Effect of Calcium Adsorption on the Properties of Phospholipid Bilayers

Small-angle X-ray scattering (SAXS), UV-Vis absorption and refractive-index detection are integrated to probe the compositional and conformational responses of membranes in nanodiscs after calcium binding.

Calcium ions (Ca^{2+}) play important roles in many cellular processes. As a messenger, Ca^{2+} regulates diverse signaling processes through interactions with membrane proteins or directly with cellular membranes.[1] Up to the present, the corresponding mechanisms of these Ca^{2+}-involved biological processes await resolution. Especially, how membranes respond to direct Ca^{2+} adsorption remains elusive. Part of the difficulty arises from the lack of a well defined membrane platform and no single tool sensitive enough to elucidate the small structural and compositional changes in membranes after calcium adsorption. Phospholipid vesicle bilayers were previously commonly adopted as model membranes for studies of calcium-membrane interactions, but the instability of vesicle membranes towards environmental stimulations and high polydispersity of size and curvature resulted in scattered or inconsistent experimental results.

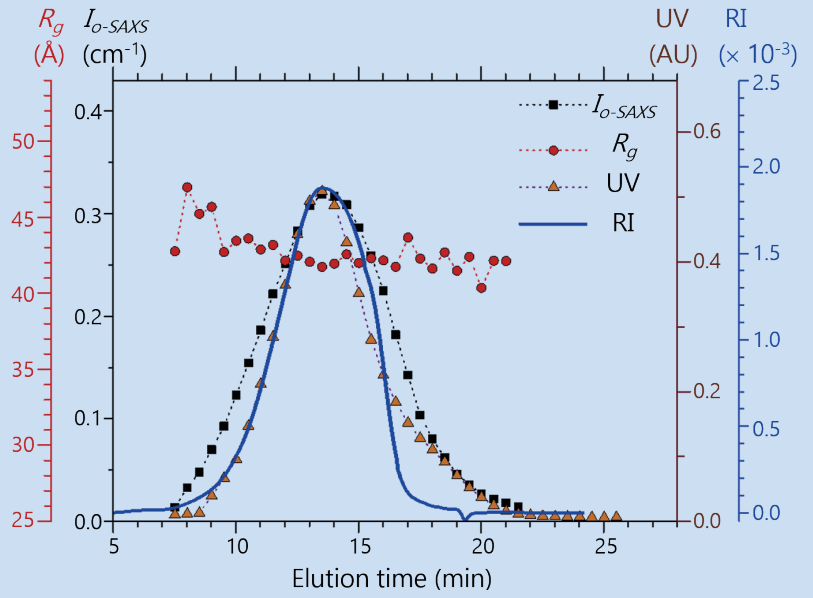

Fig. 1: Profiles of I_{UV}, I_{RI}, $I_{o\text{-}SAXS}$ and R_g extracted from the HPLC/SAXS/UV-Vis/RI data of a DMPC nanodisc with $CaCl_2$ (25 mM). The three sets of data were measured along one sample elution path at 13 °C. The UV absorption signal at 280 nm and the SAXS data were measured at the same sample position. The RI profile was measured at a later sample position, but was corrected to retrieve the peak profile at the sample position of I_{UV} and $I_{o\text{-}SAXS}$ *via* a calibration measurement with cytochrome c. [Reproduced from Ref. 3]

The rapidly increasing nanodisc technique provides stable and highly monodisperse model membranes for structural and functional characterizations of membrane proteins.[2] Nanodiscs are disc-like phospholipid bilayers surrounded with membrane scaffold protein (MSP) belts; they consist of a lipid bilayer of 100–300 phospholipids depending on the lipid type and the sequence of amphipathic MSP. The planar lipid bilayers in nanodiscs are more stable and monodisperse in size than conventional vesicle bilayers, allowing an observation of the structural changes with decreased complexity in structural analysis. The research team led by U-Ser Jeng (NSRRC) and Tsyr-Yan Yu (Academia Sinica) used MSP1D1(-)- dimyristoylphosphatidylcholine (DMPC) nanodiscs as a model membrane system and developed a unique approach to probe quantitatively the membrane compositional and structural changes upon calcium adsorption at **TLS 23A1**.

Specifically, high-performance liquid chromatography (HPLC), SAXS, UV-Vis absorption and differential refractive index (HPLC/SAXS/UV-Vis/RI) were integrated to provide full-scope measurements for nanodiscs in calcium solution.[3] **Figure 1** shows the profiles of $I_{o\text{-}SAXS}$, I_{UV} and I_{RI} measured over one HPLC sample elution of the DMPC nanodiscs with $CaCl_2$ (25 mM). Also shown is the profile of the radius of gyration (R_g) extracted from corresponding SAXS data, demonstrating a highly monodisperse $R_g = 43.9 \pm 0.1$ Å. From the peak values of the three overlapped profiles of $I_{o\text{-}SAXS}$, I_{UV} and I_{RI}, N_l (number of lipids per nanodisc) = 178 and N_c (number of absorbed Ca^{2+}) = 18.8 were deduced independently of a model. This result yields a substantial calcium-lipid binding with ratio χ_b (= N_c/N_l) 0.106. The authors found also that χ_b increases with the calcium concentration, and saturates to χ_b = 0.12 at $CaCl_2$ (75 mM), corresponding to a calcium:lipid binding ratio 1:8 for the Ca^{2+}-nanodisc complex.

To unveil the corresponding structural changes in the nanodisc upon the significant Ca^{2+} binding observed, Jeng's group analyzed the SAXS scattering profiles shown in **Fig. 2(a)** using an elliptical cylinder model (**Fig. 2(b)**) to fit the SAXS data. In this model, the phospholipid bilayer is represented with a stack of elliptical cylinders, with the core cylinder of the bilayer lipid chains sandwiched by the top and bottom slabs of the lipid heads, and laterally surrounded with a shell of MSP. As shown in **Fig. 2(a)**, all features of the SAXS data for the case $CaCl_2$ (25 mM) were adequately accounted with this model. The authors observed systematically increased SLD of the lipid head zone, ρ_H, through increased calcium adsorption into the phospholipid head region as the $CaCl_2$ concentration increased. This effect is accompanied with a swelling of the bilayer lipid chain zone (d_C altered from 27.5 to 28.0 Å). The charge interactions between bound calcium ions and the zwitterionic lipid heads inside the nanodisc also slightly contracted the area per lipid A_l, leading to more stretched acyl chains with stronger van der Waals interactions for tighter chain packing. The observed decreased roughness σ of the phospholipid bilayer is likely a consequence of the enhanced lipid-chain packing.

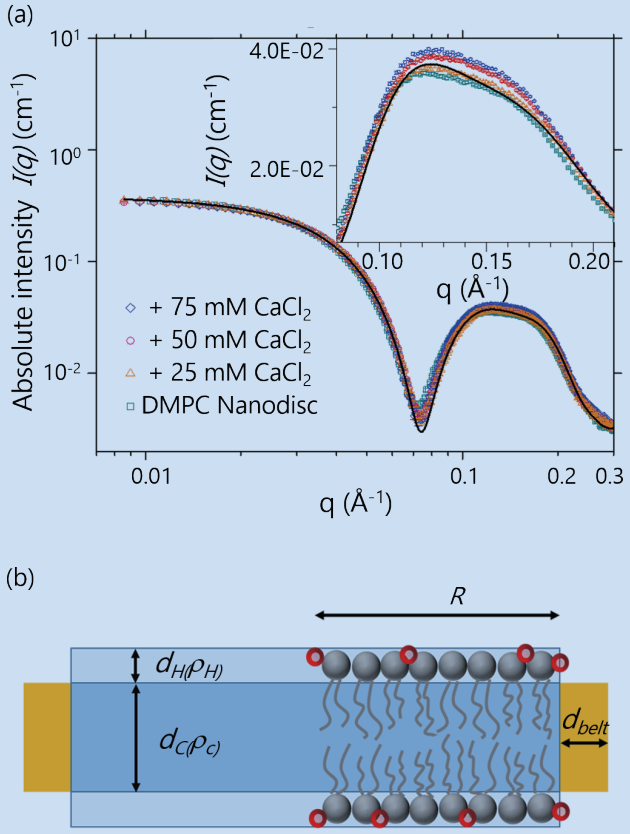

Fig. 2: (a) SAXS data measured for a DMPC nanodisc in solutions of indicated $CaCl_2$ concentrations at 13 °C. Selectively shown is the fitted curve (black) for the data with $CaCl_2$ (25 mM) using the model shown in (b). Inset: enlarged details of the broad hump zone. (b) A core-shell cylinder model for SAXS fitting of the Ca^{2+}-DMPC nanodisc complex, with core radius R, core cylinder height d_C (scattering length density ρ_C) for the bilayer hydrophobic alkyl chains, and the top and bottom discs of the same slab thickness d_H and scattering length density ρ_H for the hydrophilic lipid head-group zone. The shell cylinder of width d_{belt} represents the two MSP that stabilize the nanodisc. Small red circles are bound calcium ions. [Reproduced from Ref. 3]

In summary, Jeng's group developed an integrated analytical scheme to reveal the compositional and structural changes of the DMPC nanodisc developed by Yu's group after calcium binding based on integrated measurements of HPLC-SAXS/UV-Vis/RI. The results reveal the detailed structure of the nanodisc and the substantial calcium-lipid binding ratio that leads to delicate nanodisc charging and swelling. The substantial calcium-lipid binding ratio and leaflet potential of the Ca^{2+}-nanodisc complex support a possible signaling path via direct calcium-membrane interactions. The developed method would greatly promote the use of highly monodisperse and stable nanodiscs as a promising membrane platform to study structural changes and composition reconstitution of membranes upon incorporation of membrane proteins or small ions. (Reported by Orion Shih)

This report features the collaborative work of U-Ser Jeng's and Tsyr-Yan Yu's groups published in J. Phys. Chem. Lett. ***9****, 4287 (2018).*

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- SAXS
- Structural Biology, Life Science

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