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

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Membrane proteins and lipids

BetP oligomerization; Koshy et al, 2013

ELIC function; Henault et al, 2019

Multi-faceted effects on GPCRs; Reviewed in Renard & Byrne, 2021
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$^+$ 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 (e.g., PIs)
- Different versions of the sample lipid (e.g., POPC, DMPC etc included if appropriate)
- Try to cover as much lipid space as possible
- Example conditions

Cardiolipin + LeuT dimer formation

PS and ScBOR1p dimer

Gupta et al, 2017

Pyle et al, 2019
Screen production process

- Lipids in chloroform
- Lipids are dispensed onto a 96-well plate
- Chloroform is evaporated with N₂
- nano-DSF can be used for thermoanalysis
- Detergent-solubilised protein is added
- Lipids are resolubilised with buffer
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$_2$AR

- Known interactions with CHL. Purified in absence of CHS

Cecchetti et al, 2021
Testing membrane proteins-UapA

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

Cecchetti et al, 2021
Testing membrane proteins-Tm-PPase

- Pyrophosphatase, no known lipid dependencies
Summary

- Successfully identified CHS as stabilizing A$_{2A}$R
- For both A$_{2A}$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!!
Acknowledgements

Cristina Cecchetti  Jannik Strauss  Claudia Stohrer

Adrian Goldman

Imperial College London

UNIVERSITY OF LEEDS

Molecular Dimensions

anatrace

BBSRC