Supplementary Information

We have carried out a number of experiments on lysozyme, and using computational methods as a guide, we have attempted to accurately assess and in some cases even predict the outcome of the experimental spectra. For instance, one of the most seemingly straightforward cases involves the effects of heavy water (D_2O) on internal dynamics of proteins when compared with that of H_2O. One expects a shift in some of the peak frequencies in the THz region due to the heavier deuterium atom when contrasted with that of hydrogen (similar to that which would be observed in the Mid-Infrared region of the spectrum), but there should also be some consideration about how the exchange alters the overall flexibility of the protein since the solvent hydrogen-bonding dynamics plays an intricate role in determining the collective motions in a number of proteins. In HEWL we have suggested that the ≤ 100 cm^{-1} region of the spectrum is "mostly" governed by global backbone fluctuations that are strongly modulated by solvent dynamics and the $100 - 200$ cm^{-1} spectral region is mostly dominated by more localized, α-helical mainchain (backbone + carbonyl oxygens) dynamics that are also effected by the fast, solvent dynamics. When we exchange H_2O with D_2O in HEWL, we expect that the fluctuations arising from the mainchain dynamics should be red-shifted due to effect of the heavier deuterium atom, whereas the effects on the global backbone dynamics of the protein are a bit more complicated and harder to predict. So to initially provide some insight into the effects of heavy water on the internal protein dynamics we carry out MD simulations where it is possible to analyze distinct regions of the protein and contrast the effects of the solvent exchange. We plot the MD simulation spectrum of the velocity autocorrelation function of the mainchain dynamics (Fig. S8a) and the backbone dynamics (Fig. S8b) of lysozyme in H_2O contrasted with that in D_2O. As expected, the mainchain fluctuation is both red-shifted by approximately 60 cm^{-1} and decreased in amplitude due to the interaction with the heavier deuterium atom. The collective backbone dynamics on the other hand is particularly disrupted by the exchange. The low-frequency mode associated with the backbone motion is both severely reduced in amplitude and blue-shifted suggesting that the heavy water modifies the global flexibility of the protein. We have seen a similar disruption in protein HEWL global flexibility with both the introduction of a ligand (this work) and in partially dehydrated samples[1]. In both cases the protein global motion becomes more rigid. Additional analyses have confirmed this hypothesis and this phenomenon has also been detected in other studies[2] on globular proteins in D_2O. If we look at the comparable experimental data (Figs. S8c – d) of the

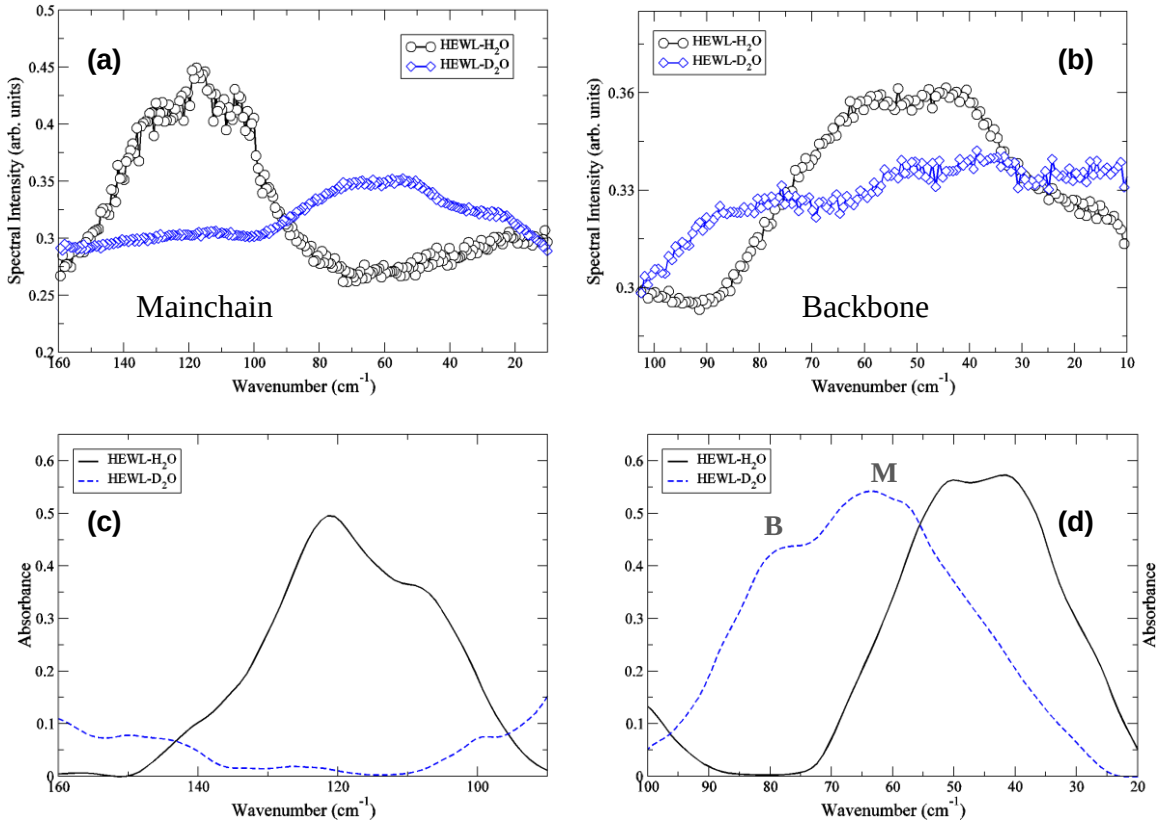

Figure S8: Velocity autocorrelation spectrum of (a) mainchain dynamics and (b) backbone dynamics of HEWL in H_2O (open black circles) and D_2O (open blue diamonds) from MD simulations. And the experimental spectrum of hydrated, film samples of HEWL in H_2O (black, solid line) and D_2O (blue, dotted line) held at 97% RH in the (c) 160 – 90 cm^{-1} spectral region and (d) the 100 – 20 cm^{-1} spectral region. The labels of B and M in (d) represent peaks that we surmise arise from backbone and mainchain fluctuations in D_2O, respectively.

hydrated, film samples, we observe the same trends in the internal dynamics of the protein using our interpretation of the detected modes; although, at first glance this is far from obvious. Beginning with the 100 -200 cm^{-1} region of the spectrum (Fig. S8c), we propose that the loss of the prominent 120 cm^{-1} peak in the D_2O spectrum is not due to the absence of the fluctuation but rather a modification of its dynamics. We surmise that the mainchain peak re-emerges in the D_2O sample at a lower frequency (Fig. S8d) in the $\leq$ 100 cm^{-1} region of the spectrum at a frequency of approximately 60 cm^{-1}. Similarly, the un-liganded backbone mode of HEWL in H_2O (Fig. S8d) contains a strong peak centered at about 45 cm^{-1} that we conjecture shifts to a higher frequency in D_2O and peaks close to 80 cm^{-1}. The comparison between simulation and experimental frequencies are not exact, but the trend is definitely discernible. Further, the MD simulation force-field specifics do not appear to be essential for their assessment. All the force-fields considered in this application (Gromacs, Amber, and Charmm) produce similar results. It should also be stated that it is clear in this discussion that we have utilized a rather simplified explanation to describe the variation in dynamics that occur with the deuteration effects in HEWL, but even in this basic test case it demonstrates that coupling experimental data with computational simulations is extremely useful in a field that is still rather in the early stages of comprehension.

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References

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2. Cioni, P. & Strambini, G. B. Effect of Heavy Water on Protein Flexibility. *Biophys. J.* **82,** 3246–3253 (2002).