Weak Long-Range Correlated Motions in a Surface Patch of Ubiquitin Involved in Molecular Recognition

R. Bryn Fenwick,† Santí Esteban-Martín,† Barbara Richter,† Donghan Lee,‡ Korvin F. A. Walter,§ Dragomir Milovanovic,‡ Stefan Becker,§ Nils A. Lakomek,§ Christian Griesinger,*,†,|| and Xavier Salvatella*‡,†,||

†Joint BSC–IRB Research Programme in Computational Biology, Institute for Research in Biomedicine (IRB Barcelona), Parc Científic de Barcelona, Baldirí Reixac 10, 08028 Barcelona, Spain
‡Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, U.K.
§Max Planck Institut fur Biophysikalische Chemie, Am Fassberg 11, 37077 Göttingen, Germany
||Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

ABSTRACT: Long-range correlated motions in proteins are candidate mechanisms for processes that require information transfer across protein structures, such as allostery and signal transduction. However, the observation of backbone correlations between distant residues has remained elusive, and only local correlations have been revealed using residual dipolar couplings measured by NMR spectroscopy. In this work, we experimentally identified and characterized collective motions spanning four β-strands separated by up to 15 Å in ubiquitin. The observed correlations link molecular recognition sites and result from concerted conformational changes that are in part mediated by the hydrogen-bonding network.

The detection of motional correlations in proteins has been hampered by difficulties in experimentally determining the fluctuations of atomic coordinates.1 However, it has been shown that NMR cross-correlated relaxation rates (CCRs) can provide a direct route to the characterization of correlated dynamics in biomolecules.2–5 We chose to study correlated backbone motions in the protein ubiquitin by analyzing a conformational ensemble that is in good agreement with CCRs measured for this protein as well as with trans-hydrogen-bond scalar couplings (\( ^{3}J_{NC} \)). Ubiquitin has a well-characterized function in targeting proteins for degradation. During this process, this protein recognizes a structurally diverse set of partners with a mechanism based on conformational selection.6

To study the presence of long-range correlated motions in the backbone of ubiquitin, we determined an ensemble of conformations that represents the structural heterogeneity of this protein on the submillisecond time scale.7 The ensemble was obtained using ensemble molecular dynamics (MD) simulations8–10 restrained by previously reported 2663 nuclear Overhauser effects (NOEs)11 and 1971 NH residual dipolar couplings (RDCs).12 The structure calculations were carried out with the CHARMM27 force field13 in explicit solvent14 using an in-house-modified version of the CHARMM molecular simulations software.15 To ensure that the structure of ubiquitin was well-reproduced, the NOEs were restrained over pairs of structures.16 The RDCs, which report on both the orientation and structural fluctuations of NH bond vectors,17 were restrained by a quadratic potential8,9 that penalizes deviations between the experimentally measured and ensemble-averaged RDCs. The five independent tensor elements that define the alignment of ubiquitin in each of the 36 alignment media12 were optimized simultaneously with the atomic coordinates. The ensemble refinement protocol was started from the X-ray structure of ubiquitin (PDB code 1UBQ18) and consisted of simulated annealing (SA) cycles [for details, see the Supporting Information (SI)].

We first identified the minimal number of conformations that fitted the NMR data.8 For this purpose, we carried out ensemble simulations starting from the X-ray structure of ubiquitin (1UBQ) using the SA protocol described in the SI and ensemble sizes ranging from 1 to 128. We then cross-validated the resulting ensembles against NMR parameters not used as restraints and obtained an optimal size of 64 (Figure S1 in the SI). Finally, to obtain a sufficiently large number of conformations to analyze correlated motions with statistical confidence (see below), we performed 12 consecutive SA cycles with an ensemble size of 64. After discarding the first two SA cycles to minimize the bias introduced by the first steps of the simulation, we obtained a 640-member ensemble that we named ensemble refinement for native proteins using a single alignment tensor (ERNST).

As shown in Table 1, ERNST is in good agreement with previously reported backbone scalar and \( ^{3}J_{HC} \) and NC' dipolar couplings11 that were not used as restraints. The level of agreement is comparable to those for static structures (PDB code 1D3Z) and dynamic ensembles (PDB code 2K39) that, unlike ERNST, were refined using some of these RDCs. ERNST is also in very good agreement with both the NMR and X-ray structures of ubiquitin and is of lower structural heterogeneity than related ensembles6 as a result of the nature of the protocol that was used to generate the ensemble.

We also analyzed the level of agreement of ERNST with NMR parameters sensitive to correlated motions. These included the cross-correlated relaxation rates \( R_{NN,L,NH} \) measured by Bodenhausen and co-workers,3 the rates \( R_{NH,Ca,H} \) measured here for ubiquitin using the experiment introduced by Vögeli and Yao20 (Table S1 in the SI), and trans-hydrogen-bond scalar couplings \( ^{3}J_{NC} \) previously reported by Cordier and Grzesiek.21 These

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parameters are useful for validating correlated motions because they average on the same time scale as RDCs and are sensitive to the degree of correlation of the motions of two distinct bond vectors. In regard to correlated motions in the β-sheet of ubiquitin, RNNH and RNCαHα values report on the relative motion of NH and CaαHα bond vectors in consecutive residues and JNCC values report on the relative motion of NH and CO bond vectors of hydrogen-bonded residues in β-strands.

Table 1 shows that cross-correlated relaxation rates involving NHs are sensitive to an accurate representation of the orientation of NH bond vectors. The only structural models of ubiquitin that gave relatively accurate values of RNNH and RNCαHα were those that were restrained with NH RDCs (1D3Z, 2K39, and ERNST). The JNCC data (Figure S2) were quite well reproduced by an unrestrained MD simulation [root-mean-square deviation (rmsd) = 0.10 Hz] and especially by ERNST. This confirms the impact of bond vector dynamics on this NMR parameter. A comparison of the performance of the different structural models available for ubiquitin indicated that ERNST gave the best agreement with the NMR parameters that are sensitive to correlated motions, justifying the use of this ensemble for the investigation of this dynamical property.

To investigate correlated motions on the submillisecond time scale, we analyzed the statistical dependence of the φ and ψ torsion angles of ubiquitin in ERNST (Figure 1A). We observed strong sequential anticorrelations (ρ ≈ −0.5) of φi and φi−1 for residues flanking amide bonds, especially in β-strands [circular correlation coefficient (ρ) ≈ −0.7]. We also observed sequential correlations of φ and ψ for the same residue in turns, helices, and loops (ρ ≈ −0.4). The former is due to the planarity of the peptide bond and is associated with the crankshaft motion observed in molecular simulations and in an analysis of the dynamics of protein G from RDCs. The latter stems from local steric effects that render φ and ψ of residues in nonextended conformations nonindependent, as shown by Pappu et al. using molecular mechanics calculations. We term the motion that leads to the φ/ψ correlation the intraresidue motion. The degree to which such steric effects limit motion in helical turns is evident from the circular variances (ρ2) as sequential correlations. These were found to be significantly lower in the loops and α-helix (ρ2(α-helix) = 0.007) than in the β-strands (ρ2(β-strand) = 0.028), consistent with the relatively high RDC-derived order parameters obtained in other analyses of the dynamics of ubiquitin.

It has been reported that invoking correlations between pairs of hydrogen-bonded residues improved the agreement with experimental JNCC data in a study of the dynamics of protein G using the 3D-GAF motional model. Similar correlations have also been observed in an MD simulation of ubiquitin. In agreement with this, we observed correlations between the backbone torsion angles of residues that are distant in sequence but connected through hydrogen bonds (shown in black in Figure 1A). In ERNST, in contrast to in silico observations for ubiquitin, such nonsequential correlations were observed between essentially all pairs of hydrogen-bonded residues in the β-sheet (β2 vs β1, β1 vs β5, β5 vs β3, and β3 vs β4) and also between hydrogen-bonded loops (loops 2 and 5). These correlations are propagated via noncovalent interactions and are generally not as strong (ρ ≈ −0.3) as sequential correlations.

A large fraction of the nonsequential short-range correlations can be rationalized qualitatively by considering the structure of the protein. When pairs of β-strands are in the antiparallel
orientation, as is the case for \( \beta_2 \) vs \( \beta_1 \), \( \beta_5 \) vs \( \beta_3 \), and \( \beta_3 \) vs \( \beta_4 \) (Figure 1B), the \( \rho \) values of hydrogen-bond donor residues and \( \psi \) values of acceptor residues (in-register) are anticorrelated. This motion, here termed \( \beta \)-lever, allows conformational changes to occur while preserving hydrogen bonds. To illustrate its prevalence, we present in Figure 2 strands \( \beta_1 \) and \( \beta_2 \) in atom detail as well as an analysis of the correlations observed in this region. In these strands, we also observed correlations between the \( \rho \) and \( \psi \) of residues that are in-register but not hydrogen-bonded, such as Lys6 and Thr12 (Figure 2A), and between the \( \rho \) and \( \phi \) (and \( \psi \) and \( \psi \)) of residues that are out-of-register, such as Lys6 and Ile13 (Figure 2C). These correlations can be attributed to concerted crankshaft and \( \beta \)-lever motions, and their signs can be predicted by consideration of the signs of these two correlations. It should be noted that for parallel \( \beta \)-strands, such as \( \beta_1 \) vs \( \beta_5 \), the \( \beta \)-lever motion causes instead a positive correlation between the torsion angles of hydrogen-bonded residues (out-of-register) that results from the change in orientation of the two strands (Figure S3).

The short-range crankshaft, intrasidue, and \( \beta \)-lever motions observed in the \( \beta \)-sheet of the ubiquitin backbone are necessary but insufficient for propagation of structural and dynamical information across the long distances required for binding allostery and signal transduction. For these biological processes to operate efficiently, these essential motions must occur in concert to link distal residues dynamically. We observed a number of statistically significant (\( p < 0.05 \)) long-range correlations (shown in red in Figure 1A). These correlations connect strands \( \beta_2 \) and \( \beta_3 \) (Ile13 and Leu67), strands \( \beta_1 \) and \( \beta_3 \) (Lys6 and Phe45) and, extraordinarily, strands \( \beta_2 \) and \( \beta_3 \) (Ile13 and Phe45; Thr14 and Phe45) that span four \( \beta \)-strands of ubiquitin and a distance of \( \sim 15 \) Å (Figure 3 and Figure S5). These properties of ERNST are robust with respect to changes in the simulation protocol (Figure S4).

To determine whether these long-range correlations were a result of the CHARMM27 force field or arose from the information on the structural heterogeneity of ubiquitin contained in the experimental data, we carried out control unrestrained simulations. The resulting in silico ensembles (see Figure S4) contained a fraction of the short-range correlations that we observed in the experimental ensemble and none of the long-range correlations shown in Figure 1. These results indicate that the experimental data act as a correction to the force field, allowing the experimental ensemble to reflect correlated motions that cannot be observed with confidence in silico.

The mechanism by which such correlations operate is likely to involve covalent and noncovalent interactions between atoms in the backbone and side chains of ubiquitin. Despite this
complexity, we were able to trace a direct path of sequential and short-range concerted motions connecting the dihedral angles of the longest-range correlation between residues Ile13 in β2 and Phe45 in β3. This path, which is presented in Figure 3B, involves the β-lever motion of Ile13 and Val5, the crankshaft motion of Val5 and Lys6, the β-lever motion of Lys6 and His68, the β-lever of His68 and Ile44, the crankshaft motion of Ile44 and Phe45, and the intraresidue correlation of Phe45.

The nonsequential correlations, connected as a path, provide channels for the relay of structural and dynamical information across protein structures. These have long been hypothesized to be ubiquitous in biology but remain challenging to detect. To investigate whether the path observed in ERNST can contribute to the function of ubiquitin, we analyzed the relationship between the correlations and the binding modes of ubiquitin binding domains (UBDs). The vast majority of UBDs bind to the surface of ubiquitin around Ile44.4 Previous studies have shown that the regions of ubiquitin most often found at the binding interface are the loop region between strands β1 and β2, the C-terminal end of strand β5, and the loop region between strands β3 and β4.7 These regions coincide with both the residues having the largest nonsequential correlations (shown in Figure 2B) and the network of hydrogen bonds that mediate the long-range correlations in Figure 3. We therefore observe that the long-range correlations present in ubiquitin link the residues that are most important for its function.

A further strong indication for the existence of long-range correlated motions in ERNST comes from the observation that a principal component analysis of the residues shown in Figure 3 and those extracted from the ensemble of crystal structures of ubiquitin complexed with recognition proteins revealed similar crankshaft and β-lever motions. The similarity between the β-strand motions in the solution ensemble of ubiquitin and the ubiquitin complexes suggests that these long-range correlations are important for protein–protein recognition.

In conclusion our analysis illustrates how long-range correlations dynamically link distal residues that contribute to the binding interface of a protein and suggests a mechanism by which conformational selection could operate in molecular recognition. The strategy used in this study is general and could be used to obtain atomic-resolution descriptions of signal transduction and binding allostery, as demonstrated by our integrated description of the dynamics, correlations, and biological function of ubiquitin.

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**ASSOCIATED CONTENT**

Supporting Information. Details of the simulation protocol and the equations used to back-calculate the NMR parameters, experimental $R_{NH,CO,H}^2$ data, additional correlation plots, correlation plots for the $^{15}N$-dec data, equations used to compute ρ and σ values, statistical analysis of the correlation of longest range, and complete ref 15. This material is available free of charge via the Internet at http://pubs.acs.org.

**AUTHOR INFORMATION**

Corresponding Author

cigr@nmr.mpipbc.mpg.de; xavier.salvatella@irbbarcelona.org

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**COMMUNICATION**