

Protein Surface and Core Dynamics Show Concerted Hydration-Dependent Activation**

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The importance of protein dynamics for biological function is increasingly recognized. Studying protein dynamics as a function of temperature provides information about the underlying energy landscape. At cryo-temperatures proteins behave like harmonic solids, with fluctuations that vary in a linear fashion with temperature.^[1] Biological macromolecules hydrated to a sufficient degree exhibit a strong onset in dynamics in the temperature range 180–250 K, as measured by neutron scattering^[1,2] and Mössbauer spectroscopy,^[3] amongst other techniques.^[4] The increase in dynamics was termed the “protein dynamical transition” and interpreted as the temperature at which the protein populates states of higher energy.^[1] Although biological activity below the transition temperature was measured in certain systems,^[5] the increased conformational flexibility above the transition temperature is generally thought to correlate with the onset of biological function.^[6] The underlying microscopic nature of the transition^[7] and its interpretation in terms of protein energy landscape is still under much debate.^[7b,8]

The importance of the surrounding solvent in protein dynamics and its role in the dynamical transition is widely recognized,^[9] but to what extent surface hydration effects propagate to the hydrophobic interior has remained elusive. In a recent report, molecular dynamics simulations showed a similar dependence on hydration of surface and buried residue dynamics.^[10] It was found that localized diffusion dominates the hydration-induced increase in picosecond-to-nanosecond timescale dynamics, and that this effect propagates into the protein core through a strong coupling of

diffusive motions between the surface and interior, effectively “softening” the protein. To address this question experimentally, we present here the separate measurement of surface and core dynamics, through a combination of specific isotope labeling and neutron scattering of a small globular protein. A protein dynamical transition is observed for two separate atomic groups: buried in the hydrophobic core and on the solvent accessible surface. We find that probes of dynamics in the interior of the protein and on the surface display comparable behaviors as a function of temperature, and a similar response to hydration.

Elastic incoherent neutron scattering can be used to assess the atomic mean square displacements [MSD] of proteins over a wide temperature range. The measured signal for a natural abundance sample is dominated by scattering from hydrogen nuclei, which are relatively evenly distributed throughout a protein, and the global average dynamics of the sample is therefore probed. The heavier isotope of hydrogen, deuterium, is a weak incoherent scatterer however, and by labeling specifically with hydrogen and deuterium it is possible to mask the contribution of the deuterated components.

In the present study, we produced two specifically H/D labeled samples, one with a probe of dynamics on the “inside” and the other with a probe on the “outside” of the protein. Calbindin D_{9k} P43G^[12] contains 90 hydrogen atoms in lysine side chains and 90 hydrogen atoms in the methyl groups of valine and leucine residues (see Section S1 in the Supporting Information). As can be seen in Figure 1 A, the methyl groups

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of valine and leucine residues are buried in the hydrophobic core of the protein. In contrast, the lysine residues are all on the surface of the protein, as can be seen in Figure 1 B. These

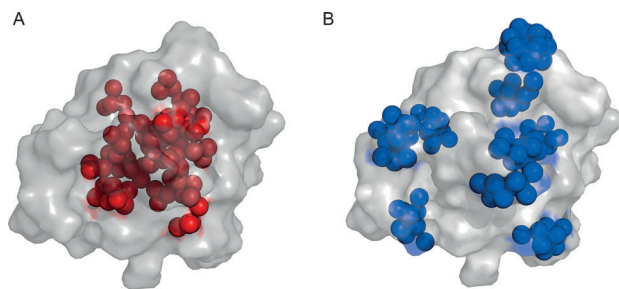


Figure 1. Molecular model (PDB ID 4ICB)^[11] of specifically labeled calbindin D_{9k} samples. Deuterated residues are shown as a gray outline and labeled hydrogen atoms as colored spheres. A) Deuterated calbindin D_{9k} with protonated methyl groups of valine and leucine residues, called Val/Leu-calbindin. B) Deuterated calbindin D_{9k} with protonated lysine residues, called Lys-calbindin. In both cases, the labeled hydrogen atoms contribute 85 % of the incoherent neutron scattering in the dry state and 77 % in the hydrated state (see Table S1).

two atomic groups therefore provide us with probes of internal and surface dynamics, respectively.

Deuterated calbindin D_{9k} with the methyl groups of valine and leucine residues protonated (Val-[γ^1, γ^2 - $^1\text{H}_6$]/Leu-[δ^1, δ^2 - $^1\text{H}_6$]-calbindin), called “Val/Leu-calbindin”, was produced using a deuterated culture medium supplemented with partially deuterated α -ketoisovaleric acid. To compare the dynamics to the lysine residues, we produced deuterated calbindin D_{9k} with protonated lysine residues (Lys-[$\alpha, \beta, \gamma, \delta, \epsilon$ - $^1\text{H}_5$]-calbindin), called “Lys-calbindin” using a deuterated medium and an excess of [^1H]-lysine. The sample quality was assessed by NMR spectroscopy, which showed that all of the labeling occurred as expected, and no scrambling to other amino acids during biosynthesis was observed (see Figure 2).^[13] A full description of the sample preparation is given in Section S2 in the Supporting Information.

The specifically labeled samples were measured as powders in two states: dry and hydrated to 0.44 g of D₂O per gram of protein using the IN16 backscattering spectrometer at the Institut Laue Langevin.^[14] IN16 probes motions on a timescale faster than 1 ns and on the Ångström length scale. The elastically scattered neutrons were recorded as a function of the temperature and the atomic MSD were extracted as described in Section S3.

Figure 3 A and B show the extracted MSD for Val/Leu-calbindin and Lys-calbindin, respectively, each in both the dry and hydrated states. In the two specifically labeled samples qualitatively similar responses to hydration are observed. At temperatures below 200 K, similar MSD are obtained for both dry and hydrated samples. Between 210 and 230 K the hydrated samples begin to show increased dynamics compared to the dry samples, and a steep increase in MSD is

observed for both hydrated samples around 250 K, characteristic of the protein dynamical transition. The results for the hydrated samples are plotted together in Figure S4 A, where their similar temperature dependence can be clearly seen. An onset of dynamics at about 250 K for both hydrated samples is also evident in the raw data from which the MSD are extracted, the elastic intensity, and is shown in Figure S5 A. The data show that probes of dynamics buried in the hydrophobic core of the protein and on the solvent exposed exterior are both sensitive to hydration, and undergo a hydration-dependent transition in the same temperature range. Previous studies of lysozyme at similar hydration levels and on equivalent instruments rule out that the observed increases in dynamics are due to rigid body motion of the entire protein, but instead indicate that the motions are confined to within 3.5 Å.^[15]

The extracted MSD for the two labeled samples presented here, are larger than previously reported values for other natural abundance proteins under the same conditions.^[9c,f,16] When natural abundance samples are measured, global dynamics of the entire proteins is probed, including backbone and all side chains and the resulting MSD are significantly smaller than the labeled groups measured here. The methyl

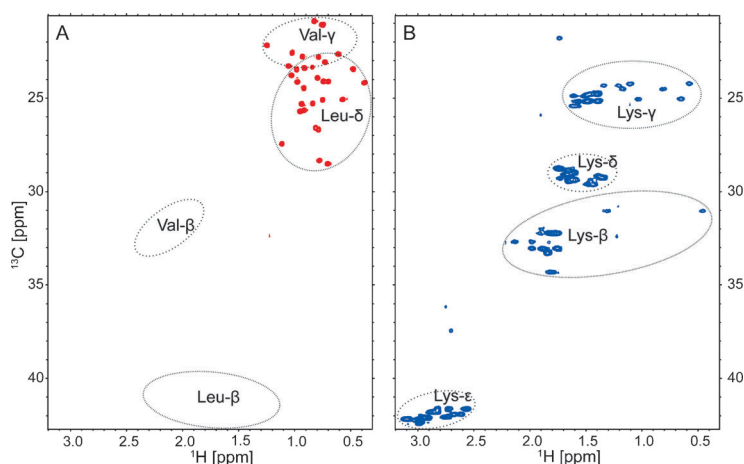


Figure 2. Natural abundance [^1H - ^{13}C]-HSQC NMR spectra of the specifically labeled samples of A) Val/Leu-calbindin and B) Lys-calbindin.^[13] In the case of Lys-calbindin the H α -positions are fully protonated as expected, but are not shown in panel B.

groups at the end of leucine and valine side chains and lysine residues are significantly more mobile than the average protein. The data presented in Figure 3 can also be compared to the dynamics of water at the surface of a protein, measured under the same conditions,^[9c,f] which displays surprisingly similar temperature dependence to the hydrated labeled samples measured here. The result shows that with the onset of water dynamics, both side chains in the interior and exterior show parallel increases in dynamics.

In both the dry and hydrated samples, the labeled methyl groups have increased dynamics compared to the lysine residues above 150 K (see Sections S4 and S5 for comparisons). An increase in global protein MSD at about 150 K was

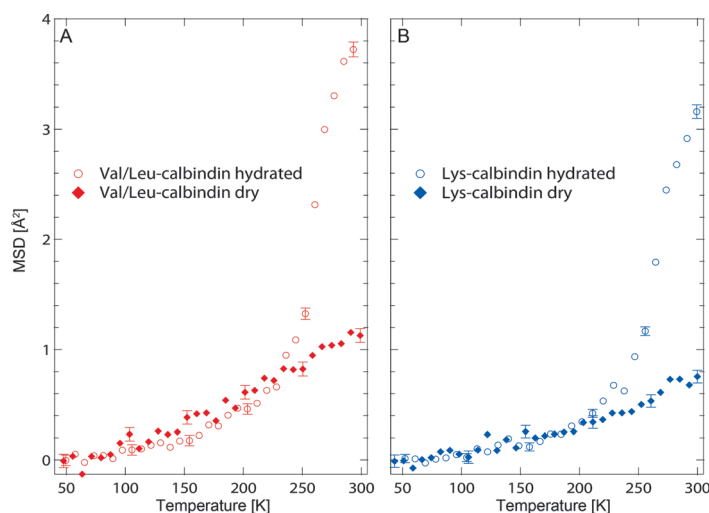


Figure 3. Mean square displacements extracted from neutron scattering A) deuterated calbindin D_{9k} with protonated methyl groups of valine and leucine residues (Val/Leu-calbindin) and B) deuterated calbindin D_{9k} with protonated lysine residues (Lys-calbindin). The hydrated samples are hydrated to 0.44 g D_2O per gram protein, corresponding to 70% of a monolayer coverage^[17] (see the Supporting Information for details).

earlier assigned to methyl group rotation,^[15,18] which enters the 1 ns timescale accessible by backscattering spectrometers at this temperature.^[19] The data from the specifically labeled Val/Leu-calbindin sample further substantiates that the increase in dynamics at 150 K is due to methyl group rotation, since above this temperature the Val/Leu-calbindin sample has increased MSD compared to the Lys-calbindin.

Above the dynamical transition temperature, protein MSD depend strongly on the hydration level of the sample^[20] and the type of solvent.^[21] Below the transition however, several studies have shown that increased hydration can result in a reduction in dynamics compared to a dry sample.^[2,20b,22] In Figure 3A, in the temperature region of 120–220 K, a slightly lower MSD of the hydrated Val/Leu-calbindin sample is observed compared to the dry sample. Although a small effect, the data seem to indicate that at these low temperatures, in the presence of hydration water, dynamics in the protein interior are dampened compared to the dry state, and water molecules exert a stiffening force on the protein. The observation again underlines how protein dynamics, and therefore the protein energy landscape, differ over a wide temperature range when the system is modified by the addition of water molecules.

A hydration-dependent transition was also observed for a subset of residues in the membrane protein bacteriorhodopsin,^[23] where residues in the proton channel were found to be sensitive to hydration. The data presented here show for the first time experimentally a response to hydration for atomic groups buried in the hydrophobic interior of the protein and on the exterior, and a parallel onset of dynamics for both. An increase in dynamics is observed in two samples where scattering is dominated either by methyl groups or lysine residues, in a temperature range where such an onset is typically called a dynamical transition. The transition is a “global” one, and not restricted to solvent accessible

residues. Our data are consistent with recently published molecular dynamics simulation results,^[10] which suggest that hydration does not simply soften proteins in a radial manner.^[24] The simulation study puts forward an atomistic interpretation to the measured dynamics presented here: the increase in dynamics with hydration, both in the core and on the surface, is predominantly due to hydrogen atoms undergoing localized diffusion, and sampling larger volumes.

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