SMALPs: past glories and future opportunities

Professor Tim Dafforn
University of Birmingham
Solubilisation of membrane proteins

MPs are not naturally solvated by water

MPs are solvated in a structurally specific fashion

The 3 challenges for membrane protein biologists

- Purification of target protein
- Stability of target protein
- Downstream analysis (function and structure)
Historical Solution

Detergents used to “solubilise” membrane proteins

Extensive screening of detergents to optimise yield/purity/function/analysis
The “evolution” of membrane protein extraction methods
The “evolution” of membrane protein extraction methods

- **Amphipols**
  - Foundation of Biochemistry (1833)
  - 1996

- **Detergents**
  - 1986
  - Emergence of Detergent-free World
  - First membrane protein structure

- **Peptide Nanodiscs**
  - 1999
  - Saponins?

- **SMALP**?
  - 2009
  - Polymer Nanodiscs?
  - Preservation of Native membrane Using SMA

- **DNA Nanodiscs**
Styrene Maleic Acid

- 30010 P from Polyscience
- 2.3:1 Styrene to Maleic acid ratio
- Mw 6.5 kDa

SMALPS in protein purification

Protein In Raw membrane

SMA Polymer

Centrifugation to remove insoluble material

HisTrap

Note: Load slowly with low (or no) Imidazole

Human Class A GPCR: Adenosine 2A Receptor

- Adenosine 2A receptor
- Expressed in *Pichia Pastoris*
- >90% yield at extraction stage

![Image of protein structure]

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Specific binding (Bmax, pmol/mg)</th>
<th>Relative yield (%)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubilised materials</td>
<td>9.6</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>IMAC eluate</td>
<td>9300</td>
<td>55</td>
<td>968</td>
</tr>
<tr>
<td>Gel filtration eluate</td>
<td>18200</td>
<td>36.7</td>
<td>1895</td>
</tr>
</tbody>
</table>

## A<sub>2A</sub>R-SMALP Pharmacology

<table>
<thead>
<tr>
<th>Drug</th>
<th>pKi (SMALP)</th>
<th>pKi (crude, membrane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZM241385</td>
<td>7.95 ± 0.45</td>
<td>7.79 ± 0.14</td>
</tr>
<tr>
<td>XAC</td>
<td>6.53 ± 0.24</td>
<td>7.16 ± 0.18</td>
</tr>
<tr>
<td>NECA</td>
<td>5.66 ± 0.26</td>
<td>5.43 ± 0.10</td>
</tr>
<tr>
<td>Theophylline</td>
<td>3.82 ± 0.30</td>
<td>4.13x ± 0.10</td>
</tr>
</tbody>
</table>
GPCR Stability

![Graphs showing stability of GPCR under different conditions.](image)

Type of membrane spanning domain

Diversity of protein-SMALPs

Bacteria
- KcsA
- AcrB
- SecYEG
- ETK
- PagP
- KCNE1
- Photoreaction centre
- Bacteriorhodopsin
- PBP2/PBP2a

Yeast
- CFTR
- Respiratory complex IV

Insect
- hENT1
- ABCG2
- MRP4
- P-glycoprotein

Mammalian
- MRP1
- GLUT1
- AE1

Functional characterisation
- Planar bilayer electrophysiology
- Ligand binding (fluor/radioactive)
- Substrate binding (FRET)
- Auto-phosphorylation
- Phospholipase activity
- Spectroscopy

Source membrane

Purification method
- Metal affinity
- Immuno-affinity
- Size exclusion
- Immuno-precipitation

Lee et al, 2016, BBA 1858: 2549–2557
Structural biology of protein-SMALPs

Negative stain EM
20-30 Å resolution

Broecker et el, Structure, 2016; Gulati et al 2014; Postis et al BBA Biomembranes 2015
Structural biology of protein-SMALPs

Rapid evolution of polymer nanodiscs

Xiran 30010
2:1 S:MA
Free Radical
CSTR
6 KDa

Sidechain Modified
SMA

SMI
SMA-ED
SMA-QA
SMA-SH

SMA
Free radical
RAFT

3:1
DIBMA

Good to see diversity…

But crucial that performance is benchmarked
Rapid evolution of polymer nanodiscs

Polydispersity
pH sensitivity
Some targets missed?
Cation sensitivity
Polymer Architecture
What can we change?

- Engineering polymer charge
- Engineering hydrophobic Moiety
- Engineering Topology
Polymer Architecture
What can we change?

<table>
<thead>
<tr>
<th>CSTR-Synthesised Polymers</th>
<th>Polymers Modified from CSTR SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>SMA-SH</td>
</tr>
<tr>
<td>DIBMA</td>
<td>SMA-ED</td>
</tr>
<tr>
<td>SMI</td>
<td>SMA-ED</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RAFT-Synthesised SMA Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dodecyltrithiocarbonate terminated</td>
</tr>
<tr>
<td>Maleimide terminated</td>
</tr>
<tr>
<td>zSMA - Phosphatidylocholine-functionalised</td>
</tr>
</tbody>
</table>
SMI: A Positively Charged Polymer

Hall et al. Nanoscale. 2018 Jun 7;10(22):10609-10619
SMILPs and GPCRs

C

$[^3]H$-ZM241385 binding/%

$[^3]H$-AVP binding/%

Total Specific

Total Specific

A2Ar

V1Ar
“That’s all well and good but I have very little protein” anon
Membrane protein research in **Miniature SMA-PAGE**

- **CELL** → **SMALP EXTRACT** → **SMA PAGE**

  - **GGDEVSSGE**
  - **HILLAGKK**
  - **AAASLVD**

**PROTEOMICS COMPLEX-OMICS**

**LIPIDOMICS**

**CRYO-EM**

SMA-PAGE separation

AcrB Complex Bam Sav1866 ZipA

A

B

C

D

SMA SDS SMA SDS SMA SDS SMA SDS
Targeted Lipidomics from SMA-PAGE

Sav1866

SMA  SDS

PE

C33:1  702.53
C34:1  714.52
C34:2  716.55
C35:2  728.53
C36:2  742.53
C37:2  756.55
SMA-PAGE to EM: Gel-2-Grid

Route A

Route B
Thanks

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BBSRC
bioscience for the future