GLYCON

UNIVERSITÄT GRAZ
UNIVERSITY OF GRAZ
Institute of Molecular Biosciences

UNI GRAZ

Glyco-DIBMA

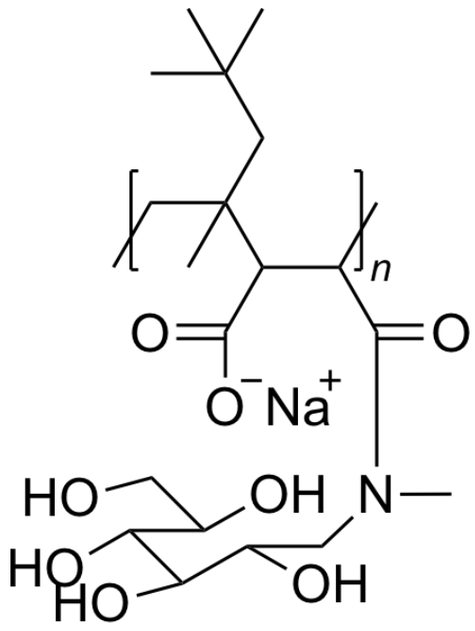
SMALP Web Meeting

28.05.2021

Bartholomäus Danielczak, Marie Rasche, Julia Lenz, Eugenio Pérez Patallo, Sophie Weyrauch, Florian Mahler, Michael Agbadaola, Annette Meister, Jonathan Oyebamiji Babalola, Carolyn Vargas, Cenek Kolar, Sandro Keller

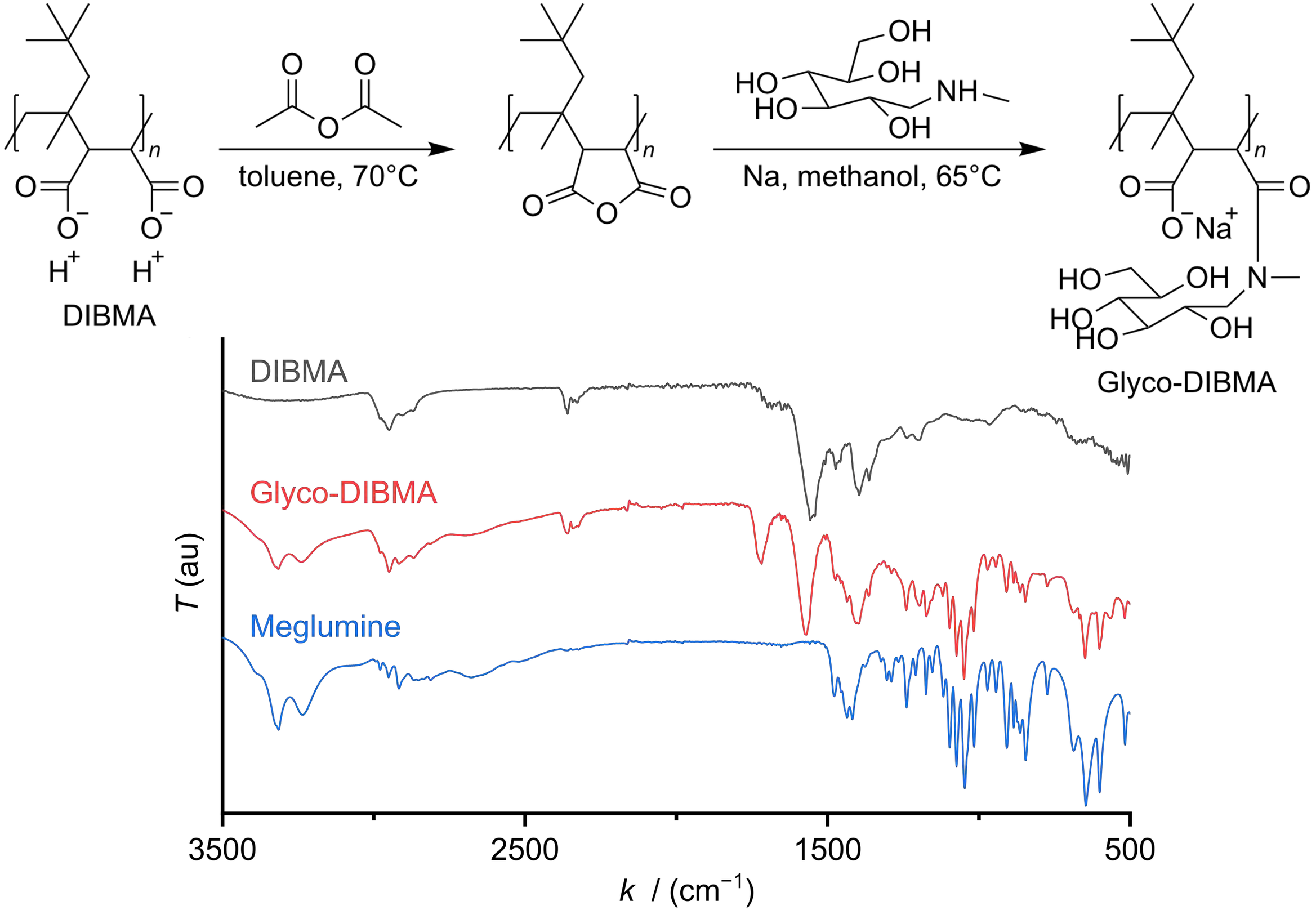
Our goal: a new polymer that…

- extracts membrane proteins and lipids with high efficiency
- forms small nanodiscs having a narrow size distribution
- is amenable to downstream analysis and manipulation
- can be safely produced in large amounts (>500 g)
- has no IP issues and is affordable (<25 €/g)

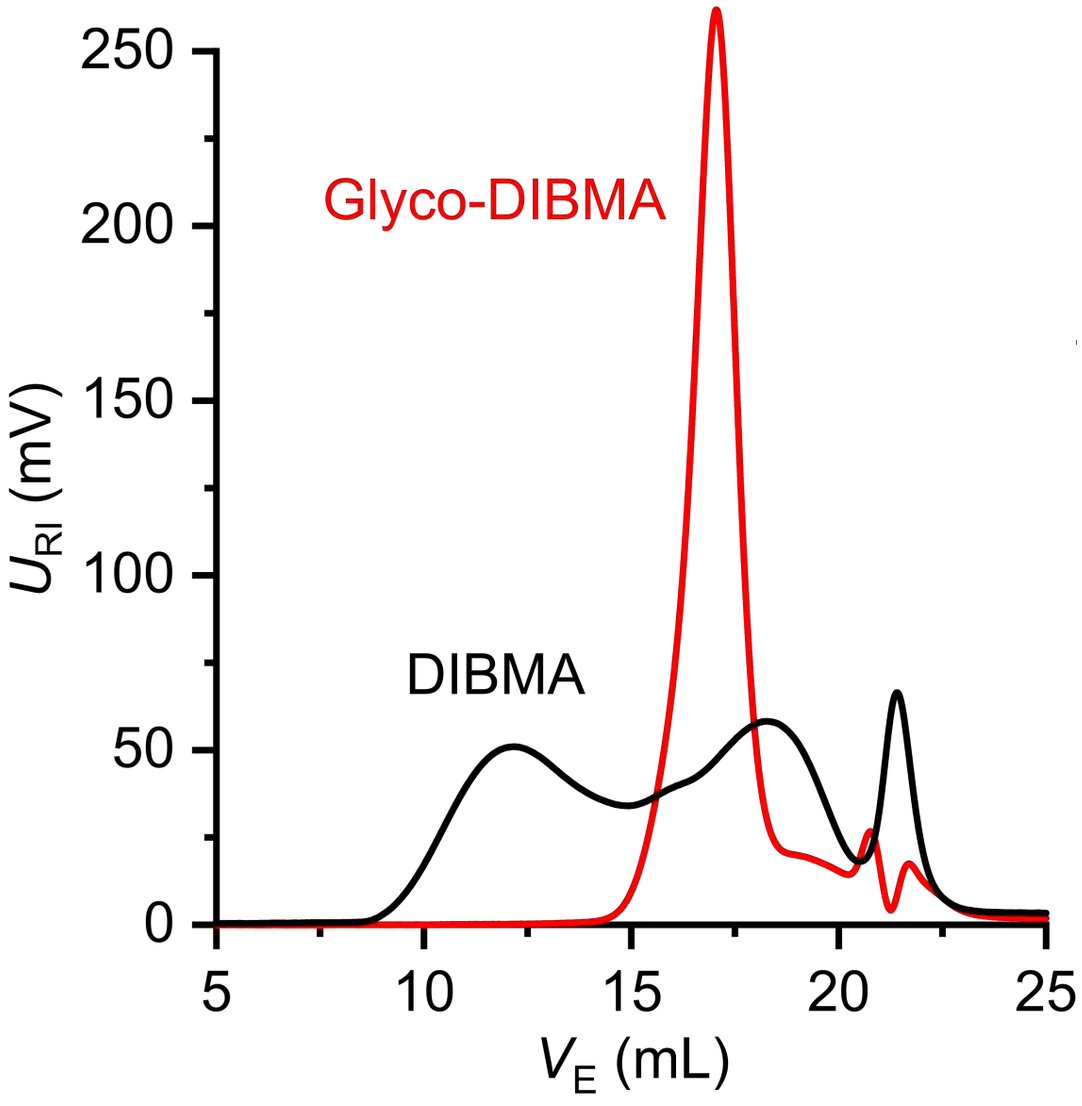


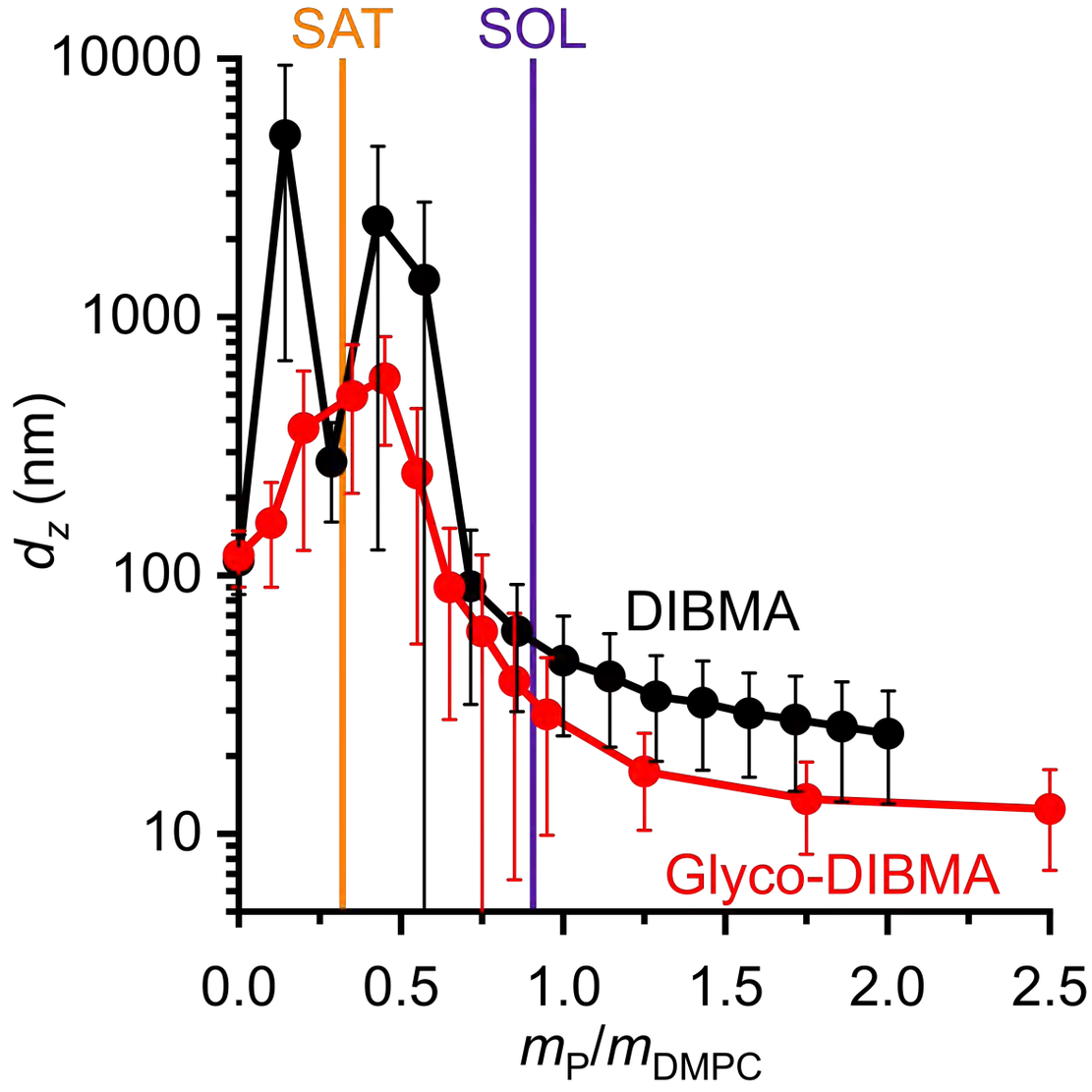
Glyco-DIBMA

Two-step synthesis & ATR-FTIR



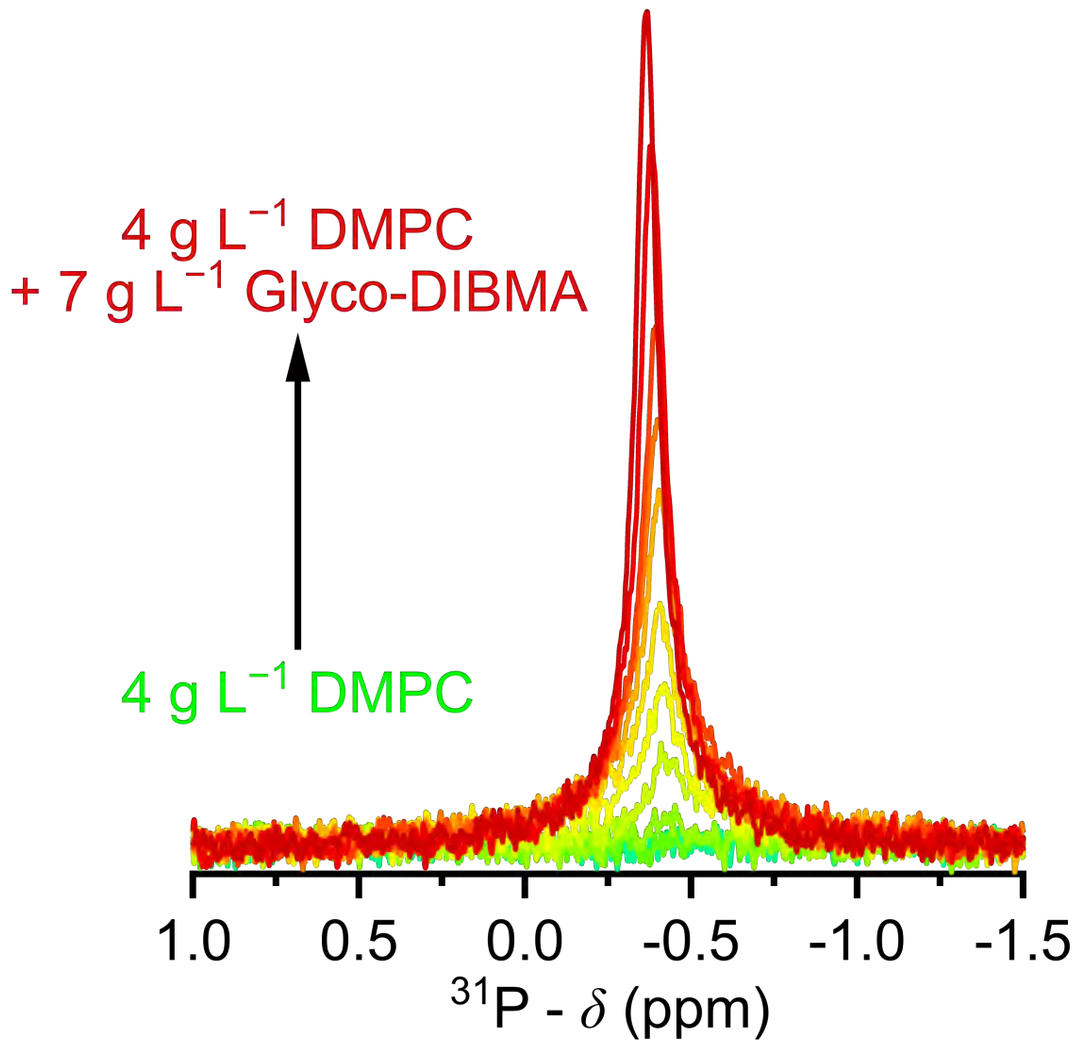
Solubilisation of DMPC by DLS & SEC

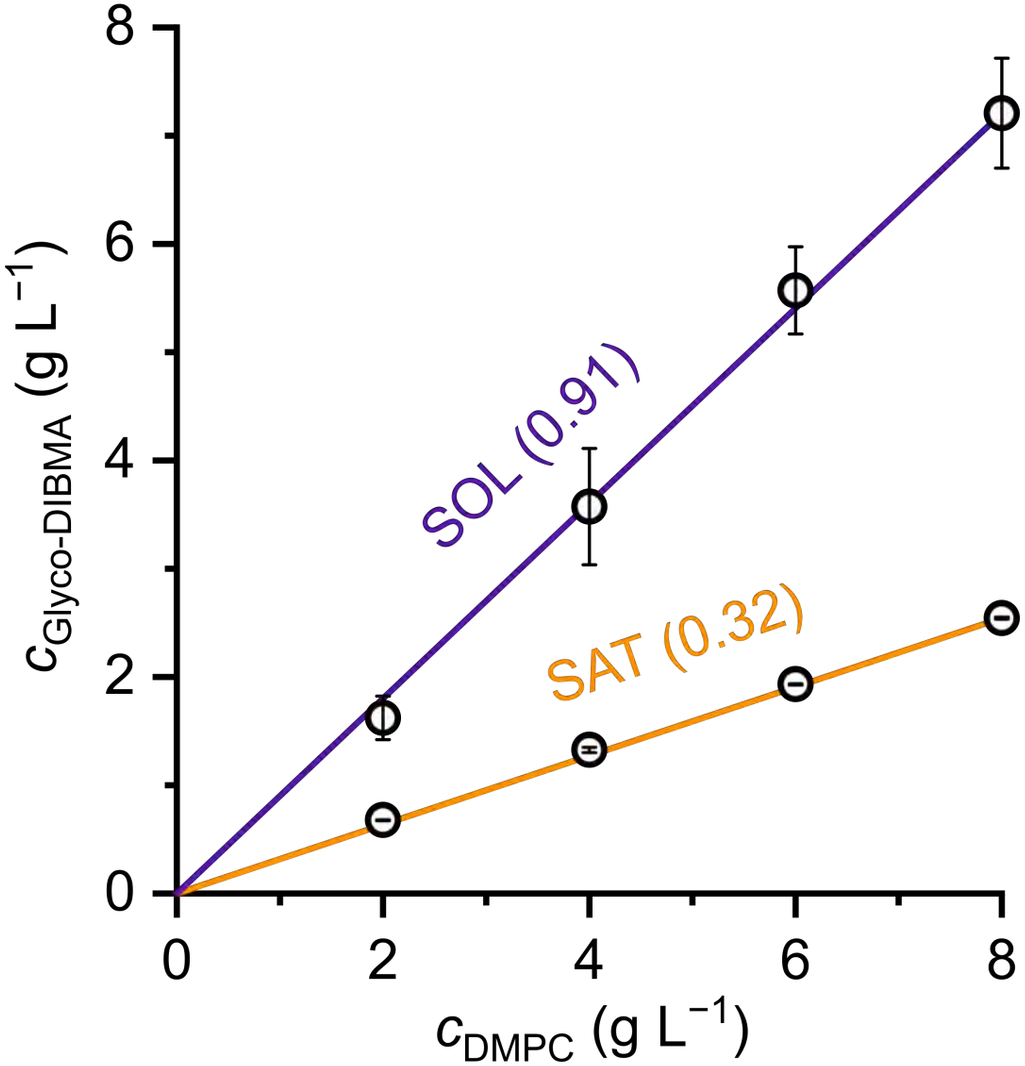




- solubilisation: Glyco-DIBMA as efficient as DIBMA
- nanodiscs: narrow size distribution of Glyco-DIBMALPs

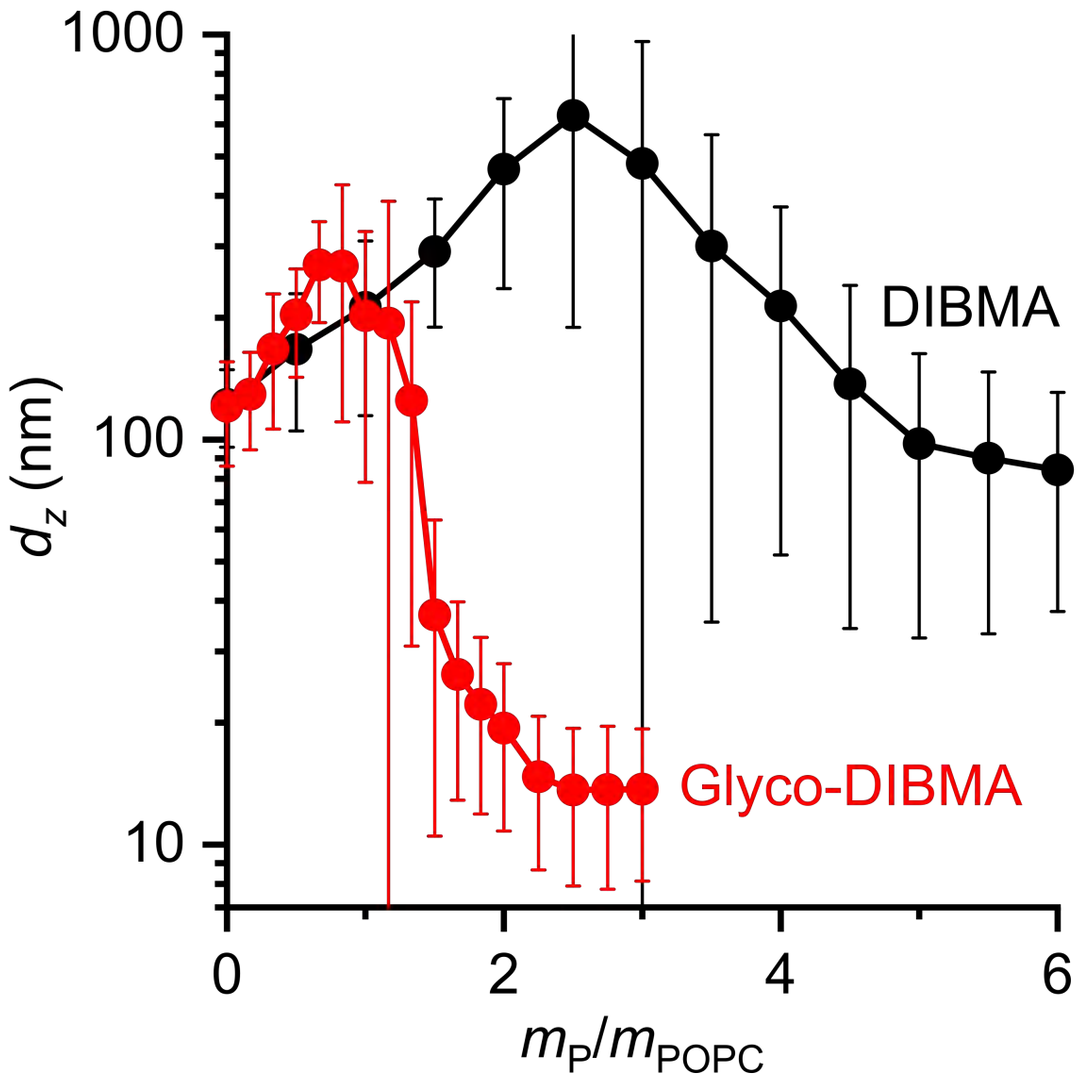
Solubilisation of DMPC by ^{31}P NMR

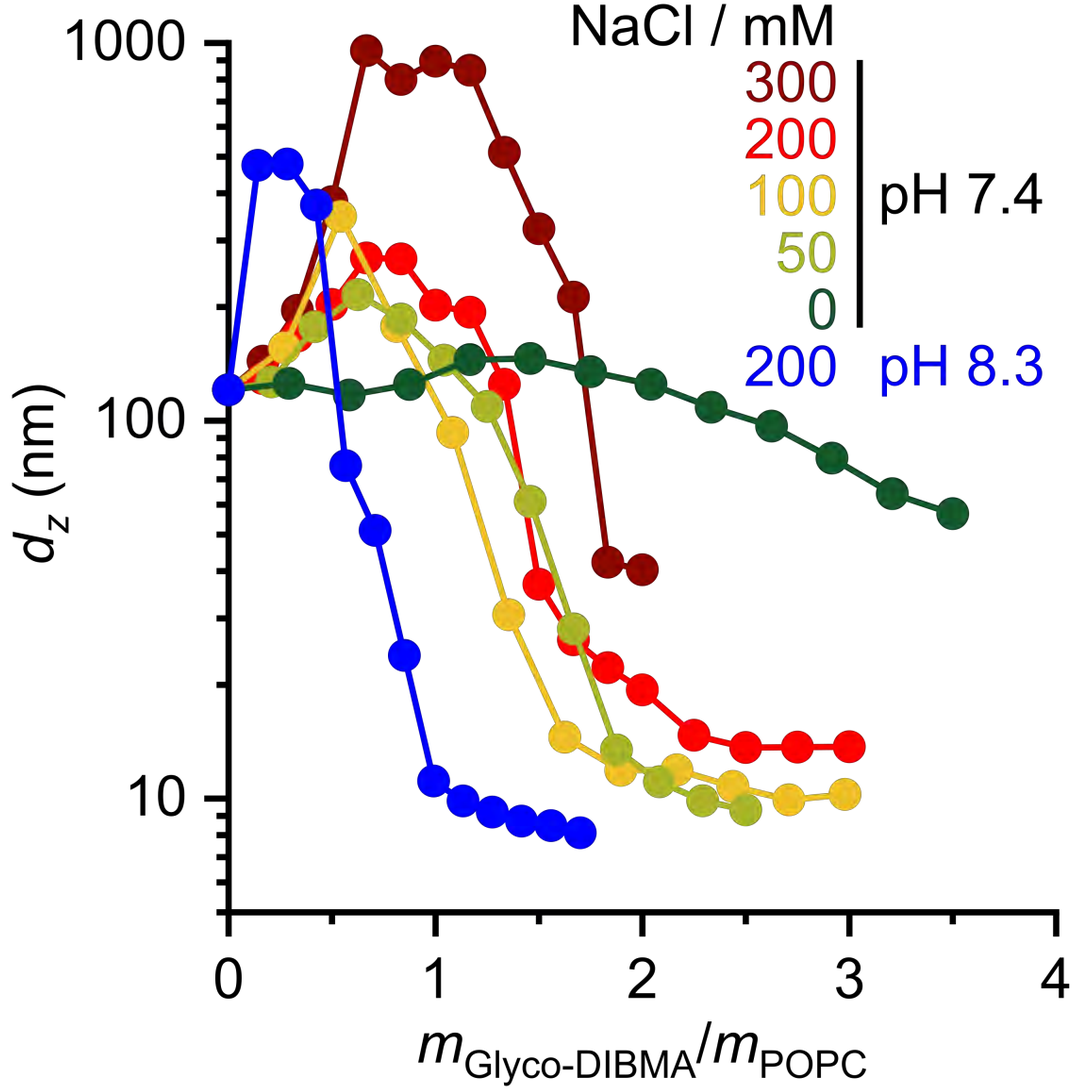




- solubilisation: Glyco-DIBMA as efficient as DIBMA
- phase diagram: three-stage model

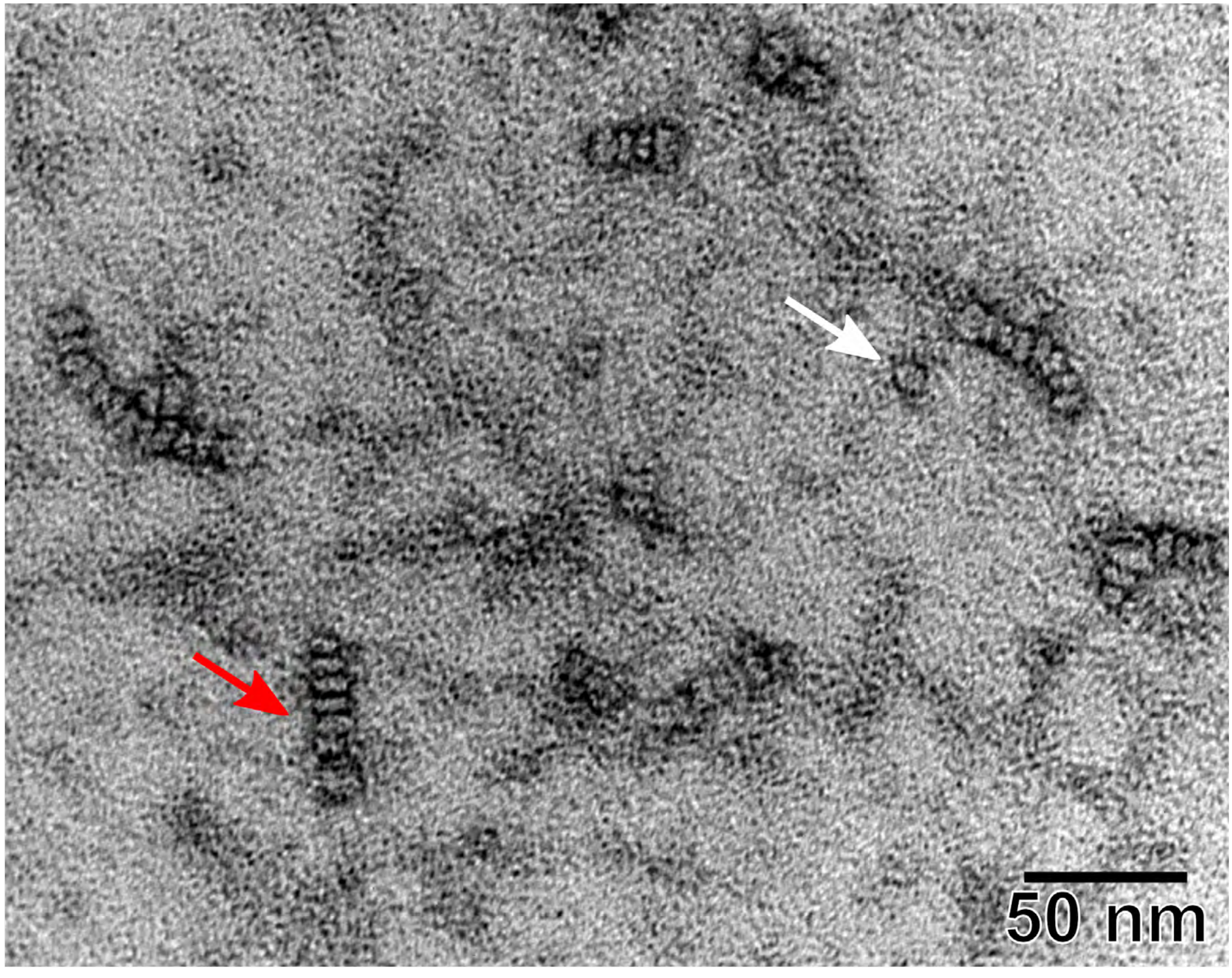
Solubilisation of POPC by DLS



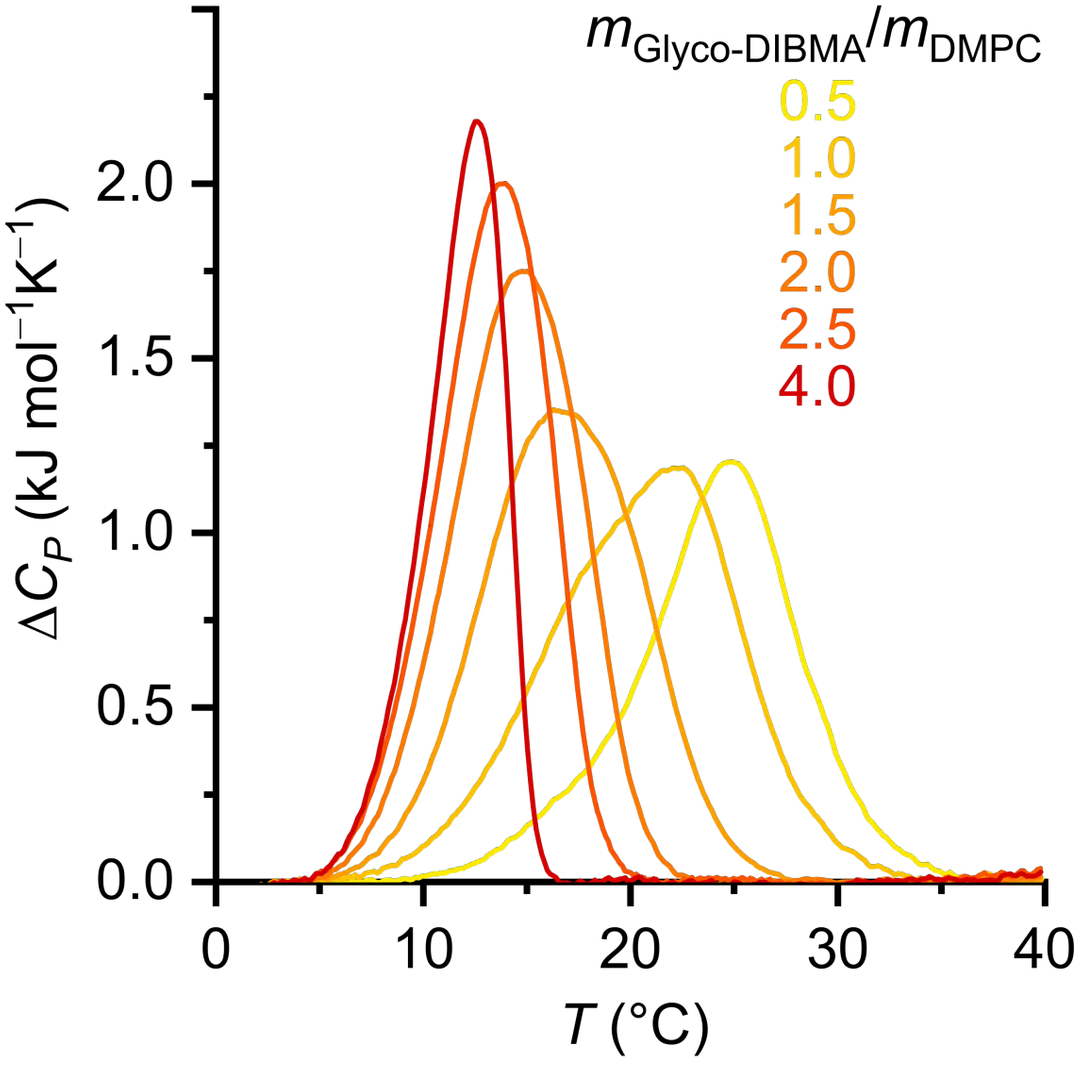


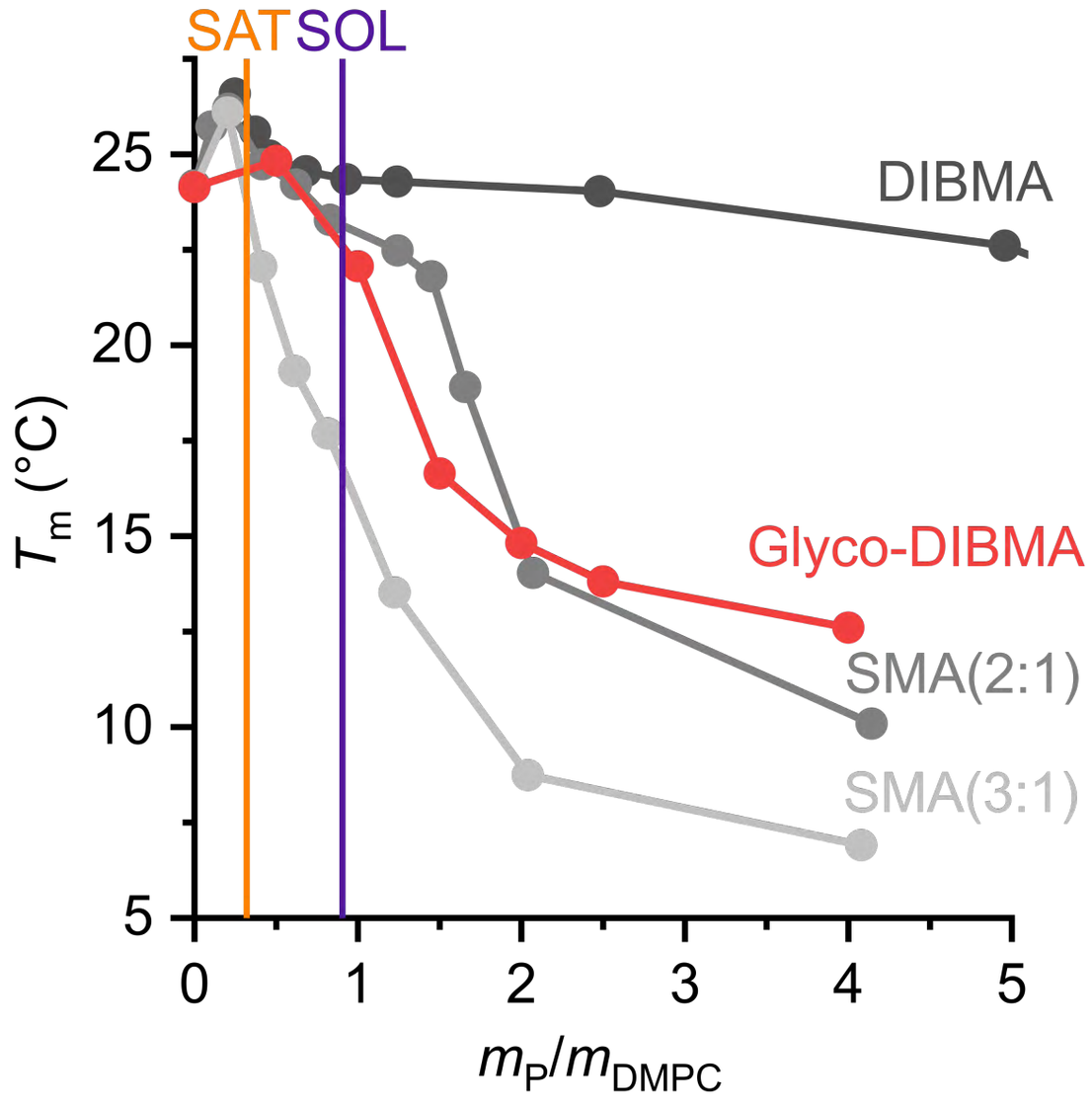
- solubilisation: Glyco-DIBMA much more efficient than DIBMA
- buffer: optimum at alkaline pH, medium ionic strength

Nanodiscs in negative-stain EM



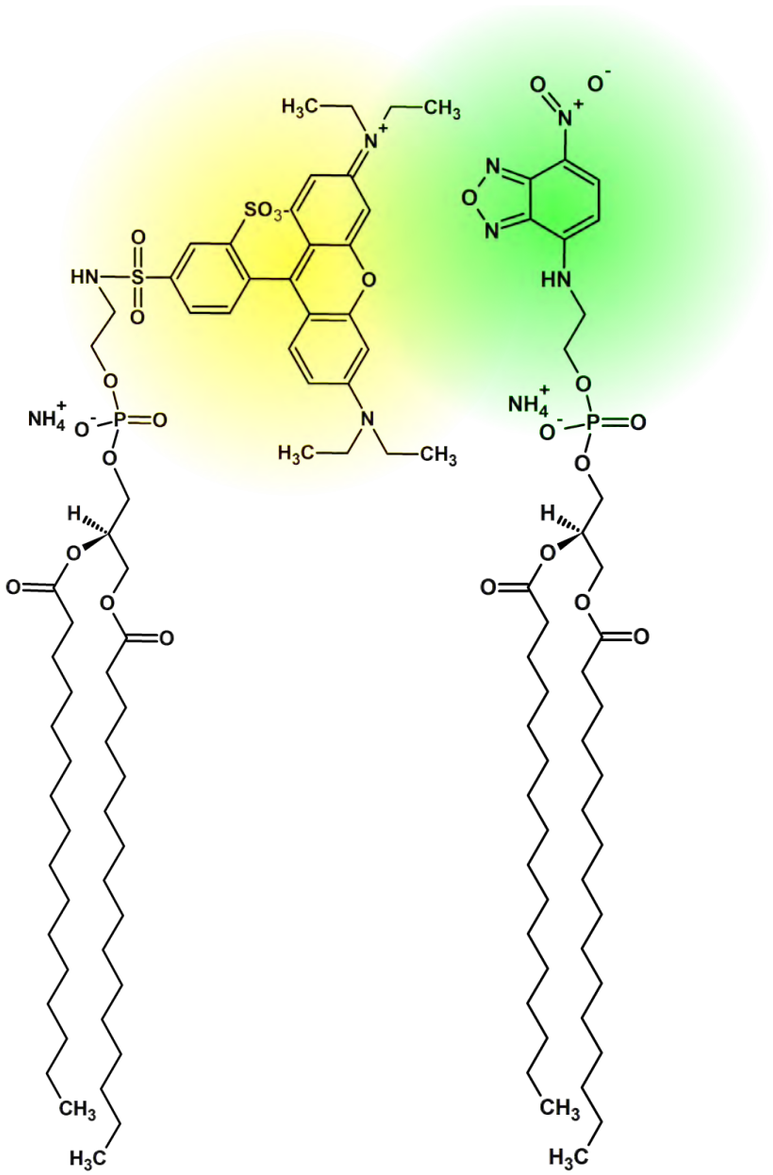
Bilayer integrity by DSC





- thermotropic phase transition: preserved in Glyco-DIBMALPs
- transition temperature: similar for Glyco-DIBMA and SMA(2:1)

Lipid exchange by stopped-flow FRET

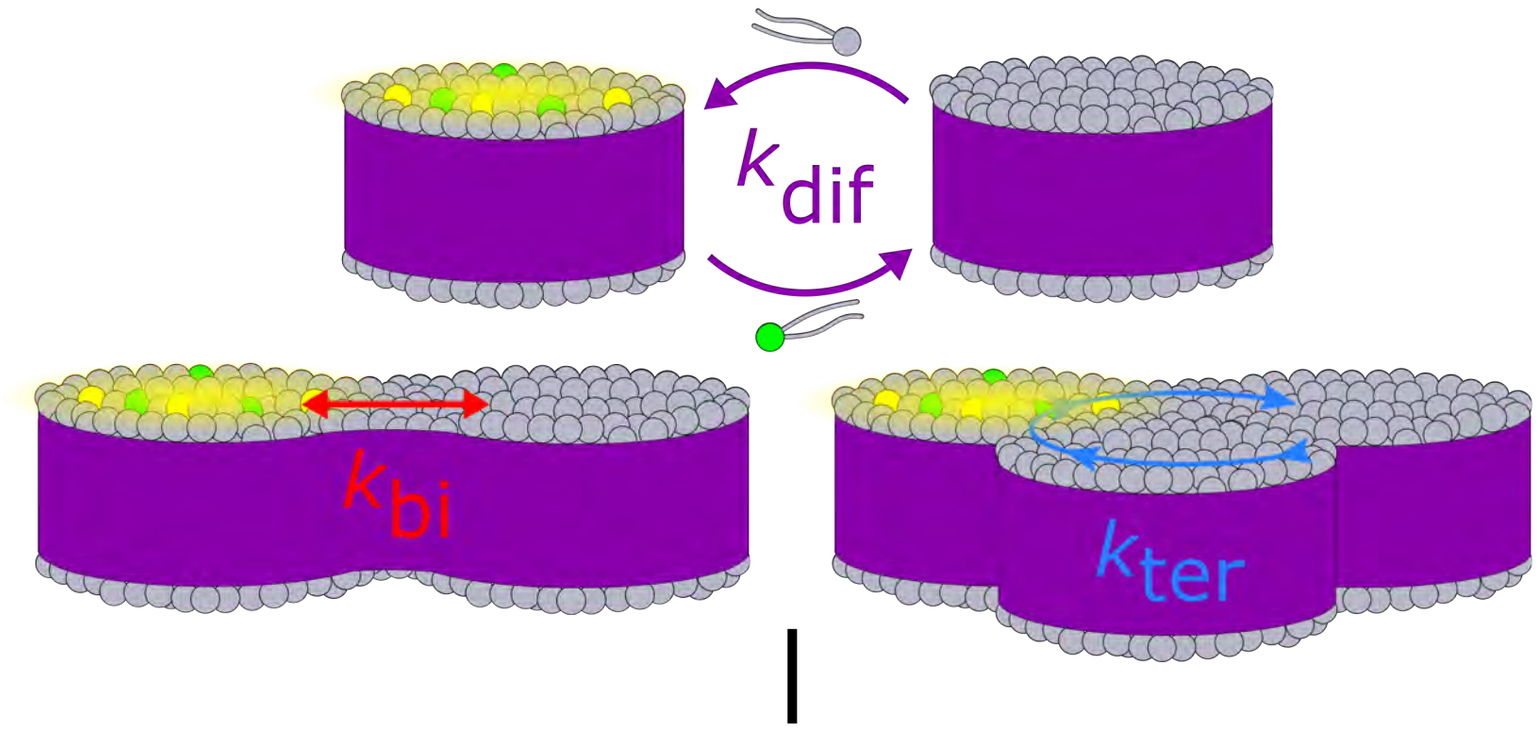


Rh-PE

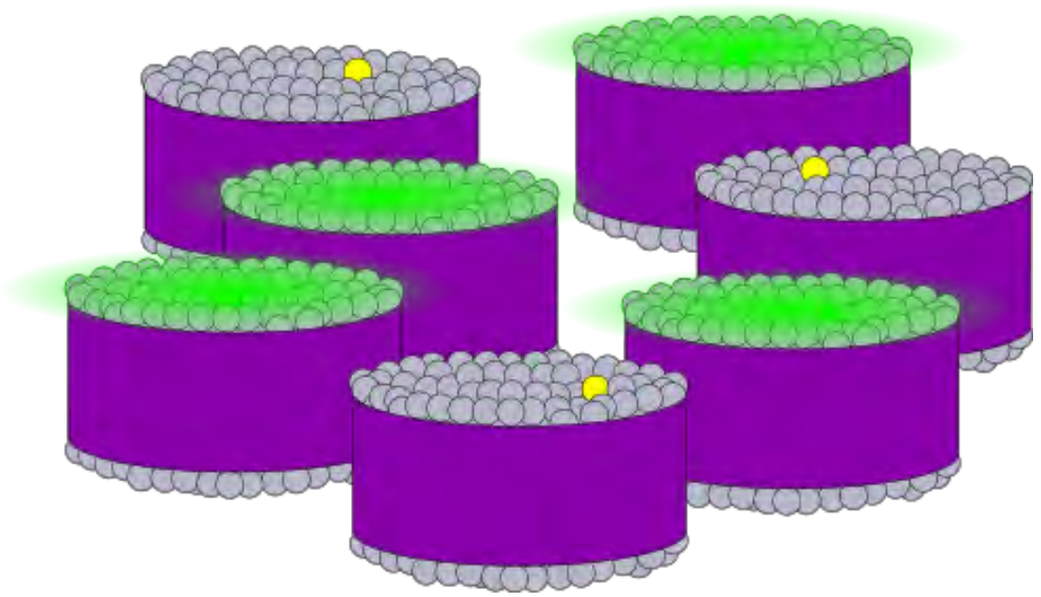
λ_{ex} = 560 nm
λ_{em} = 582 nm

NBD-PE

λ_{ex} = 475 nm
λ_{em} = 530 nm



$$F(t) = F_\infty + e^{-k_{obs}t}(F_0 - F_\infty) + mt$$



Cuevas Arenas … Keller *Sci. Rep.* **2017**, *7*, 45875

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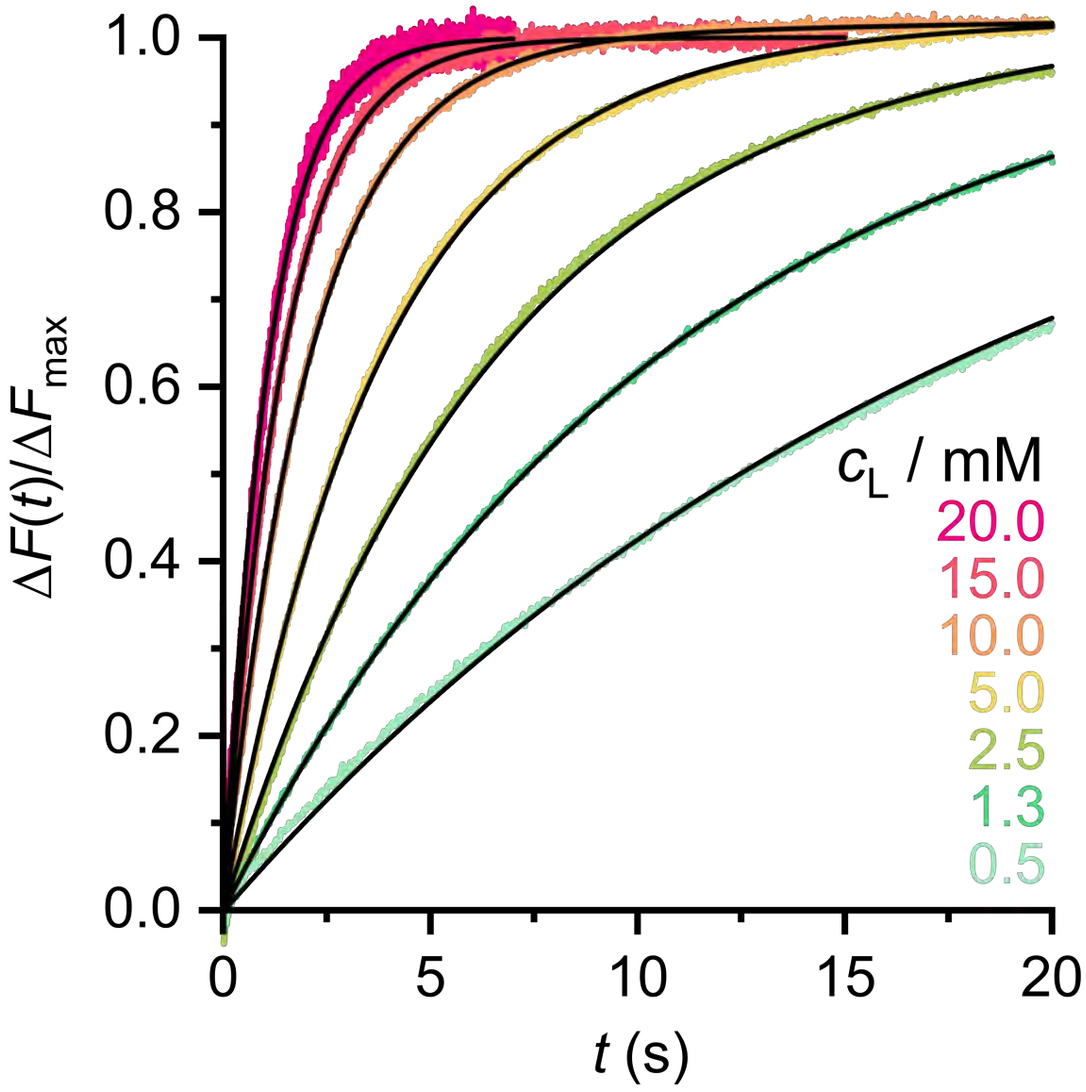
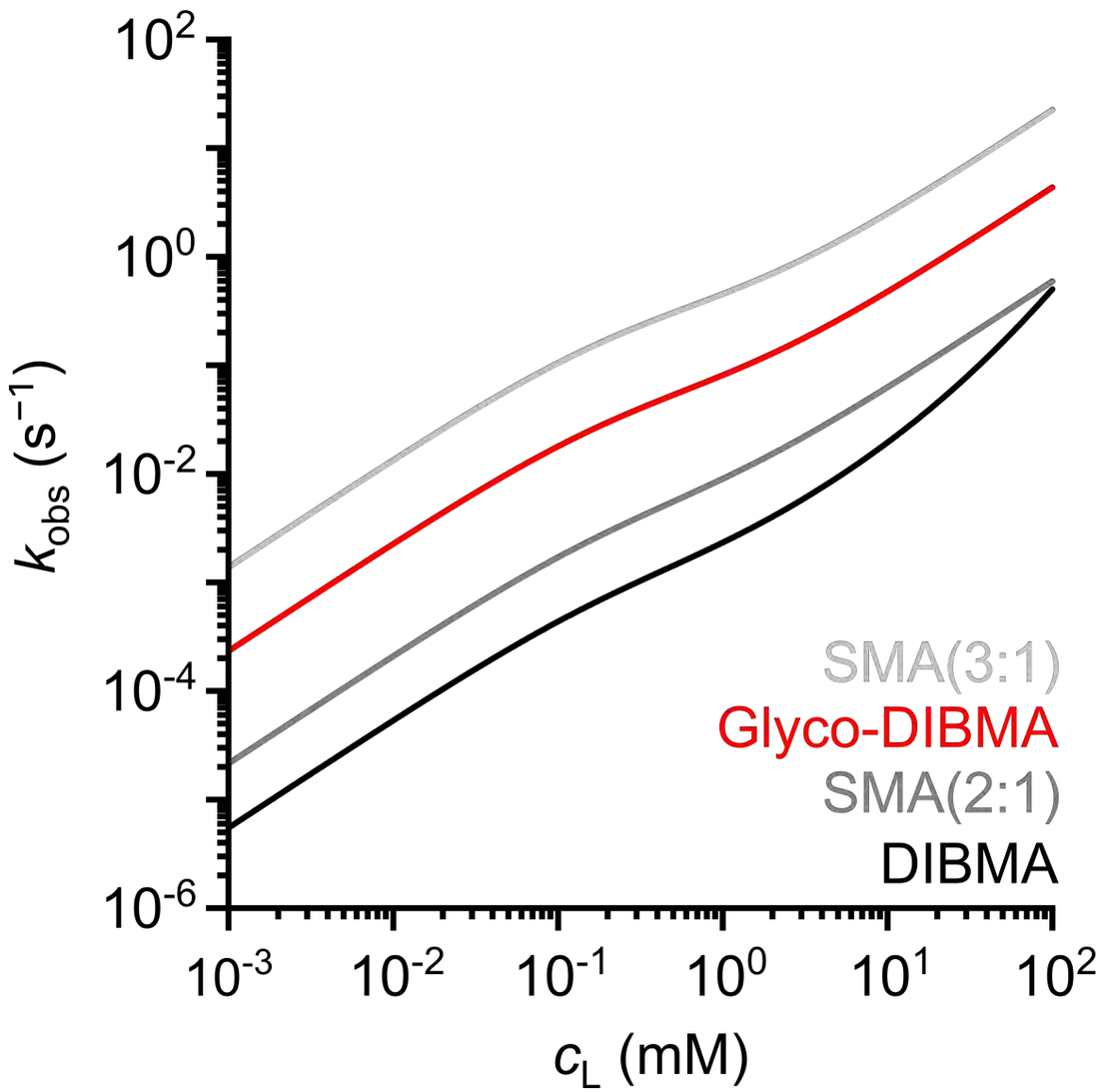
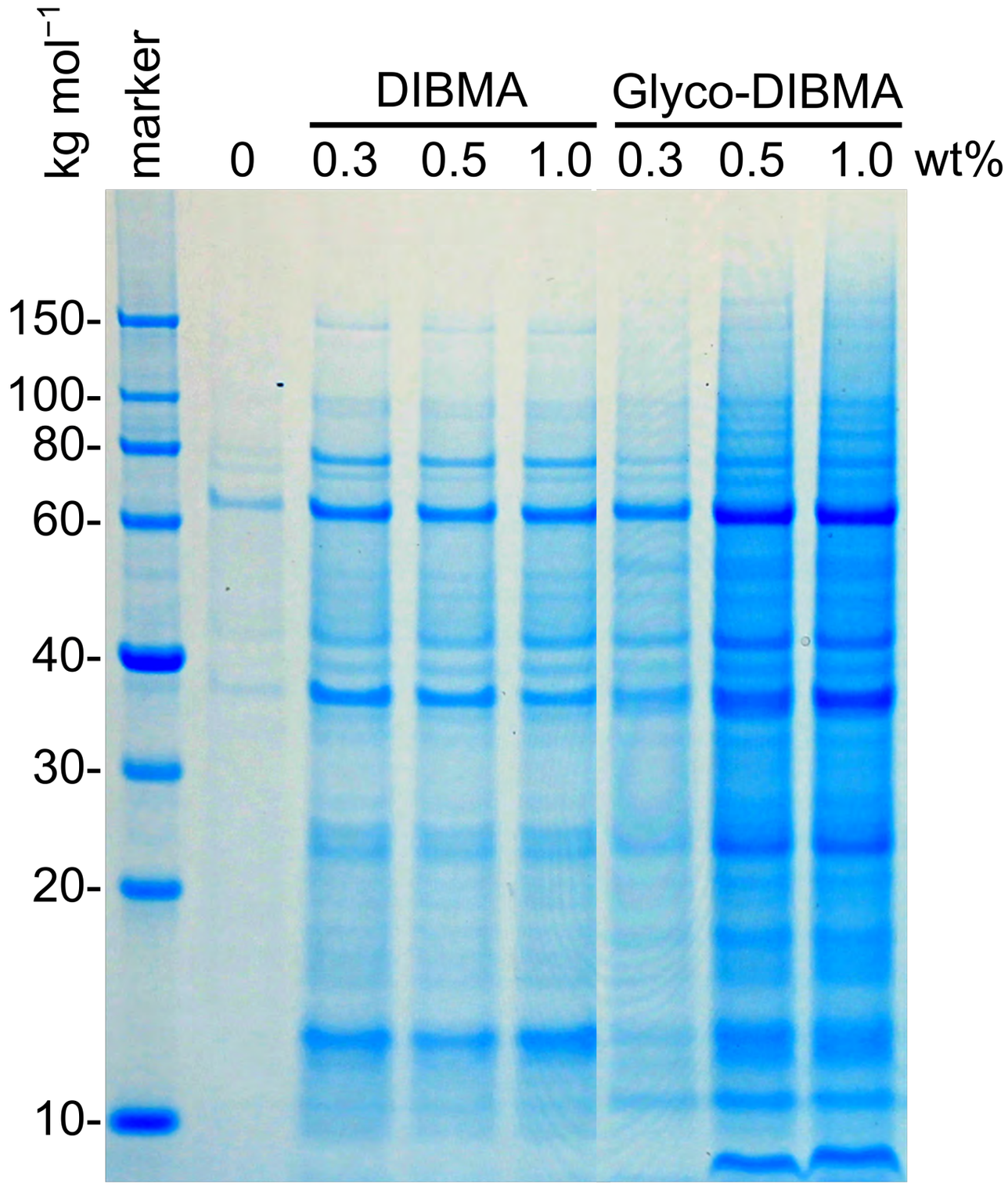


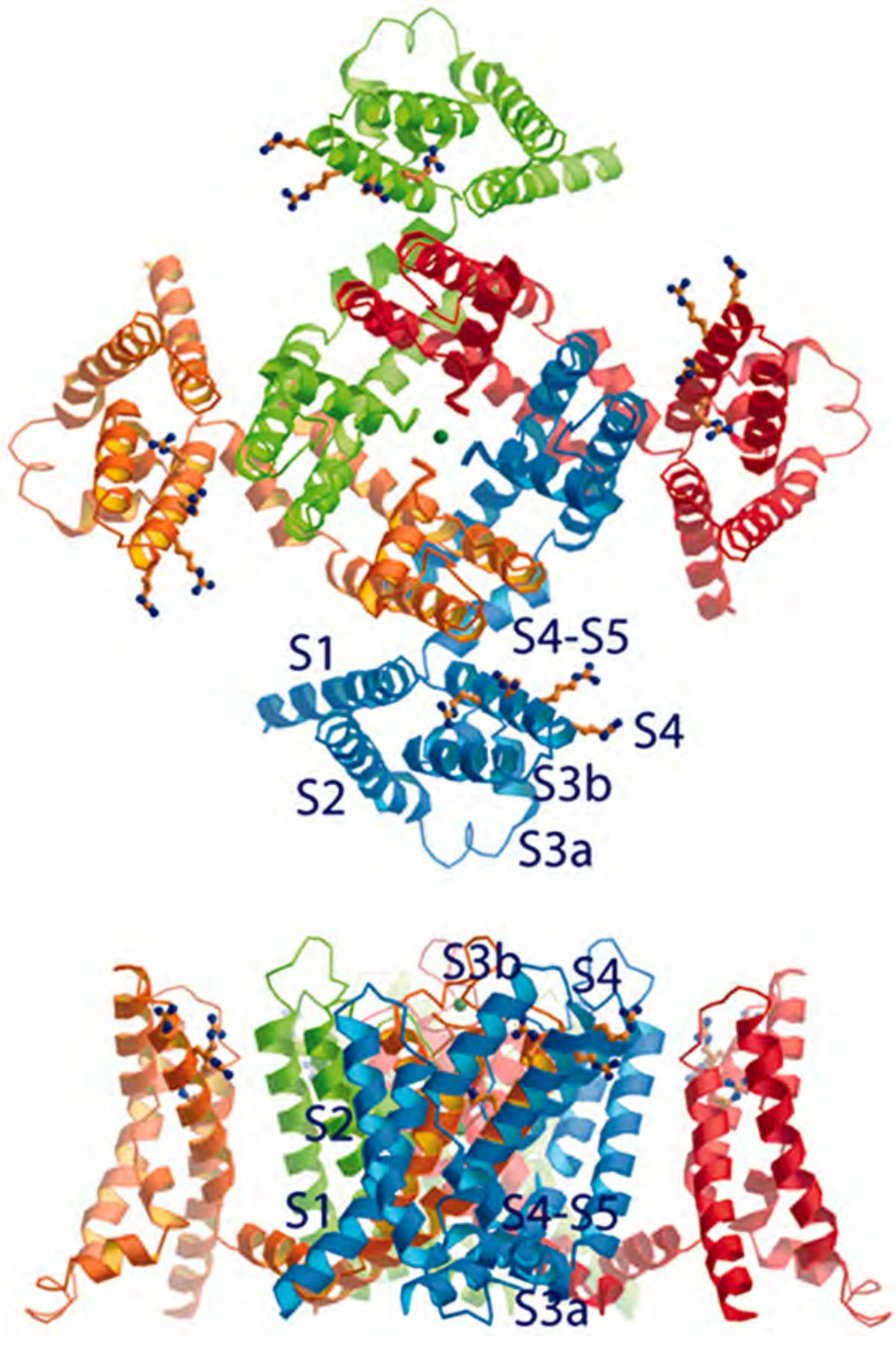


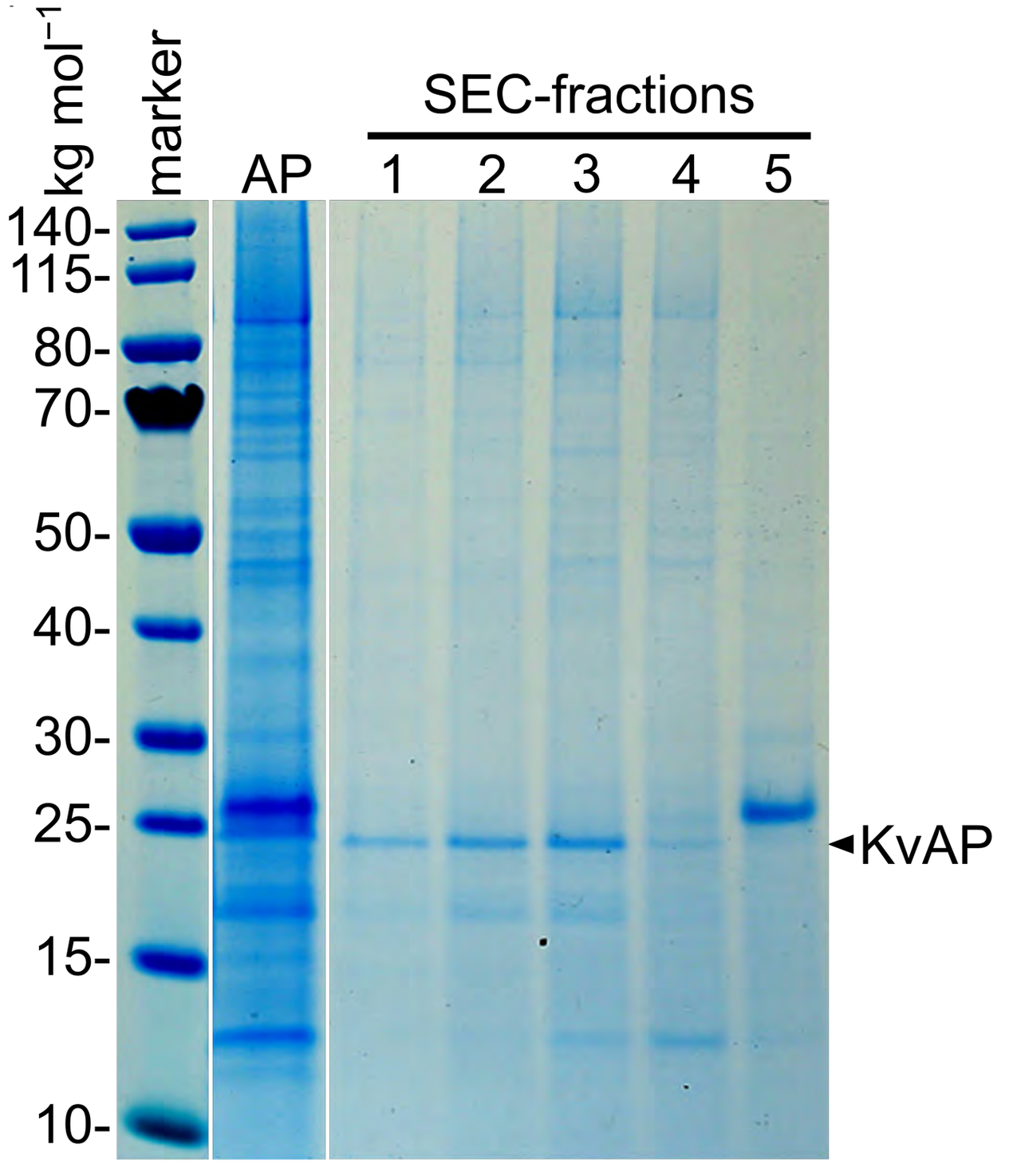
- mechanism: predominantly collisional exchange of lipids
- kinetics: faster with increasing hydrophobicity of polymer

Extraction of *E. coli* membrane proteome



Affinity & SEC purification of KvAP





Lee … MacKinnon *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 441

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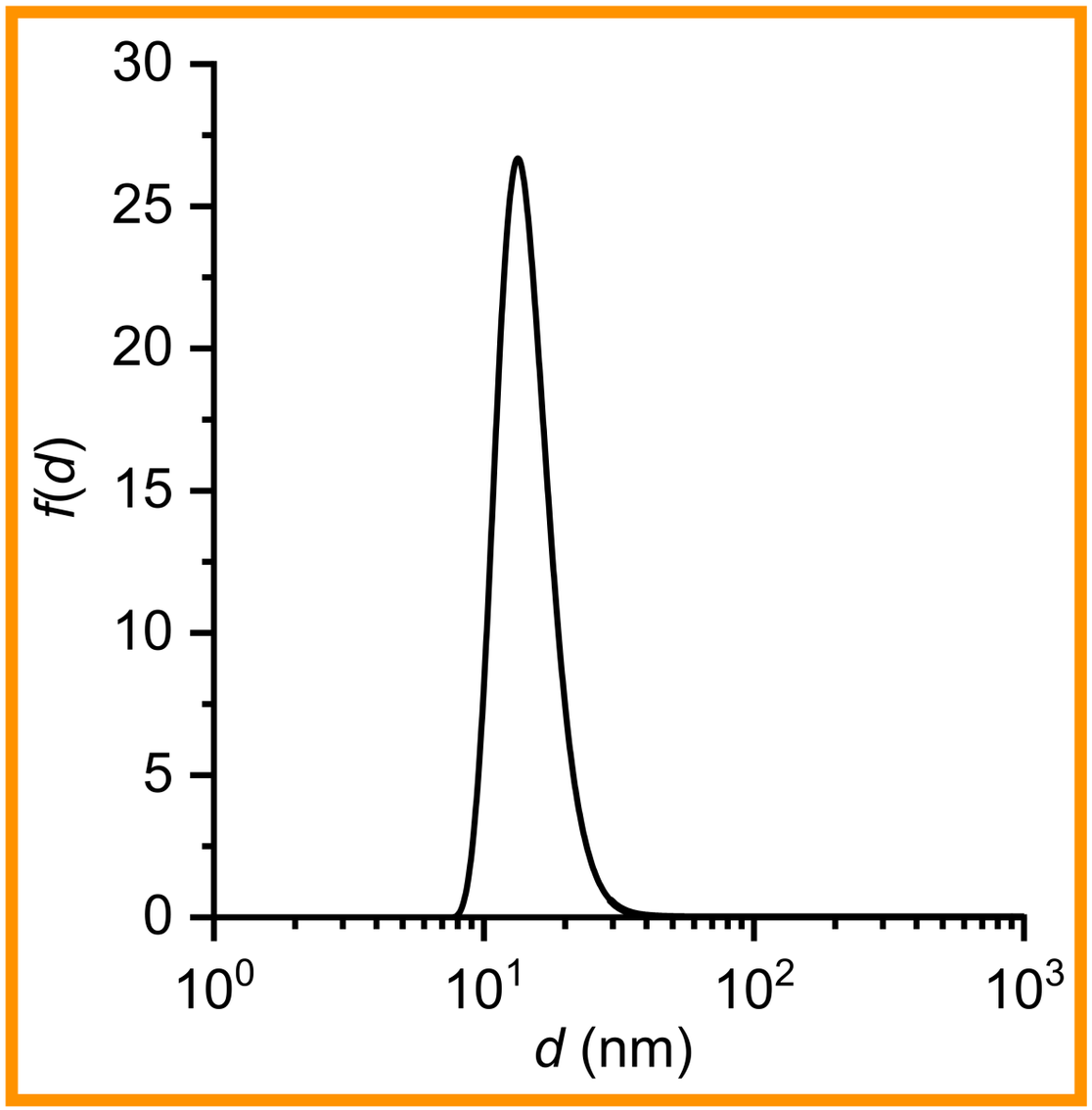
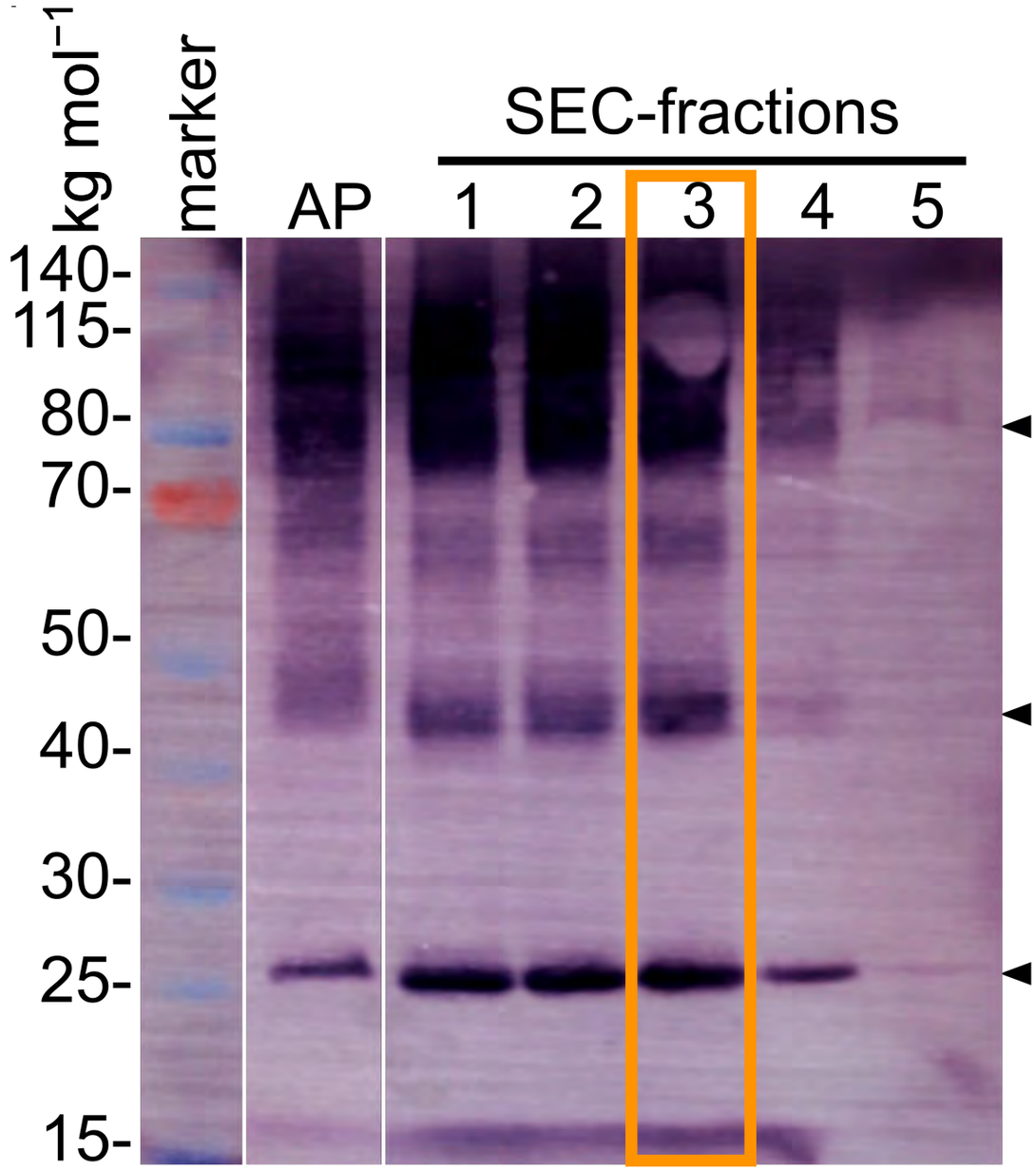
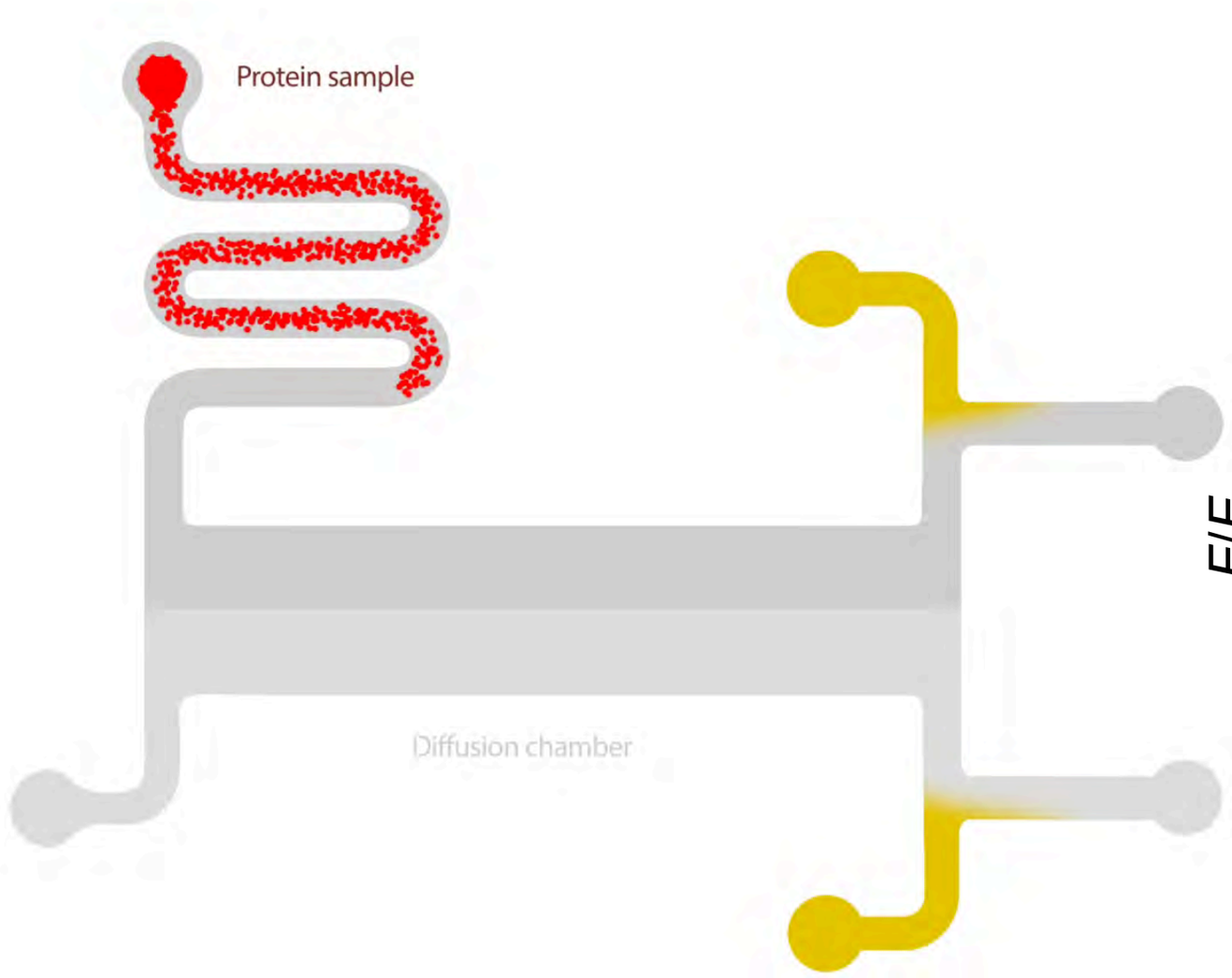
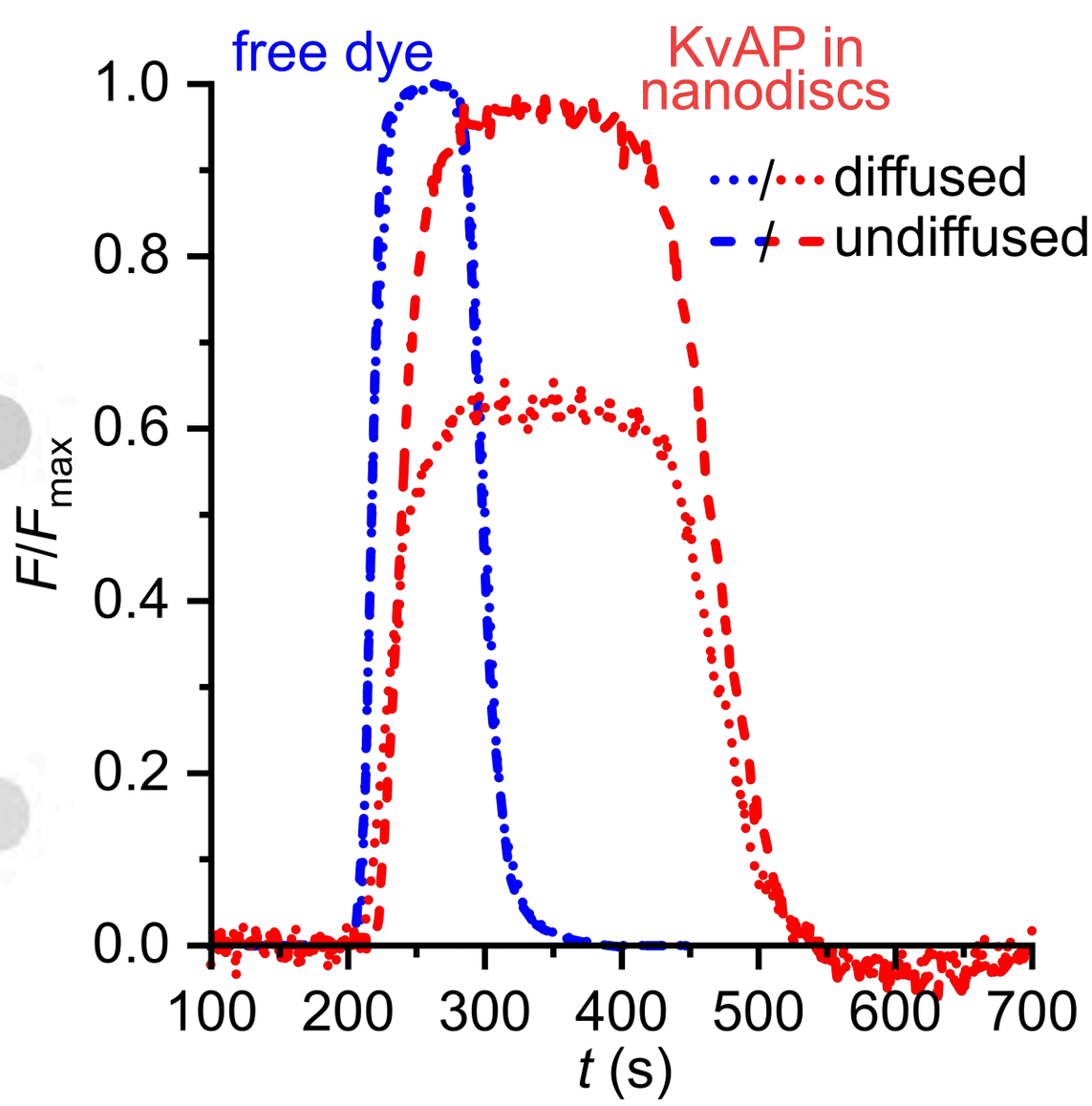




- high purity and size homogeneity after two-step purification

Microfluidic diffusional sizing (MDS)



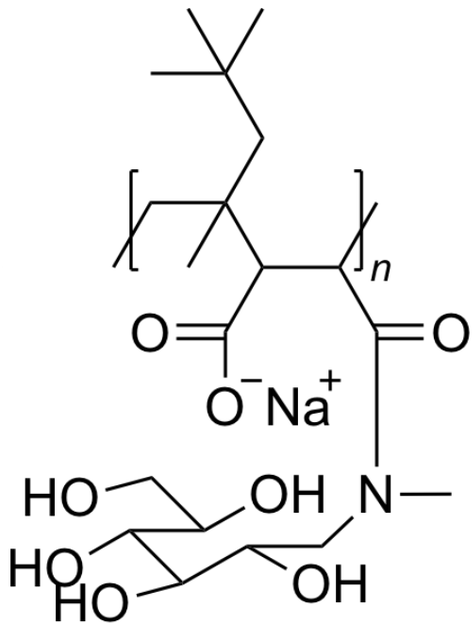


- *in situ* labelling: Atto 488 conjugated to native Cys, then SEC
- hydrodynamic size of KvAP-containing nanodiscs: 11 nm

https://www.fluidic.com

Random thoughts & emerging principles

- bacterial cells: DDM > Glyco-DIBMA = SMA(2:1) > DIBMA
- mammalian cells: Glyco-DIBMA = SMA(2:1) > DIBMA = DDM
- insect cells: strange
- lipid compatibility: anionic, unsaturated lipids & cholesterol
- polymer concentration: 0.1–3% (*w*/*v*)
- ionic strength: ideally 100–300 mM NaCl
- pH: ideally 8 or higher



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- sources: bioRxiv 437849v1 & www.glycon-biochem.eu

Carl Zeiss Stiftung

DAAD

- thanks to: Harald Kelm, Lisa Hamsch, Christiane Müller, Ann-Cathrin Schlapp, Frederik Sommer, Kai Zwara

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