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Conformational Fluctuations of UreG, an Intrinsically Disordered Enzyme

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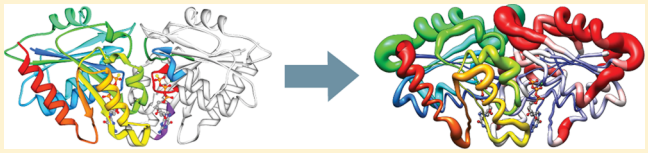
Supporting Information

ABSTRACT: UreG proteins are small GTP binding (G) proteins that catalyze the hydrolysis of GTP necessary for the maturation of urease, a virulence factor in bacterial pathogenesis. UreG proteins are the first documented cases of intrinsically disordered enzymes. The comprehension of the dynamics of folding−unfolding events occurring in this protein could shed light on the enzymatic mechanism of UreG. Here, we used the recently developed replica exchange with solute tempering (REST2) computational methodology to explore the conformational space of UreG from *Helicobacter pylori* (*Hp*UreG) and to identify its structural fluctuations. The same simulation and analysis protocol has been applied to HypB from *Methanocaldococcus jannaschii* (*Mj*HypB), which is closely related to UreG in both sequence and function, even though it is not intrinsically disordered. A comparison of the two systems reveals that both *Hp*UreG and *Mj*HypB feature a substantial rigidity of the protein regions involved in catalysis, justifying its residual catalytic activity. On the other hand, *Hp*UreG tends to unfold more than *Mj*HypB in portions involved in protein−protein interactions with metallochaperones necessary for the formation of multiprotein complexes known to be involved in urease activation.



The GTP binding proteins (G-proteins) constitute a wide class of monomeric or multimeric proteins that operate as molecular switches between GTP- and GDP-bound states. They are involved in regulatory and signaling functions[1,2] as well as in the biosynthesis of metal-based cofactors (see refs 3 and 4 for some examples). The reaction catalyzed by G-proteins is the GTP hydrolysis that yields GDP and inorganic phosphate. In general, the catalytic domain of a G-protein is constituted by a β-sheet, surrounded by flexible regions composed of α-helices and loops (Figure S1 of the Supporting Information). The stability of the active GTP-bound or inactive GDP-bound state of the G-proteins during the reaction depends on the conformation adopted by the three regions (named P-loop, switch I, and switch II) delimiting the GTP binding pocket, as well as by interactions of the G-protein with specific protein regulators.

Much attention has been paid recently to one of the bacterial members of this class, UreG, a magnesium-based enzyme involved in urease maturation,[5] and in particular to the protein from the human pathogen *Helicobacter pylori* (*Hp*UreG). *Hp*UreG is one of several UreG proteins from different biological sources that have been documented as belonging to the class of intrinsically disordered enzymes[4,6−10] displaying observable enzymatic activity while showing a largely disordered tertiary structure.[4,6,8,9] Indeed, $^{1}H-^{15}N$ HSQC nuclear magnetic resonance (NMR) spectra acquired for UreG from different bacterial species show that the protein backbone experiences exchange among multiple conformations, while on the other hand, circular dichroism (CD) spectra reveal the presence of secondary structure elements. UreG structural disorder is predicted to be localized especially in a region spanning ∼50 residues in the central portion of the sequence.[6] This disordered portion comprises both the region predicted to interact with the cognate protein UreE[11] and the switch I loop, thus influencing the structure of the GTP binding pocket. Other disordered enzymes are known, such as chorismate mutase, a case in which the enzyme is intrinsically ordered but maintains its activity also in a topologically redesigned version, obtained by site-directed mutagenesis, which features dynamic properties typical of molten globules.[12−15]

Experimental structural information for UreG is lacking. Bioinformatics-based considerations have led to the suggestion that the structured regions of *Hp*UreG might be not too dissimilar to the corresponding regions of the magnesium-based enzyme HypB protein expressed by the thermophile bacterium *Methanocaldococcus jannaschii* (*Mj*HypB).[5] Indeed, *Hp*UreG and *Mj*HypB belong to the same class of dimeric G-proteins[16] and are predicted to share a nearly identical secondary structure despite a level of sequence identity of 31% (Figure S2 of the Supporting Information).[4] The X-ray structure of *Mj*HypB in

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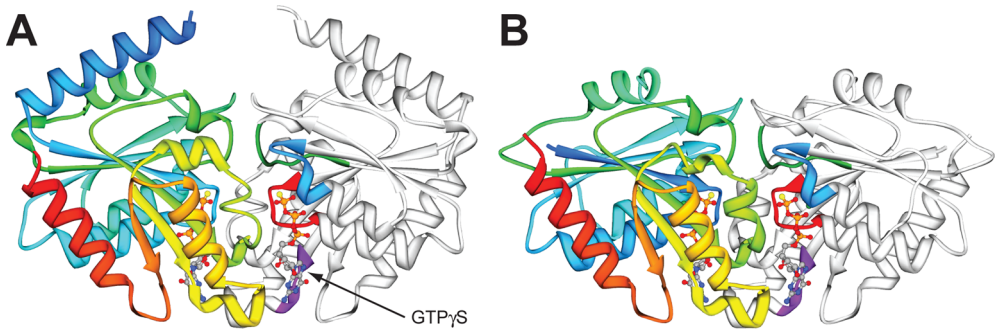

Figure 1. X-ray structure of dimeric *Mj*HypB (A, Protein Data Bank entry 2HF8) and structural model of *Hp*UreG (B, calculated as in ref 4). Left chains are colored from blue in the proximity of the N-terminus to red at the C-terminus, whereas in the right chain, the main features of the proteins are highlighted (P-loop, red; switch I, blue; switch II, dark green; guanine specificity pocket, violet).

complex with a GTP analogue[3] features a well-ordered fold (Figure 1A).

Here, we used molecular simulation methods to investigate the structural fluctuations of the *Hp*UreG model structure as it evolves from its putative folded conformation (Figure 1B)[4] to the unfolded state in explicit solvent. Comparison is also made with the MD simulation of *Mj*HypB in aqueous solution. We studied the apo form of both proteins to remove any possible effect played by metal binding on the two proteins. Indeed, *Mj*HypB appears to bind two Zn(II) ions,[3] while *Hp*UreG binds only one Zn(II) ion[4,17] at the dimer interface. Moreover, it should be noted that the occupancy of the two Zn(II) ions in the *Mj*HypB crystal structure is 0.5. Thus, it is not clear whether one should put the Zn(II) ion in one site, the other, or both. In the case of *Hp*UreG, there are no structural data available for the correct placement of the unique Zn(II) ion present in the protein. The only available information is based on a model structure derived using EXAFS data.[17] Here we chose not to bias the simulations with a subjective placement of the Zn(II) ions.

METHODS

The initial structural model of *Mj*HypB was obtained from the crystal structure of the protein in complex with GTPγS (an unreactive GTP analogue) and Mg^{2+} ions (Figure 1A, Protein Data Bank entry 2HF8).[3] The starting structural model of *Hp*UreG was predicted by homology modeling based on the X-ray structure of *Mj*HypB.[4] The modeling procedure comprised the following steps, which are described in detail in ref 4: (i) sequence alignment performed using MODELER version 9.2[18] and JPRED,[19] (ii) homology modeling using MODELER version 9.2, and (iii) validation of the best scoring model, using PROCHECK.[20]

The proteins were placed in a water box using a 10 Å buffer zone of solvent around the protein. The Amber ff99SB force field[21] for the protein and the TIP3P water model[22] were used, while known parameters were applied to GTP[23] and Mg^{2+}.[24] Each system was energy-minimized and then evolved through a replica exchange molecular dynamics (REMD) scheme.[25] This is the replica exchange with solute tempering (REST),[26] in which only the protein (i.e., the solute) is simulated at different effective temperatures. This is achieved by applying an appropriate potential energy function to each replica[27] as conducted in its most recent implementation (REST2).[28] Although the extensive sampling of conformational space of large proteins is challenging for any computational technique, we adopted REST2 because it has been previously demonstrated to optimally visit the relevant conformations for a given computational cost.[28] In particular, REST2 allows us to consistently reduce the number of replicas needed with respect to standard REMD (i.e., only 24 replicas are needed for our systems, while 100 replicas are estimated to be needed for REMD[29]) and is particularly suitable for systems as large as those considered here.[28] We exploited the λ-dynamics implementation in GROMACS version 4.5.3[30−33] to easily introduce the REST2 approach into the simulation program.[27] We ran 24 replicas for both *Mj*HypB and *Hp*UreG at different effective temperatures of the solute, from 300 to 450 K, following the REST2 technique,[27,28] and 1 atm. Temperature coupling and pressure coupling were achieved using a Nose-Hoover thermostat[34,35] and a Parrinello-Raman barostat,[36,37] respectively. The calculated properties include root-mean-square deviation (RMSD) and fluctuations (RMSF) and the plasticity of protein backbone residues calculated using protein angular dispersion.[38] Quasi-rigid domains were identified on the basis of the MD positional covariance matrix as described in refs 39 and 40. For additional details about the computational methods, see the Supporting Information. The calculations were performed on the JUROPA supercomputer (Jülich Supercomputing Centre, JSC, Jülich, Germany).

RESULTS AND DISCUSSION

Simulation of the *Mj*HypB Structure. The RMSD of the Cα atoms of the two monomers for the lowest-effective temperature replica simulation (Figure 2A) does not significantly change with respect to the starting structure. Indeed, both the secondary structure and the tertiary structure are largely maintained (Figure 2A, top panels), and the secondary structure elements are conserved throughout the simulation (Figure 2B). On the other hand, the most visited conformations during the replica exchange process progressively depart from the initial state, as illustrated in Figure 2A by the fact that the RMSD of the dimer increases steadily. This structural drift, and particularly the rapid increase in RMSD after simulation for ∼8 ns, reflects the spontaneous evolution of *Mj*HypB toward a conformational substate markedly different from the initial one. Indeed, a structural cluster analysis (see Figure S4 of the Supporting Information) identifies two dominant clusters that can be largely identified with the first and second part of the trajectory and that gather 69 and 25% of the visited conformers, respectively. The structural differences of the two cluster representatives are shown in Figure 2C and can be aptly conveyed by the relative positioning of the inertial ellipsoids representing each monomer. Upon optimal super-

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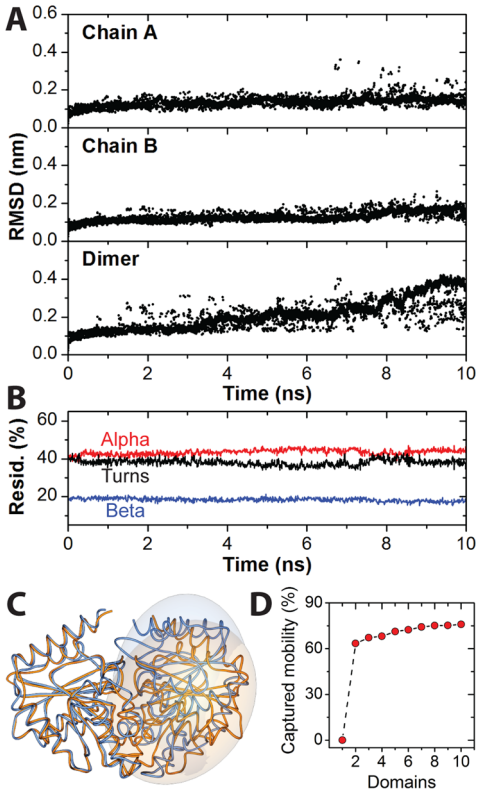

Figure 2. RMSD for the Cα atoms (A) and percentage of secondary structure elements (B) of *Mj*HypB lowest-effective temperature replica (300 K) vs time plots. (C) Ribbon diagram and inertia ellipsoid of the most representative structure of *Mj*HypB cluster 1 (orange) and cluster 2 (blue). The ellipsoids in panel C have been calculated by fixing the best fit of the left chains of *Mj*HypB to highlight the displacement of the right chains. (D) Fraction of captured mobility vs the number of quasi-rigid domains.

position of two corresponding monomers, the principal axes of the other two ellipsoids, corresponding to the other monomer, are mismatched by 42°, 47°, and 24° (Figure 2C). On the other hand, the distance between the centers of mass of the two ellipsoids remains stable during the simulation (2.89 ± 0.04 nm). These results indicate that (i) the dimer integrity is maintained during the dynamical evolution and (ii) the latter mostly entails the relative rigid rotation of the monomers around the major axis of the dimer. The latter aspect emerges very clearly as shown by a quasi-rigid domain analysis of *Mj*HypB, which indicates that the relative quasi-rigid motion of the two monomers accounts for most (65%) of the protein's RMSF (Figure 2D).

The observed capability of dimeric *Mj*HypB to bind Zn(II) at the dimer interface may be necessary to stabilize the starting conformation of the two monomers and the dimeric form of the protein.[41,42]

Simulation of the *Hp*UreG Model Structure. The Cα RMSD of *Hp*UreG increases sharply in the first nanosecond of the simulation (Figure 3A). Subsequently, the RMSD increases monotonically for both the monomers and the dimer. This situation differs from the case for *Mj*HypB, and this suggests a loss of structured features that is significantly more pronounced for *Hp*UreG. This is also revealed by a comparison of the plots of the secondary structure elements (compare Figures 2B and 3B): the average contents of α-helix, β-strand, and coil in *Hp*UreG change from 38 ± 1, 19 ± 1, and 43 ± 2%, respectively, in the first half of the simulation to 34 ± 2, 16 ± 1, and 50 ± 2%, respectively, in the second half. These final values are in agreement with the secondary structure content experimentally determined for the protein in solution using CD spectroscopy (31, 11, and 58%, respectively).[4] Large effects are visible in the overall structure of the protein backbone, as revealed by a cluster analysis of the trajectory conducted with the same protocol used for *Mj*HypB [8 vs 5 clusters in the first 10 ns, reaching 82 clusters in the second half of the simulation (see Figure S5 of the Supporting Information)]. In particular, two clusters account for ∼74% of the overall sampled conformation in the first 10 ns of the simulation; with a starting point of 9.3 ns of simulation time, the number of clusters increases dramatically, following a consequent decrease in the population of each single cluster [from <3.9%, down to single-conformation clusters (Figure S5 of the Supporting Information)]. In the case of *Hp*UreG, the mutual orientation of the two monomers changes by 13 ± 3°, 15 ± 4°, and 9 ± 5° (with maximal variations of 20°, 41°, and 40°, respectively) in the three principal axes of the inertial ellipsoids, while the distance between the relative centers of mass (2.85 ± 0.03 nm) is stable along the simulation. This indicates that *Hp*UreG samples a significantly larger conformational space than *Mj*HypB does, and that all the clusters observed in the second part of the *Hp*UreG simulation share a similar secondary structure content despite different structural properties, a result in agreement with experimental data and typical of intrinsically unstructured proteins in the molten globule state.[43]

The analysis of the RMSFs for *Hp*UreG (Figure 3C) reveals that the regions most affected by the ensemble variability comprises residues 45−92, 129−136, and 148−171 (corresponding to positions 67−114, 155−158, and 170−193, respectively, in the alignment in Figure S2 of the Supporting Information and Figure 3C). The same behavior can be observed by calculating the plasticity of protein backbone residues using protein angular dispersion[38] (Figure S6 of the Supporting Information). This region is consistent with that predicted to be disordered (residues 38−94, 128−137, and 155−176)[6] by bioinformatics tools such as PONDR VL-XT[44,45] (see Figure S7 of the Supporting Information). Indeed, the good consistency of PONDR predictions and highly mobile regions has been reported previously in another context.[46] The regions that experience "long" conformational transitions (estimated as described in ref 38) include switch I, helices 2 and 3, strand 3, and the connecting loops as well as helix 5 (Figure 3D), while other less mobile regions appear to fluctuate around a single configuration (Figure S6 of the Supporting Information). In this region, a conserved CPH motif is also found, which has been established to be involved in the binding of one Zn(II) ion at the dimer interface.[4] Helix 3 is important for the function of UreG in the in vivo urease activation process because it is predicted to be situated at the interface with UreE, the proposed metallochaperone for nickel trafficking.[11] Notably, the disordered regions do not involve the active site, except for the so-called switch I loop (residues 37−43). This property is intriguing but is not expected to be generally applicable to intrinsically disordered enzymes: in fact, in the case of engineered chorismate mutase, the catalytic region is found to be quite flexible, with protein folding associated with the substrate binding step and with subsequent catalysis.[12−15] Thus, at present, it is not clear how the increase in the level of disorder affects the catalytic activity. A comparison of the RMSF between *Mj*HypB and *Hp*UreG reveals a substantial

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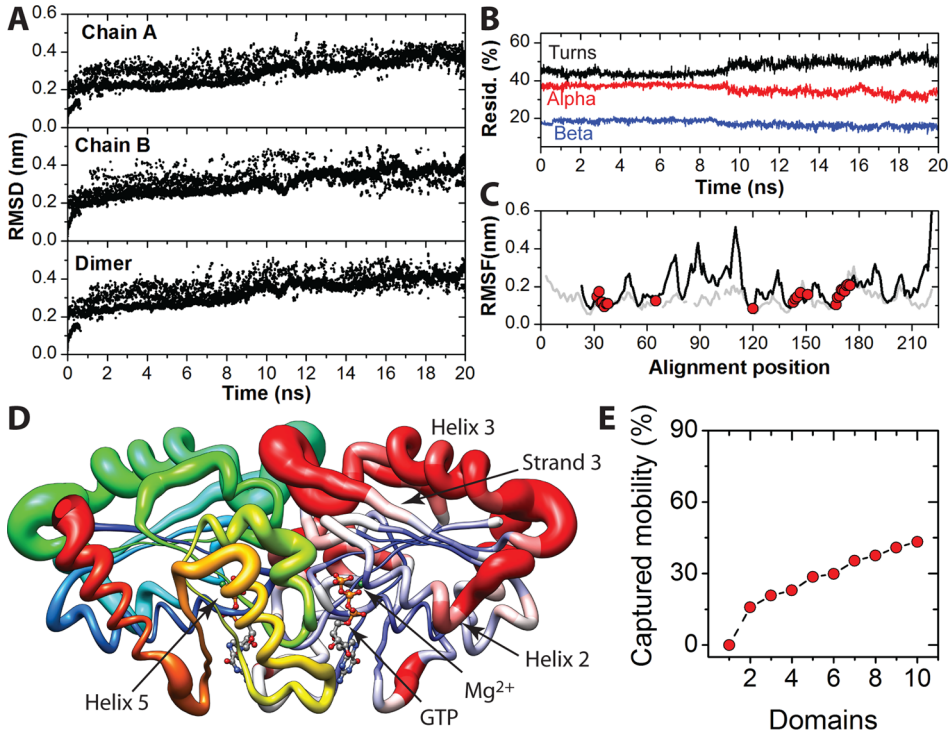

Figure 3. RMSD for the Cα atoms (A) and percentage of secondary structure elements (B) of *Hp*UreG lowest-effective temperature replica (300 K) vs time plots. (C) RMSF of *Mj*HypB (gray) and *Hp*UreG (black) vs alignment position plot (see Figure S3 of the Supporting Information). Red dots indicate residues found to be in direct contact with GTP in the *Hp*UreG model structure. (D) Sausage representation of the *Hp*UreG model structure. The diameter of the sausage is proportional to the RMSF of Cα atoms. The chain on the left is colored from blue in the proximity of the N-terminus to red at the C-terminus, whereas the chain on the right is colored from blue to red (low to high RMSF values, respectively). GTP, Mg^{2+} ions, and regions indicated in the text are indicated. (E) Fraction of captured mobility vs the number of quasi-rigid domains.

difference in the dynamic properties of the two proteins, with the exception of the residues that interact with GTP in the starting model of the folded state of the two proteins (red dots in Figure 3C). This observation can explain the low, but often observable, GTPase activity of several UreG proteins in vitro, making them the only case of intrinsically disordered enzymes so far discovered.[4,6,8,9] The observed differences in the conformational fluctuations of *Mj*HypB and *Hp*UreG are not limited to the overall amplitude, but they reflect a genuine difference in the structural plasticity of the two molecules. This is readily ascertained by repeating, for *Hp*UreG, the quasi-rigid domain analysis described above for *Mj*HypB. As can be seen from Figure 3E, the quasi-rigid character of the *Mj*HypB monomers is completely lost in *Hp*UreG. Indeed, the loss of the structural integrity of *Hp*UreG is evident by considering that the use of as many as 10 quasi-rigid domains is barely sufficient to capture half of the protein mobility, as opposed to what was observed in *Mj*HypB, for which only two quasi-rigid domains covered 65% of the internal mobility.

A striking structural difference between the two systems is the presence of an additional helix at the N-terminus of *Mj*HypB, positioned in close contact with the protein portion that corresponds to the disordered region of all members of the UreG family.[6] We thus speculate that the insertion of a similar helix into the sequence of *Hp*UreG could induce folding and stabilization of the protein, possibly also influencing the in vivo activity. The challenge is now to understand the intricate relationship between *Hp*UreG's conformational fluctuations and its function.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of the REST2 calculations and the data analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

F.M. conceived, designed, and ran the simulations, analyzed the data, and wrote the paper. E.I. designed the simulations. C.M. designed and ran the coarse-grained analysis, analyzed the data, and wrote the paper. P.C. and S.C. conceived the simulations and wrote the paper.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

*Mj*HypB, *M. jannaschii* HypB; *Hp*UreG, *H. pylori* UreG; G-protein, GTP binding protein; MD, molecular dynamics; REMD, replica exchange MD; REST, replica exchange with solute tempering; RMSD, root-mean-square deviation; RMSF, root-mean-square fluctuation; NMR, nuclear magnetic resonance; CD, circular dichroism.

■ REFERENCES

(1) Khafizov, K., Lattanzi, G., and Carloni, P. (2009) G protein inactive and active forms investigated by simulation methods. *Proteins 75* (4), 919−930.

(2) Wittinghofer, A., and Vetter, I. R. (2011) Structure-function relationships of the G domain, a canonical switch motif. *Annu. Rev. Biochem. 80*, 943−971.

(3) Gasper, R., Scrima, A., and Wittinghofer, A. (2006) Structural insights into HypB, a GTP-binding protein that regulates metal binding. *J. Biol. Chem. 281* (37), 27492−27502.

(4) Zambelli, B., Turano, P., Musiani, F., Neyroz, P., and Ciurli, S. (2009) Zn^{2+}-linked dimerization of UreG from *Helicobacter pylori*, a chaperone involved in nickel trafficking and urease activation. *Proteins 74* (1), 222−239.

(5) Zambelli, B., Musiani, F., Benini, S., and Ciurli, S. (2011) Chemistry of Ni^{2+} in Urease: Sensing, Trafficking, and Catalysis. *Acc. Chem. Res. 44* (7), 520−530.

(6) Zambelli, B., Stola, M., Musiani, F., De Vriendt, K., Samyn, B., Devreese, B., Van Beeumen, J., Turano, P., Dikiy, A., Bryant, D. A., and Ciurli, S. (2005) UreG, a chaperone in the urease assembly process, is an intrinsically unstructured GTPase that specifically binds Zn^{2+}. *J. Biol. Chem. 280* (6), 4684−4695.

(7) Neyroz, P., Zambelli, B., and Ciurli, S. (2006) Intrinsically disordered structure of *Bacillus pasteurii* UreG as revealed by steady-state and time-resolved fluorescence spectroscopy. *Biochemistry 45* (29), 8918−8930.

(8) Zambelli, B., Musiani, F., Savini, M., Tucker, P., and Ciurli, S. (2007) Biochemical studies on *Mycobacterium tuberculosis* UreG and comparative modeling reveal structural and functional conservation among the bacterial UreG family. *Biochemistry 46* (11), 3171−3182.

(9) Real-Guerra, R., Stanisçuaski, F., Zambelli, B., Musiani, F., Ciurli, S., and Carlini, C. R. (2012) Biochemical and structural studies on native and recombinant *Glycine max* UreG: A detailed characterization of a plant urease accessory protein. *Plant Mol. Biol. 78* (4−5), 461−475.

(10) Zambelli, B., Cremades, N., Neyroz, P., Turano, P., Uversky, V. N., and Ciurli, S. (2012) Insights in the (un)structural organization of *Bacillus pasteurii* UreG, an intrinsically disordered GTPase enzyme. *Mol. BioSyst. 8* (1), 220−228.

(11) Bellucci, M., Zambelli, B., Musiani, F., Turano, P., and Ciurli, S. (2009) *Helicobacter pylori* UreE, a urease accessory protein: Specific Ni^{2+}- and Zn^{2+}-binding properties and interaction with its cognate UreG. *Biochem. J. 422* (1), 91−100.

(12) Vamvaca, K., Vogeli, B., Kast, P., Pervushin, K., and Hilvert, D. (2004) An enzymatic molten globule: Efficient coupling of folding and catalysis. *Proc. Natl. Acad. Sci. U.S.A. 101* (35), 12860−12864.

(13) Pervushin, K., Vamvaca, K., Vogeli, B., and Hilvert, D. (2007) Structure and dynamics of a molten globular enzyme. *Nat. Struct. Mol. Biol. 14* (12), 1202−1206.

(14) Vamvaca, K., Jelesarov, I., and Hilvert, D. (2008) Kinetics and thermodynamics of ligand binding to a molten globular enzyme and its native counterpart. *J. Mol. Biol. 382* (4), 971−977.

(15) Roca, M., Messer, B., Hilvert, D., and Warshel, A. (2008) On the relationship between folding and chemical landscapes in enzyme catalysis. *Proc. Natl. Acad. Sci. U.S.A. 105* (37), 13877−13882.

(16) Leipe, D. D., Wolf, Y. I., Koonin, E. V., and Aravind, L. (2002) Classification and evolution of P-loop GTPases and related ATPases. *J. Mol. Biol. 317* (1), 41−72.

(17) Martin-Diaconescu, V., Bellucci, M., Musiani, F., Ciurli, S., and Maroney, M. J. (2012) Unraveling the *Helicobacter pylori* UreG zinc binding site using X-ray absorption spectroscopy (XAS) and structural modeling. *J. Biol. Inorg. Chem. 17* (3), 353−361.

(18) Marti-Renom, M. A., Stuart, A. C., Fiser, A., Sanchez, R., Melo, F., and Sali, A. (2000) Comparative protein structure modeling of genes and genomes. *Annu. Rev. Biophys. Biomol. Struct. 29*, 291−325.

(19) Cuff, J. A., Clamp, M. E., Siddiqui, A. S., Finlay, M., and Barton, G. J. (1998) JPred: A consensus secondary structure prediction server. *Bioinformatics 14* (10), 892−893.

(20) Laskowski, R. A., Mac Arthur, M. W., Moss, D. S., and Thornton, J. M. (1993) PROCHECK: A program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr. 26*, 283−291.

(21) Hornak, V., Abel, R., Okur, A., Strockbine, B., Roitberg, A., and Simmerling, C. (2006) Comparison of multiple Amber force fields and development of improved protein backbone parameters. *Proteins 65* (3), 712−725.

(22) Jorgensen, W. L., Chandrasekhar, L., Madura, J. D., Impey, R. W., and Klein, M. L. (1983) Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys. 79* (2), 926−935.

(23) Meagher, K. L., Redman, L. T., and Carlson, H. A. (2003) Development of polyphosphate parameters for use with the AMBER force field. *J. Comput. Chem. 24* (9), 1016−1025.

(24) Oelschlaeger, P., Klahn, M., Beard, W. A., Wilson, S. H., and Warshel, A. (2007) Magnesium-cationic Dummy Atom Molecules Enhance Representation of DNA Polymerase β in Molecular Dynamics Simulations: Improved Accuracy in Studies of Structural Features and Mutational Effects. *J. Mol. Biol. 366* (2), 687−701.

(25) Sugita, Y., and Okamoto, Y. (1999) Replica-exchange molecular dynamics method for protein folding. *Chem. Phys. Lett. 314* (1−2), 141−151.

(26) Liu, P., Kim, B., Friesner, R. A., and Berne, B. J. (2005) Replica exchange with solute tempering: A method for sampling biological systems in explicit water. *Proc. Natl. Acad. Sci. U.S.A. 102* (39), 13749−13754.

(27) Terakawa, T., Kameda, T., and Takada, S. (2011) On easy implementation of a variant of the replica exchange with solute tempering in GROMACS. *J. Comput. Chem. 32*, 1228−1234.

(28) Wang, L., Friesner, R. A., and Berne, B. J. (2011) Replica Exchange with Solute Scaling: A More Efficient Version of Replica Exchange with Solute Tempering (REST2). *J. Phys. Chem. B 115* (30), 9431−9438.

(29) Patriksson, A., and van der Spoel, D. (2008) A temperature predictor for parallel tempering simulations. *Phys. Chem. Chem. Phys. 10* (15), 2073−2077.

(30) Berendsen, H. J. C., van der Spoel, D., and van Drunen, R. (1995) GROMACS: A message-passing parallel molecular dynamics implementation. *Comput. Phys. Commun. 91* (1−3), 43−56.

(31) Lindahl, E., Hess, B., and van der Spoel, D. (2001) GROMACS 3.0: A package for molecular simulation and trajectory analysis. *J. Mol. Model. 7* (8), 306−317.

(32) Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., and Berendsen, H. J. C. (2005) GROMACS: Fast, flexible, and free. *J. Comput. Chem. 26* (16), 1701−1718.

(33) Hess, B., Kutzner, C., van der Spoel, D., and Lindahl, E. (2008) GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and

Scalable Molecular Simulation. *J. Chem. Theory Comput. 4* (3), 435−447.
(34) Hoover, W. G. (1985) Canonical dynamics: Equilibrium phase-space distributions. *Phys. Rev. A 31* (3), 1695−1697.
(35) Nosé, S. (2002) A molecular dynamics method for simulations in the canonical ensemble. *Mol. Phys. 100* (1), 191−198.
(36) Parrinello, M., and Rahman, A. (1981) Polymorphic transitions in single crystals: A new molecular dynamics method. *J. Appl. Phys. 52* (12), 7182−7190.
(37) Nosé, S., and Klein, M. L. (1983) Constant pressure molecular dynamics for molecular systems. *Mol. Phys. 50* (5), 1055−1076.
(38) Caliandro, R., Rossetti, G., and Carloni, P. (2012) Local Fluctuations and Conformational Transitions in Proteins. *J. Chem. Theory Comput. 8* (11), 4775−4785.
(39) Aleksiev, T., Potestio, R., Pontiggia, F., Cozzini, S., and Micheletti, C. (2009) PiSQRD: A web server for decomposing proteins into quasi-rigid dynamical domains. *Bioinformatics 25* (20), 2743−2744.
(40) Potestio, R., Pontiggia, F., and Micheletti, C. (2009) Coarse-grained description of protein internal dynamics: An optimal strategy for decomposing proteins in rigid subunits. *Biophys. J. 96* (12), 4993−5002.
(41) Sydor, A. M., Liu, J., and Zamble, D. B. (2011) Effects of metal on the biochemical properties of *Helicobacter pylori* HypB, a maturation factor of [NiFe]-hydrogenase and urease. *J. Bacteriol. 193* (6), 1359−1368.
(42) Xia, W., Li, H., Yang, X., Wong, K. B., and Sun, H. (2012) Metallo-GTPase HypB from *Helicobacter pylori* and its interaction with nickel chaperone protein HypA. *J. Biol. Chem. 287* (9), 6753−6763.
(43) Uversky, V. N., and Dunker, A. K. (2010) Understanding protein non-folding. *Biochim. Biophys. Acta 1804* (6), 1231−1264.
(44) Li, X., Romero, P., Rani, M., Dunker, A. K., and Obradovic, Z. (1999) Predicting Protein Disorder for N-, C-, and Internal Regions. *Genome Inf. Ser. 10*, 30−40.
(45) Romero, P., Obradovic, Z., Li, X., Garner, E. C., Brown, C. J., and Dunker, A. K. (2001) Sequence complexity of disordered protein. *Proteins 42* (1), 38−48.
(46) Li, L., Uversky, V. N., Dunker, A. K., and Meroueh, S. O. (2007) A computational investigation of allostery in the catabolite activator protein. *J. Am. Chem. Soc. 129* (50), 15668−15676.