Supporting Information

Influence of Glycerol on the Cooling Effect of Pair Hydrophobicity in Water: Relevance in Protein's Stabilization at Low Temperature¹

Vikas Dubey^a, Snehasis Daschakraborty^{*a}

¹Electronic supplementary information (ESI) available: The Supporting Information contains the following. (1) Weighed histogram showing sufficiency of sampling windows, (2) Figures showing convergence of PMF, (3) Alternate way of preseting PMF curves, (4) Decomposition of entropy and enthalpy components of PMF for T=280 K and 300 K, (5) Solvent contribution towards PMF for T=280 K and 300 K, (6) Table showing data of free energy, entropy and enthalpy at different minima of PMF, (7) RDF between methane solute C and solvent water O_W in pure water at four different temperatures, (8) RDF between the methane solute and solvent molecules at T=280 K and 300 K, and (9) Number of H-bond per solvent water molecule as a function of methane-methane separation for pure water at three different temperatures.

^aDepartment of Chemistry, Indian Institute of Technology Patna, Bihar 801106, India. Email: <u>snehasis@iitp.ac.in</u>



Figure S1. Weighed histogram showing sufficiency of sampling windows



Figure S2. Convergence of PMF: Comparison of PMFs obtained from gradually increasing trajectory lengths.



Figure S3. Convergence of PMF: Comparison between PMFs arising from the first 50 ns of the trajectory and that obtained from the last 50 ns trajectory.



Figure S4. PMF as a function of the distance between the pair of methane molecules, dissolved either in pure water or in the glycerol/water binary mixture, varying composition (x_G =0.05, and 0.10), each at 4 different temperatures (T=320 K, 300 K, 280 K, and 260 K). The following is the procedure by which we put the PMFs on one another. First, we obtain the PMF using WHAM analysis technique setting PMF value zero at *r*=0.0 nm, which is a default setting of the WHAM analysis. Then we corrected the entropic contribution to the PMF due to the rotation of the solutes by adding the term $2k_BT \ln(r)$ to the free energy obtained from WHAM analysis. Uncertainty in the PMF was computed by bootstrap analysis. The resulting PMF was finally scaled to zero at *r*=1.3 nm.



Figure S5. PMF between the pair of methane solute molecules and its two components, entropy (-*TS*) and enthalpy (*H*) in (a) pure water, water/glycerol mixture with (b) x_G =0.05, and (c) x_G =0.1 at 280 K temperature.



Figure S6. PMF between the pair of methane solute molecules and its two components, entropy (-*TS*) and enthalpy (*H*) in (a) pure water, water/glycerol mixture with (b) x_G =0.05, and (c) x_G =0.1 at 300 K temperature.



Figure S7. Solvent contribution of the PMF, W_{solv} , and its two components (entropy (-*TS*) and enthalpy (H_{solv})) as functions of methane-methane separation r in solvent with three different compositions: (a) x_G =0.0, (b) x_G =0.05, and (c) x_G =0.1 at T=280 K temperature. H_{solv} and its two components, $H_{sol-solv}$ and H_{rem} , at various r values for (d) x_G =0.0 (e) x_G =0.05, and (f) x_G =0.1 at 300 K temperature.



Figure S8. Solvent contribution of the PMF, W_{solv} , and its two components (entropy (-*TS*) and enthalpy (H_{solv})) as functions of methane-methane separation r in solvent with three different compositions: (a) x_G =0.0, (b) x_G =0.05, and (c) x_G =0.1 at 300 K temperature. H_{solv} and its two components, $H_{sol-solv}$ and H_{rem} , at various r values for (d) x_G =0.0 (e) x_G =0.05, and (f) x_G =0.1at T= 300 K.

x_G	T (K)	1^{st} minima ($r_{\text{CH}_4\text{-}\text{CH}_4}$ = 0.39 nm)			$1^{\text{st}} \text{ maxima}$ $(r_{\text{CH}_4-\text{CH}_4}=0.54 \text{ nm})$			2^{nd} minima ($r_{CH_4-CH_4} = 0.69$ nm)			2^{nd} maxima ($r_{CH_4-CH_4}=0.85-0.88$ nm)			3 rd minima (<i>r</i> _{CH4} -CH ₄ = 1.00-1.15 nm)		
		G	-TS	Н	G	-TS	Н	G	-TS	Н	G	-TS	Н	G	-TS	Н
0.0	260	-1.60	-3.66	2.06	0.57	2.84	-2.27	-0.56	0.95	-1.51	0.62	-1.84	2.46	-0.15	0.29	-0.44
	280	-1.89	-8.10	6.21	0.84	-0.43	1.27	-0.50	0.71	-1.21	0.48	-1.21	1.69	-0.16	0.35	-0.51
	300	-2.44	-7.23	4.79	0.78	0.91	-0.13	-0.52	1.47	-1.99	0.39	-0.01	0.40	-0.18	1.17	-1.35
0.05	260	-2.00	-5.34	3.34	0.81	1.37	-0.56	-0.49	1.15	-1.64	0.26	1.91	-1.65	-0.34	2.62	-2.97
	280	-2.41	-5.50	3.09	0.97	-0.86	1.82	-0.40	-0.65	0.24	0.47	-2.29	2.76	-0.17	2.23	-2.41
	300	-2.77	-7.76	4.99	0.96	-0.63	1.59	-0.44	2.33	-2.78	0.89	-2.32	2.68	-0.06	-0.95	1.01
0.1	260	-2.82	-1.81	-1.01	0.38	4.99	-4.60	-1.13	7.56	-8.69	-0.07	4.22	-4.28	-0.39	4.27	-4.66
	280	-2.96	-1.03	-1.93	0.81	4.18	-3.37	-0.55	4.38	-4.93	0.26	-1.76	2.02	-0.12	0.12	-0.24
	300	-3.03	-6.51	3.48	1.11	-0.69	1.80	-0.33	2.57	-2.89	0.17	1.31	-1.14	-0.17	3.00	-3.17

Table S1: Free energy, Entropy, and Enthalpy contributions at three PMF minima and two maxima. (All energy units are in kJ mol⁻¹)



Figure S9. Radial distribution function (RDF) between methane solute C and solvent water O_W in pure water at four different temperatures.



Figure S10. Radial distribution functions between the methane solute (C atom) and the solvent molecules (water's O_W and glycerol's C_G atom) for three different solvent compositions $x_G=0.0$, 0.05, and 0.1, at T=280 K.



Figure S11. Radial distribution functions between the methane solute (C atom) and the solvent molecules (water's O_W and glycerol's C_G atom) for three different solvent compositions $x_G=0.0$, 0.05, and 0.1, at T=300 K.



Figure S12. Number of H-bond per solvent water molecule as a function of methane-methane separation for pure water at three different temperatures. The interfacial layer extends up to r=5.5 Å and the bulk regime starts from r=11 Å distance from the solute.