Supporting Information

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SI Text

Both earlier experiments and our simulations found that the W62G mutant lysozyme displays a significantly larger disruption of structure in 8 M urea than the wild-type (WT). Fig. 1 shows the C_α rmsd from the native structure and the nonpolar solvent-accessible surface area (SASA) for the WT and W62G mutant with time (1) (only the first-100-ns data are shown for clearer differences). The increases in W62G are larger than in the WT indicating that the extent of destabilization is greater in the mutant (1). These measures show that the WT and W62G both unfold in 8 M urea. In addition, compared with the WT, the mutant exposed a larger polar-SASA but a similar charged-SASA (data not shown). Secondary structure analysis (Fig. 1*C*) showed that the β-sheets in W62G are disrupted very rapidly, in about 25 ns. It also lost more helix secondary structures than the WT (2). Because W62G unfolds more globally in 8 M urea than the WT we focus the rest of the analysis of urea–protein interactions by using only the results for the mutant.

Negligible Effect on Water Local Structure and Dynamics. To assess whether urea alters the water structure in 8 M urea, which was proposed as an indirect mechanism by which urea can denature proteins through weakening the hydrophobic effect (3), we calculated a number of properties that probe microscopic changes in water structure. All of the calculations in this subsection have been done in bulk region of the protein solution for both the pure water and 8 M urea solutions.

Radial Distribution Function. Atomic radial distribution functions were calculated to describe the influence on the water structure by adding urea into water, as shown in Fig. 2*A*. The water molecules in 8 M urea have very similar oxygen–oxygen and oxygen–hydrogen distributions as pure water does, indicating that urea does not disturb the water arrangements. Urea oxygen was also found to be able to at least partly mimic water oxygen. Such behavior is shown by the positional agreement of first peak in the oxygen–oxygen distribution between urea–water and water–water, as well as in the distribution between urea oxygen–water hydrogen and water oxygen–water hydrogen. The minor difference of oxygen atoms between urea and water is in the second hydration shell. However, the distribution between urea hydrogen and water oxygen is shifted toward greater distance with diminished peak heights relative to the distribution between water hydrogen and water oxygen, indicating that urea hydrogen atoms make weaker hydrogen bonds than water hydrogen atoms. These calculations show that urea mixes well with water with only a minor effect on water structure and the hydrogen bond network, which agrees well with some previous observations from other groups (4–6). Some early experiments suggest that urea molecules might cause the second shell around water molecules to be compressed, similar to what happens when neat water is pressurized (7); however, we did not find much evidence for this second shell compression. There is no significant second peak in the radial distribution function for water molecules in 8 M urea.

Hydrogen Bond Distance Distribution. To investigate the effect of urea on the water hydrogen bond network, we computed the distributions of hydrogen bond distances formed between various solvent molecules in 8 M urea and in pure water, respectively. Fig. 2*B* shows that the hydrogen bonds formed by water molecules have the same distribution in 8 M urea as in pure water. Replacing water oxygen O_W with urea oxygen O_U in hydrogen bonding does not change the distribution. However, when urea hydrogen H_U is involved in hydrogen bonding with O_U and O_W, respectively, both distributions become broader and shift to a longer hydrogen bond distance. This observation indicates that the ability of H_U to form hydrogen bonds is weaker than water hydrogen H_W, consistent with the above shifted $H_U - O_W$ radial distribution function. However, the urea oxygen has a comparable capability to form hydrogen bonds with water oxygen. Overall, we observed similar hydrogen-bonding behaviors for urea to what Bennion and Daggett did (8), but we further decomposed the urea-involved hydrogen bonds into three categories: $H_U - O_W$, $H_W - O_U$, and $H_U - O_U$, and found that urea hydrogen weakens the hydrogen bonding but urea oxygen does not.

Triple-Angle Distribution Function. To investigate further the effect of urea on the water geometrical coordination, we calculated the triple-angle distribution functions $f(\cos\theta_{ijk})$ for water molecules in 8 M urea and for pure water. Here, water i and water k are the nearest neighbors of water j, that is, the oxygen atoms of water i and k are both within 3.3 Å of water j oxygen atom. θ_{ijk} is the angle between the vector $\vec{r_{ij}}$ and $\vec{r_{kj}}$. By sampling all of the possible angles formed by the nearest neighbors of each water molecule in the bulk region, we obtained the distributions shown in Fig. 2*C*. There is only a minor decrease of the broad peak at $\cos\theta_{ijk} \simeq -0.2$, which in pure water indicates that the tetrahedral H-bond network is present at short distances. In the mean time, a minor increase is found for the sharp peak at $\cos\theta_{ijk} \simeq 0.5$, which corresponds to near-neighbor interstitial molecules. Our observation indicates that the local water structure was not significantly distorted in 8 M urea relative to pure water.

Orientational Relaxation Time. The structural properties investigated above show that urea has only a slight effect on the water local structure and the water hydrogen bond network. Then, how about the water dynamics?

To study the effect of urea on water dynamics, we calculated the orientational correlation functions for water molecules, $C_l(t)$ ($l = 1,2$), which can be defined as

$$C_l(t) = < P_l[\mathbf{e}(t)\cdot\mathbf{e}(0)]> \qquad [1]$$

where P_l is the Lengendre polynomial ($l = 1,2$) and $\mathbf{e}$ is the unit vector along one of the OH bonds of water. As shown in Fig. 3 for $l = 1$ (*A*) and $l = 2$ (*B*), the orientational relaxation of O–H vector of water in 8 M urea (red) appears to be slightly slower than that in pure water (black). It is found that those water molecules in 8 M urea, which are restricted to form at least two hydrogen bonds simultaneously with a urea molecule (see the water molecule marked with "*" in Fig. 3*C*), make the most contribution to this slight water dynamics slowdown (shown as green for these "restricted" in Fig. 3 *A* and *B*).

The orientational relaxation time τ_l was obtained by using three different methods: time integration ($\langle\tau_l\rangle$), single-exponential fit ($\langle\tau_l^{\dagger}\rangle$) and biexponential fit ($\langle\tau_l^{s,f}\rangle$). The values of τ_l for pure water, water in 8 M urea, and restricted water in 8 M urea, are reported on Table 1. Although the absolute values of τ_1 and τ_2 obtained by three methods above can be different, the general trend is the same, that is, the overall orientational relaxation time of water in 8 M urea is close to that of pure water, with only the restricted water showing a longer relaxation time. The second-order orientational relaxation

time of pure water in our study (τ_2 = 0.79 ps) is smaller than the experimental value (≈2 ps) of NMR measurements (9, 10), but it is very similar to τ_2 of TIP3P water (0.65–0.85 ps) in molecular dynamics simulations (11, 12). Similarly, our orientational relaxation time of bulk water in 8 M urea (τ_2 = 0.80 ps) is smaller than the value (2.5 ps) found in the midinfrared pump-probe study (13), but it is comparable to that of the pure water in our simulation. Even though the detailed time constants are slightly different from the experiment, the conclusion is the same, that is, the majority of water in a urea–water mixture reorient with a similar time constant as in pure water, indicating that water dynamics is not changed much by the presence of urea. A possible explanation is that urea has the right size to replace a water dimer to fit into the water hydrogen bond network (14). For the restricted water molecules, the average orientation time τ_2 increases by 16%, and if we only consider the slow motion, the relaxation time from the biexponential fitting increases by 66%. However, this relaxation time for the slow motion is still not as slow as the 15 ps found in the experiment (13), where a small fraction of water in 8 M urea was proposed to engage in 2 simultaneous hydrogen bonds with urea (exactly the same as we specified in our calculations). This discrepancy in the relaxation time for those restricted water molecules might be related to the force field we have used.

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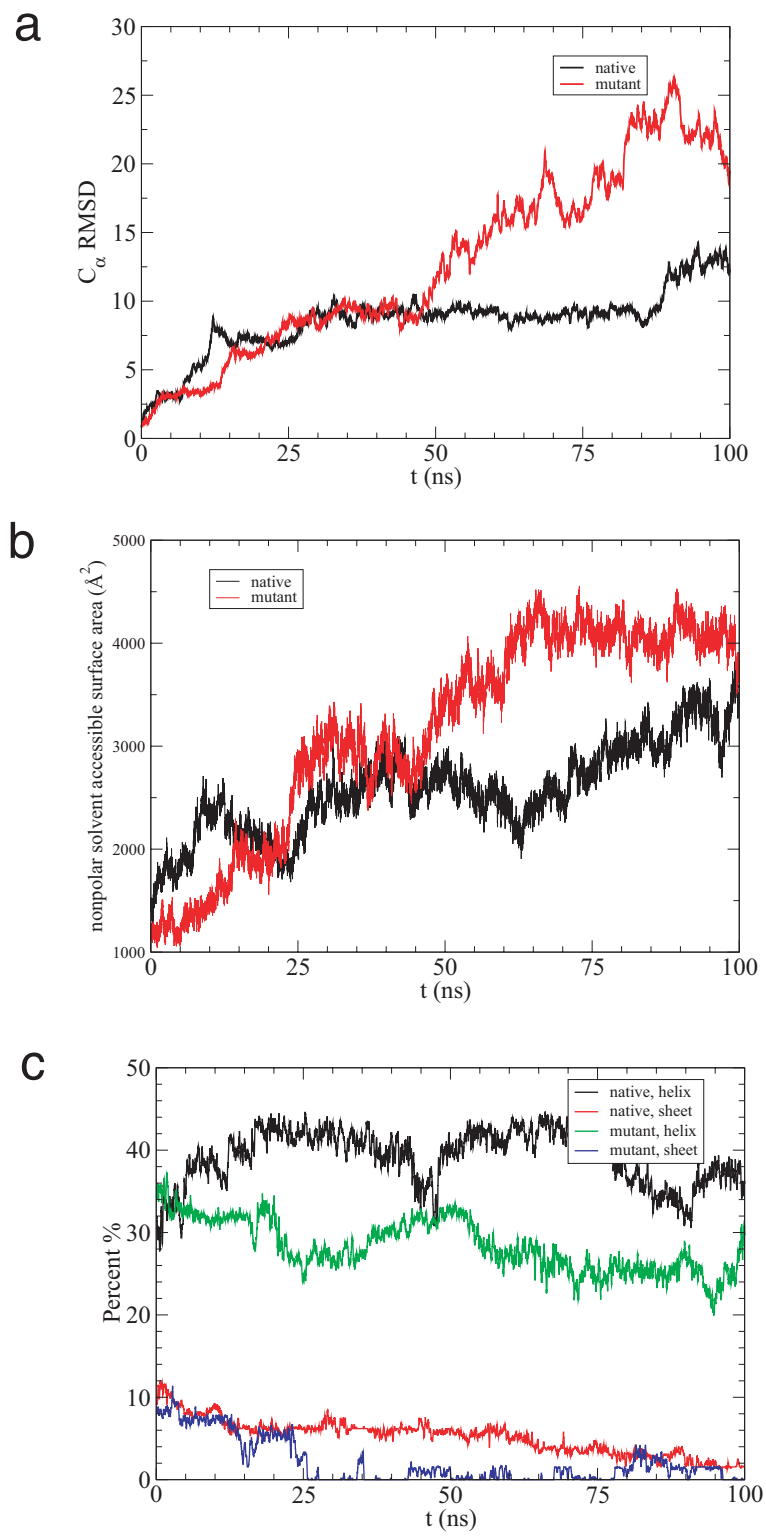

Fig. S1. Comparison between the wild-type and W62G mutant lysozyme in 8 M urea (only the first 100-ns data are shown for clearer differences). (*a*) C_α rmsd from the crystal structure for the wild-type and mutant lysozymes. (*b*) Nonpolar solvent-accessible surface area for the wild-type and mutant lysozymes. (*c*) The percentage of residues as a secondary structure (helix, β-sheet) for the wild-type and mutant lysozyme. Black and green, helix; red and blue, r β-sheet.

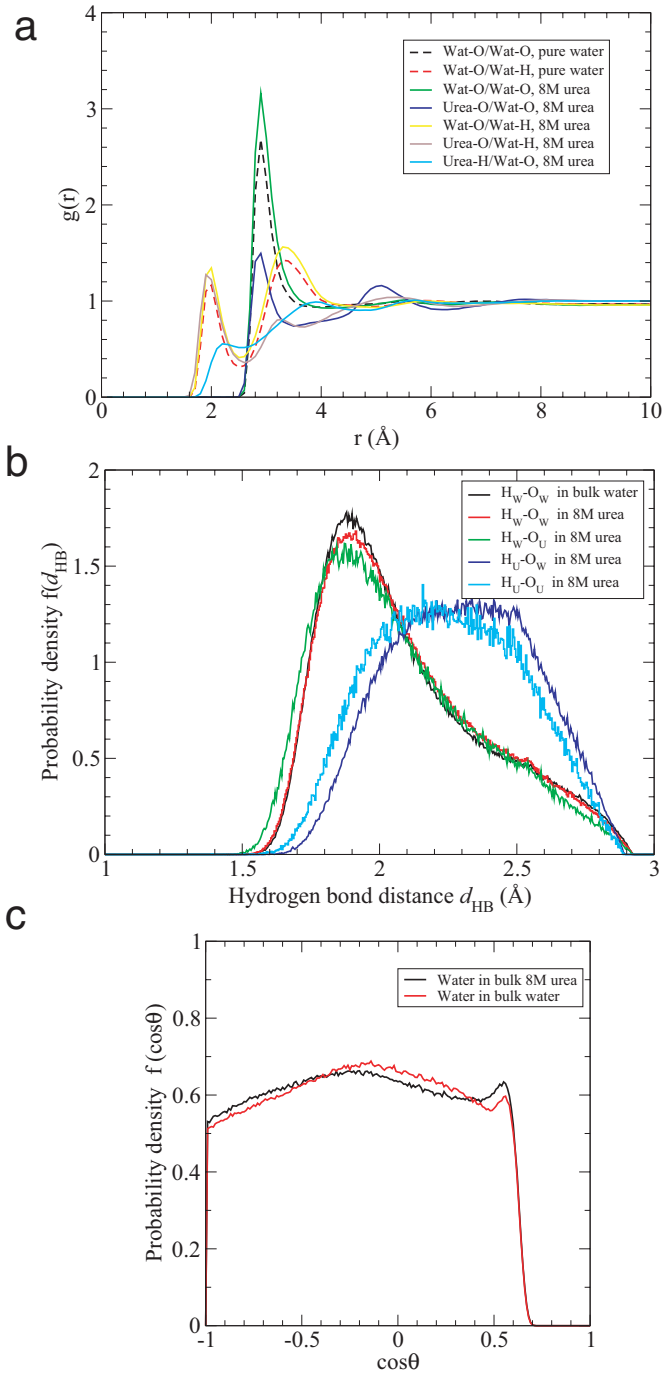

Fig. S2. Comparison of water structure in 8 M urea with that in pure water. (*a*) Pair radial distribution functions for atoms between water and urea, and between water molecules. The dashed curves are for pure water and the solid curves are for the solvents in bulk region of the system with mutant lysozyme in 8 M urea. (*b*) Hydrogen bond distance distributions for mutant lysozyme in 8 M urea and in pure water. The data are obtained with respect to the solvent in bulk region during the first 10 ns. The definition of hydrogen bonds (15) is that the distance between donor and acceptor is no greater than 3.5 Å and the angle of donor–H–acceptor is no smaller than 120°. (*c*) Triple-angle distribution for bulk water molecules in the systems of mutant lysozyme in 8 M urea (black) and in pure water (red), respectively. Water molecules are considered in bulk region when water oxygen atoms are not within 3.5 Å of polar atoms and not within 4.5 Å of nonpolar atoms.

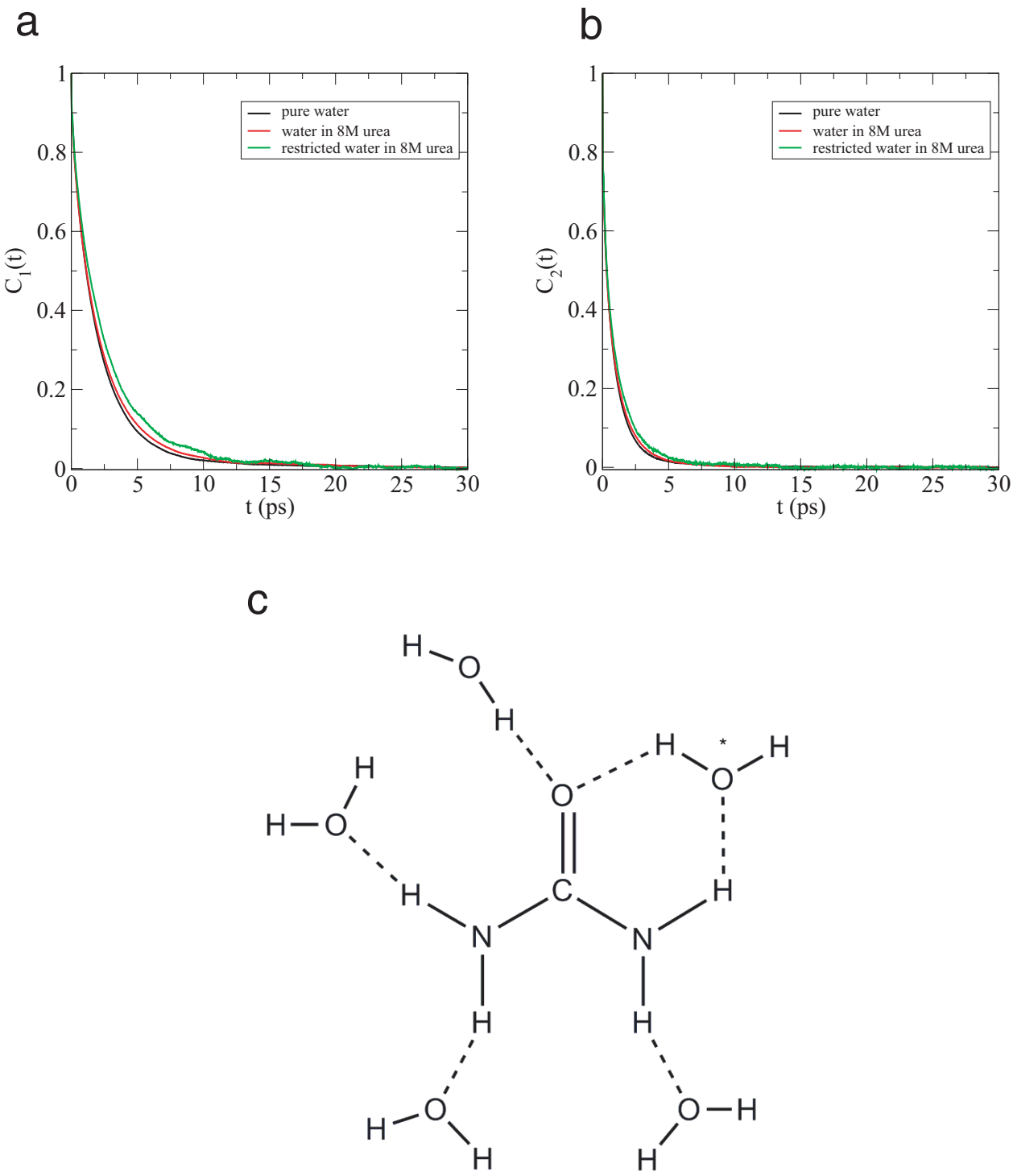

Fig. S3. The time dependence of the O–H vector orientational correlation functions, $C_1(t)$ (*a*) and $C_2(t)$ (*b*). The black curve is for pure water. The red curve is for the water in bulk region (not within 6.0 Å of protein) of the system with mutant lysozyme in 8 M urea. The green curve is for the water in the same region as that shown in red, except that 1 water molecule is only concerned when it forms at least 2 hydrogen bonds simultaneously with one urea. (*c*) Solvation structure of the urea molecule from the experiment (13). The water molecule * in the solvation shell shares 2 hydrogen bonds with urea (13).

Table S1. Values of orientational relaxation times τ_l (l = 1,2) for three different kinds of water: pure water, water in the bulk region of the system with mutant lysozyme in 8 M urea, and the water restricted to have at least 2 hydrogen bonds simultaneously with one urea in the bulk region of 8 M urea

	Pure water	Water in 8 M urea	Restricted water in 8 M urea
$\langle\tau_1\rangle$	2.05	2.19	2.48
$\tau_1^{\dagger}$	2.24	2.43	2.68
τ_1^{s}	9.87 (0.045)	8.01 (0.078)	5.02 (0.25)
τ_1^{f}	1.98 (0.82)	2.04 (0.77)	1.95 (0.62)
$\langle\tau_2\rangle$	0.79	0.80	0.93
$\tau_2^{\dagger}$	1.24	1.38	1.47
τ_2^{s}	3.62 (0.05)	2.03 (0.21)	3.36 (0.11)
τ_1^{f}	0.89 (0.62)	0.77 (0.46)	1.02 (0.55)

$\langle\tau_l\rangle$, $\tau_l^{\dagger}$, and $\tau_l^{s,f}$ are obtained from the orientational correlation functions as defined in Eq. **1** by time integration, single-exponential fits, and biexponential fits, respectively. The value enclosed in the parenthese is the weight of the corresponding time constant τ_l^{s} or τ_l^{f}. τ_l is expressed in picoseconds.