Supplementary Information:

# Probing molecular motion by double-quantum (<sup>13</sup>C,<sup>13</sup>C) solid-state NMR spectroscopy: Application to ubiquitin

Robert Schneider<sup>1</sup>, Karsten Seidel<sup>1</sup>, Manuel Etzkorn<sup>1</sup>, Adam Lange<sup>1</sup>, Stefan Becker<sup>1</sup>, and Marc Baldus<sup>2\*</sup>

<sup>1</sup>Max-Planck-Institute for Biophysical Chemistry, Department for NMR-based Structural Biology, Am Fassberg 11, 37077 Göttingen, Germany and

<sup>2</sup>Bijvoet Center for Biomolecular Research, Utrecht University, Padualaan 8, 3584 CH Utrecht, The

Netherlands

Current address: KS: Polymer Physics (GKP/R), BASF SE, 67056 Ludwigshafen, Germany; ME: Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, 240 Longwood Ave, Boston, MA 02115

\*To whom correspondence should be addressed: m.baldus@uu.nl

#### **Supplementary Methods**

#### Details of spin system simulations

In GAMMA simulations, the two spins contributing to the 2Q coherence of interest and any <sup>13</sup>C spins directly bonded to them were considered. This yielded 2-spin simulations for the TEE ester tail, 3-spin simulations for Ala and Ser and 4-spin simulations for the other unbranched amino acids as well as for the tyrosine moiety of TEE. For residues with longer sidechains (e.g. Lys), separate 4-spin simulations were conducted for sidechain 2Q coherence buildups (e.g. considering C $\beta$ , C $\gamma$ , C $\delta$ , and C $\epsilon$  for fitting an experimental ( $C\gamma$ ,  $C\delta$ ) buildup). Extended geometry was assumed, with Euler angles ( $\alpha$ ,  $\beta$ ,  $\gamma$ ) for the PAS  $\rightarrow$  MOL transformation set to (0°,90°,0°), (68°,90°,0°), and (0°,90°,0°) for dipolar tensors connecting spins (1,2), (2,3), and (3,4) of a 4-spin system, respectively. In simulations of a bent Lys sidechain conformation (see Fig. SI 3 a), these Euler angles were set to  $(0^{\circ},90^{\circ},0^{\circ})$ ,  $(78^{\circ},90^{\circ},0^{\circ})$ , and (156°,90°,0°), respectively. All dipolar couplings were considered in the setup of the system Hamiltonian for 2- to 4-spin systems. Bond lengths for aliphatic carbons were set to 1.52 Å (corresponding to a dipolar coupling constant  $d_{CC}$  of 2160 Hz in the static case), and an additional scalar coupling of 40 Hz between directly bonded nuclei was considered. For aromatic carbons, a bond length of 1.4 Å was assumed. Chemical shifts used for individual nuclei were experimental resonance assignments in case of TEE simulations or average values from the BioMagResBank<sup>1</sup> (BMRB) database in case of amino acid simulations.

For amino acids with branched sidechains, i.e. Ile, Leu, and Val, 5-spin systems had to be considered. For example, for fitting Ile (C $\beta$ ,C $\gamma$ 1) or (C $\beta$ ,C $\gamma$ 2) buildups, a spin system consisting of C $\alpha$ , C $\beta$ , C $\gamma$ 1, C $\gamma$ 2, and C $\delta$ 1 was simulated. Corresponding Euler angles ( $\alpha$ , $\beta$ , $\gamma$ ) for the PAS  $\rightarrow$  MOL transformation of dipolar coupling tensors were as follows:

| Dipolar tensor | α   | β  | γ |
|----------------|-----|----|---|
| (Cα,Cβ)        | 0   | 90 | 0 |
| (Cβ,Cγ1)       | -68 | 90 | 0 |
| (Cβ,Cγ2)       | 51  | 37 | 0 |
| (Cγ1,Cδ1)      | 0   | 90 | 0 |

Only one-bond dipolar couplings were considered in 5-spin simulations for computational reasons, which had no effect on initial rate buildup characteristics (data not shown). As above, an additional scalar coupling contribution of 40 Hz between bonded nuclei was included in the calculations.

For simulations of an amino acid side chain including chemical shift anisotropy (Fig. SI 3 b), extended geometry was again assumed as above. Isotropic chemical shift values were chosen to correspond to aspartic acid or asparagine (C' 176 ppm, C $\alpha$  52.7 ppm, C $\beta$  37 ppm, C $\gamma$  178.7 ppm). Principal axis values ( $\sigma_{xx}, \sigma_{yy}, \sigma_{zz}$ ) for CSA tensors were taken from ref. 2 and were set to (in ppm) (253,178,97) for C', (29,58,71) for C $\alpha$ , (49,44,18) for C $\beta$ , and (255,181,100) for C $\gamma$ . C' and C $\gamma$  values were adapted from values for alanine in ref. 2 to match BMRB isotropic shifts for aspartic acid / asparagine. C', C $\alpha$  and C $\gamma$  tensor orientations were as described in refs. 3,4. That is, for the C $\alpha$  CSA tensor, the axis with the largest principal value formed an angle of 76° with the C $\alpha$ –H $\alpha$  bond, and the one with the intermediate principal value formed an angle of 60° with the C $\alpha$ –N bond. For C' and C $\gamma$  CSA tensors, the axis with the intermediate principal value was perpendicular to the peptide bond plane. This led to Euler angles ( $\alpha,\beta,\gamma$ ) for the PAS  $\rightarrow$  MOL transformation of C', C $\alpha$  and C $\gamma$  CSA tensors of (90°,77°,-60°), (51°,67°,90°), and (90°,20°,-120°), respectively. C $\beta$  CSA tensor orientation was varied around a sphere in 20 steps, leading to the different buildup curves in Figure SI 3 b.

## **Supplementary Figures**



**Figure SI 1:** Cutouts of 2D (2Q,1Q) correlation experiments on TEE for SPC5 DQ excitation and reconversion times each of 564  $\mu$ s (**a**) and 923  $\mu$ s (**b**).



**Figure SI 2:** Theoretical signal evolution for spin 1 (2-spin system, (**a**)) and spin 2 (4-spin system, (**b**)) in a 2D (2Q,1Q) correlation experiment for variable order parameters *S*. Intensities shown are calculated for double-quantum coherences between spins 1 and 2 (a) and between spins 2 and 3 (b), respectively, and are normalized to the maximum. 2QE and 2QR times of length  $t_{mix}$  each were simulated for the SPC5 sequence<sup>5</sup>. In (b), all one- and multi-bond dipolar (<sup>13</sup>C,<sup>13</sup>C) couplings were scaled by the same order parameter.



**Figure SI 3:** (a) Comparison of simulated (2Q,1Q) crosspeak buildups for a lysine sidechain (Cβ,Cγ,Cδ,Cε) 4-spin system with extended geometry (solid lines) and with bent geometry (dashed lines). CβCγ-Cβ (left) and CγCδ-Cγ (right) crosspeak buildups are shown. (b) Comparison of (2Q,1Q) crosspeak buildup simulations for an aspartic acid / asparagine (C',Cα,Cβ,Cγ) 4-spin system with and without chemical shift anisotropy. An extended geometry was again assumed. Buildups for CαCβ-Cα (left) and CαCβ-Cβ (right) crosspeaks are shown. Dashed black lines with crosses represent buildups obtained if all nuclei have isotropic chemical shift tensors (C' 176 ppm, Cα 52.7 ppm, Cβ 37 ppm, Cγ 178.7 ppm). Colored lines are buildups resulting from assuming anisotropic chemical shift tensors for all four nuclei (see Supplementary Methods). Cβ CSA tensor orientation was varied around a sphere in 20 steps, leading to the different buildup curves.



**Figure SI 4:** (a) Plot of Ubi-P (Cβ,Cγ)  $S_{CC}$  order parameters from Table SI 1. Open symbols indicate values with fit RMSDs > 0.1 or that were obtained from signals not fully resolved in spectra. Solid line indicates an  $S_{CC}$  value of 0.80 below which the most mobile 17% of determined  $S_{CC}$  values are found. Secondary structure of ubiquitin as found in the crystal structure (PDB: 1UBQ) is sketched at the top. (b) Comparison of Ubi-P (Cβ,Cγ)  $S_{CC}$  (filled symbols) and Ubi-M  $S_{HC}$  order parameters<sup>6</sup> (open symbols).  $S_{HC}$  values plotted are averaged over Cβ and Cγ nuclei if both values are available. Solid line as in (a); dotted line denotes the upper limit ( $S_{HC} = 0.53$ ) for the most mobile 21% of (Cβ,Cγ) pairs detected in Ubi-M.



**Figure SI 5:** Per-residue percentage of expected correlations (( ${}^{1}H, {}^{1}H$ ) distances up to 3.5 Å, only nonmethyl  ${}^{13}C$  nuclei) which are absent from the Ubi-P CHHC spectrum with 180 µs mixing time. Red bars denote residues where backbone or sidechain nuclei with elevated dynamics are involved in absent CHHC correlations (see Table SI 3). Gray bars denote absent correlations involving residue Gly53 for which no (C $\alpha$ ,C $\beta$ ) *S<sub>CC</sub>* order parameter can be determined. Other absent correlations are shown as black bars.



**Figure SI 6:** Sum of C', C $\alpha$ , and C $\beta$  chemical-shift differences between Ubi-P and Ubi-M as determined in ref. 7 (bars, bottom), as shown in Figure 5 in the main text. Above, Ubi-P (C $\alpha$ ,C $\beta$ )  $S_{CC}$  (top) and Ubi-M (C $\alpha$ ,C $\beta$ )  $S_{HC}$  (second from top) order parameters are given as in Figure 4 in the main text, with the most mobile 20% of residues in each dataset marked by red circles, and their cutoff values (dashed lines). Third panel from top shows the normalized relative difference of (C $\alpha$ ,C $\beta$ )  $S_{CC}$  and  $S_{HC}$  order parameters,  $\Delta S_{rel,norm}$ , as defined in Methods in the main text. Residues with  $\Delta S_{rel,norm}$  values above the average (dashed line) are marked in red. Residues marked by red bars in the bottom panel and in Figure 5 in the main text exhibit either  $S_{CC}$  or  $S_{HC}$  order parameters (or both) below the 20% cutoff or  $\Delta S_{rel,norm}$  values above the average. No mobility data could be obtained for residues denoted by gray bars; available dynamics data for residues with black bars do not indicate heightened mobility.



**Figure SI 7: (a)** Average backbone RMSD per residue of the 10 calculated Ubi-P structures with lowest overall energy to their mean coordinates. (b) Average backbone RMSD per residue of the 10 lowest-energy calculated Ubi-P structures to the ubiquitin crystal structure (PDB: 1UBQ). Bars for residues 71-76 for which no restraints are available are truncated at 10 Å. (c) Number of CHHC restraints per residue obtained by PASD, counting only restraints unique in terms of the <sup>13</sup>C nuclei involved (total 208 unambiguous, 1186 ambiguous). Interresidue restraints are counted in both residues involved. Black: unambiguous restraints; gray: ambiguous restraints.



**Figure SI 8:** (a) Sum of C', C $\alpha$ , and C $\beta$  chemical-shift differences between Ubi-M as determined in ref. 8 and Ubi-P. (b) Sausage plot of the ensemble of 20 lowest-energy structures of Ubi-M from ref. 8 (gray, PDB: 2JZZ) aligned with the ensemble of 10 lowest-energy Ubi-P structures discussed in the main text, also as sausage plot (blue). Only residues 1-70 are shown. Residues with largest RMSD deviations are labeled, as well as the N-terminus. (c) Backbone RMSD per residue between the mean structures of the Ubi-P and Ubi-M<sup>8</sup> ensembles. Bars for residues 71-76 for which no restraints are available are truncated at 10 Å.



**Figure SI 9:** Fractional accessible surface area (ASA) per residue as determined for the ubiquitin crystal structure (PDB: 1UBQ) by the VADAR web server<sup>9</sup> used with standard settings. Fractional ASA is defined as the observed ASA for a residue (in Å<sup>2</sup>) divided by its ASA in an extended Gly-Xaa-Gly peptide. The Pearson correlation coefficient of fractional ASA with (C $\alpha$ ,C $\beta$ ) *S<sub>CC</sub>* values along the ubiquitin sequence is 0.16.



**Figure SI 10: (a)** Number of close crystal contacts per residue for the ubiquitin crystal structure (PDB: 1UBQ). Addition of protons to 1UBQ and construction of the crystal unit cell with neighboring molecules were performed using PyMOL<sup>10</sup>. The graph shows the number of protons in a neighboring

ubiquitin molecule that are within 6 Å of any proton of a given ubiquitin residue, divided by the number of protons in that residue. (b) Sum of C', C $\alpha$ , and C $\beta$  chemical-shift differences between Ubi-P and Ubi-M as determined in ref. 7, color-coded for low  $S_{CC}$  or  $S_{HC}$  order parameters or high  $\Delta S_{rel,norm}$  values as shown in Figure 5 in the main text and in Figure SI 6. Gray boxes indicate regions with large preparation-dependent changes in ssNMR shifts or dynamics for which comparably few crystal contacts are detected. However, no direct correlation between intermolecular interactions and elevated dynamics and / or chemical-shift changes is apparent. See main text for details.

# **Supplementary Tables**

| Residue    | Mean (Cα,Cβ) S <sub>CC</sub> | Error  | Mean (Cβ,Cγ) S <sub>CC</sub> | Error  |
|------------|------------------------------|--------|------------------------------|--------|
| M1         | 0.80                         | 0.1    | 0.80                         | 0.05   |
| Q2         | -                            | -      | -                            | -      |
| 13         | 0.85                         | 0.05   | 0.78                         | 0.10   |
| F4         | 1.00                         | 0.05   | -                            | -      |
| V5         | -                            | -      | 0.90                         | 0.05   |
| K6         | -                            | -      | -                            | -      |
| T7         | 1.00                         | < 0.05 | 0.98                         | 0.05   |
| 18         | 0.90                         | 0.1    | -                            | -      |
| <u>т</u> 9 | 0.98                         | 0.05   | _                            | _      |
| G10        | -                            | 0.00   | _                            | _      |
| K11        |                              |        |                              | -      |
| T10        | -                            | -      | -                            | -      |
| 112        | -                            | -      | -                            | -      |
| 113        | 0.78                         | 0.05   | -                            | -      |
| 114        | -                            | -      | -                            | -      |
| L15        | 0.90                         | 0.1    | 0.80                         | 0.05   |
| E16        | -                            | -      | -                            | -      |
| V17        | 0.90                         | 0.1    | 0.95                         | 0.05   |
| E18        | 0.83                         | 0.05   | -                            | -      |
| P19        | 0.93                         | 0.05   | 0.80                         | 0.1    |
| S20        | -                            | -      | -                            | -      |
| D21        | -                            | -      | -                            | -      |
| T22        | 1.00                         | 0.05   | 0.93                         | 0.05   |
| 123        | 0.88                         | 0.05   | -                            | -      |
| E24        | 0.88                         | 0.05   | -                            | -      |
| N25        | 1.00                         | 0.05   | -                            | -      |
| V26        | 0.93                         | 0.05   | 0.98                         | 0.25   |
| K27        | 0.93                         | 0.05   | 0.80                         | 0.05   |
| A28        | 1.00                         | < 0.05 | -                            | -      |
| K29        | -                            | -      | 0.80                         | 0.05   |
| 130        | 0.90                         | 0.05   | 0.85                         | 0.05   |
| 031        | 0.95                         | 0.05   | -                            | -      |
| 032        | -                            | 0.00   | _                            | _      |
| K33        |                              |        |                              | -      |
| E24        | 0.79                         | 0.05   | 0.05                         | - 0.10 |
| L34<br>C25 | 0.76                         | 0.05   | 0.95                         | 0.10   |
| 035        | -                            | -      | -                            | -      |
| 130        | 0.75                         | 0.05   | -                            | 0.05   |
| P3/        | 0.93                         | 0.05   | -                            | 0.05   |
| P38        | 0.90                         | 0.05   | -                            | 0.05   |
| D39        | -                            | -      | -                            | -      |
| Q40        | -                            | -      | -                            | -      |
| Q41        | -                            | -      | -                            | -      |
| R42        | -                            | -      | -                            | -      |
| L43        | 0.85                         | 0.05   | 0.78                         | 0.05   |
| 44         | 0.83                         | 0.05   | -                            | -      |
| F45        | 1.00                         | 0.1    | -                            | -      |
| A46        | 1.00                         | < 0.05 | -                            | -      |
| G47        | -                            | -      | -                            | -      |
| K48        | -                            | -      | -                            | -      |
| Q49        | -                            | -      | -                            | -      |
| 1.50       | 0.98                         | 0.05   | 0.83                         | 0.05   |
| LOU        |                              | ~      |                              |        |

| Residue | Mean (Cα,Cβ) S <sub>CC</sub> | Error  | Mean (Cβ,Cγ) S <sub>cc</sub> | Error  |
|---------|------------------------------|--------|------------------------------|--------|
| D52     | -                            | -      | -                            | -      |
| G53     | -                            | -      | -                            | -      |
| R54     | 0.83                         | 0.05   | -                            | -      |
| T55     | 0.95                         | 0.05   | 1.00                         | < 0.05 |
| L56     | 0.93                         | 0.05   | 0.83                         | 0.1    |
| S57     | 1.00                         | 0.05   | -                            | -      |
| D58     | -                            | -      | -                            | -      |
| Y59     | -                            | -      | -                            | -      |
| N60     | 1.00                         | < 0.05 | -                            | -      |
| l61     | 0.90                         | 0.05   | 0.85                         | 0.1    |
| Q62     | -                            | -      | -                            | -      |
| K63     | 0.83                         | 0.05   | 0.80                         | 0.20   |
| E64     | 0.90                         | 0.05   | 0.85                         | 0.05   |
| S65     | 1.00                         | 0.05   | -                            | -      |
| T66     | -                            | -      | -                            | -      |
| L67     | 0.90                         | 0.05   | 0.73                         | 0.05   |
| H68     | -                            | -      | -                            | -      |
| L69     | 0.90                         | 0.05   | 0.63                         | 0.15   |
| V70     | -                            | -      | 0.85                         | 0.05   |
| L71     | -                            | -      | -                            | -      |
| R72     | -                            | -      | -                            | -      |
| L73     | -                            | -      | -                            | -      |
| R74     | -                            | -      | -                            | -      |
| G75     | -                            | -      | -                            | -      |
| G76     | -                            | -      | -                            | -      |

**Table SI 1:** Backbone ( $C\alpha$ , $C\beta$ ) and sidechain ( $C\beta$ , $C\gamma$ )  $S_{CC}$  order parameters determined for Ubi-P. Listed are all determined  $S_{CC}$  order parameters for ( $C\alpha$ , $C\beta$ ) and ( $C\beta$ , $C\gamma$ ) pairs. If the *S* values of simulations fitting best to individual crosspeak buildups ( $C\alpha$  and  $C\beta$  or  $C\beta$  and  $C\gamma$ , respectively) are different, the average is given. Errors were determined as described in Methods in the main text. For threonine residues, ( $C\beta$ , $C\gamma$ ) corresponds to ( $C\beta$ , $C\gamma$ 2); for all residues with  $C\gamma$ 1 and  $C\gamma$ 2 nuclei, the ( $C\beta$ , $C\gamma$ 1)  $S_{CC}$  value is given.

| ( <sup>1</sup> H, <sup>1</sup> H) distance restraints     |              |
|---|--------------|
| Total   | 1210         |
| Unambiguous restraints                                    | 368          |
| Unique ( <sup>13</sup> C, <sup>13</sup> C) pairs involved | 208          |
| Intraresidue  | 68           |
| Sequential  | 33           |
| Medium-range  | 37           |
| Long-range  | 70           |
| Ambiguous restraints                                      | 842          |
| Unique ( <sup>13</sup> C, <sup>13</sup> C) pairs involved | 1186         |
| Intraresidue  | 170          |
| Sequential  | 174          |
| Medium-range  | 249          |
| Long-range  | 593          |
| Distance violations (> 0.5 Å)                             | 0            |
| Dihedral angle restraints                                 | 92           |
| Dihedral violations (> 5°)                                | 2            |
| Energies (kcal/mol)                                       |              |
| Total   | 1098.6 ± 4.2 |
| СННС  | 28.0 ± 1.3   |
| Dihedral  | 11.6 ± 2.1   |
| Coordinate precision and accuracy (residu                 | es 1-70)     |
| Average RMSD to mean coordinates (Å)                      |              |
| Backbone atoms  | 0.49         |
| Heavy atoms   | 0.87         |
| Average RMSD to 1UBQ coordinates (Å)                      |              |
| Backbone atoms  | 1.79         |
| Heavy atoms   | 2.54         |
| Ramachandran statistics (excluding Pro,G                  | ly)          |
| Residues in most favored regions (%)                      | 86.7         |
| Residues in allowed regions (%)                           | 9.4          |
| Residues in generously allowed regions (%)                | 2.6          |
| Residues in disallowed regions (%)                        | 1.4          |

**Table SI 2:** Structural statistics for the 10 calculated Ubi-P structures with lowest overall energy. The residue difference i - j of two nuclei involved in medium-range distance restraints is  $2 \le i - j \le 4$  and  $i - j \ge 5$  for long-range restraints.

| Correlation |   | n          | Comment   |
|-------------|---|------------|---|
| 13 ILE CB   | - | 33 LYS CD  | lle13 (C $\alpha$ ,C $\beta$ ) S <sub>CC</sub> < 0.85; mobile Lys sidechain   |
| 19 PRO CA   | - | 56 LEU CB  |   |
| 19 PRO CB   | - | 57 SER CA  |   |
| 19 PRO CB   | - | 57 SER CB  |   |
| 22 THR CB   | - | 24 GLU CB  |   |
| 22 THR CB   | - | 53 GLY CA  | Gly53 possibly mobile   |
| 26 VAL CA   | - | 29 LYS+ CG | mobile Lys sidechain  |
| 26 VAL CA   | - | 29 LYS+ CD | mobile Lys sidechain  |
| 33 LYS+ CG  | - | 34 GLU CG  | Glu34 (C $\alpha$ ,C $\beta$ ) S <sub>CC</sub> < 0.85; mobile Lys sidechain<br>(Lys33 (C $\gamma$ ,C $\delta$ ) correlation not found in (2Q,1Q) spectra) |
| 57 SER CA   | - | 57 SER CB  |   |

**Table SI 3:** List of non-methyl ( ${}^{13}C$ ,  ${}^{13}C$ ) correlations expected in the Ubi-P CHHC spectrum with 180  $\mu$ s mixing time (associated ( ${}^{1}H$ ,  ${}^{1}H$ ) distance in the crystal structure (PDB: 1UBQ) below 3.5 Å) but absent from the spectrum. Right column indicates for which of these mobile moieties are (possibly) involved (red or gray bars in Fig. SI 5).

### References

Ulrich, E. L.; Akutsu, H.; Doreleijers, J. F.; Harano, Y.; Ioannidis, Y. E.; Lin, J.; Livny, M.; Mading, S.; Maziuk, D.; Miller, Z.; Nakatani, E.; Schulte, C. F.; Tolmie, D. E.; Wenger, R. K.; Yao, H.; Markley, J. L. *Nucleic Acids Research* 2007, *36*, D402-D408.

(2) Wei, Y. F.; Lee, D. K.; Ramamoorthy, A. *Journal of the American Chemical Society* **2001**, *123*, 6118-6126.

(3) Sun, H. H.; Sanders, L. K.; Oldfield, E. *Journal of the American Chemical Society* **2002**, *124*, 5486-5495.

(4) Yao, X. L.; Hong, M. Journal of the American Chemical Society 2002, 124, 2730-2738.

(5) Hohwy, M.; Rienstra, C. M.; Jaroniec, C. P.; Griffin, R. G. *Journal of Chemical Physics* **1999**, *110*, 7983-7992.

(6) Lorieau, J. L.; McDermott, A. E. *Journal of the American Chemical Society* **2006**, *128*, 11505-11512.

(7) Seidel, K.; Etzkorn, M.; Heise, H.; Becker, S.; Baldus, M. *ChemBioChem* **2005**, *6*, 1638-1647.

(8) Manolikas, T.; Herrmann, T.; Meier, B. H. *Journal of the American Chemical Society* **2008**, *130*, 3959-3966.

(9) Willard, L.; Ranjan, A.; Zhang, H. Y.; Monzavi, H.; Boyko, R. F.; Sykes, B. D.; Wishart, D. S. *Nucleic Acids Research* **2003**, *31*, 3316-3319.

(10) DeLano, W. L. DeLano Scientific, Palo Alto, CA, USA 2002.