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Electronic Supplementary Information

Insight into the Amplification by Methylated Urea of the Anion Specificity of Macromolecules

Lvdan Liu,[a] Yang Shi,[a] Chang Liu,[a] Tao Wang,[a] Guangming Liu*[a] and Guangzhao Zhang*[b]

[a]*Department of Chemical Physics, Hefei National Laboratory for Physical Sciences at the Microscale, University of Science and Technology of China, Hefei, P. R. China 230026*
[b]*Faculty of Materials Science and Engineering, South China University of Technology, Guangzhou, P. R. China 510640*

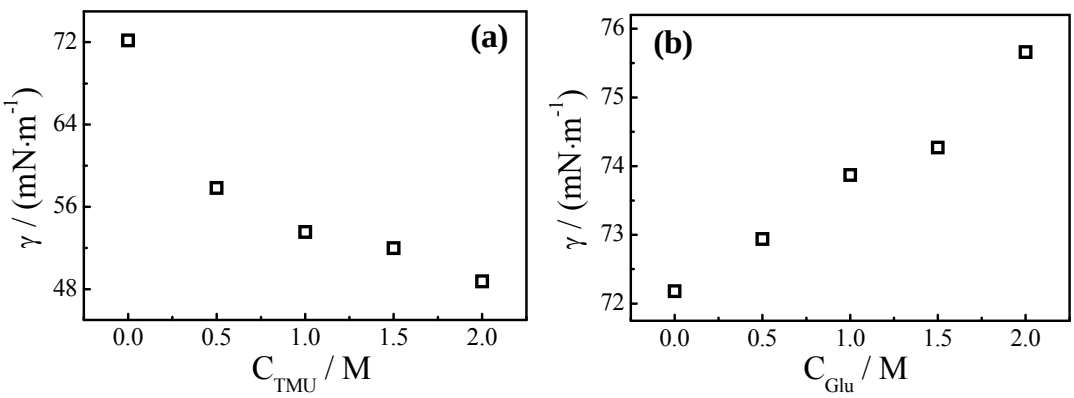

Figure S1. Change in surface tension (γ) of the salt-free solutions as a function of the molar fraction of TMU (C_{TMU}) or Glu (C_{Glu}). (a) TMU. (b) Glu.

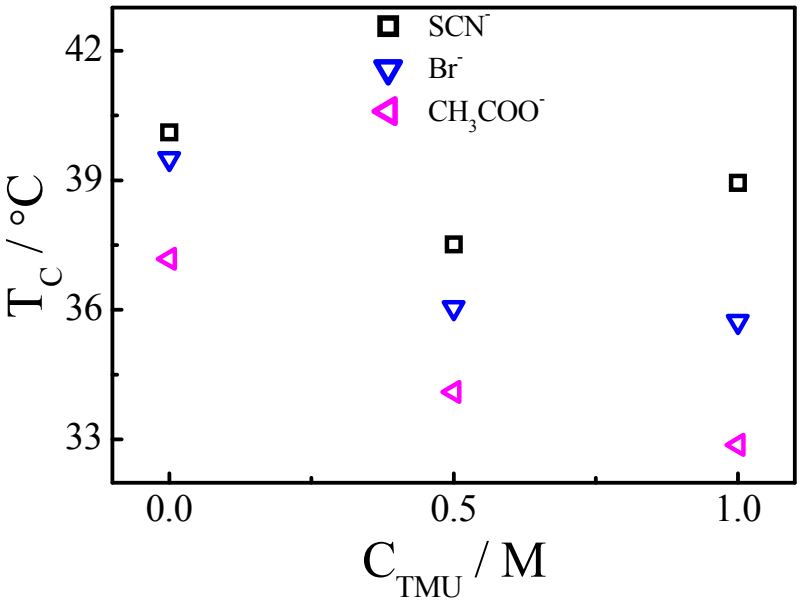

Figure S2. Change in cloud point (T_C) of lysozyme in phosphate buffer (pH 12.1, 0.2 M) as a function of the molar fraction of TMU (C_{TMU}) for SCN^-, Br^-, and CH_3COO^- with Na^+ as the common cation, where the lysozyme concentration is fixed at 3.0 mg mL^{-1} and the external salt concentration is fixed at 0.5 M.

Figure S2 shows the specific anion effect on cloud point (T_C) of lysozyme during the heat-induced protein aggregation. Here, we choose three typical anions to investigate the anion specificity. SCN^- and CH_3COO^- are strong chaotrope and kosmotrope, respectively. Br^- locates between them in the Hofmeister series. For Br^- and CH_3COO^-, T_C gradually decreases with the increasing C_{TMU}. For the case of SCN^-, T_C decreases as the C_{TMU} increases from 0 to 0.5 M, and then it increases with the further increase of C_{TMU} from 0.5 M to 1.0 M. At the same C_{TMU}, T_C decreases following the order $SCN^- > Br^- > CH_3COO^-$, namely, the anion specificity is observed here. Obviously, the anion specificity is amplified with the addition of TMU, which is similar to the observation for the specific anion effect on the LCST behavior of PNIPAM (see the main text). The added TMU molecules will interact with the previously buried hydrophobic groups of the protein molecules via hydrophobic interactions and accelerate the unfolding of protein molecules. As a result, the hydrophobic groups will distribute on the protein surface induced by the unfolding of protein structure, which will enhance the protein-protein hydrophobic interactions and favor the aggregation of protein molecules.[S1,S2] Therefore, T_C generally decreases with the C_{TMU}. On the other hand, the capability of anions to salt out hydrocarbons increases following the series $SCN^- < Br^- < CH_3COO^-$.[S3] This is why T_C decreases following the series $SCN^- > Br^- > CH_3COO^-$ in the TMU-free solution. Meanwhile, the effectiveness of anions to promote the hydrophobic interactions between TMU molecules and the hydrophobic groups of lysozyme also increases following the series $SCN^- < Br^- < CH_3COO^-$ and

this effect would become more obvious with the increasing C_{TMU}. Thus, the anion specificity regarding the protein aggregation (reflected by the cloud point) will be amplified with the addition of TMU. Also, the TMU-induced unfolding of protein structure may expose more polar and nonpolar groups outside the protein, which might also enlarge the anion-specific interactions between the anions and the groups and amplify the anion specificity. In addition, the increase of T_C with the increasing C_{TMU} from 0.5 to 1.0 M for SCN^- might be due to the strong salting-in effect generated by the SCN^-.[S3,S4]

Sugar type	Anion type / C_{sugar} / M	LCST / °C (SCN^-)	LCST / °C (salt-free)	LCST / °C (Br^-)	LCST / °C (CH_3COO^-)
Glucose	0	34.7	32.9	28.7	23.0
	0.5	28.1	28.1	21.5	15.9
	1.0	17.2	21.9	12.4	5.6
Sucrose	0	34.7	32.9	28.7	23.0
	0.5	29.8	29.4	23.1	17.8
	1.0	20.8	24.7	15.9	9.3
Maltose	0	34.7	32.9	28.7	23.0
	0.5	30.3	28.6	24.0	18.5
	1.0	25.7	24.4	19.5	13.5

Table S1. Change in LCST of PNIPAM as a function of the molar concentration of the three types of kosmotropic osmolytes (i.e., glucose, sucrose, and maltose) (C_{sugar}), where the PNIPAM concentration is fixed at 1.0 mg mL^{-1} and the salt concentration is fixed at 0.5 M. Here, we only choose three typical anions (i.e., SCN^-, Br^-, and CH_3COO^-) to investigate that whether the anion specificity can be amplified by the sugars. The highest concentration of sucrose and maltose used in the measurements is 1.0 M because of the limit of solubility of disaccharide in water.

References

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