# In-depth characterization of polymer-stabilized lipid nanodiscs

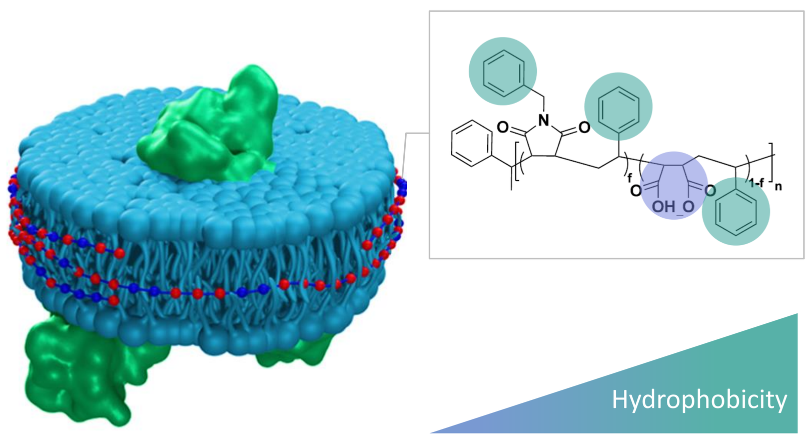

**Zahn Stanvliet [1]**, **Gestél Kuyler [1]**, **Elaine Barnard [1]**, **Eva Bittrich [2]**, **Susanne Boye [2]**, **Ralf Schweins [3]**, **Bert Klumperman [1]**, **Albena Lederer [1,2]**

[1] Department of Chemistry and Polymer Science, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa
[2] Department Advanced Macromolecular Structure Analysis, Leibniz-Institut für Polymerforschung Dresden e.V. , Hohe Str. 6, D-01069 Dresden, Germany
[3] DS/LSS Group, Institute Laue-Langevin, 6 Rue Jules Horowitz, F-38042 Grenoble Cedex 9, France

*E-mail: zstanvliet@sun.ac.za*

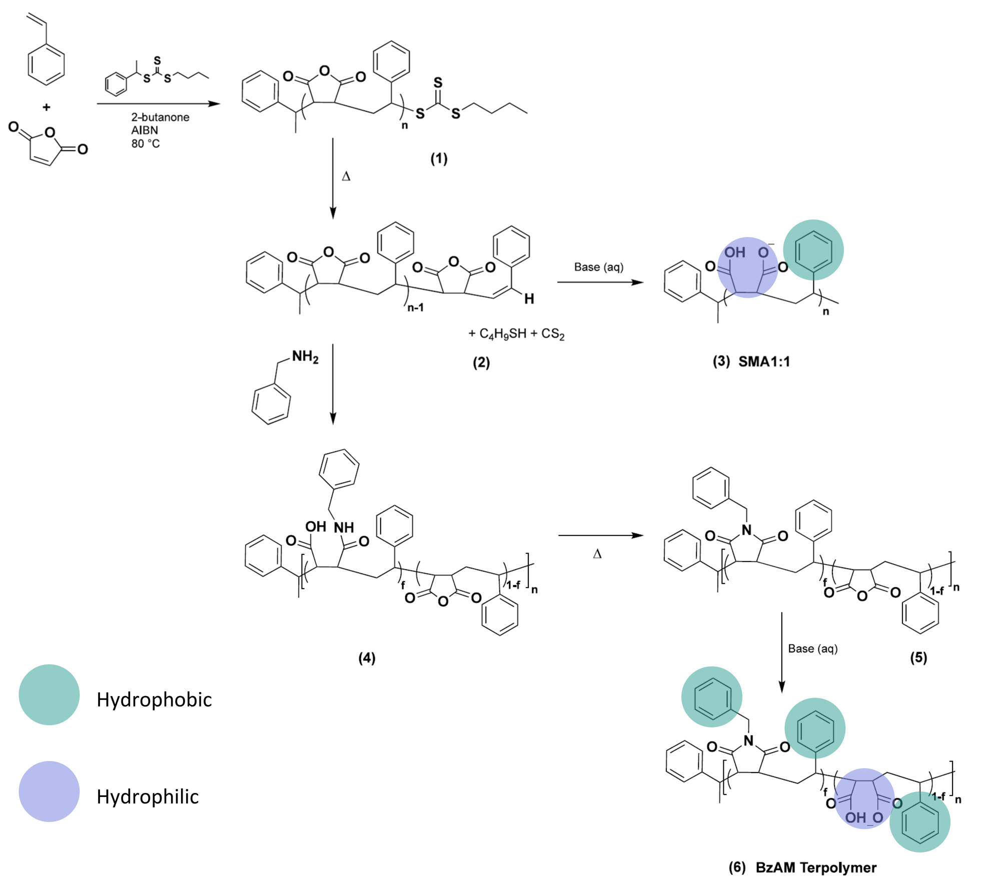

## Membrane protein solubilization

- The isolation and analysis of membrane proteins are frequently challenged by the limitations of conventional detergent-based techniques.
- Amphiphilic polymers offer an alternative by forming nanoscale discs that help maintain the structural and functional integrity of MPs.
- A new series of poly(styrene-co-maleic acid-co-(N-benzyl)maleimide) (BzAM) terpolymers was developed to replicate and enhance the performance of the industry-standard poly(styrene-co-maleic acid) (SMA).[1-2]
- These enable a systematic investigation of how polymer characteristics influence membrane solubilization.




## Preparation of polymer-stabelized lipid nanodiscs

### Synthesis



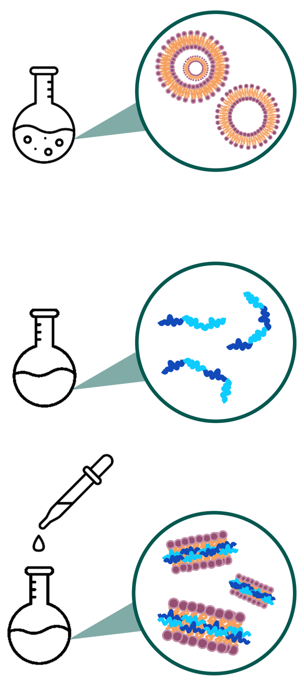

Summary of polymer and lipid-only nanodisc properties under various biophysical conditions including pH, $[Mg^{2+}]$ and $[Ca^{2+}]$, (mM), and average lipid-only nanodisc diameter (nm).

| Sample | Soluble pH range | $[Mg^{2+}]$ (mM) | $[Ca^{2+}]$ (mM) | Average lipid-only nanodisc diameter (nm) |
|---|---|---|---|---|
| SMA2000 | 5.5–10 | 5 | 5 | 5.4 (±0.4) |
| SMA1000 | 4–10 | 10 | 5 | 16.5 (±9.8) |
| 0.05 BzAM | 5–10 | 10 | 5 | 11.5 (±1.3) |
| 0.10 BzAM | 5–10 | 8 | 5 | 6.2 (±0.5) |
| 0.15 BzAM | 5.5–10 | 8 | 5 | 6.6 (±0.7) |
| 0.20 BzAM | 5.5–10 | 5 | 5 | 7.3 (±1.1) |
| 0.25 BzAM | 6–10 | 5 | 5 | 7.3 (±0.9) |
| 0.30 BzAM | 6–10 | 5 | 5 | 6.6 (±0.3) |
| 0.35 BzAM | 7–10 | 5 | 2 | 7.0 (±1.6) |
| 0.40 BzAM | 7–10 | <5 | 2 | 7.2 (±0.9) |

- A series of terpolymers were synthesized from an alternating base copolymer (poly(styrene-alt-maleic anhydride), SMAnh) using RAFT polymerization.[2]
- Anhydride groups are partially modified and in turn the hydrophobicity in gradually increased across the series.

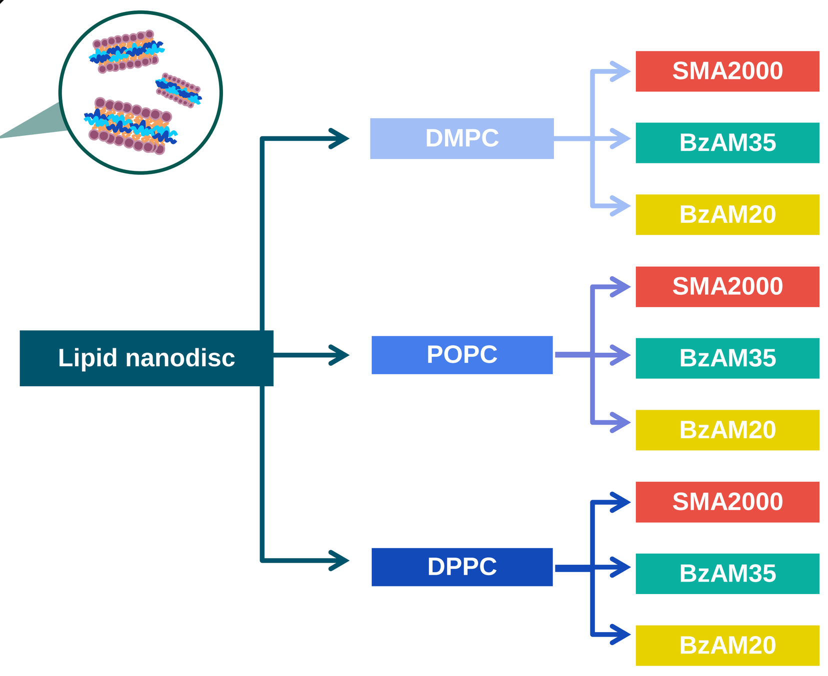
### Self-assembly

1. **Prepare liposome stock solution – 10 mg/mL**
   - Buffer: 50 mM sodium phosphate + 150 mM NaCl
   - Lipids (powder) + buffer + vortex for 60s
   - Extrude through 200 nm membrane 27-fold
2. **Prepare polymer stock solution – 0.31% - 5% w/v**
   - Polymer (powder) + buffer
3. **Combine equal volumes of polymer and liposome stock solution**



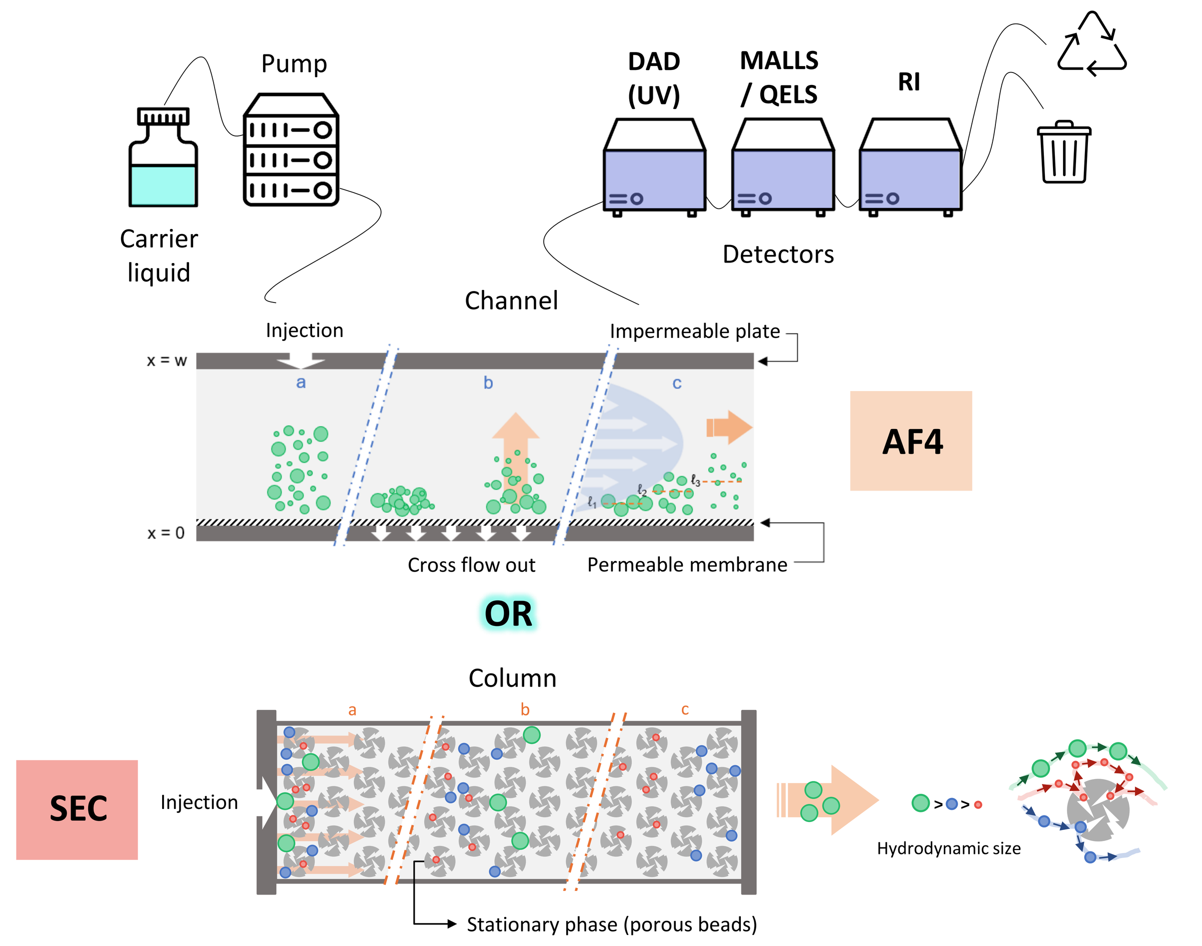
This study investigates the effect of different polymer compositions and lipid systems on nanodisc size and formation efficiency.

We examine the relationship between polymer hydrophobicity, aggregate size, and nanodisc size, as well as the influence of acyl chain length and unsaturation in lipids.




## Separation and characterization techniques

### Multi-detection asymmetric flow field-flow fractionation (AF4) and size exclusion chromatography (SEC)

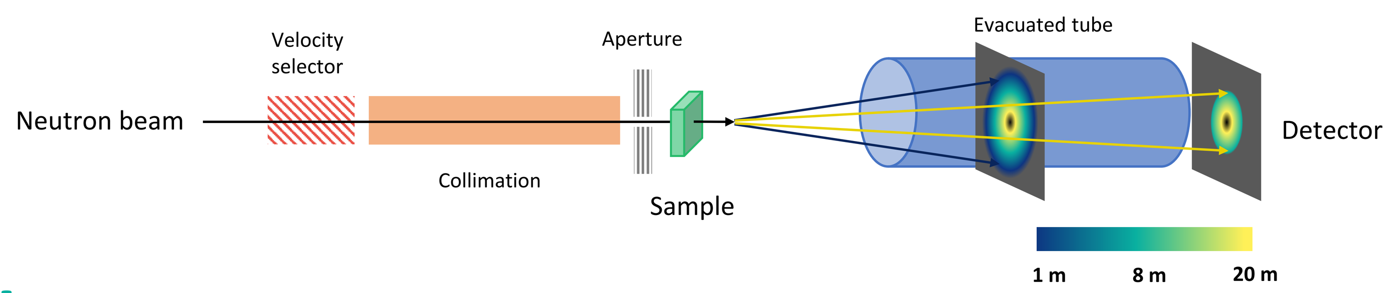



Characterization with AF4 and SEC will allow comparison of nanodisc sizes to those obtained from routinely used dynamic light scattering (DLS).

Each technique offers distinct advantages and limitations for nanodisc size determination.[3-4]

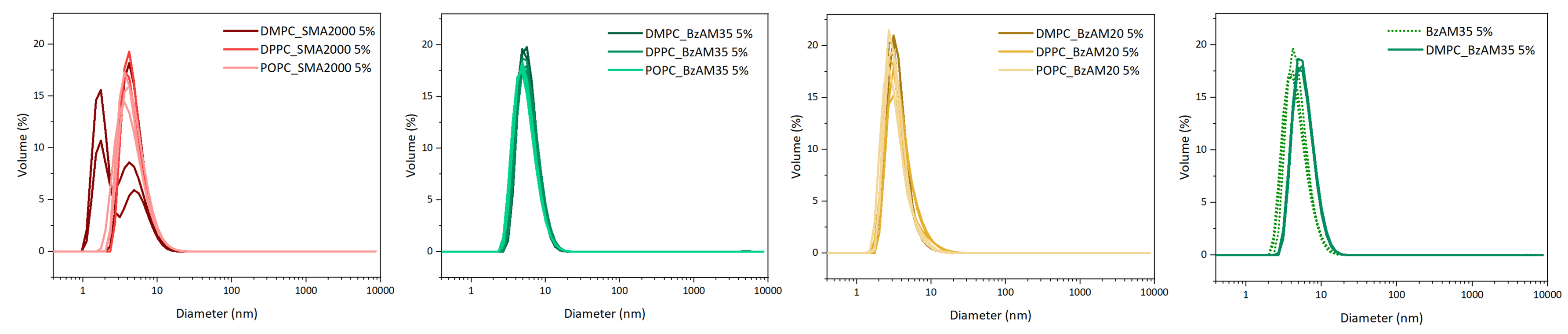
SANS enables investigation of structures at a scale ranging from 1 nm to 1000 nm, providing insights into the fine details of the nanodisc architecture.

### Small angle neutron scattering (SANS)

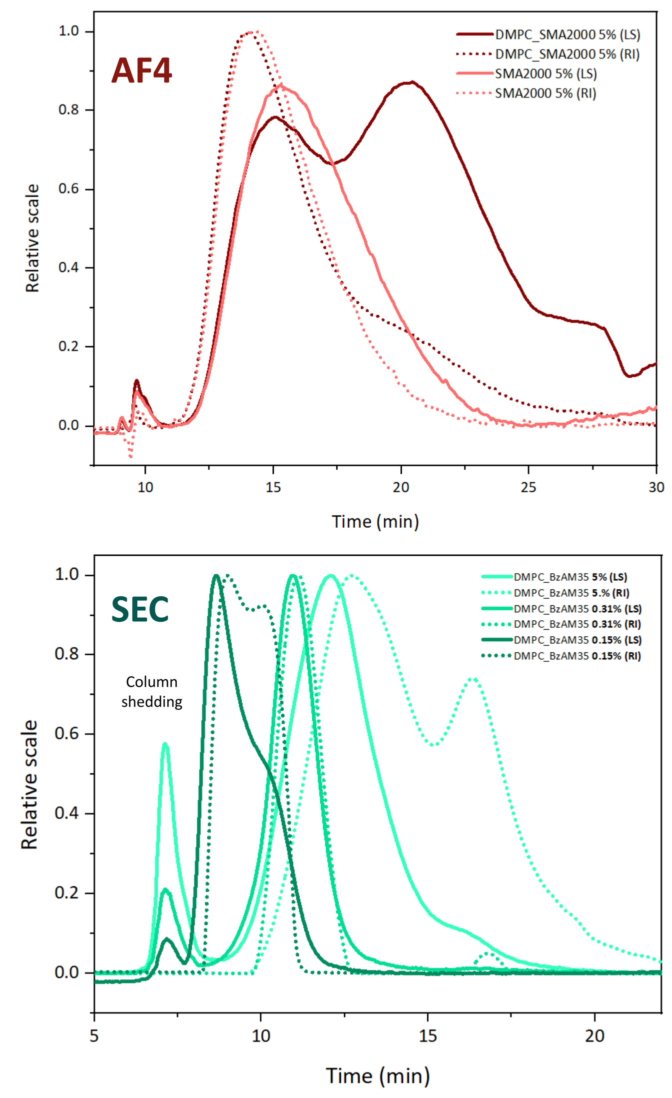



## Results

### Dynamic light scattering (DLS)

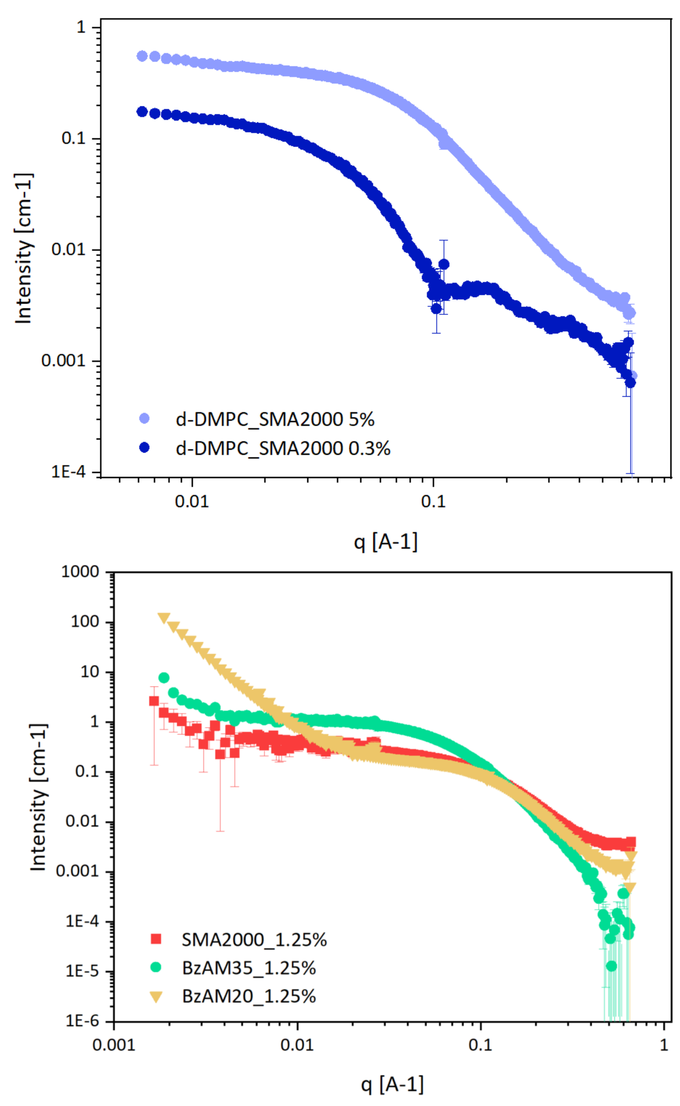



### AF4 versus SEC




- SEC provides better separation between nanodisc and polymer.
- Nanodiscs increase in size with decreasing polymer concentration.

### SANS




- Decreasing the polymer concentration enables us to obtain the internal structure.
- BzAM20 forms aggerates in solution compared to SMA2000 and BzAM35.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