

Supplementary Information

Osmolytes Modify Protein Dynamics and Function of Tetrameric Lactate Dehydrogenase upon Pressurization

Samy R. Al-Ayoubi,^a Paul Hendrik Schummel,^a Aline Cisse,^{b,c} Tilo Seydel,^b Judith Peters,^{b,c} and Roland Winter^{a*}

^aPhysical Chemistry I – Biophysical Chemistry, Faculty of Chemistry and Chemical Biology, TU Dortmund University, Otto-Hahn-Str. 4a, 44227 Dortmund, Germany

^bInstitut Laue Langevin, F-38042 Grenoble Cedex 9, France

^cUniversity Grenoble Alpes, LiPhy, F-38000 Grenoble, France

Additional figures

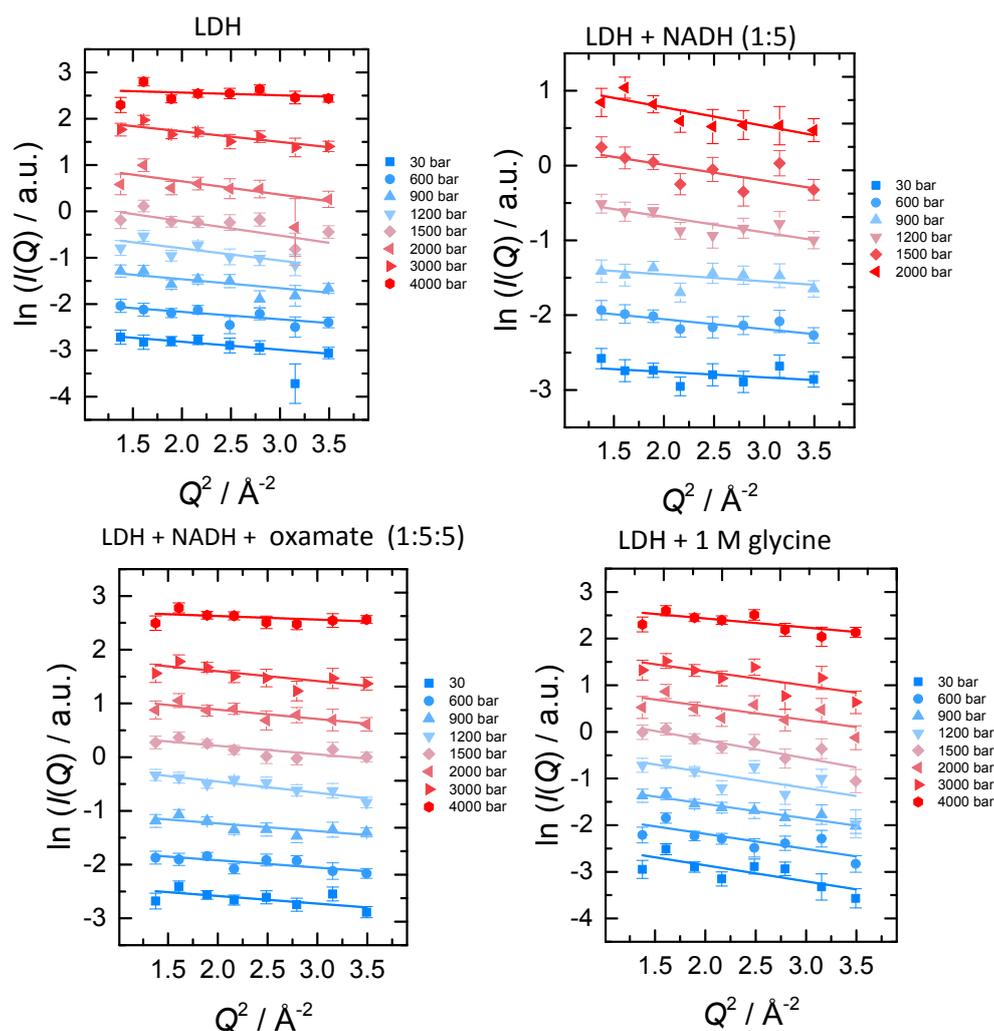


Fig. SI 1. EINS data plotted as $\ln I$ vs. Q^2 in the range from $Q^2 = 1.3 \text{ \AA}^{-2}$ to 3.5 \AA^{-2} at various pressure points (25 °C).

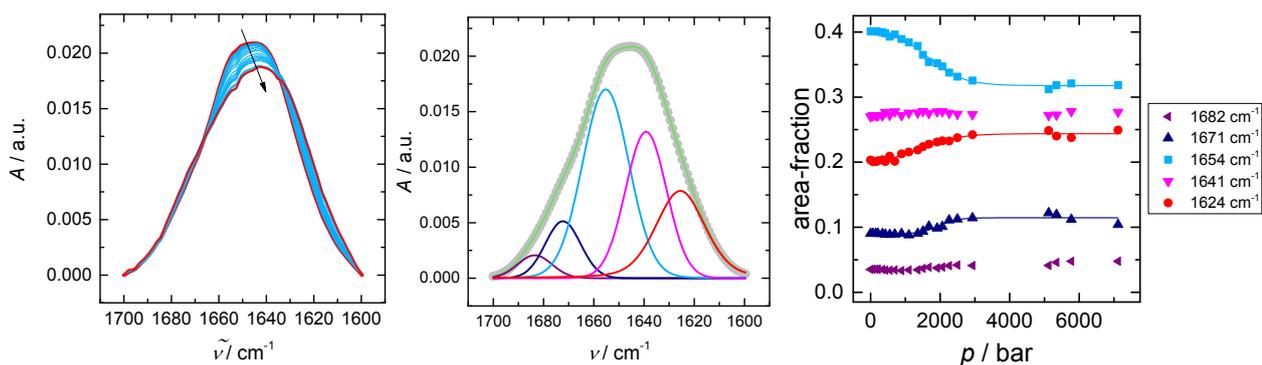


Figure SI 2. (A) Pressure dependence of the amide I' band of LDH measured by FTIR spectroscopy. (B) Amide I' band of LADH in comparison with the fitted spectrum (green line) using the underlying subbands at positions noted on the right-hand side. (C) Pressure dependent development of the area fractions of the subbands shown in (B).

Additional quasi-elastic incoherent neutron scattering results

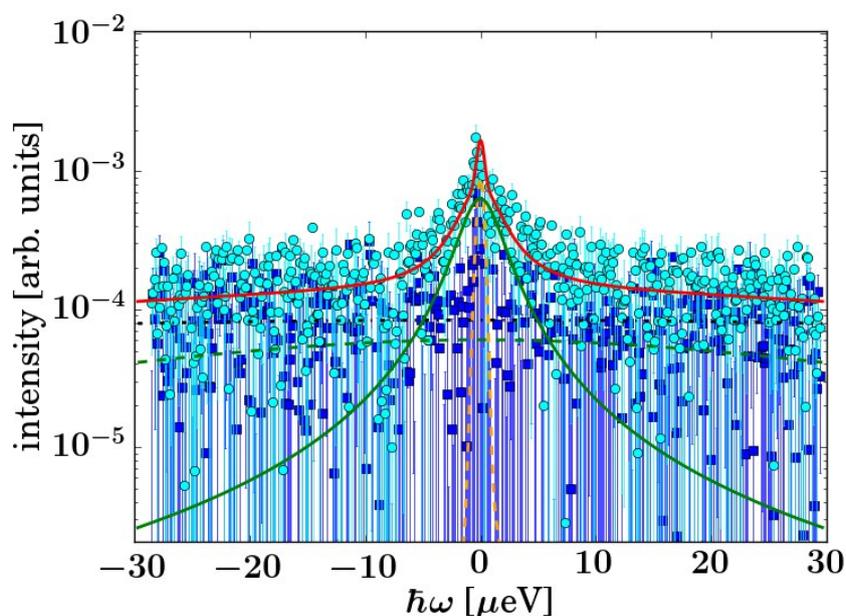


Figure SI 3. Example of a fit of the corrected neutron scattering intensity data of LDH on a semi-logarithmic scale as a function of energy transfer. The blue squares are the solvent measurements at 20 bar, and the cyan points are the sample measurements at 20 bar, for $Q = 0.945 \text{ \AA}^{-1}$. The data were binned (2 points per bin) for better visualization, but the fits were performed on non-binned data. The dashed dotted black line represents the fit of the solvent contribution, $L(\gamma_{\text{D}_2\text{O}}, \omega)$. The continuous green line corresponds to the global diffusion fit of $L(\gamma, \omega)$, and the dashed green line to the internal diffusion one, $L(\gamma + \Gamma, \omega)$. The dotted orange line is the additional elastic contribution $\delta(\omega)$. The final overall fit is represented by the continuous red line.

Figure SI 3 presents an example of a fit of a quasi-elastic spectrum at $Q = 0.945 \text{ \AA}^{-1}$ being part of the global fit of all Q -values simultaneously. The goodness of fit, χ^2 , is below 0.5 for all pressure values.

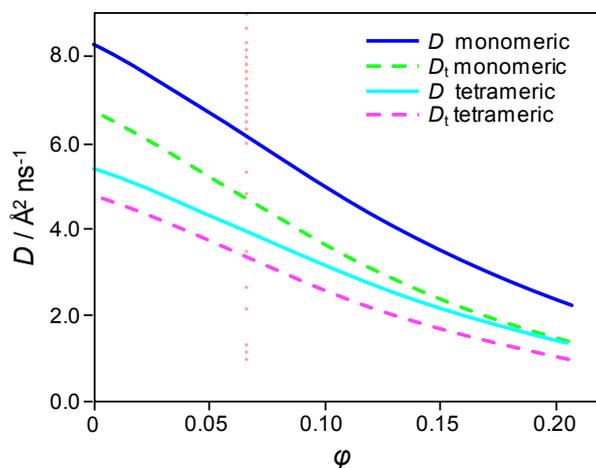


Figure SI 4. Translational diffusion coefficients D_t and experimentally observable diffusion coefficients $D = D(D_r, D_t)$ of LDH calculated for the monomeric and tetrameric state as a function of the dry volume fraction ϕ . The dilute-limit values ($\phi = 0$) have been obtained using HYDROPRO. The ϕ -dependence was calculated by applying colloid hard-sphere models as outlined in Roosen-Runge et al., PNAS 108 (2011) 11815-11820, and $D = D(D_r, D_t)$ was evaluated as also explained in that reference, where D_r is the rotational diffusion constant.

Via our QENS experiment we observe the apparent center-of-mass diffusion coefficient $D = D(D_t, D_r)$ (Figure 3) consisting of the translational $D_t = D_t(\phi)$ and rotational $D_r = D_r(\phi)$ contributions, depending on the protein volume fraction, ϕ . Following C. Beck et al., *J. Phys. Chem. B* 122, 8343 (2018) and references therein, we calculate both D_t and D_r using HYDROPRO for the limiting case of an infinitely dilute sample, i.e. $\phi = 0$. Subsequently, employing theoretical models for colloidal hard spheres, we calculate $D_t = D_t(\phi)$, $D_r = D_r(\phi)$, and $D = D(\phi)$ (Figure SI 4), employing the radial hydrogen density distribution obtained by adding the protons to the pdb files using the GROMACS tool *pdb2gmx*. We calculated theoretical global diffusion coefficients for tetramers and monomers of the LDH protein based on the crystal structure of LDH (PDB code 5QNB) using HYDROPRO, in which the protein molecules are assumed to be rigid (Figure SI 4). Using Eqs. (6) and (7), we obtain $R_h = 2.96$ nm and $R = 2.20$ nm, i.e. $R_h/R = 1.34$ for the LDH tetramer, which corresponds to the observable diffusion coefficient $D = 3.92 \text{ \AA}^2/\text{ns}$ for the experimental volume fraction ϕ (vertical line in Figure SI 4). This theoretical D value for LDH tetramers is of similar magnitude as the value for D obtained from the QENS data at ambient pressure ($D \approx 3.3 \text{ \AA}^2/\text{ns}$, Figure SI 4). Upon pressurization, the D -value initially increases (to $D \approx 4 \text{ \AA}^2/\text{ns}$) which would correspond to an essential tetramer-to-dimer and not a tetramer-to-monomer dissociation scenario, in agreement with high-pressure SAXS data (which are ca. 0.2 nm larger).⁴⁴ We emphasize that the QENS experiment probes hydrodynamic properties during the coherence time of a few nanoseconds associated with the energy resolution of the spectrometer. In contrast, SAXS probes time-averaged information. It is therefore not surprising if the pictures obtained from QENS and SAXS differ slightly, for instance in the case that the protein assemblies in the solution are transient in time.

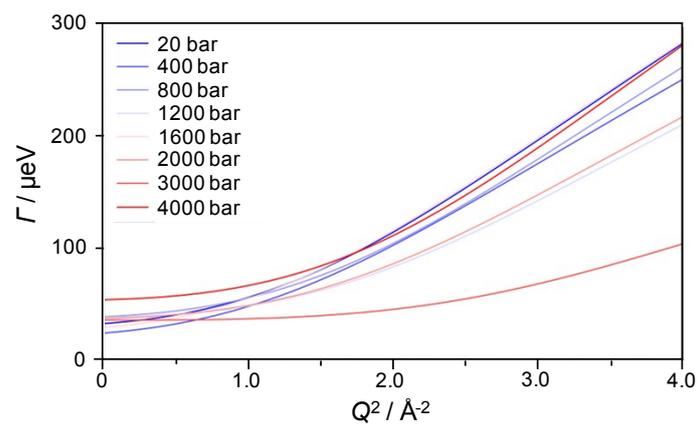


Figure SI 5. Linewidth $\Gamma(Q)$ of the internal diffusion Lorentzian of LDH for selected pressures at $T = 25$ °C. (Cf. main article, Eqs. (3) and (5).)