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TECHNISCHE UNIVERSITÄT KAISERSLAUTERN

Solubilisation of Membrane Proteins into Functional Lipid-Bilayer Nanodiscs Using Amphiphilic Copolymers

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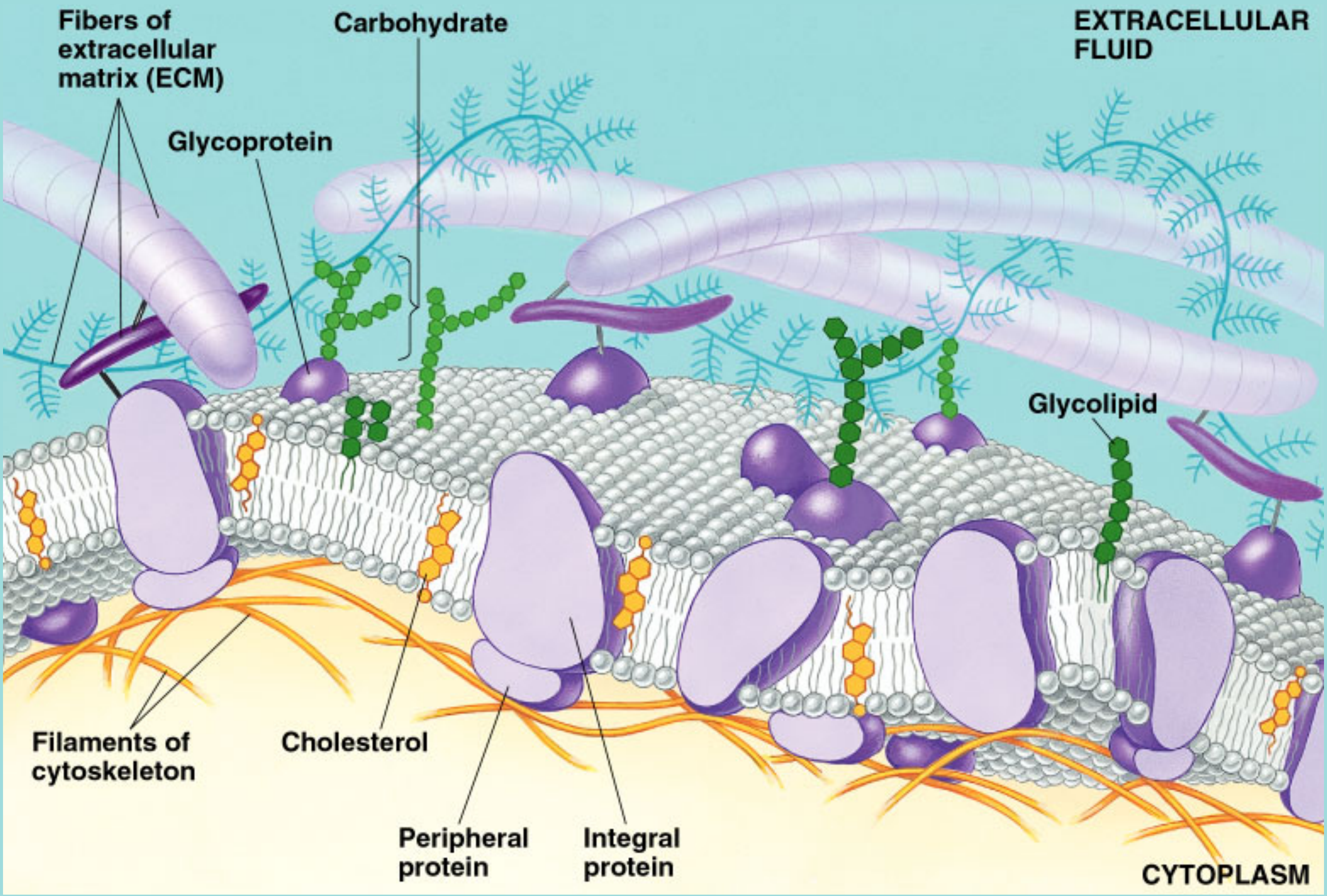
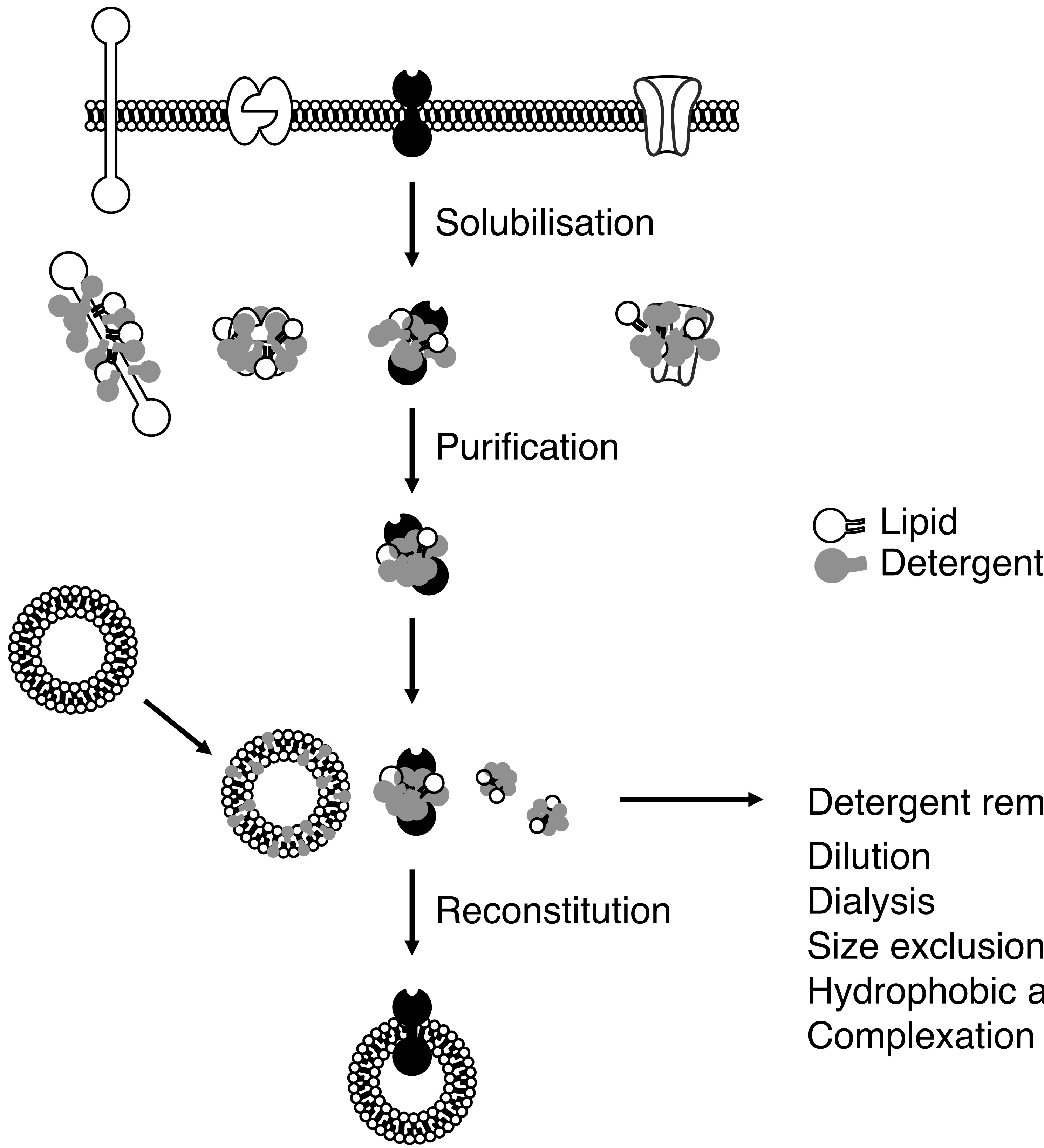
Biomembranes



In vitro approach to membrane proteins

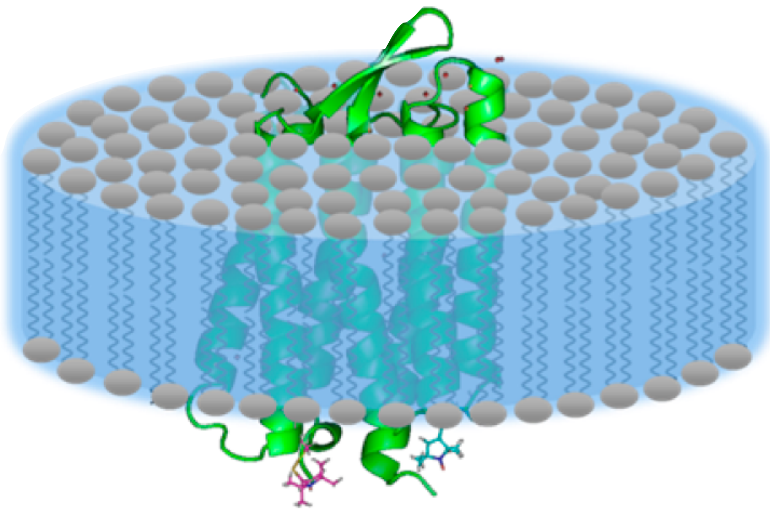


Detergent removal:

Dilution
Dialysis
Size exclusion chromatography
Hydrophobic adsorption (BioBeads)
Complexation with cyclodextrin

Overview

Nanodiscs for membrane proteins



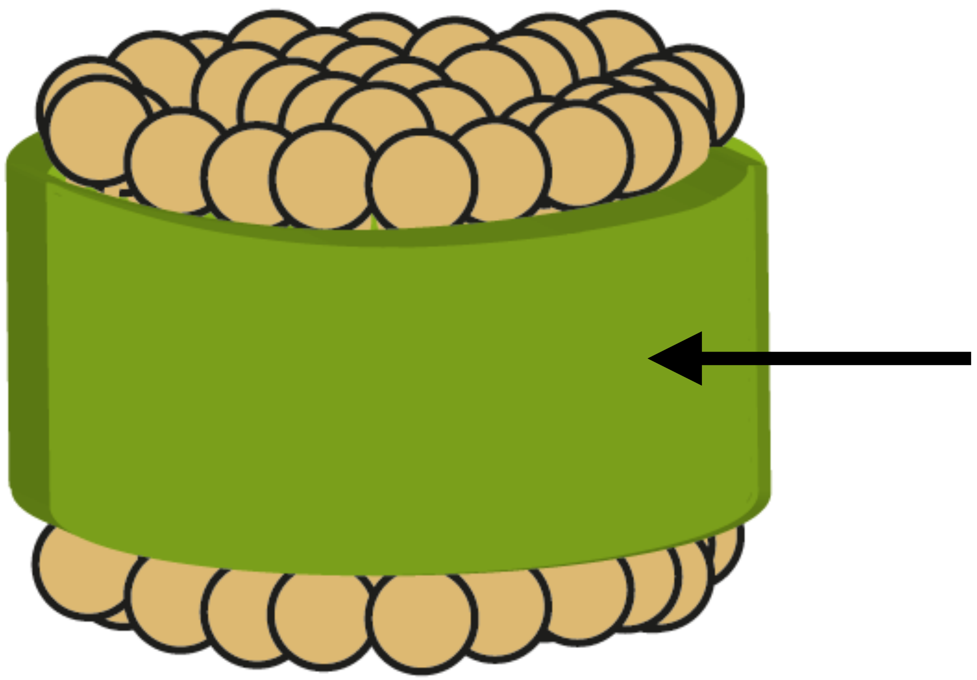
Polymer-encapsulated nanodiscs

Solubilisation of membrane proteins

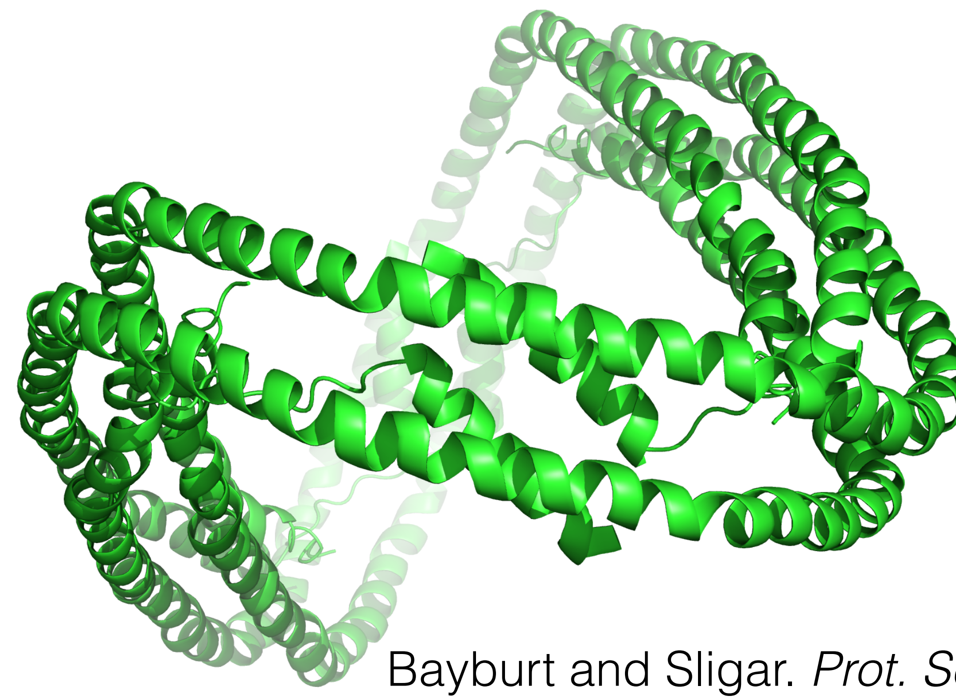
Kinetics of lipid exchange

Nanodiscs for membrane proteins

Nanodiscs: small lipid-bilayer patches

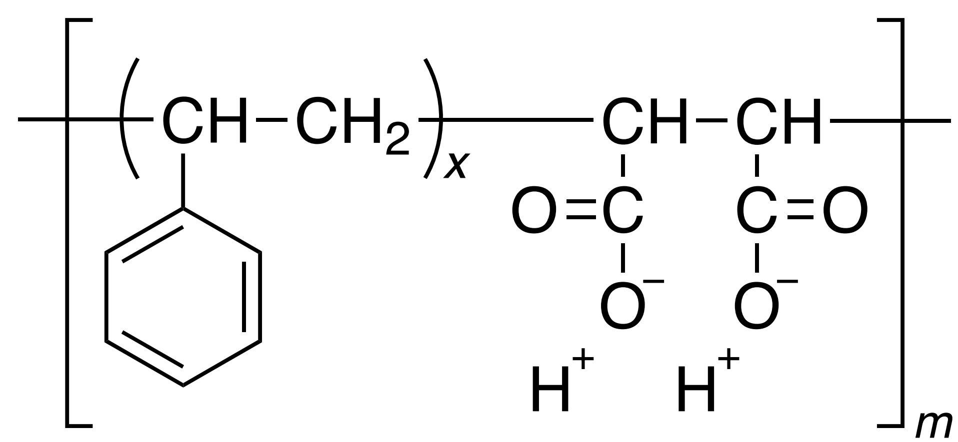


Nanodisc



Bayburt and Sligar. *Prot. Sci.* **2003**, *12*, 2476

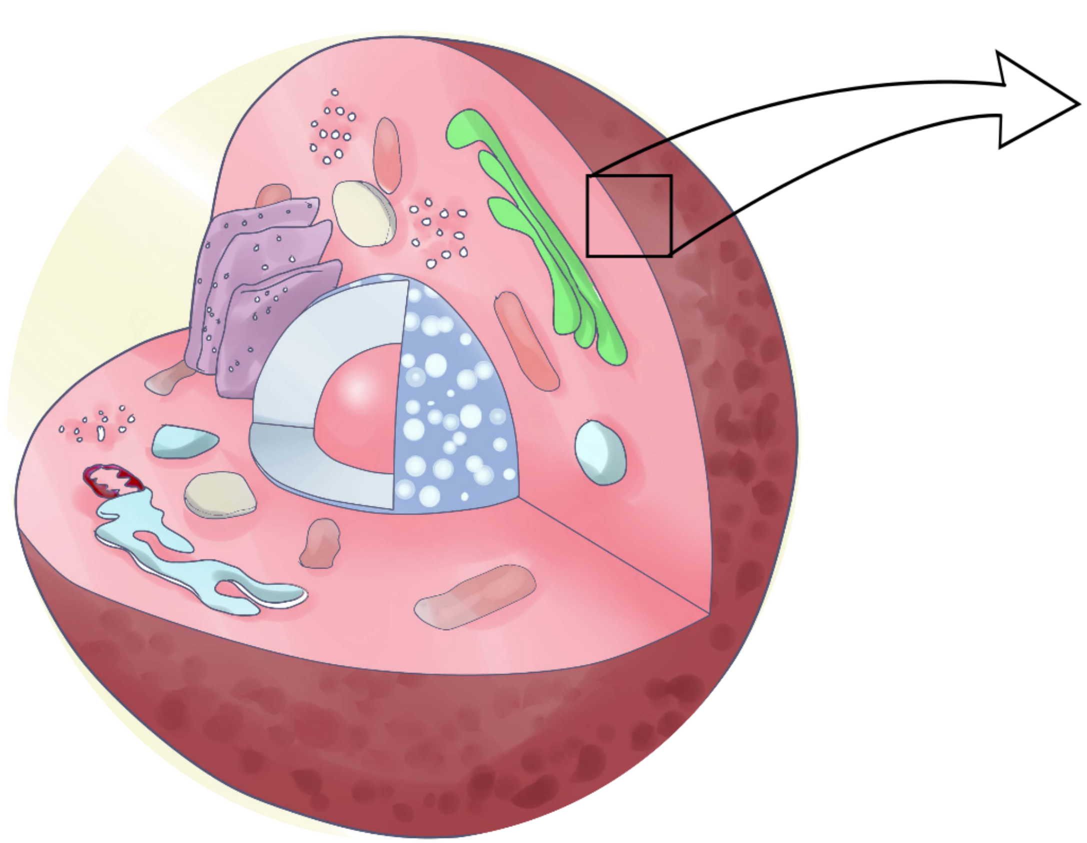
Membrane scaffold protein (MSP)

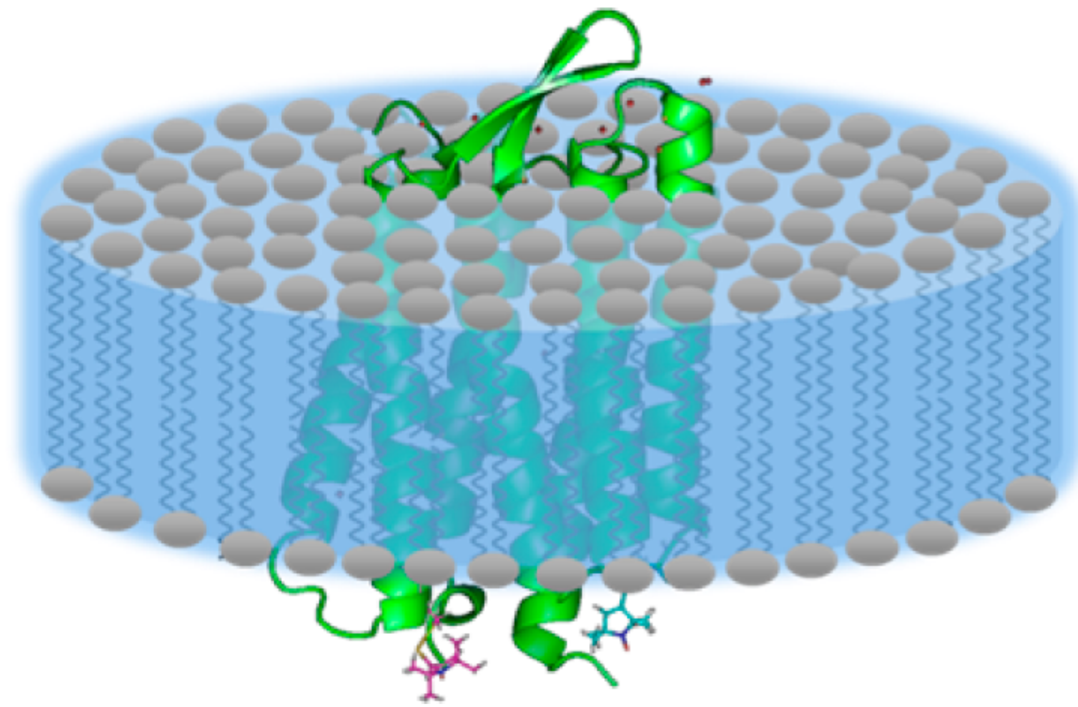


Styrene/maleic acid copolymer (SMA)

Knowles et al. *J. Am. Chem. Soc.* **2009**, *131*, 7484

Direct formation of polymer nanodiscs





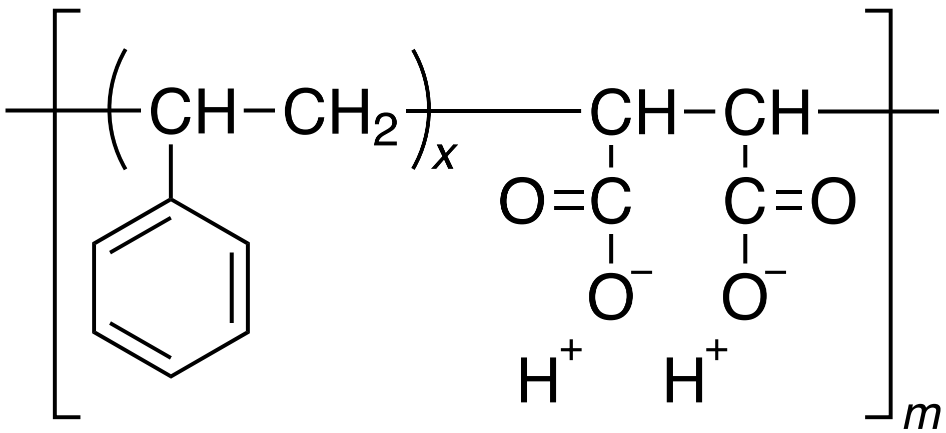
Orwick-Rydmark et al. *Nano Lett.* **2012**, *12*, 4687

Main differences compared with MSP nanodiscs:

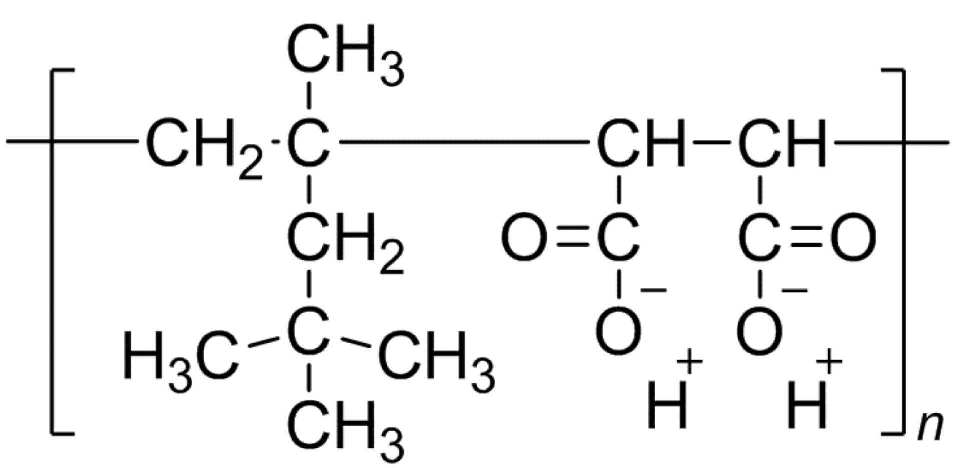
- proteins and surrounding lipids are extracted directly
- nanodisc size can be tuned by polymer/lipid ratio
- polymer adapts to bilayer height and allows fast exchange

Polymer-encapsulated nanodiscs

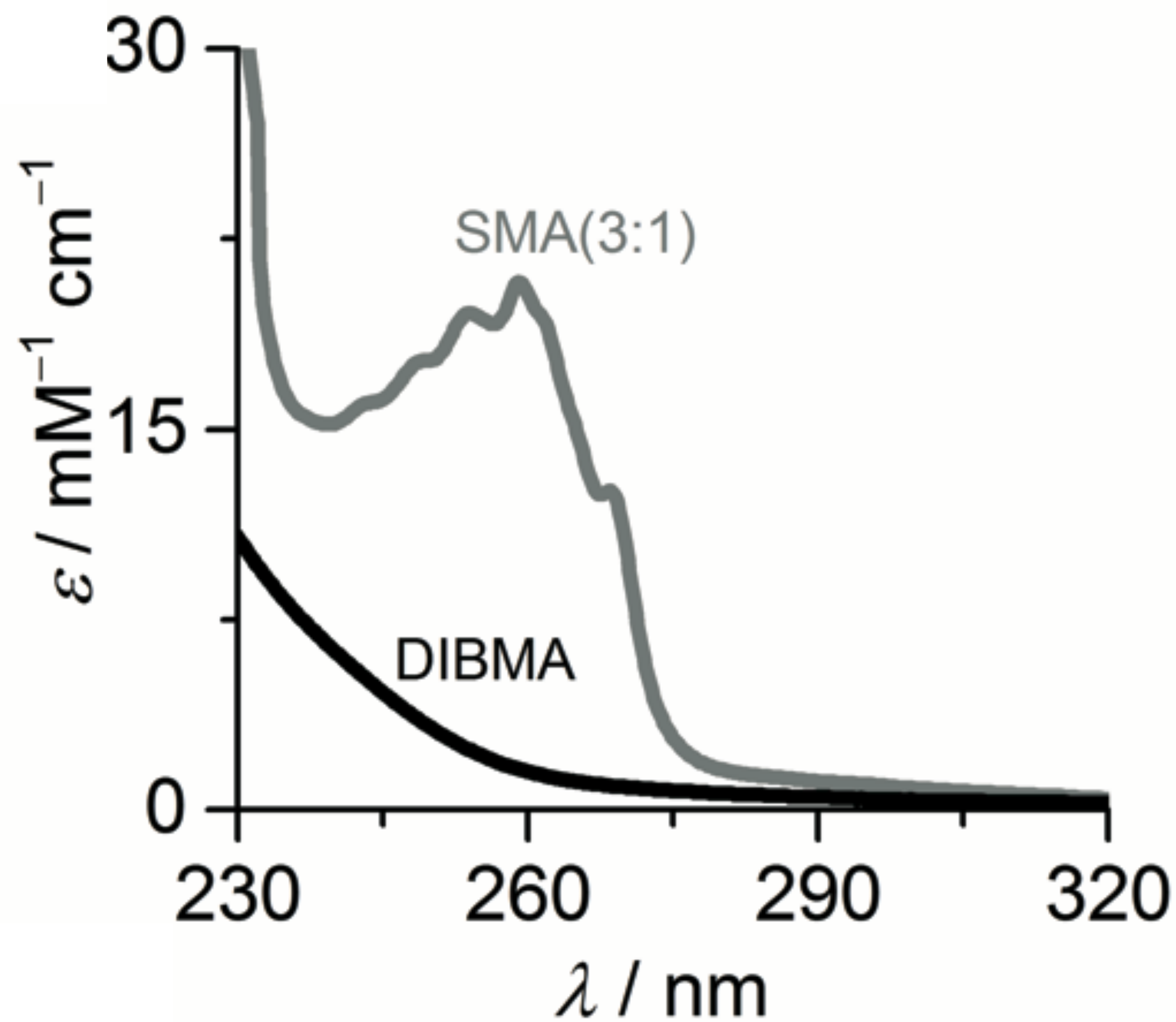
Aromatic vs. aliphatic copolymers



SMA: poly(styrene-*co*-maleic acid)
M_w = 10 kg/mol, M_n = 4 kg/mol (Polyscope)

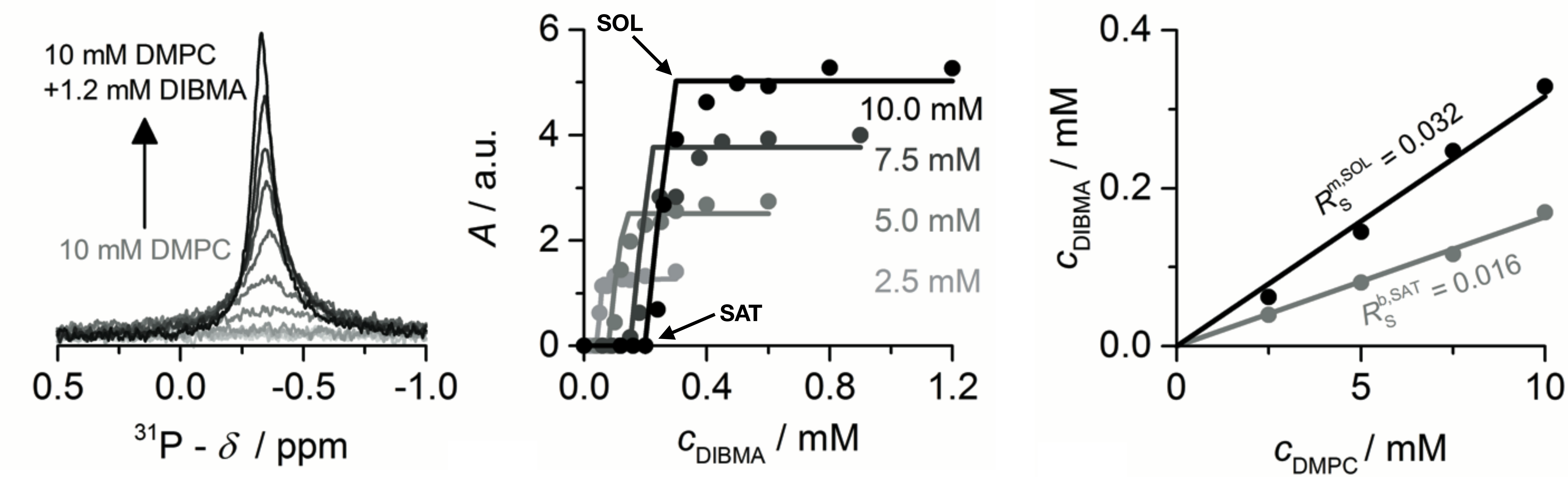


DIBMA: poly(diisobutylene-*alt*-maleic acid)
M_w = 15 kg/mol, M_n = 8 kg/mol (BASF)



Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919

Solubilisation of DMPC vesicles



Thermodynamics of solubilisation from phase diagram:

$$K_{pol}^{b\to m} = X_{pol}^{SOL} / X_{pol}^{SAT}$$

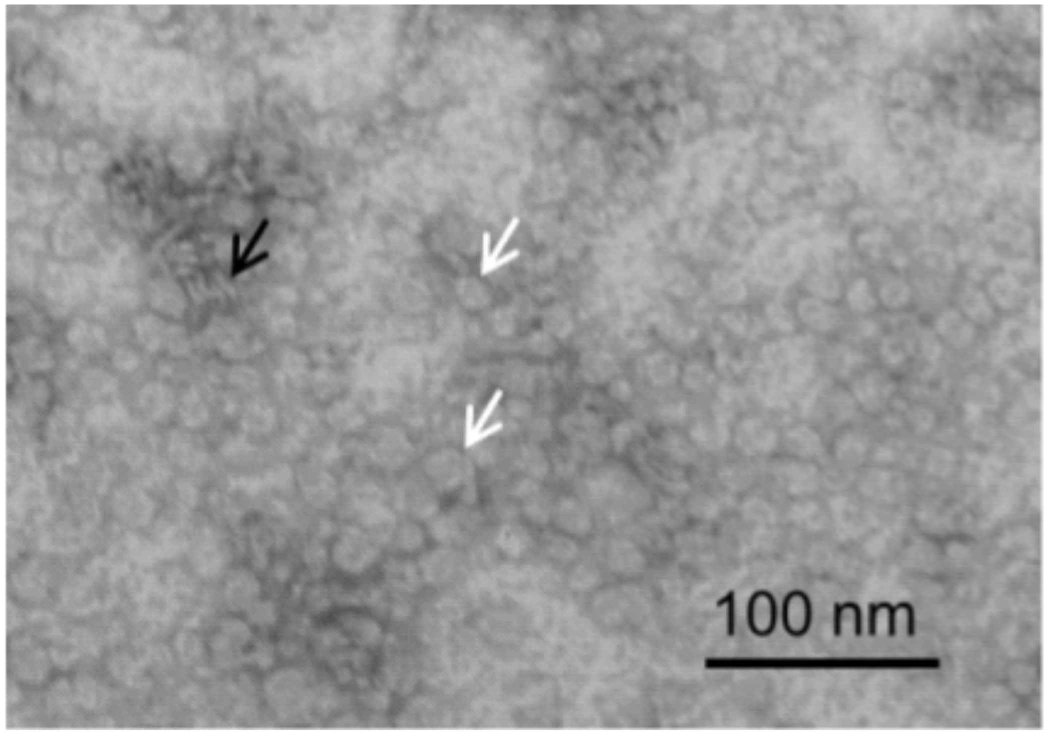
$$\Delta G_{DIBMA}^{\circ,b\to m} = -1.62 \text{ kJ/mol} \quad \Delta G_{DMPC}^{\circ,b\to m} = +0.037 \text{ kJ/mol}$$

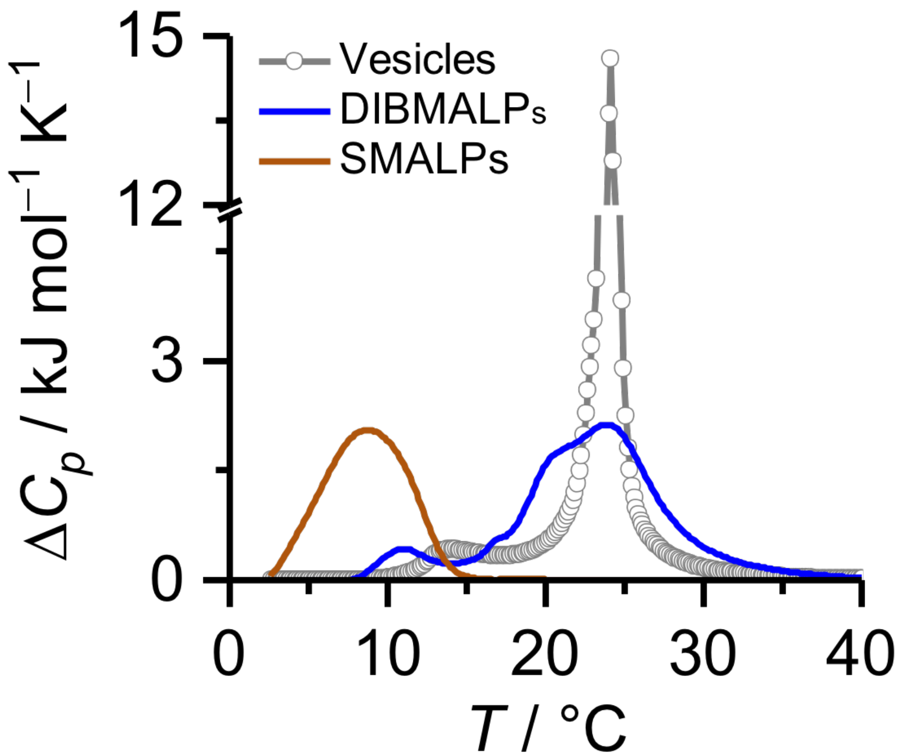
$$K_{lip}^{b\to m} = \left(1 - X_{pol}^{SOL}\right) / \left(1 - X_{pol}^{SAT}\right)$$

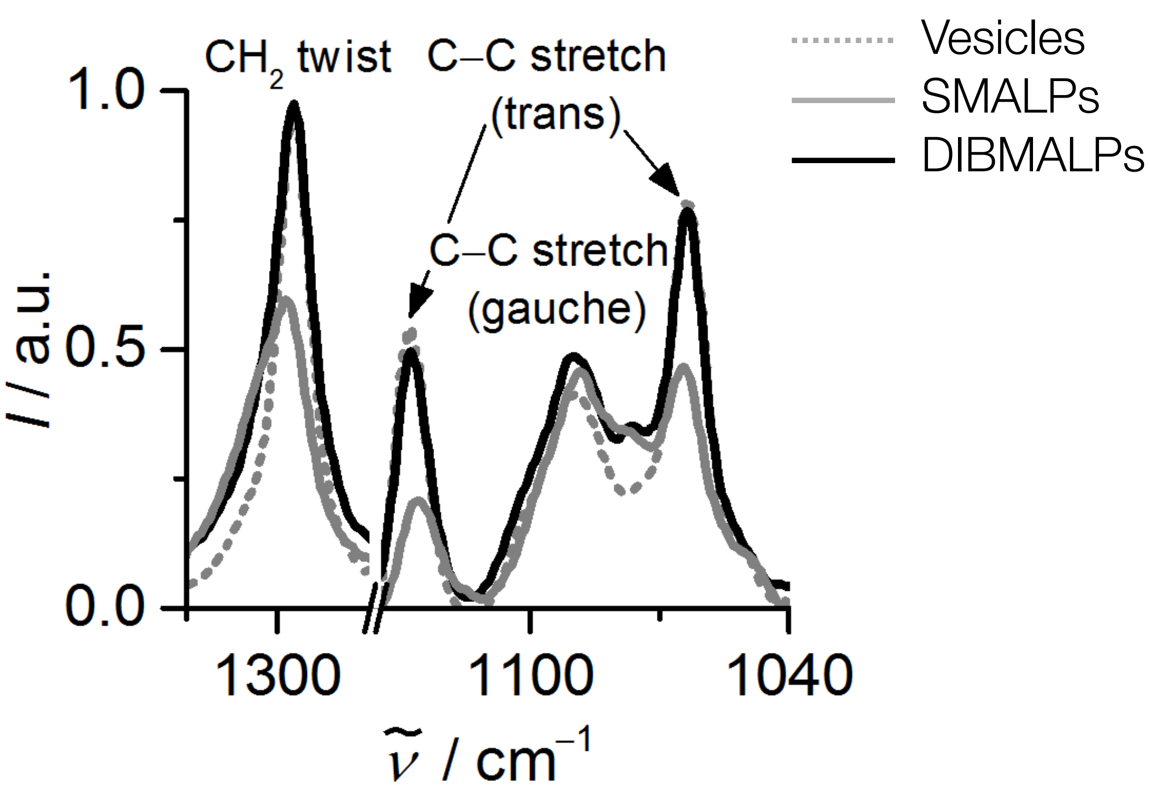
$$\Delta G_{SMA}^{\circ,b\to m} = -1.51 \text{ kJ/mol} \quad \Delta G_{DMPC}^{\circ,b\to m} = +0.068 \text{ kJ/mol}$$

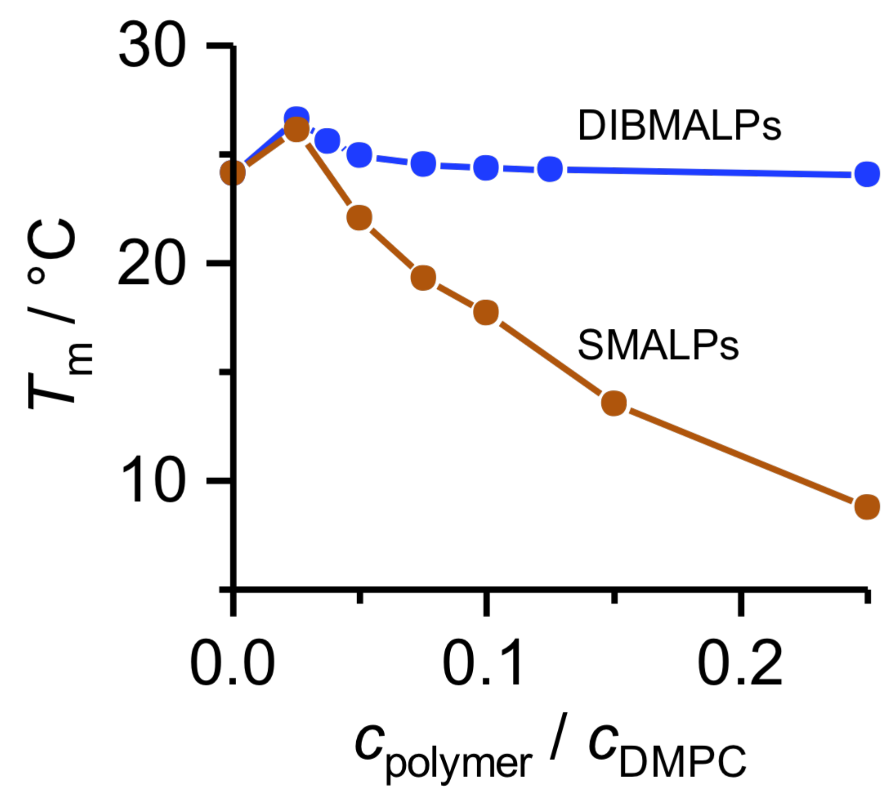
Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919

Mild effects on membrane lipid order









Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919

Compatible with divalent cations

$c_{Ca^{2+}}$ / mM

0 1 2 3 4 5

SMALPs

$c_{Ca^{2+}}$ / mM

DIBMALPs

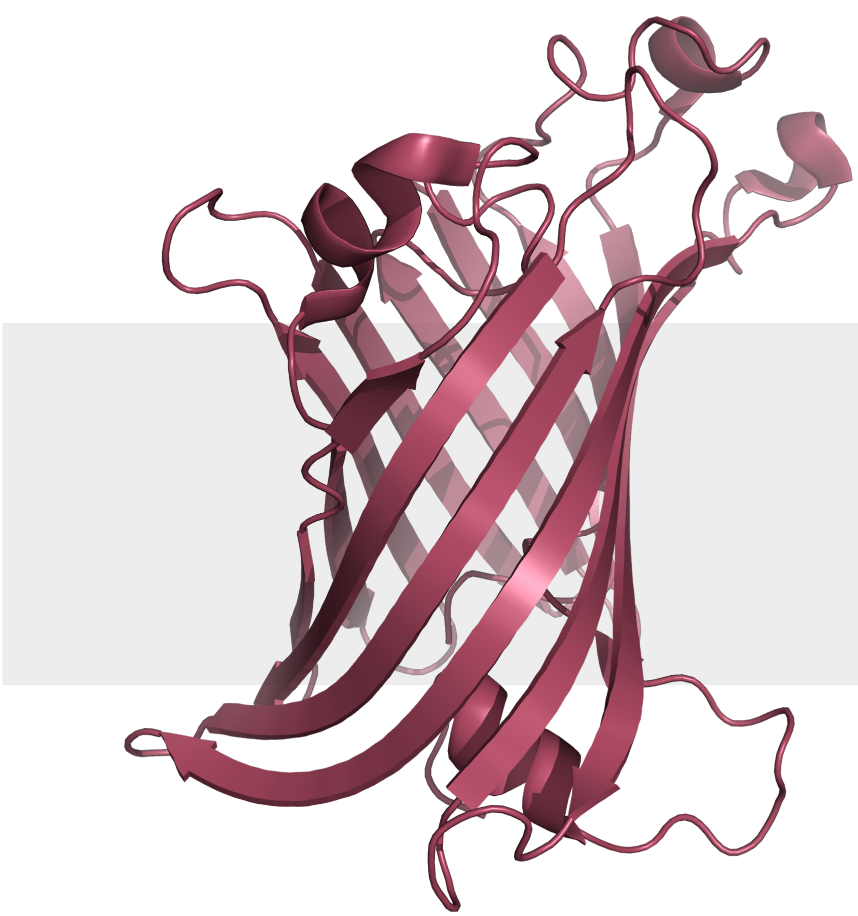
0 5 10 15 20 25 30 35 40 50

Solubilisation of membrane proteins

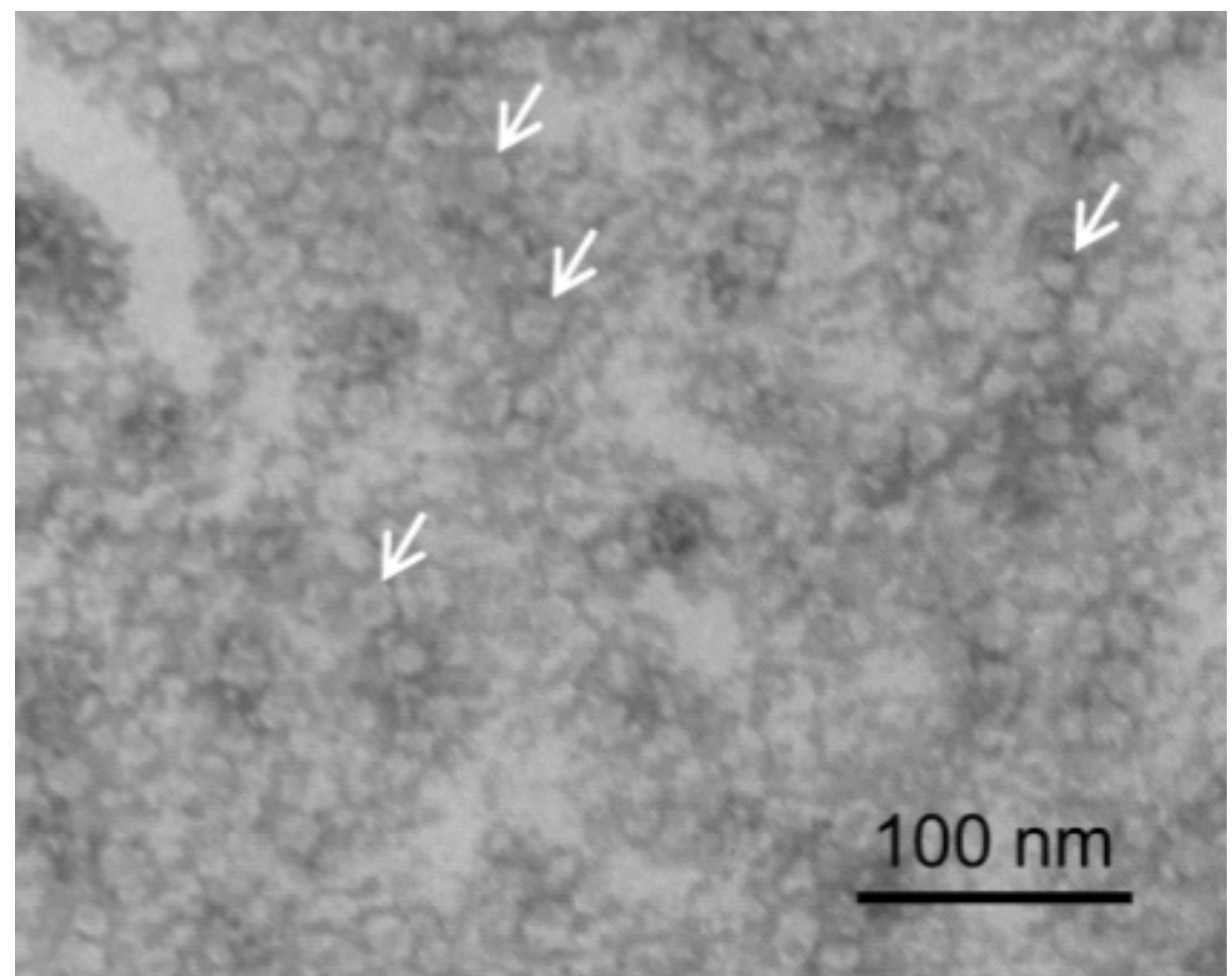
Model protein

Outer membrane phospholipase A (OmpLA):

- integral membrane enzyme
- differential migration on SDS-PAGE (folded vs. unfolded)

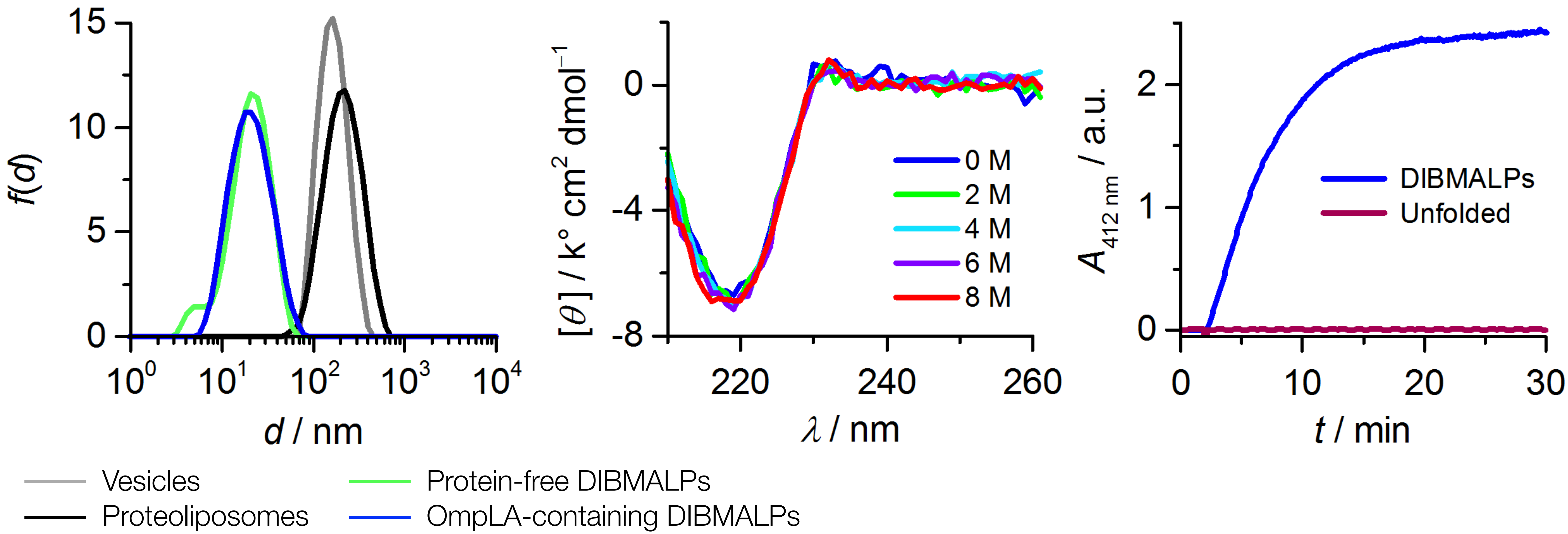


Frotscher et al. *Angew. Chem. Int. Ed.* **2015**, *54*, 5069



Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919

Solubilisation of OmpLA in DLPC

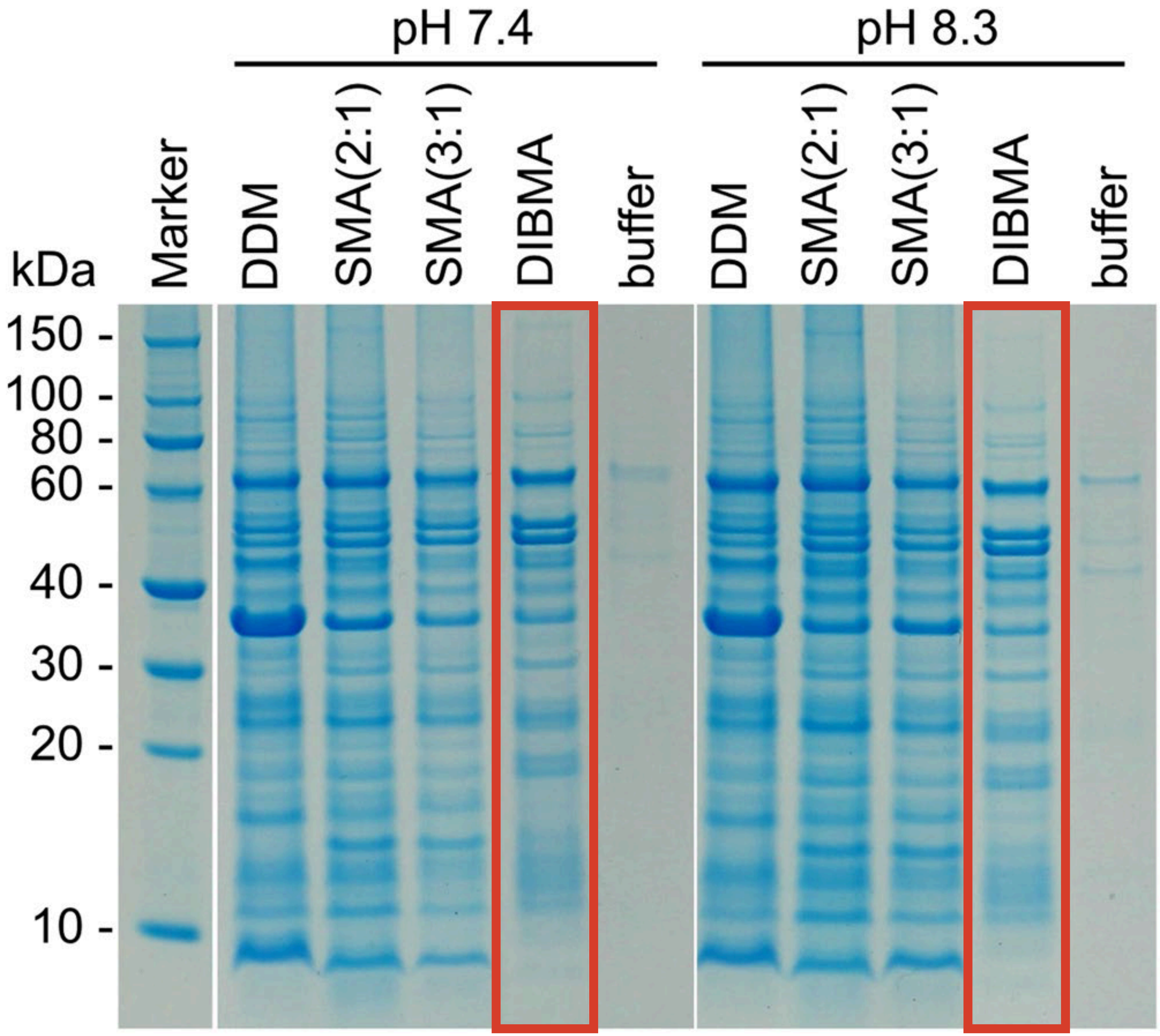


Nanodisc formation from proteoliposomes:

- as efficient as from protein-free DLPC vesicles
- OmpLA remains natively folded and enzymatically active

Oluwole et al. *Angew. Chem. Int. Ed.* **2017**, *56*, 1919

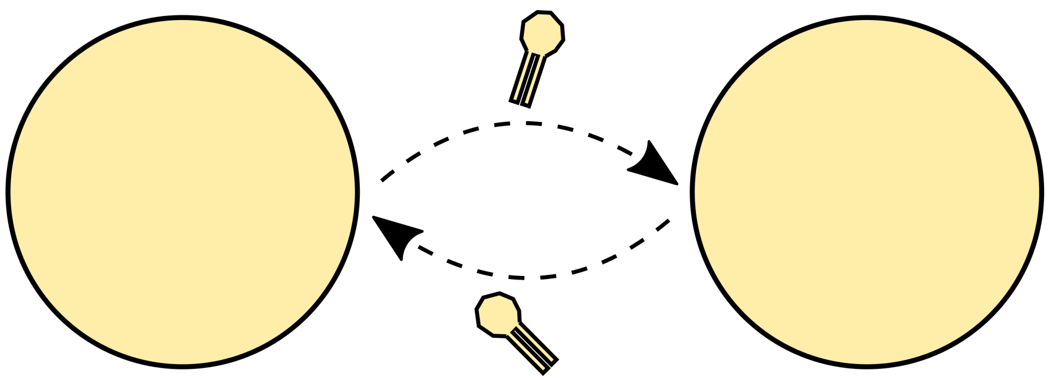
Solubilisation of *E. coli* membranes

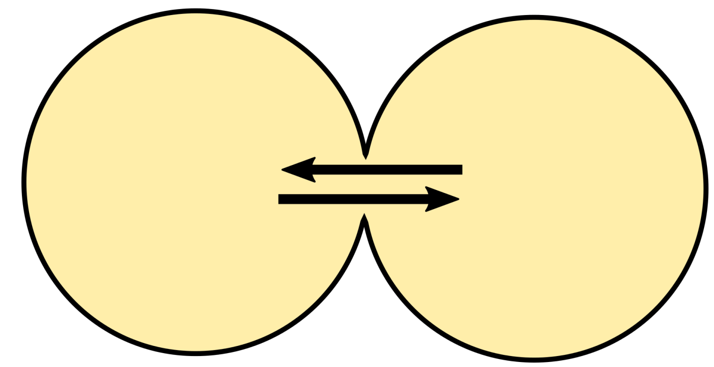


Grethen et al. *Sci. Rep.* **2017**, *7*, 11517

Kinetics of lipid exchange

Two major mechanisms of lipid transfer





Monomer diffusion:

- Slow
- Saturable:

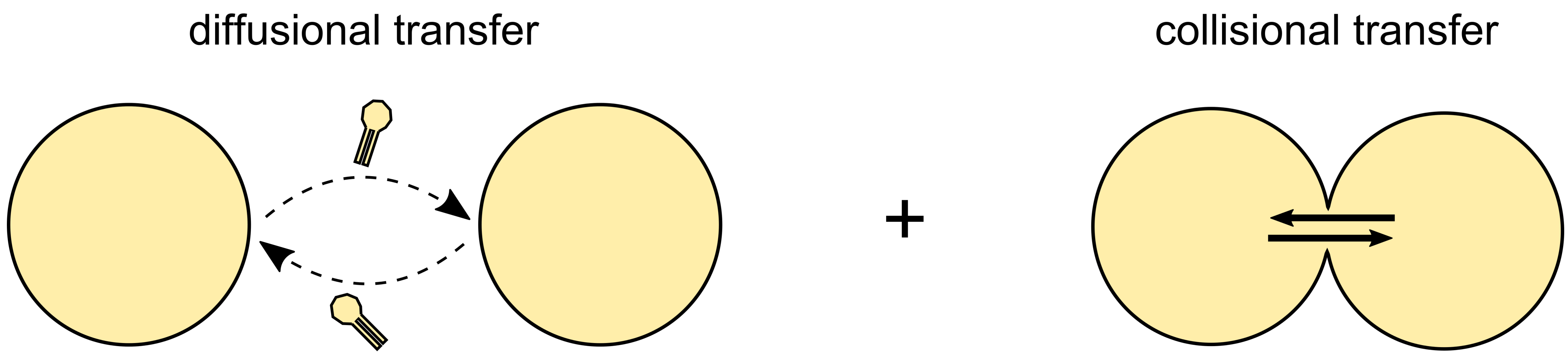
$$k_{\mathrm{obs,dif}}(c_{\mathrm{L}}) = \frac{k_{\mathrm{dif}} c_{\mathrm{L}}}{c_{\mathrm{L}}^{\circ} + c_{\mathrm{L}}}$$

Particle collisions:

- Slow to fast
- Second-order kinetics:

$$k_{\mathrm{obs,col}}(c_{\mathrm{L}}) = k_{\mathrm{col}} c_{\mathrm{L}}$$

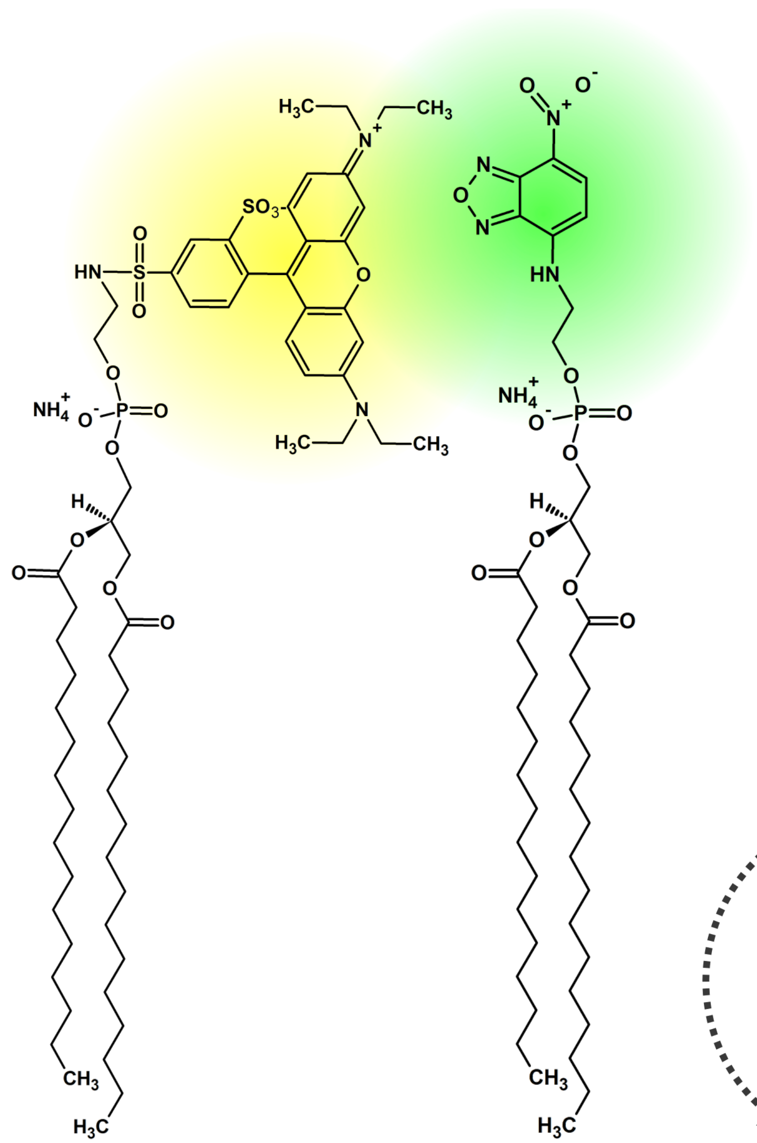
Two major mechanisms of lipid transfer



Superposition of both mechanisms:

$$k_{\mathrm{obs}}(c_{\mathrm{L}}) = k_{\mathrm{obs,dif}}(c_{\mathrm{L}}) + k_{\mathrm{obs,col}}(c_{\mathrm{L}}) = \frac{k_{\mathrm{dif}} c_{\mathrm{L}}}{c_{\mathrm{L}}^{\circ} + c_{\mathrm{L}}} + k_{\mathrm{col}} c_{\mathrm{L}}$$

Lipid transfer by TR-FRET: approach



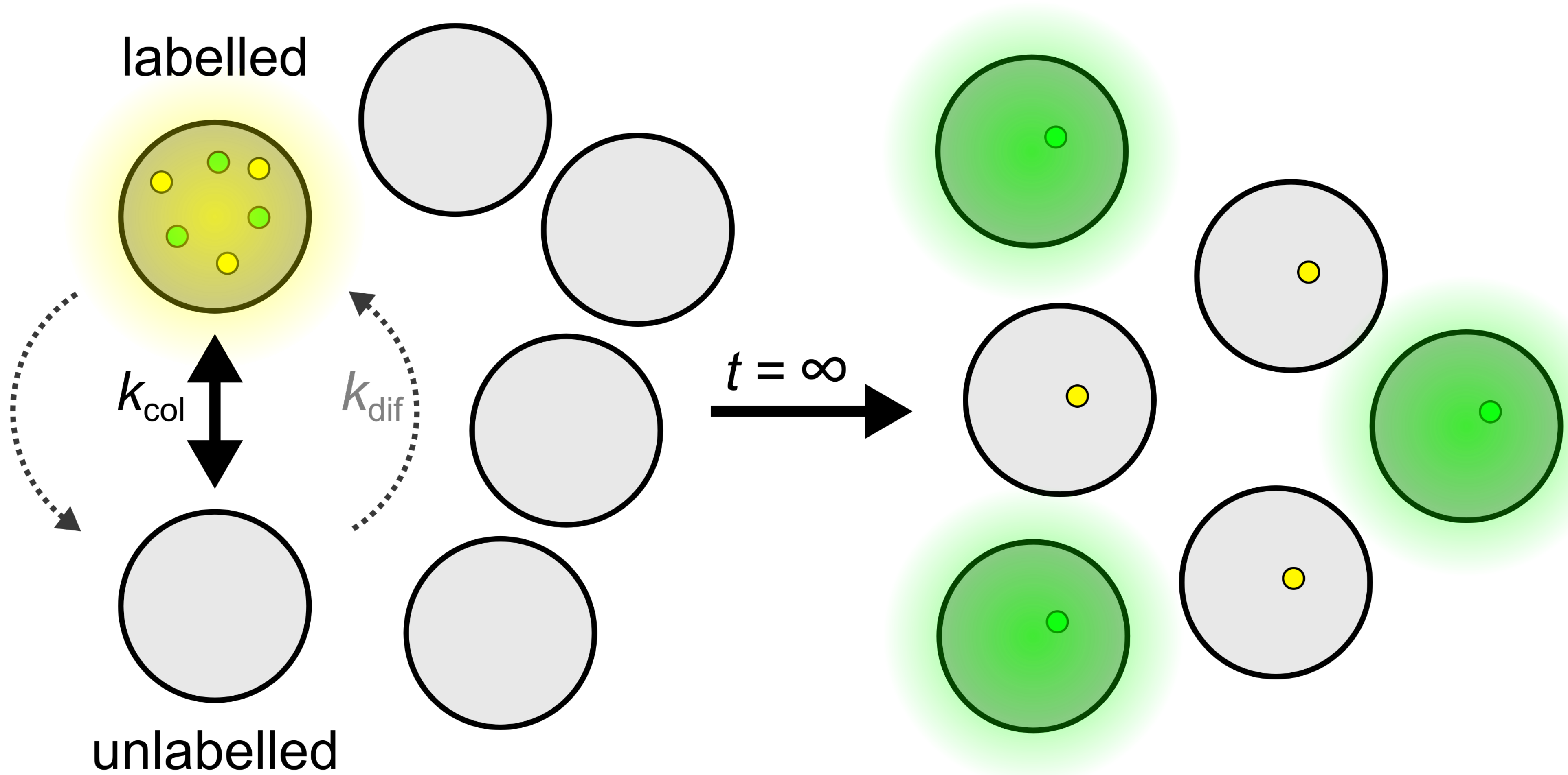
Rh-PE

λ_{ex} = 560 nm
λ_{em} = 582 nm

NBD-PE

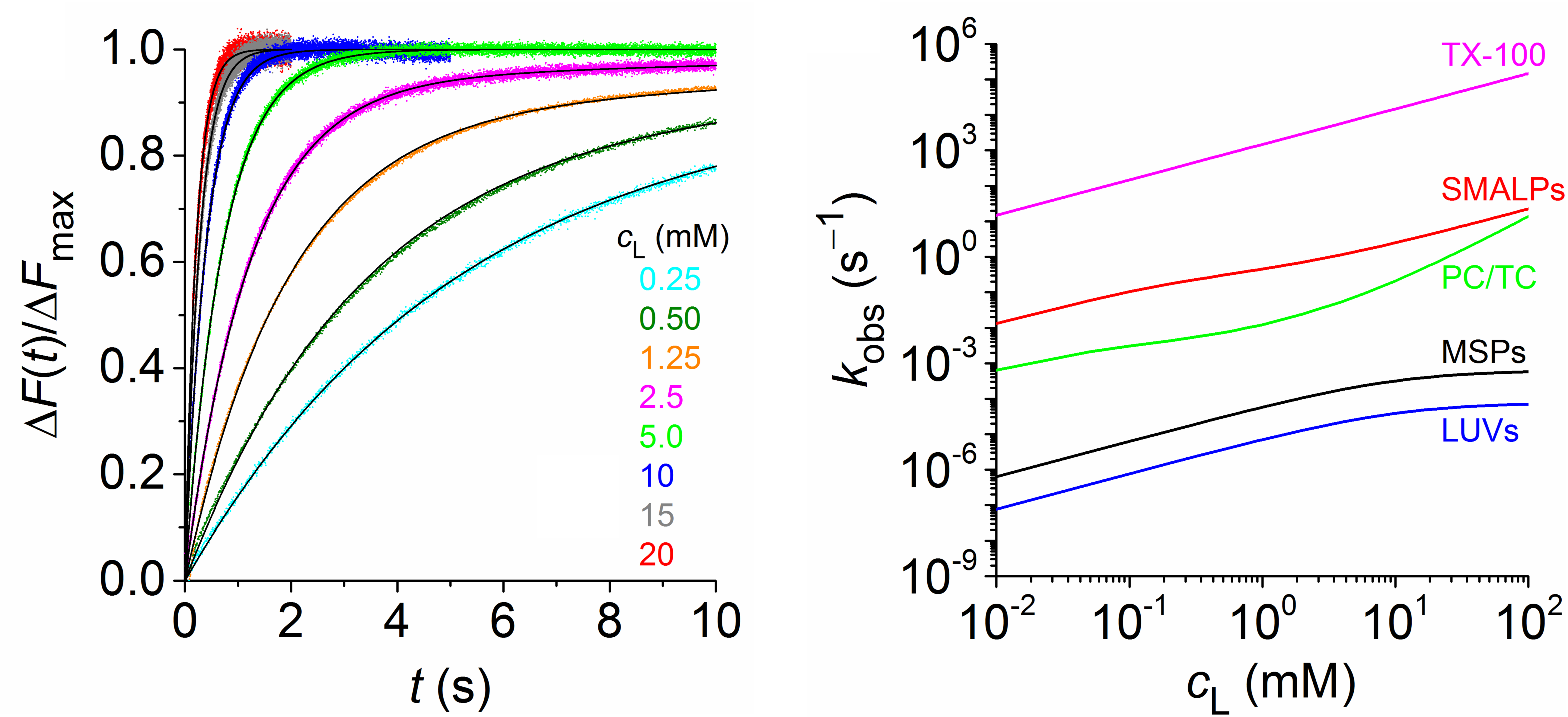
λ_{ex} = 475 nm
λ_{em} = 530 nm

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



Cuevas Arenas et al. *Sci. Rep.* **2017**, *7*, 45875

Lipid transfer by TR-FRET: results



$$k_{col} = 222\ \mathrm{M}^{-1}\mathrm{s}^{-1}$$

$$k_{dif} = 0.29\ \mathrm{s}^{-1}$$

$$k_{col,SMALPs} = 3.2 \cdot 10^{8}\,\mathrm{M}^{-1}\mathrm{s}^{-1}$$

Cuevas Arenas et al. *Sci. Rep.* **2017**, *7*, 45875

Take-home messages

Lipid-bilayer nanodiscs encapsulated by amphiphilic copolymers offer new opportunities for studying membrane proteins in a near-native environment.

Aliphatic DIBMA is a milder alternative to SMA, as it is less perturbing to lipid order and compatible with UV spectroscopy and divalent cations.

SMALPs and DIBMALPs exchange lipids by collisional transfer and, thus, represent equilibrium rather than kinetically trapped structures.

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