Osmolytes can Destabilize Proteins in Cells by Modulating Electrostatics and Quinary Interactions

Xiangfei Song,[a,b,c,⊥] Liaoyuan An,[a,b,c,⊥] Mengting Wang, [a,b,c] Jingfei Chen,[a,b] Zhijun Liu,[d] and Lishan Yao[a,b,*]
[a] Key Laboratory of Biofuels and [b] Shandong Provincial Key Laboratory of Synthetic Biology, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, 266101, China
[c] University of Chinese Academy of Sciences, Beijing, 100049, China
[d] National Facility for Protein Science, Zhangjiang Lab, Shanghai Advanced Research Institute, CAS, 201210, China

Supporting Information

⊥These two authors contributed equally to the work.

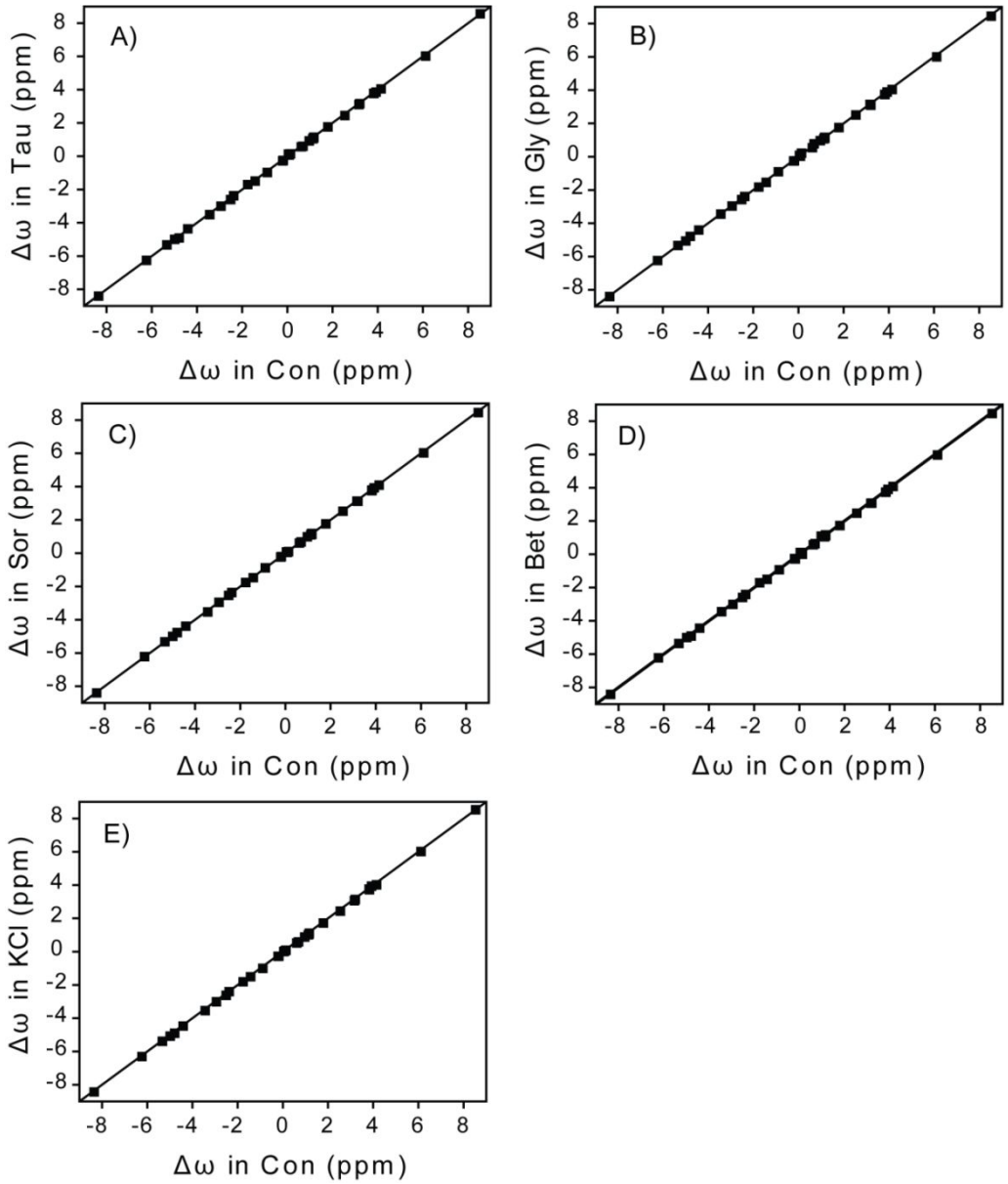

Figure S1. Correlation between Δω, the chemical shift difference between unfold state and fold state *in vitro* in the absence (Con) or presence of different small molecules.

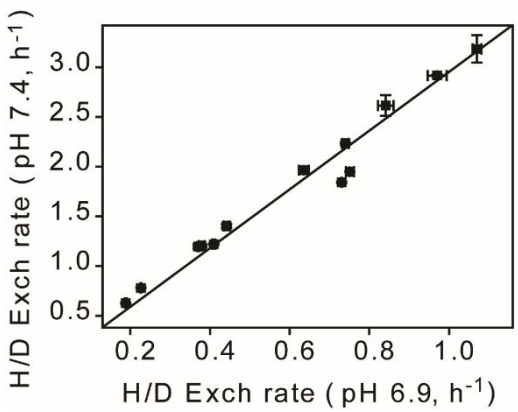

Figure S2. Correlation between the H/D exchange rates of 13 slowest exchanged

amides of MutX in two pH conditions. The best fitted line is $y = 2.94x$.

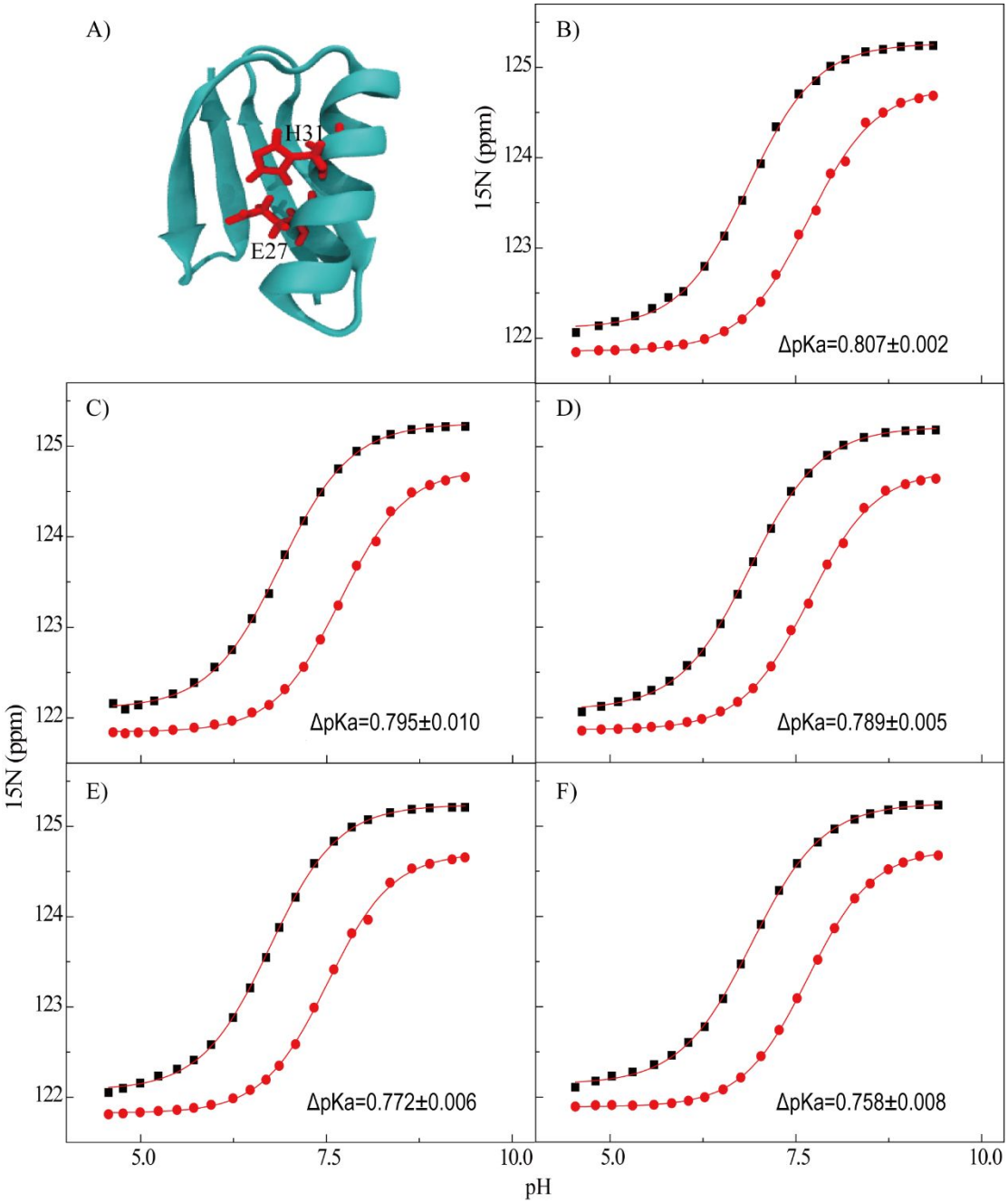

Figure S3. (A) Salt bridge H31-E27 in the structure of GB3. ^{15}N chemical shift of H31 of H31-E27 (red) and H31-Q27 (black) at 298 K and different pHs without osmolyte (B), or in the presence of 500 mM sorbitol (C), glycerol (D), betaine (E) and taurine (F). The chemical shift was fitted to the Henderson-Hasselbalch equation to yield corresponding p*K*a values.

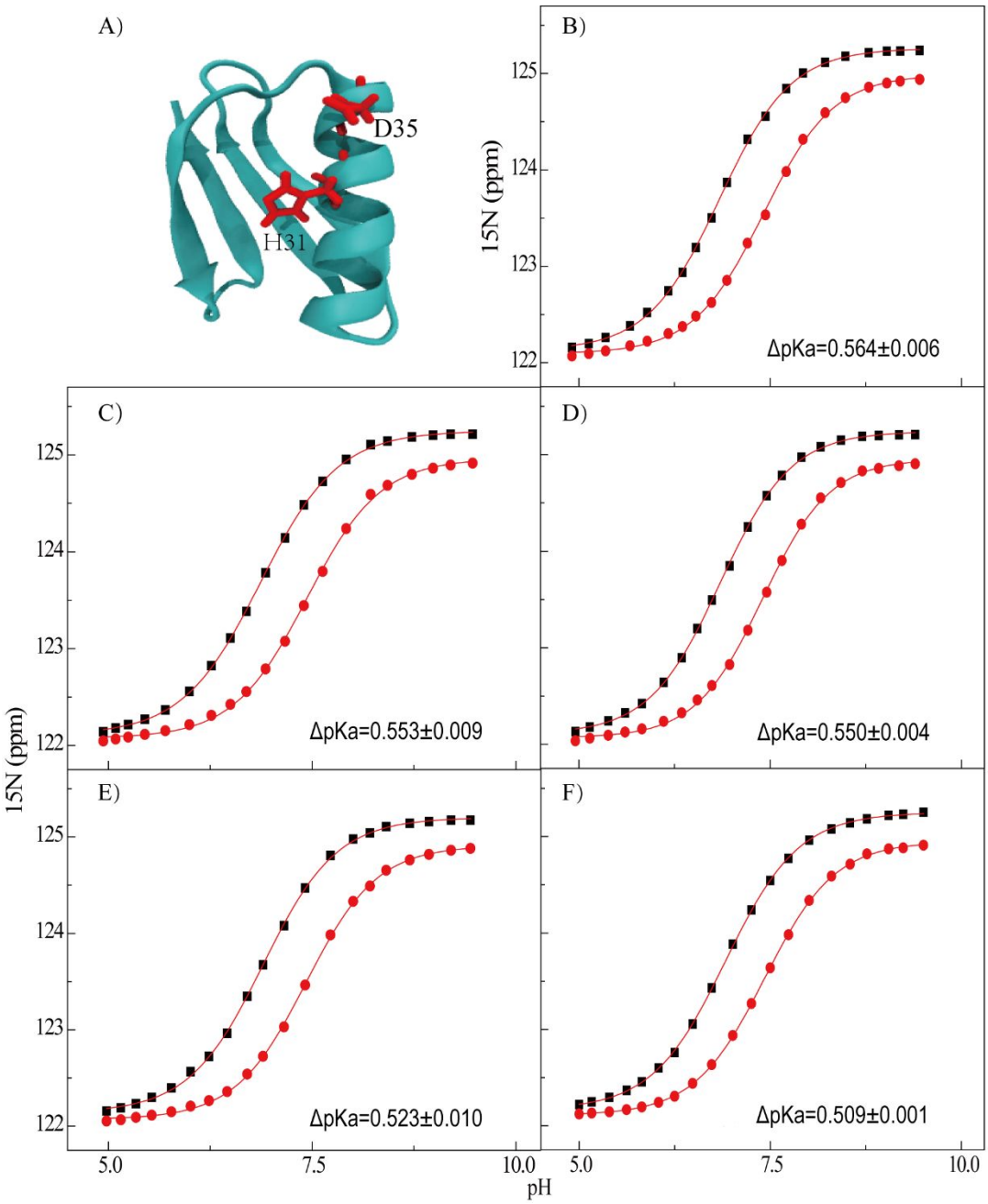

Figure S4. (A) Salt bridge H31-D35 in the structure of GB3. ^{15}N chemical shift of H31 of H31-D35 (red) and H31-N35 (black) at 298 K and different pHs without osmolyte (B), or in the presence of 500 mM sorbitol (C), glycerol (D), betaine (E) and taurine (F). The chemical shift was fitted to the Henderson-Hasselbalch equation to yield corresponding pK_a values.

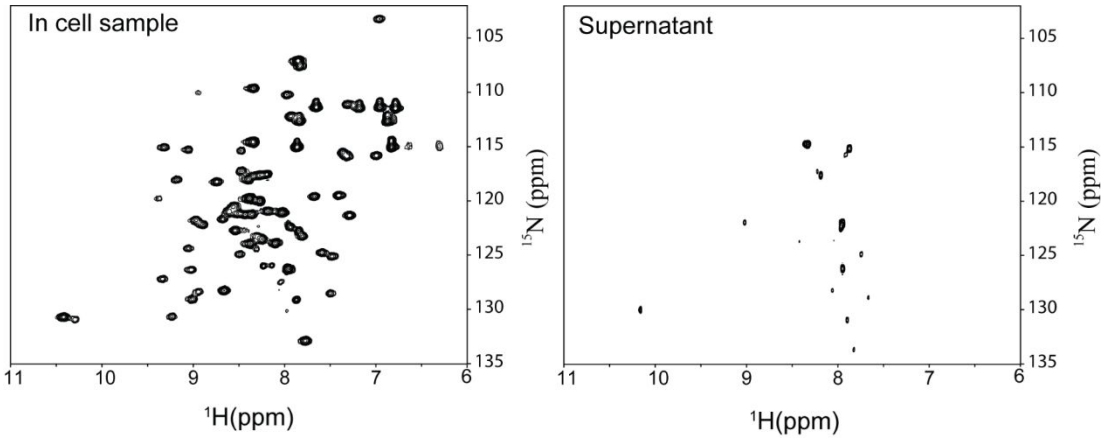

Figure S5. ^{1}H-^{15}N HSQC spectra of the GB3 mutant MutX in *E. coli* cells and in supernatant after 3.5 h of NMR CPMG measurement. The in-cell sample was dissolved in 40 mM bis–tris propane, and 40 mM HEPES, pH 7.8.

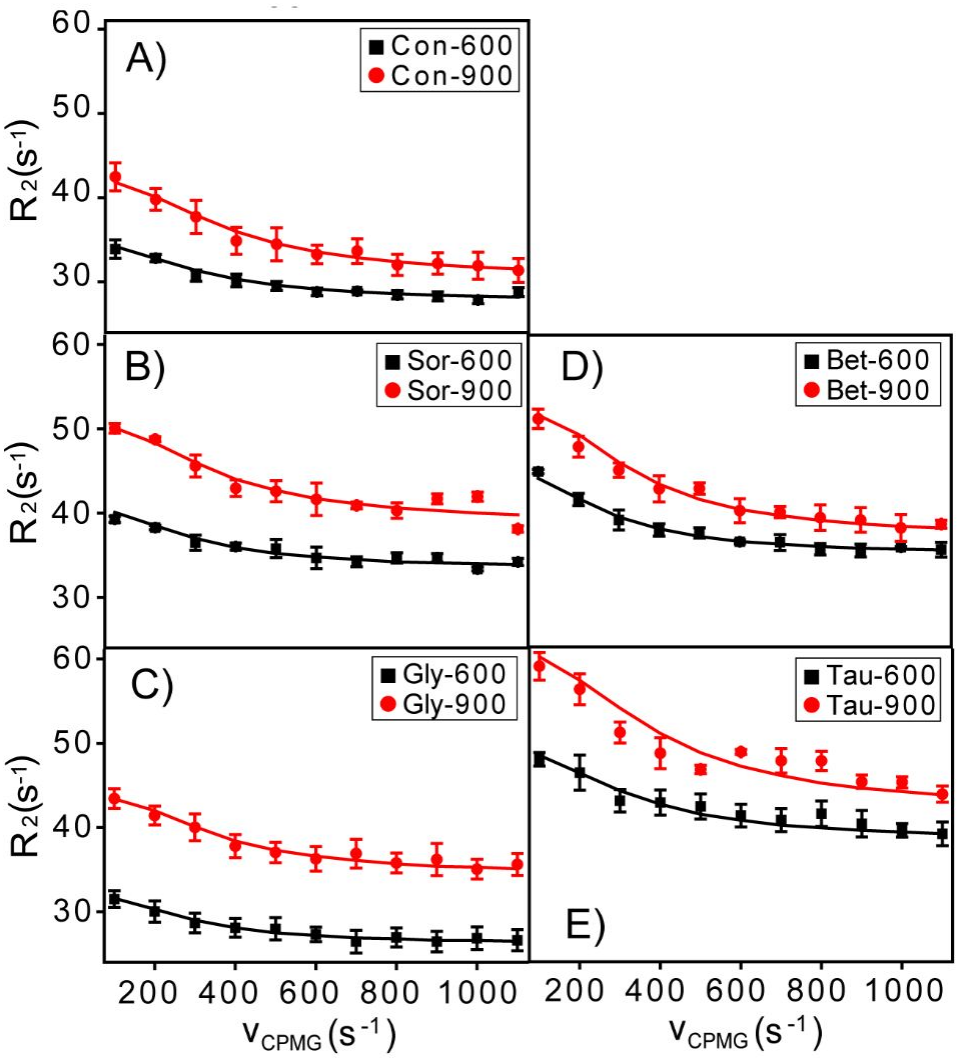

Figure S6. Global fitting of ^{15}N CPMG profiles for A48 at 600 and 900 MHz magnetic fields in cells with or without osmolytes.

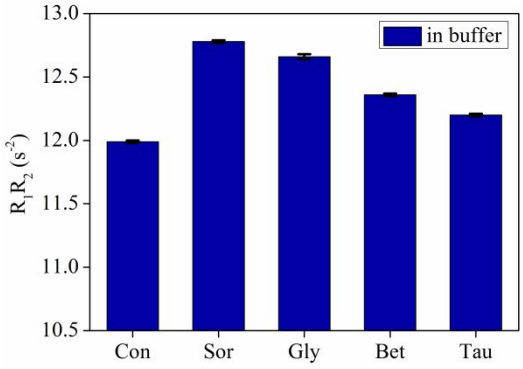

Figure S7. Product of average R_1R_2 of MutX in *in vitro* in the absence or presence of 400 mM osmolytes.