Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supplementary Material

The shift in urea orientation at protein surfaces at low pH is compatible with a direct mechanism of protein denaturation

Ivan Pires de Oliveira[1,2] and Leandro Martínez[1,*]

[1]Institute of Chemistry and Center for Computing in Engineering & Science, University of Campinas, Campinas, SP, Brazil.

[2]Department of Pharmacology, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil.

*Correspondence to: leandro@iqm.unicamp.br

Table S1. Number and characteristic survival times of hydrogen bonds between urea and BCL, considering all protein atoms except those forming direct contacts with protonable acidic residues in the crystallographic structure. The properties of the the H-bonds do not change, considering fluctuations, upon protonation, supporting the locality of the protonation effects. D: Deprotonated superficial acidic residues; P: Protonated superficial acidic residues. The mean and standard deviations of 10 simulations of each system are shown. A residue was considered in contact, and excluded from the current computation of H-bonds, if any of its atoms was closer than 3 Å from the side-chain of the superficial acidic residues in the crystallographic BCL conformation.

System		Number of H-bonds		Characteristic survival time / ps	
		BCL as donor	BCL as acceptor	BCL as donor	BCL as acceptor
1M	D	2.00±0.18	2.35±0.17	1.31±0.08	0.50±0.01
	P	2.13±0.16	2.29±0.13	1.34±0.11	0.60±0.21
3M	D	4.81±0.49	5.46±0.33	1.48±0.11	0.51±0.01
	P	5.01±0.42	5.42±0.42	1.44±0.09	0.52±0.01
6M	D	8.75±0.66	10.21±0.51	1.41±0.05	0.55±0.01
	P	9.23±0.84	10.08±0.58	1.47±0.07	0.55±0.01
12M	D	14.56±0.85	16.32±0.55	1.52±0.04	0.57±0.01
	P	14.84±1.14	16.12±0.90	1.52±0.05	0.57±0.01

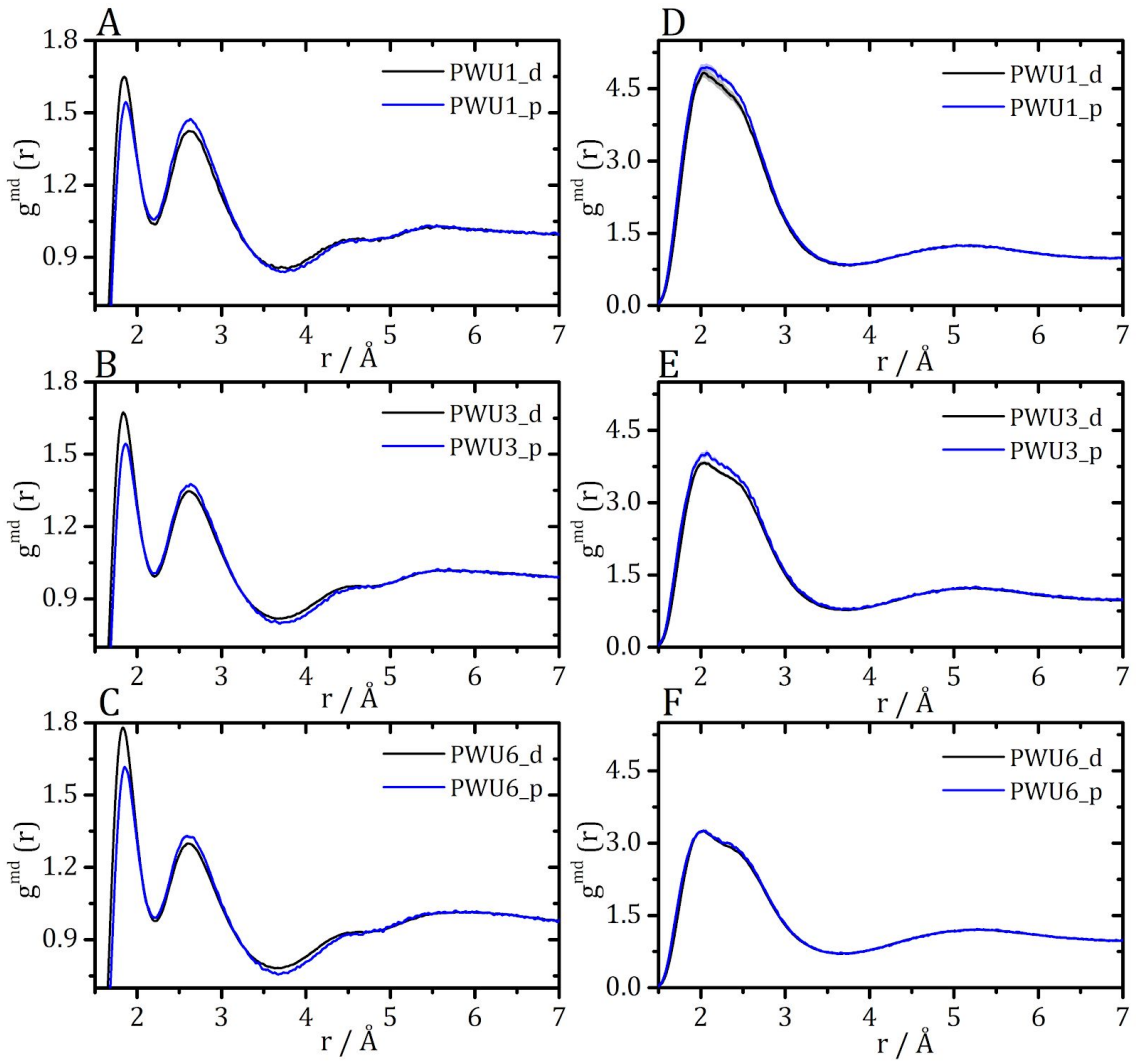

Fig. S1 Minimum distance distribution functions ($g^{md}(r)$) for water considering the protonation of Asp/Glu on the surface of the BCL enzyme. $g^{md}(r)$ for protein-water in (A) an aqueous urea solution at ~1 mol L^{-1} (PWU1 system), (B) aqueous urea solution at ~3 mol L^{-1} (PWU3 system) and (C) urea aqueous solution at ~6.5 mol L^{-1} (PWU6 system). (Complementary to Fig. 2 of the main manuscript.)

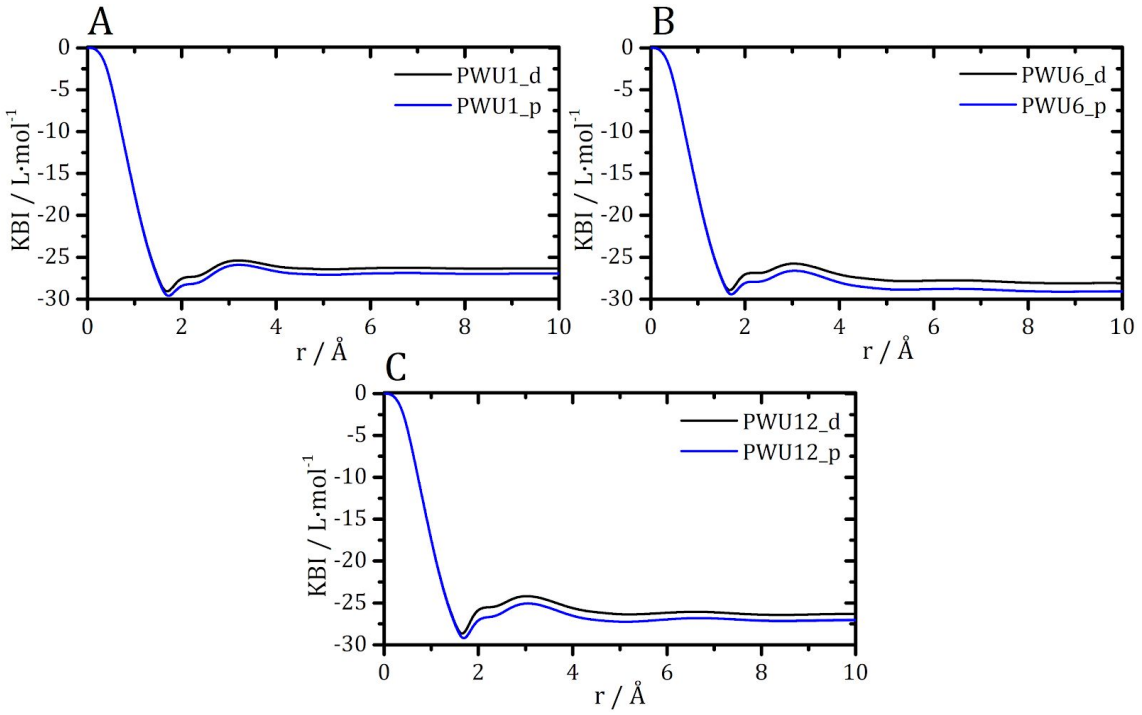

Fig. S2 Kirkwood-Buff integrals (KBI) according the protonation state of acid residues for protein-water in (A) ~1 mol L^{-1} urea, (B) ~6.5 mol L^{-1} urea, and (C) ~11.8 mol L^{-1} urea. (Complementary to Fig. 3 of the main manuscript.)

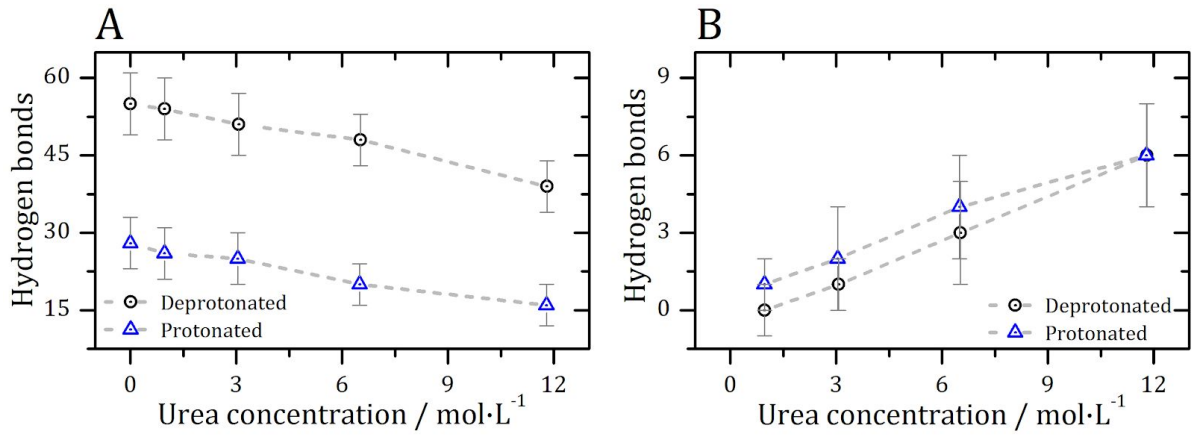

Fig. S3 Number of hydrogen bonds between aspartic and glutamic acids of the protein surface with (A) water and (B) urea. The protonation affects significantly the number of hydrogen bonds with water molecules, but not with urea. Following protonation, the protein loses ~25 hydrogen bonds with water molecules, independently of the concentration of urea. The effect of protonation of acidic side-chains in the number of hydrogen bonds with urea is small and within the fluctuations of the simulation. The variation of the number of hydrogen bonds shown in Figs. S4 and S5 is completely explained by the variation on these residues.

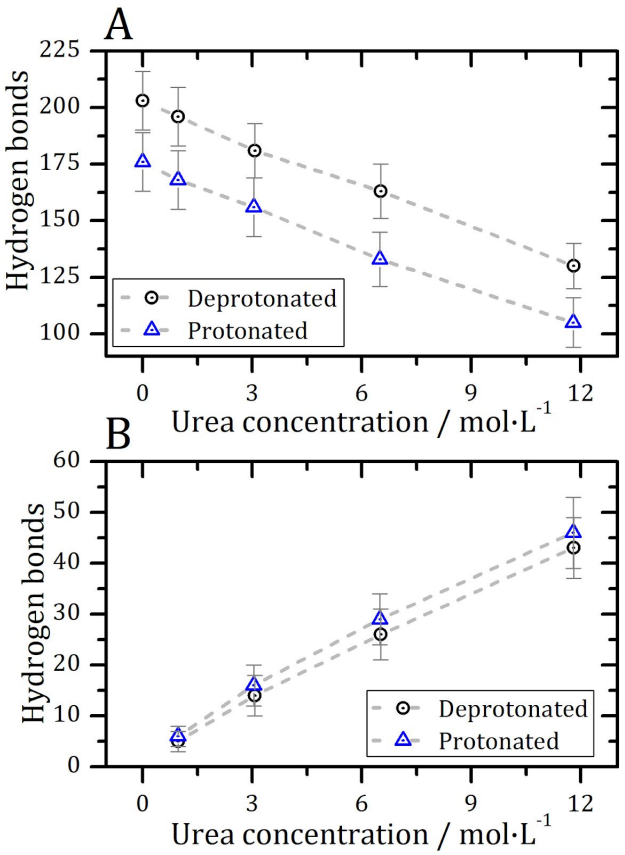

Fig. S4 Number of hydrogen bonds between the protein and (A) water and (B) urea. The protonation of acidic residues has a greater effect on the number or protein-water hydrogen bonds than it has on protein-urea ones.

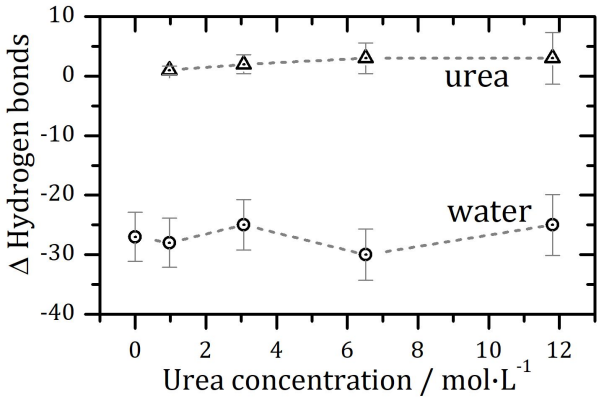

Fig. S5 The protonation of acidic side chains promotes a loss of about 25 protein-water hydrogen bonds and the gain of 2-3 protein-urea hydrogen bonds, independently of the concentration of urea. This indicates that the interactions of urea with the acidic side chains is saturated already in the the most dilute urea solutions.

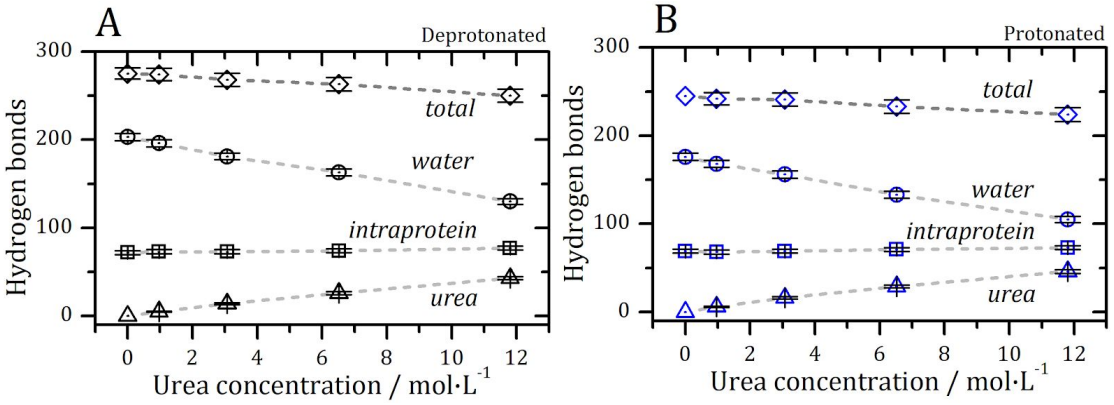

Fig. S6 Total number of hydrogen bonds between of the protein with different solution components in the deprotonated and protonated states. The total number of hydrogen bonds experienced by the protein decreases with increasing urea concentration particularly at the expense of protein-water hydrogen bonds.

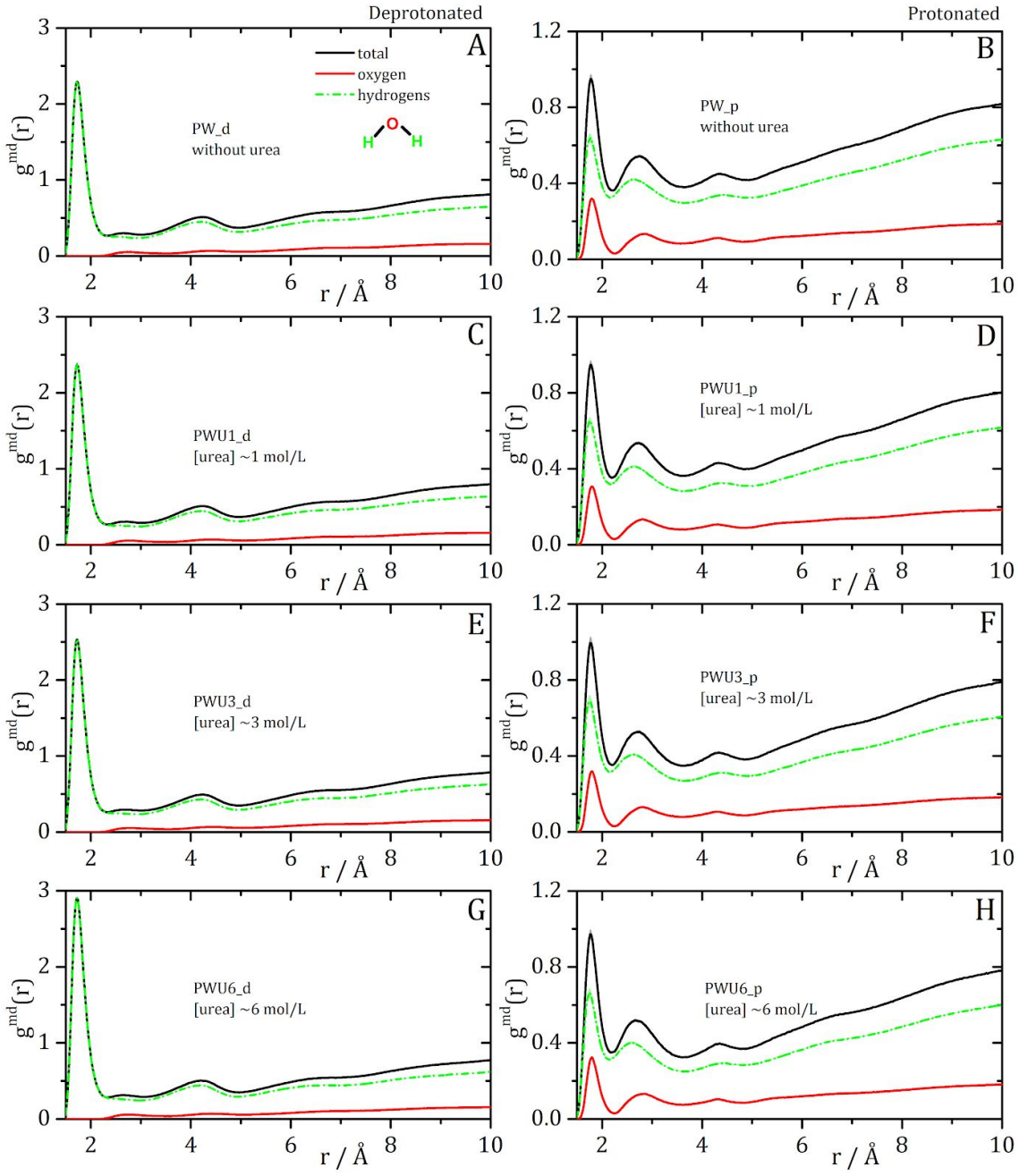

Fig. S7 Asp/Glu-water MDDFs and atomic contributions for aqueous solutions of urea (except (A) and (B), only water) with the concentrations not shown in Fig. 5 of the main manuscript.

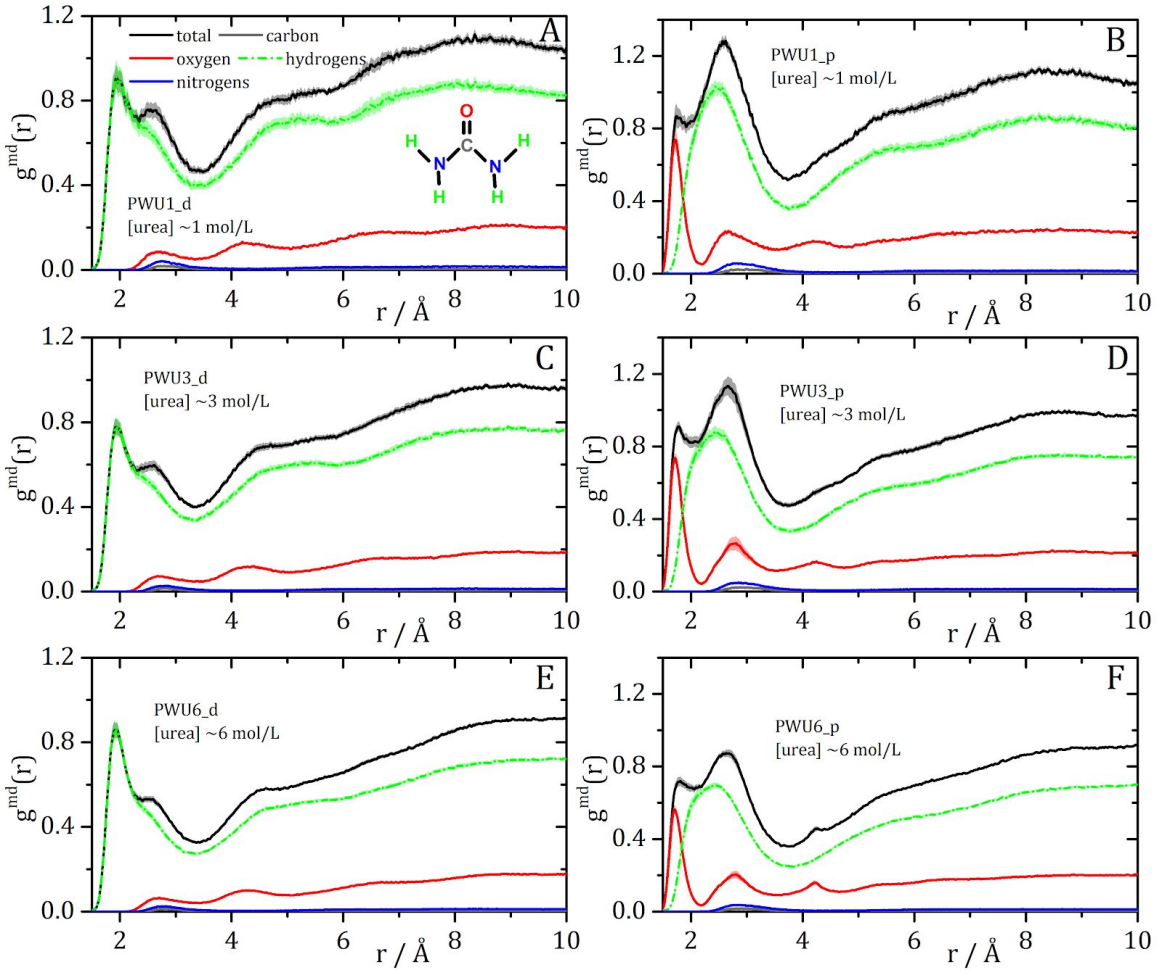

Fig. S8 Asp/Glu-urea MDDFs and atomic contributions for aqueous solutions of urea with the concentrations not shown in Fig. 5 of the main manuscript.

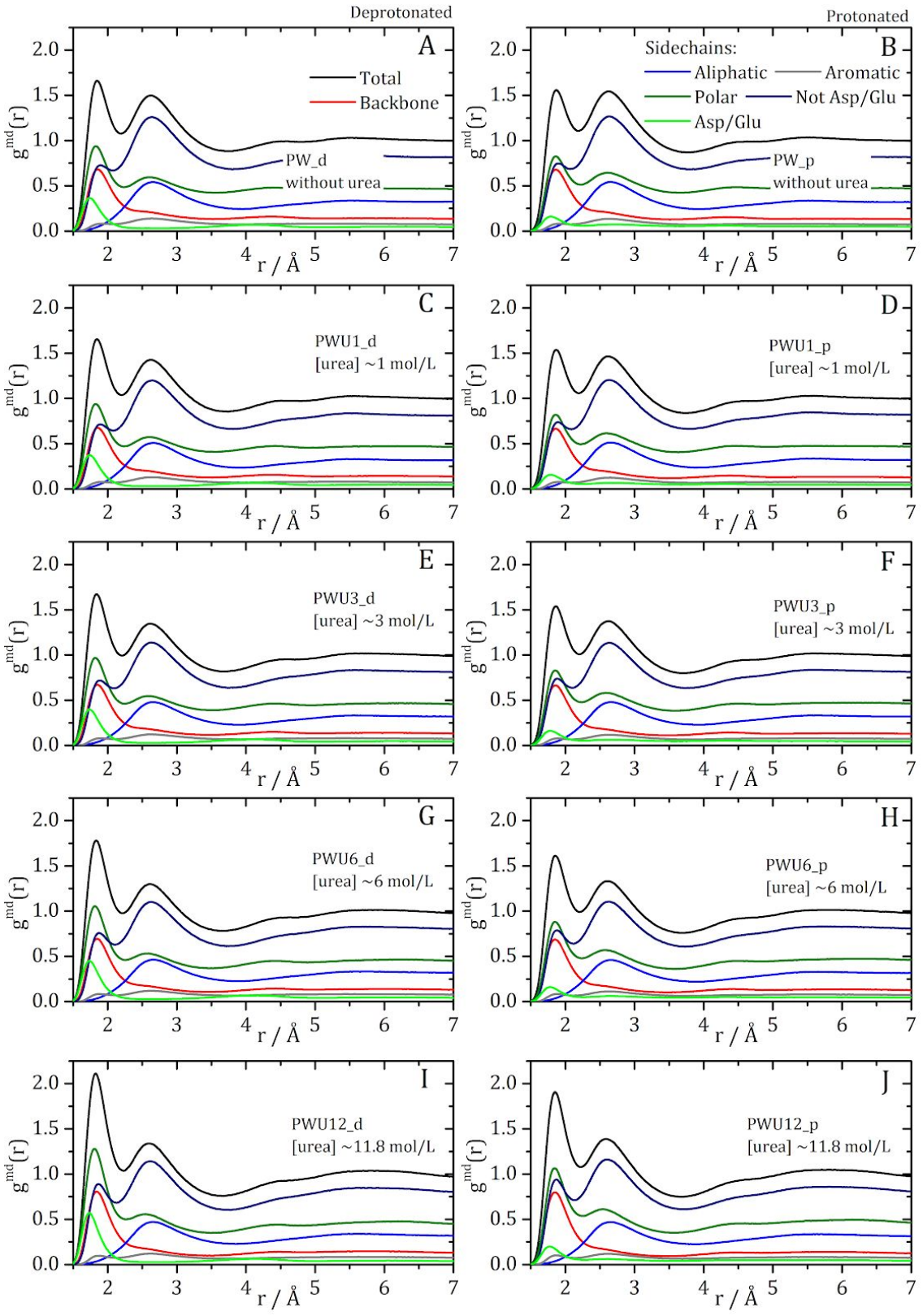

Fig. S9 Contributions of different protein subsets of atoms to the water-protein minimum-distance distribution functions: Noticeable differences upon protonation are only observed for Asp and Glu residues. All other interactions are essentially independent of the protonation state of these residues.

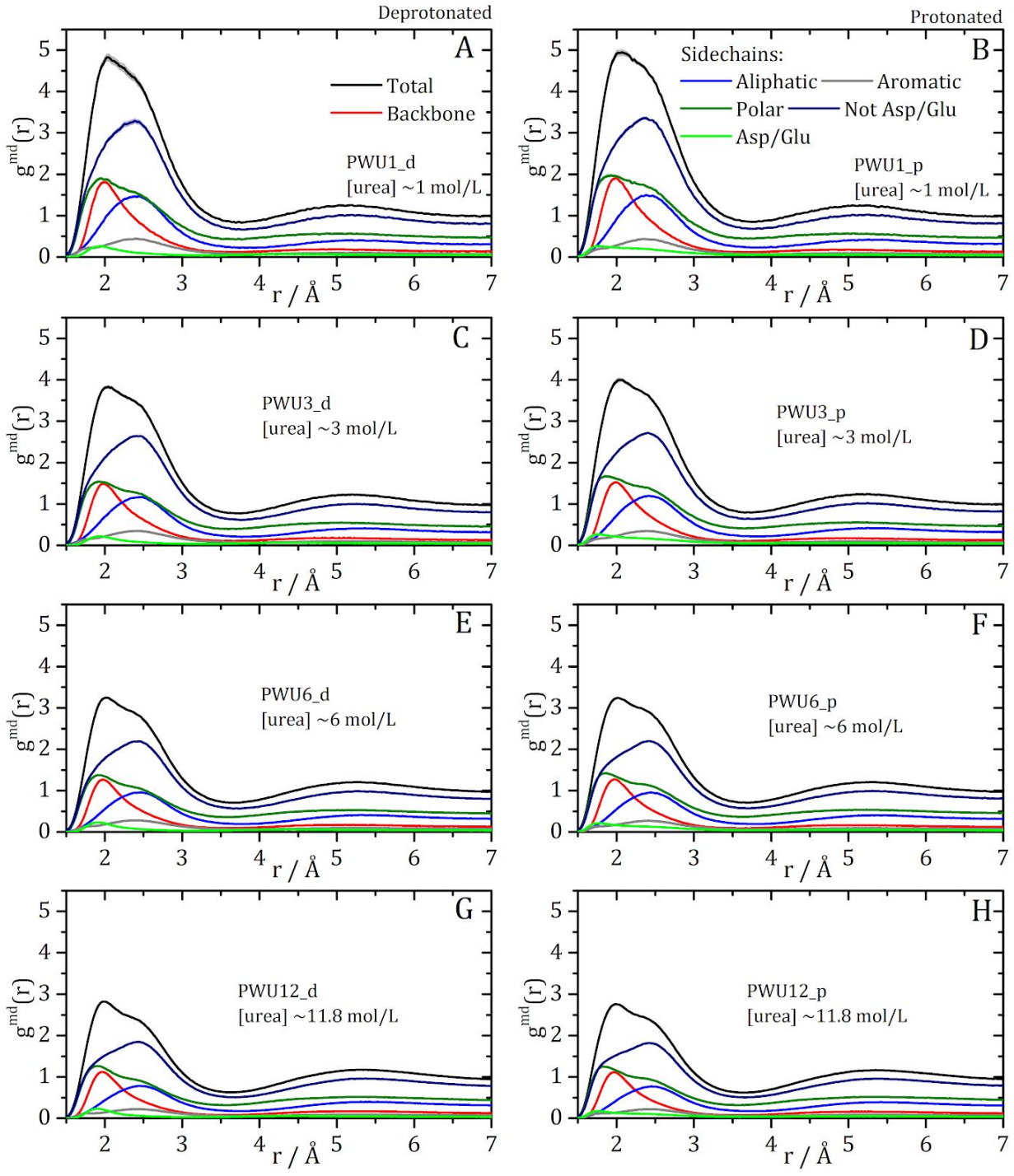

Fig. S10 Contributions of different protein subsets of atoms to the urea-protein minimum-distance distribution functions: Noticeable differences upon protonation are only observed for Asp and Glu residues. All other interactions are essentially independent of the protonation state of these residues.

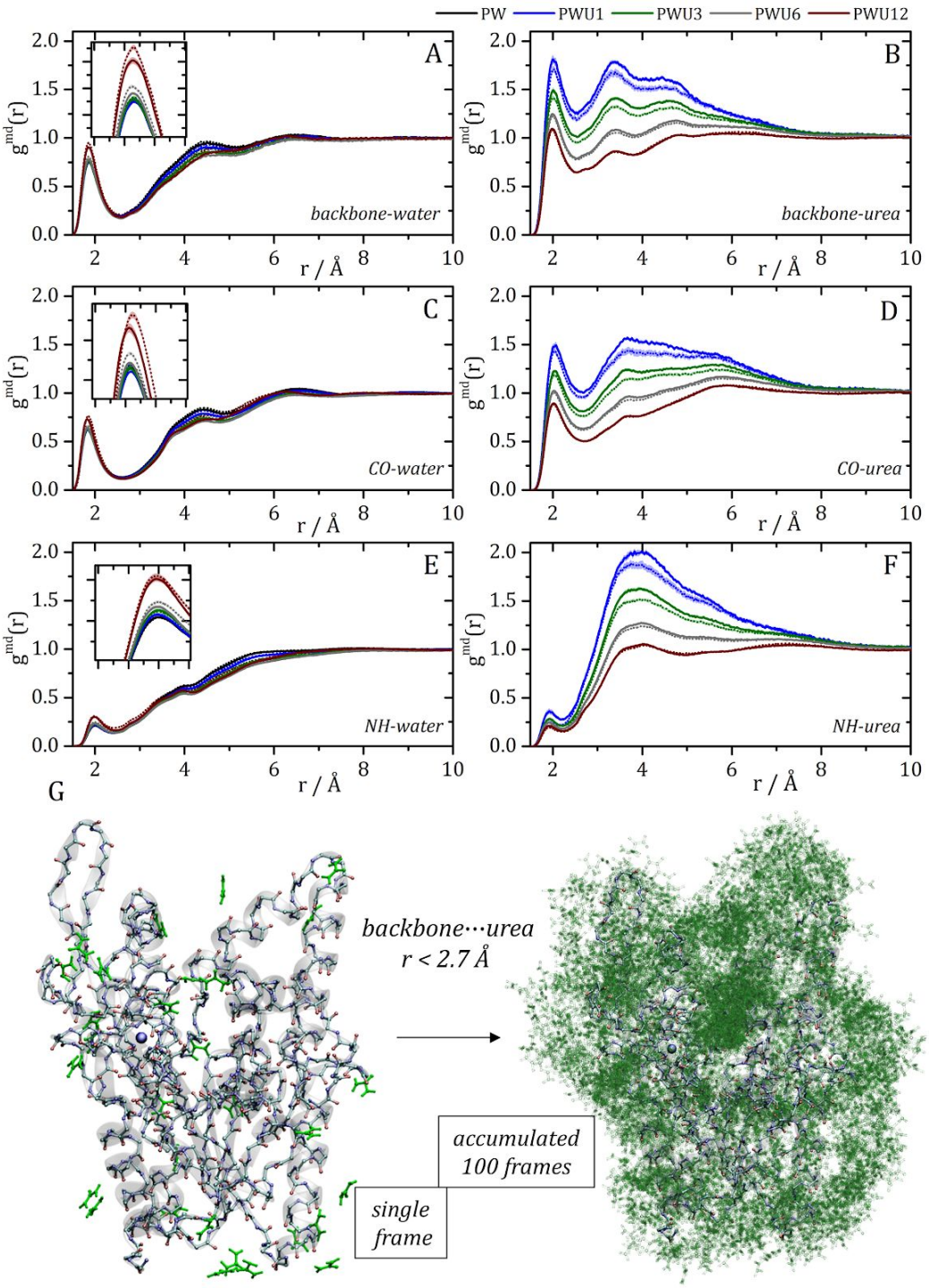

Fig. S11 Minimum-distance distribution functions (MDDFs or $g^{md}(r)$) between (A) water and (B) urea and the components of the protein backbone; (C) and (D) MDDFs with the backbone carbonyl group. (E) and (F) MDDFs with the amine group. Error bars are shown by colored shadows. Deprotonated and protonated systems are represented by dashed and solid lines, respectively. (G) Structures on the bottom show the accumulation of urea molecules on the backbone vicinities (r < 2.7Å) for single frame and for accumulated frames.

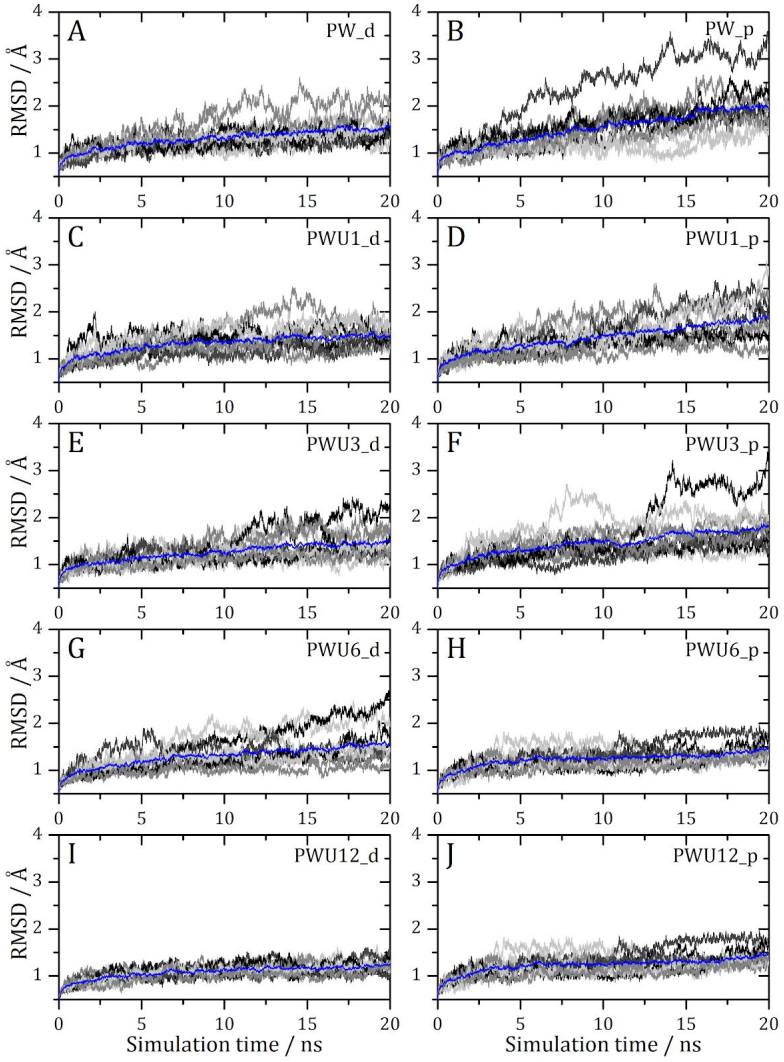

Fig. S12 Root mean square deviation (RMSD) of the BCL (*Burkholderia cepacia* lipase) enzyme considering only Cα atoms from the backbone for all simulated systems (deprotonated-***d*** and protonated-***p*** conditions). (A) and (B) without urea; (C) and (D) aqueous urea solution at ~1 mol L^{-1} (PWU1 system), (E) and (F) urea at ~3 mol L^{-1} (PWU3 system), (G) and (H) urea at ~6.5 mol L^{-1} (PWU6 system), and (I) and (J) urea at ~12 mol L^{-1} (PWU12 system). Blue lines are the mean of 10 independent simulations. No significant difference was observed in systems with or without urea in this time-scale. The simulation of the systems in these conditions is deliberate, as we would not want to couple the solvation effects with the non-equilibrium denaturation of the protein structure. Therefore, the solvation analysis were performed assuming the native state of the protein in all cases.

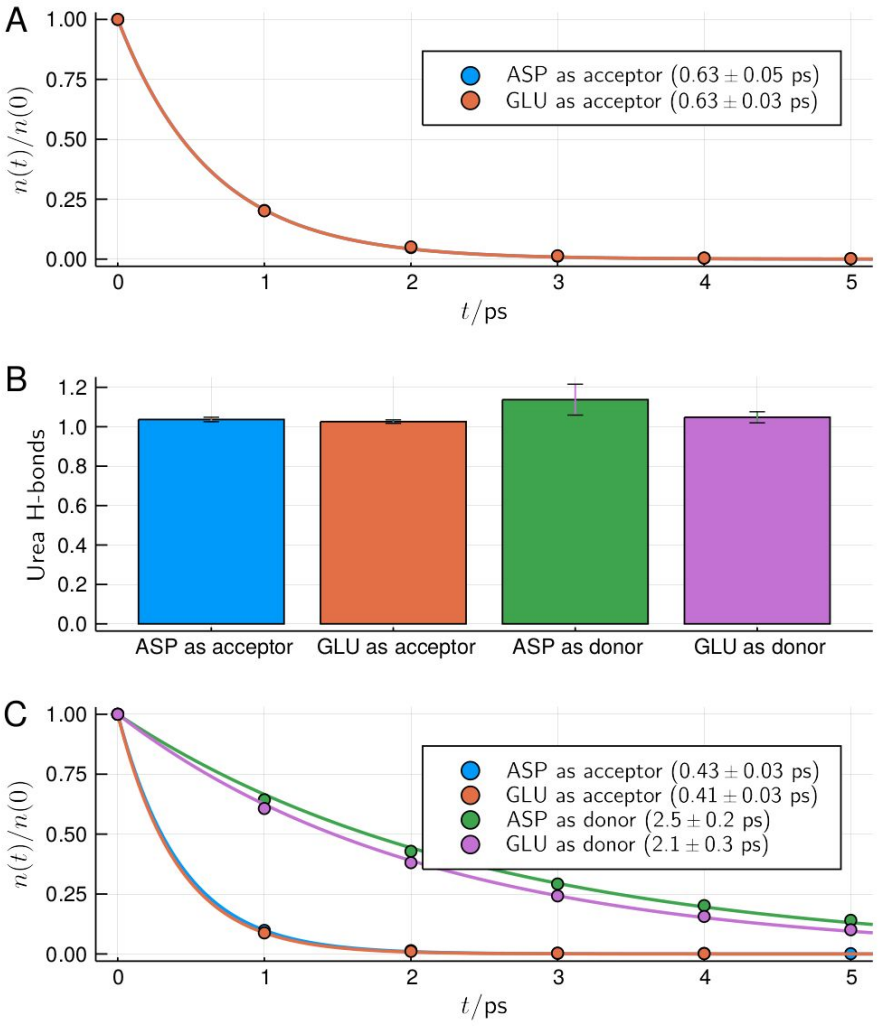

Fig. S13 Survival times and type of hydrogen bonds between urea and the side-chain of acidic residues for the protein in ~1 mol L^{-1} solution of urea. (A) Survival times of hydrogen bonds for the deprotonated acidic residues. (B) Number of hydrogen bonds with the acidic residues in the protonated form in which the residues act as hydrogen acceptors or donor acceptors of hydrogen (the deprotonated form of the residues can only act as hydrogen acceptors). (C) Survival times of the hydrogen bonds with the side chains of acidic residues in the protonated form. In (A) and (C) characteristic times obtained from mono-exponential fits are shown.

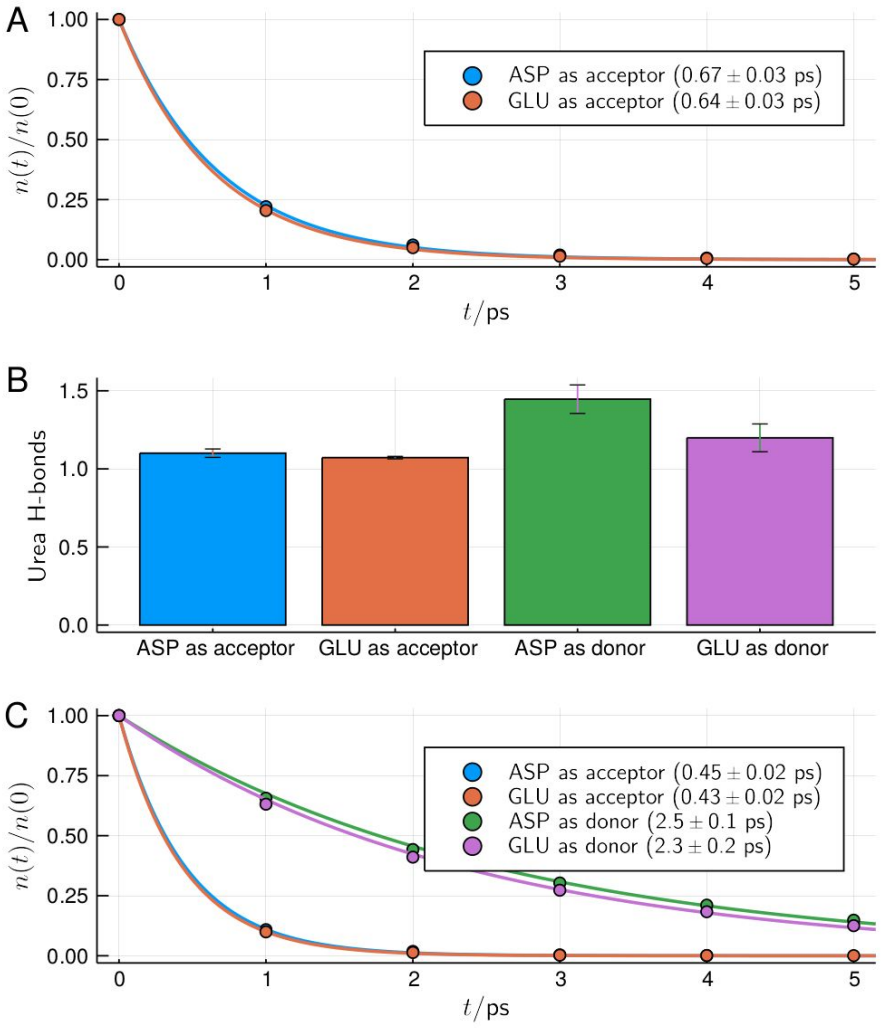

Fig. S14 Survival times and type of hydrogen bonds between urea and the side-chain of acidic residues for the protein in ~3 mol L^{-1} solution of urea. (A) Survival times of hydrogen bonds for the deprotonated acidic residues. (B) Number of hydrogen bonds with the acidic residues in the protonated form in which the residues act as hydrogen acceptors or donor acceptors of hydrogen (the deprotonated form of the residues can only act as hydrogen acceptors). (C) Survival times of the hydrogen bonds with the side chains of acidic residues in the protonated form. In (A) and (C) characteristic times obtained from mono-exponential fits are shown.

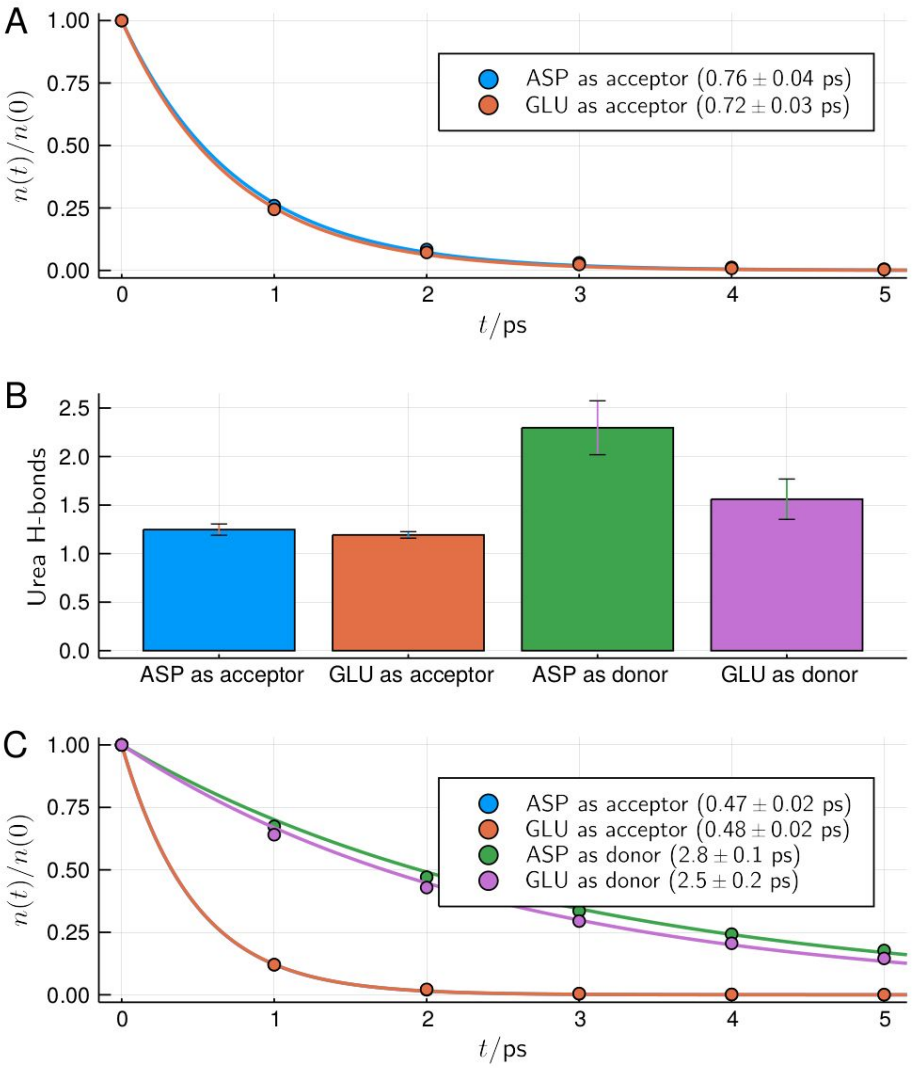

Fig. S15 Survival times and type of hydrogen bonds between urea and the side-chain of acidic residues for the protein in ~12 mol L^{-1} solution of urea. (A) Survival times of hydrogen bonds for the deprotonated acidic residues. (B) Number of hydrogen bonds with the acidic residues in the protonated form in which the residues act as hydrogen acceptors or donor acceptors of hydrogen (the deprotonated form of the residues can only act as hydrogen acceptors). (C) Survival times of the hydrogen bonds with the side chains of acidic residues in the protonated form. In (A) and (C) characteristic times obtained from mono-exponential fits are shown.

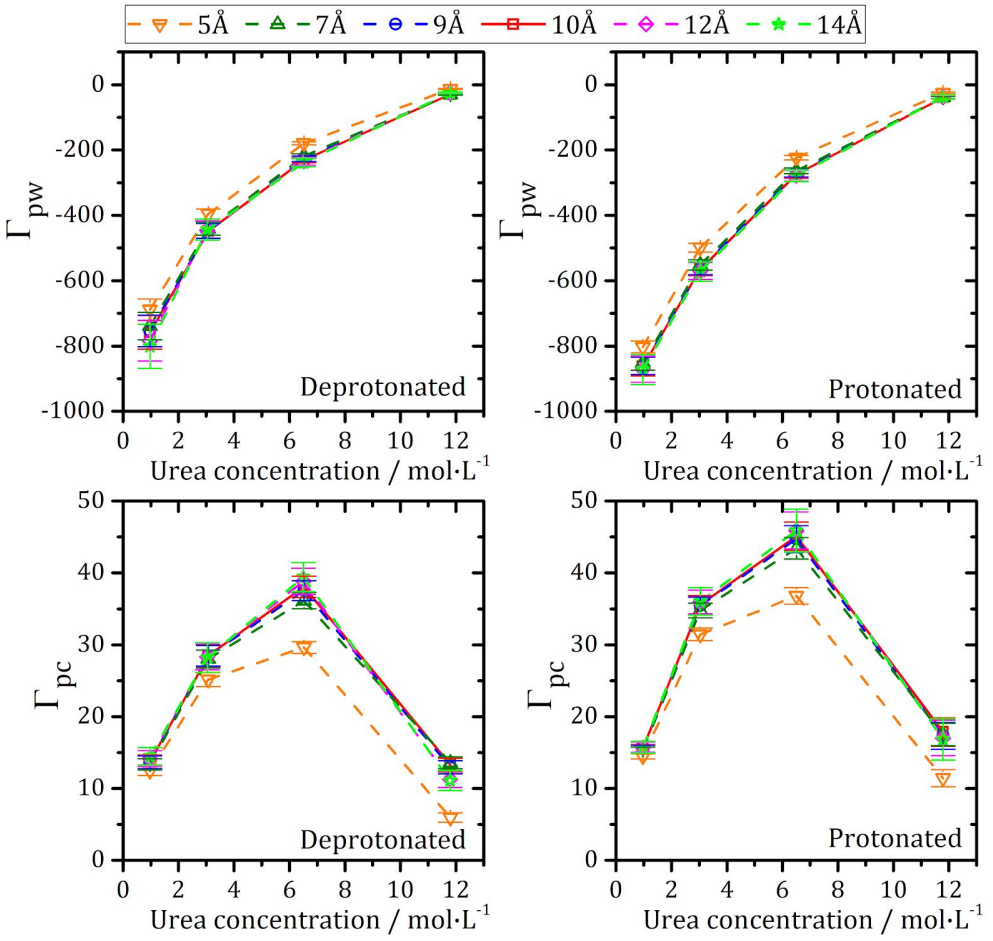

Fig. S16 Preferential interaction parameters computed for different choices of the distance defining the "protein domain". The protein domain (in this case, minimum-distance) must be large enough such that the presence of the protein does not affect the structure of the solvent, but not too large such that the fluctuations of the number of molecules inside the domain cannot be properly sampled. The small variability of the preferential interaction parameters computed for distances greater than 7 Å shows that 10 Å for the protein domain is a safe choice for this distance.

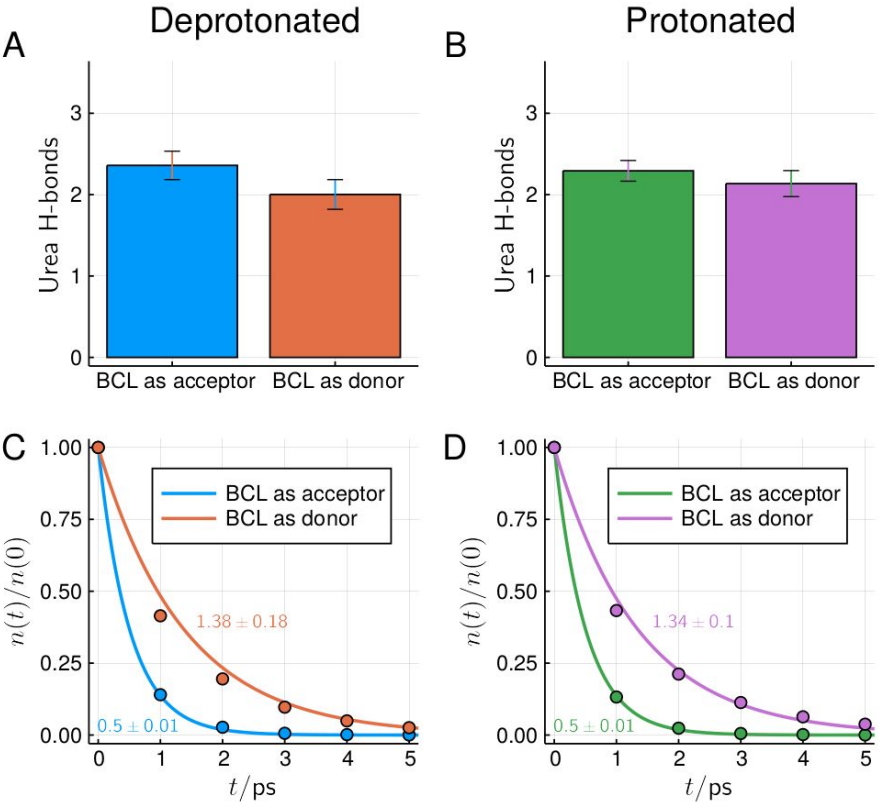

Fig. S17 Number and survival times of hydrogen bonds between BCL and urea at ~1M, considering only residues not forming direct contacts with superficial acidic residues in the crystallographic structure (corresponding to the data in Table S1).

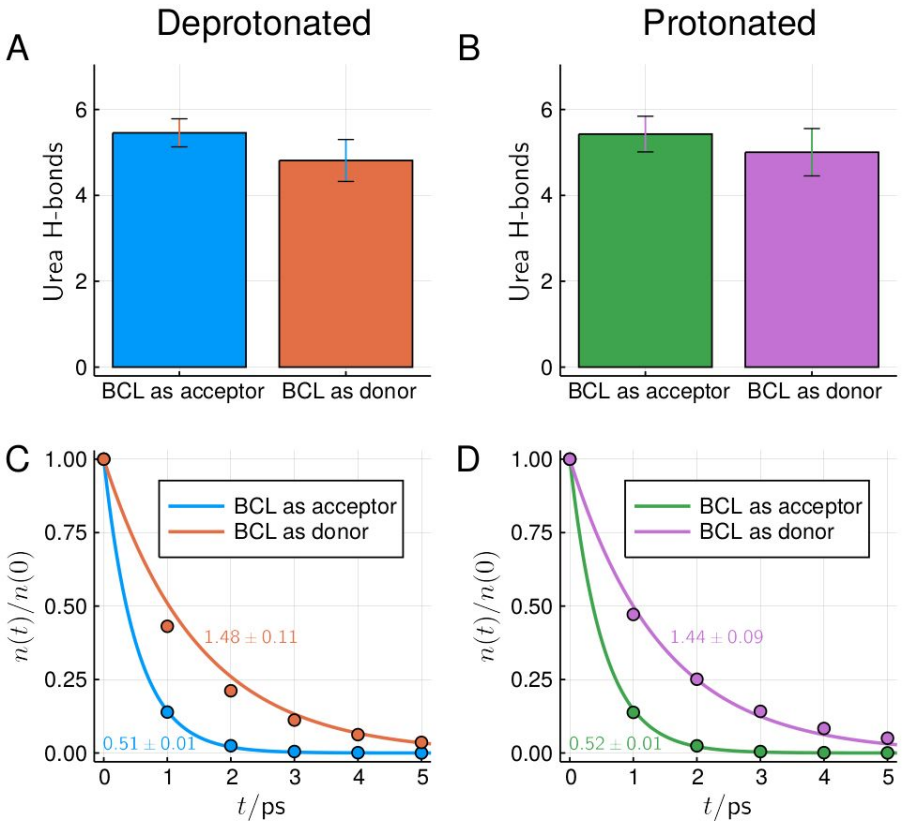

Fig. S18 Number and survival times of hydrogen bonds between BCL and urea at ~3M, considering only residues not forming direct contacts with superficial acidic residues in the crystallographic structure (corresponding to the data in Table S1).

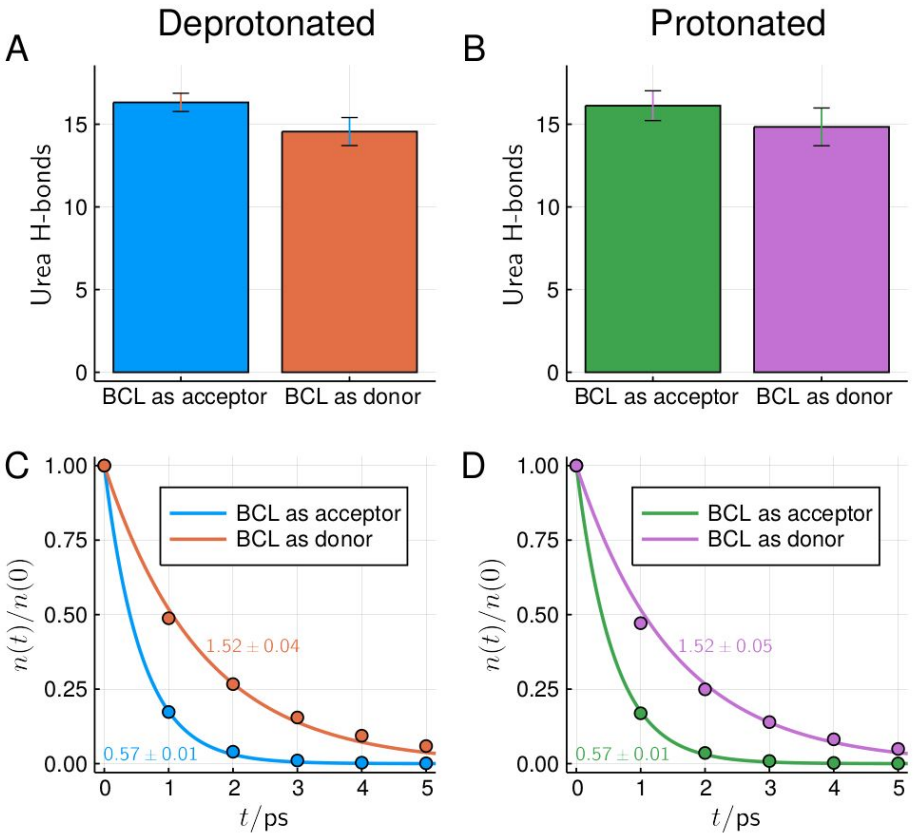

Fig. S19 Number and survival times of hydrogen bonds between BCL and urea at ~12M, considering only residues not forming direct contacts with superficial acidic residues in the crystallographic structure (corresponding to the data in Table S1).

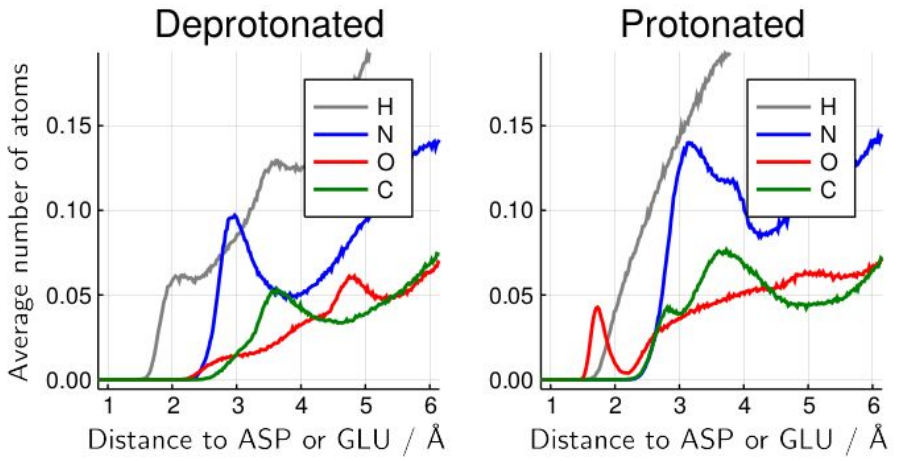

Fig. S20 Average number of atoms of urea molecules, of each type, distant from the side-chain of protonable acidic residues for the ~1 mol L^{-1} urea solution. The inversion of the configuration of urea molecules is visible by the presence of urea oxygen atoms (red) close to the acidic residues , in the protonated form but not in the deprotonated form. There is also a significant reduction in the number of urea hydrogen atoms in the immediate vicinity of these residues.

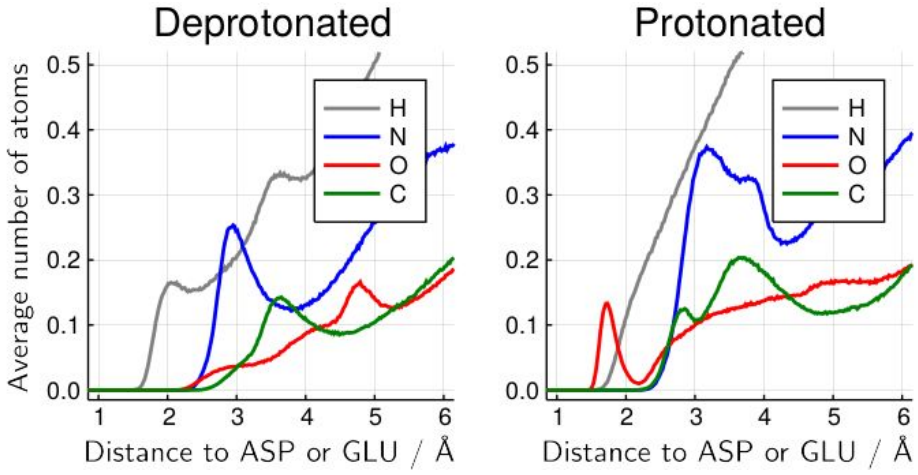

Fig. S21 Average number of atoms of urea molecules, of each type, distant from the side-chain of protonable acidic residues for the ~3 mol L^{-1} urea solution. The inversion of the configuration of urea molecules is visible by the presence of urea oxygen atoms (red) close to the acidic residues , in the protonated form but not in the deprotonated form. There is also a significant reduction in the number of urea hydrogen atoms in the immediate vicinity of these residues.

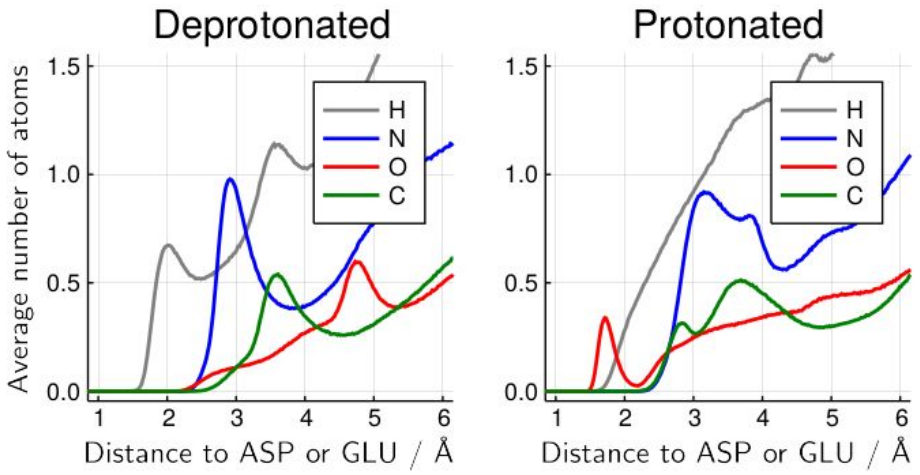

Fig. S22 Average number of atoms of urea molecules, of each type, distant from the side-chain of protonable acidic residues for the ~12 mol L^{-1} urea solution. The inversion of the configuration of urea molecules is visible by the presence of urea oxygen atoms (red) close to the acidic residues, in the protonated form but not in the deprotonated form. There is also a significant reduction in the number of urea hydrogen atoms in the immediate vicinity of these residues.

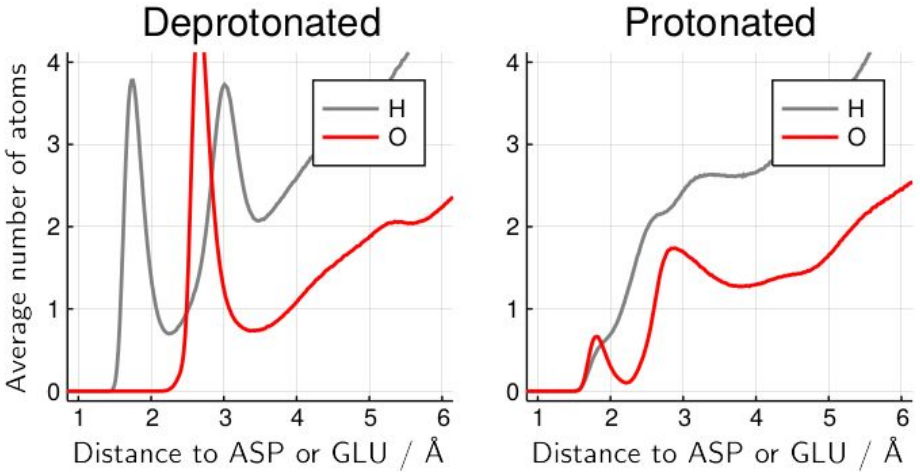

Fig. S23 Average number of atoms of water molecules, of each type, distant from the side-chain of protonable acidic residues for the ~1 mol L^{-1} urea solution. The inversion of the configuration of water molecules is visible by the presence of water oxygen atoms (red) close to the acidic residues, in the protonated form but not in the deprotonated form. Water is also significantly more structured around charged residues than around neutral ones.

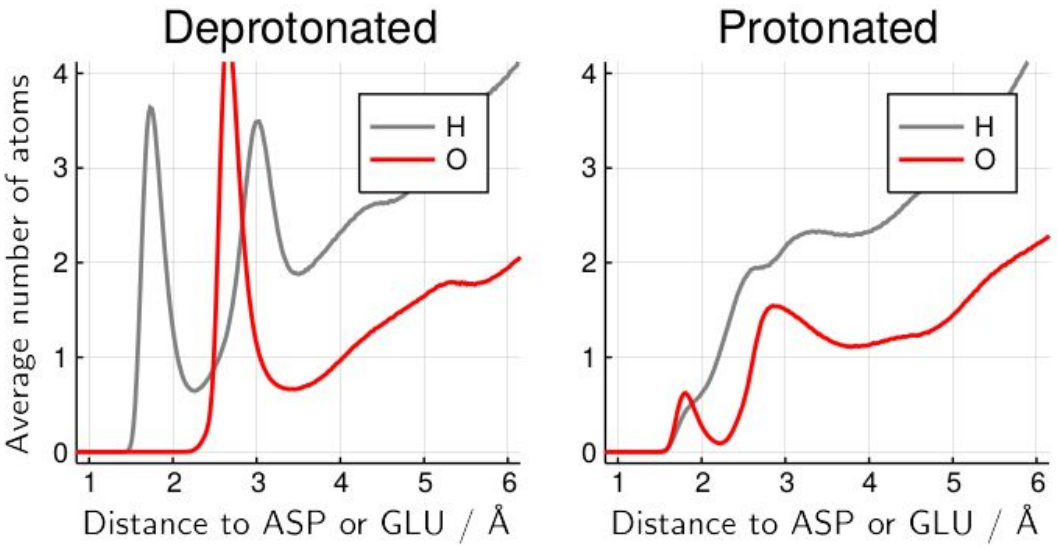

Fig. S24 Average number of atoms of water molecules, of each type, distant from the side-chain of protonable acidic residues for the ~3 mol L^{-1} urea solution. The inversion of the configuration of water molecules is visible by the presence of water oxygen atoms (red) close to the acidic residues, in the protonated form but not in the deprotonated form. Water is also significantly more structured around charged residues than around neutral ones.

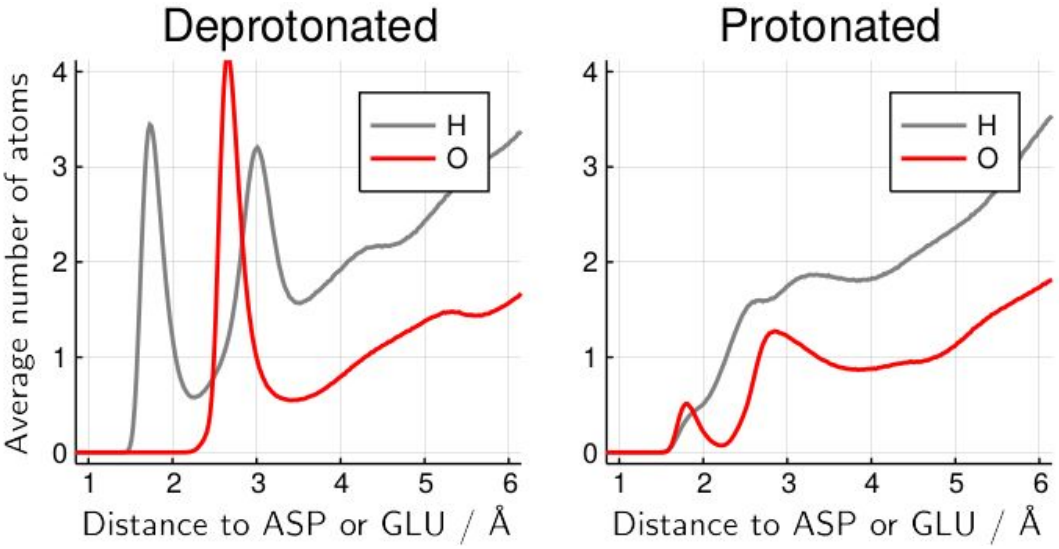

Fig. S25 Average number of atoms of water molecules, of each type, distant from the side-chain of protonable acidic residues for the ~6 mol L^{-1} urea solution. The inversion of the configuration of water molecules is visible by the presence of water oxygen atoms (red) close to the acidic residues, in the protonated form but not in the deprotonated form. Water is also significantly more structured around charged residues than around neutral ones.

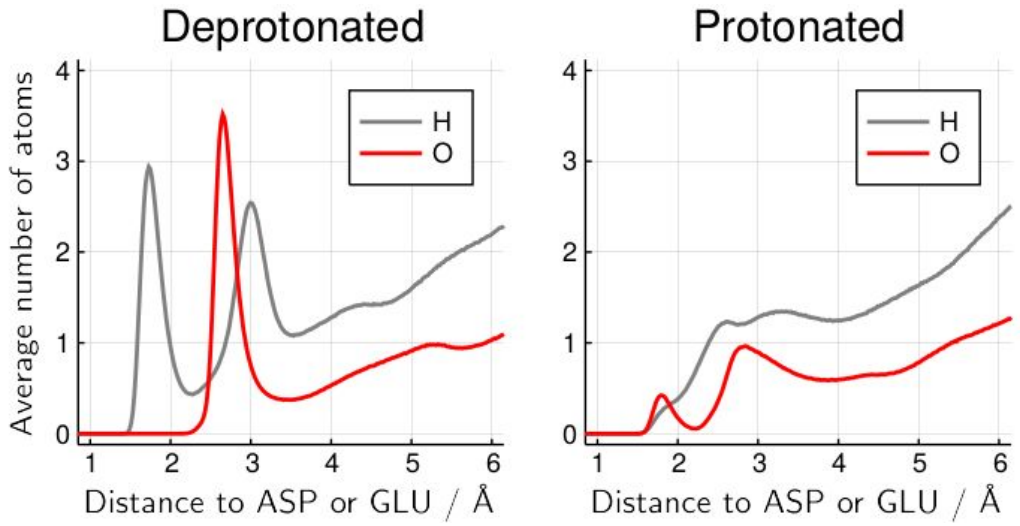

Fig. S26 Average number of atoms of water molecules, of each type, distant from the side-chain of protonable acidic residues for the ~12 mol L^{-1} urea solution. The inversion of the configuration of water molecules is visible by the presence of water oxygen atoms (red) close to the acidic residues, in the protonated form but not in the deprotonated form. Water is also significantly more structured around charged residues than around neutral ones.