Novel polymers for memtein solubilization and analysis

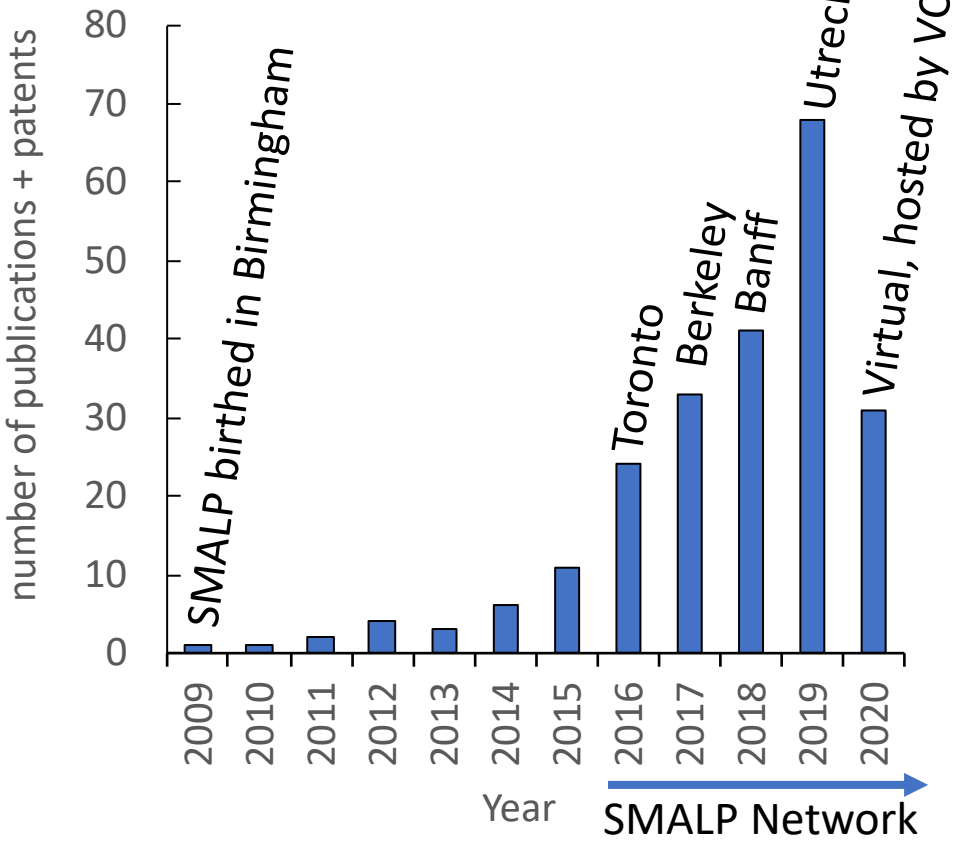
Michael Overduin

Department of Biochemistry

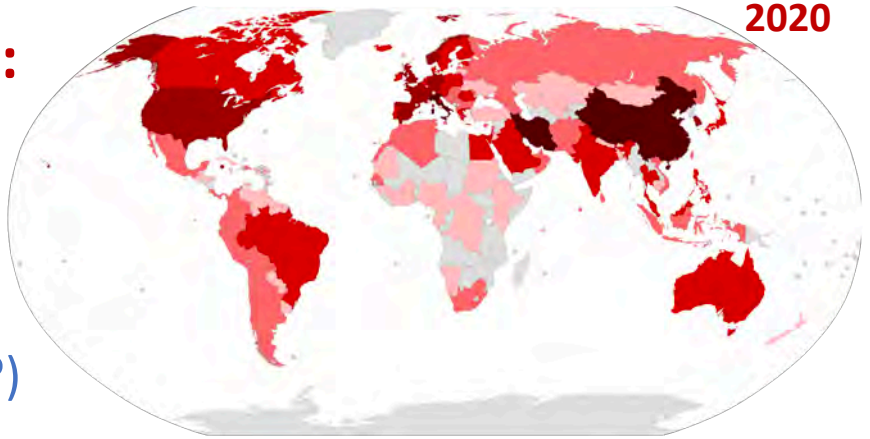
SMALP

UNIVERSITY OF ALBERTA

SMALP Network Meetings: supporting collaborations & partnerships



Future SMALP meetings:



3rd Fridays by Zoom on:

June 19, 2020: 12-1pm EST

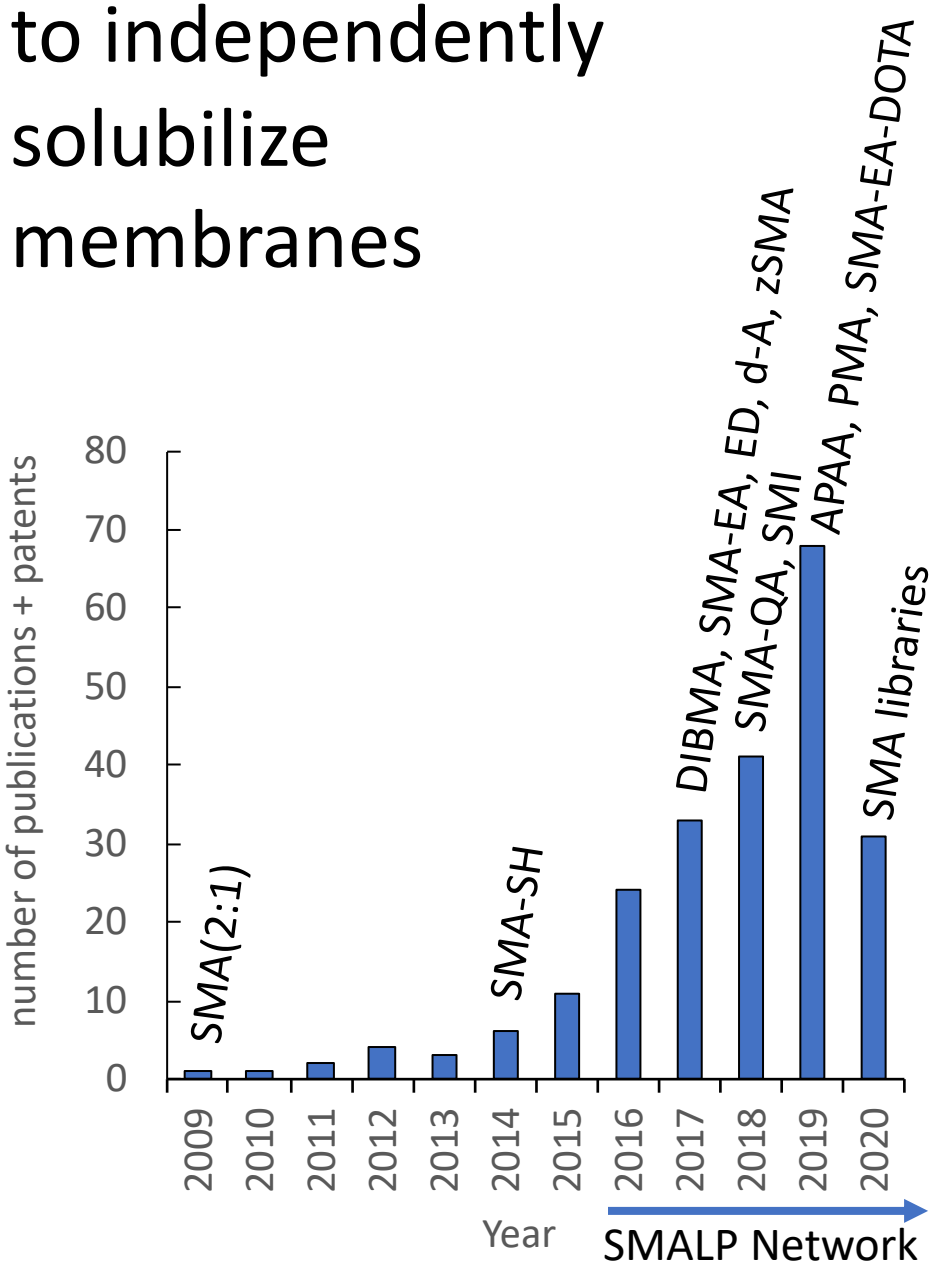
Sept 18, 2020: 12-1pm EST

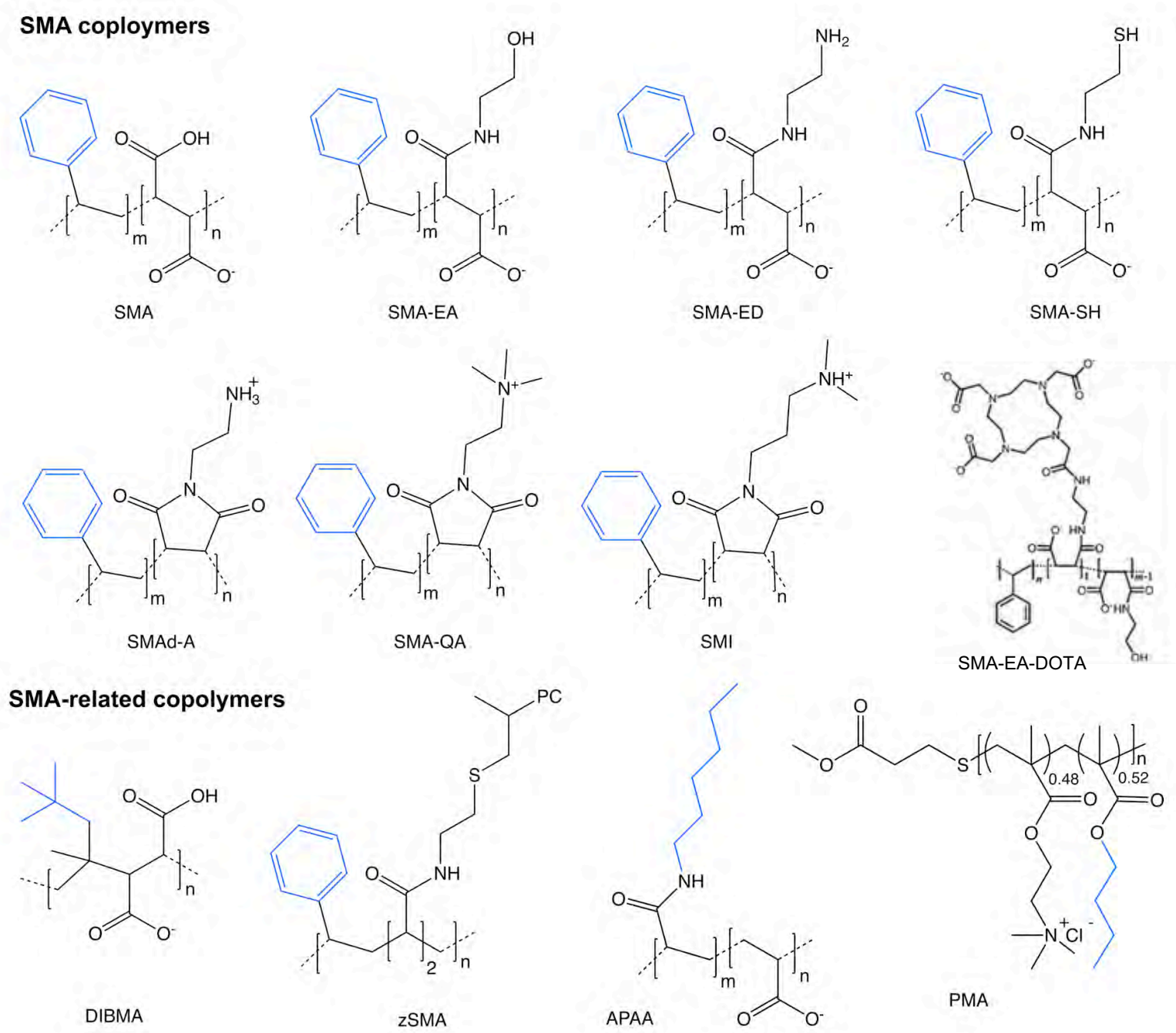
Spring 2021 in Birmingham (?)

Invited:

- Speakers with major new developments
- Delegates of this meeting
- Registration will open May at www.smalp.net

20+ copolymers shown to independently solubilize membranes



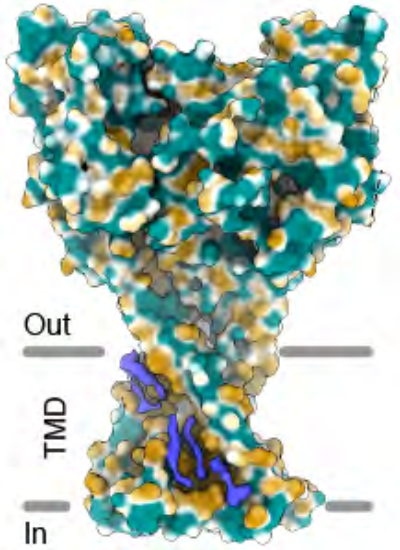


See: www.smalp.net/publications.html

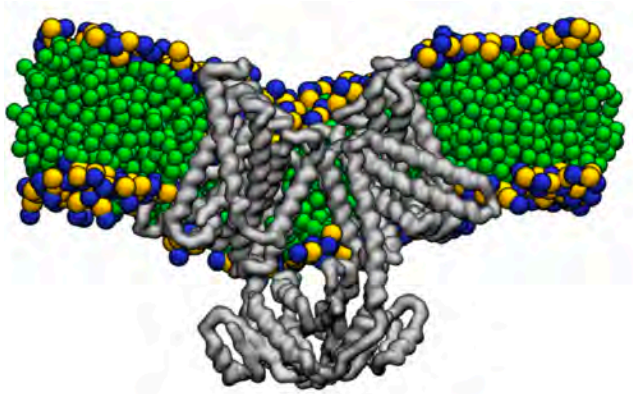
Memtein* cryo-EM structures using SMA(2:1) reveal protein multimers, PTMs, bound ligands and lipids

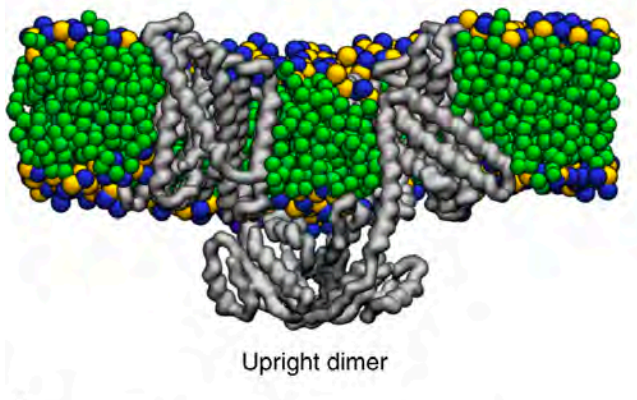
* **mem**brane:pro**tein** complex

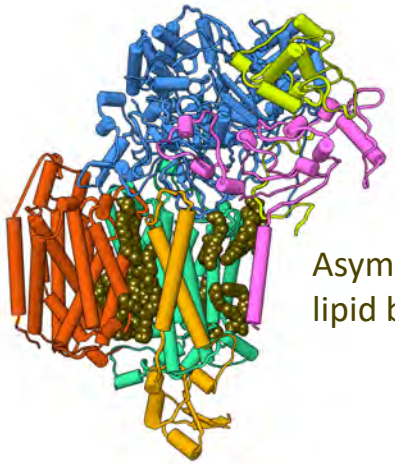
Acid-sensing ion channel 1
in SMALP 30010
Yoder N… Gouax E. 2020. **bioRxiv**



potassium importer KimA:bilayer models in SMALP 30010
Tascón I 2020. **Nat Commun**. 11:626

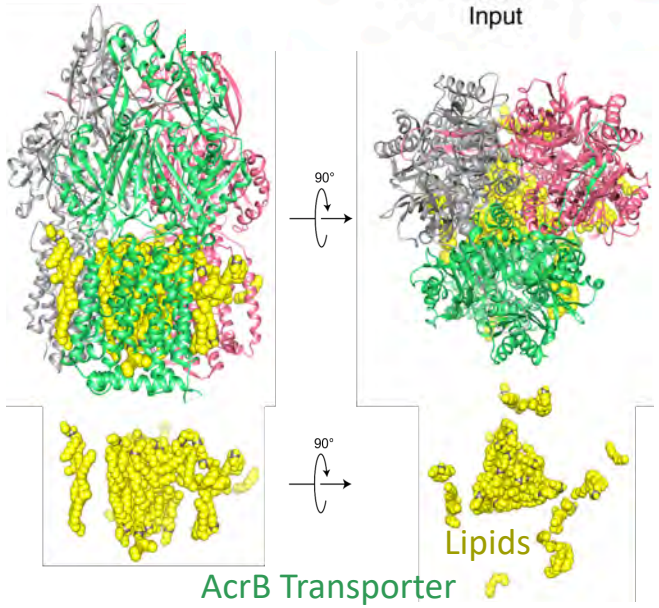




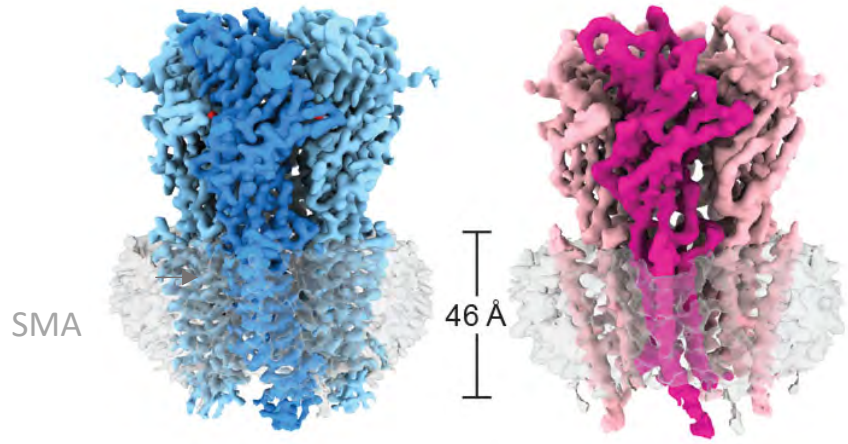


Asymmetric
lipid bilayer

Alternative Complex III
in SMA2000 and SMALP 30010
Sun, C. 2018 **Nature**, 557, 123

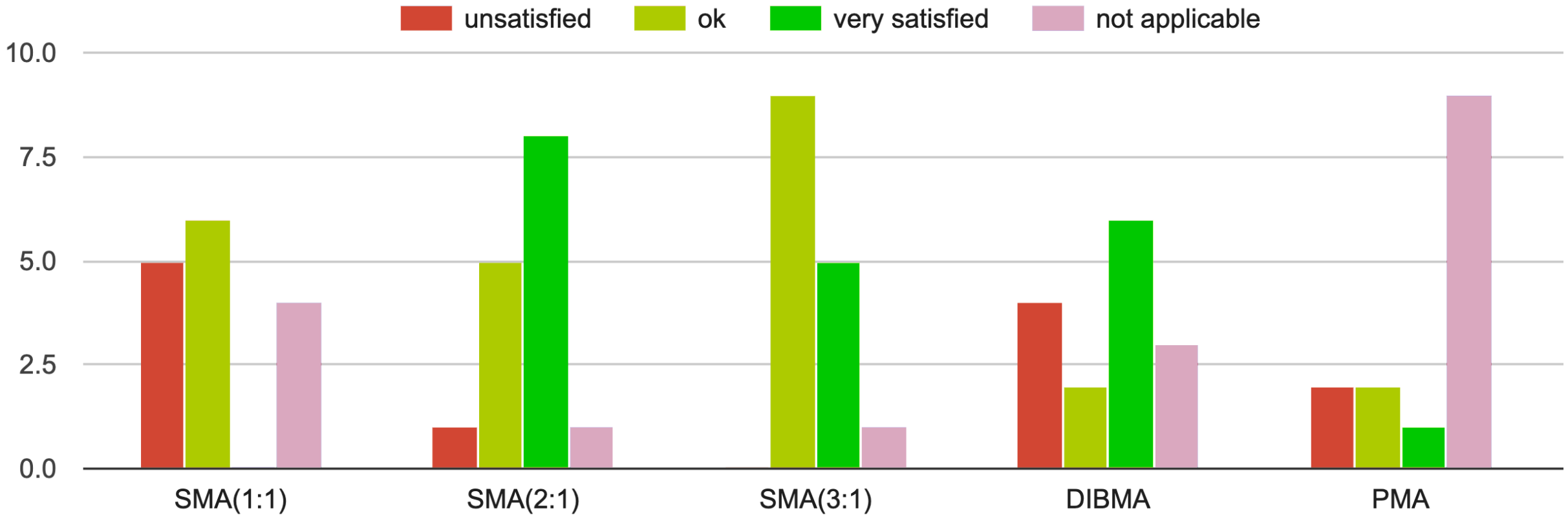


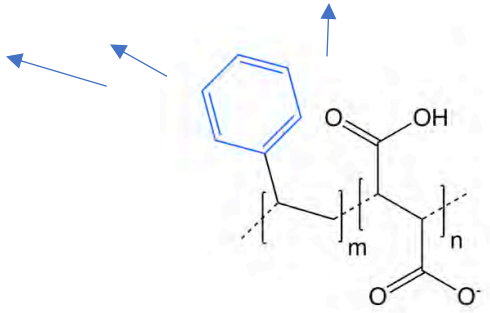
AcrB Transporter
in SMA2000
Qiu, W. 2018 **PNAS USA**, *115*, 12985

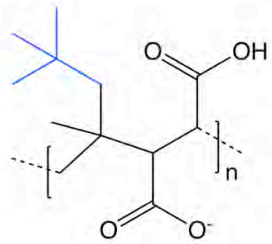


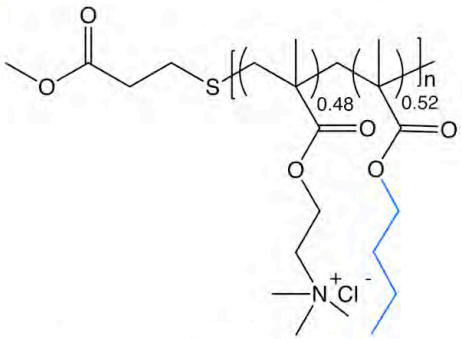
Open and Super Open Glycine Receptor Pentamer
in SMALP 30010
Yu, J … Gouax E. 2019. **bioRxiv**

Polymer Satisfaction: SMA(2:1) is best followed by SMA(3:1) and DIBMA (16 responses)









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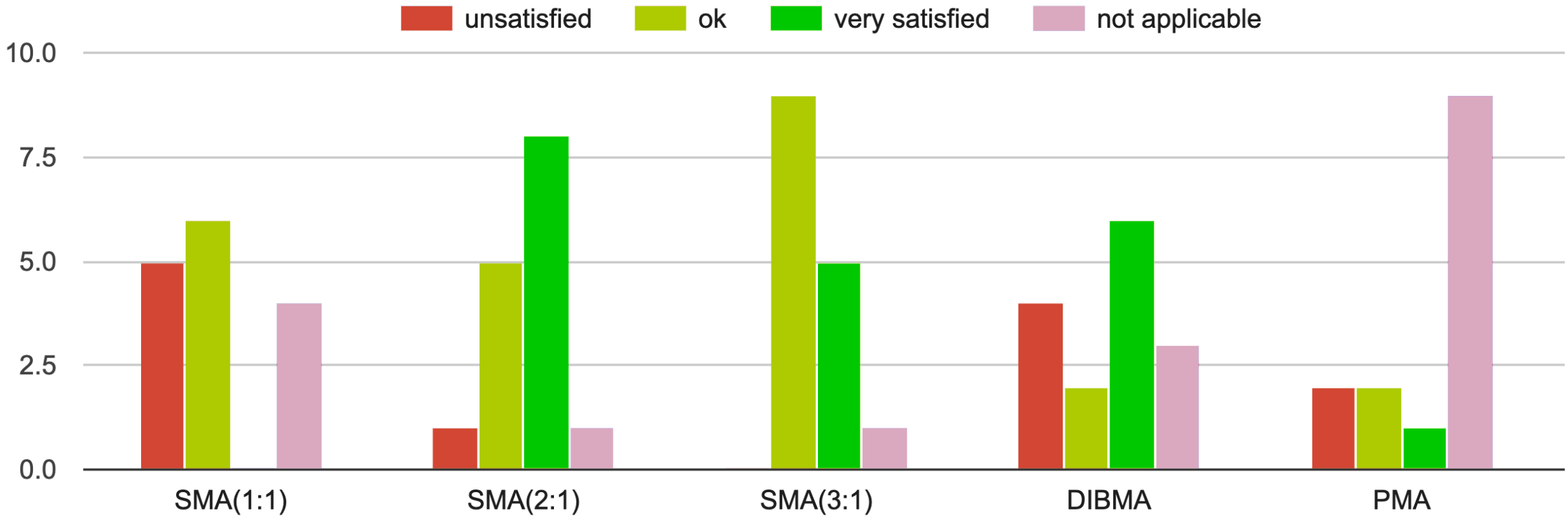
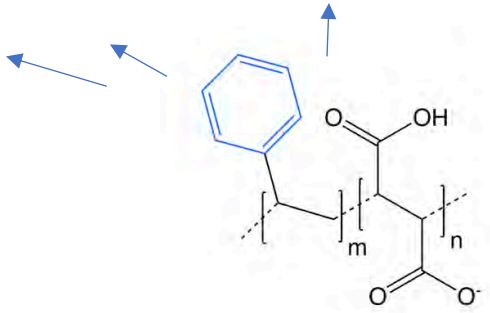
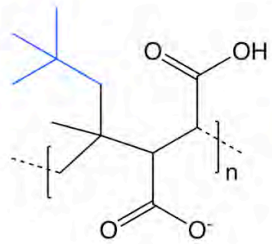
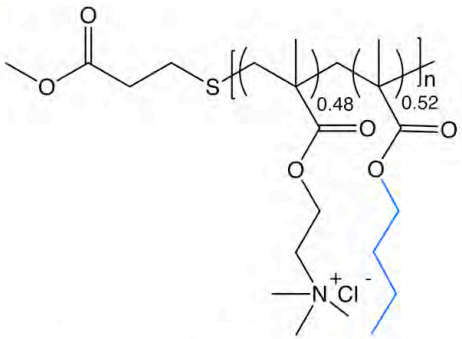






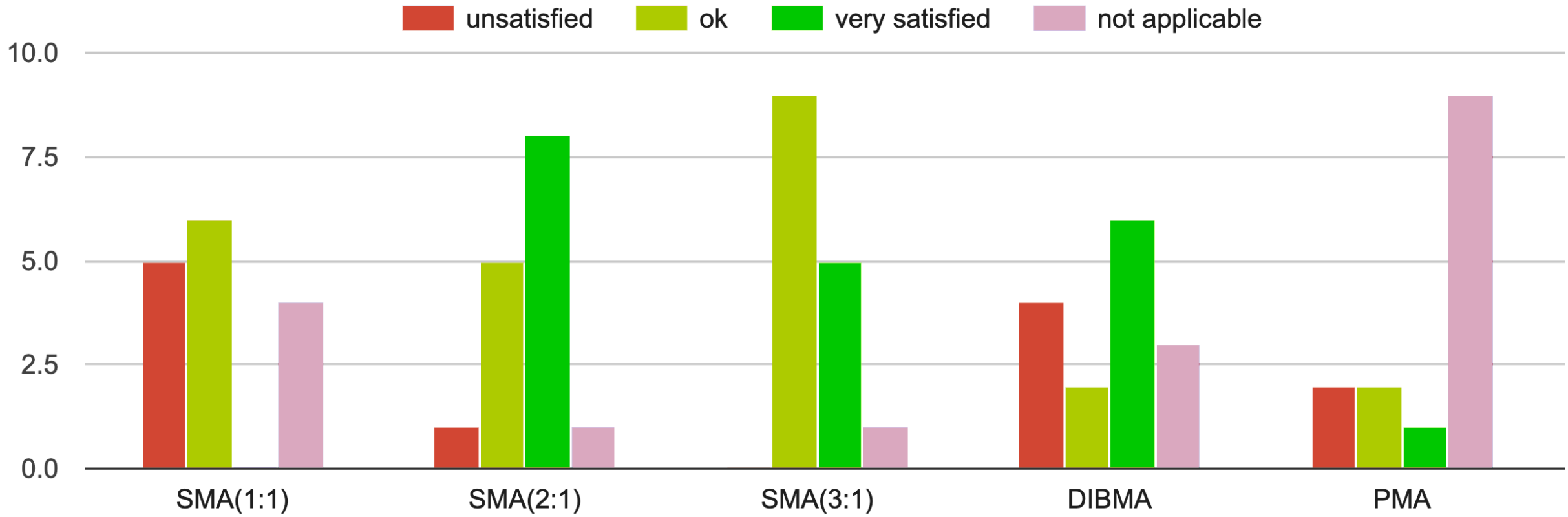


Survey is still running at www.smalp.net

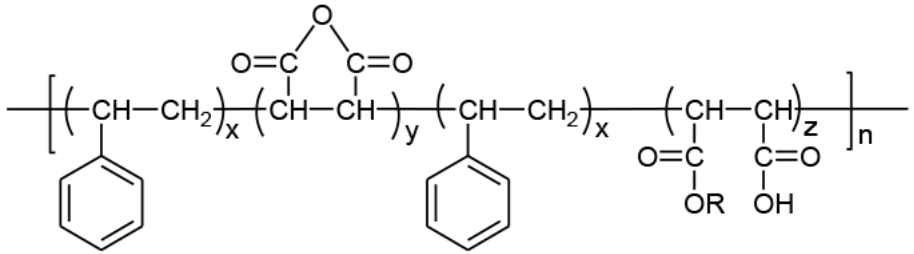
To join a collaborative study with ~10 groups on polymer performance and difficult targets contact:

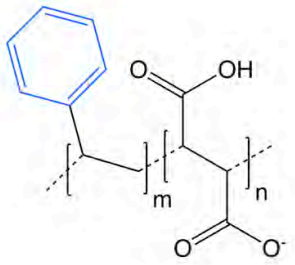
Karen Edler at k.edler@bath.ac.uk

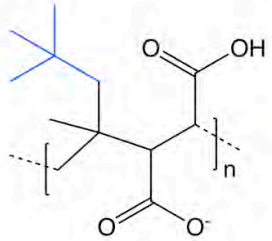
Polymer Satisfaction: there is room for improvements and new polymers

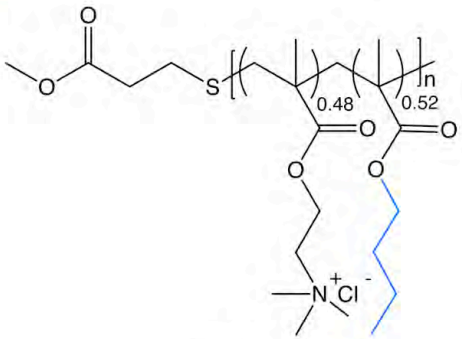


Other polymers used for making nanodiscs:



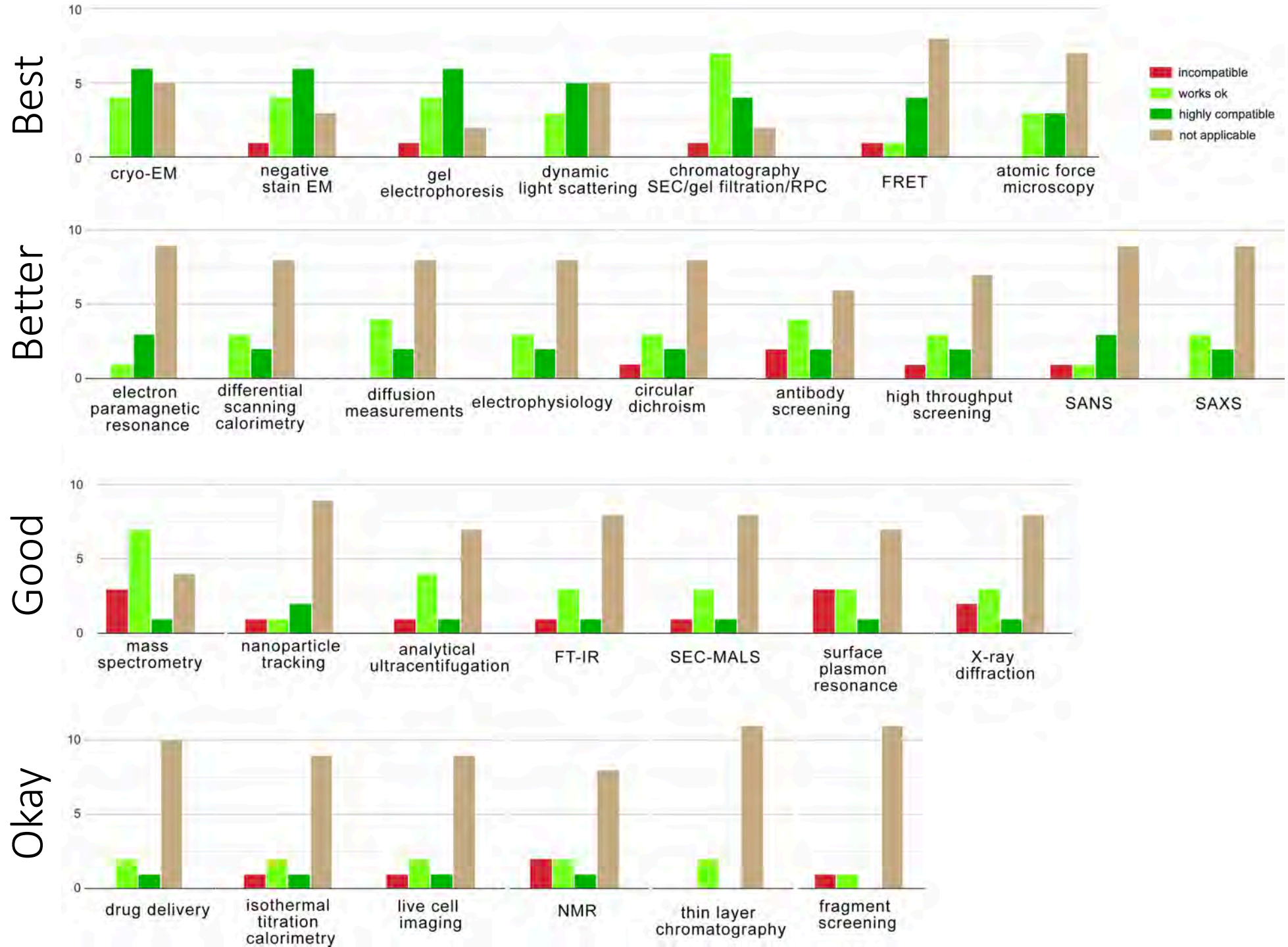




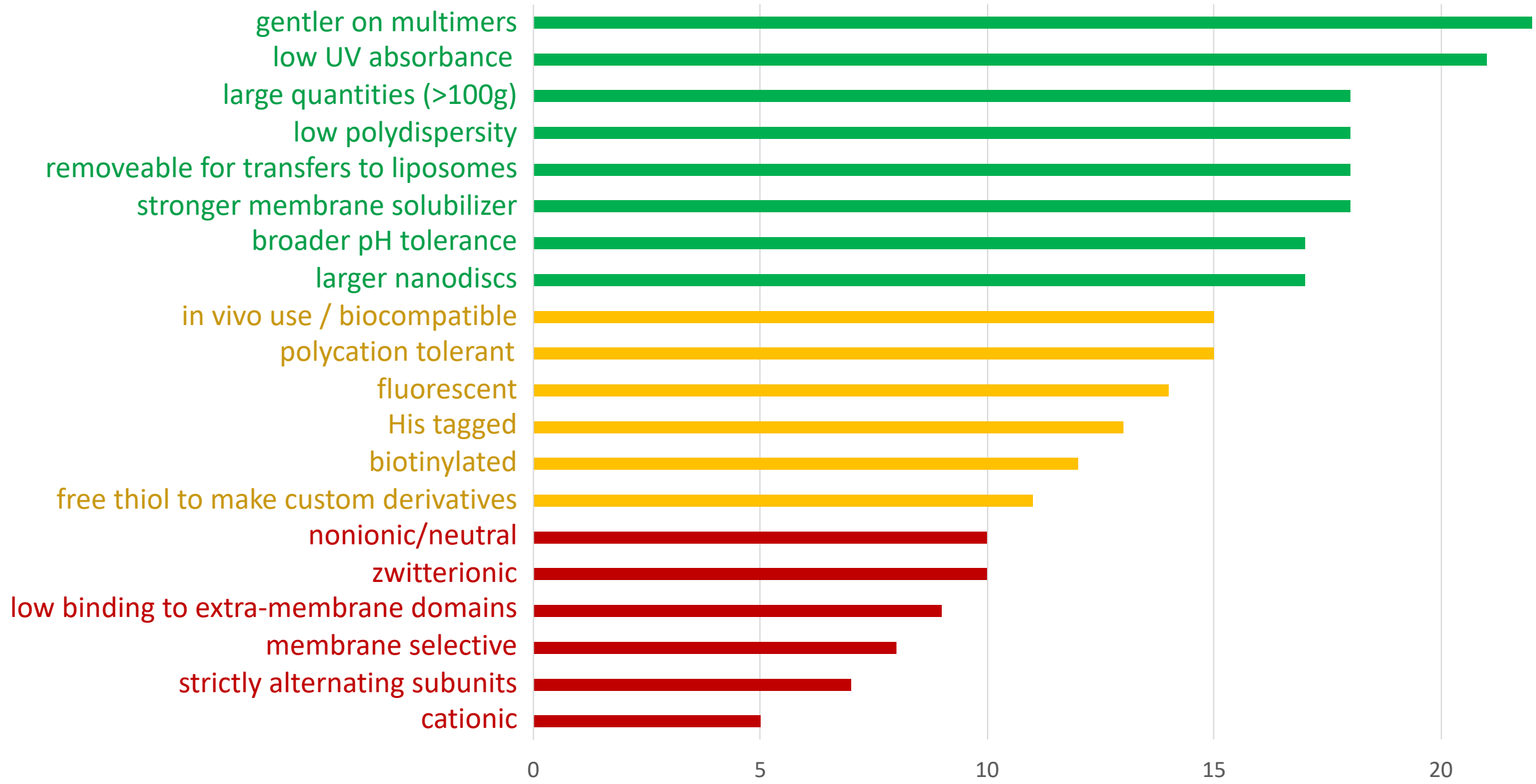


SMA1440, SMA 2625, SMA-QA, NCMNS, Apolipoproteins/MSP, Saposin, telodendrimers

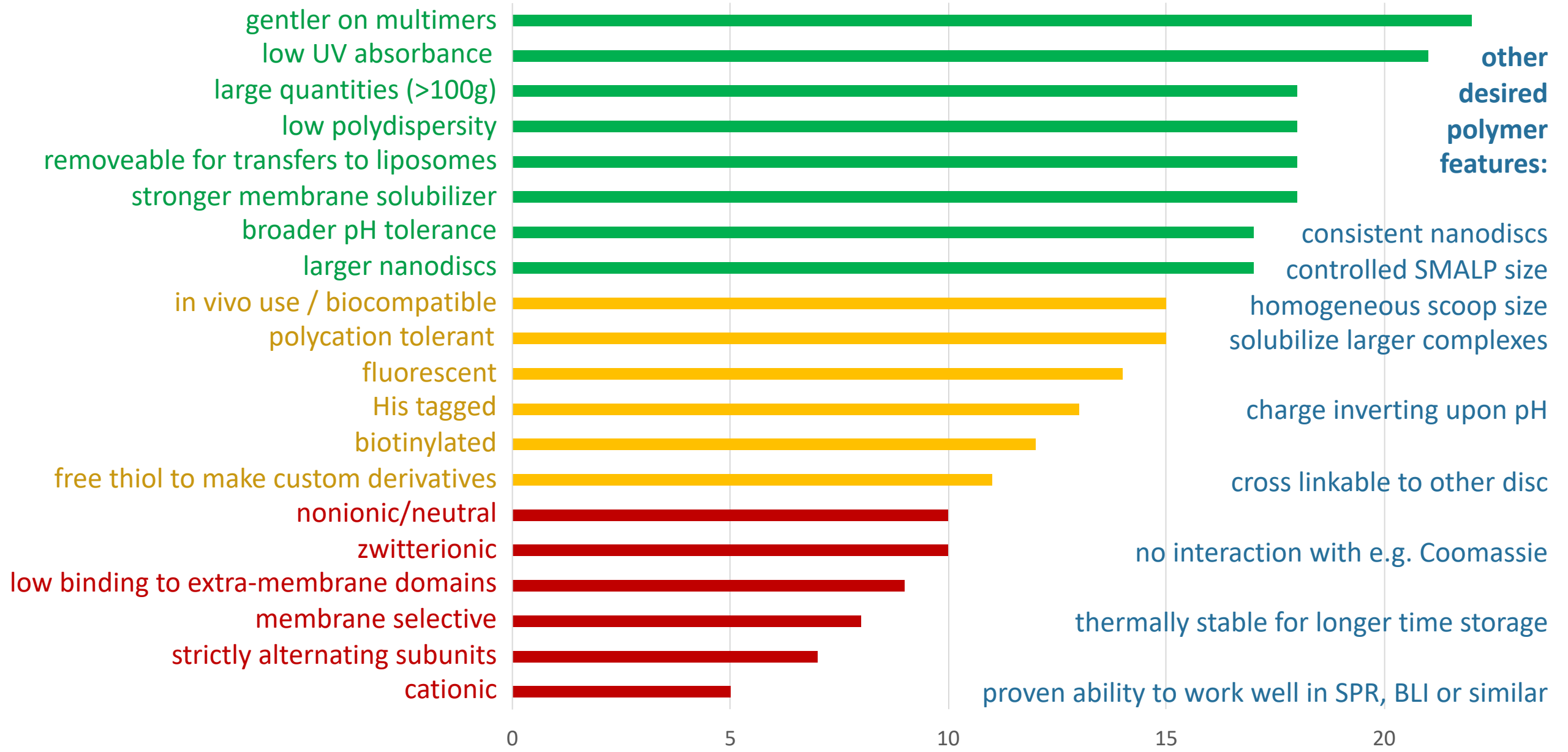
Current polymers are compatible with most (but not all) methods



Polymer Properties: Most Essential Highly Desirable Desirable



Polymer Properties: researchers want more consistency, capacity & control



Designing SMA copolymers with desired properties

Drivers:

1. reduce heterogeneity
2. generate larger discs
3. keep multimers intact
4. enhanced solubility
5. scalable production

Solutions:

alternating residues

reduce charge/repulsion

reduce hydrophobic bulk

add more polar character

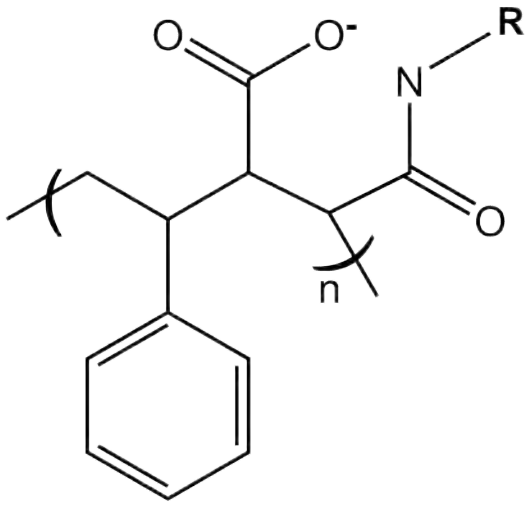
use SMA as base copolymer

New Copolymers & Derivatives

SMALP library of >40 SMA(1:1) variants

with various “R” groups, configurations

and alternatives to styrene (patent filed)



Needed for SMALP Industry Club

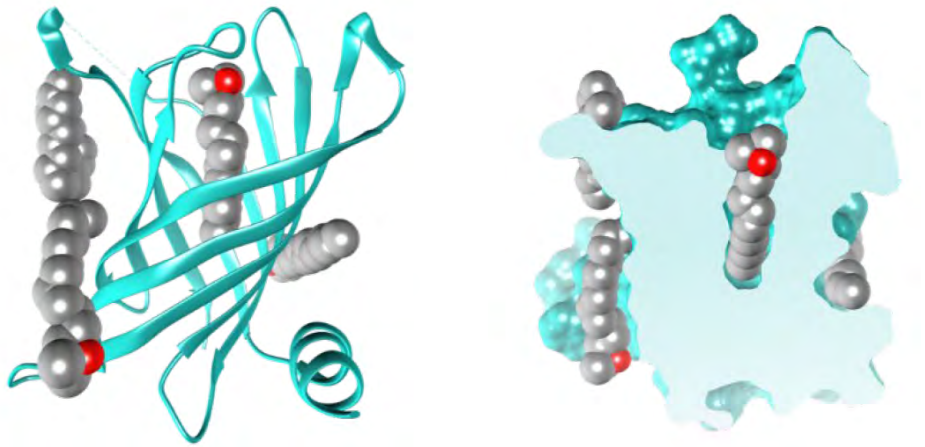
Commercial partners & academic collaborators

- apply for joint grants
- scale production of new polymers
- test critical targets inc. GPCRs, ion channels
- develop assays / equipment
- global distribution

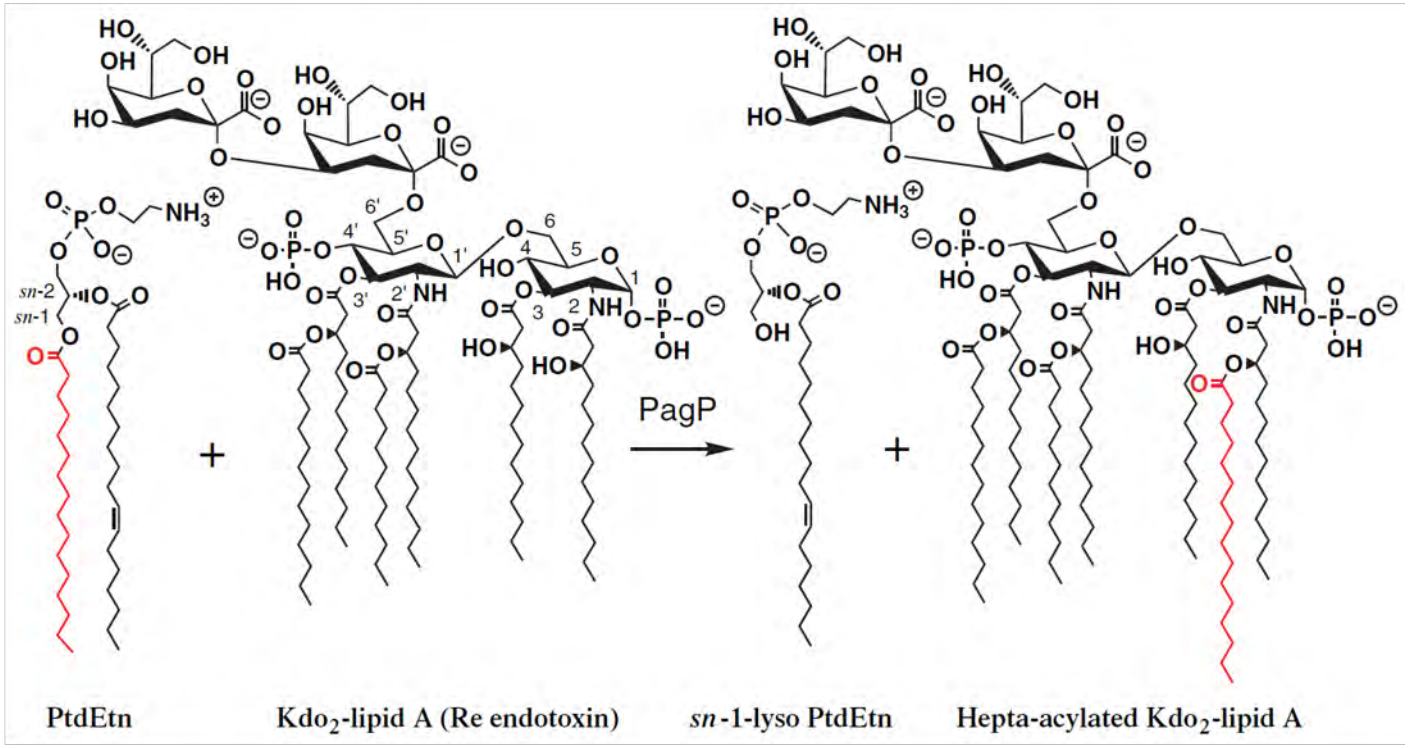
Contact Michael at overduin@ualberta.ca

Testing new SMA copolymer derivatives in 4 systems:

1. Synthetic DMPC lipid
2. His_6 tagged PagP palmitoyl transferase expressed into *E.coli* outer membrane
3. Daptomycin multimers
4. Prion minifibrils associated with neuronal membranes of infected rodents

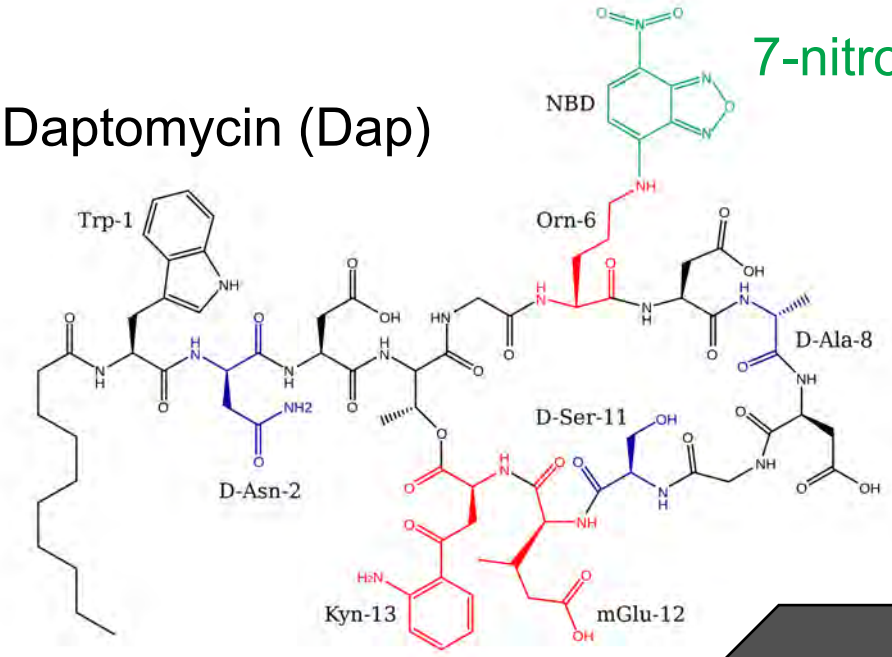


PagP:**LDAO** crystal structure
Ahn VE, Embo J, 23 (2004) 2931-2941

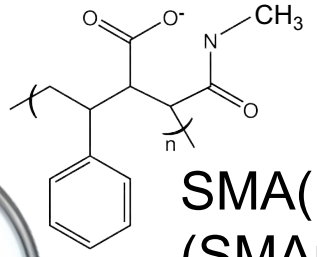


Daptomycin is used to treat Gram+ infections by an unclear mechanism

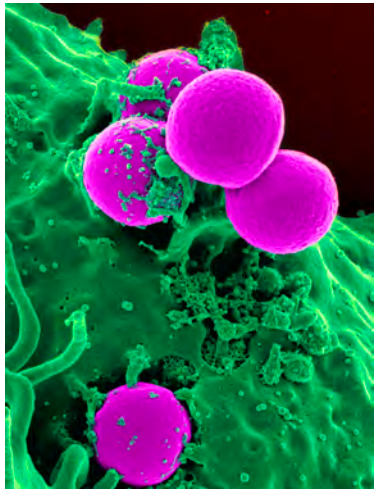
Daptomycin (Dap)



7-nitro2,1,3-benzoxadiazol fluorophore (NBD)



SMA(1:1)methylamine (SMAma)



multiple drug-resistant bacteria

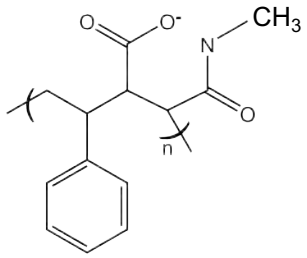
Ca^{2+}-dependent phosphatidyl-glycerol binding

cell membrane insertion, holes and cell death

SMAma:lipid:Ca^{2+} ratio determines nanodisc size

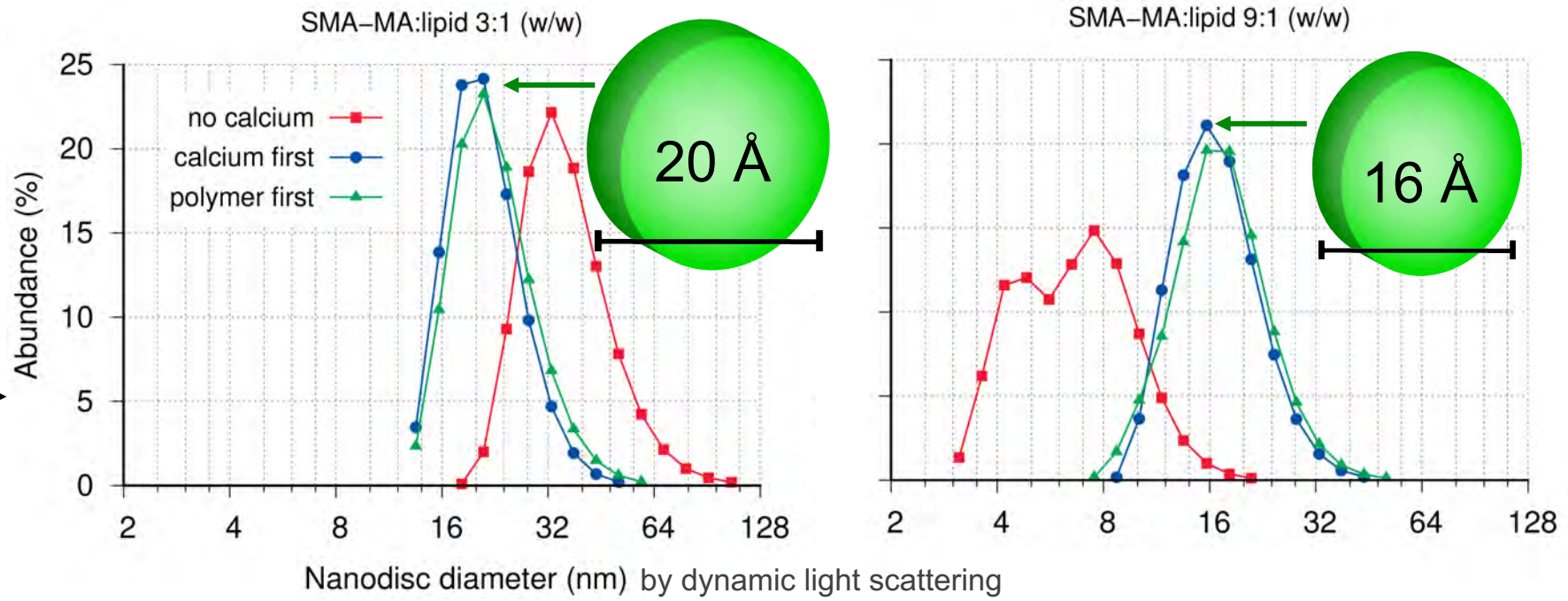
Nanodiscs (~20 nm) are formed 3:1 w/w SMAma:lipid (DMPG,DMPC) in 3 mM calcium. Without calcium disc diameters average ~32 nm.

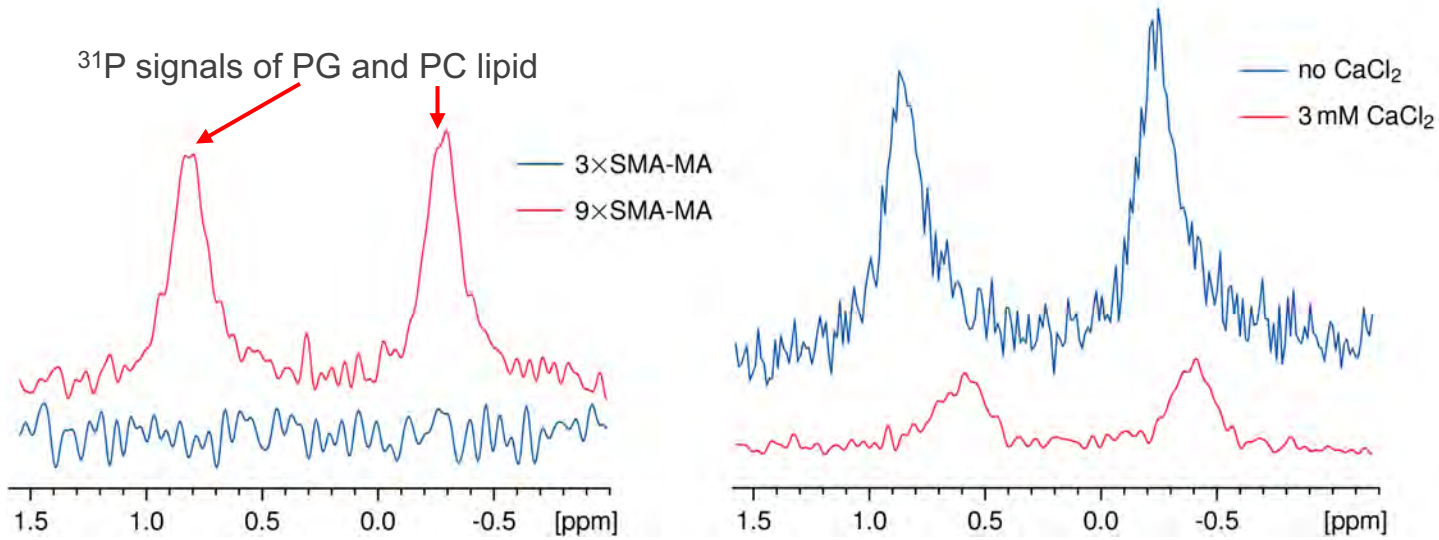
Adding SMAma to 9:1 reduces disc diameters to ~16 nm, while calcium addition increases disc diameters →→



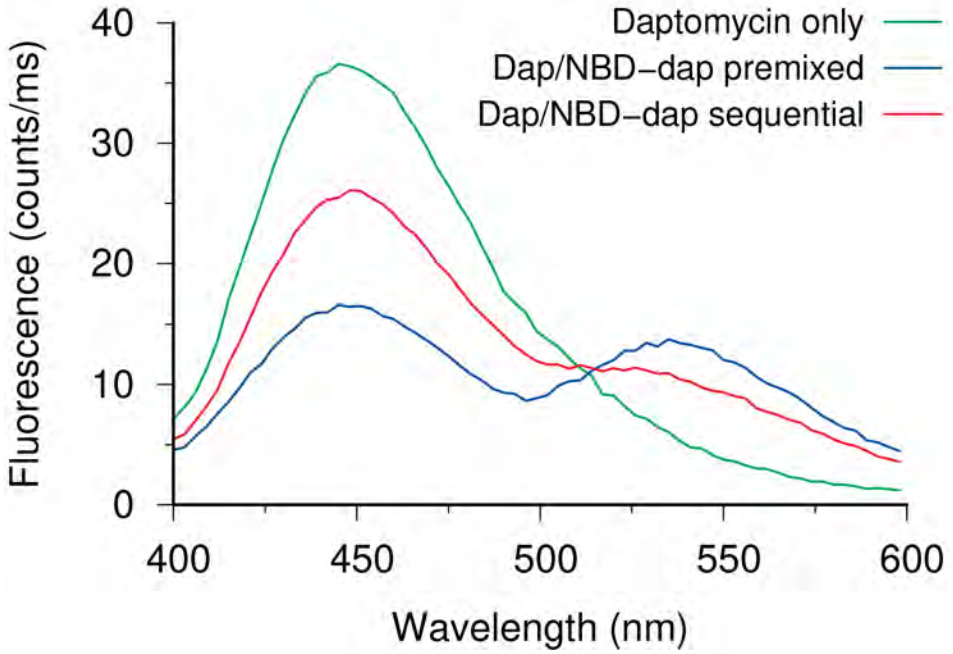
Only the smaller PG/PC nanodiscs formed by a 9 fold excess of SMAma yield detectable ^{31}P NMR signals.

The ^{31}P NMR signals broaden and shift downfield after adding 3mM calcium due to increased disc sizes and deshielding. →→

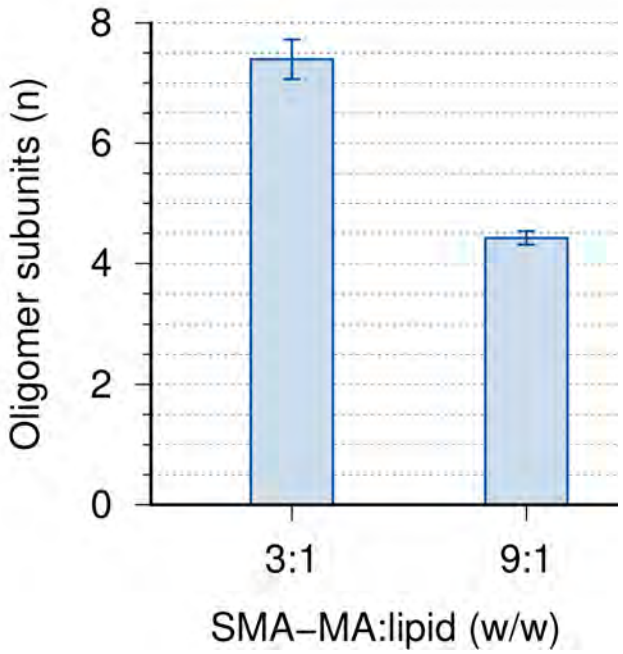




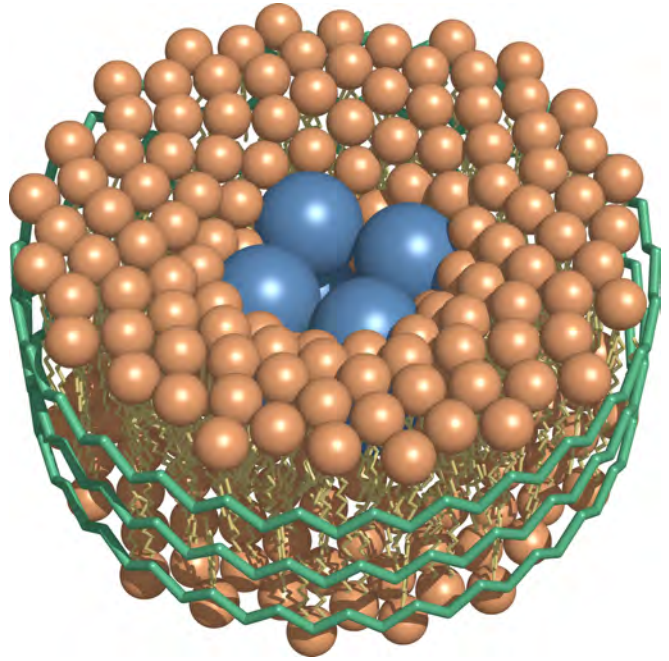
Daptomycin octamers and tetramers stabilized in nanodiscs made of 3:1 and 9:1 ratios of SMA(1:1)methylamine:lipid



FRET between native daptomycin (Dap) and NBD-daptomycin (NBD-dap) in hybrid oligomers on SMA:DMPC/DMPG nanodiscs



Subunit stoichiometry of Dap oligomers in nanodiscs formed from 3:1 and 9:1 ratios of SMAma:lipid

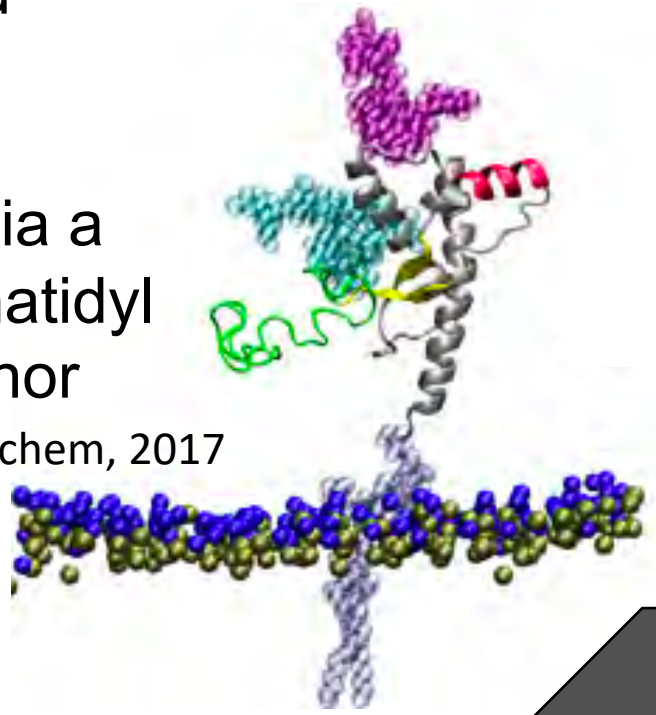


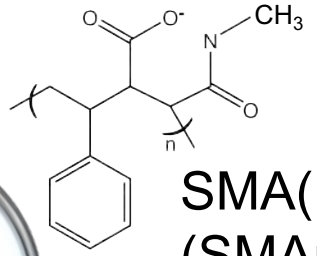
Model of stacked **Dap tetramer** in **SMAma:lipid** nanodisc

Prion diseases including CJD are caused by aggregation of Prion:Lipid

Glycosylated prion protein bound to membrane via a glycophosphatidyl -inositol anchor

CJ Cheng, J Neurochem, 2017



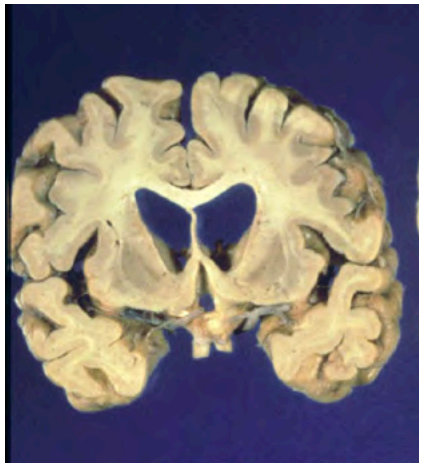


SMA(1:1)methylamine (SMAma)

healthy brain

prion oligomers aggregate to form fibrils

Creutzfeldt Jakob Disease (CJD)



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Paige Grant
Anna Jutla

NANUC:
Pascal Mercier
Rustem Shaykhutdinov

Prions: Holger Wille, Brian P. Tancowny,
Claudia Acevedo-Morantes, Xiongyao Wang, U Alberta

Polymers:
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Chanelle Brown, Rich Gandour, Virginia Tech
Bert Klumperman, U Stellenbosch
Stefan Scheidelaar, Polyscience

Daptomycin:
Michael Palmer & David Beriashvili, U Waterloo

PagP: Russell Bishop, McMaster, Canada

SMALP: Tim Dafforn

Campus Alberta Neuroscience

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