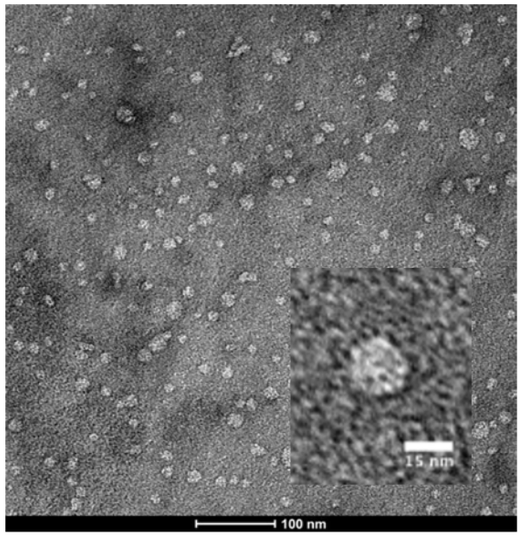
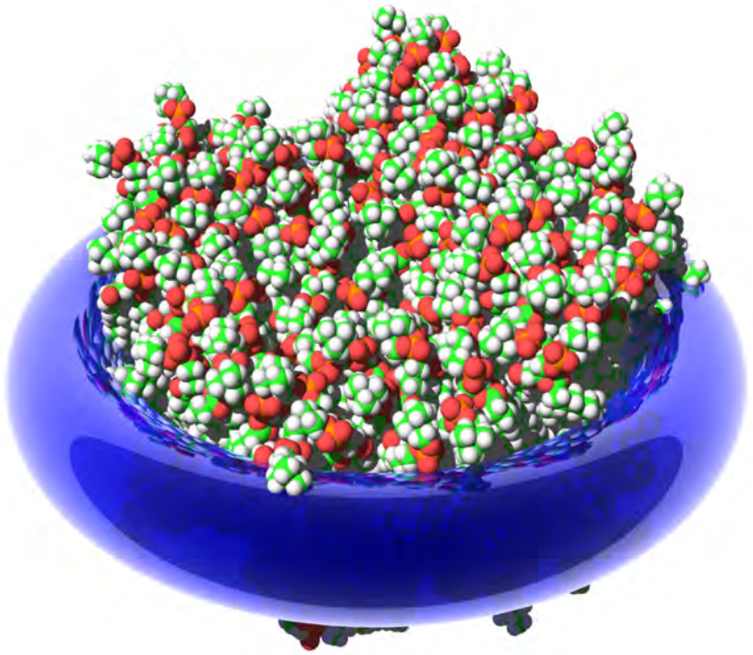
SMALPs: past glories and future opportunities

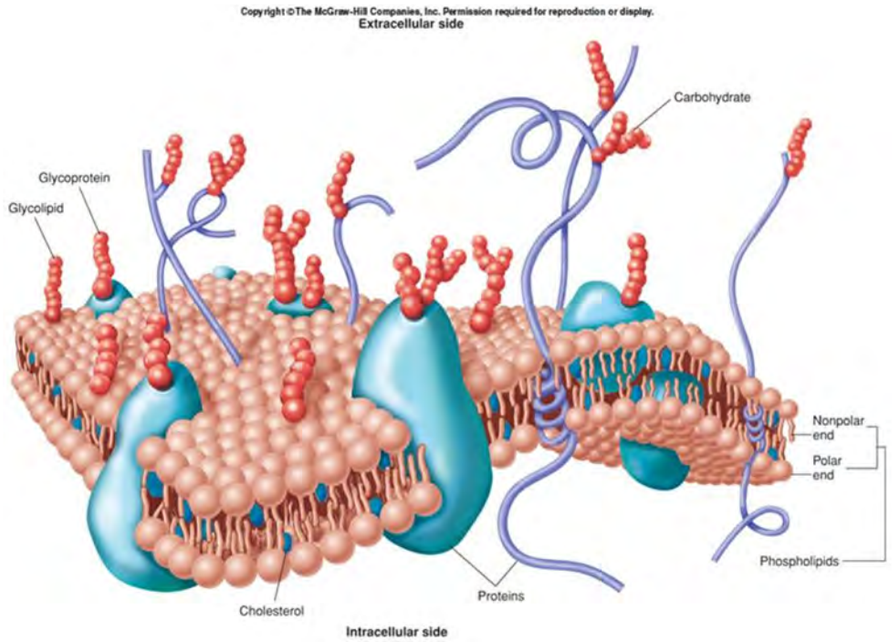


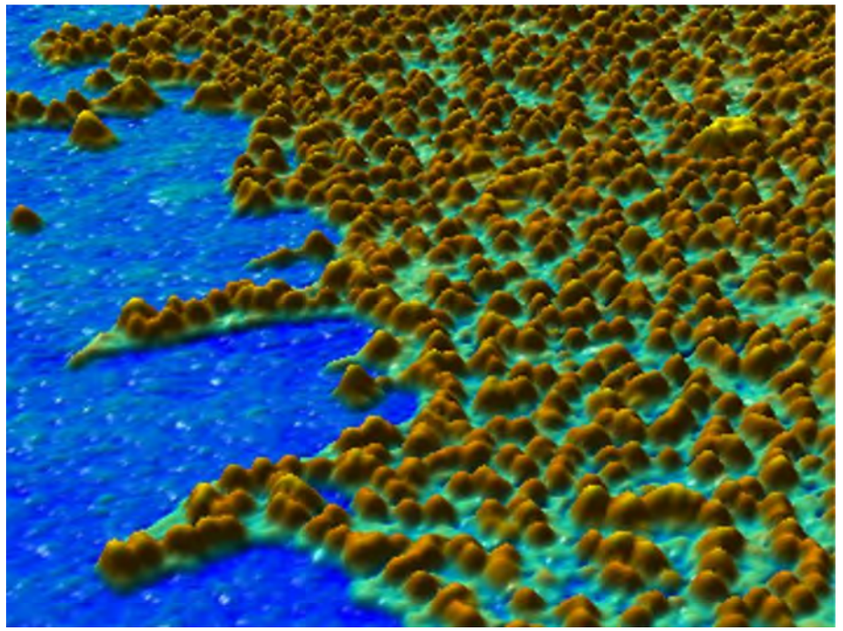


Professor Tim Dafforn
University of Birmingham

Solubilisation of membrane proteins

- MPs are not naturally solvated by water
- MPs are solvated in a structurally specific fashion





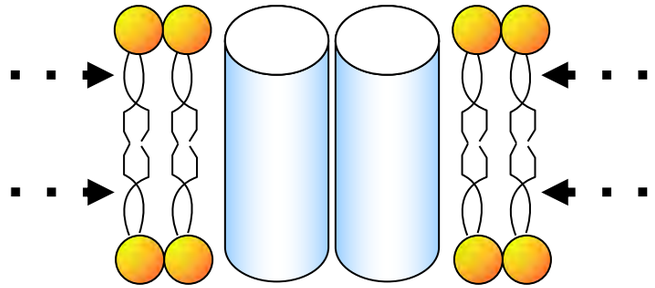
J. Membrane Biol. 180, 205–212 (2001): By H. Schillers, T. Danker, M. Madeja, H. Oberleithner

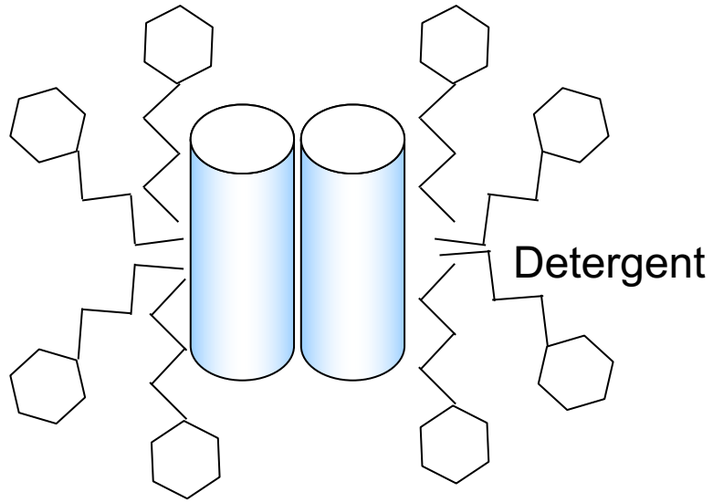
The 3 challenges for membrane protein biologists

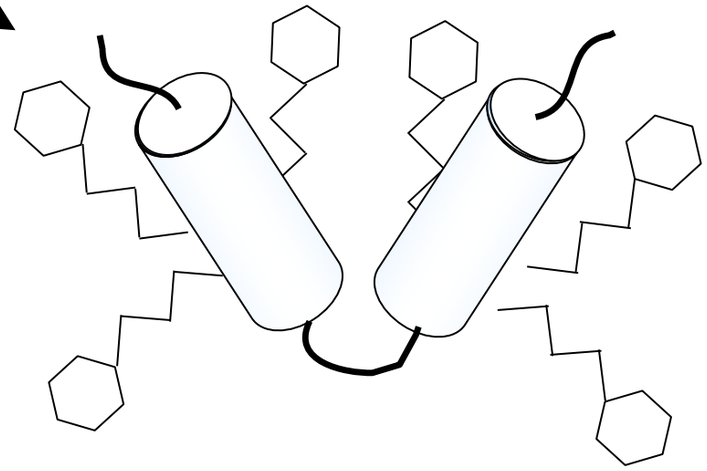
- Purification of target protein
- Stability of target protein
- Downstream analysis (function and structure)

Historical Solution

- Detergents used to “solubilise” membrane proteins

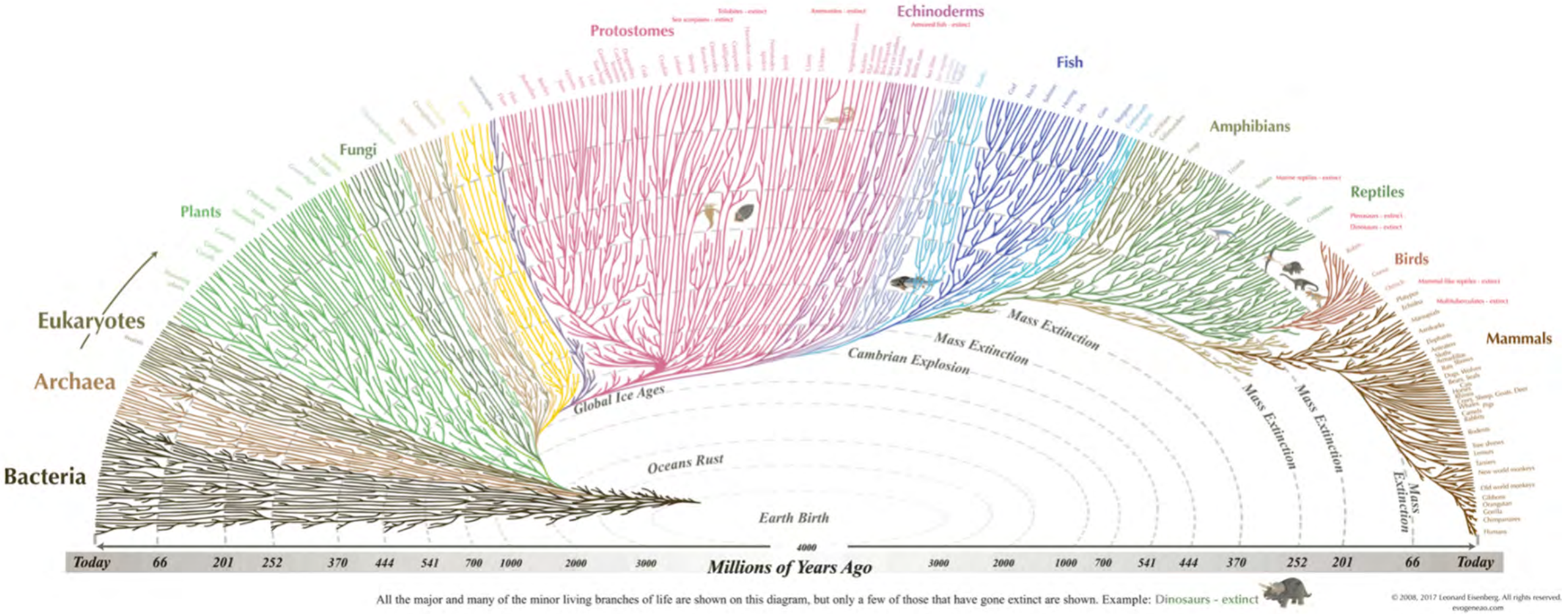




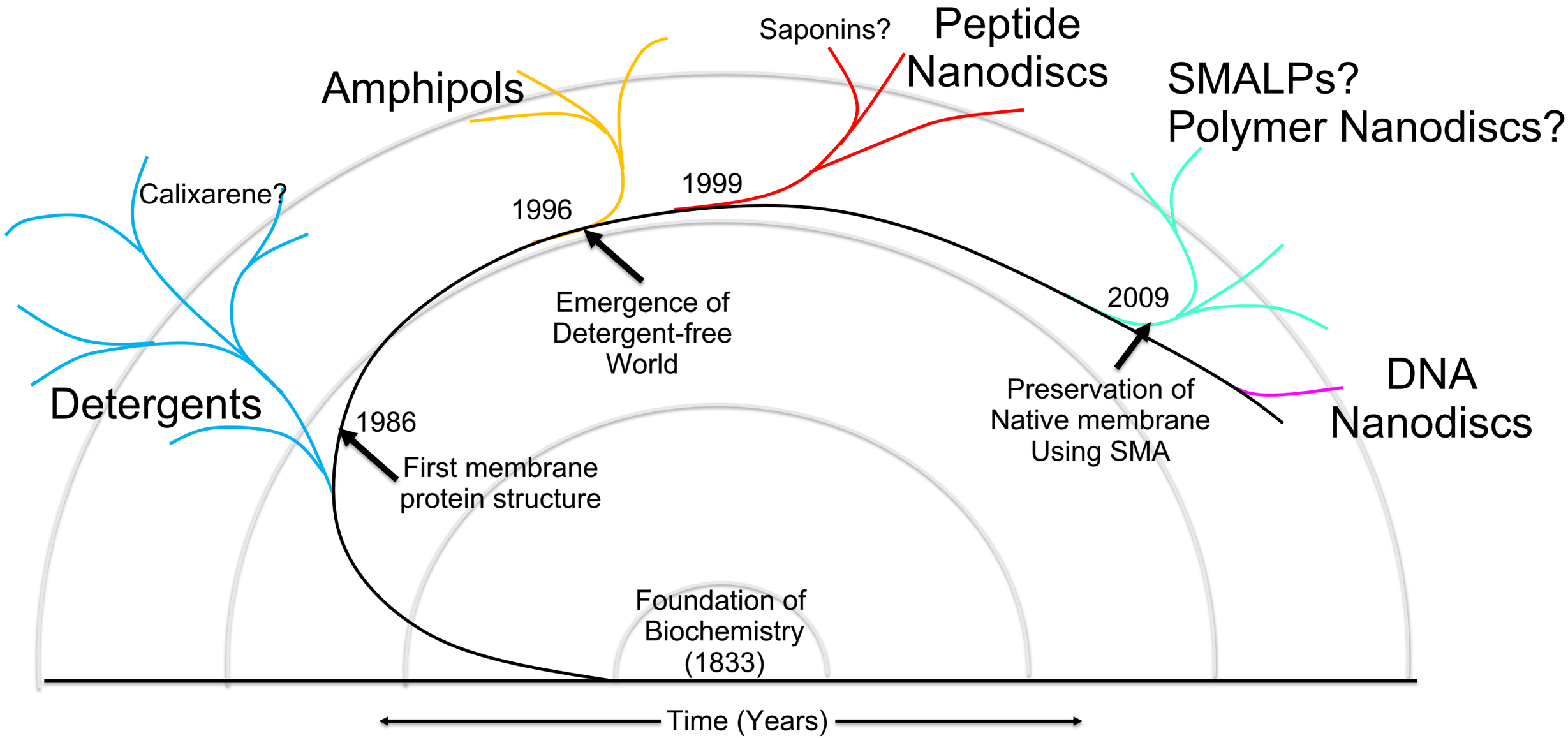


Extensive screening of detergents to optimise yield/purity/function/analysis

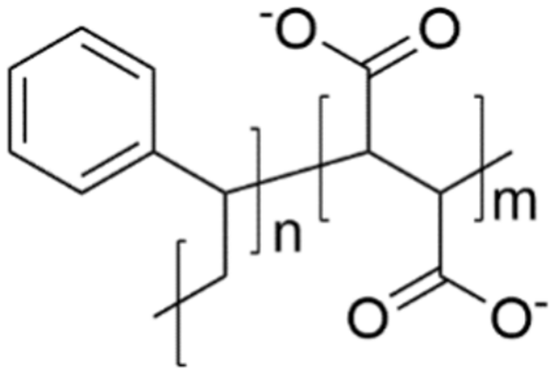
The “evolution” of membrane protein extraction methods



The “evolution” of membrane protein extraction methods



Styrene Maleic Acid



Styrene Maleic Acid co-polymer

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

J Am Chem Soc. 2009 Jun 10;131(22):7484-5

SMALPS in protein purification

Protein In Raw membrane

SMA Polymer

Centrifugation to remove insoluble material

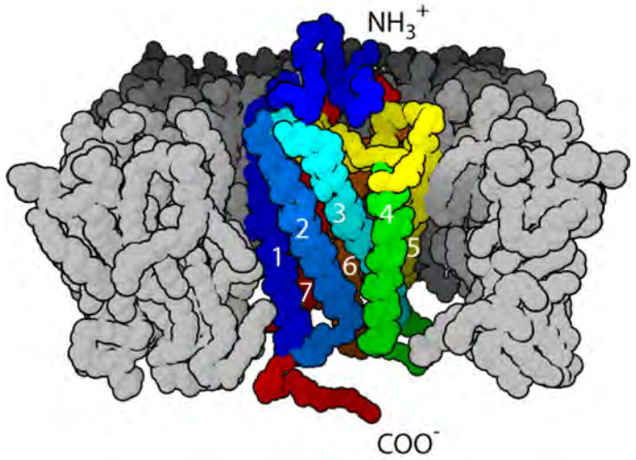
HisTrap

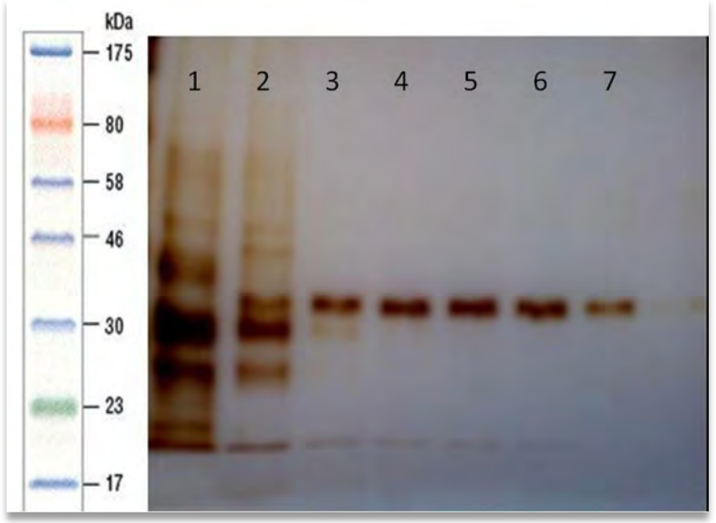
Note: Load slowly with low (or no) Imidazole

Lee SC,. Nat Protoc. 2016 Jul;11(7):1149-62.

Human Class A GPCR: Adenosine 2A Receptor

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

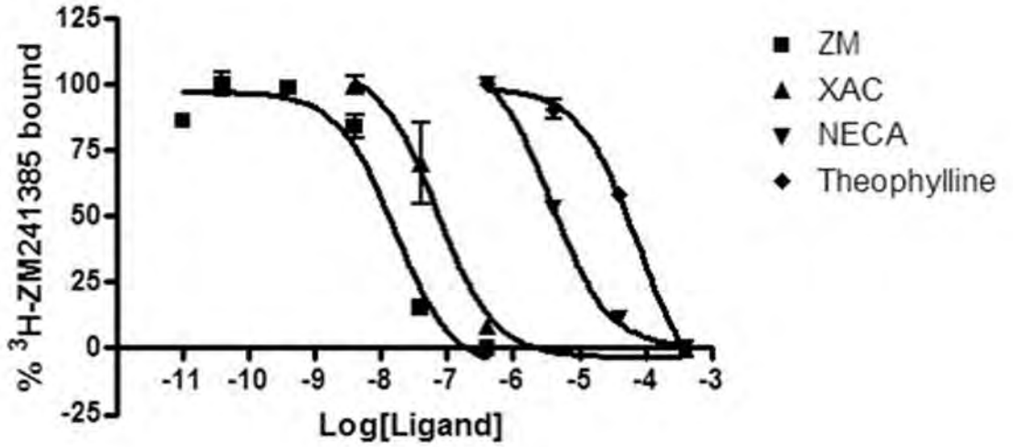




Jamshad M
Biosci. Rep. 2015 Apr 16;35(2).

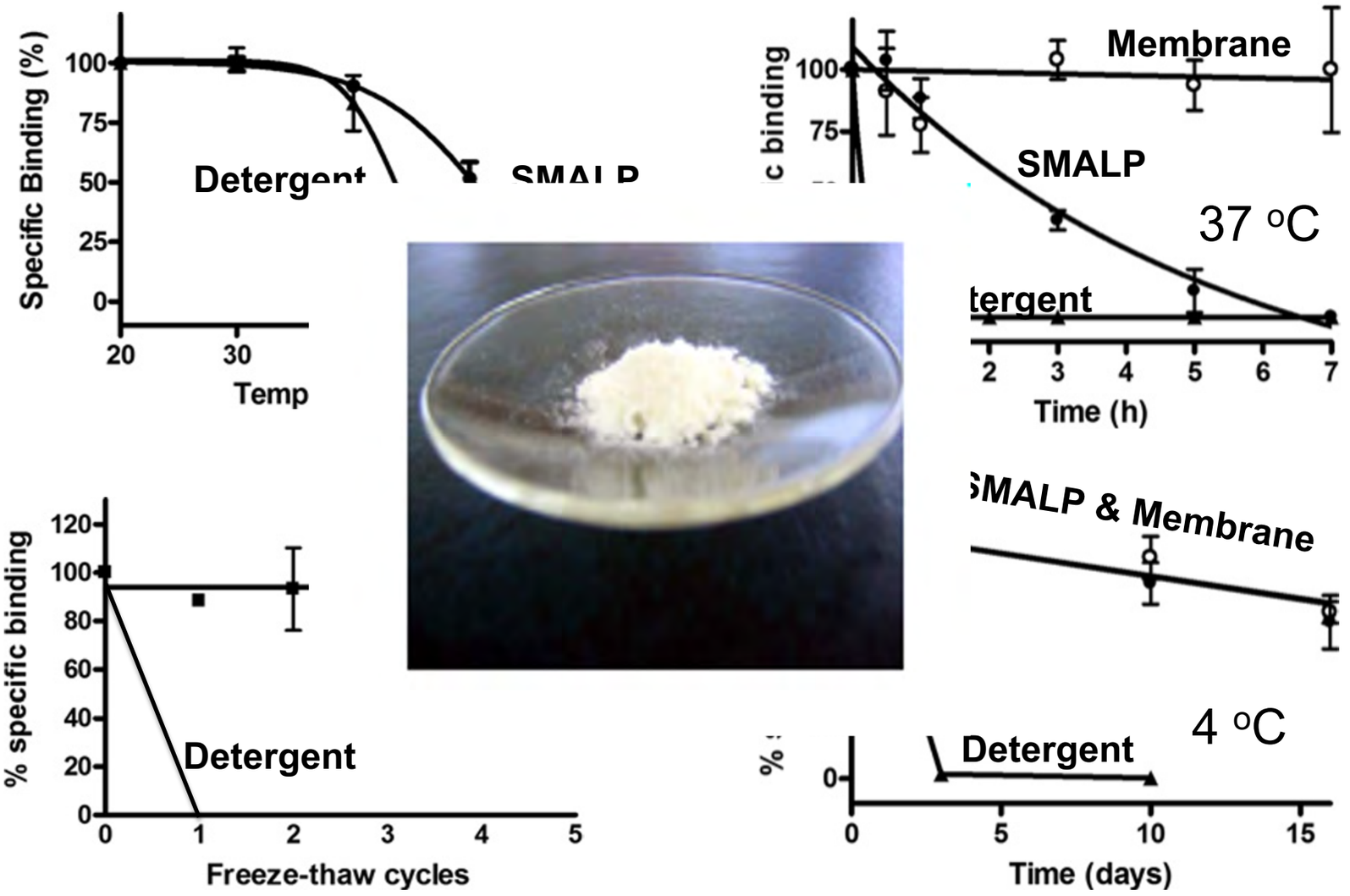
Purification step	*Specific binding (Bmax,pmol/mg)*	*Relative yield (%)*	*Purification (fold)*
Solubilised materials	***9.6***	***-***	***1***
IMAC eluate	***9300***	***55***	***968***
Gel filtration eluate	***18200***	***36.7***	***1895***

A_{2A}R-SMALP Pharmacology

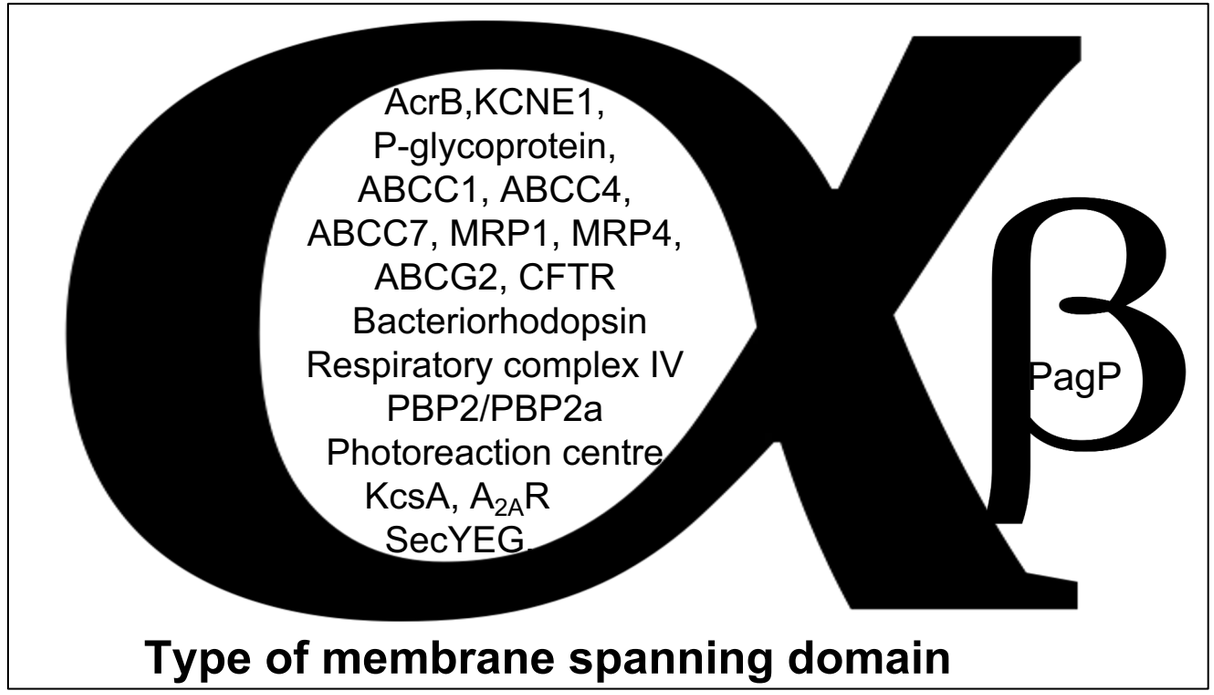


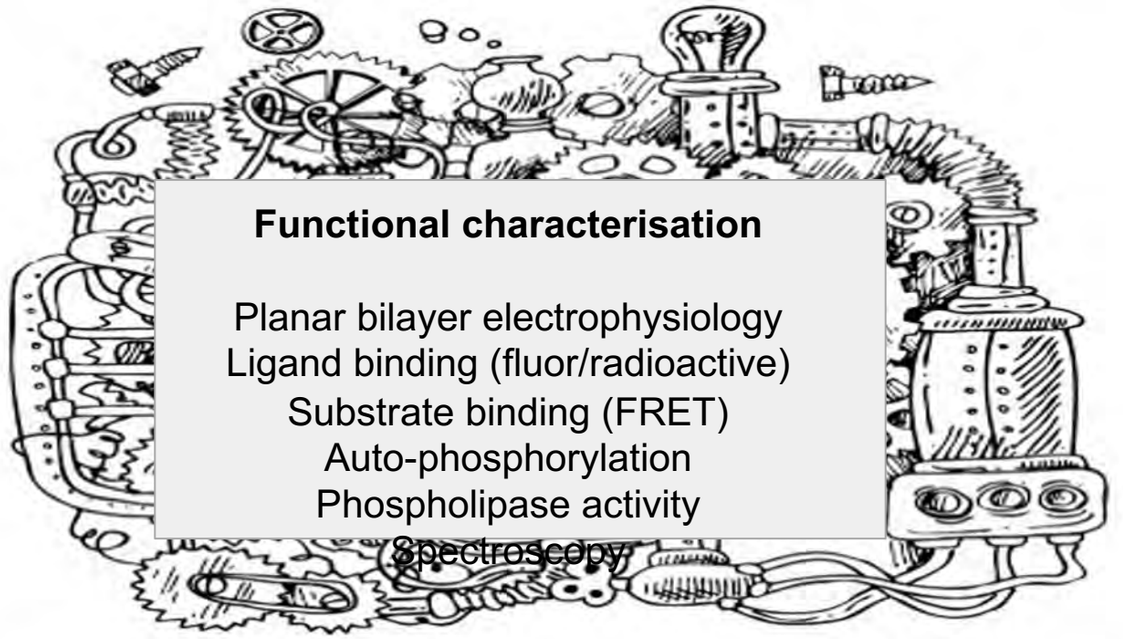
Drug	*pKi (SMALP)*	*pKi (crude, membrane)*
ZM241385	*7.95 ± 0.45*	*7.79 ± 0.14*
XAC	*6.53 ± 0.24*	*7.16± 0.18*
NECA	*5.66 ± 0.26*	*5.43 ± 0.10*
Theophylline	*3.82 ± 0.30*	*4.13x ± 0.10*

GPCR Stability

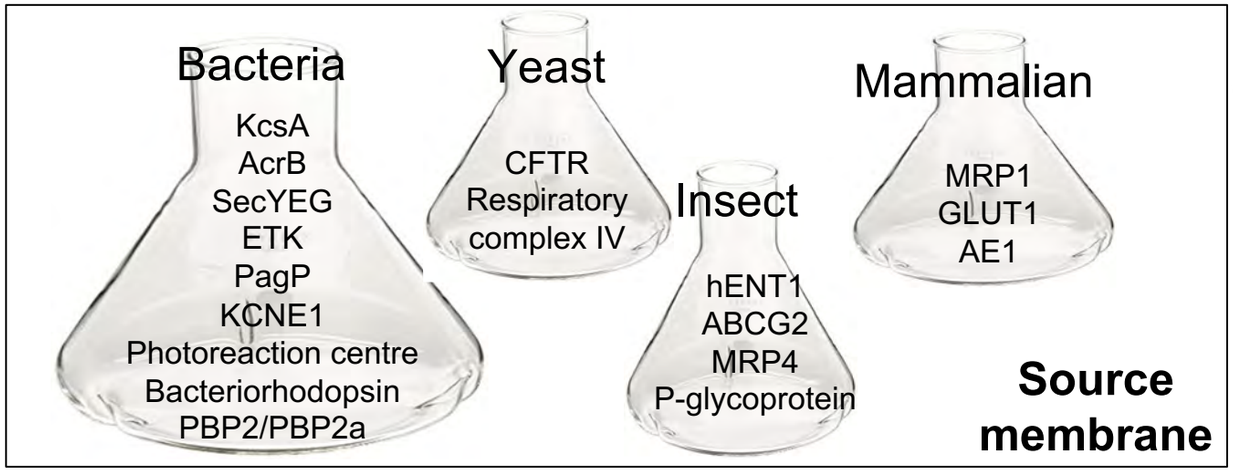


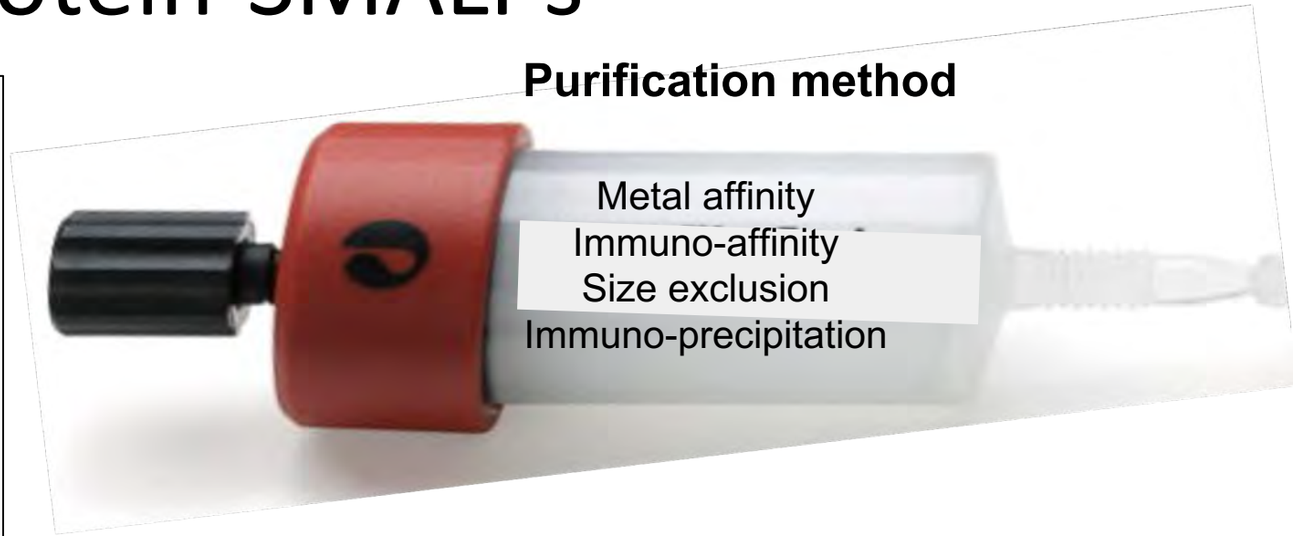
Jamshad *et al.* Biosci Rep. 2015 Apr 16;35(2).





Diversity of protein-SMALPs

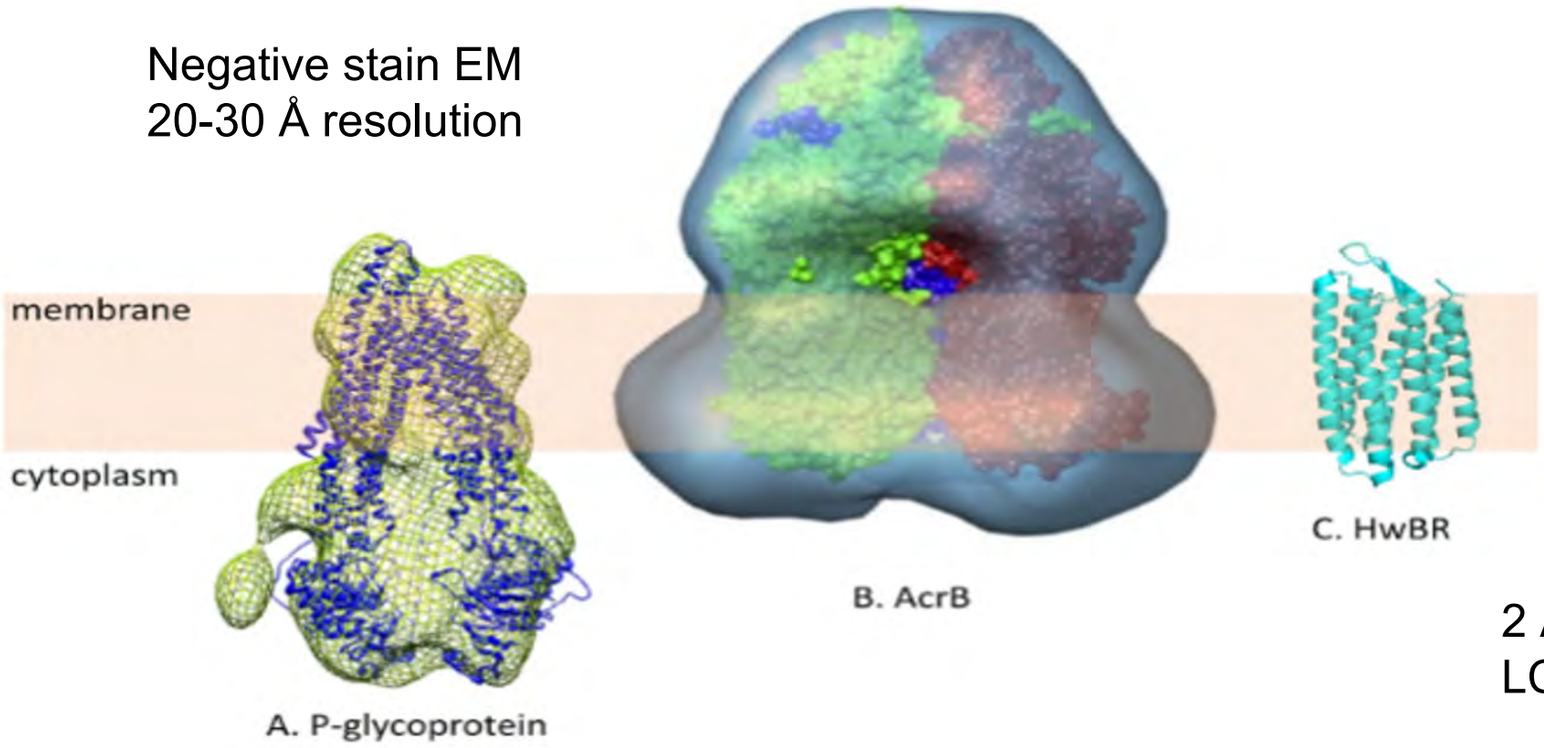




Lee et al, 2016, BBA 1858: 2549–2557

Structural biology of protein-SMALPs

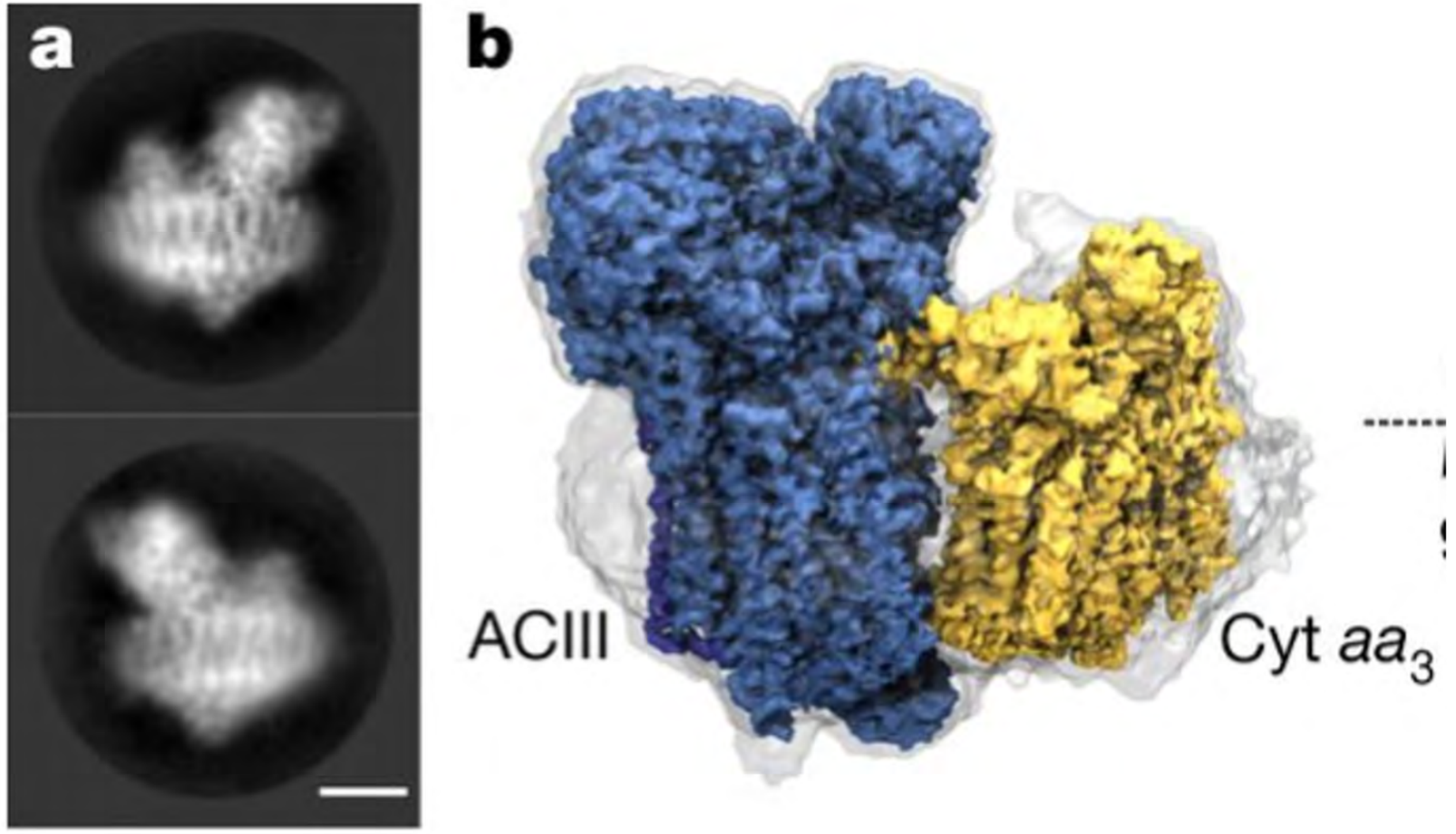
Negative stain EM
20-30 Å resolution



2 Å resolution
LCP crystallisation

Broecker et el, Structure, 2016; Gulati et al 2014; Postis et al BBA Biomembranes 2015

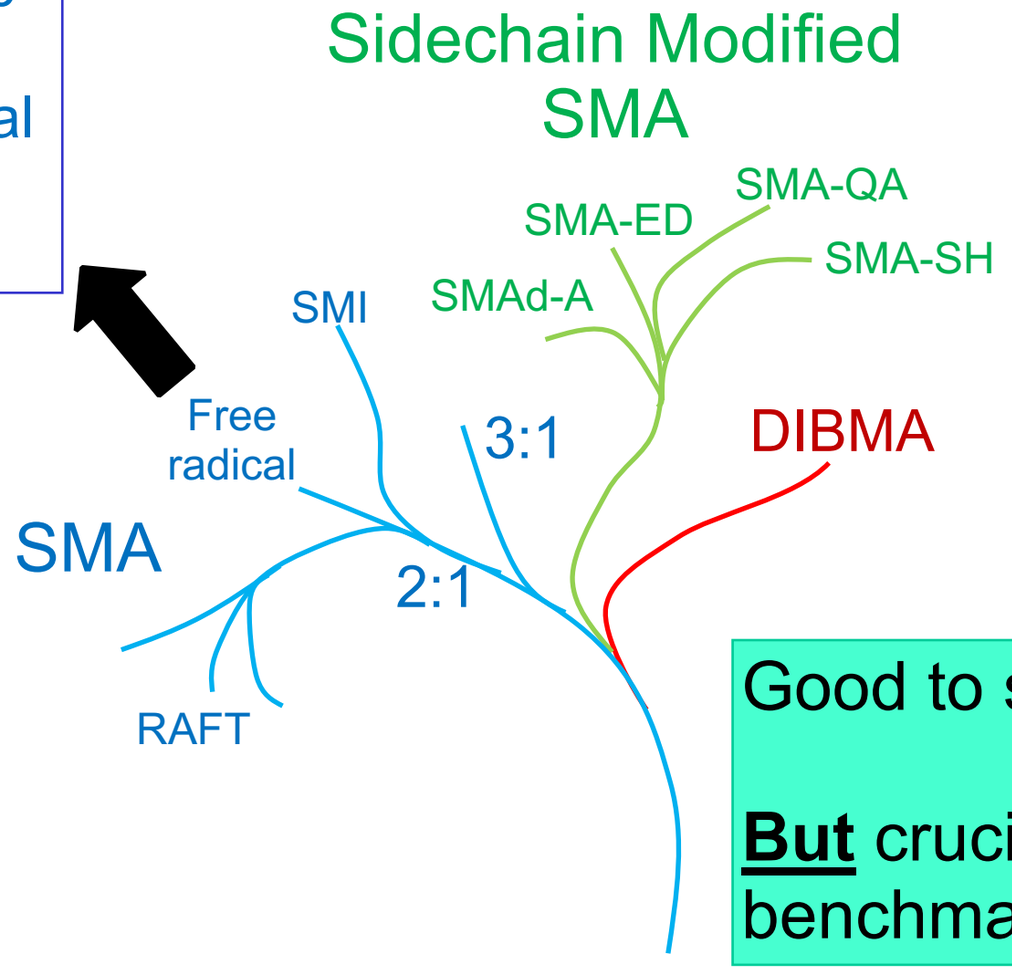
Structural biology of protein-SMALPs



Chang Sun *et al* Nature vol 557, page 123–126 (2018)

Rapid evolution of polymer nanodiscs

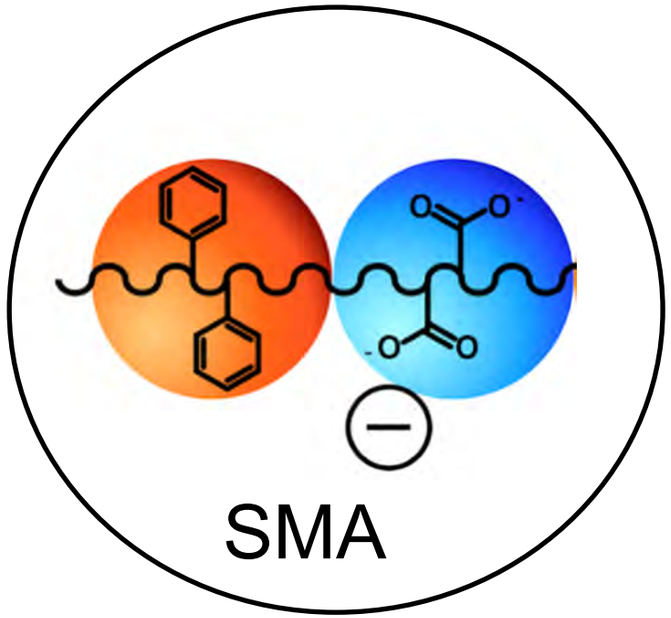
Xiran 30010
2:1 S:MA
Free Radical
CSTR
6 KDa



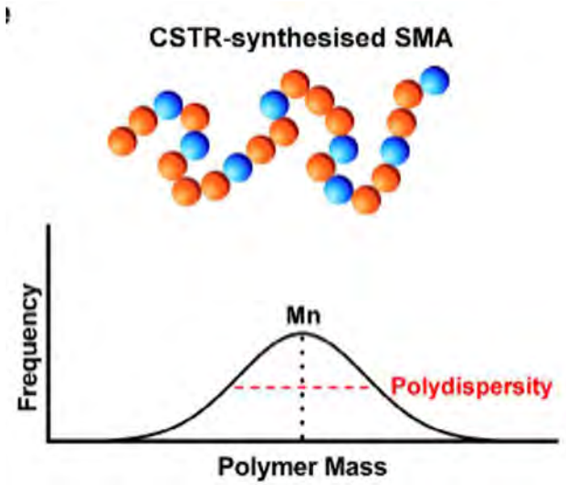
Good to see diversity…

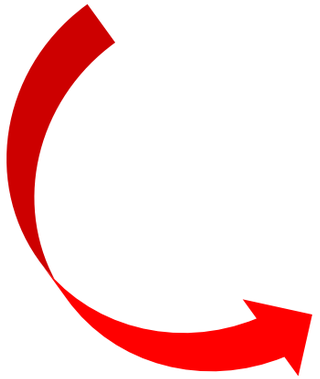
<u>But</u> crucial that performance is benchmarked

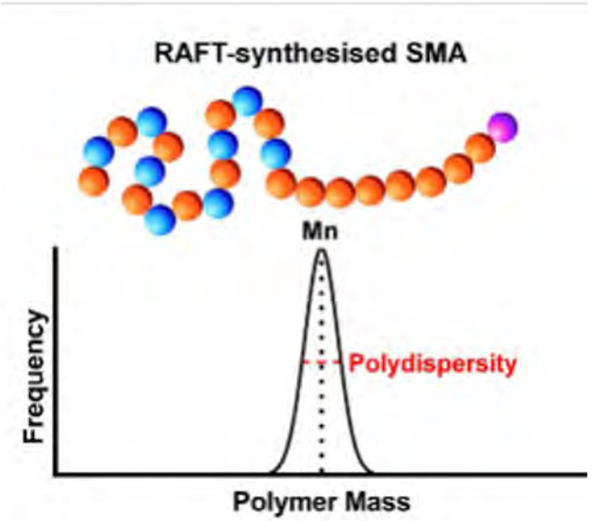
Rapid evolution of polymer nanodiscs



Polydispersity
pH sensitivity
Some targets missed?
Cation sensitivity

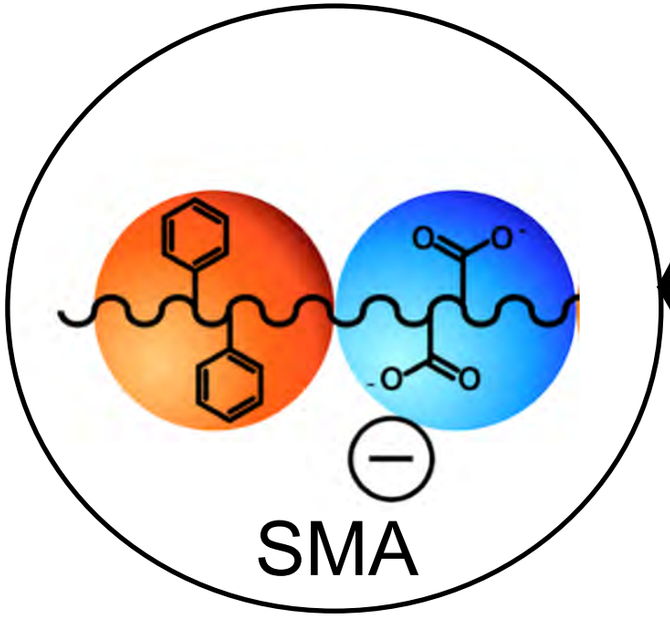


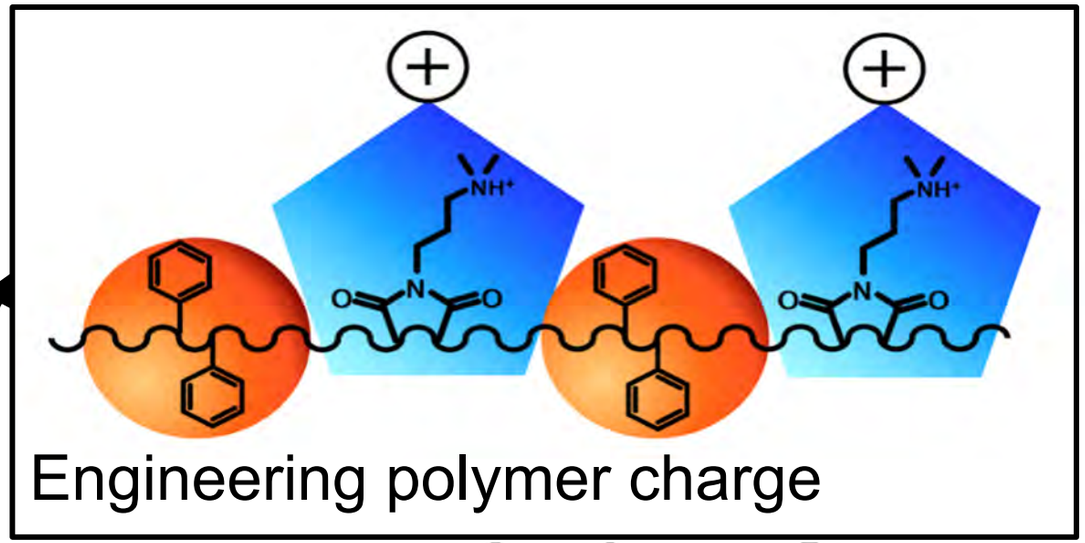


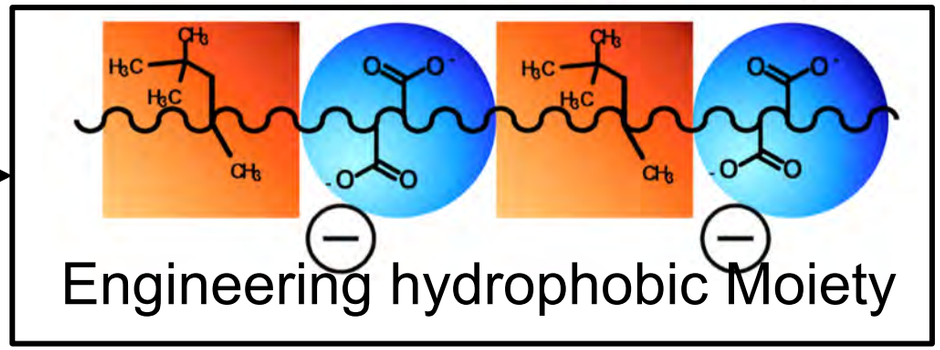


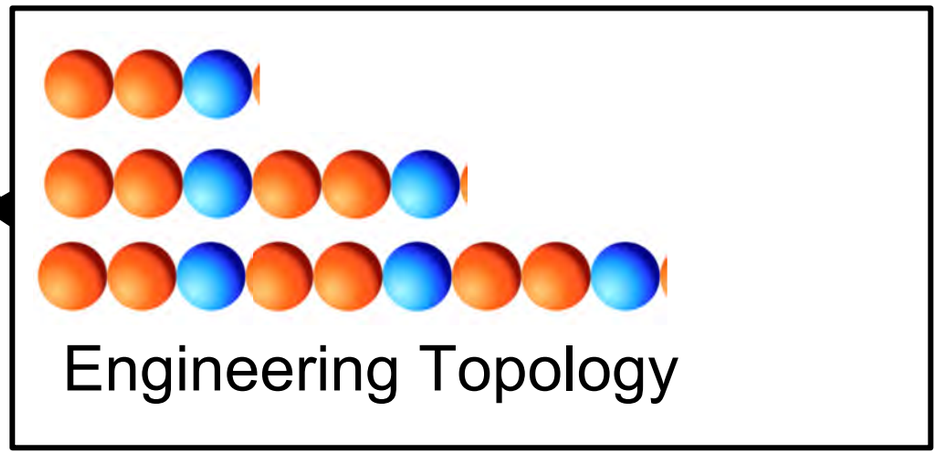
Polymer Architecture

What can we change?





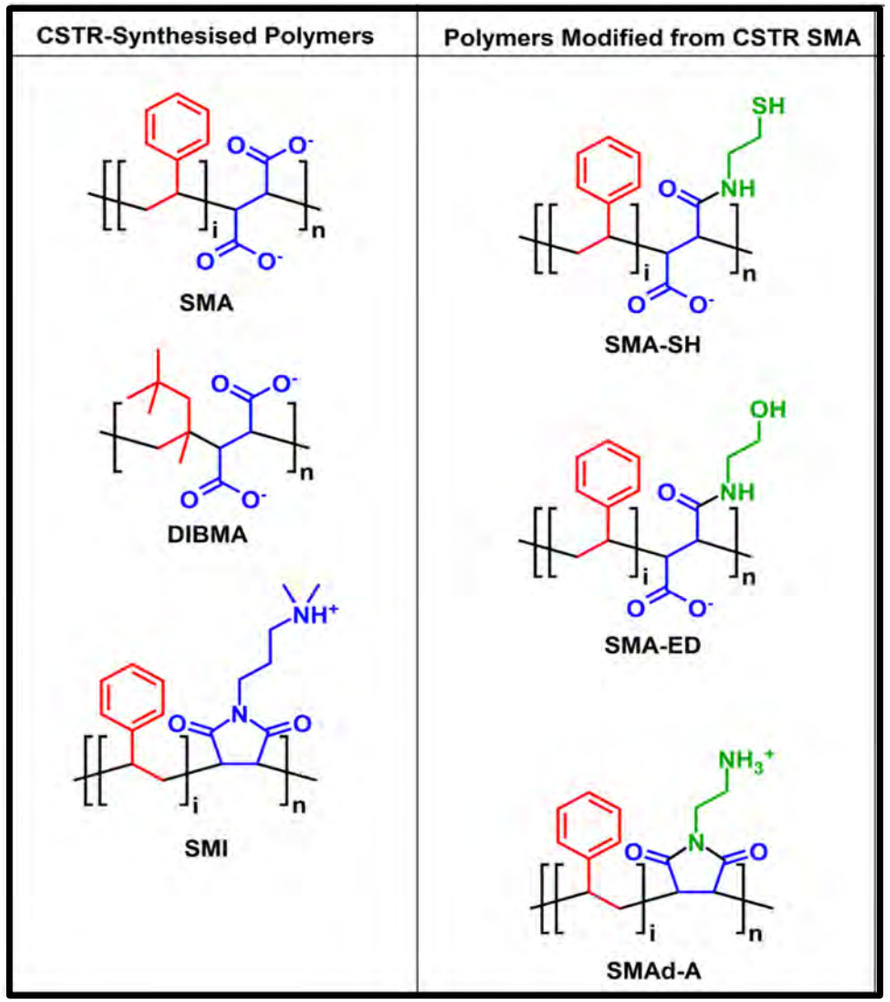


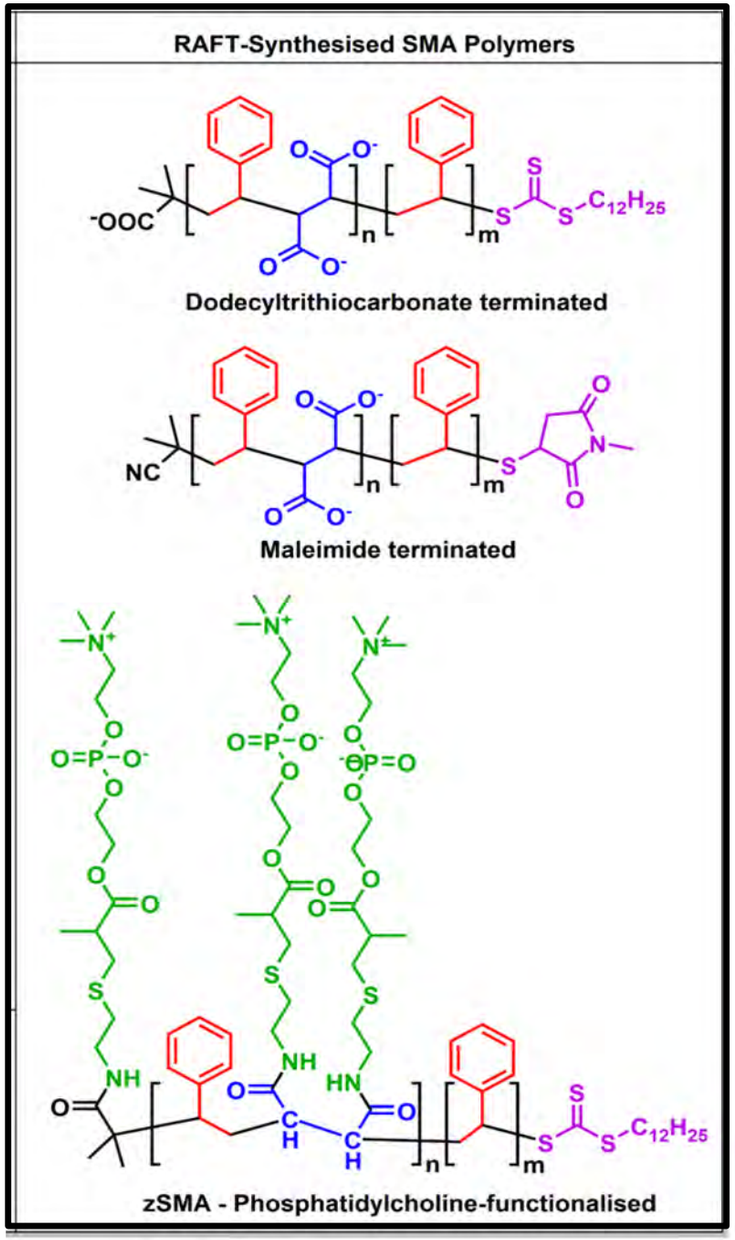


Polymer Architecture

What can we change?

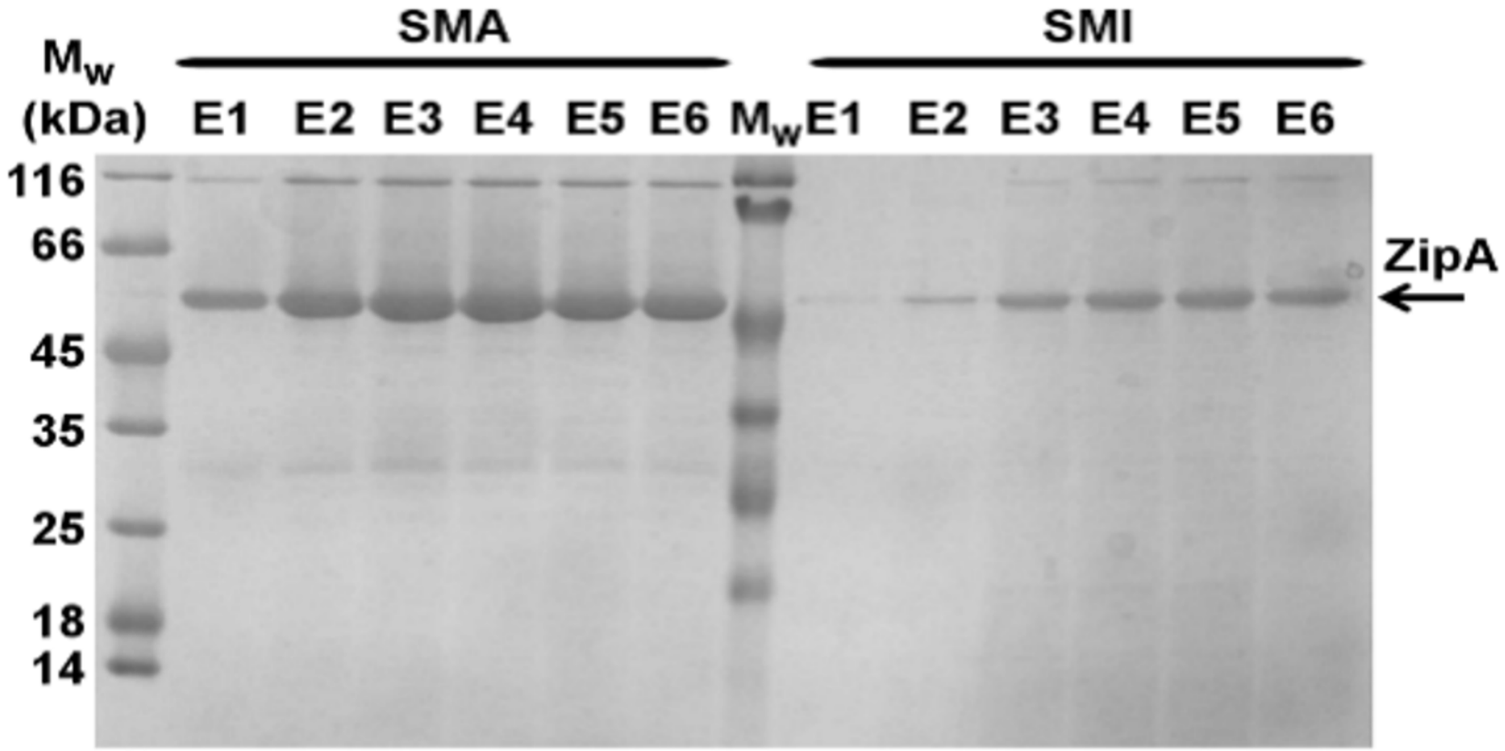
- Hydrophobic group
- Hydrophilic group
- Functionalisation of hydrophilic group
- RAFT agent termination





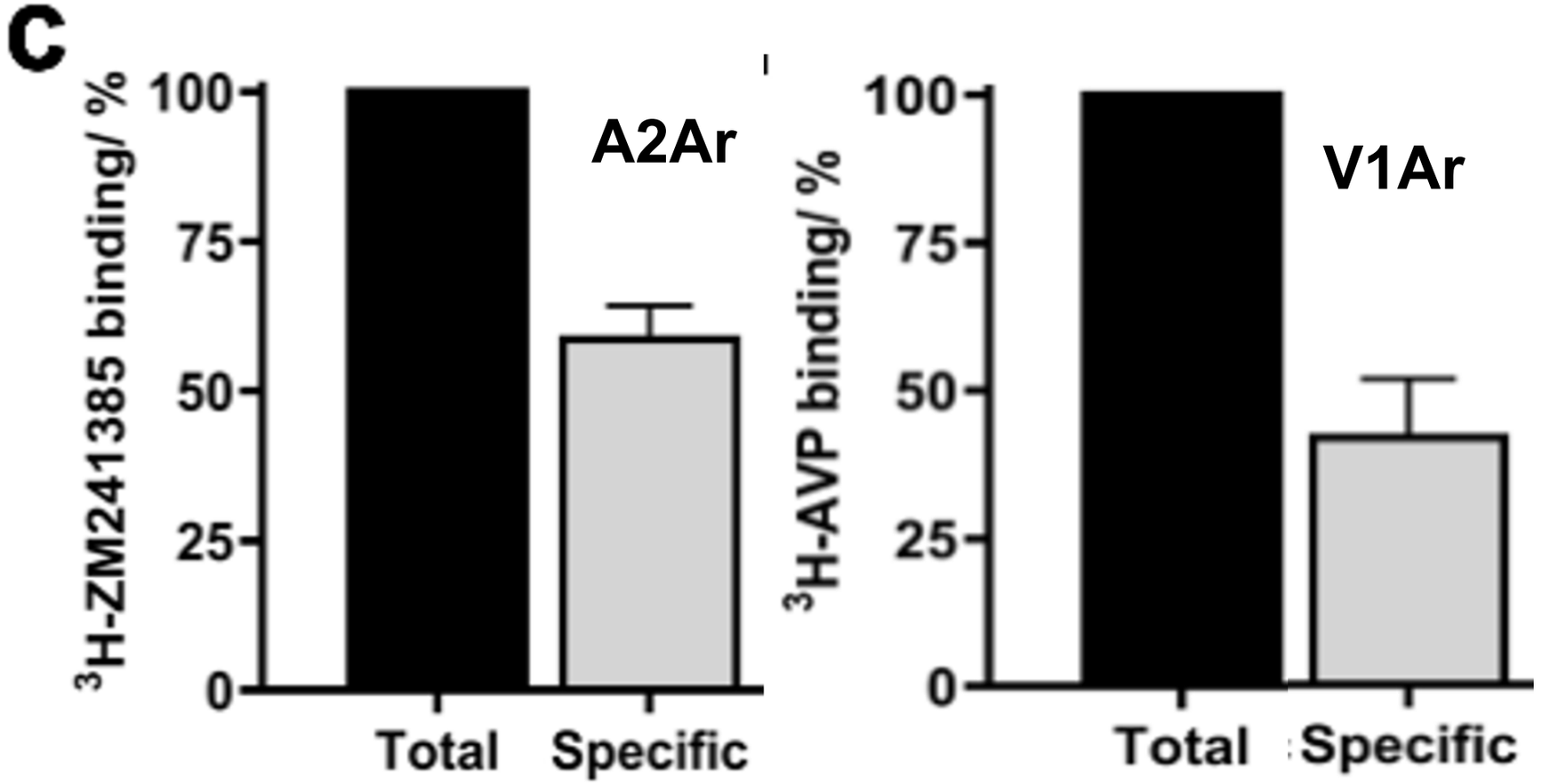
Stroud *et al.* Methods. 2018 Mar 31. pii: S1046-2023(17)30488-7.

SMI: A Positively Charged Polymer



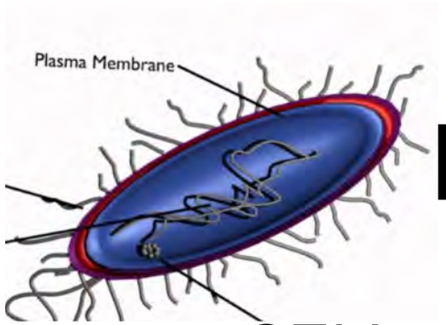
Stroud *et al.* Methods. 2018 Mar 31. pii: S1046-2023(17)30488-7.
Hall *et al.* Nanoscale. 2018 Jun 7;10(22):10609-10619

SMILPs and GPCRs

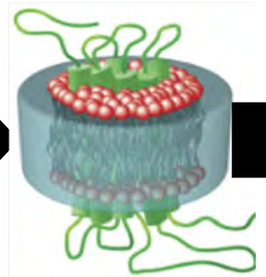


“That’s all well and good but I have very little protein” *anon*

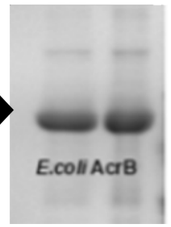
Membrane protein research in **Miniature**
SMA-PAGE



CELL



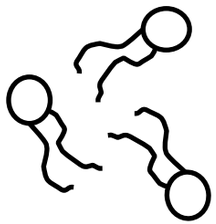
SMALP
EXTRACT



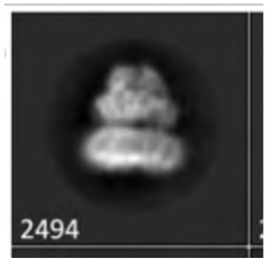
SMA
PAGE

GGDEVSSE
HILLAGKK
AAASLVDC

PROTEOMICS
COMPLEX-OMICS



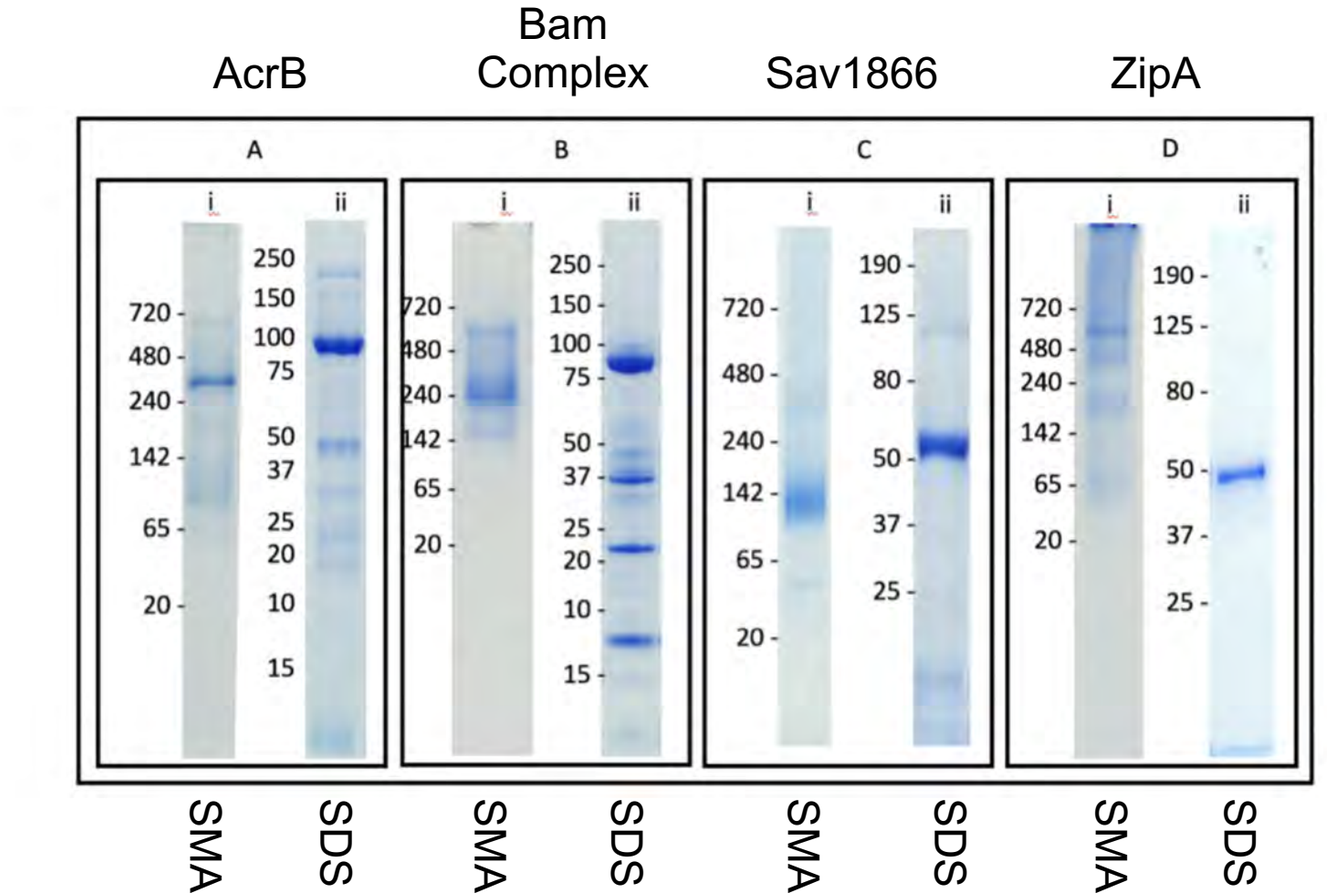
LIPIDOMICS



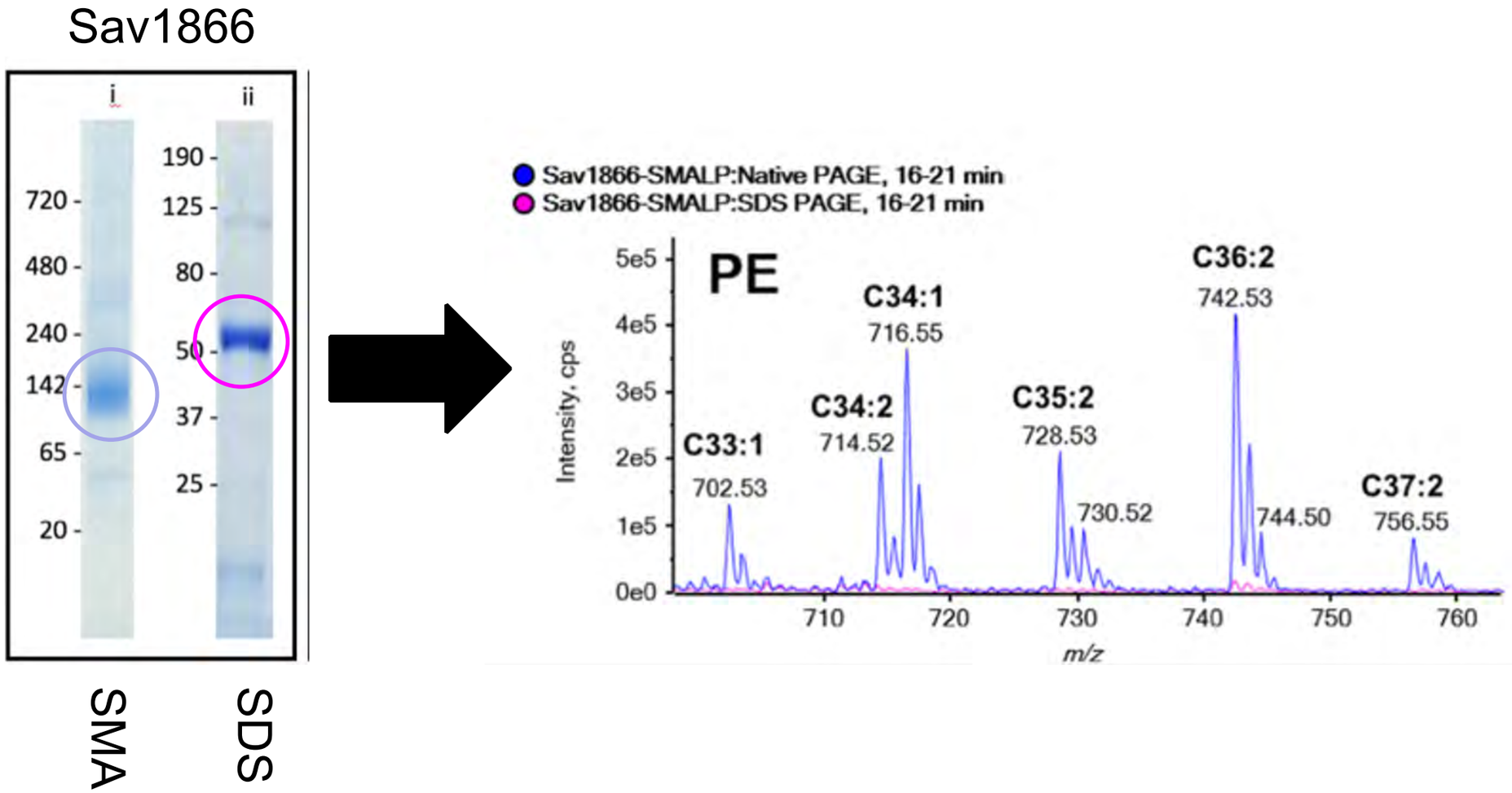
CRYO-EM

Pollock *et al.* Biochim Biophys Acta Biomembr. 2019 pii: S0005-2736(19)30108-7.

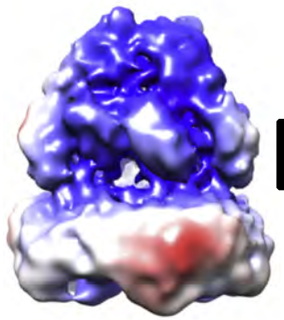
SMA-PAGE separation

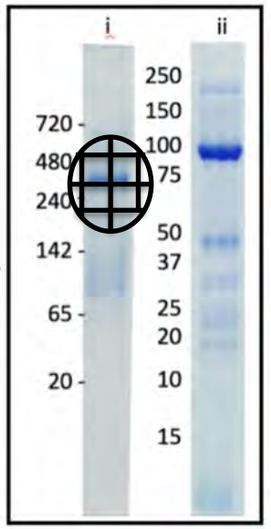


Targeted Lipidomics from SMA-PAGE



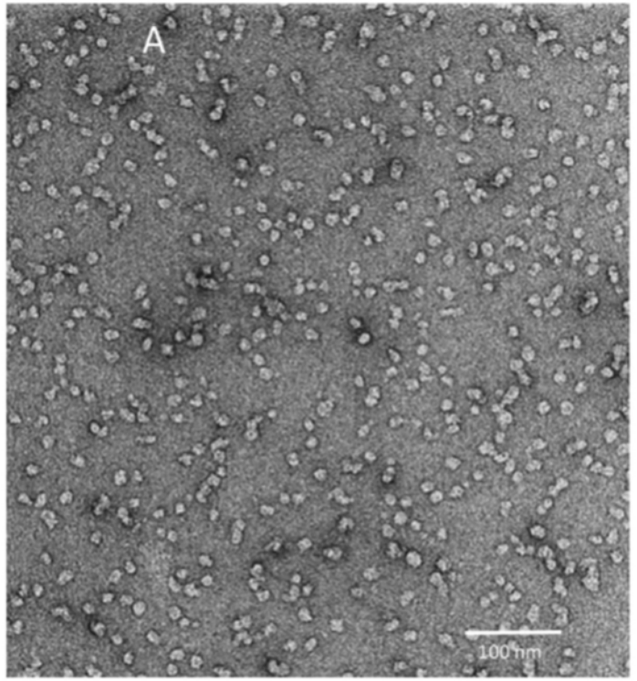
SMA-PAGE to EM: Gel-2-Grid



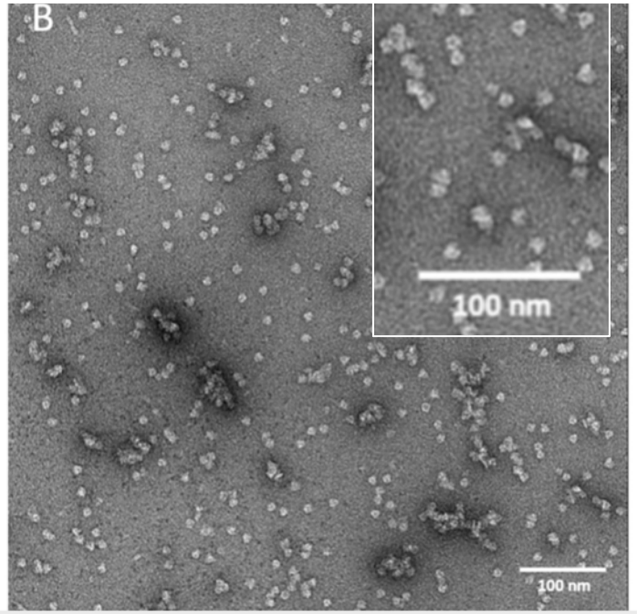


SMA-PAGE
Of AcrB

Route A



Route B



Thanks

University of Birmingham
Yu-pin Lin
Sarah Lee
Tim Knowles
Michael Overduin
Naomi Pollock
Mohammed Jamshad
Rosemary Parslow
Steve Hall
Zoe Stroud
Mark Wheatley

University of Bath
Karen Edler & Tom Arnold

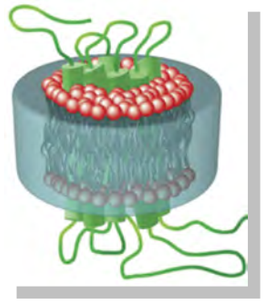
University of Aston
David Poyner
Alice Rothnie
Roslyn Bill

University of Warwick
Ian Hands-Portman
David Roper
Corinne Smith

University of Stellenbosch
Bert Klumperman

University of Leeds
Vincent Postis
Stephen Muench
Steve Baldwin

Malvern Cosmeceutics Ltd
Steve Tong



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bioscience for the future