Determination of hydration of the hydrophobic layer of the artificial macromolecular ion channels by neutron scattering

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Ion channels are proteins responsible for functions such as muscle contraction and apoptosis by selectively allowing the passage of specific ions. Dysfunction of ion channels is known to cause various diseases. Recently, artificial ion channels have gained attention as potential therapeutic agents for channelopathies or as anticancer drugs (Roy *et al*., ChemBioChem, 2021). Meanwhile, We have discovered that amphiphilic polymers with poly(propylene oxide) (PPO) as the hydrophobic chain form polymer vesicles with high molecular permeability in aqueous solutions. Furthermore, in hybrid vesicles incorporating these polymers into phospholipid liposomes, the incorporated polymers function as artificial molecular channels (*Adv. Mater.*, 2017; *JACS*, 2020). PPO, being a polyether with high affinity for cations, suggests that polymers with PPO as the hydrophobic chain could function as effective artificial ion channels. Therefore, we developed an amphiphilic block copolymer with oligo(tri(ethylene glycol) glutamate, known for its high ion affinity, as the hydrophilic chain and PPO as the hydrophobic chain. We then examined the ion permeability of the vesicles formed by this polymer. The results showed that, unlike DOPC liposomes, which have very low permeability to Na^+ and K^+ ions, the resulting polymer vesicles rapidly permeated these ions. Additionally, in hybrid vesicles incorporating this polymer into phospholipid liposomes, ion permeation was also observed, indicating their potential as artificial polymer ion channels.

Natural ion channels have water molecules within the permeation pathway, facilitating smooth ion passage through ligand exchange with oxygen atoms. Our previous research using neutron scattering revealed that the PPO hydrophobic layer contains water (*Macromolecules*, 2020). We believe this water and the ether oxygen atoms in PPO create an environment favorable for ion permeation, leading to the observed high permeability. However, the optimal water content needed to further improve ion permeability and selectivity is still unknown, making the rational design of artificial polymer ion channels challenging.

In this study, we evaluated the water content of the bilayer hydrophobic region of vesicles composed of oligo(tri(ethylene glycol) glutamate)-*block*-PPO and polymers with varying hydrophobic chain compositions using neutron scattering. The SANS profile of the vesicles is shown in Fig. 1. Analysis of the obtained curves using a bilayer cross-sectional model revealed that the SLD of PPO, which forms the hydrophobic core, was significantly higher than the theoretical SLD value. This suggests the presence of heavy water within the hydrophobic region. Assuming that the hydrophobic region consists only of PPO and heavy water, the calculated water content was approximately 25%.

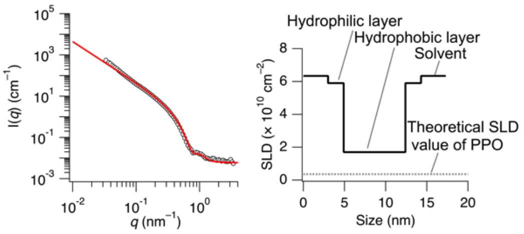

Fig. 1. (left) SANS profile of the polymer vesicles in D_2O at 25 ºC (circles) and the theoretical curves obtained using the cross-sectional bilayer membrane moldes (red line). (right) SLD profle of the polymer vesicle membranes (solid line) and the theoretical SLD value of poly(propylene oxide) (dotted line).