Experimental report

26/07/2024

Proposal: **EASY-1187** **Council:** 4/2023

Title: Functional insertion of a self-assembling capsule into solid-supported triblock-copolymer membrane for specific guest molecule uptake

Research area: Soft condensed matter

This proposal is a new proposal

Main proposer: **Moritz MUTHWILL**

Experimental team: Moritz MUTHWILL

Local contacts: Nicolo PARACINI

Samples: Hybrid membrane

Instrument	Requested days	Allocated days	From	To
FIGARO	24	24	01/09/2023	02/09/2023

Abstract:

The aim of this project is to functionally insert a macromolecular hexameric capsule into a solid-supported triblock-copolymer membrane. The capsule self-assembles from resorcinarene monomers (Rs) in apolar, water-saturated solutions such as CHCl3, and is able to specifically encapsulate cationic guests in its cavity. For functional insertion, the capsule must be able to assemble inside the block-copolymer membrane and take up a guest molecule from the aqueous phase. The difficulty is the sensitivity of the capsule to polar solvents, since they lead to its disassembly. In a bulk experiment in CHCl3 solution we found that the capsule disassembles in presence of the hydrophilic polymer block, but not in presence of the hydrophobic block. We used QCM-D to deposit the hybrid polymer-Rs membranes by a solvent-assisted approach and to quantify the uptake of a cationic guest from the aqueous flow. We found homogeneous hybrid membranes (also characterised by AFM), containing functional capsules with specific guest uptake ability, proved by QCM-D and confirmed by LC-MS. Since we suspect the capsules located in the hydrophobic layer, we would like to confirm this with neutron reflectometry.

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This report is part of the following publication:

Muthwill, M. S.; Bina, M.; Paracini, N.; Coats, J. P.; Merget, S.; Yorulmaz Avsar, S.; Messmer, D.; Tiefenbacher, K.; Palivan, C. G. Planar Polymer Membranes Accommodate Functional Self-Assembly of Inserted Resorcinarene Nanocapsules. *ACS Applied Materials & Interfaces* **2024**, *16* (10), 13291-13304. DOI: 10.1021/acsami.3c18687.

Investigations of membrane structure and guest encapsulation by neutron reflectometry

To gain insights on the membrane structure and organization, NR measurements were conducted in liquid environment. NR allows for structural analysis of thin layers at an interface with sub-nanometer resolution and is sensitive to the chemical composition, thickness, water content, and roughness of individual layers. The deposition protocol used here was similar to the one used for QCM-D, with two minor modifications to accommodate the changed setup: The flow rate during solvent exchange was increased (from 0.1 to 2 mL min^{-1}), and the polymer concentration was likewise increased (from 0.5 to $\geq$ 1 g L^{-1}). Neutron reflectivity was measured in PBS using 4 SLD contrasts of varying D_2O:H_2O ratio (D_2O, 4MW, SMW, H_2O). The 4 contrasts were fitted to a common slab model of the interface in order to obtain structural information about individual block lengths, water content and roughness (Figure 1). The model was designed according to the expected ABA triblock copolymer membrane structure: a monolayer consisting of an inner hydrophobic layer (B, $PDMS_{50}$) and two identical outer hydrophilic layers (A, $PMOXA_7$) of equal length. Between the membrane and the silicon wafer, a layer of native SiO_2 was included. Roughness of the interface between successive slabs was modelled as gaussian smearing,[1] and two roughness parameters were used in total, one for the Si substrate and SiO_2 layer, and one for three slabs representing the polymer monolayer. Since the membrane was in an aqueous environment, a hydration value was calculated individually for each layer, which is expressed as volume fraction (VF) of water. Due to an insufficient intrinsic neutron contrast (SLD difference) between the polymer blocks and the Rs monomers as well as between the polymer blocks and the assembled capsule-guest complex, the Rs monomer and capsule-guest complex were not included into the SLD calculations of the polymer layer.

Interestingly, the total membrane thicknesses in PBS measured by NR was higher than that obtained from QCM-D measurements, with 19.37 ± 0.02 nm for the copolymer membrane and 18.46 ± 0.02 nm for the hybrid membrane (Figure 1A+B). Note that when measured by NR, the hybrid membrane is 0.9 nm thinner than the copolymer membrane, while the hybrid membrane is 1.5 nm thicker than the copolymer membrane when measured by QCM-D. When comparing the hydrophobic blocks lengths only, the difference between both membranes is much smaller. In the copolymer membrane, the PDMS has a block length of 15.86 ± 0.01 nm but only 15.43 ± 0.01 nm in the hybrid membrane, resulting in a difference of 0.42 ± 0.01 nm. This shows that the hydrophilic blocks have a relatively high impact on the total thickness difference in NR, with 1.76 ± 0.01 nm and 1.51 ± 0.01 nm per block for the copolymer and hybrid membranes, respectively. For a $PMOXA_7$ block in stretched conformation in water, we expect a length of max. 2.58 nm. Therefore, the measured PMOXA block lengths are in a reasonable range, and it is likely that PMOXA is not completely stretched but rather in a coiled conformation causing intertangling with neighboring strands.

Modelling the fitted reflectivity data to the expected copolymer membrane structure in contact with the water bulk phase yielded the profile of the SLD across the interface. This was converted into the corresponding profile of the VFs (Figure 1C+D) of the different components, which is split up into VF_{water} and the VF of the polymer blocks. VF_{PDMS} is equivalent to the surface coverage of the membrane, since for a defect-free membrane we expect $VF_{PDMS} = 1$ (corresponding to a surface coverage of 100%) and $VF_{water} = 0$, and vice versa for a bare substrate.[2] The initial surface coverages (in PBS) were 69% and 82% for copolymer and hybrid membrane, respectively. After adding the guest of the Rs capsule ($[Ru(BPY)_3]^{2+}$), NR 4 contrast characterization was repeated with the Rs-containing hybrid membrane and the copolymer membrane (control) (Figure 1A+B). Addition of the guest had similar relative effects on thickness: a decrease in PDMS thickness (-7% for the copolymer and -5% for the hybrid membrane),

an increase in PMOXA thickness (+21% for the copolymer and +5% for the hybrid membrane), and a decrease in total thickness (-2% for the copolymer and -3% for the hybrid membrane). The relative changes in VF_{PDMS} were however different: -6% for the copolymer membrane and +7% for the hybrid membrane (Figure 1C+D). The different evolution of VF_{PDMS} after addition of guest suggests that in the Rs-containing membrane, the guest is taken up into the PDMS layer, as indicated by the increase in the volume fraction of the hydrophobic layer and the simultaneous decrease in VF_{water}. In case of the copolymer membrane, where no Rs is present, no increase in hydrophobic volume is observed. Conversely, the opposite is true, i.e. the hydrophobic volume decreases and a simultaneous increase of the water content in the layer is detected, potentially as consequence of the osmotic changes or unspecific interactions between guest and membrane. Due to the presence of C_4-Rs in the hybrid membrane, the observed effect is compatible with capsule self-assembly and guest encapsulation in the PDMS layer. The increase of the PDMS volume and the simultaneous, partial displacement of bulk water is coherent with the scenario in which the uptake of guest into the PDMS layer happens through guest encapsulation by C_4-Rs and concomitant migration of C_4-Rs from the hydrophilic-hydrophobic interface to the hydrophobic PDMS layer. Even though the guest or the C_4-Rs monomer and capsule were not detected directly, using this indirect approach we were able to infer the presence of C_4-Rs in the hybrid membrane as well as their ability to encapsulate a guest, and thus to assemble inside the hydrophobic PDMS layer.

The lower initial surface coverage of the copolymer membrane (69%) compared to the hybrid membrane (82%) can be explained by the different polymer concentrations used for their deposition (1 g L^{-1} for copolymer membrane and 2 g L^{-1} for the hybrid membrane). During the optimization of the deposition protocol for the changed setup, we observed a polymer concentration-dependent surface coverage for the copolymer membrane, yielding a surface coverage of 87% for a concentration of 2 g L^{-1}. This confirms our findings by QCM-D of highly covered SSPMs with similar coverage for copolymer and hybrid membrane. The 2D detector images of the D_2O measurements show the presence of off-specular scattering alongside the specular reflectivity signal which arises from in-plane inhomogeneities in the SLD of the monolayer. This is observed when there are μm-sized D_2O-filled regions in the monolayer which is consistent with the non-homogeneous distribution of water in the monolayer. The off-specular signal did indeed become weaker with increasing coverages in agreement with the proposed origin of the signal. Some more optimization is required in order to obtain defect-free block copolymer membranes with coverages reaching 100% as observed for lipid membranes.[3]

References

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(2) Parra-Ortiz, E.; Malekkhaiat Haffner, S.; Saerbeck, T.; Skoda, M. W. A.; Browning, K. L.; Malmsten, M. Oxidation of Polyunsaturated Lipid Membranes by Photocatalytic Titanium Dioxide Nanoparticles: Role of pH and Salinity. *ACS Appl Mater Interfaces* **2020**, *12* (29), 32446-32460. DOI: 10.1021/acsami.0c08642.

(3) Michalak, D. J.; Losche, M.; Hoogerheide, D. P. Charge Effects Provide Angstrom-Level Control of Lipid Bilayer Morphology on Titanium Dioxide Surfaces. *Langmuir* **2021**, *37* (13), 3970-3981. DOI: 10.1021/acs.langmuir.1c00214.

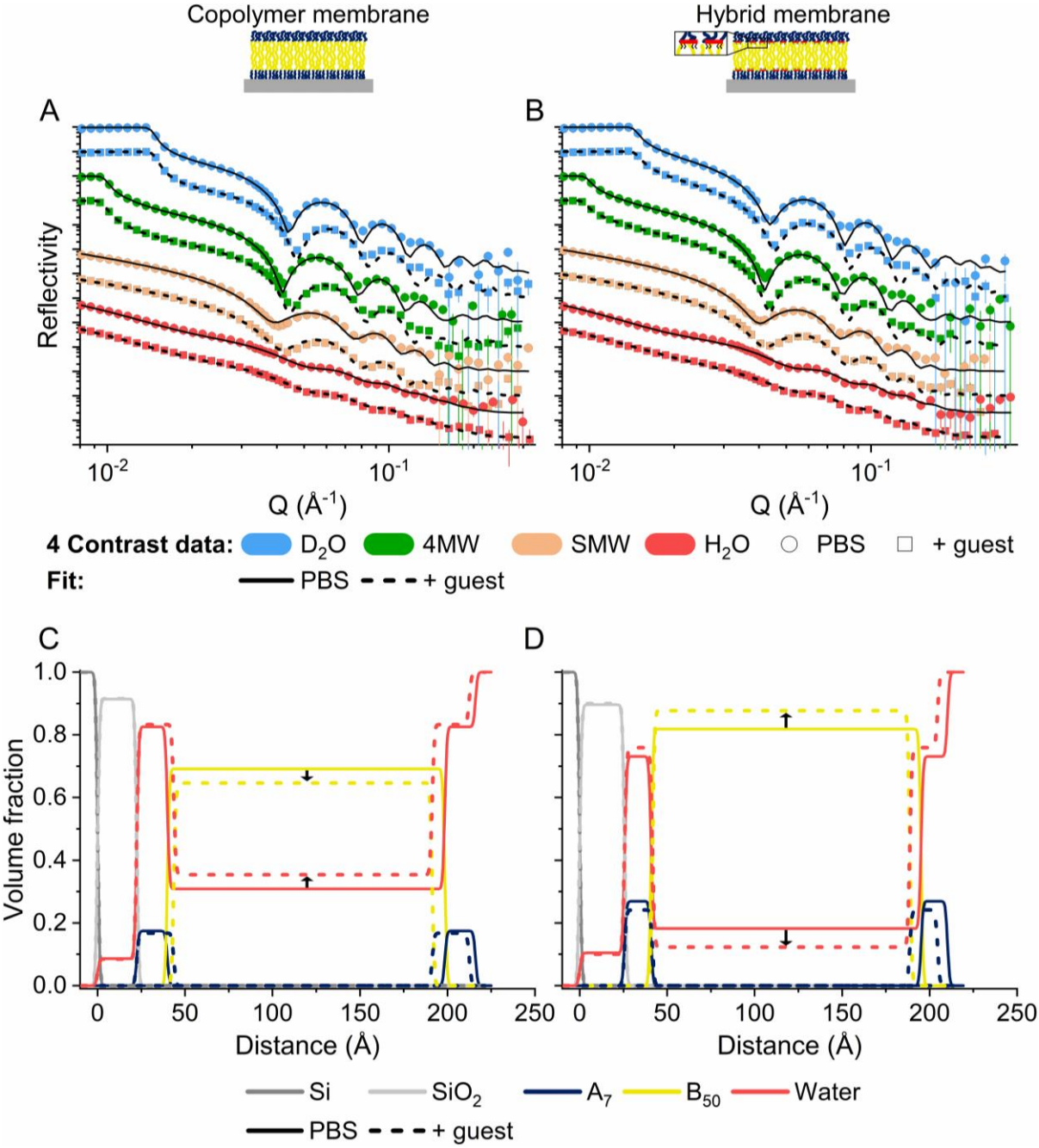

Figure 1. Characterization of membranes by NR comparing them in PBS (no guest) and after addition of guest. **A+B**: Reflectivity data of membranes measured in PBS at 4 contrasts of D_2O/H_2O (in log-log scale). For better visibility, an offset of 10 was used between two datasets, starting from D_2O at a reflectivity of 10^0 for lowest Q. **C+D**: Volume fractions (VF) resulting from modelling of reflectivity data. Hydrophilic block ($PMOXA_7$): A_7, hydrophobic block ($PDMS_{50}$): B_{50}. Arrows indicate the change of the VF_{PDMS} together with $VF_{Water\ (PDMS)}$ after addition of guest. **A+C**: Copolymer membrane. **B+D**: Hybrid membrane. Membranes were investigated in PBS. Guest for Rs capsule ($[Ru(BPY)_3]Cl_2$) was added in excess (3 mM in PBS). The opposing effect of guest addition on the hydrophobic volume in copolymer and hybrid membrane confirms the guest uptake into the PDMS layer of the hybrid membrane by encapsulation.