High-resolution relaxometry of ubiquitin

Supporting Information

Nanosecond timescale motions in proteins revealed
by high-resolution NMR relaxometry

Cyril Charlier,†,‡ Shahid Nawaz Khan,†,‡ Thorsten Marquardsen,§ Philippe Pelupessy,† Volker Reiss,§ Dimitris Sakellariou,†,|| Geoffrey Bodenhausen,†,⊥ Frank Engelke,§ and Fabien Ferrage*,†

†Ecole Normale Supérieure, Département de Chimie, UMR 7203 CNRS-UPMC-ENS, Laboratoire des Biomolécules, 24 rue Lhomond, 75231 Paris Cedex 05, France
§Bruker BioSpin GmbH, Silberstreifen 4, D 76287 Rheinstetten, Germany
|| Laboratoire Structure et Dynamique par Résonance Magnétique, UMR 3299-SIS2M CEA/CNRS, IRAMIS, DSM, CEA Saclay, F-91191, Gif-sur-Yvette Cedex, France
††Institut des Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, BCH, 1015 Lausanne, Switzerland

1 Corresponding author:
Fabien.Ferrage@ens.fr
Tel: +33 1 44 32 33 43
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1. Detailed presentation of the shuttle system

Probe

The probe uses two coils to generate radiofrequency (rf) fields ($B_1$). We chose saddle coils that allow a vertical shuttle motion of the sample and resemble coils used in standard high-resolution probes. The outer coil is matched to the $^1$H frequency, in order to minimize mismatching of the $^1$H channel that may arise from inaccurate positioning of the shuttle and from interactions between the sample and the electric component of the rf field. This outer coil is doubly tuned for $^1$H (observation) and for $^2$H (field-frequency lock). The inner coil is doubly tuned for $^{13}$C and $^{15}$N and oriented in such a manner that the rf fields of the inner and outer coils are orthogonal; see Fig. S1 B and C.

Special care was taken (Fig. S1B) to attenuate vibrations arising from shocks when the shuttle is stopped suddenly at the upper and lower ends of its displacement. Careful investigations of these effects were necessary to obtain lineshapes of similar quality as in standard high-resolution NMR spectroscopy. A shielded z-gradient coil (Fig. S1 A and B) allows one to use standard NMR experiments.

![Figure S1: Schematic views of the upper part of the shuttle probe. A. Complete view. B. Exploded view. C. Coil assembly with amorphous quartz shuttle container. (1) Shuttle touchdown pad. (2) Lower attenuating connector. (3) Lower insert. (4) Thermal glass shield. (5) Guiding glass tube and outer rf coil. (6) Guiding glass tube and inner rf coil. (7) Shuttle protection glass tube. (8) Glass gauge. (9) Vibration damper. (10) Upper insert and second attenuating connector. (11) Z-gradient coil.](image)

A vertical metal tube runs from the bottom of the probe to a position just below the rf coils. This tube is used to insert or eject the shuttle container and to guide the shuttle stopper and
the shuttle touchdown pad (Fig. S1B (1) top.) The shuttle stopper is fixed with two springs at the bottom of the coaxial tube. The shuttle stopper and spring serve as shock absorbers that center the shuttle container with respect to the rf coils (Fig. S1C). The “lower attenuating connector” marks the transition between the central tube and the detection area at the top of the probe (Fig. S1B (2).) A sensor was integrated in the lower attenuating connector to detect the mechanical position of the shuttle container. A shuttle protection glass tube ensures that the shuttle container is properly aligned (Fig. S1B (7).) The interconnection between the top of the probe and the shuttle transfer system consists of an upper insert and a second attenuating connector (Fig. S1B (10).) An O-ring (Fig. S1B (9)) inserted between the gradient system (Fig. S1B (11)) and the upper insert further improves vibration damping.

The design of the rf circuit is similar to many high-resolution probes. Low-susceptibility and/or susceptibility-compensated materials were selected, particularly for the coil and capacitor wires and all surrounding components. As a result, the spectral resolution and line shape were comparable to those of a standard 600 MHz high-resolution probe. However, as expected, the NMR sensitivity of the shuttle probe is reduced by about an order of magnitude due to the lower filling factor and reduced volume of the sample.

**Shuttle transfer system**

The shuttle transfer system allows one to stop the shuttle at a predetermined position at a chosen height in the stray field above the magnetic center. The shuttle guide consists of a tube connected to the top of the probe at its lower end, and to the top of the cryostat of the main magnet at its upper end. At the top, a second tube, coaxial with the shuttle guide, is equipped with a ‘stopper’ that prevents the shuttle container to move beyond a well-defined position. This inner tube has been isolated from the outer one and a damping system has been designed to reduce vibrations and shock-waves from the shuttle motion and sudden stop. Inside the stopper a second optical sensor has been integrated to detect the precise position of the shuttle in the low field. The position of the stopper was measured before and after the experiments to ensure that the value of the magnetic field $B_0^{\text{low}}$ was constant during the course of the experiment.
Shuttle controller unit

The pneumatic shuttle control consists of a main unit and two satellite units (Fig. S2). The main unit is equipped with a microcontroller board which allows communication with the NMR workstation and the spectrometer console, controls the valves and pressures, and allows one to determine the position of the sample. In addition, a pneumatically driven vacuum pump generates a suitable (under) pressure. The two satellites are equipped with a proportional pressure valve that controls the shuttle motion and an optical sensor to detect the end positions of the shuttle. The main unit is designed to be installed either next to the NMR workstation or to the spectrometer console, while the two satellite units should be close to the magnet, one at the bottom, close to the shuttle probe, and the other one at the top, close to the shuttle transfer system.

A simple script file is used to program the shuttle controller. This file lists pressure settings for the transfers and at the two static positions. The pressure settings are kept constant during each phase. The shuttle motion is activated by the TCU (timing control unit) of the spectrometer and information from the optical sensors about the positions (top for low field, bottom for high field) is sent back to the spectrometer. The shuttle controller creates a report in the form of a table with the timing of all shuttle motions that can be displayed by the NMR workstation.
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Shuttle container

The shuttle system was developed to withstand fast motions and strong shocks caused by sudden stops. This is particularly important for the sample container (Fig. S3). A special synthetic amorphous quartz was chosen for the glass parts (4) - (7) and a high performance polyimide resin for the caps (1) + (2). Two O-rings (3) were integrated in the caps as dampers to reduce shocks to the glass body. All of these materials have a low magnetic susceptibility to reduce distortions of NMR signals.

Figure S3: Schematic view of shuttle container. A. Exploded view of all parts; B. Assembled shuttle container with glass parts and sample in blue; (1) End plug; (2) Glass connector; (3) Shock and vibration damper; (4) Bottom glass plug; (5) Shuttle glass tube; (6) Inner glass capillary; (7) Top glass capillary; (V1) active sample volume. (V2) Total sample volume. C. Details of the upper part of the shuttle container: (V3) Sample reservoir and bubble catcher; (8) Glue seal.
The total sample volume (V2) is about is 110 µL, while the active volume (V1) of the sample is about 60 µL. Once filled, the container is sealed with glue. The shuttle container has a “bubble catcher” with a volume of about 10 µL to confine air bubbles that may appear in the sample and to accommodate thermal expansion of the sample. Bubbles appearing in the active volume of the sample can be easily centrifuged into the bubble catcher through a thin capillary. In our hands the samples are stable for several hours, and bubbles are predominantly confined in the bubble catcher.

2. Temperature Control

The sample temperature was monitored using chemical shifts differences of a few selected pairs of residues in $^1$H-$^{15}$N HSQC spectra of ubiquitin: $\delta(^1$H N L8)-$\delta(^1$H N V5), $\delta(^1$H N L8)-$\delta(^1$H N I44), $\delta(^1$H N L8)-$\delta(^1$H N H68). These differences in chemical shifts were then fitted to a linear function of the temperature. The table below summarizes the temperatures of all experiments performed in our study.

| Experiment | Field (T) | Concentration (mM) | Temperature (K) |
|------------|-----------|--------------------|-----------------|
| R1         | 0.5       | 3                  | 296.33          |
| R1         | 0.74      | 3                  | 297.09          |
| R1         | 1         | 3                  | 297.20          |
| R1         | 1.4       | 3                  | 296.06          |
| R1         | 2         | 3                  | 296.26          |
| R1         | 3         | 3                  | 296.59          |
| R1         | 5         | 3                  | 296.95          |
| R1         | 14.1      | 0.2                | 296.39          |
| R1         | 14.1      | 0.2                | 296.45          |
| NOE        | 14.1      | 0.2                | 296.52          |
| $\eta_{xy}$ | 14.1  | 0.2                | 296.39          |
| $\eta_z$ | 14.1      | 0.2                | 296.56          |
| R1         | 14.1      | 3                  | 296.62          |
| R2         | 14.1      | 3                  | 296.41          |
| NOE        | 14.1      | 3                  | 296.42          |
| $\eta_{xy}$ | 14.1  | 3                  | 296.64          |
| $\eta_z$ | 14.1      | 3                  | 296.64          |
| R1         | 18.8      | 3                  | 296.38          |
| R2         | 18.8      | 3                  | 296.46          |
| NOE        | 18.8      | 3                  | 296.38          |
| $\eta_{xy}$ | 18.8  | 3                  | 296.49          |
| $\eta_z$ | 18.8      | 3                  | 296.46          |
The experiments at \( B_0 = 22.3 \) T (950 MHz for \(^1\text{H}\)) were acquired at \( T = 298.5 \) K, about 2 K higher than all other experiments. In order to correct for this temperature difference we used experiments at 14.1T as a reference. Using a full set of relaxation rates (\( R_1, R_2, \text{NOE}, \) longitudinal \( \eta_z \) and transverse \( \eta_{xy} \) cross-correlations) we estimated the overall correlation times at \( B_0 = 14.1 \) T and \( B_0 = 22.3 \) T. We assigned the variation of \( \tau_c \) to the change of viscosity of water with temperature. Taking this effect into account, we corrected the experimental relaxation rates observed at 22.3 T prior to analysis of the relaxation rates in terms of dynamics.

### 3. The ICARUS protocol

ICARUS is a MATLAB package for the Iterative Correction and Analysis of Relaxation rates Under Shuttling which permits a quantitative analysis in terms of local dynamics of longitudinal relaxation rates (\(^{15}\text{N} R_1\)) recorded at a series of low magnetic fields. In order to achieve this, ICARUS is adapted from a well-known program developed for the analysis of high-field nitrogen-15 relaxation, using model-free or extended model-free spectral density functions. This package uses both \textsc{ROTDIF}\(^1\) to obtain overall tumbling parameters and \textsc{DYNAMICS}\(^2\) to fit microdynamics parameters (\( S^2, \tau_c, \tau_{loc}, S^2, \tau_t \)).

#### Spectral density functions

The spectral density functions \( J(\omega) \) used are:

(i) Model-free: \( J(\omega) = \frac{S^2 \tau_c}{(1 + \omega^2 \tau_c^2)} + \frac{(1 - S^2) \tau_e}{(1 + \omega^2 \tau_e^2)} \) (S1)

where \( S^2 \) is the generalized order parameter, \( \tau_c \) the overall isotropic rotational correlation time of the molecule, and \( \tau_e = \tau_c \tau_{loc}/(\tau_c + \tau_{loc}) \) where \( \tau_{loc} \) is a single effective correlation time that describes all internal motions.
(ii) Extended model-free:  \[ J(\omega) = \frac{S^2\tau_c}{(1 + (\omega \tau_c)^2)} + \frac{(1-S^2)\tau_f^{\text{eff}}}{(1 + (\omega \tau_f^{\text{eff}})^2)} + \frac{(S^2-S^2)\tau_s^{\text{eff}}}{(1 + (\omega \tau_s^{\text{eff}})^2)} \] (S2)

with  \( \tau_f^{\text{eff}} = \tau_c \tau_f / (\tau_c + \tau_f) \) and  \( \tau_s^{\text{eff}} = \tau_c \tau_s / (\tau_c + \tau_s) \) where  \( \tau_f \) (respectively  \( \tau_s \)) is the correlation time for fast (respectively slow) internal motions. The generalized order parameter can be expressed as a product  \( S^2 = S_f^2 S_s^2 \) where  \( S_f^2 \) and  \( S_s^2 \) are generalized order parameters describing fast and slow motions. The models used in simulations of spin dynamics by ICARUS always correspond to the model selected in the previous step by DYNAMICS.

**Spin System**

All simulations of spin dynamics were carried out for the following spin system comprising one nitrogen-15 and 3 protons with the following distances and interactions:

![Spin System Diagram]

The relaxation matrix  \( \hat{R} \) was approximated by:

\[
\hat{R} = \begin{pmatrix}
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
-\theta_H & \rho_H & \sigma_{NH} & \delta_H & \sigma & 0 & 0 \\
-\theta_N & \sigma_{NH} & \rho_N & \delta_N & 0 & 0 & 0 \\
-\theta_{NH} & \delta_H & \delta_N & \rho_{NH} & 0 & \sigma & 0 \\
-\theta_{H}^l & \sigma & 0 & 0 & \rho_{H}^l & 0 & 0 \\
-\theta_{NH}^l & 0 & 0 & \sigma & 0 & \rho_{NH}^l & 0 \\
-\theta_{H}^w & \sigma & 0 & 0 & 0 & \rho_{H}^w & 0 \\
-\theta_{NH}^w & 0 & 0 & \sigma & 0 & 0 & \rho_{NH}^w
\end{pmatrix} \] (S3)

with 3:
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\[ \rho_H = \frac{1}{10} c_{NH}^2 \left[ J(\omega_H - \omega_N) + 3J(\omega_H) + 6J(\omega_H + \omega_N) \right] \]
\[ + \frac{2}{15} \left[ c_{Hxx}^2 + c_{Hy}^2 - c_{Hxx}c_{Hy} \right] J(\omega_H) \]
\[ + \frac{2}{10} c_{HH}^2 J(0) + 3J(\omega_H) + 6J(2\omega_H) \]

\[ \rho_N = \frac{1}{10} c_{NH}^2 \left[ J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_N - \omega_N) \right] + \frac{2}{15} c_{NN}^2 J(\omega_N) \]

\[ \rho_{NH} = \frac{3}{10} c_{NH}^2 J(\omega_N) + \frac{2}{15} c_{NH}^2 J(\omega_H) + \frac{3}{10} c_{NH}^2 J(\omega_H) \]
\[ + \frac{2}{15} \left[ c_{Hxx}^2 + c_{Hy}^2 - c_{Hxx}c_{Hy} \right] J(\omega_H) \]
\[ + \frac{2}{10} c_{HH}^2 J(0) + 3J(\omega_H) + 6J(2\omega_H) \]

\[ \rho_{NN}^i = \frac{1}{10} c_{NH}^2 \left[ 3J(\omega_N) \right] + \frac{2}{15} c_{NH}^2 J(\omega_N) + \frac{1}{10} c_{NH}^2 [3J(\omega_H)] \]
\[ + \frac{2}{15} \left[ c_{Hxx}^2 + c_{Hy}^2 - c_{Hxx}c_{Hy} \right] J(\omega_H) \]
\[ + \frac{1}{10} c_{HH}^2 J(0) + 3J(\omega_H) + 6J(2\omega_H) \]

\[ \rho_{HH}^i = \frac{1}{10} c_{NH}^2 \left[ 3J(\omega_H) \right] + \frac{2}{15} c_{NH}^2 J(\omega_H) + \frac{1}{10} c_{NH}^2 [3J(\omega_H)] \]
\[ + \frac{2}{15} \left[ c_{Hxx}^2 + c_{Hy}^2 - c_{Hxx}c_{Hy} \right] J(\omega_H) \]
\[ + \frac{1}{10} c_{HH}^2 J(0) + 3J(\omega_H) + 6J(2\omega_H) \]

\[ \rho_{NN}^w = \frac{1}{10} c_{NH}^2 \left[ 3J(\omega_N) + \frac{2}{15} c_{NH}^2 J(\omega_N) + \frac{1}{10} c_{NH}^2 (3J(\omega_H)) \right] \]
\[ + \frac{2}{15} \left[ c_{Hxx}^2 + c_{Hy}^2 - c_{Hxx}c_{Hy} \right] J(\omega_H) \]
\[ + \frac{1}{10} c_{HH}^2 J(0) + 3J(\omega_H) + 6J(2\omega_H) \]

\[ \rho_{HH}^w = \frac{1}{10} c_{NH}^2 \left[ 3J(\omega_H) + \frac{2}{15} c_{NH}^2 J(\omega_H) + \frac{1}{10} c_{NH}^2 (3J(\omega_H)) \right] \]
\[ + \frac{2}{15} \left[ c_{Hxx}^2 + c_{Hy}^2 - c_{Hxx}c_{Hy} \right] J(\omega_H) \]
\[ + \frac{1}{10} c_{HH}^2 J(0) + 3J(\omega_H) + 6J(2\omega_H) \]
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\[
\sigma_{NH} = \frac{1}{10} c_{NH}^2 \left[ 6J(\omega_H + \omega_N) - J(\omega_H - \omega_N) \right]
\]

\[
\delta_H = -\frac{4}{10} c_{NH} \left[ c_{Hxx} J(\omega_H)_{xx} + c_{Hyy} J(\omega_H)_{yy} \right]
\]

\[
\delta_N = -\frac{4}{10} c_{NH} c_I(\omega_N) c
\]

\[
\sigma = \frac{1}{10} c_{HH}^2 [J(2\omega_H) - J(0)]
\]

\[
\theta_N = \theta_H = \theta_{NH} = \theta_{iH} = \theta_{wH} = \theta_{wNH} = \theta_{wNH} = 0;
\]

\[
J(\omega_H)_{xx} = \frac{3(\cos(\beta_{Hxx}))^2 - 1}{2} J(\omega_H); J(\omega_H)_{yy} = \frac{3(\cos(\beta_{Hyy}))^2 - 1}{2} J(\omega_H)
\]

(S4)

where \(c_{NH} = \mu_0 \gamma_H \gamma_N h / (8\pi^2 d_{NH}^3)\), \(c_{HH} = (\mu_0 \gamma_H^2 h / 8\pi^2 d_{HH}^3)\), \(c_N = \Delta \sigma_N \omega_N\), \(c_{Hxx} = \omega_H (\sigma_{Hxx} - \sigma_{Hzz})\) and \(c_{Hyy} = \omega_H (\sigma_{Hyy} - \sigma_{Hzz})\); \(\mu_0\) is the permittivity of free space; \(\gamma_H (\gamma_N)\) is the gyromagnetic ratio of the proton (respectively of the nitrogen-15 nucleus); \(h\) is Planck’s constant; \(\omega_H (\omega_N)\) is the Larmor frequency of the proton (respectively of the nitrogen-15); \(\Delta \sigma_N (= 160 \times 10^6 \text{ppm})\) is the average value of the anisotropy of the \(^{15}\text{N}\) chemical shift (CSA); \(\sigma_{Hxx} (= 14.6 \times 10^6 \text{ppm})\), \(\sigma_{Hyy} (= 8.2 \times 10^6 \text{ppm})\) and \(\sigma_{Hzz} (= 2.1 \times 10^6 \text{ppm})\) are the main components of the \(^1\text{H}\) CSA tensors; \(d_{NH} (= 1.02 \text{Å})\) is the internuclear nitrogen-hydrogen distance; \(d_{HH} (= 2.1 \text{Å})\) is the effective distance between the proton H and the other two protons (\(^1\text{H}_i\) and \(^1\text{H}_w\)). \(\beta_{Hxx} (= 90\pi/180)\) and \(\beta_{Hyy} (= 99\pi/180)\) are the angles between the NH vector and the respective components of the \(^1\text{H}\) CSA tensors.

**First step of ICARUS**

In a first step, ROTDIF and DYNAMICS are used to obtain hydrodynamic and microdynamic parameters from high-field relaxation data (\(^{15}\text{N} R_1, R_2\) and NOE at 14.1 T, 18.8 T and 22.3 T) only (TABLE S2. see below). Using ROTDIF, the parameters of the overall rotational diffusion tensor for an axially symmetric model were estimated from relaxation rates at 14.1 T (0.2 mM or 3 mM), 18.8 T (3 mM) and 22.3 T (3 mM) (Table S2.).
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### Table S2: Parameters for the overall rotational diffusion tensor

| Magnetic Field | 14.1 T | 14.1 T | 18.8 T | 22.3 T* |
|----------------|--------|--------|--------|---------|
| Concentration  | 0.2 mM | 3 mM   | 3 mM   | 3 mM    |
| $\tau_c = (6\text{Tr}(D))^3$ | 4.22 ± 0.15 ns | 4.89 ± 0.1 ns | 4.84 ± 0.2 ns | 4.84 ± 0.16 ns |
| $D_{\text{par}}/D_{\text{per}}$ | 1.22 ± 0.08 | 1.22 ± 0.04 | 1.18 ± 0.08 | 1.20 ± 0.07 |
| $\theta$       | 112° ± 12° | 112° ± 7° | 120° ± 15° | 119° ± 12° |
| $\phi$         | 157° ± 29° | 157° ± 15° | 155° ± 36° | 157° ± 27° |

* Analysis carried out with temperature-corrected rates as explained in text.

**Iterative analysis with ICARUS**

The dynamic parameters obtained in the first step are used next to calculate the evolution of each spin system during the transfer between the high- and low-field positions as well as during the relaxation and stabilization delays (see Figure 3).

The elements of the relaxation matrix depend on the magnetic field. The transfer durations shown in table S3 and Figure S4 have been determined by using optical sensors. The position of the shuttle is described by a trajectory with a short lag time at the starting position followed by motion at a constant speed of about 11 m/s. There is an additional pre-shuttling delay of 26 ms in high field. In the simulations, the time-dependence of the populations is integrated along the trajectory from the high- to the low-field positions.

To simulate relaxation during the transfer, a time-dependent relaxation matrix is derived as a function of the position of the shuttle. The same approach is employed for relaxation during the back-transfer from the low- to the high-field position, with a somewhat lower speed, also assumed to be constant, of about 6.5 m/s. Relaxation during the stay at the low-field position is also simulated. An additional 40 ms delay accounts for the minimum duration of the stay at the low-field position. The last step is the calculation of the relaxation of the spin system during the 100 ms stabilization delay once the shuttle has returned to the high field position.
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**Figure S4:** Correlation between the height above the high-field position and the shuttling time. The speed of the shuttle is higher for the upward motion \((v = 11 \text{ m/s})\) than for the downward motion \((v = 6.5 \text{ m/s})\). The correlation confirms that the velocity is constant after the initial time-lag. (In practice, relaxometry data were often recorded with slightly different pneumatic settings, which explains small variations between these data and those presented in Table S3.)

| \(B_0 (\text{T})\) | Position (cm) | Shuttling time (ms) |
|-------------------|---------------|---------------------|
| 5                 | 27.1          | 41.1                |
| 3                 | 31.1          | 44.1                |
| 2                 | 34.4          | 46.4                |
| 1.4               | 37.35         | 48.3                |
| 1                 | 39.8          | 49.9                |
| 0.75              | 42.55         | 51.6                |
| 0.5               | 46.2          | 53.7                |

All simulated relaxation decays were fitted to mono-exponential functions. The deviations with respect to calculated longitudinal relaxation rates of nitrogen-15 nuclei are used to correct the experimental relaxation rates. These rates are used, along with high field relaxation data \((^{15}\text{N} R_1\text{ and } ^{15}\text{N}\{^1\text{H}\} \text{ NOE})\) as input for DYNAMICS in the subsequent steps of the ICARUS analysis.

The convergence of the analysis is fast for most residues, although up to four steps may be required for all residues to converge. The final set of microdynamic parameters is given in
Table S11. A flow-chart describing the program is shown in Figure S5.

**Error evaluation**

The determination of relaxation rates in low fields is more challenging than in high fields. Our analysis takes some of the complexity of the spin systems into account, with approximate evaluations of cross-relaxation pathways involving a manifold of interactions (proton-proton dipole-dipole couplings, CSA tensors…). It is unlikely that spectral noise is the main source of errors. In order to evaluate systematic errors, we implemented a jack-knife procedure. We have carried out the ICARUS analysis for 7 sets of data. Each data set included the longitudinal relaxation rates $R_1$ and NOE’s at high fields (14.1, 18.8, and 22.3 T) as well as the longitudinal relaxation rates at 6 of the 7 low fields. Figures 4, S6-9 and Table S10 show averages of the values obtained in the 7 ICARUS analyses of the jack-knife procedure. The errors are equal to the standard deviations of these datasets multiplied by $\sqrt{7-1}$.

**Graphical output**

After the final iteration of ICARUS, the user can visualize the updated results of DYNAMICS and the latest set of corrections. Microdynamic parameters $S^2$ and $S^2_f$, $\tau_{loc}$, $R_{ex}$, and the model selected in DYNAMICS are indicated. In order to verify the quality of the analysis, the $R_1$ values that have been ‘back-calculated’ by ICARUS can be compared with experimental rates corrected for all residues at all fields (one figure for each field $B_0$). The other series of plots displays $R_1$ values calculated by ICARUS and corrected experimental data at all fields for all residues.
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**Figure S5**: Flowchart for ICARUS.
4. Order parameters and timescales of motions

Order parameters

Figure S6: Comparison of order parameters in human ubiquitin. (Black) Order parameters derived from the analysis of an accelerated molecular dynamics trajectory\(^4\). (Green) Order parameters from the analysis of relaxation rates measured at 10 different fields.

Figure S7: Comparison of order parameters in human ubiquitin. (Red) Order parameters \(S^2\) obtained from our analysis of relaxation rates at 14.1 T only. (Blue) Order parameters derived from the analysis of a different set of nitrogen-15 relaxation rates at 14.1 T.\(^4\) (Green) Order parameters of fast motions for an extended model-free spectral density function in the analysis by DYNAMICS of relaxation rates recorded at 14.1 T only.
Timescales of local motions

Figure S8: Timescales $\tau_{\text{loc}}$ of local motions determined by the analysis of relaxation rates measured at 10 different fields. The timescales displayed correspond either to $\tau_{\text{loc}}$ for basic model-free spectral density functions, or to $\tau_s$ for extended model-free spectral density functions. The large error bars reflect the instabilities of the selected model when applying a jack-knife analysis.

Figure S9: Timescales $\tau_{\text{loc}}$ of local motions determined by the analysis of relaxation rates measured at 10 different fields. The data are the same as in Fig. S8, we only show residues for which $\tau_{\text{loc}}$ is significant, with $\Delta \tau_{\text{loc}} < \tau_{\text{loc}}/3$. 

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Transverse relaxation

Figure S10: Contribution of chemical exchange rates $R_{ex}$ to transverse relaxation at 14.1 T obtained with three different methods: (Green) method based on the measurement of longitudinal and transverse cross-relaxation due to cross-correlation of fluctuations of the $^{15}$N chemical shift anisotropy and the dipolar coupling with the attached proton$^5$; (Red) $R_{ex}$ fitted by a conventional analysis of relaxation at 14.1, 18.8, and 22.3 T with the program DYNAMICS; (Blue) $R_{ex}$ calculated from transverse relaxation rates with the microdynamic parameters obtained in the ICARUS analysis of relaxation rates observed at 10 magnetic fields.
Comparison of order parameters

**Figure S11:** Comparison of the order parameters of backbone N-H vectors in ubiquitin obtained through a variety of methods. The order parameters obtained from the analysis of relaxation at 10 magnetic fields are shown on the x-axis for all plots. The y-axis displays order parameters obtained by the following methods: (a) the analysis of residual dipolar couplings measured in 23 different alignment media with the SCRM approach;\(^6\) (b) the analysis of 36 sets of dipolar couplings with the SF-GAF approach;\(^7\) (c) an accelerated molecular dynamics trajectory of ubiquitin;\(^8\) and (d) our analysis of relaxation at 14.1 T.

**Table S4:** Statistics

| Method              | RMSD  | Correlation coefficient | Regression         |
|---------------------|-------|-------------------------|---------------------|
| SCRM vs Relaxometry | 0.0730| 0.725                   | y = 0.161+0.753x    |
| GAF vs Relaxometry  | 0.0771| 0.660                   | y = 0.235+0.726x    |
| AMD vs Relaxometry  | 0.0481| 0.913                   | y = 0.086+0.948x    |
Effective distance to neighboring protons

\[ \sum 1/d_i^6 \text{ (Å}^6\text{)} \]

Figure S12: Total effect of proton-proton dipolar interactions for amide protons in ubiquitin. We show, for each amide proton the sum \( \sum 1/d_i^6 \), where \( d_i \) are the distances with all other protons in the protein (the first model in the pdb file 1d3z was used). For comparison, horizontal lines show the corresponding value \( 2/(d_{HH})^6 \) for the effective distances in the spin system with two neighboring protons used in the ICARUS procedure. Note that the value used in ICARUS, \( d_{HH} = 2.1 \text{ Å} \), yields \( 2/(d_{HH})^6 = 0.023 \text{ Å}^6 \), which is very close to \( 0.025 \text{ Å}^6 \), the median of the distribution of values of \( \sum 1/d_i^6 \) in ubiquitin.
High-resolution relaxometry of ubiquitin

**Figure S13:** Dependence of order parameters upon the effective distance of neighboring protons employed in the ICARUS analysis. (Top) Order parameters obtained with $d_{HH} = 2.1$ Å (same as Fig. 4) are shown in purple, other curves represent order parameters obtained with $d_{HH} = 1.7, 1.8, 1.9, 2.2, 2.4,$ and $2.6$ Å. With few exceptions, the order parameters increase with increasing values of $d_{HH}$. (Bottom) Order parameters obtained with $d_{HH} = 2.1$ Å (same as Fig. 4) are shown in blue, with error intervals obtained from the jackknife analysis of ICARUS. The grey region shows the interval between the maximum and minimum order parameters obtained in the ICARUS analysis with the following values of $d_{HH} = 1.7, 1.8, 1.9, 2.0, 2.2, 2.4,$ and $2.6$ Å.
5. Relaxation data and parameters of local dynamics

Figure S14: Relaxation decays and mono-exponential fits of a few signals at three representative magnetic fields. Results of a single relaxation experiment (total duration 11 to 13 hours) are shown for residues Thr9 (left); Asp52 (center); and Gly75 (right) at the following magnetic fields $B_{0 \text{low}}$: 5 T (top); 1.4 T (center); and 0.5 T (bottom). The solid lines show the results of mono-exponential fit of the relaxation decays.
High-resolution relaxometry of ubiquitin

**Table S5:** High field $^{15}$N relaxation rates for 3mM ubiquitin (pH = 4.5; see table S1 for sample temperature)

| Residue | $R_1$ (s$^{-1}$) | $R_2$ (s$^{-1}$) | NOE |
|---------|----------------|----------------|-----|
| 2       | 1.907 ± 0.029  | 6.775 ± 0.102  | 0.731 ± 0.011 |
| 3       | 2.058 ± 0.031  | 6.752 ± 0.101  | 0.766 ± 0.011 |
| 4       | 2.106 ± 0.032  | 7.038 ± 0.106  | 0.739 ± 0.011 |
| 5       | 1.973 ± 0.030  | 6.432 ± 0.096  | 0.727 ± 0.011 |
| 6       | 2.046 ± 0.031  | 6.662 ± 0.100  | 0.766 ± 0.011 |
| 7       | 2.014 ± 0.030  | 6.604 ± 0.099  | 0.720 ± 0.011 |
| 8       | 1.986 ± 0.030  | 5.936 ± 0.089  | 0.630 ± 0.009 |
| 9       | 1.876 ± 0.028  | 5.889 ± 0.088  | 0.594 ± 0.009 |
| 10      | 1.963 ± 0.029  | 5.930 ± 0.089  | 0.588 ± 0.009 |
| 11      | 1.809 ± 0.027  | 5.787 ± 0.087  | 0.581 ± 0.009 |
| 12      | 1.863 ± 0.028  | 6.057 ± 0.091  | 0.678 ± 0.010 |
| 13      | 1.991 ± 0.030  | 6.634 ± 0.100  | 0.713 ± 0.011 |
| 14      | 1.933 ± 0.029  | 6.771 ± 0.102  | 0.774 ± 0.012 |
| 15      | 2.072 ± 0.031  | 6.528 ± 0.098  | 0.759 ± 0.011 |
| 16      | 1.832 ± 0.027  | 6.270 ± 0.094  | 0.725 ± 0.011 |
| 17      | 2.025 ± 0.030  | 6.829 ± 0.102  | 0.768 ± 0.012 |
| 18      | 1.890 ± 0.028  | 6.832 ± 0.102  | 0.706 ± 0.011 |
| 19      | 1.997 ± 0.030  | 6.702 ± 0.101  | 0.728 ± 0.011 |
| 20      | 2.057 ± 0.031  | 6.669 ± 0.100  | 0.733 ± 0.011 |
| 21      | 2.109 ± 0.032  | 8.377 ± 0.126  | 0.776 ± 0.012 |
| 22      | 2.074 ± 0.031  | 10.104 ± 0.152 | 0.759 ± 0.011 |
| 23      | 2.084 ± 0.031  | 6.900 ± 0.103  | 0.748 ± 0.011 |
| 24      | 2.126 ± 0.032  | 7.198 ± 0.108  | 0.778 ± 0.012 |
| 25      | 2.066 ± 0.031  | 6.987 ± 0.105  | 0.791 ± 0.012 |
| 26      | 2.080 ± 0.031  | 6.956 ± 0.104  | 0.751 ± 0.011 |
| 27      | 2.056 ± 0.031  | 6.952 ± 0.104  | 0.764 ± 0.011 |
| 28      | 1.965 ± 0.029  | 6.661 ± 0.100  | 0.741 ± 0.011 |
| 29      | 1.944 ± 0.029  | 6.590 ± 0.099  | 0.741 ± 0.011 |
| 30      | 1.955 ± 0.029  | 7.010 ± 0.105  | 0.762 ± 0.011 |
| 31      | 1.725 ± 0.026  | 6.399 ± 0.096  | 0.732 ± 0.011 |
| 32      | 2.048 ± 0.031  | 6.495 ± 0.097  | 0.750 ± 0.011 |
| 33      | 2.026 ± 0.030  | 6.776 ± 0.102  | 0.738 ± 0.011 |
| 34      | 2.044 ± 0.031  | 6.585 ± 0.099  | 0.731 ± 0.011 |
| 35      | 2.001 ± 0.030  | 6.534 ± 0.098  | 0.763 ± 0.011 |
| 36      | 1.992 ± 0.030  | 6.601 ± 0.099  | 0.742 ± 0.011 |
| 37      | 1.996 ± 0.030  | 6.633 ± 0.099  | 0.724 ± 0.011 |
| 38      | 2.020 ± 0.030  | 6.926 ± 0.104  | 0.729 ± 0.011 |
| 39      | 2.015 ± 0.030  | 6.472 ± 0.097  | 0.726 ± 0.011 |
High-resolution relaxometry of ubiquitin

|   |        |        |        |        |
|---|--------|--------|--------|--------|
| 47 | 1.934 ± 0.029 | 6.252 ± 0.094 | 0.748 ± 0.011 |
| 48 | 1.930 ± 0.029 | 6.986 ± 0.105 | 0.748 ± 0.011 |
| 49 | 1.859 ± 0.028 | 6.126 ± 0.092 | 0.656 ± 0.010 |
| 50 | 2.029 ± 0.030 | 6.713 ± 0.101 | 0.724 ± 0.011 |
| 51 | 1.880 ± 0.028 | 6.572 ± 0.099 | 0.713 ± 0.011 |
| 52 | 1.795 ± 0.027 | 6.636 ± 0.100 | 0.755 ± 0.011 |
| 53 | 1.920 ± 0.029 | 6.922 ± 0.104 | 0.748 ± 0.011 |
| 54 | 1.996 ± 0.030 | 7.020 ± 0.105 | 0.729 ± 0.011 |
| 55 | 2.124 ± 0.032 | 6.861 ± 0.103 | 0.766 ± 0.011 |
| 56 | 2.060 ± 0.031 | 6.654 ± 0.100 | 0.760 ± 0.011 |
| 57 | 2.017 ± 0.030 | 6.500 ± 0.098 | 0.767 ± 0.012 |
| 58 | 2.042 ± 0.031 | 6.783 ± 0.102 | 0.754 ± 0.011 |
| 59 | 2.035 ± 0.031 | 6.797 ± 0.102 | 0.758 ± 0.011 |
| 60 | 1.795 ± 0.027 | 5.666 ± 0.085 | 0.602 ± 0.009 |
| 61 | 1.892 ± 0.028 | 6.830 ± 0.102 | 0.754 ± 0.011 |
| 62 | 2.100 ± 0.031 | 6.659 ± 0.100 | 0.745 ± 0.011 |
| 63 | 2.036 ± 0.031 | 6.851 ± 0.103 | 0.770 ± 0.012 |
| 64 | 1.963 ± 0.029 | 6.634 ± 0.100 | 0.734 ± 0.011 |
| 65 | 2.033 ± 0.030 | 6.504 ± 0.098 | 0.791 ± 0.012 |
| 66 | 1.979 ± 0.030 | 6.683 ± 0.100 | 0.773 ± 0.012 |
| 67 | 2.072 ± 0.031 | 7.572 ± 0.114 | 0.746 ± 0.011 |
| 68 | 1.963 ± 0.029 | 6.265 ± 0.094 | 0.718 ± 0.011 |
| 69 | 1.612 ± 0.024 | 2.921 ± 0.044 | 0.091 ± 0.001 |
| 70 | 1.270 ± 0.019 | 1.924 ± 0.029 | -0.319 ± -0.005 |
| 71 | 0.871 ± 0.013 | 1.295 ± 0.019 | -0.785 ± -0.012 |
High-resolution relaxometry of ubiquitin

Table S5 (continued): High-field $^{15}$N relaxation data for 3 mM ubiquitin (pH = 4.5; see table S1 for sample temperature)

| Residue | $R_1$ (s$^{-1}$) | $R_2$ (s$^{-1}$) | NOE |
|---------|------------------|------------------|-----|
| 2       | 1.458 ± 0.022    | 7.698 ± 0.115    | 0.785 ± 0.0035 |
| 3       | 1.575 ± 0.024    | 7.866 ± 0.118    | 0.826 ± 0.0039 |
| 4       | 1.617 ± 0.024    | 7.615 ± 0.114    | 0.832 ± 0.0042 |
| 5       | 1.490 ± 0.022    | 7.399 ± 0.111    | 0.811 ± 0.0038 |
| 6       | 1.580 ± 0.024    | 7.652 ± 0.115    | 0.821 ± 0.0043 |
| 7       | 1.552 ± 0.023    | 7.296 ± 0.109    | 0.805 ± 0.0037 |
| 8       | 1.575 ± 0.024    | 7.152 ± 0.107    | 0.718 ± 0.0031 |
| 9       | 1.514 ± 0.023    | 7.015 ± 0.105    | 0.657 ± 0.0032 |
| 10      | 1.534 ± 0.023    | 6.667 ± 0.100    | 0.689 ± 0.0031 |
| 11      | 1.482 ± 0.022    | 6.767 ± 0.101    | 0.643 ± 0.0026 |
| 12      | 1.474 ± 0.022    | 7.355 ± 0.110    | 0.730 ± 0.0033 |
| 13      | 1.554 ± 0.023    | 7.822 ± 0.117    | 0.801 ± 0.0044 |
| 14      | 1.482 ± 0.022    | 7.541 ± 0.113    | 0.816 ± 0.0039 |
| 15      | 1.584 ± 0.024    | 7.133 ± 0.107    | 0.814 ± 0.0037 |
| 16      | 1.360 ± 0.020    | 7.435 ± 0.112    | 0.784 ± 0.0037 |
| 17      | 1.533 ± 0.023    | 8.011 ± 0.120    | 0.797 ± 0.0039 |
| 18      | 1.418 ± 0.021    | 7.817 ± 0.117    | 0.796 ± 0.0041 |
| 19      | 1.539 ± 0.023    | 7.649 ± 0.115    | 0.805 ± 0.0036 |
| 20      | 1.579 ± 0.024    | 8.696 ± 0.130    | 0.811 ± 0.0037 |
| 21      | 1.660 ± 0.025    | 11.580 ± 0.174   | 0.825 ± 0.0043 |
| 22      | 1.610 ± 0.024    | 10.524 ± 0.158   | 0.830 ± 0.0037 |
| 23      | 1.623 ± 0.024    | 8.086 ± 0.121    | 0.837 ± 0.0033 |
| 24      | 1.609 ± 0.024    | 8.253 ± 0.124    | 0.838 ± 0.0033 |
| 25      | 1.577 ± 0.024    | 7.967 ± 0.119    | 0.819 ± 0.0034 |
| 26      | 1.618 ± 0.024    | 8.003 ± 0.120    | 0.824 ± 0.0035 |
| 27      | 1.590 ± 0.024    | 7.873 ± 0.118    | 0.827 ± 0.0030 |
| 28      | 1.522 ± 0.023    | 7.427 ± 0.111    | 0.813 ± 0.0033 |
| 29      | 1.506 ± 0.023    | 7.776 ± 0.117    | 0.807 ± 0.0041 |
| 30      | 1.496 ± 0.022    | 7.686 ± 0.115    | 0.825 ± 0.0039 |
| 31      | 1.330 ± 0.020    | 7.287 ± 0.109    | 0.816 ± 0.0044 |
| 32      | 1.585 ± 0.024    | 7.644 ± 0.115    | 0.806 ± 0.0028 |
| 33      | 1.569 ± 0.024    | 7.750 ± 0.116    | 0.813 ± 0.0038 |
| 34      | 1.549 ± 0.023    | 7.460 ± 0.112    | 0.805 ± 0.0036 |
| 35      | 1.480 ± 0.022    | 7.358 ± 0.110    | 0.818 ± 0.0040 |
| 36      | 1.485 ± 0.022    | 7.329 ± 0.110    | 0.811 ± 0.0046 |
| 37      | 1.547 ± 0.023    | 7.557 ± 0.113    | 0.807 ± 0.0042 |
| 38      | 1.546 ± 0.023    | 7.542 ± 0.113    | 0.844 ± 0.0043 |
| 39      | 1.560 ± 0.023    | 7.239 ± 0.109    | 0.799 ± 0.0039 |
| 40      | 1.478 ± 0.022    | 7.544 ± 0.113    | 0.783 ± 0.0039 |
High-resolution relaxometry of ubiquitin

|   | T1 (s) | T2 (ms) | T1/T2 | T1/τc | T2/τc |
|---|---|---|---|---|---|
| 48 | 1.50 ± 0.023 | 7.379 ± 0.111 | 0.790 ± 0.0034 |
| 49 | 1.477 ± 0.022 | 7.179 ± 0.108 | 0.742 ± 0.0030 |
| 50 | 1.595 ± 0.024 | 7.487 ± 0.112 | 0.795 ± 0.0039 |
| 51 | 1.44 ± 0.022 | 7.532 ± 0.113 | 0.771 ± 0.0043 |
| 52 | 1.352 ± 0.020 | 7.937 ± 0.119 | 0.785 ± 0.0029 |
| 54 | 1.482 ± 0.022 | 8.350 ± 0.125 | 0.813 ± 0.0036 |
| 55 | 1.538 ± 0.023 | 8.263 ± 0.124 | 0.803 ± 0.0043 |
| 56 | 1.637 ± 0.025 | 7.891 ± 0.118 | 0.837 ± 0.0037 |
| 57 | 1.582 ± 0.024 | 7.828 ± 0.117 | 0.827 ± 0.0030 |
| 59 | 1.553 ± 0.023 | 7.617 ± 0.114 | 0.804 ± 0.0037 |
| 60 | 1.571 ± 0.024 | 7.778 ± 0.117 | 0.823 ± 0.0035 |
| 61 | 1.593 ± 0.024 | 7.083 ± 0.106 | 0.819 ± 0.0039 |
| 62 | 1.403 ± 0.021 | 7.150 ± 0.107 | 0.643 ± 0.0034 |
| 63 | 1.442 ± 0.022 | 7.665 ± 0.115 | 0.811 ± 0.0030 |
| 64 | 1.596 ± 0.024 | 7.679 ± 0.115 | 0.812 ± 0.0043 |
| 65 | 1.580 ± 0.024 | 7.634 ± 0.115 | 0.824 ± 0.0031 |
| 66 | 1.459 ± 0.022 | 7.602 ± 0.114 | 0.820 ± 0.0039 |
| 67 | 1.525 ± 0.023 | 7.625 ± 0.114 | 0.817 ± 0.0043 |
| 68 | 1.499 ± 0.022 | 8.454 ± 0.127 | 0.818 ± 0.0041 |
| 70 | 1.558 ± 0.023 | 8.212 ± 0.123 | 0.822 ± 0.0046 |
| 71 | 1.526 ± 0.023 | 5.315 ± 0.080 | 0.768 ± 0.0034 |
| 74 | 1.448 ± 0.022 | 2.762 ± 0.041 | 0.323 ± 0.0018 |
| 75 | 1.242 ± 0.019 | 1.722 ± 0.026 | 0.020 ± 0.0017 |
| 76 | 0.852 ± 0.013 | 0.702 ± 0.011 | -0.345 ± 0.0016 |
High-resolution relaxometry of ubiquitin

Table S5 (continued): High-field $^1$Hrelaxation rates for 3 mM ubiquitin (pH = 4.5; see table S1 for sample temperature)

| residue | $R_1$ (s$^{-1}$) | $R_2$ (s$^{-1}$) | NOE |
|---------|------------------|------------------|-----|
| 2       | 1.332 ± 0.007    | 8.327 ± 0.042    | 0.810 ± 0.008 |
| 3       | 1.440 ± 0.007    | 8.343 ± 0.043    | 0.850 ± 0.009 |
| 4       | 1.450 ± 0.007    | 8.630 ± 0.038    | 0.855 ± 0.009 |
| 5       | 1.307 ± 0.007    | 7.629 ± 0.041    | 0.852 ± 0.009 |
| 6       | 1.429 ± 0.007    | 8.291 ± 0.041    | 0.845 ± 0.008 |
| 7       | 1.405 ± 0.007    | 8.231 ± 0.037    | 0.824 ± 0.008 |
| 8       | 1.463 ± 0.007    | 7.468 ± 0.038    | 0.772 ± 0.008 |
| 9       | 1.398 ± 0.007    | 7.552 ± 0.037    | 0.709 ± 0.007 |
| 10      | 1.401 ± 0.007    | 7.360 ± 0.037    | 0.718 ± 0.007 |
| 11      | 1.392 ± 0.007    | 7.426 ± 0.036    | 0.704 ± 0.007 |
| 12      | 1.325 ± 0.007    | 7.277 ± 0.044    | 0.768 ± 0.008 |
| 13      | 1.391 ± 0.007    | 8.849 ± 0.042    | 0.827 ± 0.008 |
| 14      | 1.350 ± 0.007    | 8.439 ± 0.039    | 0.838 ± 0.008 |
| 15      | 1.445 ± 0.007    | 7.844 ± 0.037    | 0.842 ± 0.008 |
| 16      | 1.245 ± 0.006    | 7.462 ± 0.043    | 0.818 ± 0.008 |
| 17      | 1.382 ± 0.007    | 8.563 ± 0.043    | 0.831 ± 0.008 |
| 18      | 1.282 ± 0.006    | 8.648 ± 0.039    | 0.824 ± 0.008 |
| 19      | 1.357 ± 0.007    | 7.777 ± 0.041    | 0.832 ± 0.008 |
| 20      | 1.409 ± 0.007    | 8.249 ± 0.055    | 0.836 ± 0.008 |
| 21      | 1.509 ± 0.008    | 10.935 ± 0.076   | 0.859 ± 0.009 |
| 22      | 1.475 ± 0.007    | 15.127 ± 0.042   | 0.861 ± 0.009 |
| 23      | 1.456 ± 0.007    | 8.312 ± 0.046    | 0.850 ± 0.009 |
| 24      | 1.452 ± 0.007    | 9.134 ± 0.043    | 0.861 ± 0.009 |
| 25      | 1.422 ± 0.007    | 8.616 ± 0.042    | 0.863 ± 0.009 |
| 26      | 1.455 ± 0.007    | 8.381 ± 0.044    | 0.851 ± 0.009 |
| 27      | 1.446 ± 0.007    | 8.737 ± 0.041    | 0.857 ± 0.009 |
| 28      | 1.381 ± 0.007    | 8.281 ± 0.039    | 0.830 ± 0.008 |
| 29      | 1.348 ± 0.007    | 7.785 ± 0.046    | 0.832 ± 0.008 |
| 30      | 1.331 ± 0.007    | 9.115 ± 0.041    | 0.851 ± 0.009 |
| 31      | 1.213 ± 0.006    | 8.115 ± 0.040    | 0.852 ± 0.009 |
| 32      | 1.470 ± 0.007    | 8.001 ± 0.042    | 0.836 ± 0.008 |
| 33      | 1.406 ± 0.007    | 8.370 ± 0.041    | 0.841 ± 0.008 |
| 34      | 1.401 ± 0.007    | 8.215 ± 0.038    | 0.836 ± 0.008 |
| 35      | 1.366 ± 0.007    | 7.692 ± 0.041    | 0.837 ± 0.008 |
| 36      | 1.327 ± 0.007    | 8.114 ± 0.039    | 0.849 ± 0.008 |
| 37      | 1.348 ± 0.007    | 7.738 ± 0.044    | 0.854 ± 0.009 |
| 38      | 1.412 ± 0.007    | 8.761 ± 0.039    | 0.859 ± 0.009 |
| 39      | 1.393 ± 0.007    | 7.788 ± 0.037    | 0.818 ± 0.008 |
| 40      | 1.380 ± 0.007    | 7.357 ± 0.043    | 0.809 ± 0.008 |
High-resolution relaxometry of ubiquitin

|   |   |   |   |   |
|---|---|---|---|---|
| 48 | 1.387 ± 0.007 | 8.613 ± 0.037 | 0.820 ± 0.008 |
| 49 | 1.316 ± 0.007 | 7.315 ± 0.041 | 0.775 ± 0.008 |
| 50 | 1.412 ± 0.007 | 8.242 ± 0.040 | 0.832 ± 0.008 |
| 51 | 1.269 ± 0.006 | 7.906 ± 0.041 | 0.817 ± 0.008 |
| 52 | 1.234 ± 0.006 | 8.122 ± 0.045 | 0.812 ± 0.008 |
| 54 | 1.346 ± 0.007 | 8.998 ± 0.049 | 0.829 ± 0.008 |
| 55 | 1.387 ± 0.007 | 9.727 ± 0.043 | 0.828 ± 0.008 |
| 56 | 1.493 ± 0.007 | 8.547 ± 0.041 | 0.846 ± 0.008 |
| 57 | 1.472 ± 0.007 | 8.254 ± 0.044 | 0.842 ± 0.008 |
| 59 | 1.374 ± 0.007 | 7.818 ± 0.042 | 0.850 ± 0.009 |
| 60 | 1.445 ± 0.007 | 8.398 ± 0.042 | 0.843 ± 0.008 |
| 61 | 1.431 ± 0.007 | 8.329 ± 0.035 | 0.837 ± 0.008 |
| 62 | 1.293 ± 0.006 | 7.007 ± 0.041 | 0.682 ± 0.007 |
| 63 | 1.305 ± 0.007 | 8.294 ± 0.040 | 0.817 ± 0.008 |
| 64 | 1.445 ± 0.007 | 7.998 ± 0.042 | 0.851 ± 0.009 |
| 65 | 1.451 ± 0.007 | 8.433 ± 0.040 | 0.846 ± 0.008 |
| 66 | 1.327 ± 0.007 | 8.007 ± 0.042 | 0.845 ± 0.008 |
| 67 | 1.378 ± 0.007 | 8.431 ± 0.040 | 0.835 ± 0.008 |
| 68 | 1.329 ± 0.007 | 8.087 ± 0.052 | 0.841 ± 0.008 |
| 70 | 1.430 ± 0.007 | 10.393 ± 0.039 | 0.838 ± 0.008 |
| 71 | 1.413 ± 0.007 | 7.739 ± 0.016 | 0.798 ± 0.008 |
| 74 | 1.415 ± 0.007 | 3.152 ± 0.011 | 0.432 ± 0.004 |
| 75 | 1.237 ± 0.006 | 2.165 ± 0.007 | 0.181 ± 0.002 |
| 76 | 0.858 ± 0.004 | 1.432 ± 0.004 | -0.196 ± 0.004 |
## High-resolution relaxometry of ubiquitin

**Table S6:** High-field \(^{15}\)N relaxation rates for 0.2 mM ubiquitin (pH = 4.5; see table S1 for sample temperature)

| Residue | \(R_1\) (s\(^{-1}\)) | \(R_2\) (s\(^{-1}\)) | NOE |
|---------|----------------|----------------|------|
| 2       | 2.042 ± 0.006 | 5.972 ± 0.064 | 0.689 ± 0.020 |
| 3       | 2.131 ± 0.007 | 6.095 ± 0.072 | 0.700 ± 0.023 |
| 4       | 2.350 ± 0.009 | 6.556 ± 0.085 | 0.721 ± 0.027 |
| 5       | 2.103 ± 0.008 | 5.809 ± 0.073 | 0.787 ± 0.024 |
| 6       | 2.207 ± 0.008 | 5.967 ± 0.077 | 0.723 ± 0.024 |
| 7       | 2.196 ± 0.007 | 5.970 ± 0.070 | 0.711 ± 0.023 |
| 8       | 2.133 ± 0.006 | 5.402 ± 0.057 | 0.601 ± 0.018 |
| 9       | 1.906 ± 0.007 | 5.352 ± 0.067 | 0.557 ± 0.019 |
| 10      | 2.075 ± 0.006 | 5.113 ± 0.058 | 0.556 ± 0.018 |
| 11      | 1.930 ± 0.005 | 5.077 ± 0.049 | 0.587 ± 0.016 |
| 12      | 1.982 ± 0.006 | 5.433 ± 0.062 | 0.632 ± 0.020 |
| 13      | 2.155 ± 0.008 | 6.173 ± 0.080 | 0.780 ± 0.026 |
| 14      | 2.093 ± 0.007 | 5.983 ± 0.069 | 0.709 ± 0.022 |
| 15      | 2.219 ± 0.008 | 5.729 ± 0.071 | 0.718 ± 0.023 |
| 16      | 1.941 ± 0.006 | 5.556 ± 0.062 | 0.703 ± 0.022 |
| 17      | 2.171 ± 0.008 | 6.403 ± 0.076 | 0.688 ± 0.023 |
| 18      | 2.145 ± 0.007 | 5.976 ± 0.073 | 0.706 ± 0.023 |
| 19      | 2.103 ± 0.007 | 5.919 ± 0.067 | 0.692 ± 0.020 |
| 20      | 2.197 ± 0.007 | 5.757 ± 0.070 | 0.739 ± 0.022 |
| 21      | 2.278 ± 0.008 | 7.109 ± 0.085 | 0.750 ± 0.022 |
| 22      | 2.232 ± 0.007 | 8.959 ± 0.082 | 0.776 ± 0.022 |
| 23      | 2.242 ± 0.006 | 6.052 ± 0.060 | 0.742 ± 0.020 |
| 24      | 2.245 ± 0.007 | 6.637 ± 0.065 | 0.787 ± 0.021 |
| 25      | 2.183 ± 0.006 | 6.150 ± 0.062 | 0.768 ± 0.021 |
| 26      | 2.191 ± 0.007 | 6.362 ± 0.067 | 0.728 ± 0.020 |
| 27      | 2.206 ± 0.005 | 6.202 ± 0.052 | 0.741 ± 0.018 |
| 28      | 2.088 ± 0.006 | 5.883 ± 0.057 | 0.722 ± 0.019 |
| 29      | 2.063 ± 0.008 | 6.067 ± 0.075 | 0.662 ± 0.025 |
| 30      | 2.118 ± 0.007 | 6.252 ± 0.074 | 0.707 ± 0.021 |
| 31      | 1.849 ± 0.008 | 5.819 ± 0.092 | 0.670 ± 0.038 |
| 32      | 2.196 ± 0.005 | 5.768 ± 0.048 | 0.718 ± 0.016 |
| 33      | 2.168 ± 0.007 | 5.990 ± 0.070 | 0.718 ± 0.023 |
| 34      | 2.129 ± 0.007 | 5.793 ± 0.069 | 0.711 ± 0.023 |
| 35      | 2.149 ± 0.008 | 5.768 ± 0.074 | 0.802 ± 0.026 |
| 36      | 2.184 ± 0.009 | 5.742 ± 0.085 | 0.697 ± 0.027 |
| 37      | 2.161 ± 0.008 | 5.698 ± 0.078 | 0.744 ± 0.026 |
| 38      | 2.296 ± 0.008 | 6.456 ± 0.077 | 0.663 ± 0.024 |
| 39      | 2.156 ± 0.007 | 5.830 ± 0.071 | 0.660 ± 0.021 |
| 40      | 2.062 ± 0.007 | 5.677 ± 0.069 | 0.731 ± 0.022 |
High-resolution relaxometry of ubiquitin

| 48 | 2.060 ± 0.006 | 6.281 ± 0.060 | 0.708 ± 0.021 |
| 49 | 1.995 ± 0.005 | 5.418 ± 0.050 | 0.625 ± 0.018 |
| 50 | 2.194 ± 0.008 | 5.693 ± 0.075 | 0.748 ± 0.024 |
| 51 | 2.027 ± 0.008 | 5.857 ± 0.083 | 0.774 ± 0.027 |
| 52 | 1.921 ± 0.005 | 5.844 ± 0.051 | 0.737 ± 0.017 |
| 54 | 2.042 ± 0.007 | 6.203 ± 0.068 | 0.751 ± 0.023 |
| 55 | 2.157 ± 0.009 | 6.388 ± 0.085 | 0.737 ± 0.025 |
| 56 | 2.267 ± 0.008 | 5.988 ± 0.073 | 0.759 ± 0.022 |
| 57 | 2.211 ± 0.006 | 6.006 ± 0.056 | 0.794 ± 0.018 |
| 59 | 2.156 ± 0.008 | 5.820 ± 0.074 | 0.778 ± 0.025 |
| 60 | 2.134 ± 0.007 | 5.882 ± 0.064 | 0.702 ± 0.019 |
| 61 | 2.175 ± 0.007 | 6.058 ± 0.069 | 0.745 ± 0.023 |
| 62 | 1.891 ± 0.006 | 4.981 ± 0.066 | 0.516 ± 0.019 |
| 63 | 2.016 ± 0.005 | 5.948 ± 0.050 | 0.749 ± 0.018 |
| 64 | 2.208 ± 0.009 | 5.717 ± 0.080 | 0.719 ± 0.024 |
| 65 | 2.192 ± 0.006 | 5.942 ± 0.059 | 0.787 ± 0.020 |
| 66 | 2.086 ± 0.008 | 5.724 ± 0.078 | 0.705 ± 0.024 |
| 67 | 2.184 ± 0.009 | 5.798 ± 0.084 | 0.700 ± 0.024 |
| 68 | 2.163 ± 0.008 | 5.941 ± 0.077 | 0.732 ± 0.025 |
| 70 | 2.166 ± 0.009 | 6.689 ± 0.088 | 0.764 ± 0.028 |
| 71 | 2.115 ± 0.006 | 5.707 ± 0.058 | 0.716 ± 0.022 |
| 74 | 1.611 ± 0.003 | 2.829 ± 0.029 | 0.069 ± 0.012 |
| 75 | 1.265 ± 0.003 | 1.853 ± 0.028 | -0.287 ± 0.012 |
| 76 | 0.856 ± 0.002 | 1.141 ± 0.022 | -0.866 ± 0.013 |
High-resolution relaxometry of ubiquitin

| residue | 5 T | 3 T | 2 T | 1.4 T |
|---------|-----|-----|-----|-------|
|         | $R_s$ (s$^{-1}$) | $R_i$ (s$^{-1}$) | $R_s$ (s$^{-1}$) | $R_i$ (s$^{-1}$) |
| 2       | 4.169 ± 0.089 | 5.796 ± 0.108 | 7.364 ± 0.158 | 8.539 ± 0.151 |
| 3       | 4.469 ± 0.107 | 6.066 ± 0.128 | 7.598 ± 0.190 | 9.534 ± 0.197 |
| 4       | 4.531 ± 0.140 | 6.074 ± 0.150 | 7.719 ± 0.217 | 9.630 ± 0.258 |
| 5       | 4.122 ± 0.103 | 5.769 ± 0.127 | 7.453 ± 0.189 | 9.079 ± 0.191 |
| 6       | 4.418 ± 0.113 | 5.943 ± 0.138 | 7.310 ± 0.194 | 9.608 ± 0.218 |
| 7       | 4.259 ± 0.102 | 5.788 ± 0.118 | 7.744 ± 0.178 | 9.180 ± 0.183 |
| 8       | 4.025 ± 0.081 | 5.374 ± 0.094 | 6.927 ± 0.136 | 8.270 ± 0.125 |
| 9       | 3.702 ± 0.075 | 4.982 ± 0.084 | 6.179 ± 0.114 | 7.286 ± 0.113 |
| 10      | 3.672 ± 0.067 | 4.985 ± 0.080 | 6.245 ± 0.109 | 7.443 ± 0.112 |
| 11      | 3.475 ± 0.060 | 4.806 ± 0.067 | 5.759 ± 0.088 | 7.083 ± 0.086 |
| 12      | 3.779 ± 0.077 | 5.448 ± 0.092 | 6.378 ± 0.125 | 8.028 ± 0.132 |
| 13      | 4.058 ± 0.111 | 5.872 ± 0.136 | 7.345 ± 0.196 | 9.223 ± 0.204 |
| 14      | 4.206 ± 0.094 | 5.818 ± 0.112 | 7.358 ± 0.160 | 9.198 ± 0.168 |
| 15      | 4.242 ± 0.102 | 5.951 ± 0.125 | 7.259 ± 0.177 | 9.298 ± 0.195 |
| 16      | 4.156 ± 0.091 | 5.724 ± 0.107 | 7.234 ± 0.156 | 8.775 ± 0.156 |
| 17      | 4.296 ± 0.108 | 5.963 ± 0.132 | 7.637 ± 0.195 | 9.044 ± 0.198 |
| 18      | 4.476 ± 0.127 | 6.201 ± 0.132 | 7.577 ± 0.190 | 9.454 ± 0.204 |
| 19      | 4.226 ± 0.092 | 5.869 ± 0.107 | 7.233 ± 0.155 | 9.015 ± 0.152 |
| 20      | 4.335 ± 0.104 | 6.054 ± 0.122 | 7.283 ± 0.174 | 8.986 ± 0.176 |
| 21      | 4.480 ± 0.124 | 6.373 ± 0.159 | 7.368 ± 0.226 | 9.338 ± 0.232 |
| 22      | 4.568 ± 0.105 | 6.209 ± 0.127 | 7.508 ± 0.185 | 8.983 ± 0.182 |
| 23      | 4.507 ± 0.098 | 6.036 ± 0.117 | 7.586 ± 0.174 | 9.467 ± 0.168 |
| 24      | 4.669 ± 0.105 | 6.058 ± 0.126 | 7.790 ± 0.188 | 9.579 ± 0.184 |
| 25      | 4.428 ± 0.101 | 6.160 ± 0.126 | 7.853 ± 0.187 | 9.188 ± 0.175 |
| 26      | 4.753 ± 0.112 | 6.106 ± 0.133 | 7.510 ± 0.194 | 9.909 ± 0.195 |
| 27      | 4.479 ± 0.090 | 6.034 ± 0.108 | 7.557 ± 0.161 | 9.087 ± 0.146 |
| 28      | 4.090 ± 0.088 | 5.767 ± 0.105 | 7.144 ± 0.149 | 8.831 ± 0.148 |
| 29      | 4.229 ± 0.121 | 5.993 ± 0.142 | 7.535 ± 0.209 | 9.450 ± 0.224 |
| 30      | 4.258 ± 0.123 | 6.359 ± 0.151 | 8.600 ± 0.242 | 9.934 ± 0.234 |
| 31      | 3.720 ± 0.126 | 5.679 ± 0.113 | 6.940 ± 0.159 | 8.229 ± 0.350 |
| 32      | 4.162 ± 0.078 | 5.711 ± 0.091 | 7.057 ± 0.130 | 8.544 ± 0.124 |
| 33      | 4.452 ± 0.109 | 6.085 ± 0.130 | 7.600 ± 0.189 | 8.873 ± 0.189 |
| 34      | 4.351 ± 0.106 | 5.931 ± 0.128 | 7.659 ± 0.190 | 8.960 ± 0.188 |
| 35      | 4.347 ± 0.116 | 6.214 ± 0.137 | 7.530 ± 0.196 | 9.170 ± 0.204 |
| 36      | 4.436 ± 0.121 | 5.873 ± 0.148 | 7.776 ± 0.219 | 9.532 ± 0.240 |
| 37      | 4.434 ± 0.120 | 6.077 ± 0.148 | 7.874 ± 0.224 | 9.840 ± 0.237 |
| 38      | 4.206 ± 0.113 | 5.992 ± 0.143 | 7.641 ± 0.217 | 10.185 ± 0.239 |
| 39      | 4.102 ± 0.099 | 5.711 ± 0.120 | 6.980 ± 0.167 | 8.782 ± 0.168 |
High-resolution relaxometry of ubiquitin

|   | 4.128 ± 0.096 | 5.669 ± 0.115 | 6.932 ± 0.163 | 8.544 ± 0.160 |
|---|---------------|---------------|---------------|---------------|
| 47| 4.075 ± 0.084 | 5.627 ± 0.102 | 7.338 ± 0.148 | 8.785 ± 0.152 |
| 48| 3.831 ± 0.073 | 5.397 ± 0.087 | 6.941 ± 0.123 | 7.811 ± 0.114 |
| 49| 4.430 ± 0.119 | 6.142 ± 0.143 | 7.766 ± 0.213 | 8.868 ± 0.205 |
| 50| 4.224 ± 0.102 | 5.935 ± 0.138 | 7.445 ± 0.203 | 9.336 ± 0.219 |
| 51| 4.236 ± 0.078 | 5.847 ± 0.096 | 7.151 ± 0.135 | 8.602 ± 0.125 |
| 52| 4.166 ± 0.094 | 5.835 ± 0.115 | 7.053 ± 0.158 | 8.760 ± 0.166 |
| 53| 4.305 ± 0.119 | 6.082 ± 0.140 | 7.565 ± 0.204 | 9.161 ± 0.207 |
| 54| 4.490 ± 0.123 | 6.020 ± 0.148 | 7.577 ± 0.214 | 10.043 ± 0.220 |
| 55| 4.363 ± 0.091 | 5.966 ± 0.103 | 7.058 ± 0.141 | 8.986 ± 0.140 |
| 56| 4.293 ± 0.109 | 5.796 ± 0.133 | 7.347 ± 0.188 | 9.266 ± 0.198 |
| 57| 4.172 ± 0.098 | 5.675 ± 0.118 | 7.286 ± 0.176 | 8.711 ± 0.173 |
| 58| 4.489 ± 0.112 | 5.630 ± 0.131 | 7.687 ± 0.203 | 9.392 ± 0.193 |
| 59| 3.786 ± 0.086 | 5.192 ± 0.102 | 6.598 ± 0.141 | 7.869 ± 0.143 |
| 60| 4.359 ± 0.083 | 5.981 ± 0.096 | 7.574 ± 0.140 | 9.198 ± 0.130 |
| 61| 4.446 ± 0.131 | 6.203 ± 0.160 | 7.576 ± 0.238 | 9.757 ± 0.244 |
| 62| 4.159 ± 0.087 | 5.829 ± 0.104 | 7.080 ± 0.145 | 8.815 ± 0.145 |
| 63| 4.179 ± 0.106 | 5.800 ± 0.126 | 7.370 ± 0.182 | 9.020 ± 0.196 |
| 64| 4.304 ± 0.121 | 5.960 ± 0.149 | 7.404 ± 0.212 | 9.017 ± 0.223 |
| 65| 4.331 ± 0.118 | 6.142 ± 0.146 | 7.489 ± 0.205 | 8.743 ± 0.206 |
| 66| 4.452 ± 0.122 | 6.170 ± 0.150 | 7.849 ± 0.224 | 9.181 ± 0.225 |
| 67| 4.043 ± 0.078 | 5.357 ± 0.095 | 7.011 ± 0.139 | 8.357 ± 0.143 |
| 68| 2.425 ± 0.034 | 3.155 ± 0.033 | 3.844 ± 0.040 | 4.585 ± 0.038 |
| 69| 1.695 ± 0.027 | 2.216 ± 0.026 | 2.495 ± 0.028 | 2.897 ± 0.027 |
| 70| 1.106 ± 0.020 | 1.350 ± 0.018 | 1.442 ± 0.019 | 1.664 ± 0.018 |
High-resolution relaxometry of ubiquitin

Table S7 (continued): Uncorrected low-field $^1$H relaxation rates for 3 mM ubiquitin (pH = 4.5; see table S1 for sample temperature)

| residue | $R_1$ (s$^{-1}$) | $R_2$ (s$^{-1}$) | $R_3$ (s$^{-1}$) |
|---------|-----------------|-----------------|-----------------|
| 2       | 10.078 ± 0.227  | 12.766 ± 0.390  | 13.278 ± 0.546  |
| 3       | 10.904 ± 0.288  | 13.579 ± 0.493  | 14.810 ± 0.729  |
| 4       | 10.435 ± 0.386  | 11.813 ± 0.603  | 14.744 ± 0.851  |
| 5       | 10.798 ± 0.282  | 12.676 ± 0.475  | 14.936 ± 0.727  |
| 6       | 11.938 ± 0.320  | 12.602 ± 0.528  | 13.585 ± 0.734  |
| 7       | 10.747 ± 0.272  | 12.500 ± 0.475  | 13.677 ± 0.607  |
| 8       | 9.776 ± 0.194   | 11.654 ± 0.312  | 13.098 ± 0.452  |
| 9       | 8.494 ± 0.167   | 9.448 ± 0.260   | 10.733 ± 0.350  |
| 10      | 8.314 ± 0.165   | 11.283 ± 0.285  | 12.019 ± 0.358  |
| 11      | 8.522 ± 0.131   | 9.754 ± 0.204   | 11.095 ± 0.285  |
| 12      | 9.215 ± 0.187   | 11.467 ± 0.300  | 13.026 ± 0.445  |
| 13      | 10.596 ± 0.304  | 12.016 ± 0.492  | 14.297 ± 0.724  |
| 14      | 9.173 ± 0.233   | 12.452 ± 0.399  | 13.812 ± 0.571  |
| 15      | 10.668 ± 0.272  | 13.393 ± 0.464  | 14.535 ± 0.700  |
| 16      | 10.133 ± 0.223  | 13.153 ± 0.391  | 12.659 ± 0.539  |
| 17      | 10.148 ± 0.278  | 12.210 ± 0.466  | 14.611 ± 0.721  |
| 18      | 12.195 ± 0.340  | 13.623 ± 0.557  | 15.289 ± 0.762  |
| 19      | 10.711 ± 0.226  | 11.731 ± 0.372  | 13.815 ± 0.575  |
| 20      | 10.702 ± 0.274  | 12.863 ± 0.451  | 14.294 ± 0.649  |
| 21      | 10.847 ± 0.364  | 14.190 ± 0.617  | 14.571 ± 0.890  |
| 22      | 10.800 ± 0.287  | 13.184 ± 0.486  | 15.270 ± 0.703  |
| 23      | 11.651 ± 0.271  | 14.434 ± 0.455  | 14.048 ± 0.645  |
| 24      | 13.037 ± 0.318  | 15.125 ± 0.536  | 15.640 ± 0.740  |
| 25      | 11.281 ± 0.276  | 11.510 ± 0.435  | 15.917 ± 0.738  |
| 26      | 11.478 ± 0.289  | 13.276 ± 0.499  | 15.291 ± 0.764  |
| 27      | 10.671 ± 0.232  | 12.250 ± 0.378  | 14.780 ± 0.594  |
| 28      | 9.891 ± 0.216   | 12.756 ± 0.368  | 13.280 ± 0.541  |
| 29      | 12.065 ± 0.350  | 13.496 ± 0.577  | 15.906 ± 0.808  |
| 30      | 11.492 ± 0.338  | 13.059 ± 0.575  | 16.538 ± 1.019  |
| 31      | 11.168 ± 0.492  | 11.880 ± 0.720  | 14.936 ± 0.630  |
| 32      | 9.839 ± 0.191   | 11.588 ± 0.316  | 13.743 ± 0.475  |
| 33      | 10.459 ± 0.286  | 14.370 ± 0.512  | 14.412 ± 0.704  |
| 34      | 11.554 ± 0.303  | 14.579 ± 0.507  | 14.923 ± 0.705  |
| 35      | 10.783 ± 0.303  | 13.572 ± 0.529  | 14.220 ± 0.746  |
| 36      | 12.209 ± 0.382  | 12.618 ± 0.586  | 14.148 ± 0.844  |
| 37      | 10.646 ± 0.326  | 14.533 ± 0.588  | 14.865 ± 0.858  |
| 38      | 11.669 ± 0.376  | 13.208 ± 0.627  | 14.194 ± 0.780  |
| 39      | 11.300 ± 0.267  | 12.753 ± 0.446  | 13.046 ± 0.597  |
| 40      | 10.243 ± 0.239  | 12.560 ± 0.388  | 12.799 ± 0.571  |
High-resolution relaxometry of ubiquitin

|    |    |    |    |    |    |
|----|----|----|----|----|----|
| 48 | 10.474 ± 0.219 | 11.444 ± 0.344 | 13.543 ± 0.521 |
| 49 | 9.301 ± 0.175  | 10.732 ± 0.281  | 12.752 ± 0.415  |
| 50 | 10.648 ± 0.314  | 12.918 ± 0.520  | 15.398 ± 0.813  |
| 51 | 11.864 ± 0.347  | 13.925 ± 0.578  | 14.604 ± 0.764  |
| 52 | 10.142 ± 0.204  | 12.340 ± 0.333  | 13.541 ± 0.500  |
| 54 | 10.968 ± 0.257  | 12.467 ± 0.410  | 13.784 ± 0.600  |
| 55 | 10.929 ± 0.298  | 14.092 ± 0.540  | 14.281 ± 0.752  |
| 56 | 12.056 ± 0.353  | 13.128 ± 0.560  | 16.882 ± 0.911  |
| 57 | 10.410 ± 0.224  | 12.107 ± 0.365  | 14.043 ± 0.523  |
| 58 | 9.814 ± 0.284   | 12.521 ± 0.481  | 14.993 ± 0.740  |
| 59 | 10.225 ± 0.264  | 13.655 ± 0.446  | 14.688 ± 0.677  |
| 60 | 11.320 ± 0.295  | 13.800 ± 0.483  | 14.122 ± 0.738  |
| 61 | 8.742 ± 0.206   | 9.988 ± 0.324   | 11.689 ± 0.474  |
| 62 | 10.361 ± 0.204  | 12.870 ± 0.348  | 13.563 ± 0.499  |
| 63 | 11.408 ± 0.362  | 14.034 ± 0.604  | 16.037 ± 0.932  |
| 64 | 10.029 ± 0.218  | 11.989 ± 0.362  | 13.103 ± 0.511  |
| 65 | 11.505 ± 0.296  | 12.599 ± 0.472  | 14.716 ± 0.671  |
| 66 | 11.717 ± 0.347  | 13.427 ± 0.578  | 14.124 ± 0.817  |
| 67 | 11.412 ± 0.325  | 12.873 ± 0.516  | 15.173 ± 0.831  |
| 68 | 11.377 ± 0.333  | 12.666 ± 0.545  | 14.223 ± 0.827  |
| 70 | 9.918 ± 0.210   | 11.286 ± 0.337  | 13.055 ± 0.476  |
| 71 | 4.698 ± 0.053   | 5.851 ± 0.081   | 6.227 ± 0.096   |
| 74 | 3.191 ± 0.037   | 3.388 ± 0.055   | 3.627 ± 0.061   |
| 75 | 1.792 ± 0.024   | 1.858 ± 0.036   | 1.992 ± 0.040   |
| 76 | 10.078 ± 0.227  | 12.766 ± 0.390  | 13.278 ± 0.546  |
High-resolution relaxometry of ubiquitin

| residue | 5T $R_i$(s$^{-1}$) | 3T $R_i$(s$^{-1}$) | 2T $R_i$(s$^{-1}$) | 1.4T $R_i$(s$^{-1}$) |
|---------|---------------------|---------------------|---------------------|---------------------|
| 2       | 4.545 ± 0.097       | 6.089 ± 0.113       | 7.740 ± 0.166       | 9.141 ± 0.162       |
| 3       | 4.897 ± 0.117       | 6.374 ± 0.134       | 7.967 ± 0.199       | 10.204 ± 0.211      |
| 4       | 4.970 ± 0.153       | 6.384 ± 0.157       | 8.097 ± 0.228       | 10.310 ± 0.276      |
| 5       | 4.502 ± 0.113       | 6.056 ± 0.133       | 7.815 ± 0.198       | 9.708 ± 0.205       |
| 6       | 4.838 ± 0.124       | 6.250 ± 0.145       | 7.688 ± 0.204       | 10.308 ± 0.234      |
| 7       | 4.654 ± 0.112       | 6.082 ± 0.124       | 8.135 ± 0.187       | 9.830 ± 0.196       |
| 8       | 4.384 ± 0.089       | 5.653 ± 0.099       | 7.297 ± 0.143       | 8.867 ± 0.134       |
| 9       | 4.022 ± 0.081       | 5.238 ± 0.089       | 6.517 ± 0.120       | 7.820 ± 0.121       |
| 10      | 3.988 ± 0.073       | 5.242 ± 0.084       | 6.579 ± 0.115       | 7.974 ± 0.120       |
| 11      | 3.773 ± 0.065       | 5.056 ± 0.070       | 6.084 ± 0.093       | 7.612 ± 0.092       |
| 12      | 4.115 ± 0.084       | 5.719 ± 0.097       | 6.703 ± 0.131       | 8.597 ± 0.142       |
| 13      | 4.431 ± 0.122       | 6.170 ± 0.143       | 7.714 ± 0.206       | 9.873 ± 0.218       |
| 14      | 4.594 ± 0.103       | 6.112 ± 0.118       | 7.731 ± 0.168       | 9.853 ± 0.180       |
| 15      | 4.643 ± 0.112       | 6.263 ± 0.131       | 7.647 ± 0.186       | 9.987 ± 0.210       |
| 16      | 4.532 ± 0.099       | 6.007 ± 0.112       | 7.590 ± 0.164       | 9.386 ± 0.167       |
| 17      | 4.694 ± 0.119       | 6.271 ± 0.139       | 8.034 ± 0.205       | 9.694 ± 0.212       |
| 18      | 4.890 ± 0.139       | 6.510 ± 0.139       | 7.945 ± 0.199       | 10.111 ± 0.218      |
| 19      | 4.616 ± 0.100       | 6.166 ± 0.113       | 7.597 ± 0.162       | 9.651 ± 0.163       |
| 20      | 4.741 ± 0.113       | 6.366 ± 0.128       | 7.659 ± 0.183       | 9.632 ± 0.189       |
| 21      | 4.915 ± 0.136       | 6.700 ± 0.168       | 7.730 ± 0.237       | 10.000 ± 0.248      |
| 22      | 5.007 ± 0.115       | 6.524 ± 0.133       | 7.873 ± 0.194       | 9.615 ± 0.194       |
| 23      | 4.942 ± 0.107       | 6.342 ± 0.123       | 7.956 ± 0.183       | 10.133 ± 0.180      |
| 24      | 5.123 ± 0.115       | 6.368 ± 0.132       | 8.172 ± 0.197       | 10.258 ± 0.197      |
| 25      | 4.850 ± 0.110       | 6.481 ± 0.132       | 8.264 ± 0.197       | 9.863 ± 0.188       |
| 26      | 5.209 ± 0.123       | 6.423 ± 0.140       | 7.898 ± 0.205       | 10.631 ± 0.210      |
| 27      | 4.904 ± 0.098       | 6.337 ± 0.113       | 7.921 ± 0.168       | 9.720 ± 0.156       |
| 28      | 4.467 ± 0.096       | 6.062 ± 0.110       | 7.514 ± 0.156       | 9.467 ± 0.158       |
| 29      | 4.624 ± 0.132       | 6.293 ± 0.149       | 7.900 ± 0.219       | 10.108 ± 0.240      |
| 30      | 4.661 ± 0.135       | 6.682 ± 0.158       | 9.024 ± 0.254       | 10.634 ± 0.250      |
| 31      | 4.051 ± 0.137       | 5.956 ± 0.118       | 7.278 ± 0.167       | 8.794 ± 0.374       |
| 32      | 4.549 ± 0.085       | 6.013 ± 0.096       | 7.445 ± 0.137       | 9.185 ± 0.133       |
| 33      | 4.869 ± 0.119       | 6.395 ± 0.137       | 7.982 ± 0.199       | 9.502 ± 0.203       |
| 34      | 4.758 ± 0.116       | 6.231 ± 0.135       | 8.038 ± 0.200       | 9.590 ± 0.201       |
| 35      | 4.757 ± 0.126       | 6.524 ± 0.144       | 7.891 ± 0.206       | 9.806 ± 0.219       |
| 36      | 4.852 ± 0.133       | 6.165 ± 0.155       | 8.150 ± 0.229       | 10.194 ± 0.256      |
| 37      | 4.850 ± 0.132       | 6.382 ± 0.155       | 8.257 ± 0.235       | 10.527 ± 0.254      |
| 38      | 4.608 ± 0.123       | 6.295 ± 0.151       | 8.011 ± 0.227       | 10.897 ± 0.255      |
| 39      | 4.479 ± 0.108       | 6.001 ± 0.126       | 7.334 ± 0.175       | 9.404 ± 0.180       |
| 40      | 4.500 ± 0.104       | 5.953 ± 0.121       | 7.278 ± 0.171       | 9.141 ± 0.171       |
High-resolution relaxometry of ubiquitin

|   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|
| 48 | 4.445 ± 0.091 | 5.912 ± 0.107 | 7.713 ± 0.156 | 9.406 ± 0.163 |
| 49 | 4.163 ± 0.080 | 5.662 ± 0.091 | 7.284 ± 0.129 | 8.346 ± 0.122 |
| 50 | 4.842 ± 0.130 | 6.456 ± 0.151 | 8.158 ± 0.224 | 9.496 ± 0.220 |
| 51 | 4.609 ± 0.111 | 6.230 ± 0.144 | 7.809 ± 0.213 | 9.984 ± 0.234 |
| 52 | 4.614 ± 0.085 | 6.130 ± 0.100 | 7.491 ± 0.142 | 9.186 ± 0.133 |
| 54 | 4.550 ± 0.102 | 6.130 ± 0.120 | 7.410 ± 0.166 | 9.382 ± 0.178 |
| 55 | 4.705 ± 0.130 | 6.392 ± 0.148 | 7.947 ± 0.214 | 9.810 ± 0.222 |
| 56 | 4.927 ± 0.135 | 6.329 ± 0.155 | 7.950 ± 0.224 | 10.756 ± 0.236 |
| 57 | 4.775 ± 0.099 | 6.263 ± 0.101 | 7.395 ± 0.148 | 9.609 ± 0.150 |
| 59 | 4.691 ± 0.119 | 6.090 ± 0.140 | 7.719 ± 0.198 | 9.922 ± 0.212 |
| 60 | 4.564 ± 0.107 | 5.958 ± 0.124 | 7.635 ± 0.184 | 9.315 ± 0.185 |
| 61 | 4.915 ± 0.123 | 5.922 ± 0.138 | 8.087 ± 0.214 | 10.076 ± 0.207 |
| 62 | 4.110 ± 0.093 | 5.444 ± 0.107 | 6.924 ± 0.148 | 8.410 ± 0.153 |
| 63 | 4.760 ± 0.091 | 6.281 ± 0.101 | 7.950 ± 0.147 | 9.842 ± 0.140 |
| 64 | 4.869 ± 0.143 | 6.524 ± 0.168 | 7.964 ± 0.250 | 10.459 ± 0.262 |
| 65 | 4.547 ± 0.095 | 6.117 ± 0.110 | 7.417 ± 0.152 | 9.422 ± 0.155 |
| 66 | 4.566 ± 0.115 | 6.091 ± 0.132 | 7.735 ± 0.191 | 9.653 ± 0.209 |
| 67 | 4.712 ± 0.133 | 6.259 ± 0.157 | 7.761 ± 0.222 | 9.645 ± 0.239 |
| 68 | 4.738 ± 0.130 | 6.448 ± 0.153 | 7.847 ± 0.215 | 9.348 ± 0.221 |
| 70 | 4.874 ± 0.133 | 6.487 ± 0.158 | 8.246 ± 0.235 | 9.838 ± 0.241 |
| 71 | 4.405 ± 0.085 | 5.628 ± 0.100 | 7.369 ± 0.146 | 8.944 ± 0.153 |
| 74 | 2.611 ± 0.037 | 3.352 ± 0.036 | 4.099 ± 0.043 | 4.931 ± 0.040 |
| 75 | 1.826 ± 0.030 | 2.377 ± 0.027 | 2.682 ± 0.030 | 3.125 ± 0.029 |
| 76 | 1.191 ± 0.022 | 1.452 ± 0.019 | 1.552 ± 0.020 | 1.793 ± 0.019 |
High-resolution relaxometry of ubiquitin

Table S8 (continued): Corrected low-field $^{15}$N relaxation rates for 3 mM ubiquitin (pH = 4.5; see table S1 for sample temperature)

| residue | 1T $R_1$(s$^{-1}$) | 0.74T $R_1$(s$^{-1}$) | 0.5T $R_1$(s$^{-1}$) |
|---------|---------------------|----------------------|----------------------|
| 2       | 11.041 ± 0.249      | 14.214 ± 0.434       | 14.922 ± 0.614       |
| 3       | 11.987 ± 0.316      | 15.204 ± 0.552       | 16.740 ± 0.824       |
| 4       | 11.477 ± 0.424      | 13.234 ± 0.676       | 16.671 ± 0.962       |
| 5       | 11.843 ± 0.309      | 14.151 ± 0.530       | 16.835 ± 0.820       |
| 6       | 13.120 ± 0.351      | 14.076 ± 0.590       | 15.305 ± 0.826       |
| 7       | 11.791 ± 0.298      | 13.950 ± 0.530       | 15.405 ± 0.683       |
| 8       | 10.708 ± 0.212      | 12.960 ± 0.347       | 14.695 ± 0.507       |
| 9       | 9.295 ± 0.183       | 10.477 ± 0.288       | 11.998 ± 0.391       |
| 10      | 9.090 ± 0.180       | 12.515 ± 0.317       | 13.449 ± 0.401       |
| 11      | 9.326 ± 0.144       | 10.805 ± 0.226       | 12.381 ± 0.318       |
| 12      | 10.094 ± 0.205      | 12.757 ± 0.333       | 14.619 ± 0.499       |
| 13      | 11.621 ± 0.333      | 13.404 ± 0.548       | 16.099 ± 0.815       |
| 14      | 10.062 ± 0.255      | 13.882 ± 0.444       | 15.537 ± 0.643       |
| 15      | 11.722 ± 0.299      | 14.941 ± 0.517       | 16.348 ± 0.787       |
| 16      | 11.104 ± 0.244      | 14.657 ± 0.436       | 14.238 ± 0.606       |
| 17      | 11.132 ± 0.305      | 13.613 ± 0.520       | 16.436 ± 0.811       |
| 18      | 13.383 ± 0.373      | 15.221 ± 0.623       | 17.247 ± 0.860       |
| 19      | 11.747 ± 0.248      | 13.084 ± 0.415       | 15.554 ± 0.648       |
| 20      | 11.747 ± 0.301      | 14.353 ± 0.503       | 16.093 ± 0.730       |
| 21      | 11.933 ± 0.401      | 15.901 ± 0.691       | 16.480 ± 1.007       |
| 22      | 11.737 ± 0.316      | 14.763 ± 0.545       | 17.261 ± 0.795       |
| 23      | 12.810 ± 0.298      | 16.165 ± 0.509       | 15.881 ± 0.730       |
| 24      | 14.343 ± 0.350      | 16.949 ± 0.601       | 17.689 ± 0.837       |
| 25      | 12.400 ± 0.304      | 12.855 ± 0.486       | 17.926 ± 0.831       |
| 26      | 11.519 ± 0.318      | 14.839 ± 0.558       | 17.240 ± 0.862       |
| 27      | 11.723 ± 0.255      | 13.707 ± 0.422       | 16.696 ± 0.671       |
| 28      | 10.849 ± 0.237      | 14.215 ± 0.410       | 14.928 ± 0.608       |
| 29      | 13.244 ± 0.385      | 15.084 ± 0.644       | 17.949 ± 0.912       |
| 30      | 12.623 ± 0.371      | 14.602 ± 0.643       | 18.667 ± 1.150       |
| 31      | 12.222 ± 0.539      | 13.221 ± 0.801       | 16.782 ± 0.707       |
| 32      | 10.802 ± 0.210      | 12.903 ± 0.352       | 15.422 ± 0.533       |
| 33      | 11.481 ± 0.314      | 16.048 ± 0.572       | 16.244 ± 0.793       |
| 34      | 12.685 ± 0.333      | 16.290 ± 0.566       | 16.833 ± 0.796       |
| 35      | 11.842 ± 0.333      | 15.181 ± 0.592       | 16.058 ± 0.842       |
| 36      | 13.408 ± 0.419      | 14.113 ± 0.656       | 15.976 ± 0.953       |
| 37      | 11.691 ± 0.358      | 16.251 ± 0.657       | 16.781 ± 0.968       |
| 38      | 12.824 ± 0.413      | 14.785 ± 0.702       | 16.040 ± 0.882       |
| 39      | 12.391 ± 0.293      | 14.221 ± 0.498       | 14.683 ± 0.672       |
| 40      | 11.222 ± 0.261      | 13.994 ± 0.433       | 14.396 ± 0.643       |
High-resolution relaxometry of ubiquitin

|   | 11.478 ± 0.241 | 12.748 ± 0.384 | 15.226 ± 0.585 |
|---|----------------|----------------|----------------|
| 48| 10.172 ± 0.191 | 11.934 ± 0.313 | 14.319 ± 0.466 |
| 49| 11.687 ± 0.345 | 14.423 ± 0.580 | 17.354 ± 0.916 |
| 50| 13.010 ± 0.381 | 15.541 ± 0.645 | 16.456 ± 0.860 |
| 51| 11.109 ± 0.223 | 13.758 ± 0.371 | 15.246 ± 0.563 |
| 52| 12.027 ± 0.282 | 13.897 ± 0.457 | 15.506 ± 0.675 |
| 53| 11.993 ± 0.327 | 15.730 ± 0.602 | 16.090 ± 0.847 |
| 54| 13.265 ± 0.389 | 14.714 ± 0.628 | 19.096 ± 1.030 |
| 55| 11.431 ± 0.246 | 13.541 ± 0.408 | 15.857 ± 0.590 |
| 56| 10.766 ± 0.312 | 13.969 ± 0.537 | 16.882 ± 0.833 |
| 57| 11.228 ± 0.289 | 15.272 ± 0.499 | 16.585 ± 0.764 |
| 58| 12.437 ± 0.324 | 15.410 ± 0.539 | 15.905 ± 0.831 |
| 59| 9.559 ± 0.225  | 11.100 ± 0.360 | 13.114 ± 0.532 |
| 60| 11.362 ± 0.224 | 14.357 ± 0.389 | 15.274 ± 0.562 |
| 61| 12.534 ± 0.398 | 15.684 ± 0.675 | 18.087 ± 1.051 |
| 62| 11.008 ± 0.239 | 13.403 ± 0.404 | 14.789 ± 0.577 |
| 63| 12.619 ± 0.325 | 14.059 ± 0.527 | 16.577 ± 0.756 |
| 64| 12.871 ± 0.381 | 15.024 ± 0.647 | 15.954 ± 0.923 |
| 65| 12.531 ± 0.357 | 14.396 ± 0.577 | 17.133 ± 0.939 |
| 66| 12.497 ± 0.365 | 14.153 ± 0.609 | 16.037 ± 0.933 |
| 67| 10.860 ± 0.230 | 12.561 ± 0.375 | 14.666 ± 0.535 |
| 68| 5.102 ± 0.057  | 6.405 ± 0.089  | 6.854 ± 0.106 |
| 69| 3.457 ± 0.040  | 3.684 ± 0.059  | 3.955 ± 0.067 |
| 70| 1.934 ± 0.026  | 2.009 ± 0.039  | 2.156 ± 0.043 |
# High-resolution relaxometry of ubiquitin

| residue | $S^2$   | $S^2_{fast}$ | $\tau_{rel}$  |
|---------|---------|--------------|---------------|
| 2       | 0.833 ± 0.00924 | 1 ±          | 0.02935 ± 0.00519 |
| 3       | 0.873 ± 0.00922 | 1 ±          | 0.00000 ± 0.00000 |
| 4       | 0.899 ± 0.00964 | 1 ±          | 0.04223 ± 0.01110 |
| 5       | 0.832 ± 0.00846 | 1 ±          | 0.02862 ± 0.00562 |
| 6       | 0.869 ± 0.00944 | 1 ±          | ±              |
| 7       | 0.851 ± 0.00937 | 1 ±          | 0.03672 ± 0.00672 |
| 8       | 0.709 ± 0.01968 | 0.8788 ± 0.0109 | 1.18833 ± 0.16592 |
| 9       | 0.761 ± 0.00821 | 1 ±          | 0.06472 ± 0.00460 |
| 10      | 0.779 ± 0.00850 | 1 ±          | 0.07630 ± 0.00560 |
| 11      | 0.735 ± 0.00802 | 1 ±          | 0.06026 ± 0.00371 |
| 12      | 0.779 ± 0.00851 | 1 ±          | 0.03777 ± 0.00395 |
| 13      | 0.848 ± 0.00865 | 1 ±          | 0.03941 ± 0.00660 |
| 14      | 0.851 ± 0.00805 | 1 ±          | ±              |
| 15      | 0.767 ± 0.02566 | 0.8791 ± 0.0110 | 3.55937 ± 0.73535 |
| 16      | 0.785 ± 0.00815 | 1 ±          | 0.02374 ± 0.00382 |
| 17      | 0.871 ± 0.00954 | 1 ±          | 0.00000 ± 0.00000 |
| 18      | 0.830 ± 0.00898 | 1 ±          | 0.04024 ± 0.00541 |
| 19      | 0.854 ± 0.00906 | 1 ±          | 0.03279 ± 0.00658 |
| 20      | 0.865 ± 0.00931 | 1 ±          | 0.03400 ± 0.00708 |
| 21      | 0.901 ± 0.01379 | 1 ±          | ±              |
| 22      | 0.906 ± 0.01342 | 1 ±          | ±              |
| 23      | 0.888 ± 0.00950 | 1 ±          | 0.02996 ± 0.00937 |
| 24      | 0.922 ± 0.00957 | 1 ±          | ±              |
| 25      | 0.893 ± 0.00975 | 1 ±          | ±              |
| 26      | 0.896 ± 0.00924 | 1 ±          | ±              |
| 27      | 0.887 ± 0.00956 | 1 ±          | ±              |
| 28      | 0.845 ± 0.00903 | 1 ±          | 0.02424 ± 0.00611 |
| 29      | 0.843 ± 0.00870 | 1 ±          | ±              |
| 30      | 0.868 ± 0.00905 | 1 ±          | ±              |
| 31      | 0.770 ± 0.00824 | 1 ±          | 0.01863 ± 0.00343 |
| 32      | 0.789 ± 0.02653 | 0.8766 ± 0.0110 | 2.39826 ± 0.80415 |
| 33      | 0.852 ± 0.01195 | 1 ±          | 0.02680 ± 0.00669 |
| 34      | 0.858 ± 0.00932 | 1 ±          | 0.03246 ± 0.00668 |
| 35      | 0.850 ± 0.00910 | 1 ±          | 0.01188 ± 0.00508 |
| 36      | 0.855 ± 0.00863 | 1 ±          | ±              |
| 37      | 0.850 ± 0.00921 | 1 ±          | 0.03352 ± 0.00668 |
| 38      | 0.848 ± 0.01211 | 1 ±          | 0.03094 ± 0.00636 |
| 39      | 0.844 ± 0.00923 | 1 ±          | 0.03185 ± 0.00626 |
| 40      | 0.814 ± 0.00873 | 1 ±          | 0.01669 ± 0.00468 |
| 41      | 0.827 ± 0.01311 | 1 ±          | 0.01873 ± 0.00526 |
| 42      | 0.780 ± 0.00790 | 1 ±          | 0.04624 ± 0.00403 |
### High-resolution relaxometry of ubiquitin

|    |    |    |    |    |    |    |
|----|----|----|----|----|----|----|
| 50 | 0.861 ± 0.00891 | 1 ± | 0.03815 ± 0.00714 |
| 51 | 0.812 ± 0.00889 | 1 ± | 0.03329 ± 0.00482 |
| 52 | 0.809 ± 0.00839 | 1 ± | 0.03449 ± 0.00704 |
| 54 | 0.827 ± 0.01255 | 1 ± | 0.01866 ± 0.00492 |
| 55 | 0.855 ± 0.01247 | 1 ± | 0.01704 ± 0.00649 |
| 56 | 0.897 ± 0.00940 | 1 ± | 0.01689 ± 0.00834 |
| 57 | 0.869 ± 0.00913 | 1 ± | 0.01704 ± 0.00649 |
| 59 | 0.853 ± 0.00918 | 1 ± | 0.01704 ± 0.00649 |
| 60 | 0.878 ± 0.00962 | 1 ± | 0.01890 ± 0.00675 |
| 61 | 0.872 ± 0.00947 | 1 ± | 0.01890 ± 0.00675 |
| 62 | 0.731 ± 0.00751 | 1 ± | 0.05206 ± 0.00326 |
| 63 | 0.843 ± 0.00957 | 1 ± | 0.00000 ± 0.00000 |
| 64 | 0.821 ± 0.02633 | 0.9015 ± 0.0106 | 1.95872 ± 0.73543 |
| 65 | 0.881 ± 0.00953 | 1 ± | 0.02788 ± 0.00581 |
| 66 | 0.842 ± 0.00881 | 1 ± | 0.02788 ± 0.00581 |
| 67 | 0.857 ± 0.00902 | 1 ± | 0.02788 ± 0.00581 |
| 68 | 0.857 ± 0.00921 | 1 ± | 0.02788 ± 0.00581 |
| 70 | 0.874 ± 0.01295 | 1 ± | 0.02666 ± 0.00802 |
| 71 | 0.819 ± 0.00933 | 1 ± | 0.02993 ± 0.00508 |
| 74 | 0.208 ± 0.01148 | 0.7810 ± 0.0104 | 1.11704 ± 0.02375 |
| 75 | 0.092 ± 0.00776 | 0.6871 ± 0.0091 | 0.88851 ± 0.01440 |
| 76 | 0.056 ± 0.00466 | 0.5334 ± 0.0079 | 0.66351 ± 0.01104 |
High-resolution relaxometry of ubiquitin

**Table S10:** Parameters of local dynamics in 3 mM ubiquitin (pH = 4.5; T = 296.5 K) derived from high-field relaxation data (14.1; 18.8; and 22.3 T).

| Residue | S<sup>2</sup> | S<sup>2</sup> <sub>H</sub> | τ<sub>uu</sub> | τ<sub>uu</sub><sup>fast</sup> |
|---------|--------------|-----------------|-------------|-----------------|
| 0.836 ± 0.890 | 0.885 ± 0.546 | 0.759 ± 0.132 | 0.356 ± 0.313 |
| 0.845 ± 0.139 | 0.966 ± 0.581 | 2.517 ± 0.544 | 1.721 ± 0.516 |
| 0.890 ± 0.187 | 0.924 ± 0.568 | 0.449 ± 0.155 | 1.659 ± 0.427 |
| 0.839 ± 0.644 | 0.858 ± 0.548 | 3.444 ± 0.731 | 3.444 ± 0.731 |
| 0.756 ± 0.396 | 0.864 ± 0.134 | 0.145 ± 0.158 | 0.145 ± 0.158 |
| 0.779 ± 0.341 | 0.861 ± 0.125 | 1.923 ± 0.618 | 0.356 ± 0.313 |
| 0.782 ± 0.238 | 0.882 ± 0.185 | 0.619 ± 0.570 | 0.619 ± 0.570 |
| 0.630 ± 0.430 | 0.780 ± 0.190 | 2.113 ± 0.424 | 0.337 ± 0.253 |
| 0.567 ± 0.482 | 0.761 ± 0.175 | 0.346 ± 0.149 | 0.346 ± 0.149 |
| 0.899 ± 0.492 | 1.000 ± | 1.972 ± 0.487 | 1.972 ± 0.487 |
| 0.725 ± 0.463 | 0.838 ± 0.167 | 0.170 ± 0.144 | 0.170 ± 0.144 |
| 0.853 ± 0.623 | 1.000 ± | 2.737 ± 0.392 | 2.737 ± 0.392 |
| 0.772 ± 0.153 | 0.899 ± 0.770 | 1.545 ± 0.780 | 0.114 ± 0.269 |
| 0.892 ± 0.516 | 1.000 ± | 0.249 ± 0.249 | 0.249 ± 0.249 |
| 0.772 ± 0.262 | 0.842 ± 0.117 | 1.187 ± 0.252 | 1.187 ± 0.252 |
| 0.867 ± 0.521 | 1.000 ± | 0.172 ± 0.168 | 0.172 ± 0.168 |
| 0.956 ± 0.579 | 1.000 ± | 0.213 ± 0.269 | 0.213 ± 0.269 |
| 0.942 ± 0.778 | 1.000 ± | 0.170 ± 0.469 | 0.170 ± 0.469 |
| 0.948 ± 0.796 | 1.000 ± | ± | ± |
| 0.919 ± 0.560 | 1.000 ± | ± | ± |
| 0.929 ± 0.733 | 1.000 ± | ± | ± |
| 0.918 ± 0.536 | 1.000 ± | 0.138 ± 0.241 | 0.138 ± 0.241 |
| 0.834 ± 0.334 | 0.898 ± 0.192 | 2.467 ± 0.736 | 2.467 ± 0.736 |
| 0.874 ± 0.125 | 0.920 ± 0.563 | 2.177 ± 0.482 | 2.177 ± 0.482 |
| 0.763 ± 0.387 | 0.849 ± 0.139 | 2.133 ± 0.529 | 2.133 ± 0.529 |
| 0.859 ± 0.543 | 1.000 ± | 0.152 ± 0.178 | 0.152 ± 0.178 |
| 0.738 ± 0.434 | 0.838 ± 0.146 | 3.527 ± 0.863 | 3.527 ± 0.863 |
| 0.673 ± 0.336 | 0.764 ± 0.129 | 2.297 ± 0.474 | 2.297 ± 0.474 |
| 0.827 ± 0.114 | 0.928 ± 0.547 | 1.738 ± 0.193 | 1.738 ± 0.193 |
| 0.819 ± 0.323 | 0.872 ± 0.125 | 1.646 ± 0.532 | 1.646 ± 0.532 |
| 0.844 ± 0.347 | 0.870 ± 0.115 | 1.420 ± 0.414 | 1.420 ± 0.414 |
| 0.854 ± 0.973 | 1.000 ± | 0.963 ± 0.283 | 0.963 ± 0.283 |
| 0.843 ± 0.637 | 1.000 ± | 0.115 ± 0.158 | 0.115 ± 0.158 |
| 0.857 ± 0.674 | 0.887 ± 0.567 | 0.489 ± 0.155 | 0.489 ± 0.155 |
| 0.890 ± 0.675 | 1.000 ± | ± | ± |
| 0.841 ± 0.967 | 0.884 ± 0.537 | 0.783 ± 0.191 | 0.783 ± 0.191 |
| 0.836 ± 0.521 | 1.000 ± | 0.298 ± 0.156 | 0.298 ± 0.156 |
| 0.873 ± 0.533 | 1.000 ± | 0.245 ± 0.200 | 0.245 ± 0.200 |
| 0.757 ± 0.197 | 0.837 ± 0.846 | 0.665 ± 0.139 | 0.665 ± 0.139 |
High-resolution relaxometry of ubiquitin

|   |     |     |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|-----|-----|
| 50 | 0.893 ± 0.261 | 0.886 ± 0.159 | 1.298 ± 0.277 | ± |
| 51 | 0.797 ± 0.176 | 0.863 ± 0.111 | 0.789 ± 0.285 | ± |
| 52 | 0.798 ± 0.673 | 1.000 ±     | 0.152 ± 0.915 | ± |
| 54 | 0.754 ± 0.397 | 0.836 ± 0.153 | 2.964 ± 0.577 | ± |
| 55 | 0.797 ± 0.525 | 0.842 ± 0.185 | 2.322 ± 0.537 | ± |
| 56 | 0.927 ± 0.583 | 1.000 ±     | ±           | ± |
| 57 | 0.876 ± 0.170 | 0.984 ± 0.592 | 1.766 ± 0.457 | ± |
| 59 | 0.868 ± 0.987 | 1.000 ±     | 0.159 ± 0.320 | ± |
| 60 | 0.786 ± 0.359 | 0.868 ± 0.119 | 2.958 ± 0.756 | ± |
| 61 | 0.839 ± 0.119 | 0.896 ± 0.543 | 1.846 ± 0.337 | ± |
| 62 | 0.668 ± 0.320 | 0.749 ± 0.166 | 1.578 ± 0.677 | 0.356 ± 0.336 |
| 63 | 0.855 ± 0.516 | 1.000 ±     | 0.135 ± 0.116 | ± |
| 64 | 0.843 ± 0.214 | 0.986 ± 0.158 | 1.626 ± 0.468 | ± |
| 65 | 0.795 ± 0.359 | 0.872 ± 0.125 | 3.134 ± 0.782 | ± |
| 66 | 0.818 ± 0.247 | 0.856 ± 0.964 | 1.337 ± 0.527 | ± |
| 67 | 0.866 ± 0.641 | 1.000 ±     | 0.158 ± 0.184 | ± |
| 68 | 0.858 ± 0.713 | 1.000 ±     | 0.896 ± 0.169 | ± |
| 70 | 0.896 ± 0.756 | 1.000 ±     | 0.133 ± 0.253 | ± |
| 71 | 0.687 ± 0.188 | 0.829 ± 0.531 | 1.598 ± 0.162 | ± |
| 74 | 0.197 ± 0.620 | 0.647 ± 0.428 | 1.457 ± 0.280 | 0.457 ± 0.145 |
| 75 | 0.618 ± 0.396 | 0.513 ± 0.388 | 1.374 ± 0.294 | 0.518 ± 0.120 |
| 76 | 0.948 ± 0.162 | 0.298 ± 0.685 | 0.495 ± 0.985 | ± |
High-resolution relaxometry of ubiquitin

Table S11: Parameters of local dynamics in 3 mM ubiquitin (pH = 4.5; T = 296.5 K) derived from the analysis of relaxation data at all fields (0.5 to 22.3 T)

| residue | $S^2$       | $S^2_f$      | $\tau_{rel}$ | $\tau_{fast}$ |
|---------|-------------|--------------|---------------|---------------|
| 2       | 0.683 ± 0.179 | 0.822 ± 0.697 | 1.939 ± 0.125 | ±             |
| 3       | 0.876 ± 0.576 | 1.000 ±      | 0.847 ± 0.365 | ±             |
| 4       | 0.892 ± 0.519 | 1.000 ±      | ±             | ±             |
| 5       | 0.772 ± 0.252 | 0.829 ± 0.123 | 1.615 ± 0.389 | ±             |
| 6       | 0.833 ± 0.153 | 0.967 ± 0.165 | 1.247 ± 5.179 | ±             |
| 7       | 0.746 ± 0.300 | 0.852 ± 0.190 | 2.140 ± 0.324 | ±             |
| 8       | 0.633 ± 0.339 | 0.837 ± 0.470 | 1.888 ± 1.546 | 0.122 ± 0.280 |
| 9       | 0.535 ± 0.229 | 0.737 ± 0.668 | 3.123 ± 0.295 | 0.319 ± 0.132 |
| 10      | 0.553 ± 0.214 | 0.776 ± 0.113 | 1.999 ± 0.264 | 0.198 ± 0.334 |
| 11      | 0.451 ± 0.267 | 0.716 ± 0.649 | 3.385 ± 0.499 | 0.323 ± 0.134 |
| 12      | 0.618 ± 0.436 | 0.759 ± 0.122 | 3.643 ± 1.565 | 0.242 ± 0.244 |
| 13      | 0.738 ± 0.322 | 0.843 ± 0.128 | 1.976 ± 0.324 | ±             |
| 14      | 0.694 ± 0.537 | 0.813 ± 0.155 | 3.652 ± 0.775 | ±             |
| 15      | 0.670 ± 0.347 | 0.855 ± 0.855 | 4.288 ± 0.491 | ±             |
| 16      | 0.719 ± 0.321 | 0.880 ± 0.236 | 1.525 ± 1.652 | 0.138 ± 0.897 |
| 17      | 0.744 ± 0.268 | 0.847 ± 0.924 | 2.579 ± 0.255 | ±             |
| 18      | 0.812 ± 0.149 | 0.854 ± 0.666 | 0.917 ± 0.151 | ±             |
| 20      | 0.732 ± 0.232 | 0.836 ± 0.867 | 2.222 ± 0.262 | ±             |
| 22      | 0.727 ± 0.182 | 0.853 ± 0.567 | 2.865 ± 0.235 | ±             |
| 23      | 0.913 ± 0.532 | 1.000 ±      | 0.929 ± 0.426 | ±             |
| 25      | 0.885 ± 0.600 | 1.000 ±      | ±             | ±             |
| 26      | 0.882 ± 0.644 | 1.000 ±      | ±             | ±             |
| 27      | 0.900 ± 0.994 | 1.000 ±      | ±             | ±             |
| 29      | 0.867 ± 0.189 | 0.942 ± 0.176 | 1.955 ± 5.963 | ±             |
| 30      | 0.759 ± 0.474 | 0.878 ± 0.122 | 4.157 ± 0.795 | ±             |
| 32      | 0.855 ± 0.541 | 1.000 ±      | 0.569 ± 0.197 | ±             |
| 33      | 0.670 ± 0.314 | 0.819 ± 0.916 | 3.458 ± 0.386 | ±             |
| 34      | 0.894 ± 0.263 | 0.854 ± 0.193 | 1.160 ± 0.399 | ±             |
| 35      | 0.819 ± 0.430 | 0.864 ± 0.169 | 2.127 ± 0.994 | ±             |
| 36      | 0.683 ± 0.347 | 0.766 ± 0.136 | 2.253 ± 0.471 | ±             |
| 39      | 0.595 ± 0.214 | 0.837 ± 0.582 | 4.375 ± 0.196 | ±             |
| 40      | 0.768 ± 0.238 | 0.856 ± 0.786 | 2.376 ± 0.329 | ±             |
| 41      | 0.795 ± 0.244 | 0.866 ± 0.926 | 1.629 ± 0.358 | ±             |
| 42      | 0.824 ± 0.621 | 0.933 ± 0.234 | 0.818 ± 2.552 | ±             |
| 43      | 0.830 ± 0.386 | 0.894 ± 0.178 | 0.578 ± 1.217 | ±             |
| 44      | 0.824 ± 0.275 | 0.867 ± 0.113 | 1.394 ± 0.396 | ±             |
| 45      | 0.868 ± 0.114 | 1.000 ±      | ±             | ±             |
| 46      | 0.723 ± 0.298 | 0.839 ± 0.994 | 2.230 ± 0.312 | ±             |
| 47      | 0.759 ± 0.152 | 0.821 ± 0.531 | 1.772 ± 0.129 | ±             |
| 48      | 0.695 ± 0.256 | 0.825 ± 0.948 | 2.128 ± 0.299 | ±             |
## High-resolution relaxometry of ubiquitin

|   |    |    |    |    |    |    |    |    |
|---|----|----|----|----|----|----|----|----|
|49 | 0.655 | ± | 0.190 | 0.800 | ± | 0.818 | 1.270 | ± | 0.962 | ± |
|50 | 0.766 | ± | 0.188 | 0.869 | ± | 0.733 | 1.753 | ± | 0.164 | ± |
|51 | 0.754 | ± | 0.548 | 0.828 | ± | 0.448 | 2.366 | ± | 2.712 | ± | 0.122 | ± | 0.256 |
|52 | 0.733 | ± | 0.354 | 0.789 | ± | 0.318 | 2.740 | ± | 3.629 | ± | 0.457 | ± | 0.140 |
|53 | 0.745 | ± | 0.268 | 0.818 | ± | 0.647 | 2.926 | ± | 0.290 | ± |
|54 | 0.758 | ± | 0.256 | 0.859 | ± | 0.775 | 1.984 | ± | 0.218 | ± |
|55 | 0.958 | ± | 0.857 | 1.000 | ± | 1.660 | ± | 1.660 | ± |
|56 | 0.843 | ± | 0.322 | 1.000 | ± | 0.636 | ± | 0.334 | ± |
|57 | 0.735 | ± | 0.345 | 0.845 | ± | 0.115 | 2.428 | ± | 0.421 | ± |
|58 | 0.842 | ± | 0.784 | 1.000 | ± | 0.636 | ± | 0.334 | ± |
|59 | 0.762 | ± | 0.185 | 0.899 | ± | 0.169 | 2.983 | ± | 5.792 | ± |
|60 | 0.623 | ± | 0.280 | 0.732 | ± | 0.148 | 2.180 | ± | 0.374 | ± | 0.342 | ± | 0.145 |
|61 | 0.745 | ± | 0.259 | 0.826 | ± | 0.966 | 2.114 | ± | 0.321 | ± |
|62 | 0.788 | ± | 0.275 | 0.888 | ± | 0.956 | 2.450 | ± | 0.378 | ± |
|63 | 0.827 | ± | 0.564 | 1.000 | ± | 0.522 | ± | 0.158 | ± |
|64 | 0.751 | ± | 0.295 | 0.827 | ± | 0.177 | 2.519 | ± | 0.538 | ± |
|65 | 0.819 | ± | 0.149 | 0.935 | ± | 0.199 | 1.367 | ± | 4.264 | ± |
|66 | 0.827 | ± | 0.484 | 0.954 | ± | 0.191 | 0.549 | ± | 2.268 | ± |
|67 | 0.776 | ± | 0.273 | 0.866 | ± | 0.838 | 3.123 | ± | 0.524 | ± |
|68 | 0.658 | ± | 0.538 | 0.818 | ± | 0.255 | 2.194 | ± | 2.669 | ± | 0.285 | ± | 0.136 |
|69 | 0.200 | ± | 0.146 | 0.646 | ± | 0.773 | 1.433 | ± | 0.517 | ± | 0.442 | ± | 0.130 |
|70 | 0.726 | ± | 0.295 | 0.515 | ± | 0.389 | 1.258 | ± | 0.146 | ± | 0.496 | ± | 0.752 |
|71 | 0.237 | ± | 0.233 | 0.353 | ± | 0.370 | 1.233 | ± | 0.142 | ± | 0.384 | ± | 0.695 |
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