Slow Internal Protein Dynamics from Water 1H Magnetic Relaxation Dispersion

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Structural flexibility is essential to protein function, but the full range of internal dynamics has not yet been explored. Solution NMR relaxation methods routinely probe motions faster than ~10 ns or slower than ~10 µs.1 Residual dipolar couplings from weakly aligned proteins indicate fluctuations in the 10−8−10−5 s window2 but do not provide the associated correlation times. To study internal protein dynamics in this relatively inaccessible time window by NMR relaxation, isotropic averaging of spin couplings by protein tumbling must be prevented. This is accomplished by direct protein−protein contacts in microcrystals, studied by solid-state 13C or 15N relaxation,3 or by covalent cross-links between fully hydrated proteins, studied by water 1H, 2H, or 17O magnetic relaxation dispersion (MRD).4,5

In MRD studies of immobilized proteins, information about protein dynamics is conveyed to the observed bulk water resonance via internal water molecules and, for 1H and 2H, also via labile OH and NH hydrogens. If they exchange with bulk water on the relaxation time scale, these intermediary species probe protein dynamics in two ways. While residing on the protein, the intermediary species is dynamically coupled to the protein and thus reports on protein motions on time scales shorter than the residence time. Exchange plays the same role for an immobile protein as tumbling does for a free protein: it eliminates the effect of slower internal motions and induces relaxation by randomizing the orientation of the residual spin−lattice coupling tensor. For internal waters,5 such exchange-mediated orientational randomization (EMOR) provides access to intermittent protein fluctuations involving highly excited conformational states. For exposed labile hydrogens, exchange is rate-limited by the chemical step so the residence time yields a protection factor but no information about protein dynamics.

The EMOR mechanism accounts quantitatively for water 1H and 2H MRD data from polysaccharide and polypeptide gels.6,7 These gels are built from compact double or triple helices, and internal motions are not evident in the MRD data, except via intermittent fluctuations that control internal-water exchange. Globular proteins are more flexible, and slow side-chain motions have been detected by 2H MRD.5 Whereas 2H relaxes by a single-spin electric-quadrupole mechanism, magnetic dipole−dipole couplings induce cross-relaxation between intermediary protons and nearby protein protons, potentially making 1H MRD a more sensitive probe of internal dynamics. To explore this possibility, we present here 1H MRD data from mammalian ubiquitin (mUb), immobilized by glutaraldehyde (GA) cross-linking of lysine side chains.8 There is currently no consensus on the mechanism of water 1H relaxation in systems containing immobilized biomolecules, including biological tissue. Our second objective here is to resolve this issue by comparing 1H MRD data from deuterated and protonated forms of ubiquitin.

We analyzed the 1H MRD data with the most general version of the EMOR approach,9,10 with the 1.8 Å resolution crystal structure (1UBQ)11 of mUb as input, as well as with an alternative, essentially phenomenological, approach.4 The latter approach rests on two major assumptions. First, a coarse-grained two-phase (2P) description10 is used, based on the assumption that coherent spin diffusion and/or cross-relaxation is so fast that the 1H magnetization of the entire protein can be characterized by a single longitudinal relaxation rate R1p(o0). The observed R1 is identified with the rate of the slowly decaying magnetization component in the 2P model,10

\[ R_1 = (R_{1p} + R_{1W} + k)/2 \]

\[ - (\{R_{1p} - R_{1W} + (f_p - f_w)k\}^2/4 + f_p f_w k^2)^{1/2} \] (1)

where \( f_p = 1 - f_w \) is the fraction of protons that belong to the protein. The relaxation rate, \( R_{1W} \), of the water phase and the protein−water magnetization exchange rate constant, \( k \), are both taken to be frequency-independent.4

The alternative model further assumes that protein 1H relaxation is induced by small-amplitude collective vibrational modes (‘fractons’) in a direct mechanism akin to spin-phonon coupling in crystalline solids.11 In this spin-fracton (SF) model,4 the internal-motion time scale is set by a spectral dimension, \( d_s \), that governs the low-frequency scaling of the vibrational density of states, \( \sigma(\omega) \propto \omega^{d_s - 1} \), and one obtains

\[ R_{1p}(\omega_0) = 3\pi M_d d_s \frac{k_B T}{h} \Omega^b - 2 \left[ \frac{1}{\omega_0^b} + \frac{1}{(2\omega_0^b)^b} \right] \] (2)

Here, \( M_d \) is the average 1H second moment of the protein, \( b = 3 - d_s(1 + 2/d_s) \), \( d_i \) is the fractal dimension of the proton distribution, and \( \Omega \) is a high-frequency mode cutoff.12

Although the 2PSF and EMOR models differ in physical content, they can both provide adequate fits to 1H MRD data. However, the two models make very different predictions about the effect of H−D substitution in the protein, and they can therefore be discriminated by comparing the 1H MRD profiles from partially deuterated (D-mUb) and fully protonated (H-mUb) ubiquitin. Analysis of 1H NMR spectra (Figure S1) from the two proteins shows that 83% of the 485 nonexchangeable hydrogen sites are deuterated in D-mUb. Because the protein is dissolved in H2O, essentially all OH and NH sites carry protons, but the hydrophobic core is extensively deuterated (Figure 1). The experimental results show that protein deuteration reduces \( R_1 \) by merely 20% (Figure 2).

In the 2PSF model, deuteration affects \( R_1 \) mainly via the reduction of \( f_p \) (by a factor 2.8) and \( M_d \) (by a factor 3.1). But these global variables only capture a part of the isotope effect. The fracton modes are thought to propagate mainly along the backbone.4 The effective \( M_d \) should then be dominated by the mostly-β-strand backbone of mUb, where H deuterium increases the nearest-neighbor H−H separation by a factor of 2, thereby reducing the effective \( M_d \) by an order of magnitude.

As a result of these H−D substitution effects, the 2PSF model predicts at least an order-of-magnitude reduction of \( R_1 \) at low
As for the 2PSF model, we determined EMOR parameters from a fit to the H-mUb profile (Figure 2b, solid curve) and then used these parameters and the known deuteration pattern to predict the D-mUb profile (Figure 2b, dashed curve). The agreement between predicted and measured $R_1$ indicates that the EMOR model captures the essential features of the relaxation mechanism. With residence times on the order of $10^{-8}$ s, the EMOR dispersion is close to the adiabatic limit where the dispersion frequency is given by the inverse dipole coupling. For relaxation induced by a pure EMOR mechanism, with OH protons exchanging from a rigid protein, we thus expect a nearly Lorentzian dispersion centered at $\sim 20$ kHz. The observation of a more extended MRD profile indicates internal motions of substantial amplitude on a $\mu$s time scale. For the fit in Figure 2b, a model-free spectral density function was used, yielding a correlation time $\tau_{\text{int}} = 2.7 \mu$s and an order parameter $\chi_{\text{int}} = 0.47$. Interestingly, $\mu$s side-chain motions in mUb have also been inferred from residual dipolar couplings.

In summary, MRD data on deuterated mUb support the EMOR model for water $^1$H relaxation in immobilized proteins but are incompatible with the 2PSF model. The present analysis also demonstrates that $^1$H MRD provides information about side-chain dynamics in the $10^{-8}$–$10^{-5}$ s time window.

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Supporting Information Available: Figure S1, Table S1, experimental methods, and data analysis protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(12) For the fit, we used $\Delta_0 = 2.5$ and $\Omega = 1560$ cm$^{-1}$ (corresponding to the amide-II band), as proposed, and $M_0 = 7.4 \times 10^5$ s$^{-1}$, based on the crystal structure (1UBQ) with allowance for motional averaging.
(13) For the EMOR analysis, the full $10 \times 10$ relaxation matrix was used.
(14) Internal motions were assigned to all dipole couplings with labile protons. Restricted rigid-body rotation of the entire protein in the gel network is expected to occur on the $\mu$s time scale and, in any case, does not agree well with the data. From $R_1$ data above 1 MHz, we infer internal motions also on shorter time scales.
Supporting Information

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**Materials and Methods**

**Ubiquitin Expression and Purification.** Unlabeled mammalian ubiquitin (H-mUb) was expressed in *E. coli* strain BL21(DE3) and purified to > 99 % as described.\(^1\) Deuterated ubiquitin (D-mUb) was expressed in a minimal medium with unlabeled glucose as the only carbon source. All components (Table S1) except the antibiotics were dissolved in 400 ml 99.8 % D\(_2\)O and sterile filtered. The solution was then diluted to 3000 ml with sterile 99.9 % D\(_2\)O or a 50:50% H\(_2\)O/D\(_2\)O mixture. Cells from overnight cultures grown in LB/H\(_2\)O medium at 37 °C were resuspended in 50 ml minimal medium with 50 % D\(_2\)O and grown to OD\(_{600} = 1\). 500 µl of this cell suspension was then inoculated into 50 ml minimal medium with 99.9 % D\(_2\)O and grown overnight. Next, 6 1-liter baffled flasks, each containing 500 ml minimal medium with 99.9 % D\(_2\)O, were inoculated with 2.5 ml cell suspension (taken at OD\(_{600} = 1.4\)). Protein production was induced by adding 50 mg isopropyl thiogalactoside per flask at OD\(_{600} = 0.6 \text{–} 0.7\), and the cultures were harvested by centrifugation 4.5 h later (OD\(_{600} = 1.1 \text{–} 1.3\)). Pellet resuspension, protein harvesting and purification were performed as described.\(^1\) As for H-mUb, the purity of D-mUb was estimated by SDS-PAGE to > 99%.
Table S1. Composition of minimal medium

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount per dm$^3$</th>
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<tr>
<td>Na$_2$HPO$_4$·2H$_2$O</td>
<td>7.5 g</td>
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<tr>
<td>KH$_2$PO$_4$</td>
<td>3 g</td>
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<tr>
<td>NaCl</td>
<td>0.5 g</td>
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<tr>
<td>NH$_4$Cl</td>
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<tr>
<td>CaCl$_2$·2H$_2$O</td>
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<td>MgCl$_2$·6H$_2$O</td>
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<tr>
<td>MgSO$_4$·7H$_2$O</td>
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<tr>
<td>FeCl$_3$</td>
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<tr>
<td>FeSO$_4$·7H$_2$O</td>
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<tr>
<td>CuSO$_4$·5H$_2$O</td>
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<tr>
<td>KI</td>
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</tr>
<tr>
<td>MnSO$_4$·4H$_2$O</td>
<td>20 µg</td>
</tr>
<tr>
<td>NiSO$_4$·6H$_2$O</td>
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</tr>
<tr>
<td>Na$_2$MoO$_4$·2H$_2$O</td>
<td>4 µg</td>
</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O</td>
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<tr>
<td>Citric acid·H$_2$O</td>
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<td>Glucose·H$_2$O</td>
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<td>Ampicillin</td>
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<tr>
<td>Chloramphenicol</td>
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Figure S1. (A) Aliphatic and (B) aromatic + NH regions of the 500 MHz $^1$H spectra of H-mUb (blue) and D-mUb (red), scaled to the same amount of protein in the two samples. The gray areas indicate the chemical shift ranges used to establish the degree of deuteration.
Deuteration Pattern. The degree of deuteration of D-mUb was assessed by comparing $^1$H NMR spectra from H-mUb and D-mUb (Fig. S1). The spectra were acquired at 500 MHz on a Varian Unity Inova instrument with 7.3 kHz spectral width, 9.6 µs 90° pulse length and 16 transients. The proteins were dissolved in D$_2$O at a concentration of ~ 2 mM. Taking into account the 5 % difference in concentration (determined by absorbance measurements) and the difference in sample volume, the H-mUb spectrum was scaled to correspond to the same number of protein molecules in the active probe volume as for the D-mUb sample. The ratio of integrated intensities in the two spectra is then equal to the ratio of protons in the two proteins with resonances in a given chemical shift range. In this way, the degree of deuteration, $X_D$, was determined for different hydrogen classes.

From integrals over the range 0.1 – 1.0 ppm, which contains 40 of the 49 methyl groups in mUb, we obtain $X_D = 0.821$. This value is taken to apply to all methyl groups (except the one in the single Met residue). From the range 3.5 – 3.9 ppm, we deduce $X_D = 0.0$ for Ser H$^\beta$ and Pro H$^\delta$ (12 hydrogens in mUb). This range also contains some H$^\alpha$ resonances, which are taken to be fully deuterated (see below). The range 1.5 – 3.0 ppm contains 170 aliphatic protons not belonging to the previous two classes or to the H$^\alpha$ class. The value obtained for this range, $X_D = 0.848$, is taken to apply to all 228 aliphatic hydrogens not in the aforementioned three classes. Based on biosynthetic pathways and previous results of protein expression in pure D$_2$O with unlabeled glucose as the sole carbon source, we use the approximate values $X_D = 1.0$ for the 82 H$^\alpha$ hydrogens and $X_D = 0.50$ for the 16 aromatic hydrogens in mUb. Altogether, this yields a mean deuteration level of 83.3 % for the 485 nonexchangeable hydrogens of mUb. This value is in the same range, 80 – 86 %, as reported for deuteration of other proteins using the same protocol.

At pH 5.3, mUb contains 146 labile OH and NH protons, including two in the single internal water molecule. Cross-linking with glutaraldehyde removes most of the 21 Lys amino protons. In the cross-linked D-mUb sample used for the MRD measurements, all of the remaining 125 labile hydrogens will be fully protonated ($X_D = 0$) since even the most protected
amide hydrogens exchange with the H$_2$O solvent on a time scale of hours at pH 5.3.\textsuperscript{7} The NH portion of the $^1$H NMR spectra in Fig. S1 provides no useful information, but it can be rationalized by considering the different extents of H→D exchange in H-mUb and D-mUb (which was exposed to H$_2$O in the course of purification).

**Protein Immobilization.** Lyophilized protein was dissolved in a 75 mM PIPES (piperazine-N-N’-bis[2-ethane-sulfonic acid]) buffer (Sigma-Aldrich, St Louis, MO) in H$_2$O (Fluka Biochimika). pH was adjusted by µl additions of NaOH or HCl, followed by centrifugation. The protein concentration of the solution was determined by complete amino acid analysis (performed at the Amino Acid Analysis Center, Dept. of Biochemistry and Organic Chemistry, Uppsala University, Sweden). Expressed as the H$_2$O/protein mole ratio, $N_W$, we thus obtained 3795 for H-mUb and 4286 for D-mUb. The $R_1$ data displayed in Fig. 2 have been normalized to $N_W = 4000$ using the relation $R_1 - R_1$(pure H$_2$O) $\propto 1/N_W$. The protein was cross-linked by addition of ice-cold 25% glutaraldehyde solution (MP Biomedicals Inc, Solon, OH) as described.\textsuperscript{1} The initial glutaraldehyde concentration in the protein samples was 0.4 M, four times the concentration of amino groups in mUb. The final pH of the cross-linked sample was 5.30 for H-mUb and 5.35 for D-mUb.

**Magnetic Relaxation Dispersion.** The water-$^1$H longitudinal relaxation rate, $R_1$, was measured from 5 kHz to 600 MHz using seven different NMR spectrometers.\textsuperscript{1} To analyze the effect of H→D substitution, we focus on the frequency range below 1 MHz, where $^{14}$N quadrupolar peaks do not interfere and the frequency-dependent relaxation enhancement from the protein is large compared to the frequency-independent bulk and surface water contribution to $R_1$. The $R_1$ data shown in Fig. 2 were measured at 20 $\pm$ 0.1 °C on a 1 T Stelar fast-field-cycling instrument with polarization at 40 MHz and detection at 16.6 MHz, a field switching time of 3 ms, and a 90° pulse length of 6.1 µs. The polarization and recovery times were set to 4 times $T_1$ and 8 transients were accumulated.
Data Analysis

Spin-Fracton Model. Fits to the $^1$H MRD data for H-mUb (Fig. 2) were performed with the aid of eqs (1) and (2), with $b$ and $k$ as the only adjustable parameters. The fractal dimension, $d_f = 2.5$, and the high-frequency mode cutoff, $\Omega = 1560$ cm$^{-1}$, were fixed at the values used in previous applications of this model. In eq (1), we used $R_{1W} = 0.50$ s$^{-1}$ (the measured $R_1$ value at 600 MHz) and $f_P = N_H/(2N_W + N_H) = 0.071$, computed from the number, $N_H = 610$, of protons in cross-linked H-mUb at pH 5.3 and the H$_2$O/protein mole ratio, $N_W = 4000$.

The average $^1$H second moment for mUb was calculated as

$$M_2 = \frac{9}{20} \left( \frac{\mu_0}{4\pi} \hbar \gamma_H^2 \right)^2 \frac{1}{N_H} \sum_{i=1}^{N_H} \sum_{j=1, j\neq i}^{N_H} S_{ij}^2 R_{ij}^{-6}$$

where $\gamma_H$ is the proton magnetogyric ratio. The proton-proton separations $R_{ij}$ were obtained from the crystal structure 1UBQ of mUb with hydrogens added in standard geometry. The rank-2 orientational order parameter $S_{ij}$ was set to 1 uniformly, except for proton pairs in methyl groups, for which $S_{ij} = -1/2$ due to rapid rotation about the C–C bond. In this way, we obtained $M_2 = 1.37 \times 10^{10}$ s$^{-2}$. This calculation assumes that the protein structure is rigid, apart from methyl group rotation. In reality, a variety of internal motions will contribute to a substantial motional averaging of the second moment. For the H-mUb fit, we therefore used $M_2 = 0.74 \times 10^{10}$ s$^{-2}$, which is 54% of the calculated $M_2$ value. This motional averaging factor is taken from a comparison of a similarly computed $M_2$ value for hen lysozyme with the $M_2$ value measured from the free induction decay. If $M_2$ is regarded as an adjustable parameter, the fit quality is somewhat improved, but the resulting second moment, $M_2 = 4.2 \times 10^{10}$ s$^{-2}$, is unphysically large. In any case, the MRD profile predicted for D-mUb is hardly affected.

The predicted $^1$H MRD profile for D-mUb was computed with $b = 0.78$ and $k = 364$ s$^{-1}$, as obtained from H-mUb fit, and the same fixed parameter values as for H-mUb, except for $f_P$ and $M_2$. Based on the 83.3% deuteration level of nonexchangeable hydrogens in D-mUb (see above),...
we obtain $N'_H = 610 - 0.833 \times 485 = 206$ and $f_p = 206 / 8206 = 0.025$. To compute the effect of H→D substitution on the second moment, we used the deuteration fractions $X_D$ determined above and placed the residual protons randomly within each hydrogen class. (Repeated calculations showed that the randomness had no significant effect.) To compute $R_{1P}(\omega_b)$ for D-mUb, we write eq (2) as

$$R_{1P}(\omega_b) = 3\pi M_2 d_S \frac{k_B T}{\hbar} \Omega_b^{-2} \left[ \frac{C_1}{\omega_0^b} + \frac{C_2}{(2\omega_0)^b} \right]$$

(S2)

where the factors $C_1$ and $C_2$ account for the effect of H→D substitution on $M_2$ and on the sum frequency $\omega_{0ij}$, which equals $2 \omega_0$ for a H–H pair and $\omega_0 (1 + \gamma_D / \gamma_H)$ for a H–D pair. Although eq (S1) with $S_{ij} = 1$ except for methyl pairs does not take motional averaging fully into account, it can be used as a reasonable approximation to compute the ratio of $M_2$ values for D-mUb and H-mUb. We thus compute the isotope factors in eq (S2) as

$$C_1 = \frac{\sum_{i=1}^{N'_H} \sum_{j=1}^{N'_H} h_j S_{ij}^2 R_{ij}^{-6}}{\sum_{i=1}^{N'_H} \sum_{j=1}^{N'_H} S_{ij}^2 R_{ij}^{-6}}$$

(S3a)

$$C_2 = \frac{\sum_{i=1}^{N'_H} \sum_{j=1}^{N'_H} h_j \left( \frac{1 + g_j}{2} \right)^{-b} S_{ij}^2 R_{ij}^{-6}}{\sum_{i=1}^{N'_H} \sum_{j=1}^{N'_H} S_{ij}^2 R_{ij}^{-6}}$$

(S3b)

with

$$h_j = \begin{cases} 1 & \text{if } j = H \\ (8/3) \left( \frac{\gamma_D}{\gamma_H} \right)^2 & \text{if } j = D \end{cases}$$

(S4a)

$$g_j = \begin{cases} 1 & \text{if } j = H \\ \gamma_D / \gamma_H & \text{if } j = D \end{cases}$$

(S4b)
Note that the sum over \( i \) only includes the \( N'_{\text{H}} \) residual protons, whereas the sum over \( j \) includes all \( N_{\text{H}} \) hydrogen sites, whether protons or deuterons. For D-mUb, we obtain \( C_1 = 0.321 \) and \( C_2 = 0.336 \).

Equations (2) and (S2) describe the effect of longitudinal vibrational modes, which, in the small-amplitude approximation, modulate only the length of the H–H vectors \( R_{ij} \).\(^{12}\) The effect of transverse modes is described by a similar expression, differing only in the cutoff frequency \( \Omega \).\(^{12}\) As the transverse \( \Omega \) is rather ill-defined, we ignore this term, which, in any case, would hardly affect the predicted MRD profile for D-mUb.

The critical feature of the spin-fracton model, without which this relaxation mechanism would be utterly insignificant, is the small value of the spectral dimension, \( d_S \), that characterizes the space in which the fractons propagate.\(^8,12\) For \( d_f = 2.5 \) and \( b = 0.78 \) (see above), \( d_S = 1.23 \), much less than the value 3 expected for a Debye solid. Such low \( d_S \) values have been rationalized with reference to the one-dimensional topology of the polypeptide chain.\(^8,12\) But if relaxation is induced primarily by vibrations along the polypeptide backbone, it would be more appropriate to average the second moment in eq (S2) over the protons in the backbone, rather than over all protons in the protein. The effect of H→D substitution would then be much larger than shown in Fig. 2a. This follows because essentially all H\(^\alpha\) positions are deuterated in D-mUb so the nearest-neighbor H–H separation along the backbone increases from \(~2.3\) Å in H-mUb to \(~4.6\) Å in D-mUb for the predominant \( \beta \)-strand secondary structure in mUb.

It is not entirely clear how the fractal dimension \( d_f \) in the 2PSF model should be interpreted. If \( d_f \) is regarded as an average over all proton sites, one finds a value smaller than 3 (as expected for a uniform proton distribution) but this reduction is essentially a trivial boundary effect. Although deuteration may alter \( d_f \) somewhat, we have used the same \( d_f = 2.5 \) for D-mUb and H-mUb. A decrease (increase) of \( d_f \) upon deuteration would shift the D-mUb dispersion (dashed curve in Fig. 2a) to lower (higher) frequency but would not increase \( R_1 \) significantly. For example, if \( d_f \) is changed from 2.5 to 2.3 (2.7), \( R_1 \) at 1 kHz is changed from 9.0 to 6.2 (9.6) s\(^{-1}\).
The high-frequency mode cutoff was fixed at $\Omega = 1560 \text{ cm}^{-1} = 2.94\times10^{14} \text{ s}^{-1}$ for both H-mUb and D-mUb. This value, proposed by Korb and Bryant,\textsuperscript{8} corresponds to the amide-II band, mainly composed of the out-of-phase N–H bending and C–N stretching modes.\textsuperscript{13} In D$_2$O, when N–H is exchanged to N–D, the amide-II frequency is down-shifted by $\sim 100 \text{ cm}^{-1}$,\textsuperscript{14} but in our H$_2$O sample, virtually all amide hydrogens are back-exchanged to N–H so there should be no significant isotope effect on $\Omega$.

In the fast-exchange limit, $k \gg |R_{1p}(\omega_0) - R_{1W}|$, eq (1) reduces to

$$R_1(\omega_0) = R_{1W} + f_P[R_{1p}(\omega_0) - R_{1W}]$$  \hspace{1cm} (S5)

Since $R_{1W} \ll R_{1p}$ in the frequency range of Fig. 2a, H→D substitution would in this limit reduce $R_1$ by a factor $\sim 9$, given essentially by the ratio of $f_P M_2$ for H-mUb and D-mUb. However, the fast-exchange limit does not apply at the lower frequencies. At 10 kHz, for example, $R_{1p} = 2000 \text{ s}^{-1}$ for H-mUb according to the spin-fracton model. At this frequency, H→D substitution is predicted to reduce $R_1$ by a factor 3.5, which is still much more than the observed 20 % (Fig. 2a). As the fast-exchange limit is approached with increasing frequency, this factor first increases to 5.2 near 200 kHz and then decreases towards 1 as $R_{1p}$ goes to zero and $R_1 = R_{1W}$. 

S9
**EMOR Model.** In the dilute regime, the relaxation rate of the observed longitudinal water $^1$H magnetization is equal to the integral relaxation rate, which can be obtained as\(^\text{15}\)

$$R_1(\omega_0) = \frac{1}{[(K + Q)^{-1}]_{1,1}} \quad (S6)$$

The exchange matrix is

$$K = \begin{bmatrix} \sum_{i=1}^{N_H} f_i \tau_i & -\frac{1}{\tau_1} & -\frac{1}{\tau_2} & \cdots & -\frac{1}{\tau_{N_H}} \\ -\frac{f_1}{\tau_1} & 1 & 0 & \cdots & 0 \\ -\frac{f_2}{\tau_2} & 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ -\frac{f_{N_H}}{\tau_{N_H}} & 0 & 0 & \cdots & 1/\tau_{N_H} \end{bmatrix} \quad (S7)$$

where $\tau_i$ is the mean residence time of protein proton $i$ and $f_i$ is the mole ratio of proton $i$ to water protons. Note that the two internal water protons are treated as a part of the protein in the same way as labile protons. The $Q$ matrix is

$$Q = \begin{bmatrix} R_{1W} & 0 \\ 0 & R_{1P} \end{bmatrix} \quad (S8)$$

where $R_{1W}$ is the frequency-independent relaxation rate of external water (with fast exchange between the bulk and surface regions) and the relaxation matrix of the $N_H = 610$ protein protons is given by

$$R_{1P} = \begin{bmatrix} \rho_1 & \sigma_{1,2} & \cdots & \sigma_{1,N_H} \\ \sigma_{2,1} & \rho_2 & \cdots & \sigma_{2,N_H} \\ \vdots & \vdots & \ddots & \vdots \\ \sigma_{N_H,1} & \sigma_{N_H,2} & \cdots & \rho_{N_H} \end{bmatrix} \quad (S9)$$
The auto-relaxation rates $\rho_i$ and cross-relaxation rates $\sigma_{ij}$ are\(^9\)

$$\rho_i = \sum_{j=1}^{N_H} \left[ 0.1 J_{ij}(\omega_{0i} - \omega_{0j}) + 0.3 J_{ij}(\omega_{0i}) + 0.6 J_{ij}(\omega_{0i} + \omega_{0j}) \right]$$  \hspace{1cm} (S10a)

$$\sigma_{ij} = 0.6 J_{ij}(\omega_{0i} + \omega_{0j}) - 0.1 J_{ij}(\omega_{0i} - \omega_{0j})$$  \hspace{1cm} (S10b)

For the difference frequencies, we used the proton chemical shifts reported for mUb\(^{16}\). Coherent spin diffusion among protein proteins was also included\(^{15}\), but it was found to have a negligible effect on $R_1$.

The spectral density function for proton pair $ij$ was expressed on the model-free form

$$J_{ij}(\omega) = D_{ij}^2 \left[ S_{ij}^2 \frac{\tau_{ij}}{1 + (\omega \tau_{ij})^2} + (1 - S_{ij}^2) \frac{\tau_{\text{int,ij}}}{1 + (\omega \tau_{\text{int,ij}})^2} \right]$$  \hspace{1cm} (S11)

with the correlation times defined as

$$\frac{1}{\tau_{ij}} = \frac{1}{\tau_i} + \frac{1}{\tau_j}$$  \hspace{1cm} (S12)

$$\frac{1}{\tau_{\text{int,ij}}} = \frac{1}{\tau_{\text{int}}} + \frac{1}{\tau_{ij}}$$  \hspace{1cm} (S13)

For the fit in Fig. 2b, we set the order parameter $S_{ij}$ to a common value $S_{\text{int}}$ for all pairs $ij$ that include at least one labile proton, whereas $S_{ij} = 1$ for other pairs. We thus model, in a crude way, a protein with internal flexibility near the surface but not in the core. The dipole coupling constant in eq (S11) was calculated as

$$D_{ij} = \frac{\mu_0}{4 \pi} \hbar \gamma_H^2 \frac{1}{R_{ij}^3}$$  \hspace{1cm} (S14)

with the H–H separations, $R_{ij}$, obtained from the crystal structure 1UBQ of mUb\(^{10}\).
The residence times, $\tau_i$, were assigned as follows. For nonexchangeable protons, $\tau_i$ was set to $\infty$. For the single internal water molecule, we used $\tau_i = 20\text{ ns}$ and $S_{ij} = 0.6$ as determined by $^{17}$O MRD.1 (When $i$ and $j$ both belong to the internal water molecule, $\tau_{ij} = \tau_i$ and eq (S12) does not apply.) For COOH protons (only 0.8 per protein at pH 5.3), we used $\tau_i = 0.2\mu$s.1,17 Neither the internal water protons nor the COOH protons contribute significantly to $R_1$ at low frequencies. On the basis of the known order of magnitude for exchange rate constants for labile protons in proteins, it is clear that proton exchange catalysis by $\text{H}_3\text{O}^+$ and $\text{OH}^-$ plays a negligible role here. At pH 5.3, the dominant mechanism of proton exchange in the hydroxyl groups of the 11 Ser, Thr and Tyr residues in mUb is acid catalysis by PIPES buffer and glutaraldehyde reaction products. We model this by writing $\tau_i = \kappa_i/k_0$, where the pseudo-first-order exchange rate constant $k_0$ is an adjustable parameter and the protection factors $\kappa_i$ were randomly assigned values in the range 1.5 – 20, as for $\text{H}_3\text{O}^+$ catalyzed proton exchange in the hydroxyl groups of BPTI.18

The fit of the EMOR model described above to the H-mUb data in Fig. 2b involved three adjustable parameters. The resulting values are $k_0 = (1.2 \pm 0.1) \times 10^5\text{ s}^{-1}$, $S_{\text{int}} = 0.47 \pm 0.01$ and $\tau_{\text{int}} = 2.7 \pm 0.4\mu$s. To predict the MRD profile for D-mUb, we used these values with the following two modifications of the EMOR protocol.9 First, the frequencies in eqs (S10) were computed as

$$\omega_{0i} = (1 + \delta_i) \omega_0 \left( 1 - \nu_i + \nu_i \frac{\gamma_D}{\gamma_H} \right)$$

(S15)

where $\omega_0$ is the nominal $^1\text{H}$ frequency, $\delta_i$ the chemical shift of proton (or deuteron) $i$, and the isotope index $\nu_i$ is 0 if $i = \text{H}$ and 1 if $i = \text{D}$. Second, the spectral densities $J_{ij}(\omega)$ in eq (S10a) were multiplied by the factors

$$C_{ij} = \left[ 1 - \nu_i + \nu_i \left( \frac{\gamma_D}{\gamma_H} \right)^2 \right] \left[ 1 - \nu_j + \nu_j \frac{8}{3} \left( \frac{\gamma_D}{\gamma_H} \right)^2 \right]$$

(S16)

In eq (S10b) for the cross-relaxation rates, $J_{ij}(\omega)$ was instead multiplied by $C_{ji}$. 

S12
References


Entry 5387 in the Biological Magnetic Resonance Data Bank (www.bmrb.wisc.edu).