Experimental report

13/04/2016

Proposal: **9-13-571** **Council:** 10/2014

Title: Probing Dynamics in Lipid Membranes with Photosensitive Cholesterol Derivatives

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: POPC/azobenzene-cholesterol/D2O

Instrument	Requested days	Allocated days	From	To
IN5	7	6	01/07/2015	07/07/2015
D16	1	1	06/07/2015	07/07/2015

Abstract:

The dynamics of lipid molecules in a membrane comprises several modes like rotations, individual and collective fluctuations, and also lateral diffusion. These depend on the thermodynamic state of the two-dimensional membrane as a confinement and exhibit their peculiar time window. We propose a project on a model active membrane consisting of a well-known basic lipid matrix (1-palmitoyl-2-oleoyl-phosphatidylcholine, POPC) hosting a lipoid effector. The effector is a cholesterol derivative - azobenzene-cholesterol - inserted into the matrix. Its hydrophobic part resembles cholesterol but it carries a photosensitive azobenzene group that in the polar region of the host bilayer. Upon light excitation, the reversible trans-cis transition of this active group exerts a momentum within the headgroup region. This disturbance is propagated into the core region, modulating the structure and the dynamics within the lipid layer. The membrane equilibrates into either of two end states, depending on the trans- or cis conformation of the effector. Here we seek to provide experimental evidence on the detailed modified dynamical properties of the membrane due to the presence of the active compound.

Experimental Report 9-13-571, IN5 and D16

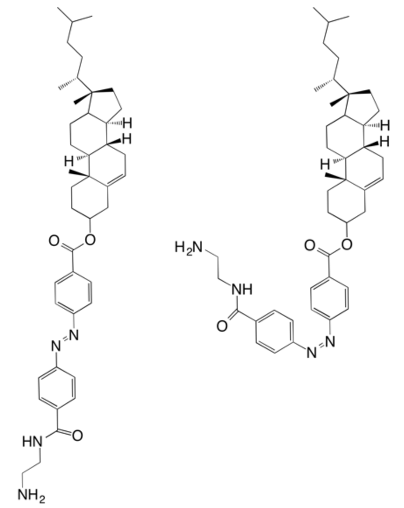
date: 1st ~ 7th, July, 2015

Proposers: J. Pieper, B. Klösgen, C. Shen, J. Peters

This experiment was done as a joint project, using the instruments IN5 and D16, and in parallel with the experiment CRG-2232 on IN13, to collect data on a set of photosensitive model membranes.

Scientific background

Cholesterol (chol) is a passive molecular effector on membrane structure, and hence on fluidity and elasticity [1-4]. Its chemically modified variety azobenzene-cholesterol (azo-chol, Fig.1, a-b) is active since it exhibits a reversible *trans*-*cis* transition of the headgroup upon illumination with proper wavelength (365nm: *trans* to *cis*; 455nm: *cis* to *trans*). The half time of the spontaneous relaxation of the excited *cis*- state is about 11 days at room temperature [5], far beyond the average measurement time on IN5 and D16 (see experimental methods below). When embedded within a lipid membrane, its conformational change will switch the host membrane between two distinct structural states [6] and hence modify also its dynamics. In the experiments reported on, lipid dynamics was studied with azo-chol, present within stacked and oriented 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) host lipid membranes, in either *trans*- or *cis*-state, at IN5 and IN13. The target time scale of the dynamics on IN5 was 5~50ps. The lamellar structures of the bilayer samples were characterized in parallel experiments at D16.



(a) *trans* (b) *cis*

Fig. 1: Structure formulae of the two states of the photoactive azo-chol.

Materials and Methods

Hydrogenated bilayer-stack samples containing ~100mg lipid and ~100mg D_2O were deposited onto sapphire substrates (0001-plane). Samples A and B consisted of pure POPC and POPC with 5mol% chol as controls, respectively; sample C contained POPC with 5mol% azo-chol. Sample C was illuminated with 365nm light to convert all azo-chol molecules into their *cis*-state (state C1). State T, with all azo-chol molecules in their *trans*-state, was obtained by another illumination of 455nm light on C1. State C2, with all azo-chol molecules back in their *cis*-state, was switched from T by an illumination of 365nm light. Samples were studied immediately after their illumination. No single measurement took more than 4 hours.

The lamellar structures of the bilayer samples at various temperatures (310, 300, 290, 280, 270 and 265K) were investigated on D16 using the neutron wavelength λ=4.51Å. Scattering signals were collected in the angle range [+2^o, +21^o] horizontally (x) and [-10^o, +10^o] vertically (y) with respect to the incident beam direction, corresponding to $0.05Å^{-1} < Q_x < 0.5Å^{-1}$ and $-0.25Å^{-1} < Q_y < 0.25Å^{-1}$. The membrane stacks were oriented at 90^o with respect to the incident beam direction. Raw data were corrected by the empty instrument background and empty cell contributions, and then radially integrated. Data reduction was carried out by using the software LAMP.

Quasielastic neutron scattering experiments (QENS) were carried out on IN5 with λ=5Å and 10Å, corresponding to energy resolutions (δE, FWHM) of ~ 100μeV and ~ 13μeV, respectively. Samples were measured at 310K and 265K while oriented at η=135^o and at 45^o with respect to the incident beam direction at each temperature. A vanadium sample (25mm·50mm·1mm, provided from IN5) was measured at 295K as reference. Raw data were reduced by the standard IN5 reduction routine, then further corrected by the empty cell spectra and normalized to the vanadium spectra, also using the software LAMP.

Preliminary Results

Diffractograms from the measurements on D16 at 310K and at 265K are shown in Fig. 2. All curves exhibit diffraction peaks between 0.05Å^{-1} and 0.1Å^{-1}. The curves shown in Fig. 2 were normalized to the maximum of their main peaks. At 265K the maximum of this peak is found around Q = 0.095Å^{-1}. The peak is shifted towards lower Q values with increasing temperature.

On IN5, the scattered intensity was measured as function of both momentum transfer Q and energy transfer ΔE. Fig. 3 shows this intensity $I_Q(Q)$ summed over -3meV<ΔE<+2meV from selected samples (sample C (C1) at 265K and at 310K, and vanadium). All three curves were measured at 135^o orientation with λ=5Å. Vanadium as an incoherent scatterer shows an almost constant intensity while the intensity from the bilayer stacks at 310K is only constant below Q≈1.8Å^{-1}. At 265K, Bragg peaks are present at 1.4Å^{-1} <Q< 1.8Å^{-1}. These peaks are probably related to ice structures [7, 8].

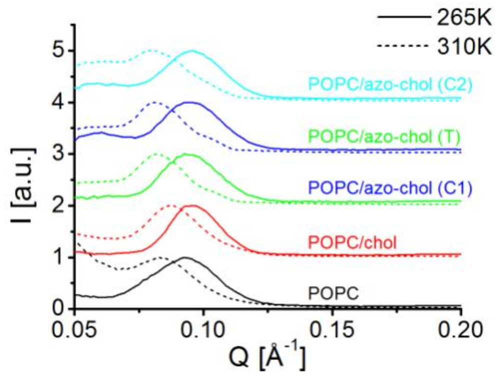


Fig. 2: Integrated diffraction curves I(Q) as measured on D16, taken at 310K and at 265K.

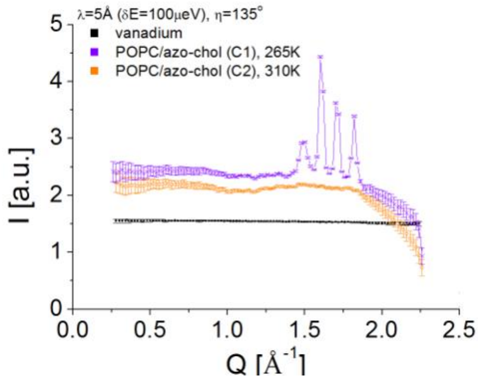
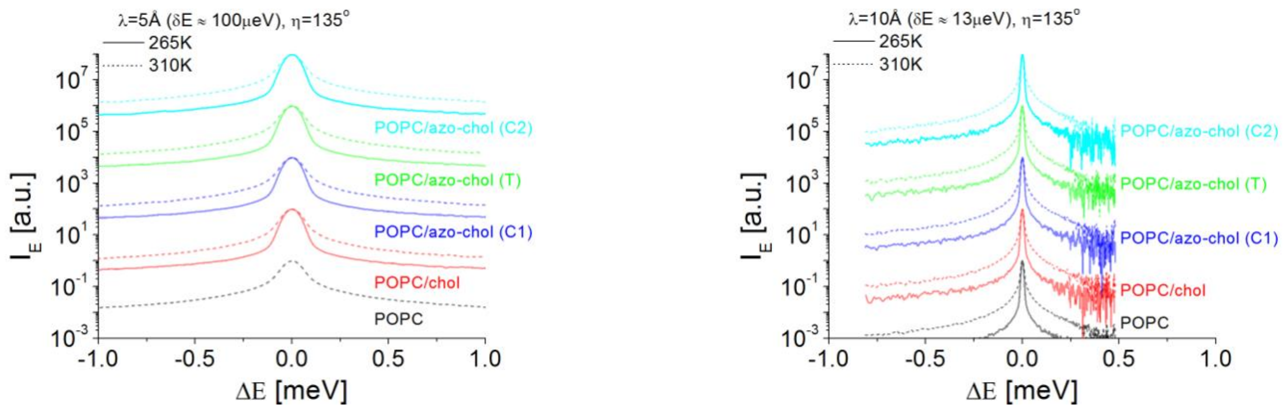


Fig. 3: Diffractograms from selected measurements on IN5. The properly corrected vanadium signal is entered for illustration.

Fig. 4 shows the QENS spectra $I_E(\Delta E)$, as obtained by summing over the whole Q-range (excluding the Bragg peak range, see Fig. 3), for λ=5Å (a) and 10Å (b). Both plots arise from measurements at 135^o orientation. All spectra were normalized to their maximum at ΔE=0meV. The QENS spectra of all samples exhibit a quasielastic-broadening. The dynamics within the samples is reflected in quasielastic broadening. The data indicate a dependency on sample state,as depending on illumination and temperature.

Detailed analysis of both experiments is going on.



(a) (b)

Fig. 4: QENS spectra measured on IN5. (a) λ=5Å; (b) λ=10Å.

References

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