J|A|C|S COMMUNICATIONS

Published on Web 09/25/2009

Short-Range Coherence of Internal Protein Dynamics Revealed by High-Precision in Silico Study

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Conformational dynamics of folded proteins plays an essential role in biologically important functional processes, such as enzymatic activity, molecular recognition, and allosteric regulation.[1,2] The comprehensive experimental characterization of protein dynamics, including mutual correlation effects, is challenging because of the large number of degrees of freedom (DOFs) and the wide range of motional time scales involved. In silico studies using atomistic molecular dynamics (MD) simulations[3] have been hampered in the past by their relatively short length, leading to poor conformational sampling statistics. The recent availability of microsecond MD trajectories[4,5] promises substantial improvement in this regard. In parallel, important progress in the quality of protein force fields has been made,[6,7] as validated using experimental data.[5,8,9] This suggests that the latest generation of MD simulations permits in silico characterization of protein dynamics that is both increasingly realistic and precise.

In this work, we analyzed motional correlations between dihedral angles at unprecedented precision using long submicrosecond MD simulations of the two proteins ubiquitin and calbindin D_{9k}. These soft internal DOFs, which constitute less than 10% of the Cartesian coordinates, account for the dominant fraction of all protein fluctuations. Without exception, all of the mobile dihedral angle pairs in ubiquitin with sizable dynamics correlations were found to be at short-range distance. In some cases, they involve sequentially remote dihedral angles that form sparse clusters, enabling the propagation of structural dynamics changes via soft torsional couplings that act over short distances with a rapid loss of coherence over longer distances.

The MD trajectory of ubiquitin, which was performed using AMBER9[10] and the AMBER99SB force field[6] in explicit SPC/E water as described previously,[8] showed stable behavior over its 0.7 μs length [see the Supporting Information (SI)]. The mutual correlations between all $310 \cdot 309/2 = 47\,895$ dihedral angle pairs, assessed using the standard Pearson correlation coefficient R, are depicted in Figure 1 for the entire 0.7 μs simulation length and compared with the first 0.01 μs segment. The standard deviation (σ) of the distribution of the 0.01 μs segment is substantially larger ($\sigma = 0.056$) than that of the 0.7 μs trajectory ($\sigma = 0.022$) reflecting the poorer precision of the shorter trajectory resulting from its limited sampling statistics (Figure 1A). For individual dihedral angles, the changes in R between the 0.01 μs and 0.7 μs trajectories can be large, as is visible in Figure 1B. These false correlations are caused by rare events, e.g., when (uncorrelated) dihedral angles accidentally flip once at a similar time point during the 0.01 μs segment.

For the 0.7 μs trajectory, 67 (45) dihedral angle pairs have $|R| \geq 0.5$, whereas the remaining 47 828 (46 890) dihedral angle pairs have $|R| < 0.5$ (the numbers in parentheses are significant at the 95% confidence level). There are 153 (93) dihedral angle pairs with $R^2 \geq 0.1$ and 47 742 (44 146) pairs with $R^2 < 0.1$. Only 1.7%, i.e. 811, of all dihedral angle pairs display correlations that are statistically significantly different from zero. Hence, on the basis of the linear cross-correlation metric (without time delay) used here, the vast majority (98.3%) of protein dihedral angle pairs are in essence uncorrelated, which is consistent with mutual information analysis (see the SI).

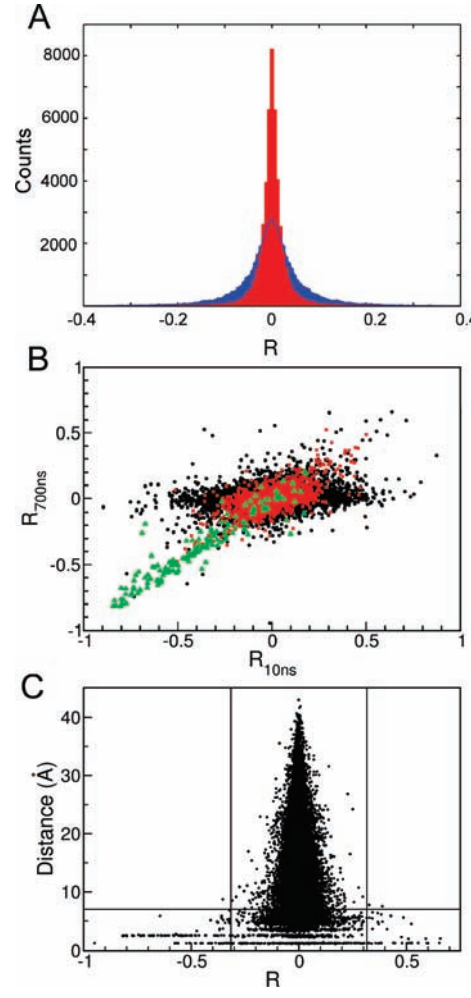

Figure 1. (A) Histogram of Pearson correlation coefficients R for all dihedral angle pairs of ubiquitin. Blue points belong to first 10 ns and red points to the full 700 ns trajectory. (B) Plot of the correlation between the R values of the 700 ns and 10 ns trajectories. Neighboring main-chain dihedral angle pairs (ψ_{i-1}, φ_i) and (φ_i, ψ_i) are shown in green, all other main-chain pairs in red, and the remaining pairs in black. (C) Relationship between R and the distance r between the midpoints of the two dihedral angles for both the backbone and side chains of the 700 ns MD simulation. The horizontal line corresponds to $r = 7$ Å and the two vertical lines to $R = \pm 0.316$ (i.e., $R^2 = 0.1$).

10.1021/ja905340s CCC: $40.75

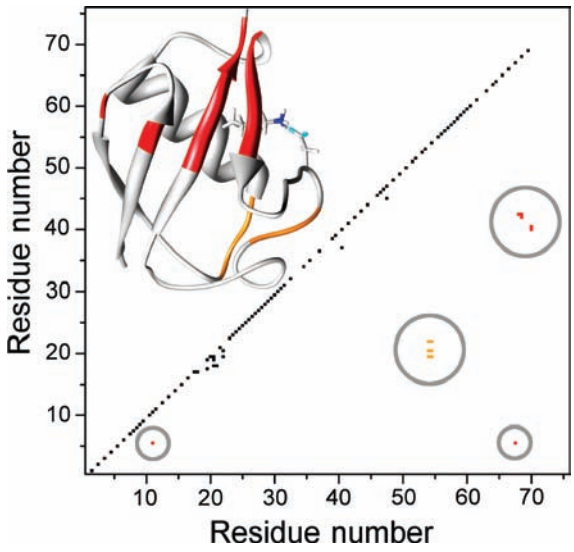

Figure 2. Pairwise correlation coefficients R between all main-chain dihedral angles of ubiquitin with $R^2 \geq 0.1$ (only the part below the diagonal is shown). The only sequentially remote side-chain−side-chain pairs with $R^2 \geq 0.1$ are χ_2(Arg42)/φ(Arg72) and χ_2(Arg42)/χ_1(Arg72). Inset: ribbon diagram of ubiquitin with dynamically correlated short-range dihedral angle clusters across the extended β-sheet (red) and across two neighboring loops (yellow). The salt bridge between Lys27 and Glu52 is shown explicitly.

All of the dihedral angle pairs with $R^2 \geq 0.1$ are at short range, with dihedral angle centers separated by a distance $r < 7$ Å (Figure 1C). The only exceptions are dihedral angle pairs φ_{20}/ψ_{22} ($r = 7.6$ Å), φ_{20}/ψ_{54} (8.7 Å), and φ_{21}/ψ_{54} (7.8 Å), which involve residues on both sides of two interacting loops (Figure 2). Correlations over medium-range distances can be mediated via a combination of bonded and nonbonded interactions. This is the case for the χ_2(Lys27)/χ_1(Glu52) pair ($R = 0.342$ and $r = 6.6$ Å), whose side-chain termini form a salt bridge as depicted in Figure 2.

The amino acid sequence relationship of main-chain dihedral angle correlations is shown in Figure 2, with the characteristic anticorrelated backbone (ψ_{i-1}, φ_i) pairs[11] along the diagonal. Remarkably few correlations belong to pairs whose dihedral angles are far apart in primary sequence. Such pairs belong to sparse, isolated clusters of spatially proximate residues (Figure 2, gray circles). The yellow cluster in Figure 2 represents dynamic correlations between residues 20−22 and residues 54−55 that belong to two interacting loops. The red clusters are part of the extended β-sheet structure of ubiquitin, and they include dynamic correlations between residues 40−42 and 70−72 and amino acid pairs (Lys6, Lys11) and (Lys6, His68). These correlations exist between adjacent β-strands, and they rapidly fade away between strands that are not in direct physical contact. However, the reverse does not apply: spatial proximity of two dihedral angles generally does not induce dynamics correlations, as the vast majority of spatially close dihedral angle pairs display weak or absent correlations (Figure 1C). The short-range nature of correlated dynamics reflects low transitivity, i.e., the tendency to transmit correlation to a third dihedral angle is weak (Table S1 in the SI), indicative of a rapid loss of coherence of dynamics through dihedral angle networks.

Transmission of information across proteins is a central feature of allosteric regulation, although the atomistic mechanisms are not well-understood.[2,12] The high-precision statistical behavior of ubiquitin illustrates how information propagates in a diffusion-like manner via a local interaction network across a wide portion of this biomacromolecule. Such soft interaction networks, uncovered by spatial clustering of precise dihedral angle correlations, may serve as templates for the (allosteric) propagation of perturbations in other protein systems.

Acknowledgment. We thank Dr. Scott A. Showalter for support with the generation of the MD trajectories. This work was supported by the NSF (Grant MCB-0918362).

Supporting Information Available: Information about MD trajectories, internal correlation times, correlation and mutual information analysis, complete list of dihedral angle correlations, Cartesian space analysis, and calbindin D_{9k} results. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA905340S