

David Glück

Electroneutral Polymer Nanodiscs

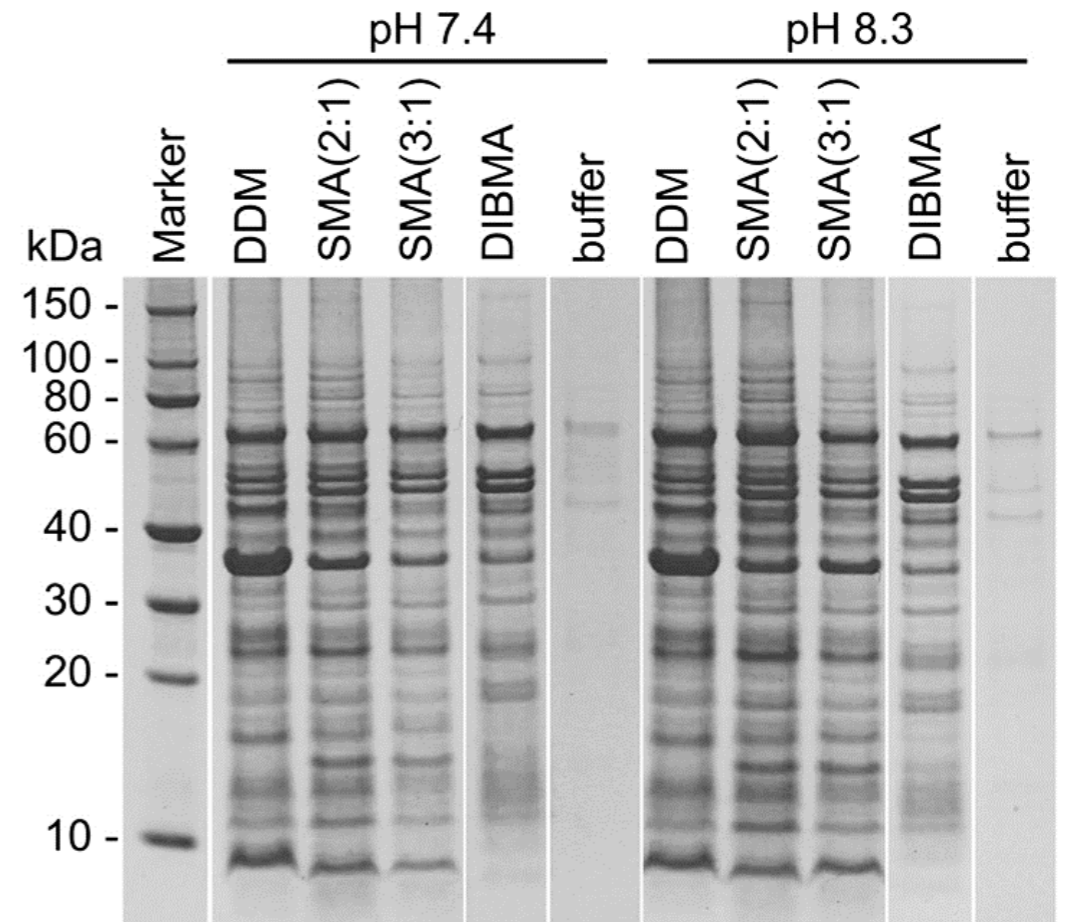
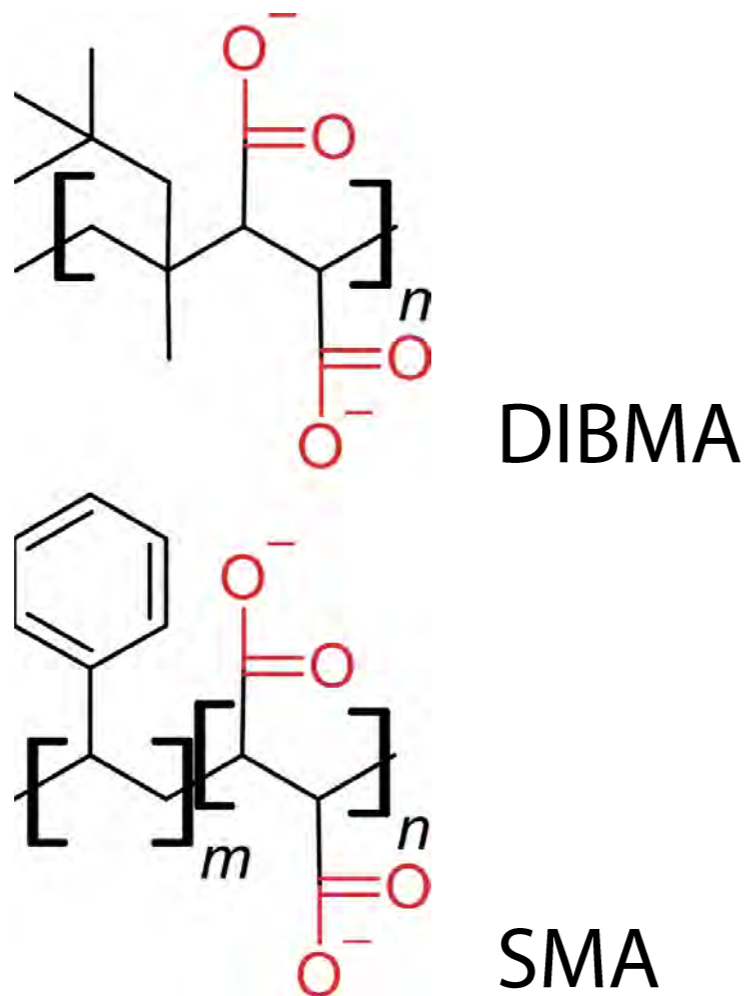
Enable Interference-Free Probing of Membrane
Proteins in a Lipid-Bilayer Environment

SMALP Meeting

08.12.2022



Why electroneutral polymers?

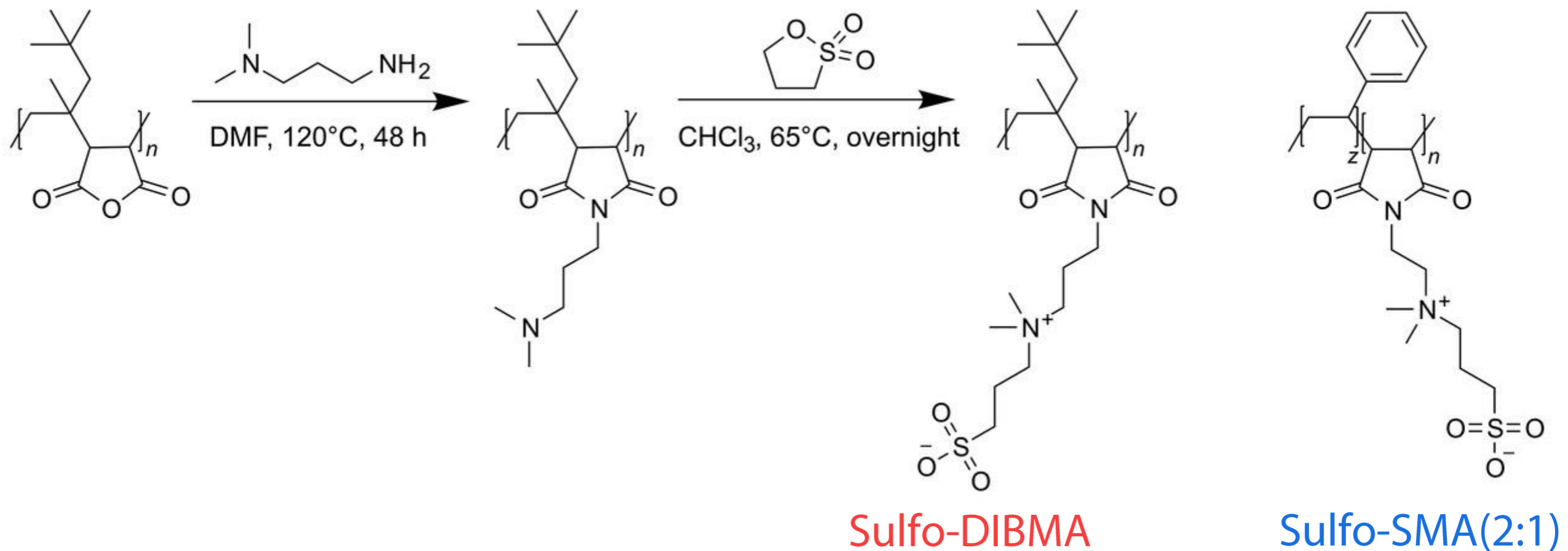


Grethen et al. *Nat. Sci. Rep.* **2017**, 7, 11517 (modified)

The anionic polymers SMA and DIBMA...

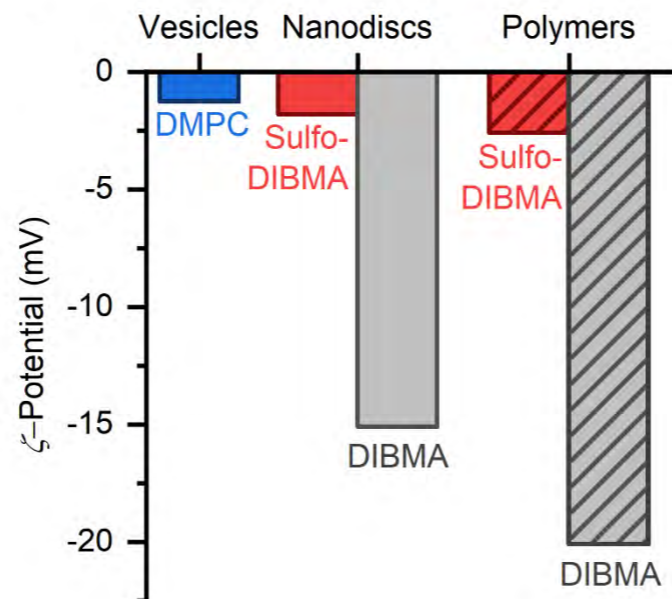
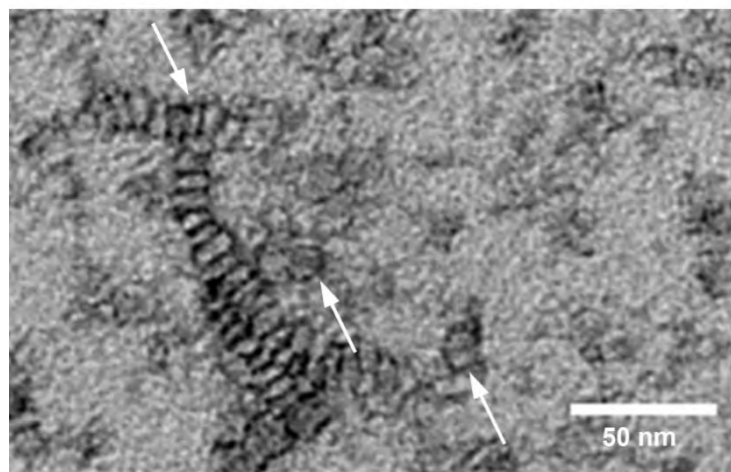
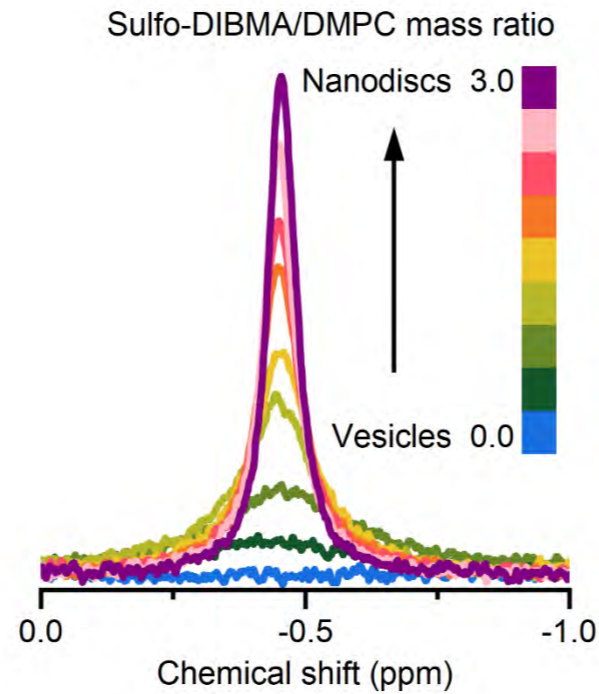
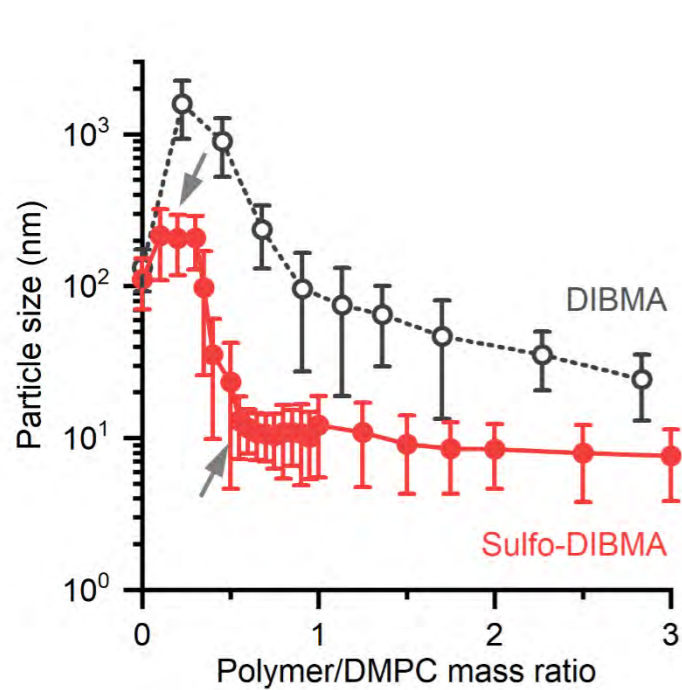
- ...depend on pH/NaCl for nanodisc formation.
- ...interact with divalent cations.
- ...interfere with charge-dependent molecular interactions.

Synthesis of sulfobetaine polymers



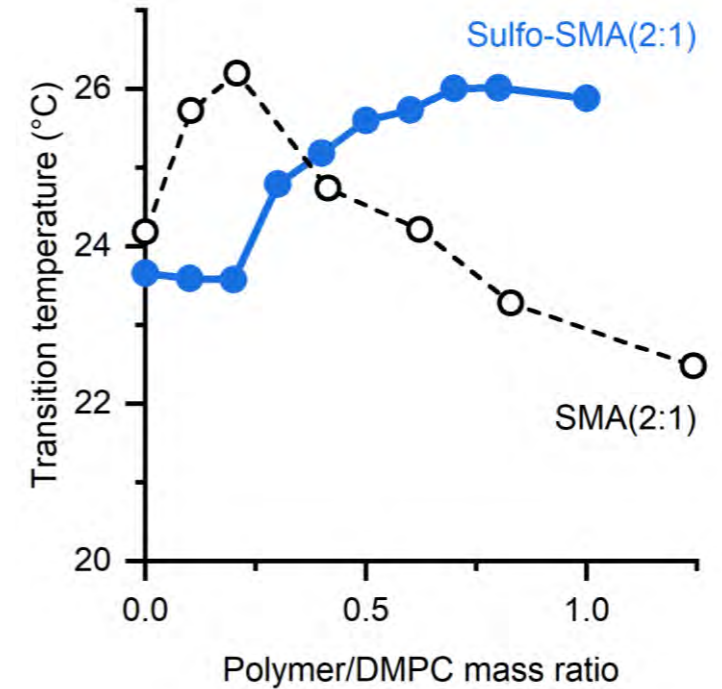
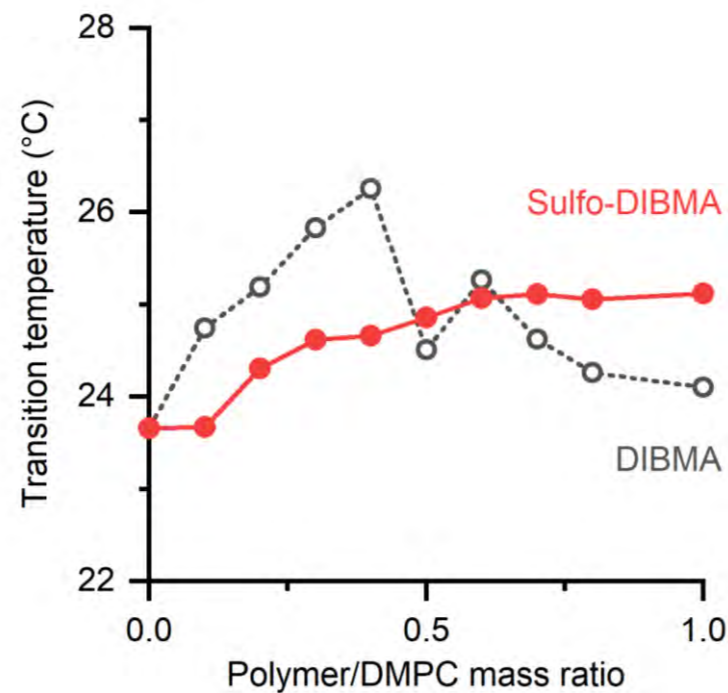
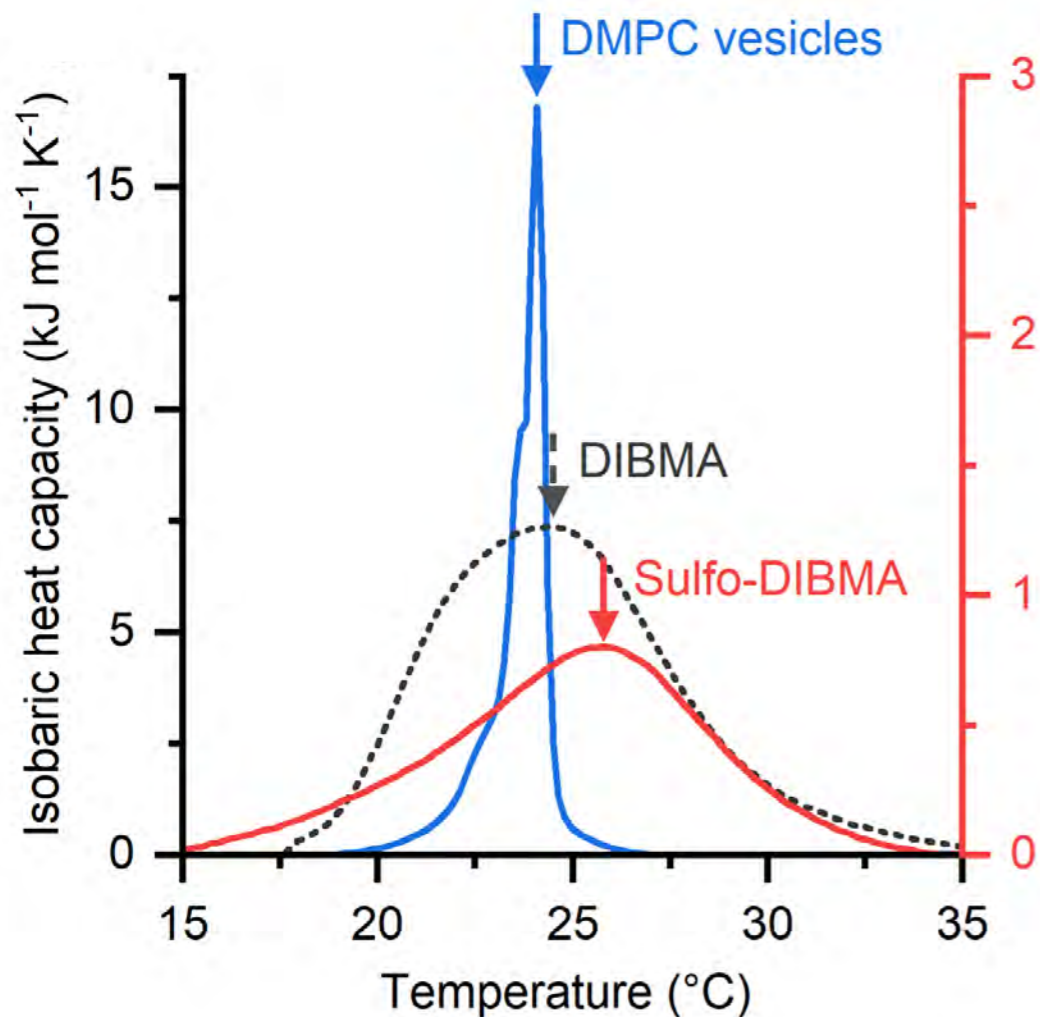
- Two-step reaction
- Quantitative yield, no post-reaction clean up
- Modular approach for customized polymers

Solubilization efficiency

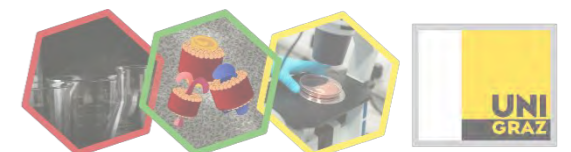


- Efficient nanodisc formation at $m_{\text{polymer}}/m_{\text{DMPC}} > 0.5$
- Smaller nanodiscs compared with DIBMA
- ζ -potential confirms electroneutrality

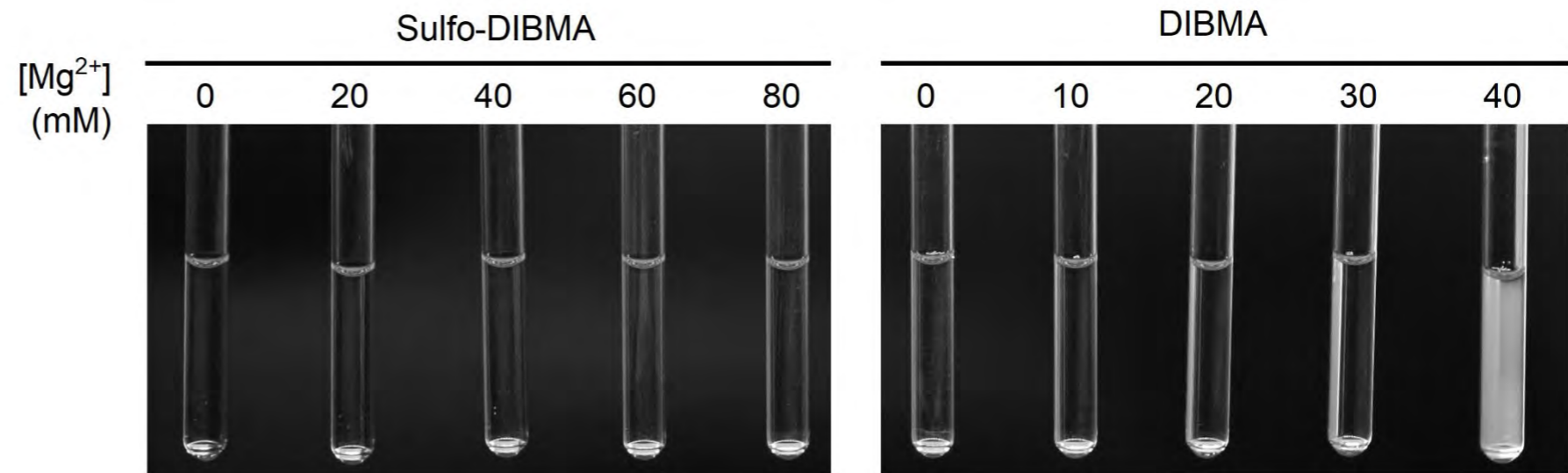
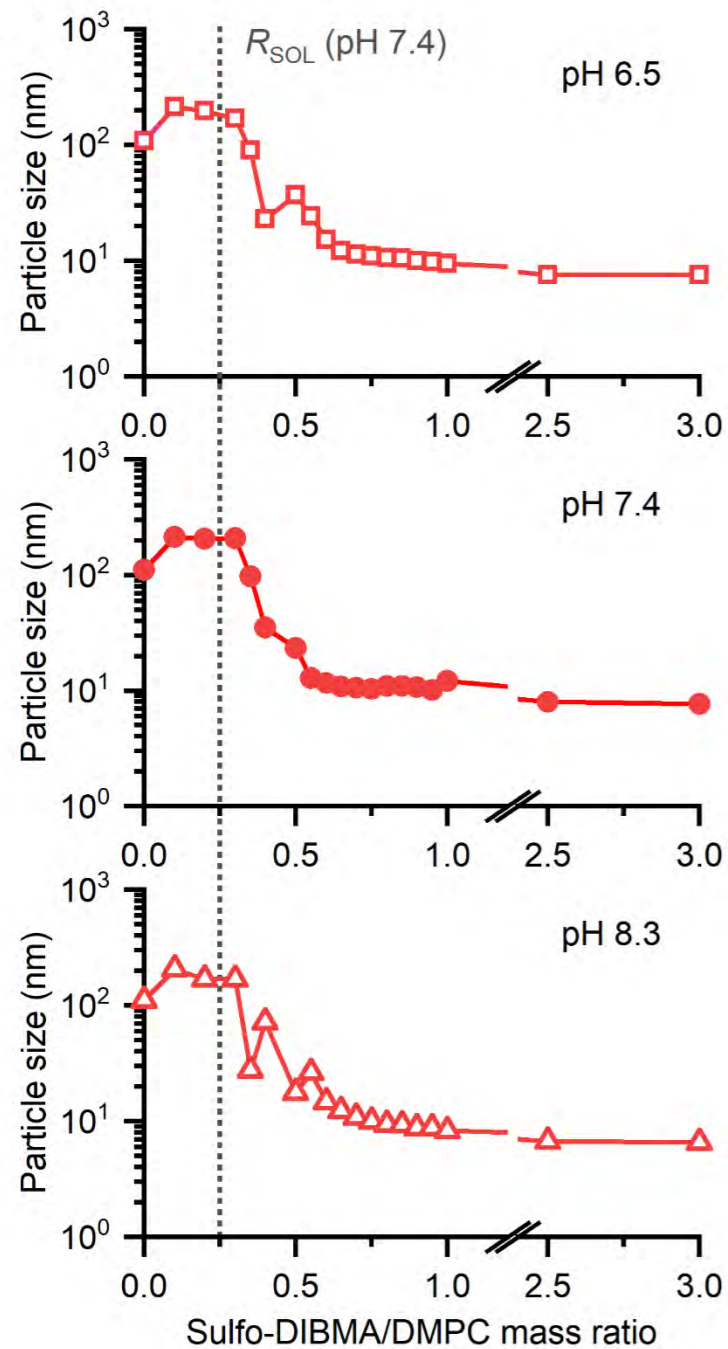
Effect on lipid melting temperature



- Differential scanning calorimetry
- T_m of DMPC slightly increased in Sulfo-nanodiscs
- Low perturbation of lipid acyl chain order

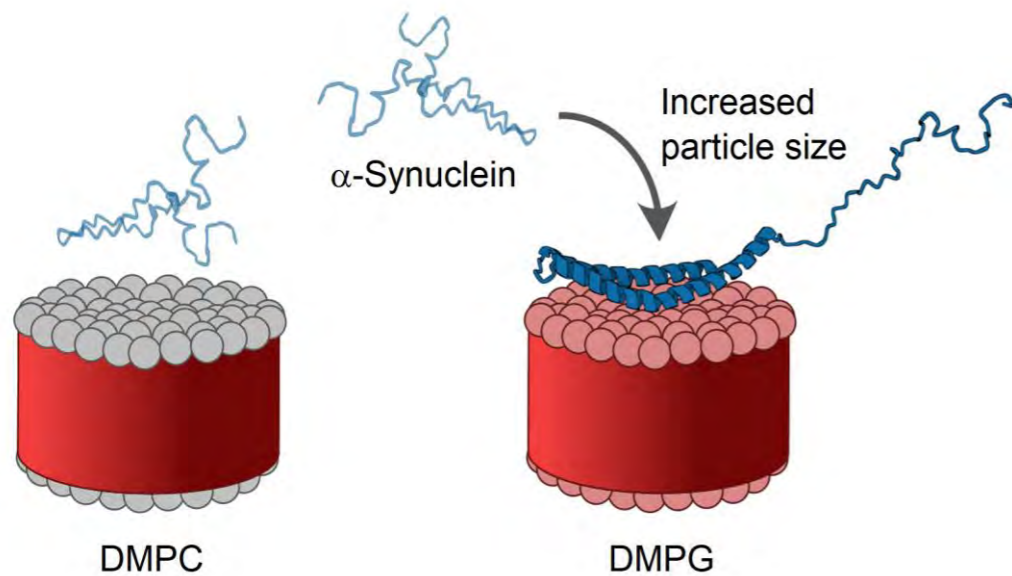


Stability of nanodiscs in challenging buffers



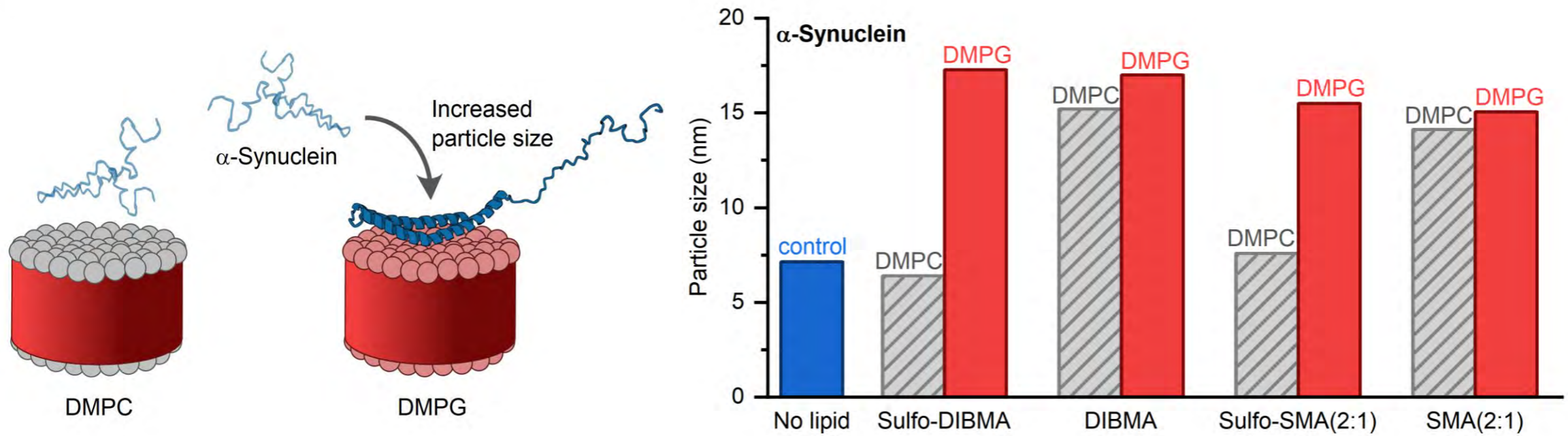
- Identical solubilization efficiency at pH 6.5, 7.4, 8.3
- Inert towards divalent cations (Mg²⁺, Ca²⁺)

Charge-dependent protein—lipid interactions



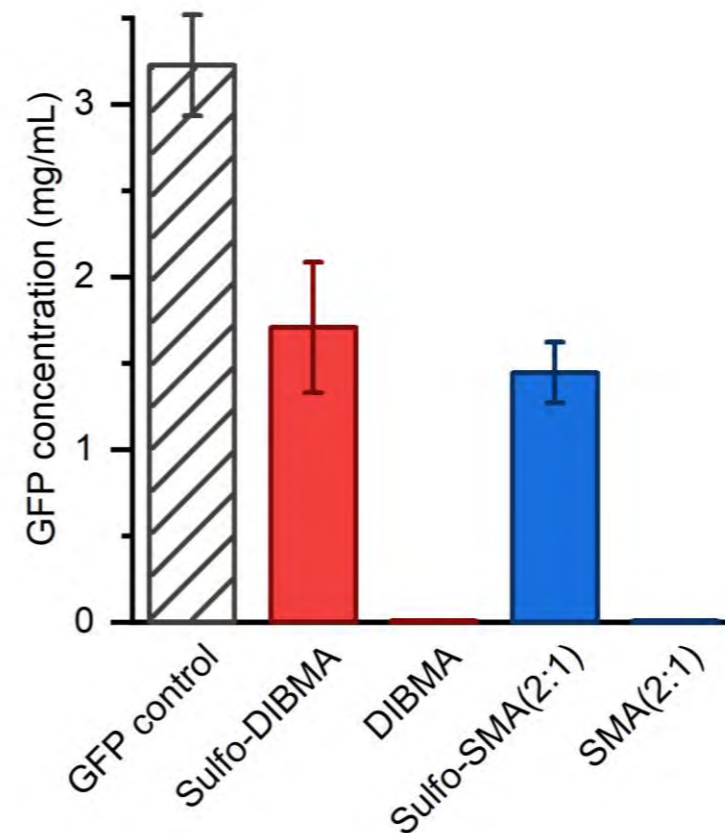
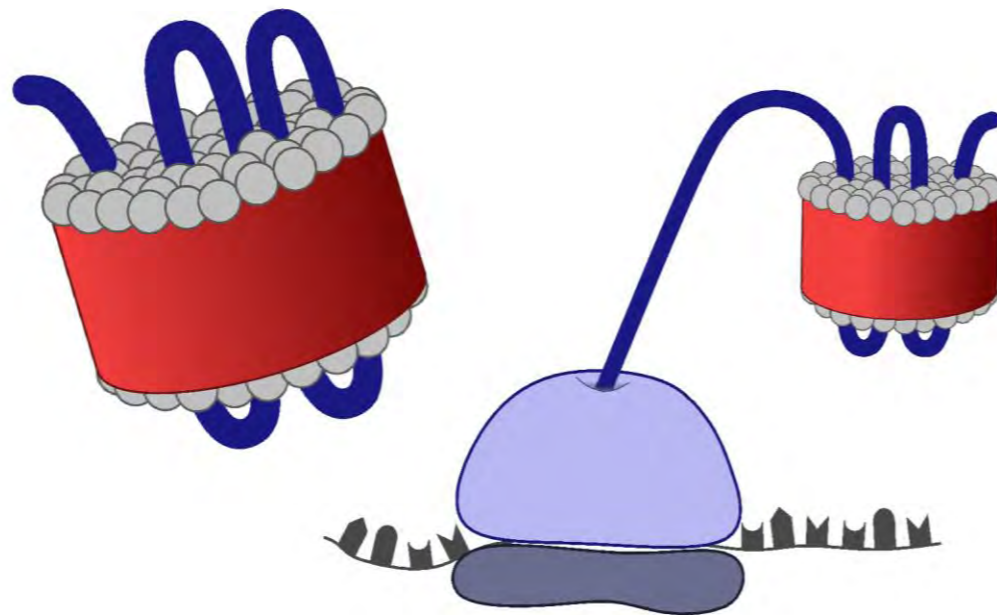
- Lab-on-a-chip method: microfluidic diffusional sizing (MDS)
- Detection of fluorescent protein samples
- Probing of charge-dependent protein—lipid interactions

Charge-dependent protein—lipid interactions



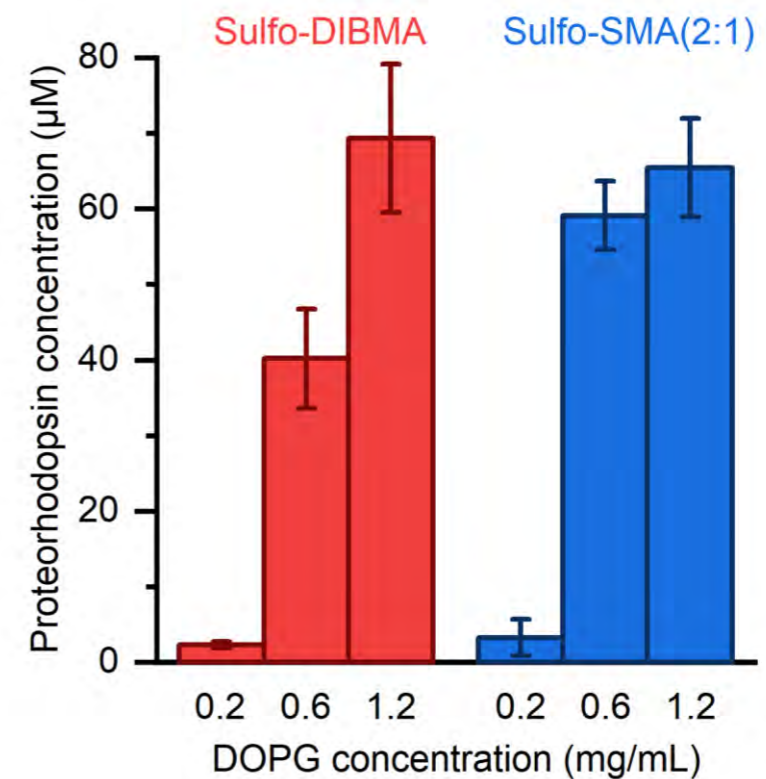
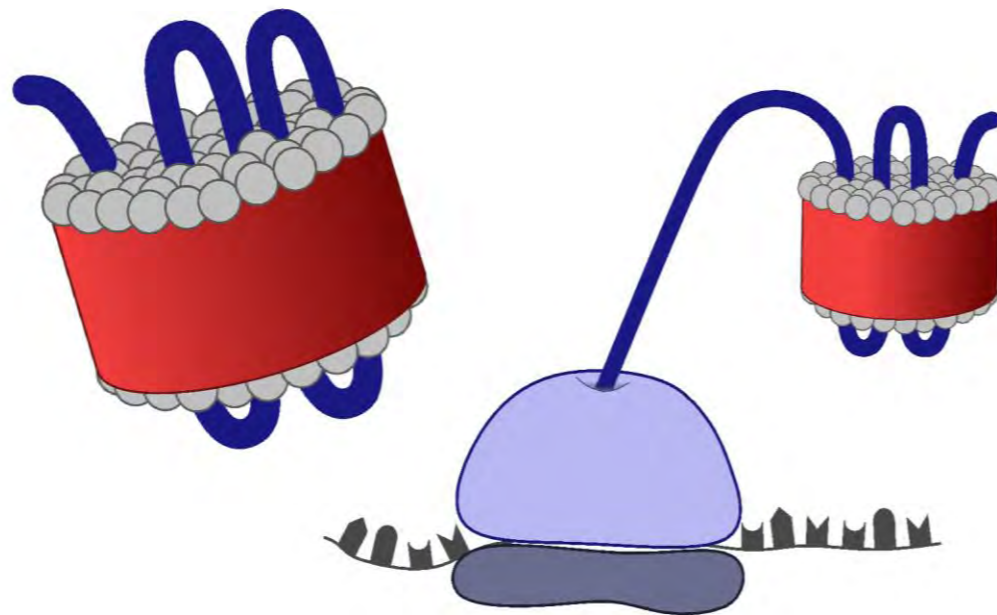
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Cell-free protein synthesis



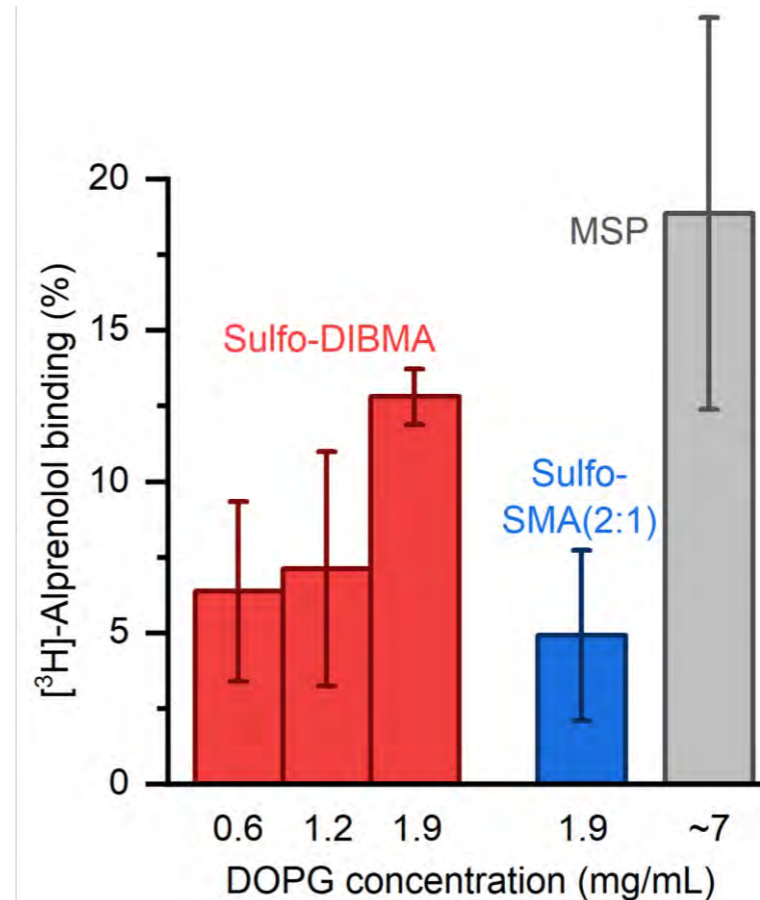
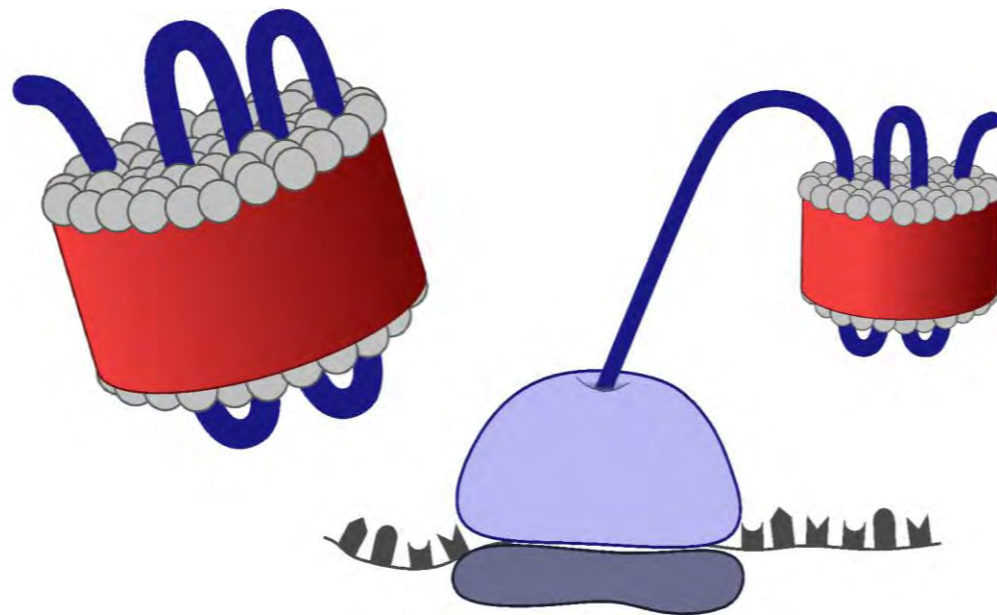
- Inhibition of GFP synthesis by anionic polymers (Mg^{2+} -sensitive)
- Sulfo-nanodiscs stabilize proteorhodopsin after translation
- Yield of functional protein

Cell-free protein synthesis



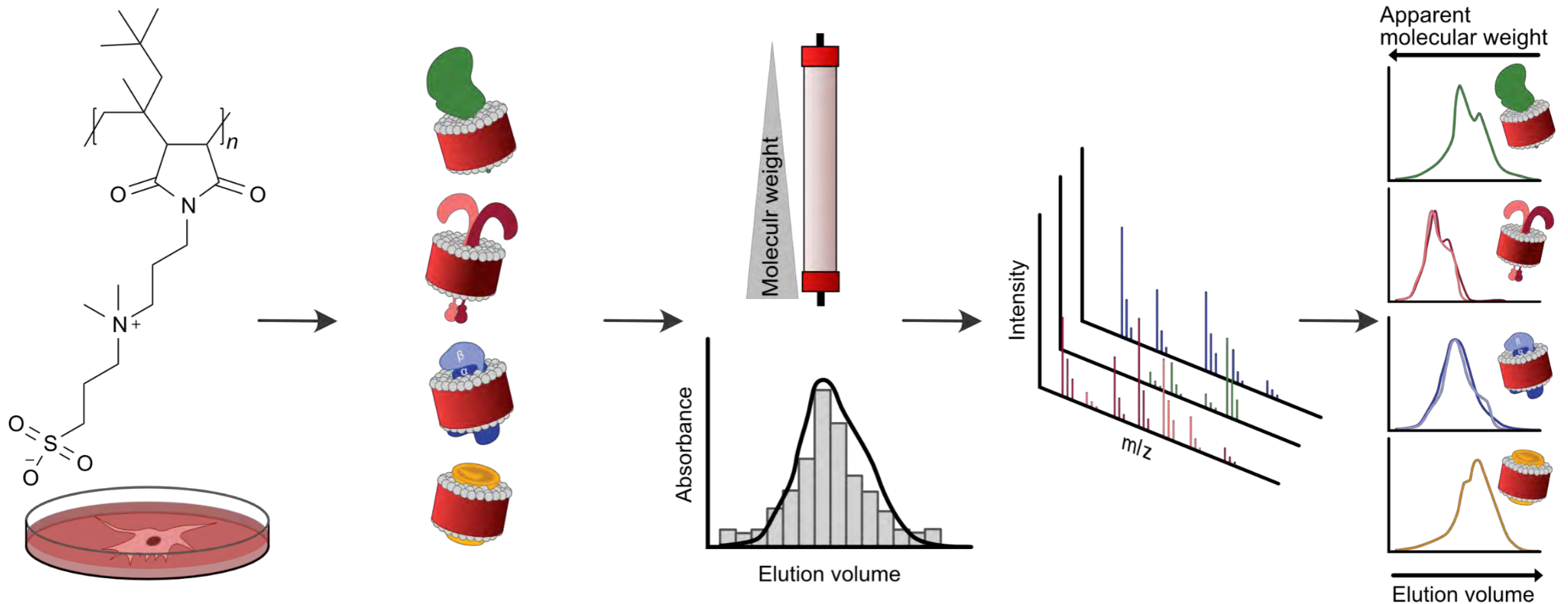
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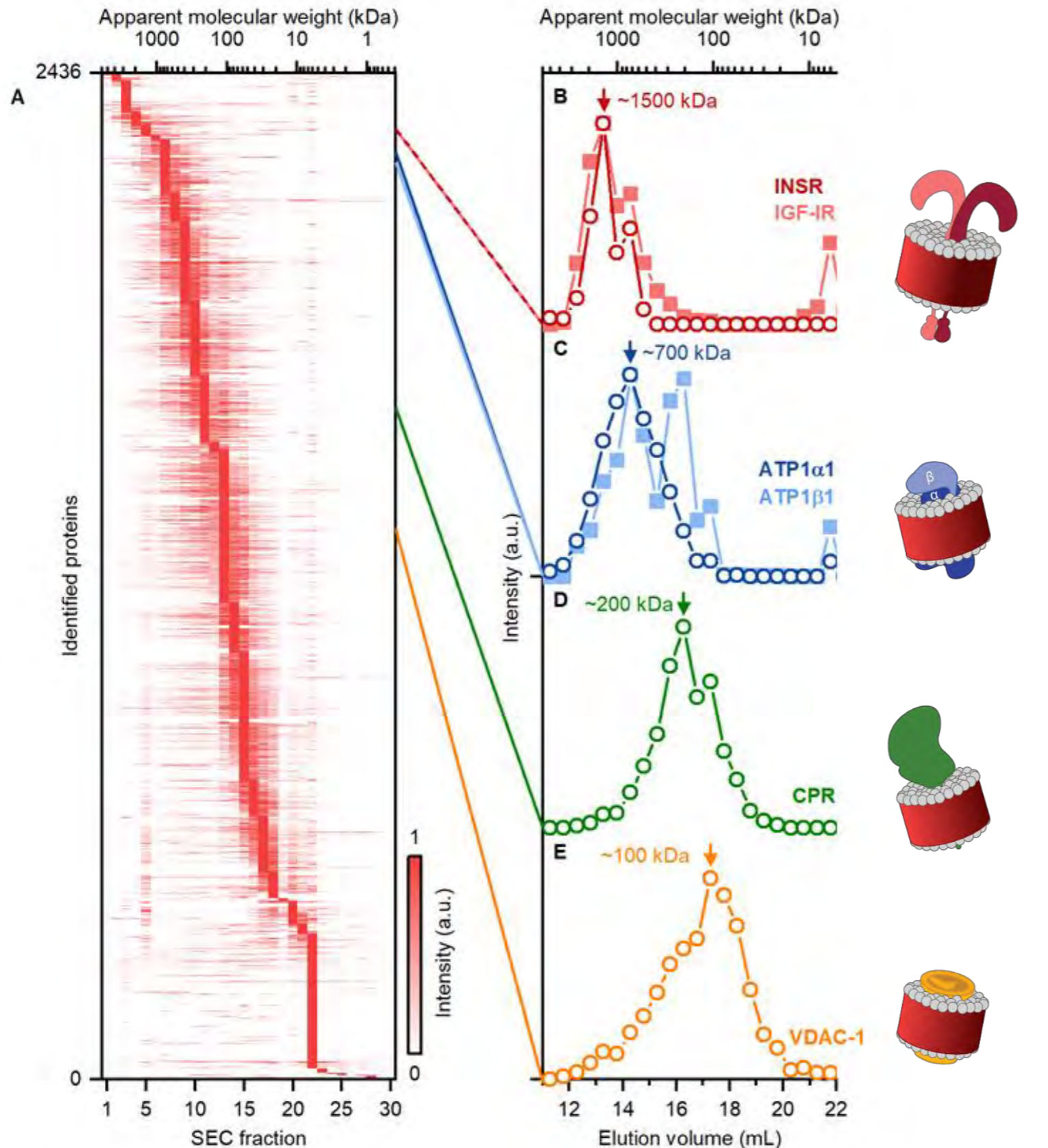
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Membrane proteome profiling



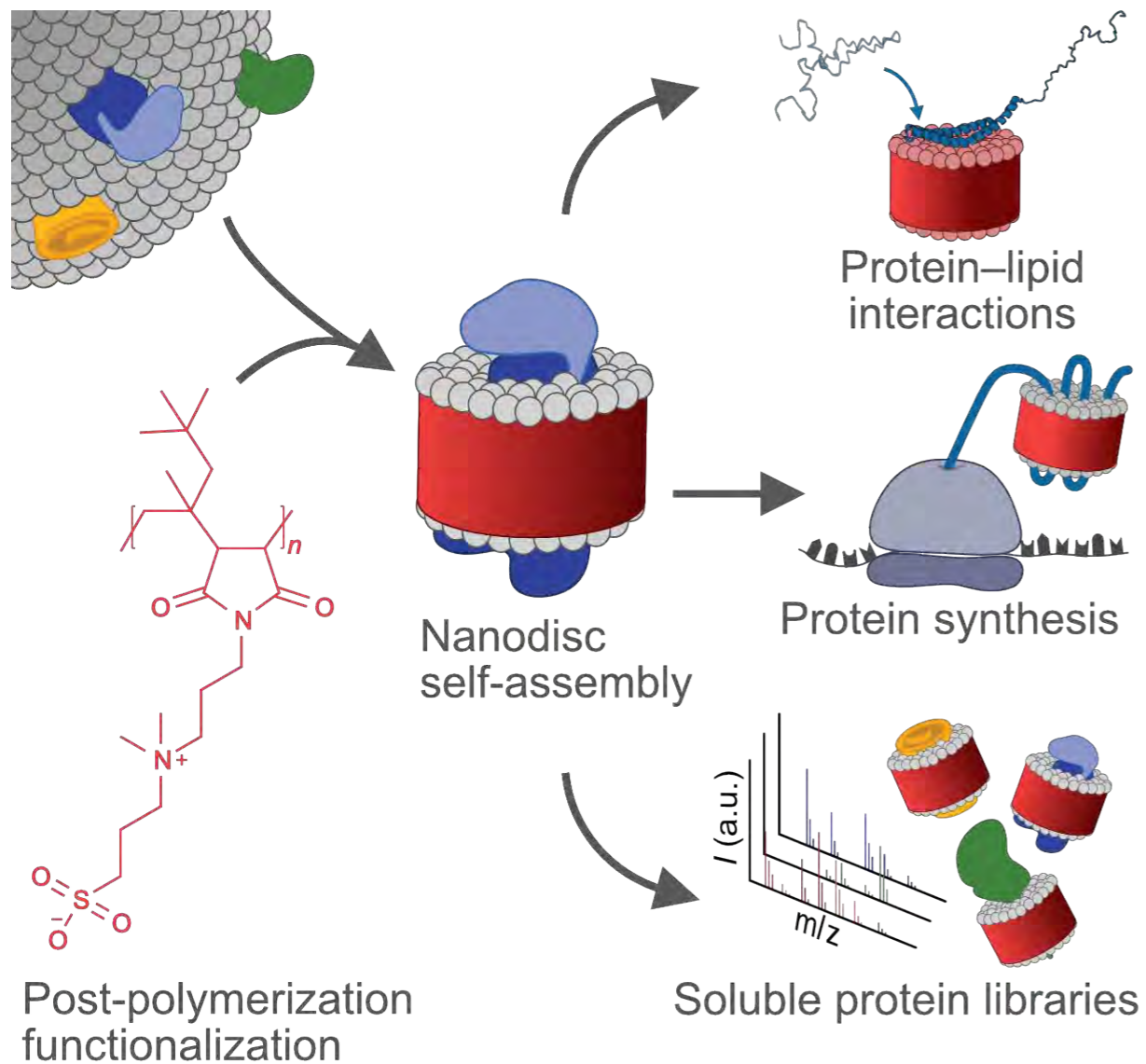
- Protein extraction from HeLa cells
- Fractionation via size exclusion chromatography (SEC)
- LC-MS/MS identification of all SEC fractions

Membrane proteome profiling



- 2403 protein elution profiles
- Co-elution of protein complex subunits
- Label-free profiling of membrane proteome

Summary



Electroneutral sulfo-nanodiscs...

... are **monodisperse** at low $R_{\text{polymer/lipid}}$

... allow probing **charge-sensitive protein-lipid interactions**

... stabilize membrane proteins after **cell-free synthesis**

... extract **soluble membrane protein libraries** from cell membranes

Acknowledgements

Coauthors of this study

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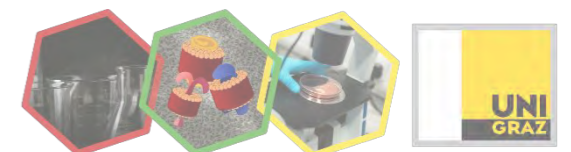
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External collaborators

Cenek Kolar
Harald Kelm



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RESEARCH ARTICLE

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Electroneutral Polymer Nanodiscs Enable Interference-Free Probing of Membrane Proteins in a Lipid-Bilayer Environment

*David Glueck, Anne Grethen, Manabendra Das, Ogochukwu Patricia Mmeka, Eugenio Pérez Patallo, Annette Meister, Ritu Rajender, Stefan Kins, Markus Räsche, Julian Victor, Ci Chu, Manuel Etzkorn, Zoe Köck, Frank Bernhard, Jonathan Oyebamiji Babalola, Carolyn Vargas, and Sandro Keller**

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