Experimental report

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Proposal: 8-04-734 **Council:** 10/2014

Title: Molecular Dynamics of Cyanobacterial Thylakoid Membranes

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Cyanobacterial Thylakoid Membranes

Instrument	Requested days	Allocated days	From	To
D16	1	1	07/07/2015	08/07/2015
IN6	6	4	02/07/2015	06/07/2015

Abstract:

Protein dynamics due to structural fluctuations of small molecular subgroups on the picosecond time scale has been shown to play an important role in physiological processes in nature. Prominent examples for such dynamics-function correlations include proton transfer in bacteriorhodopsin of halobacterium salinarum, ligand binding to myoglobin, and photosynthetic electron transfer in plant photosystem II. In the case of photosystem II membrane fragments of green plants, quasielastic neutron scattering (QENS) has established that the onset of diffusive molecular motions at ~240 K, and at a relative humidity of ~45% is strictly correlated with the temperature- and hydration-dependent electron transfer efficiency from an electron donor referred to as QA to a transiently bound acceptor molecule named QB. Such a characteristic dependence of a functional process on temperature and hydration may indicate a crucial role of molecular dynamics in the underlying molecular mechanism. We propose to investigate the dynamics of cyanobacterial thylakoid membranes by QENS experiments on IN6 in order to search for a correlation between internal dynamics and function.

Molecular dynamics of photosynthetic PSII membrane in solution

Proposal No.: 8 – 04 – 734

Experimental team:
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Instruments: IN6 (02.07.2015 – 06.07.2015); D16 (08.07. - 09.07.2015)

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The sample used in the present study is the oxygen-evolving membrane fragments from the grana stacks of plant thylakoids dissolved in the buffer solution containing D_2O. The proper hydration level of the PSII membrane samples was verified by independent neutron diffraction experiments on D16. QENS experiments were carried out with an incident neutron wavelength of 5.12 Å corresponding to a Q-range of 0.4 – 2.0 Å^{-1}. The elastic energy resolution (FWHM) was determined by standard vanadium runs and equalled 88 μeV (Q = 1.0 Å^{-1}). The temperature was varied from 50 K to 300 K for both the buffer and the sample in the buffer solution. To ensure a proper correction of temperature dependent QENS spectra, empty cell runs were recorded for several representative T-values in the studied range and interpolated for the other intermediate temperatures. The buffer and protein contributions have been separated in a way similar to what was presented in ref. [1]. Owing to the sucrose content (137 mg/ml) in the buffer solution, the transmission of the latter amounted to 0.826. As a result, the transmission of the membrane stacks in solution was equal to 0.790 and the contribution of multiply scattered neutrons was not negligible. Moreover, after the procedure of buffer subtraction the effect of multiple scattering is even more pronounced than for the individual spectra of the buffer and the sample (Fig. 1). Thus, multiple scattering correction has to be carried out for a more sophisticated analysis.

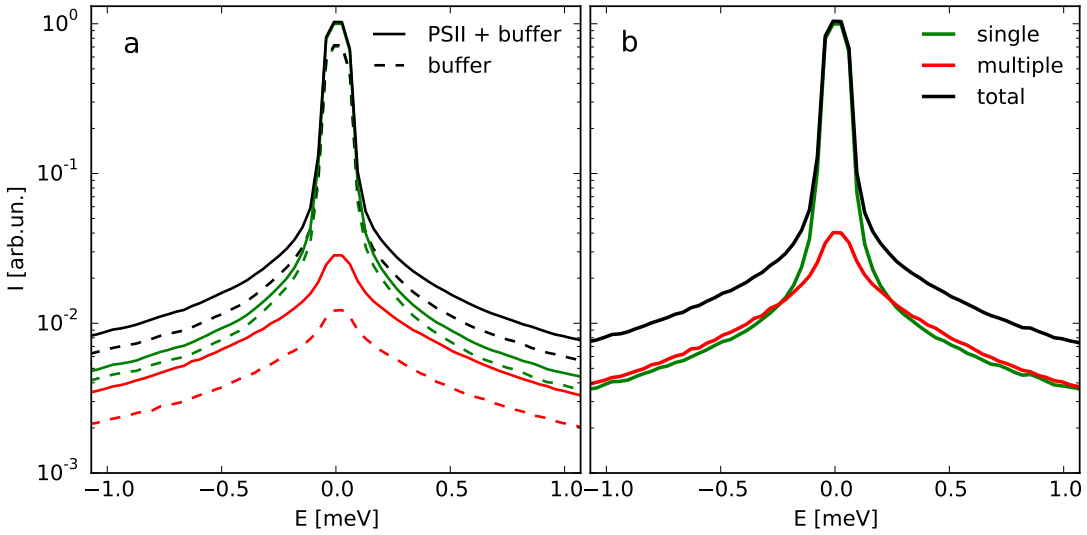

Fig. 1. McStas [2] simulation results. **Panel a**: The contributions of single (green) and multiply (red) scattered neutrons have been calculated and presented with the total (black) QENS spectra of the protein complex in the buffer solution and the buffer sample. **Panel b:** The difference spectrum of the membrane fragments with the single and multiple components.

The theoretical scattering function used to fit the difference spectra above the freezing point of the buffer is the convolution of $S_{\mathrm{i}}(Q, E)$ describing internal protein dynamics like

rotation or libration of subgroups, $S_{\mathrm{g}}(Q,E)$ related to the global motion of the protein, and $S_{\mathrm{v}}(Q,E)$ accounting for fast vibrational motions [3].

$$S_1(Q,E) \cong S_{\mathrm{v}}(Q,E) \otimes S_{\mathrm{g}}(Q,E) \otimes S_{\mathrm{i}}(Q,E) = \\ = \exp(-\langle u_{\mathrm{v}}^2\rangle Q^2) \left[\frac{A(Q)}{\pi} \frac{\Gamma_{\mathrm{g}}(Q)}{\Gamma_{\mathrm{g}}(Q)^2 + E^2} + \frac{1-A(Q)}{\pi} \frac{\Gamma_{\mathrm{g}}(Q) + \Gamma_{\mathrm{i}}(Q)}{(\Gamma_{\mathrm{g}}(Q) + \Gamma_{\mathrm{i}}(Q))^2 + E^2} \right] \quad (1)$$

where Γ_{g} and Γ_{i} are the linewidths of the Lorenzian functions describing the global and internal dynamics, respectively; $A(Q)$ is the elastic incoherent structure factor; $\langle u_{\mathrm{v}}^2\rangle$ is the mean square displacement of vibrational motions. The results of the fits at 300 K are presented in Fig. 2, left panel. Both $\Gamma_{\mathrm{g}}(Q)$ and $\Gamma_{\mathrm{i}}(Q)$ exhibit a non-linear dependence on Q^2 and approach definite non-zero values as small Q. In the case of $\Gamma_{\mathrm{i}}(Q)$ this is an indication of a motion in confinement, whereas the little value of $\Gamma_{\mathrm{g}}(Q)$ at $Q \to 0$ can be a result of resolution limitations ($\Gamma_{\mathrm{g}}(Q) \ll \mathrm{HWHM}_{\mathrm{res}}$) and multiple scattering effects.

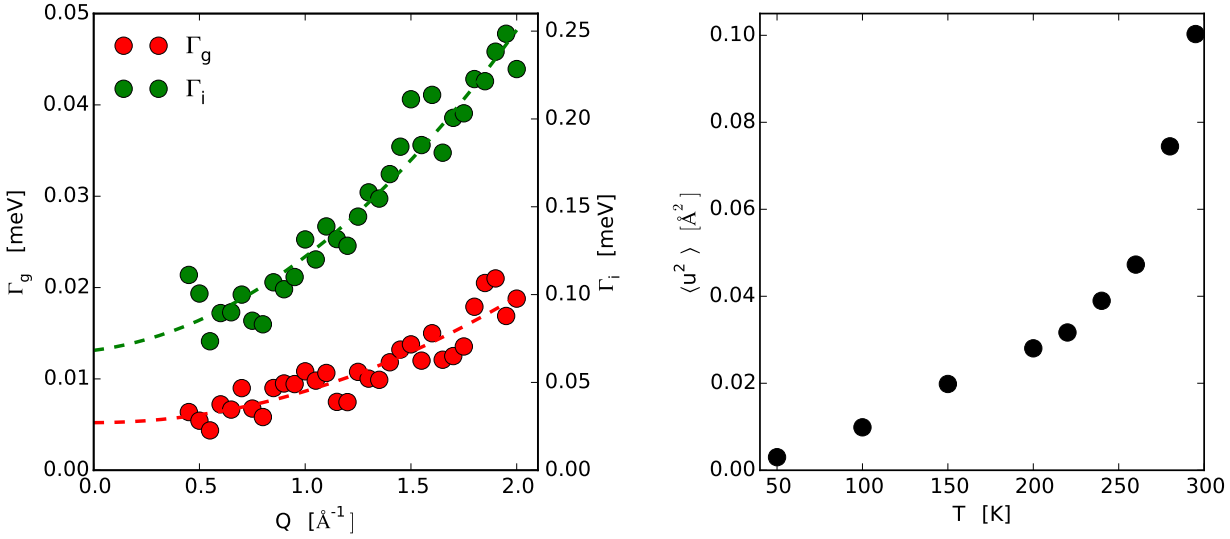

Fig. 2. Left panel: Linewidths (HWHM) of the Lorentzian contributions in eq 1 characterizing slower global dynamics (red) and faster internal motions (green). **Right panel:** Temperature dependence of mean square displacement (eq 3)

At $T < 280$ K the global motions in the protein are essentially suppressed ($\Gamma_{\mathrm{g}}(Q) \to 0$), so that the fit model (eq 1) is reduced to the following expression:

$$S_2(Q,E) = \exp(-\langle u_{\mathrm{v}}^2\rangle Q^2) \left[A(Q)\delta(E) + \frac{1-A(Q)}{\pi} \frac{\Gamma_{\mathrm{i}}(Q)}{\Gamma_{\mathrm{i}}(Q)^2 + E^2} \right] \quad (2)$$

The Q-dependence of Γ_{i} at 260 K $< T <$ 280 K is similar to what presented in Fig. 2, although internal dynamics consistently slow down. With the further temperature decrease it becomes impossible to resolve this type of motion because of $A(Q) \to 1$. Therefore, this temperature range was characterized in terms of the mean square displacement.

$$\langle u^2\rangle = \langle u_{\mathrm{v}}^2\rangle - \ln A(Q)/Q^2 \quad (3)$$

The temperature dependence of $\langle u^2\rangle$ (Figure 2,right panel) is proportional to T up to 220 K, which is typical for harmonic vibrational motions. The deviation of this linear behaviour above 220 K is the onset of internal dynamics on picosecond time scale.

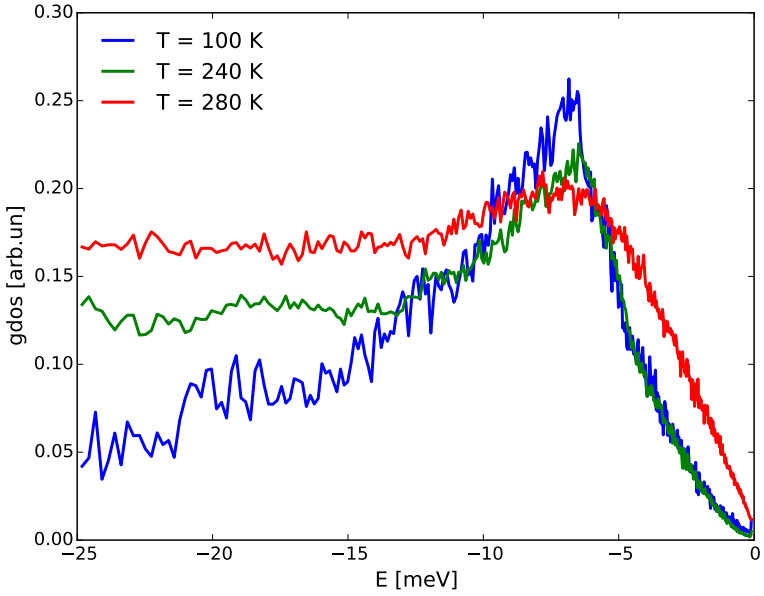

Fig. 3. Generalized density of states (GDOS) at selected temperatures

The generalized density of states (GDOS) was calculated from the inelastic part of the spectra and is displayed in Fig. 3. The broad plateau observed at $E > 10$ meV originates from the protein, because it cannot be removed by varying the buffer content in the mixture. On the contrary, the maximum at 6.8 meV is present in the pure buffer spectra as well. However, in order to simultaneously remove this feature from all the spectra, it is necessary to assume that the buffer mass contribution is different for different temperatures.

To sum up, the further QENS studies on dynamics of proteins in solution require a careful procedure of buffer subtraction and multiple scattering correction.

[1] Pieper, J.; Renger, G. *Photosynthesis Research* **102** (2009) 281–293
[2] **McStas** – a neutron ray-trace simulation package. `http://mcstas.org/`
[3] Stadler, A. M.; van Eijck, L.; Demmel, F.; Artmann, G. *Journal of the Royal Society, Interface* **8** (2011) 590–600