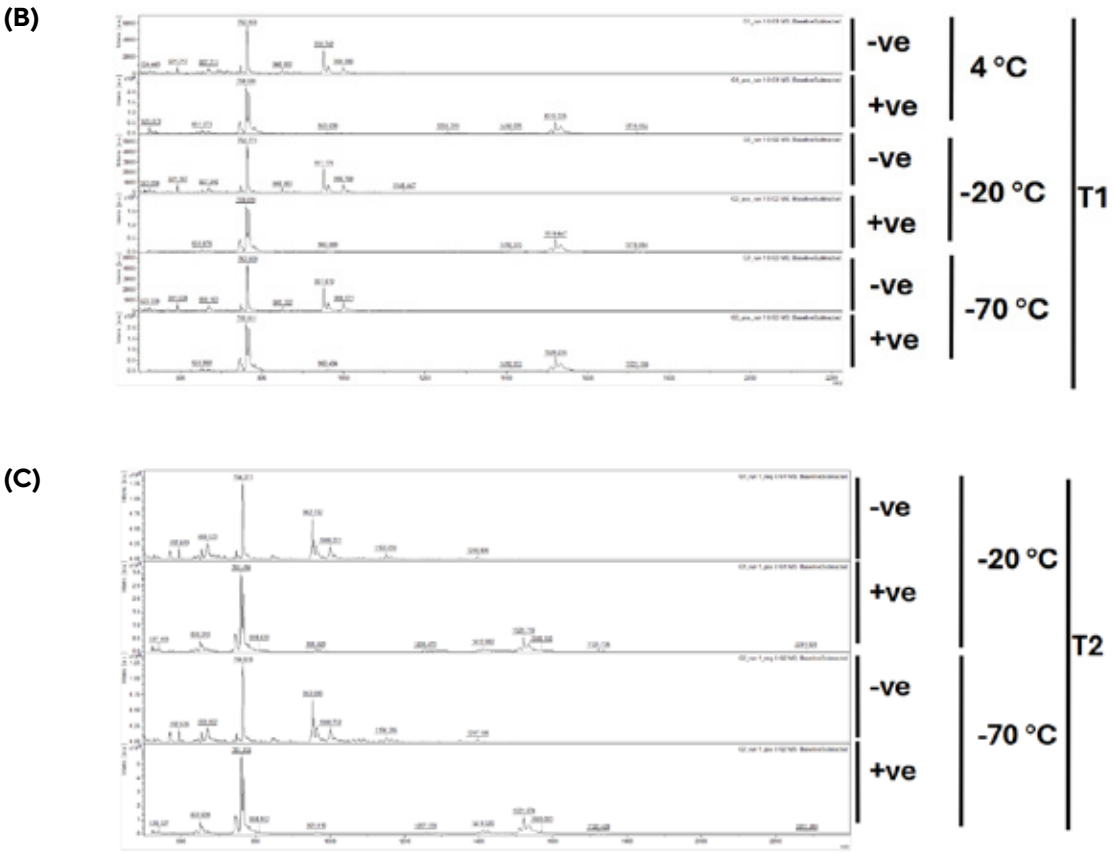


Figure 12. Representative mass spectra showing analysis of the 0.2 POPC, 0.1 POPS from the RAMP Lipid Screen, across 3 time points and storage at 3 temperatures.

(A) shows lipids at T0 (freshly solubilised), **(B)** at T1 (following 1 month storage) and **(C)** T2 (following 3 months storage). All lipids were measured on a MALDI Biotyper® (Bruker). Data was analysed using FlexAnalysis (Bruker) in both positive and negative ionisation modes. Spectra were visually compared to investigate the effects of different storage temperatures and durations on the condition of the lipids.

User Guide

RAMP Lipid Screen

6.0 Screen conditions

Table

Well	lipid I	mg	lipid II	mg	lipid III	mg	molar ratio
A1	DOPE	0.3	-				1
A2	DOPE	0.3	-				1
A3	DOPE	0.3	-				1
A4	POPE	0.3	-				1
A5	POPE	0.3	-				1
A6	POPE	0.3	-				1
A7	DPPE	0.3	-				1
A8	DPPE	0.3	-				1
A9	DPPE	0.3	-				1
A10	POPC	0.3	-				1
A11	POPC	0.3	-				1
A12	POPC	0.3	-				1
B1	DOPC	0.3	-				1
B2	DOPC	0.3	-				1
B3	DOPC	0.3	-				1
B4	DMPC	0.3	-				1
B5	DMPC	0.3	-				1
B6	DMPC	0.3	-				1
B7	DPPC	0.3	-				1
B8	DPPC	0.3	-				1
B9	DPPC	0.3	-				1
B10	LMPC	0.3	-				1
B11	LMPC	0.3	-				1
B12	LMPC	0.3	-				1
C1	POPG	0.3	-				1
C2	POPG	0.3	-				1
C3	POPG	0.3	-				1
C4	DPPG	0.3	-				1
C5	DPPG	0.3	-				1

Molecular Dimensions

A CALIBRE SCIENTIFIC COMPANY

anatrace

A CALIBRE SCIENTIFIC COMPANY

User Guide

RAMP Lipid Screen

Well	lipid I	mg	lipid II	mg	lipid III	mg	molar ratio
C5	DPPG	0.3	-				1
C6	DPPG	0.3	-				1
C7	POPS	0.3	-				1
C8	POPS	0.3	-				1
C9	POPS	0.3	-				1
C10	DOPS	0.3	-				1
C11	DOPS	0.3	-				1
C12	DOPS	0.3	-				1
D1	10:0 PA	0.3	-				1
D2	10:0 PA	0.3	-				1
D3	10:0 PA	0.3	-				1
D4	16:0-18:1 PA	0.3	-				1
D5	16:0-18:1 PA	0.3	-				1
D6	16:0-18:1 PA	0.3	-				1
D7	EPL	0.3	-				1
D8	EPL	0.3	-				1
D9	EPL	0.3	-				1
D10	CHS	0.3	-				1
D11	CHS	0.3	-				1
D12	CHS	0.3	-				1
E1	CHL	0.3	-				1
E2	CHL	0.3	-				1
E3	CHL	0.3	-				1
E4	Cardiolipin*	0.3	-				1
E5	Cardiolipin*	0.3	-				1
E6	Cardiolipin*	0.3	-				1
E7	18:1CL	0.3	-				1
E8	18:1CL	0.3	-				1
E9	18:1CL	0.3	-				1
E10	PBL	0.3	-				1
E11	PBL	0.3	-				1
E12	PBL	0.3	-				1

Calibre SCIENTIFIC

Molecular Dimensions

Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257

anatrace

434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280

Molecular Dimensions

A CALIBRE SCIENTIFIC COMPANY

anatrace

A CALIBRE SCIENTIFIC COMPANY

User Guide

RAMP Lipid Screen

Well	lipid I	mg	lipid II	mg	lipid III	mg	molar ratio
F1	Sphingomyelin*	0.3	-				1
F2	Sphingomyelin*	0.3	-				1
F3	Sphingomyelin*	0.3	-				1
F4	Ganglioside Total*	0.3	-				1
F5	Ganglioside Total*	0.3	-				1
F6	Ganglioside Total*	0.3	-				1
F7	Yeast Extract Polar*	0.3	-				1
F8	Yeast Extract Polar*	0.3	-				1
F9	Yeast Extract Polar*	0.3	-				1
F10	POPC	0.18	POPG	0.06	POPE	0.06	3:1:1
F11	POPC	0.18	POPG	0.06	POPE	0.06	3:1:1
F12	POPC	0.18	POPG	0.06	POPE	0.06	3:1:1
G1	POPC	0.24	POPS	0.06	-		4:1
G2	POPC	0.24	POPS	0.06	-		4:1
G3	POPC	0.24	POPS	0.06	-		4:1
G4	POPG	0.23	POPE	0.07	-		3:1
G5	POPG	0.23	POPE	0.07	-		3:1
G6	POPG	0.23	POPE	0.07	-		3:1
G7	POPG	0.07	POPE	0.23	-		1:3
G8	POPG	0.07	POPE	0.23	-		1:3
G9	POPG	0.07	POPE	0.23	-		1:3
G10	DMPC	0.23	CHL	0.07	-		2:1
G11	DMPC	0.23	CHL	0.07	-		2:1
G12	DMPC	0.23	CHL	0.07	-		2:1
H1	POPC	0.27	CHL	0.03	-		5:1
H2	POPC	0.27	CHL	0.03	-		5:1
H3	POPC	0.27	CHL	0.03	-		5:1
H4	CHL	0.1	POPC	0.1	Sphingomyelin	0.1	2:1:1.5
H5	CHL	0.1	POPC	0.1	Sphingomyelin	0.1	2:1:1.5
H6	CHL	0.1	POPC	0.1	Sphingomyelin	0.1	2:1:1.5
H7	POPC	0.15	POPE	0.15	-		1:1
H8	POPC	0.15	POPE	0.15	-		1:1
H9	POPC	0.15	POPE	0.15	-		1:1
H10	Blank	-					0
H11	Blank	-					0
H12	Blank	-					0

*The conditions are new additions compared to the original version of the screen as described in Cecchetti *et al.*, (2021).

Calibre SCIENTIFIC

Molecular Dimensions

Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257

anatrace

434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280

User Guide

RAMP Lipid Screen

7.0 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.

Cioccolo, S., Barritt, J.D., Pollock, N., Hall, Z., Babuta, J., Sridhar, P., Just, A., Morgner, N., Dafforn, T., Gould, I. and Byrne, B. (2024). The Mycobacterium lipid transporter MmpL3 is dimeric in detergent solution, SMALPs and reconstituted nanodiscs. RSC Chemical Biology, [online] 5(9), pp.901–913. doi:https://doi.org/10.1039/d4cb00110a.

Heerklotz H. (2008) Interactions of surfactants with lipid membranes. Q. Rev. Biophys. 41, 205–264. https://doi.org/10.1017/S0033583508004721.

Pyle, E., Kalli, A.C., Amillis, S., Hall, Z., Lau, A.M.,

Hanyaloglu, A.C., Diallinas, G., Byrne, B. and Politis, A. (2018). Structural Lipids Enable the Formation of Functional Oligomers of the Eukaryotic Purine Symporter UapA. Cell Chemical Biology, 25(7), pp.840-848.e4. doi:https://doi.org/10.1016/j.chembiol.2018.03.011.

Martens, C., Shekhar, M., Borysik, A. J., Lau, A. M., Reading, E., Tajkhorshid, E., Booth, P. J., and Politis, A. (2018) Direct Protein-Lipid Interactions Shape the Conformational Landscape of Secondary Transporters. Nat. Commun. 2018, 9, 4151.

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.

FAQ

Q: What is the RAMP Lipid Screen?

A: The RAMP Lipid Screen is a 96-well plate containing 31 different lipids and lipid mixtures, designed for high-throughput identification of lipids that stabilise membrane proteins (MPs) in detergent-based solutions.

Q: What are the benefits of using the RAMP Lipid Screen?

A: The RAMP Lipid Screen offers several benefits, including:

- **Stabilisation of MPs:** Identify lipids that stabilise MPs, improving the accuracy of downstream analysis.
- **Cost-effectiveness:** Reduce the cost and reagent waste associated with lipid screening.
- **Efficiency:** Enables high-throughput screening of a wide range of lipids.
- **Versatility:** Compatible with various stability assessment methods and downstream applications.

Q: How does the RAMP Lipid Screen compare to purchasing individual lipids?

A: The RAMP Lipid Screen is more cost-effective. The estimated price to purchase all individual lipids is 8x-10x higher.

Q: What is the shelf life of the RAMP Lipid Screen?

A: The shelf life of the unopened RAMP Lipid Screen is 18 months, but when opened and solubilized in detergent (3% DDM), the lipids are stable for at least two weeks at 4°C, or for at least 3 months at -70 °C or -20 °C.

Q: How do I store the plate before use?

A: Store at -20°C.

Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257

434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280

Molecular Dimensions
A CALIBRE SCIENTIFIC COMPANY

anatrace
A CALIBRE SCIENTIFIC COMPANY

User Guide

RAMP Lipid Screen

Q: What are the shipping conditions?

A: The kit is shipped at room temperature. As the lipids are in dry format and purged with Argon/nitrogen before sealing, there should be no concern about the stability of the product even if left at room temperature for a few days.

Q: How many proteins can be analysed with the RAMP Lipid Screen?

A: Each plate contains enough lipid for 3 proteins to be analysed by nano-DSF in triplicate in three different detergents.

Q: What types of lipids are included in the RAMP Lipid Screen?

A: The RAMP Lipid Screen covers a wide lipid space, including commonly used individual lipids and lipid mixtures. See datasheet for the full list of lipids.

Q: What instruments can be used with the RAMP Lipid Screen?

A: The RAMP Lipid Screen can be used with a variety of instruments to generate stability data, including:

- NanoTemper's Prometheus
- Protein Stable's SUPR-DSF
- Thermal Shift assay
- Other instruments suitable for assessing membrane protein stability

Q: Are all the lipids compatible with dyes used in thermal shift experiments?

A: Not all lipids in the RAMP Lipid Screen are compatible with dyes commonly used in thermal shift assays, such as SYPRO Orange and CPM. Here is list of the compatible lipids:

- DPPE
- POPC
- DOPC
- DMPC
- DPPC
- LMPC
- POPG
- DPPG
- 10:0 PA
- 16:0-18:1 PA
- CHS
- CHL
- CL
- 18:1 CL
- Sphingomyelin
- POPG/POPE
- DMPC/CHL
- POPC/CHL
- CHL/POPC/SM

Q: What applications can the RAMP Lipid Screen be used for?

- Stability testing
- Crystallisation
- Functional assays
- Reconstitution studies

Calibre SCIENTIFIC
Molecular Dimensions
Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
enquiries@moleculardimensions.com
+44 (0)114 224 2257

anatrace
434 W. Dussel Dr. Maumee, OH 43537
customerservice@anatrace.com
800.252.1280