ABSTRACT: Water plays a major role in biosystems, greatly contributing to determine their structure, stability, and function. It is well known, for instance, that proteins require a minimum amount of water to be fully functional. Despite many years of intensive research, however, the detailed nature of protein–hydration water interactions is still partly unknown. The widely accepted “protein dynamical transition” scenario is based on perfect coupling between the dynamics of proteins and that of their hydration water, which has never been probed in depth experimentally. I present here high-resolution elastic neutron scattering measurements of the atomistic dynamics of lysozyme in water. The results show for the first time that the dynamics of proteins and of their hydration water are actually decoupled at low temperatures. This important result challenges the “protein dynamical transition” scenario and requires a new model to link protein dynamics to the dynamics of its hydration water.

In 60 BC, Thales hypothesized that water is the primary essence of life, and nowadays this hypothesis is widely accepted. Since a few decades ago, it has become well established that water molecules actively interact with, and support, the biochemistry of different classes of biomolecules. In the case of proteins, for instance, the water molecules adsorbed at the protein surface play a major biological role, and it is well known that proteins require a minimum amount of water to be fully biologically active. These water molecules form the so-called protein hydration layers, usually consisting of one or two layers adsorbed at the protein surface and accounting for ~20% of the hydrated protein weight (Figure 1a). These water molecules display physicochemical properties that are apparently different from molecules in pure bulk water. For instance, they are prevented from crystallizing just below 0 °C because the competition of their mutual interaction and their hydrogen bonding to the protein makes it difficult for them to arrange into the typical tetrahedral ice structure (Figure 1b, cycle 1). Remarkably, proteins’ denaturation restores water ability to crystallize (Figure 1b, cycle 2).

Because the biological functions of proteins involve changes of conformation and sometimes chemical reactions, it is natural to expect a connection of these functions with dynamical properties of the coupled system made of proteins with their hydration water. An important thread in this discussion started in 1989 with the seminal neutron scattering work of Doster and coworkers. Pointing to a sharp increase in the atomic mean-square displacement of a hydrated model protein at around $T_{\text{PDT}} \approx 220$ K, the authors referred to this transition as the “protein dynamical transition” (PDT). Because proteins seem to become biologically active around this temperature and because this transition was not observed in the absence of hydration water, the authors proposed to link the PDT to the functioning of proteins. The common way to measure such PDT experimentally is by elastic neutron scattering, which is collected as a function of the sample-temperature in what is called “fixed-window scan”. This plot shows an abrupt decrease for the hydrated protein with respect to the dry one at $T_{\text{PDT}}$ (Figure 2). Consequently, the research community has focused on understanding the protein–water relaxation mechanisms underlying the PDT and, in turn, the biochemical function.

To the best of my knowledge, the only attempt to propose a unified model of protein dynamics was made by Frauenfelder and coworkers. In their paper they proposed that protein motions are modulated by the hydration shell and by the bulk solvent. Essentially, they proposed that (i) large-scale protein motions are coupled to fluctuations in the bulk solvent that are controlled by the solvent viscosity, which are absent in a solid environment, whereas (ii) internal protein motions are coupled to the beta fluctuations of the hydration shell, they are controlled by hydration, and are absent in dehydrated proteins. This intriguing scenario in which water molecules drive protein dynamics and, in turn, their functions has to be considered together with the relaxation dynamics of the protein hydration water itself. This protein hydration water relaxation has been measured by Chen and coworkers. By increasing the temperature, it revealed a crossover from Arrhenius (strong glass-like) to non-Arrhenius (fragile glass-like) behavior at $T_{\text{PDT}} \approx 220$ K. The picture that emerges is even more intriguing: The change in the hydration–water dynamics at $T_{\text{PDT}}$ triggers the...
protein internal motions that also became active at \( T_{\text{FSC}} \). This would imply that at \( T_{\text{FSC}} \) a sudden variation in the protein dynamics should occur as well. The scenario emerging from this logical implication is supported by the fact that \( T_{\text{PDT}} \approx T_{\text{FSC}} \approx 220 \text{ K} \), which is a strong coupling between the dynamics of proteins and of their hydration water.

The coupling between the transition in the hydration—water dynamics and the PDT has been observed in all neutron-scattering experiments on this subject so far. Again, as above-mentioned, the elastic neutron scattering intensity versus temperature represents one of the most used observables to study such protein—hydration water coupled dynamics, which allows the dynamics of proteins and of their hydration water to be probed independently. To achieve this aim, two elastic-scattering profiles of the hydrated protein are collected in my experiment: one by hydrating with H\(_2\)O and the other with D\(_2\)O, in both cases with an amount of water corresponding to the protein hydration water as sketched in Figure 1a (i.e., between about 0.3 and 0.4 g of water per gram of protein). The elastic spectrum of the protein in the dry state also has to be collected to provide a baseline. The rationale is that neutrons are very sensitive to hydrogen atoms and much less sensitive to deuterium, enabling a distinction between the isotopes. The biochemical function, however, is only slightly affected by the use of heavy water instead of water. As a result, the D\(_2\)O signal from a protein hydrated in D\(_2\)O is negligible, and relaxations in the elastic spectra can be related to the protein itself, which also contains a high number of hydrogen atoms. In this way it is possible to probe the relaxation dynamics of protein alone, yet hydrated, and extract \( T_{\text{PDT}} \). In the case of a protein hydrated in H\(_2\)O, the contributions of water and protein have very similar weights in the signal (i.e., the densities of hydrogen atoms in the protein and in hydration water are nearly equivalent). As a result, by measuring the protein hydrated in H\(_2\)O, relaxations in the elastic spectra arise from relaxations of either the protein or its hydration water. By comparison of the spectra of the protein in D\(_2\)O with that in H\(_2\)O, it is possible to determine the relaxation process of hydration water alone and, in turn, extract its transition temperature.

With the current state of the art, all experiments so far show proteins and their hydration water to have the same transition temperature.
temperature, which strongly supports the PDT scenario described above. That is, the dynamics of proteins and of their hydration water are strongly coupled. In Figure 2, the classic example of the model protein, lysozyme, is shown.

On the contrary, there is a collection of experimental and computational results that is indeed in disagreement with the above-pictured PDT scenario. Several of these out-of-scenario results are based on the fact that no changes in the dynamical behavior of proteins seem to occur at all at the PDT temperature, but they do seem to agree on a sort of dynamical crossover in the relaxation dynamics of the protein hydration water. Furthermore, a recent work also shows that proteins exhibit a dynamical transition even in the dry state. In summary, the PDT scenario is supported by the experimentally measured perfect coupling between the dynamics of proteins and their hydration water, as shown in Figure 2, while the decoupling suggested by other studies points to some new scenario.

I aim to shed light on this 30 year old puzzle by using elastic neutron scattering at the highest achievable resolution for this type of studies, that is, 0.3 μeV in place of the more usual 1 μeV used so far. Lysozyme still represents the protein component. The rationale is that (i) if the relaxation times of two (or more) processes are identical (i.e., perfect coupling of their dynamics), then their coupling has to be independent of the time resolution of the experiment; however, (ii) if their relaxation times are different but so similar that they cannot be separated at a given resolution, then an higher resolution can see their decoupling. For more details of elastic neutron scattering spectroscopy and energy resolution, refer to refs 27 and 28. However, a good analogy is the following: Imagine two objects moving with two different but very similar velocities in the same direction. The longer the observation time, the better the chance of seeing a difference in their motions. However, if the two velocities are identical, then no difference in their motions can be seen at any observation time (Figure 3).

Figure 4. High-resolution elastic neutron data showing the decoupling between the dynamics of protein and of its hydration water. Total elastic neutron-scattered intensity (Q-range = [0.2±1.9]|Å⁻¹|) versus temperature for D₂O hydrated lysozyme (green), H₂O hydrated lysozyme (blue), and dry lysozyme (red). The Q-range perfectly matches the one accessed in Figure 2 by IN10. The comparison in panel a clearly shows the decoupling between the dynamics of lysozyme and of its hydration water. The protein starts to relax at $T_P = 195$ K, decoupled from the transition in the dynamics of hydration water, which only starts to relax 16 K lower, that is, $T_W = 179$ K. (b,c) High counting-statistic of the experiment also allows us to probe the effect of hydrating with heavy water. In this case the protein dynamics is slightly slower when hydrated in isotopically normal water.

Figure 5. Measured mean-square displacements showing the decoupling between the dynamics of protein and of its hydration water.
Consistently with the elastic intensities of Figure 4, the dynamical decoupling occurs in the measured mean-square displacements as well (Figure 5).

In conclusion, with the limited energy resolutions used in the past (up to ~1 μeV on the best high-resolution spectrometers), proteins and their hydration water appeared to undergo a dynamical transition at the same temperature. This prompted the erroneous conclusion of a perfect-coupled water–protein relaxation dynamics, supporting, in turn, the conjecture that transitions in hydration–water dynamics are responsible for protein dynamics. My high-resolution experimental data (Figure 4 and 5) reveal for the first time that there is decoupling between the relaxation of a model protein and the relaxation of its hydration water. This is an important milestone toward the solution of this 30 year old puzzle.

■ AUTHOR INFORMATION
Corresponding Author
*E-mail: antonio.benedetto@ucd.ie.

ORCID
Antonio Benedetto: 0000-0002-9324-8995

Notes
The author declares no competing financial interest.

■ ACKNOWLEDGMENTS
A.B. thanks Pietro Ballone and Gordon J. Kearley for fruitful discussions. A.B. acknowledges support from (i) the European Community under the Marie-Curie Fellowship Grants HYDRA (No. 301463) and PSI-FELLOW (No. 290605) and from (ii) Science Foundation Ireland (SFI) under the Start Investigator Research Grant 15-SIRG-3538, with additional support provided by the School of Physics, University College Dublin, Ireland, and the Laboratory for Neutron Scattering, Paul Scherrer Institute (PSI), Switzerland. A.B. acknowledges the Institut Laue-Langevin (Grenoble, France) for the access to IN10 and IN16B spectrometers and Drs. Miguel Gonzales and Bernard Frick, in particular, for the help during the neutron beam-time. A.B. acknowledges Drs. Ekaterina Pompjakshina and Antonietta Gasperina (PSI) for the access to the differential scanning calorimetry laboratory and the biolab, respectively.

■ REFERENCES
(29) See http://www.ill.eu/instruments-support/instruments-groups/instruments/in16b.

■ NOTE ADDED AFTER ASAP PUBLICATION
This paper was published ASAP on September 25, 2017. The paper was reposted on September 27, 2017 with a revised abstract graphic.