

# Cooperative long range protein-protein dynamics in Purple Membrane

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We present experimental evidence for a cooperative long range protein-protein interaction in the purple membrane (PM). Dynamics has been quantified by determining the spectrum of the acoustic phonons in the 2d Bacteriorhodopsin (BR) protein lattice. Data were compared to an analytical model. The effective spring constant for the collective interaction between protein trimers was determined to be  $k = 53.49$  N/m. The finding might be relevant for protein function in PM.

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The high protein concentration in biological membranes might lead to long-range protein-protein interactions, on which there have been speculations, already some time ago [1]. Motions in proteins occur on various length and time scales [2, 3], and the functional behavior of membrane proteins is likely to depend on the lipid bilayer composition and physical properties, such as hy-

drophobic thickness and elastic moduli. How the variety of inter- and intra-protein motions, occurring over different time and length scales, interact to result in a functioning biological system remains an open field for those working at the interface of physics and biology. The dynamical coupling between proteins, i.e., *cooperative protein dynamics*, might be important for the understanding of macromolecular function in a cellular context because it might lead to an effective inter-protein communication. Here, we report collective inter-protein excitations in a biological membrane, the purple membrane (PM), investigated by coherent inelastic neutron scattering. The results allowed a quantification of the effective coupling constant.

Purple Membrane (PM) occurs naturally in the form of a two-dimensional crystal, consisting of 75% (wt/wt) of a single protein, Bacteriorhodopsin (BR), that functions as a light-activated proton pump, and 25% various lipid species (mostly phospho- and glyco-lipids) [4]. BR is a proton transporting membrane protein, formed of seven trans-membrane  $\alpha$ -helices arranged around the photosensitive retinal molecule. The protein in the lipid matrix is organized in trimers that form a highly ordered 2d hexagonal lattice with lattice parameter  $a \approx 62$  Å, as depicted in Fig. 1 (c). The structure of PM is well established by electron microscopy, neutron and x-ray diffraction experiments reviewed in [4, 5, 6, 7, 8, 9]. Here we present the unprecedented determination of collective protein-protein dynamics, i.e., acoustic phonons, of the 2d protein lattice in PM using inelastic neutron scattering.

The experiments were performed on the IN12 cold-triple-axis spectrometer at the Institut Laue Langevin (Grenoble, France). IN12 turned out to be highly suited for elastic and inelastic investigations in oriented biological samples because of its flexibility, good energy resolution and extremely low background. It allows the

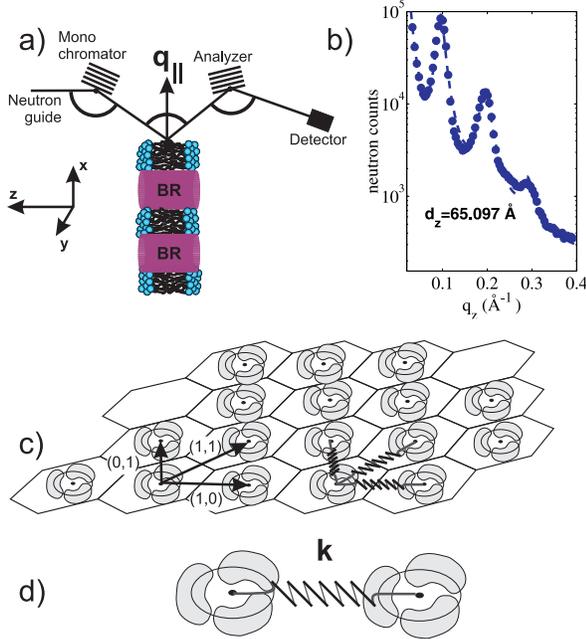


FIG. 1: (Color online). (a) Sketch of the triple-axis scattering geometry.  $q_{\parallel}$  is the in-plane component of the scattering vector  $\vec{Q}$ . (b) Reflectivity curve measured at  $T = 30^{\circ}\text{C}$ . The dotted line is a fit of Lorentzian peak profiles including a  $q^{-4}$  term. (c) BR trimers are arranged on a hexagonal lattice of lattice constant  $a \approx 62$  Å. (d) The interaction between the protein trimers is depicted as springs with effective spring constant  $k$ .

measurement of diffraction and inelastic scattering in the same run without changing the set-up, which is crucial to assign dynamical modes to structural properties and molecular components. IN12 was equipped with its vacuum box to avoid air scattering at small scattering angles and with vertically focusing monochromator and analyzer to increase the neutron flux at the sample position. There was no horizontal focusing, but the beam was collimated to 40' – monochromator – 30' – sample – 30' – analyzer – 60' – detector. All scans were done in W-configuration with fixed  $k_f = 1.25 \text{ \AA}^{-1}$  resulting in a  $q$  resolution of  $\Delta q = 0.005 \text{ \AA}^{-1}$  and an energy resolution of  $\Delta\omega = 25 \text{ \mu eV}$ . The use of a spectrometer to measure diffraction (at energy transfer  $\Delta\omega = 0$ ) has a particular advantage when working with (partially) deuterated biological samples because the common elements C, O, N, and D still have non-zero contributions to the incoherent scattering cross section. As the downstream analyzer cuts out the (within the instrumental resolution) elastically scattered neutrons, the quasielastic contribution to the background arising from incoherent scattering is reduced and the signal to noise ratio drastically improved.

Deuterated PM was produced and hydrated by  $\text{H}_2\text{O}$  in order to suppress the contribution of the membrane water to the phonon spectrum in inelastic coherent neutron scattering experiments and to then obtain information on the collective membrane dynamics. Because of the minuteness of the coherent inelastically scattered signals, the preparation of appropriate samples and experimental set-ups is challenging in this type of experiments. We used a completely deuterated PM to enhance the collective protein–protein excitations over other contributions to the inelastic scattering cross section. 200 mgs of deuterated PM suspended in  $\text{H}_2\text{O}$  was centrifuged down and the obtained pellet spread onto a  $40 \times 30$  mm aluminum sample holder. This was then partially dried to 0.5 g water per gram of membrane over silica gel in a desiccator. The PM patches naturally align along the surface of the sample holder as they dry. The silica gel was then replaced by water, and the sample left to hydrate to a lamellar spacing of  $d_z = 65 \text{ \AA}$  at 303 K (30°C).

A sketch of the scattering geometry is shown in Fig. 1(a). The experiments were carried out on PM stacks, i.e., PM samples with a regular repeat distance  $d_z$ . The mosaicity of the sample (the distribution of normal vectors with respect to the substrate) was checked by rocking scans to about 17 degs (not shown). The sample was aligned by centering the first reflectivity Bragg peak at  $q_z \approx 0.1 \text{ \AA}^{-1}$ . For the inelastic scans,  $\vec{q}$  was then put in the plane of the membranes ( $q_{\parallel}$ ). Figure 1(b) shows a reflectivity curve measured at  $T = 30^\circ\text{C}$ . From the three well developed Bragg peaks, the lamellar spacing  $d_z$  is determined to be  $d_z = 65.1 \text{ \AA}$ . A lamellar spacing of  $65 \text{ \AA}$  corresponds to an average inter-membrane water layer of  $16 \text{ \AA}$ , since the thickness of a dry PM fragment is  $49 \text{ \AA}$ .

The in-plane diffraction pattern of the 2d hexago-

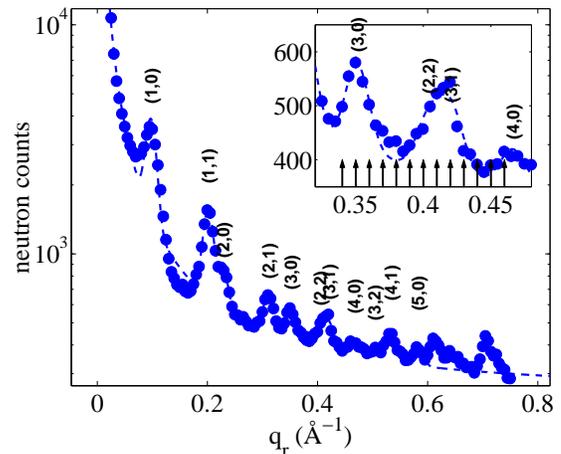


FIG. 2: (Color online). Diffraction pattern of the 2d protein lattice from  $q_{\parallel} = 0$  to  $0.75 \text{ \AA}^{-1}$ . The inset shows the third Brillouin zone in magnification. The arrows mark the positions of inelastic scans at constant  $q_{\parallel}$  values.

nal protein lattice has been measured and is shown in Fig. 2. Although the normal vectors of the membranes are well aligned with respect to the substrate, the  $(x, y)$ -orientation of the membrane layers is statistical and the signal a superposition of the different domains (powder average). All reflections can be indexed by a hexagonal unit cell with a lattice parameter of  $61.78 \pm 0.73 \text{ \AA}$ . Correlations and motions in membranes are often well separated in reciprocal space because of the largely different length and time scales involved. The prominent distances in PM, such as lipid–lipid and BR–BR monomers and trimers for instance lead to spatially well separated signals. The same holds for the different time scales involved from the picosecond (molecular reorientations) to the nano- or microsecond (membrane undulations, large protein motions). The use of oriented samples further allows to separate correlations in the plane of the membranes, and perpendicular to the bilayers. Dynamics between different protein trimers should be dominant where the 2d BR diffraction pattern occurs. Because elastic and inelastic scattering at small momentum transfers was dominated by the rather strong (1,0) and (1,1) reflections, systematic inelastic scans were taken at  $q_{\parallel}$ -values in the third Brillouin zone of the 2d pattern, between  $0.34 \text{ \AA}^{-1}$  and  $0.46 \text{ \AA}^{-1}$ , to determine the in-plane excitation spectrum of the 2d protein lattice. The inset in Fig. 2 shows the Bragg peaks in the third Brillouin zone in magnification and marks the positions of inelastic scans at constant  $q_{\parallel}$  values. Figure 3 shows an example of a constant- $q_{\parallel}$  scan at  $q_{\parallel} = 0.39 \text{ \AA}^{-1}$ . The total signal consists of a Gaussian central peak due to instrumental resolution, quasielastic broadening, which is described by a Lorentzian peak shape, and a pair of excitations at about  $\omega_0 = \pm 0.7 \text{ meV}$ , which were fitted using a damped harmonic oscillator. Because of the pronounced and sym-

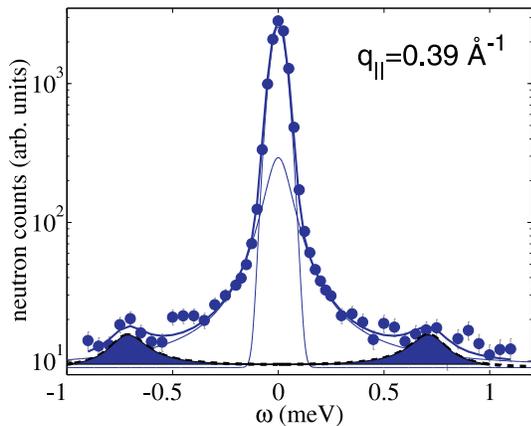


FIG. 3: (Color online). Energy scan at  $q_{||} = 0.39 \text{ \AA}^{-1}$ .

metric inelastic signals, we are sure that the observed peaks are not spurious effects. The quasielastic signal most likely contains contributions from coherent and incoherent scattering, i.e., from auto- and pair-correlation functions.

Propagating modes are experimentally difficult to observe in the scattering function  $S(q, \omega)$  as they often show up as a small peak or a soft shoulder in the tails of the central mode and quasielastic broadening, rather than as a clear excitation maximum. In contrast, in the spectrum defined by  $C_l(q, \omega) = (\omega^2/q^2)S(q, \omega)$ , the multiplication by  $\omega^2$  suppresses the central mode and quasielastic scattering and makes the excitations (sound modes) more easily visible for analysis. Systematic inelastic scans for  $q_{||}$  values between  $0.34 \text{ \AA}^{-1}$  and  $0.46 \text{ \AA}^{-1}$  are shown in Figure 4(a). As discussed above, the figure shows a  $(q_{||}, \omega)$  map of  $C_l(q, \omega)$  to emphasize the propagating modes. The corresponding Bragg reflections are also marked in the figure.

In order to interpret the data we analytically modeled the excitation spectrum of the 2d protein lattice. In a very simple approach, the protein trimers were taken as the centers of a primitive hexagonal lattice with lattice constant  $a = 62 \text{ \AA}$ , and the acoustic phonon spectrum was calculated. The model is depicted in Fig. 1(c). The basic hexagonal translations are marked by arrows. The interaction between the protein trimers is contained in springs with an effective (longitudinal) spring constant  $k$  (Fig. 1(d)).

The result for  $C_l(q, \omega)$  is shown in Fig. 4(b). The statistical average leads to a superposition of the two phonon branches. Phonon energies were scaled to match the experiment. Because the proteins trimers were treated as points with an effective mass of  $M_{tr}$ , the calculation does not include any contributions from intra-protein or intra-trimer dynamics, i.e., possible optical phonons. Figure 4(c) overlays experiment and theory. Note that in the experiment, a pronounced excitation is mainly pro-

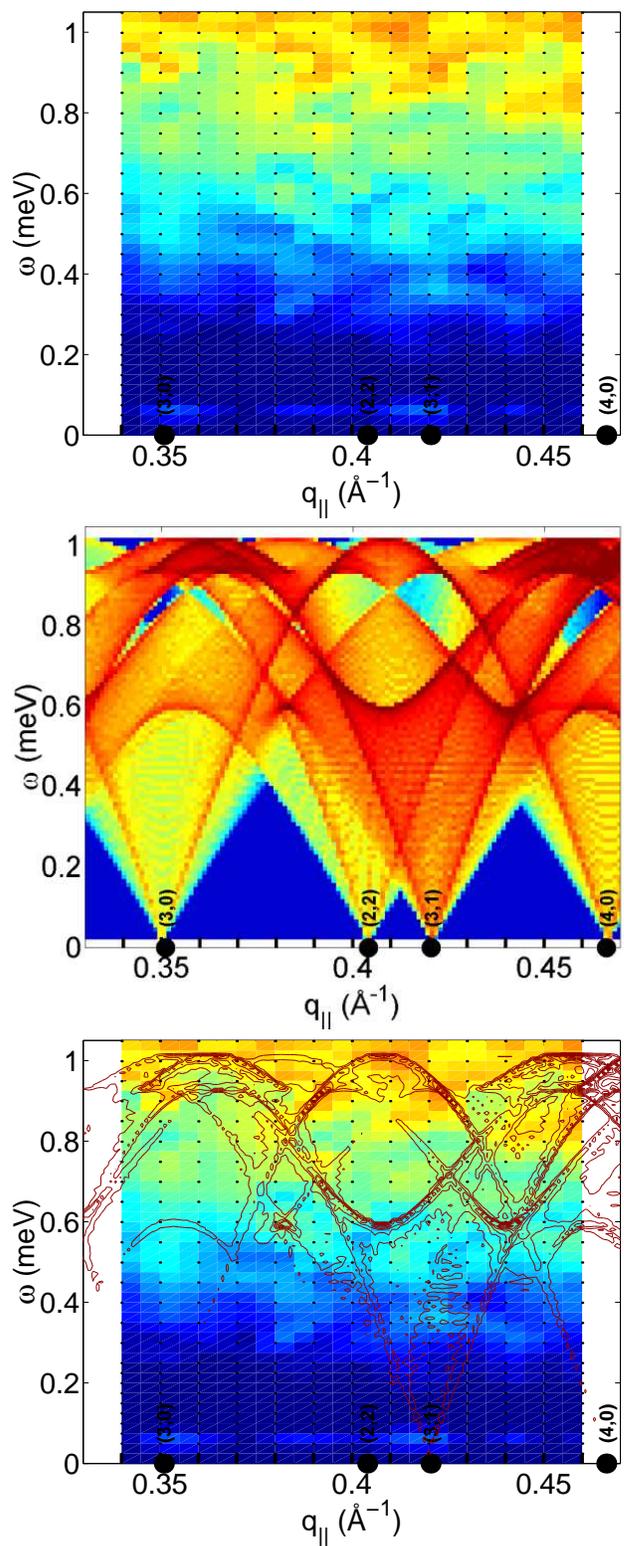


FIG. 4: (Color online). (a)  $C_l(q, \omega)$  map of the third Brillouin zone. (b) Theoretical excitation spectrum  $C_l(q, \omega)$ . (c) Overlay of experiment and calculations.

duced when the scan direction (along  $\omega$ ) is perpendicular to the gradient of the corresponding phonon branch. The data therefore emphasize flat regions in the phonon branches and (anti)crossings of different branches. While it is difficult to assign phonon branches in the raw data in Fig. 4(a) by eye without an underlying model, data and calculation in Fig. 4(c) show a remarkable agreement. So does the scan in Fig. 3 perfectly reproduce the high-intensity feature in the calculations at  $q_{\parallel} = 0.39 \text{ \AA}^{-1}$  and  $\hbar\omega = 0.7 \text{ meV}$ . Note that in contrast to the calculations there are basically no phonon branches visible in the experiment at energies below about 0.3 meV, firstly because they can not be well resolved in the experiment because of their rather steep slope. Secondly, these excitations should show up as shoulders of the central peak, only, rather than as pronounced inelastic peaks.

The commonly assumed interaction mechanism between inclusions in membranes is a lipid-mediated interaction due to local distortions of the lipid bilayer [10, 11, 12, 13, 14], with a strong dependence on the bilayer properties, in particular elastic properties. The PM might, however, be a special case because there are very few lipids between neighboring BR proteins [15]. While the nature of the interaction still will be mainly elastic, it is not likely to be purely lipid-mediated but for the most part a direct protein-protein interaction. The strength of the interaction can be determined from the data in Fig. 4. The energy of the zone-boundary phonon at the M-point of the hexagonal Brillouin zone (for instance at a  $q_{\parallel}$  value of  $0.35 \text{ \AA}^{-1}$ ) relates to the coupling constant by  $M_{tr}\omega^2 = 6k$ . Because this energy is determined as  $\hbar\omega = 1.02 \text{ meV}$ , the effective protein-protein spring constant  $k$  is calculated to  $k = 53.49 \text{ N/m}$  [19]. On the microscopic level, displacing the BR trimer by 1 Å yields a force between neighboring trimers of 5.3 nN. There is therefore strong protein-protein communication in PM. Using the same approach, the spring constant for graphite for comparison is calculated to 27,000 N/m for the in-plane interaction, and 3.5 N/m for out-of-plane interactions. The force constant that we measure in PM thus is 1-2 orders of magnitude larger than the effective van-der-Waals force constant in graphite, but 2-3 orders of magnitude weaker than a C-C bond. The mechanism might be relevant to model the photocycle in PM where the BR proteins undergo small structural changes [16, 17], which then propagate to neighboring proteins by the coupling  $k$ . Because the energy of the excitation is about 1 meV  $\approx 12 \text{ K}$ , this process is highly populated at room temperature (96%) and might therefore be highly relevant for biological function.

In conclusion we present experimental evidence for a cooperative long range protein-protein interaction in purple membrane. The effective spring constant for the interaction between protein trimers can be determined from the acoustic phonon branches as  $k = 53.49 \text{ N/m}$ . The finding might be relevant for protein function,

i.e., for the photocycle of BR proteins, where the BR monomers undergo small structural changes. Future experiments will address cooperative intra-protein-trimer and also intra-monomer dynamics. To make a clear relationship to protein function, protein dynamics of activated proteins, i.e., proteins undergoing the photocycle will be studied.

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weighs  $m_{tr} = 1.34 \cdot 10^{-22}$  kg