# DYNAMICS OF LYSOZYME UNDER PRESSURE SEEN BY MOLECULAR SIMULATION AND NEUTRON SCATTERING

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## 1. Motivation

The motivation of the study presented here is to investigate how hydrostatic pressure influences the internal dynamics of proteins below the denaturation threshold. Combining molecular dynamics simulations and quasielastic neutron scattering, we study the internal dynamics of lysozyme in solution for pressures between 1 atm and 3 kbar. Both techniques give access to the internal dynamics of condensed matter on the nanosecond time scale and length scales between 1 Å and 100 Å. The analysis of the simulations yields valuable complementary information to the experimental data which helps to interpret the latter and to develop models for the internal dynamics of proteins.

## 2. Simulation

We performed Molecular Dynamics simulations of one single lysozyme molecule in a solvent of 3403 water molecules, yielding a total number of 12169 atoms in the simulation box. In order to mimic the experimental conditions of constant temperature and pressure, all simulations have been performed in the thermodynamic *NpT* ensemble [1,2] using an integration time step of 1 *fs* and applying periodic boundary conditions. We used the AMBER94 potential [3] to describe the interactions in the system. To be able to compute purely quasielastic neutron scattering spectra, global protein motions have been subtracted from the trajectories, which have been saved for further analysis. The length of the production runs varied between 1 and 1.2 nanoseconds, yielding a frequency resolution of $\Delta f \approx 5.10^8$ Hz ≅ 2.06 μeV. The molecular dynamics simulations and the analyses have been performed with the programs MMTK and nMoldyn, respectively [4,5].

## 3. Experiments

The experiments described in the following have been performed on the time-of-flight spectrometer IN5 of the Institut Laue-Langevin in Grenoble [6]. The experiments were performed on a lysozyme solution of 60 mg/ml, using a deuterated acetate buffer (50 mM and pH 4.6). We used a pressure cell made of a titane-zirconium alloy whose scattering is purely incoherent. The experiments were performed at pressures of 1 atm and 3 kbar, with a resolution of 8 μeV (HWHM). The preparation of the samples and the data analysis is briefly described in [6]. and a more detailed description will be published in a forthcoming article [7]. In contrast to the simulations, the experimental spectra contain contributions from internal protein motions *and* fro

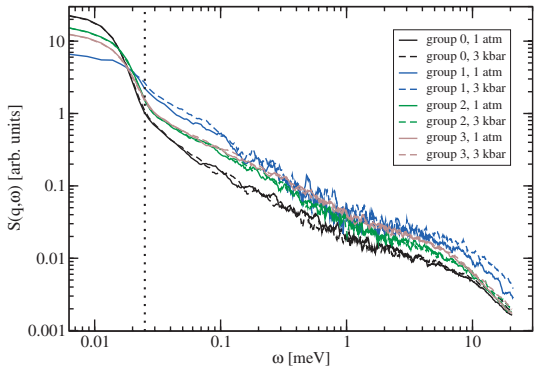


Figure 1. Experimental QENS spectra for lysozyme in solution from IN5. The broken line indicates the range of the contribution due to global translational protein motions.

## 4. Results

We found that the experimental quasielastic spectra and their simulated counterparts do not show a significant change upon the application of pressures up to 3kbar (see Fig.1). The detector groups 0,1,2,3 correspond, respectively, to $q_{el}$ = 3.9, 6.7, 9.7, 11.9 $nm^{-1}$ A zoom on the experimental structure factor for the extreme pressures of 1 bar and 3 kbar at small energy transfers is shown in Fig.2. One recognises that the intensity of the quasielastic is slightly increased at 3 kbar. To understand the result we write the measured dynamic structure factor as a convolution product: $S_{meas}(q,\omega) = R(\omega) \otimes S_{cm}(q,\omega) \otimes S_{int}(q, \omega)$. Here $S_{cm}(q, \omega)$ and $S_{int}(q, \omega)$ are, respectively, the dynamic structure factors describing translational diffusion and internal motions in the protein, and $R(\omega)$ is the resolution function of the instrument. One supposes that internal and global protein motions are uncorrelated.It is worthwhile noting that rotational diffusion can be neglected onthe time scale of the spectrometers we used. The dynamic structure describing internal motions splits into an elastic part

and a contribution which describes diffusive and vibrational motions,

$$S_{\text{int}}(q,\omega) + EISF(q)\delta(\omega) + S'_{\text{int}}(q,\omega) \quad (1)$$

Here EISF(q) is the elastic incoherent structure factor Consequently, $S_{meas}(q, \omega)$ takes the form

$$S_{meas}(q,\omega) = R(\omega) \otimes S_{cm}(q,\omega) \otimes (EISF(q)\delta(\omega) + S'(q,\omega)) \quad (2)$$

The inset of Fig. 2 shows the normalised EISF obtained from the simulations described above at $p$ = 1 atm and $p$ = 3 kbar. At high pressure the EISF decreases less rapidly with $q$, which indicates that the atomic fluctuations are reduced. This effect can explain qualitatively the slight increase of $S_{meas}(q,\omega = 0)$. A more detailed study, accounting for a narrowing of $S_{cm}(q,\omega)$ due to a reduction of the protein diffusion constant in water under pressure

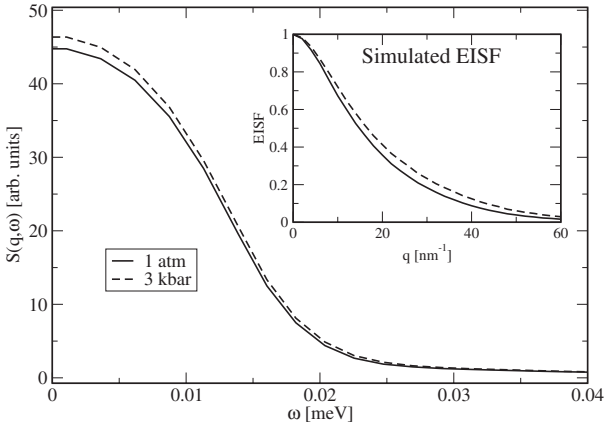


Figure 2. Comparison of $S(q,\omega)$ for $p$ = 1 atm and $p$ = 3 kbar at small energy transfers and $q$ = 4 nm$^{-1}$. The inset shows the *simulated* normalized EISF at the same pressures.

To investigate the influence of pressure on the internal dynamics of lysozyme and the influence on the position fluctuations, we analysed the frequency spectrum of the average velocity autocorrelation function (density of states) of the hydrogen atoms. The latter yield the dominant contribution to the neutron scattering spectra. The DOS is related to the mean-square position fluctuations

$$\left\langle \mathbf{u}^2 \right\rangle(\omega_c) = \lim_{t\to\omega} \frac{6}{\pi} \int_0^{\omega_c} d\omega \frac{1-\cos\omega t}{\omega^2} g_{vv}(\omega) \quad (3)$$

One recognises that the low frequency part of the DOS contributes most to the position fluctuation. Fig. 3 pressure has an impact on the low frequency part of the DOS (inset), which leads in turn to a reduction of the position fluctuations and confirms the effect seen in the EISF and in the experimental spectra. Pressure has thus an impact on the internal dynamics and the position fluctuations of lysozyme, but the effect is not pronounced in the quasielastic

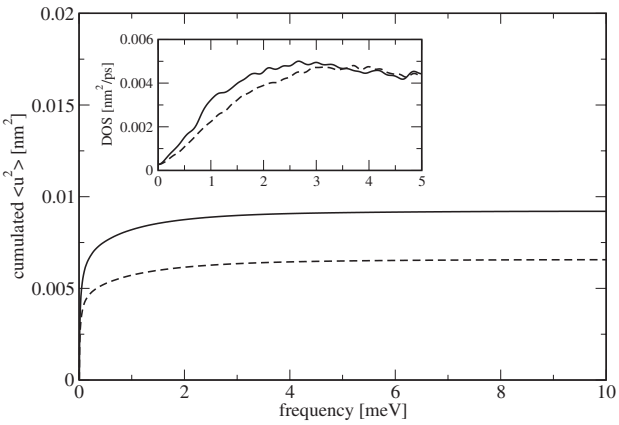


Figure 3. Cumulated position fluctuation as a function of frequency and corresponding density of states (inset).

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