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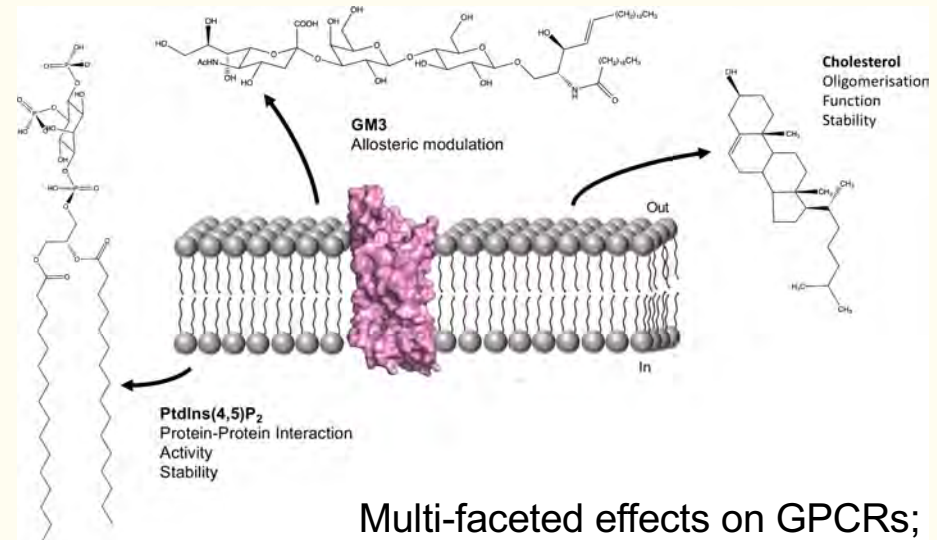
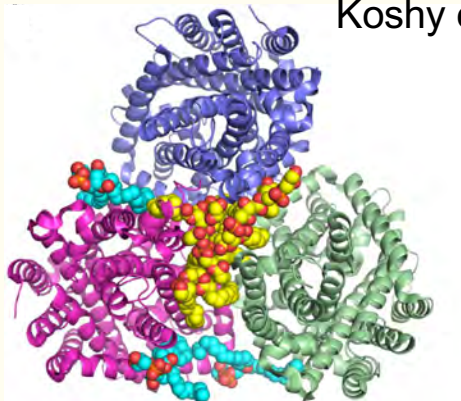
# A novel high-throughput screen for identifying lipids that stabilise membrane proteins in detergent based solution

Bernadette Byrne

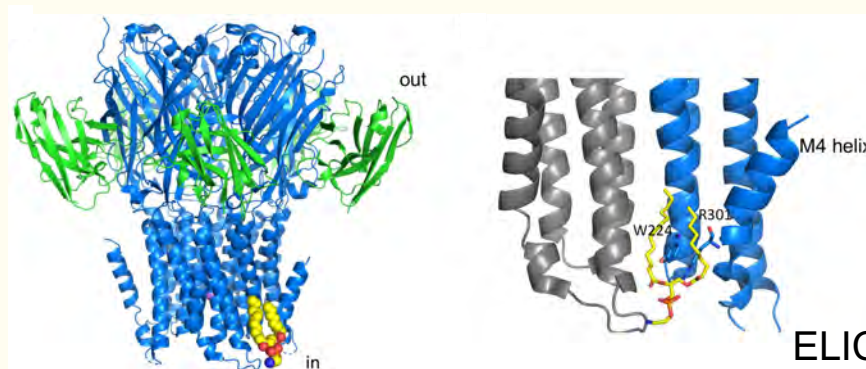
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# Membrane proteins and lipids

BetP oligomerization;  
Koshy et al, 2013



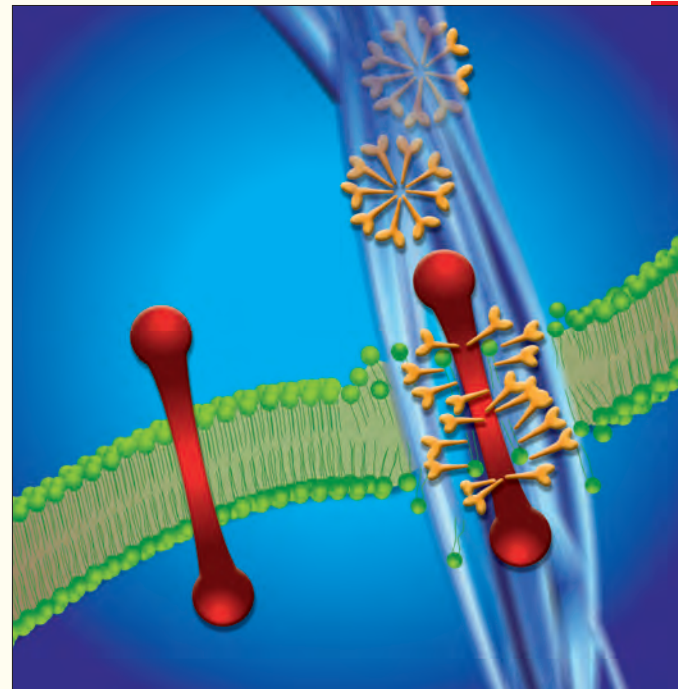
Multi-faceted effects on GPCRs;  
Reviewed in Renard & Byrne, 2021



ELIC function;  
Henault et al, 2019

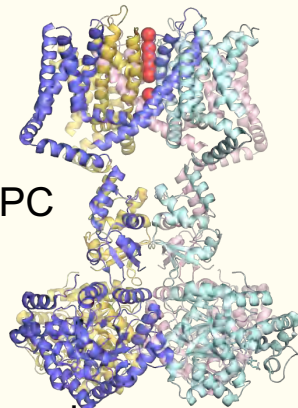
## Lipid loss

- Detergents disrupt the membrane-replacing lipid
- Lipids are lost during isolation stages



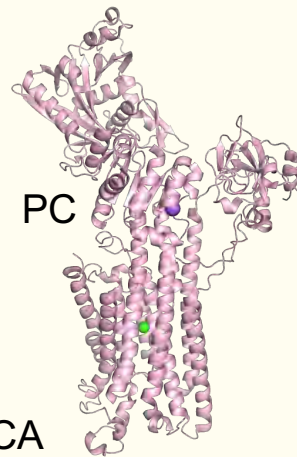
## Relipidation, but which lipids?

- Adding lipids during purification and/or crystallization has been successful



PG, PE and PC

Shaker K<sup>+</sup> channel  
Long et al, 2005; PDB 2A79



PC

SERCA  
Toyoshima et al, 2001; PDB 1SU4

- If not sure what to add can use commercially available additive screen, good for crystallization, but less so for nanodisc preparation

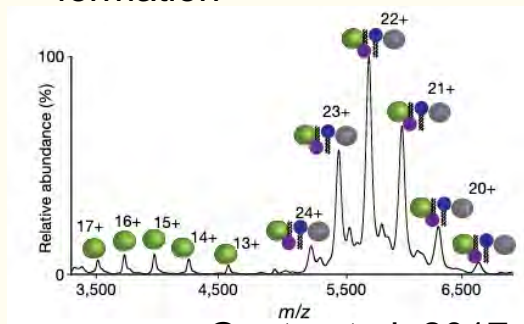
## Designing a cost effective lipid screen

- Collaborative project (part of EU ITN)
  - Imperial, University of Leeds, Molecular Dimensions, Anatrace
- Put together a database of lipids known to have roles in structure and function
  - LipidMaps
  - PDB
  - Literature searches
- Business case

## Designing a cost effective lipid screen

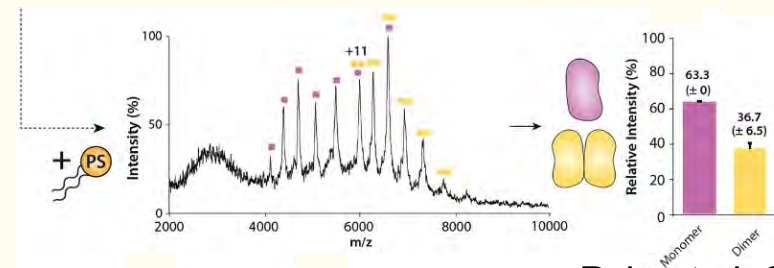
- 96 well plate format
- 32 different conditions in triplicate
- Exclude expensive lipids (eg PIs)
- Different versions of the sample lipid (eg POPC, DMPC etc included if appropriate)
- Try to cover as much lipid space as possible
- Example conditions

Cardiolipin + LeuT dimer formation



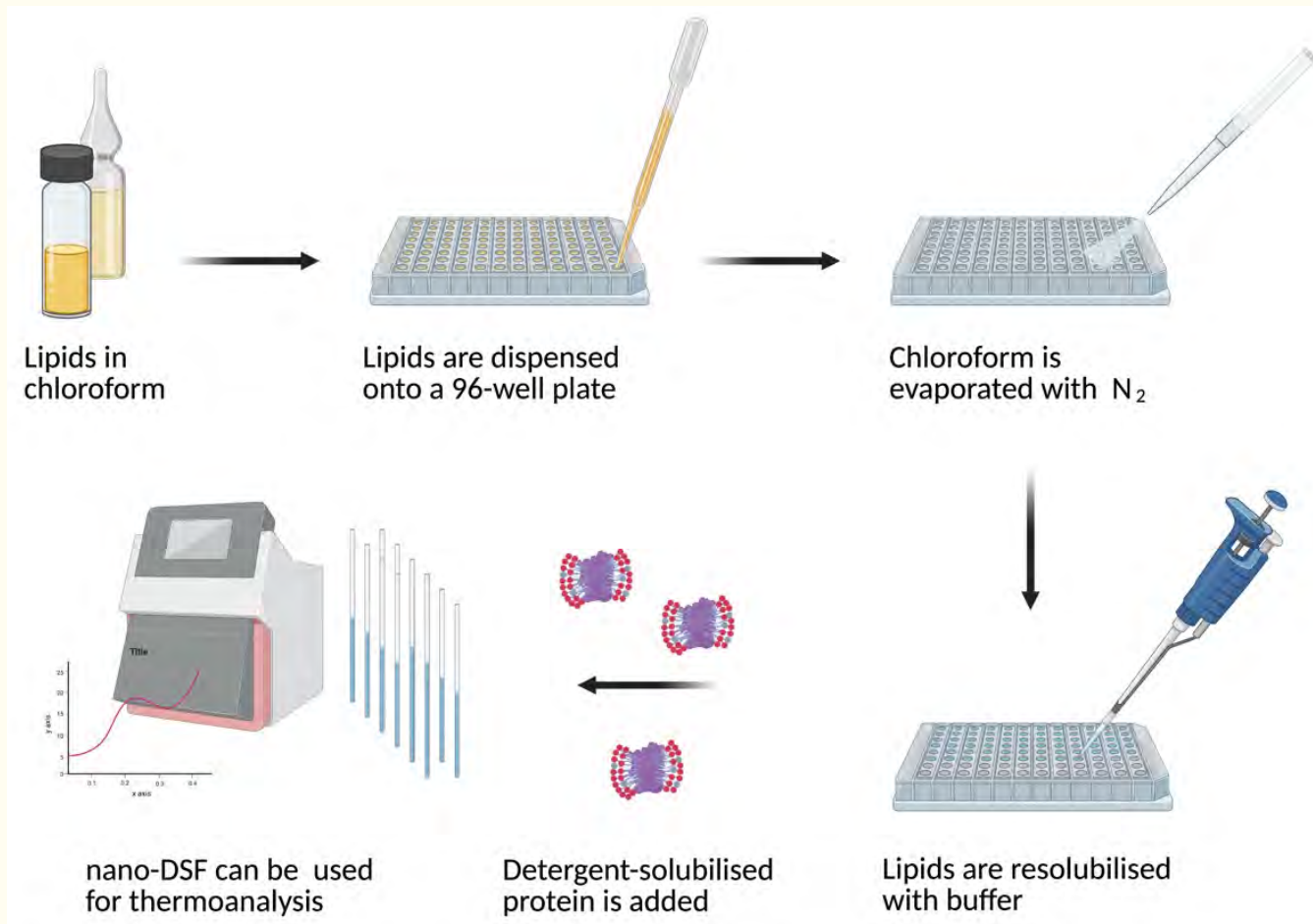
Gupta et al, 2017

PS and ScBOR1p dimer



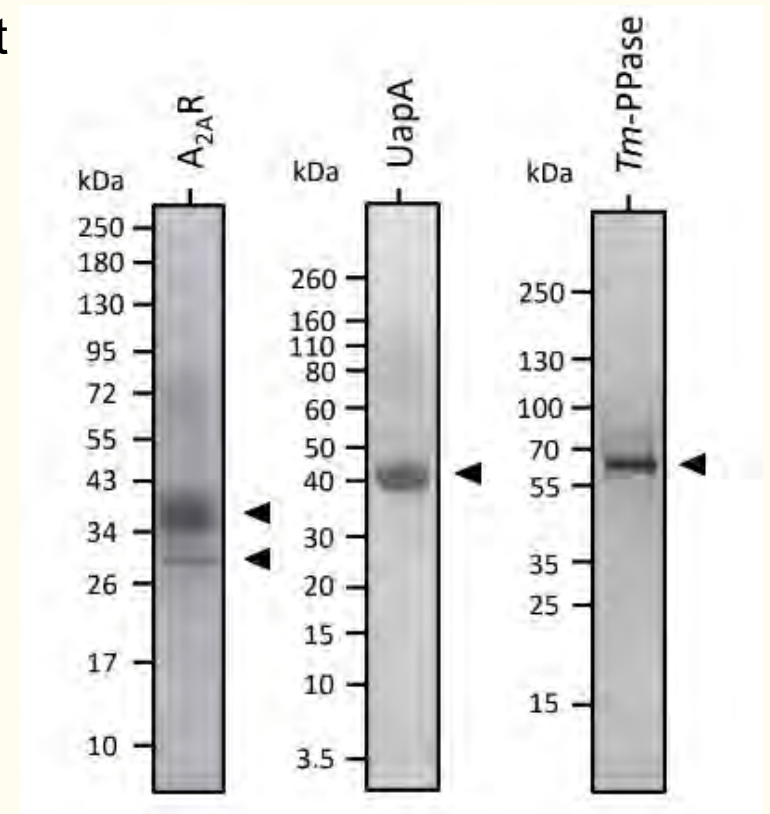
Pyle et al, 2019

# Screen production process



## Three Test proteins

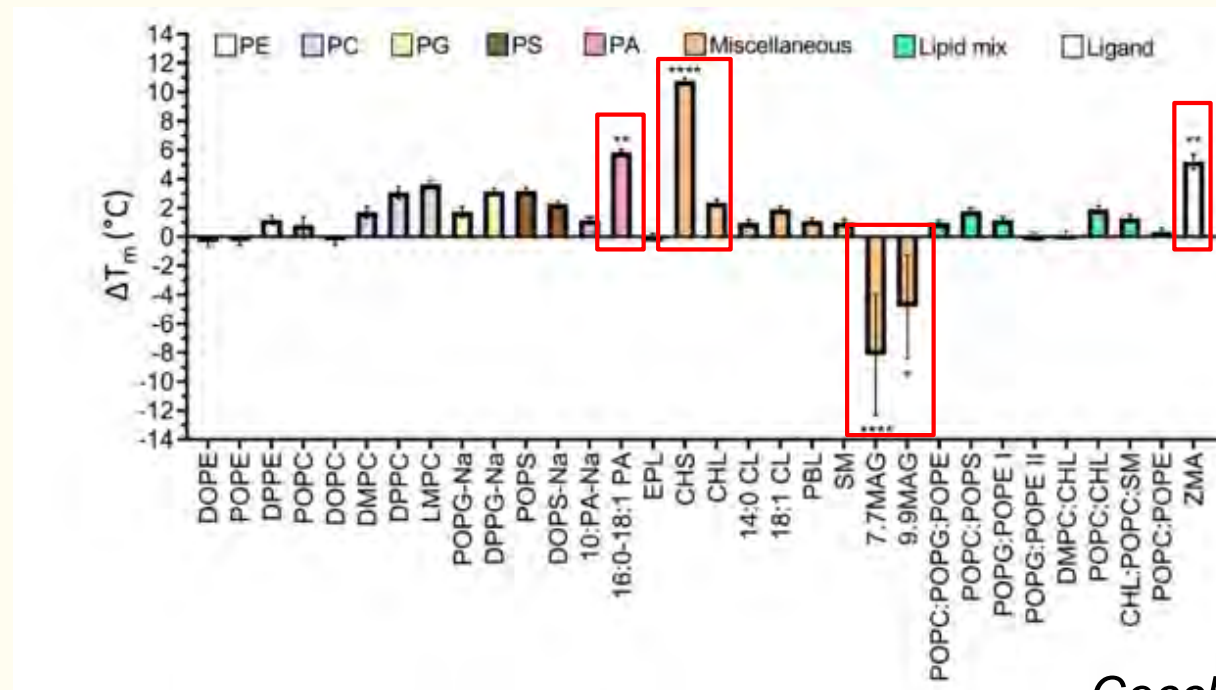
- Human  $A_{2A}R$  + BRIL, 7 TMs, expressed in insect cells
- *Aspergillus nidulans*, UapA-G411V $_{\Delta 1-11}$ , 14 TMs, expressed in *S. cerevisiae*
- *Thermatoga maritima* pyrophosphatase, Tm-Ppase, expressed in *S.cerevisiae*





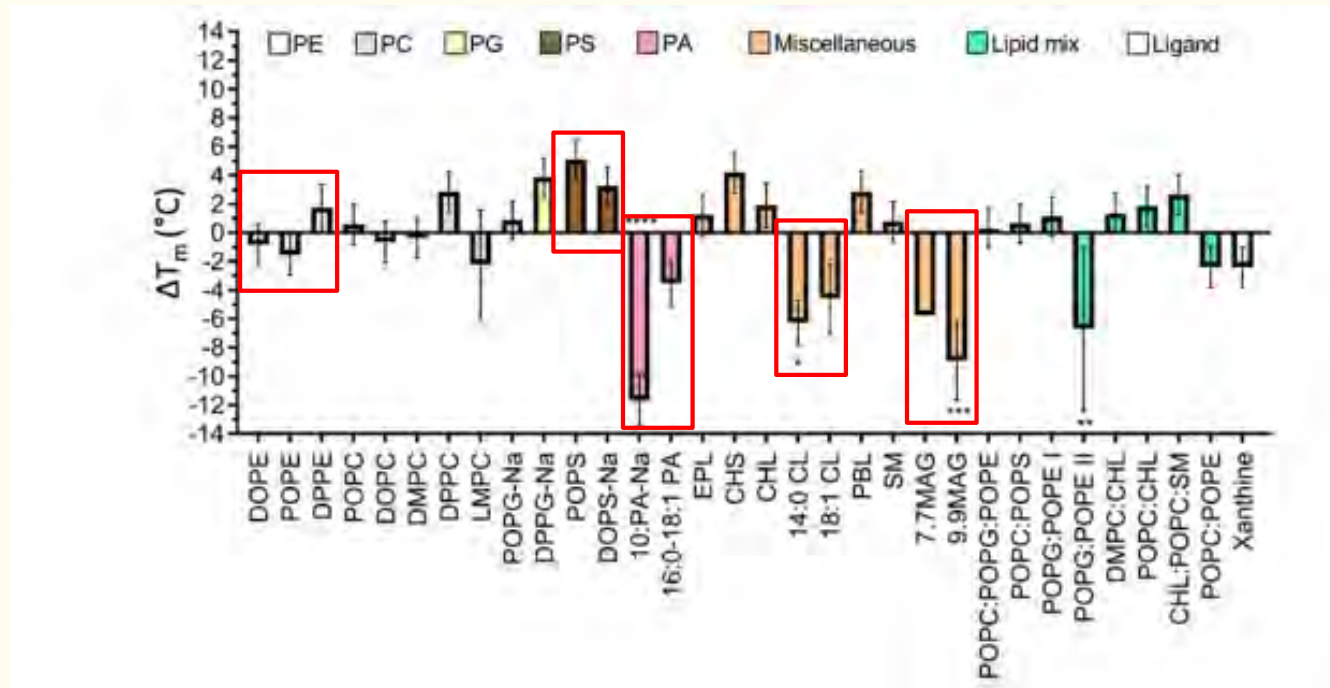
## Testing membrane proteins-hA<sub>2</sub>AR

- Known interactions with CHL. Purified in absence of CHS



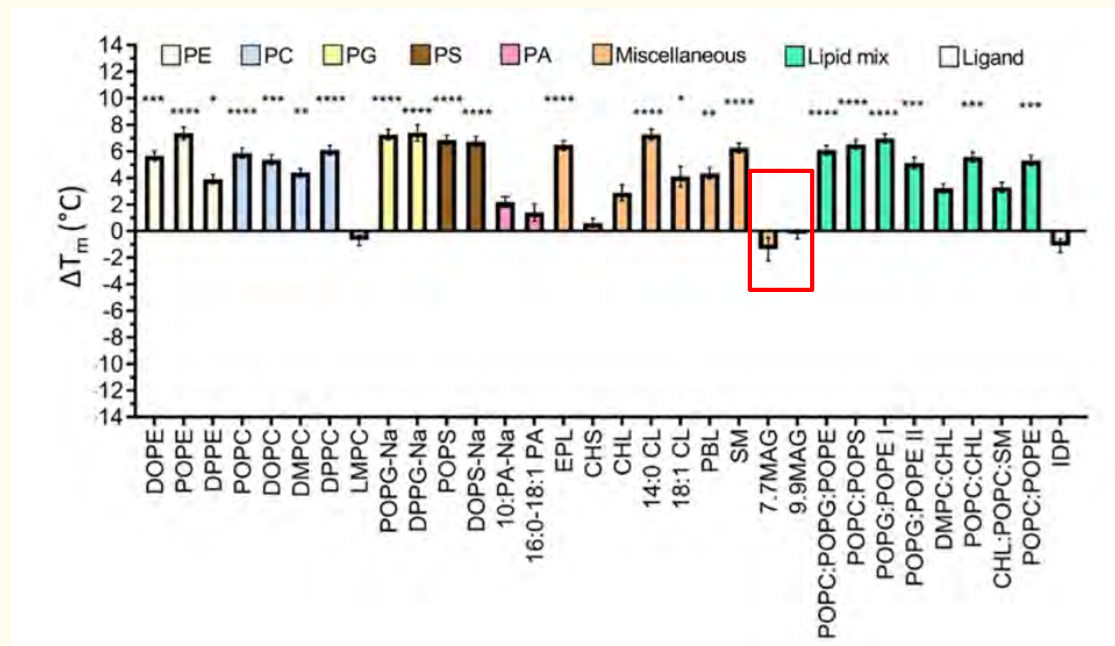
## Testing membrane proteins-UapA

- PE and PI known to have a role in dimer formation



# Testing membrane proteins-Tm-PPase

- Pyrophosphatase, no known lipid dependencies



## Summary

- Successfully identified CHS as stabilizing A<sub>2A</sub>R
- For both A<sub>2A</sub>R and UapA identified novel lipids worth further investigation
- For TmPPase revealed a non-specific lipid effect
  
- Demonstrated the validity of the screen
- Identified that the MAGs are not particularly useful

## Further work

- Optimise screen conditions (replace MAGs)
- Long term stability of the screen
- Screening with a larger number/variety of membrane proteins
- Use with other stability analysis methods
- Assess use of screen as a source of lipids for crystallization screening and nanodisc reconstitution
- All dependent on funding!!

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## Acknowledgements



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