Supporting Information for

Evaluating long-range orientational ordering of water around proteins: Signature of a tug-of-war scenario

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Contents of the Supporting Information

Supporting-Results

SR1. Ion Distribution in the First Hydration Layer

Charge	Concentration(M)	Total -Ions	1st Hydration Layer (%)
+8	0.154	634	0.09%
-8	0.154	634	0.17%
0	0.154	626	0.05%

SR2: Propensity Calculation

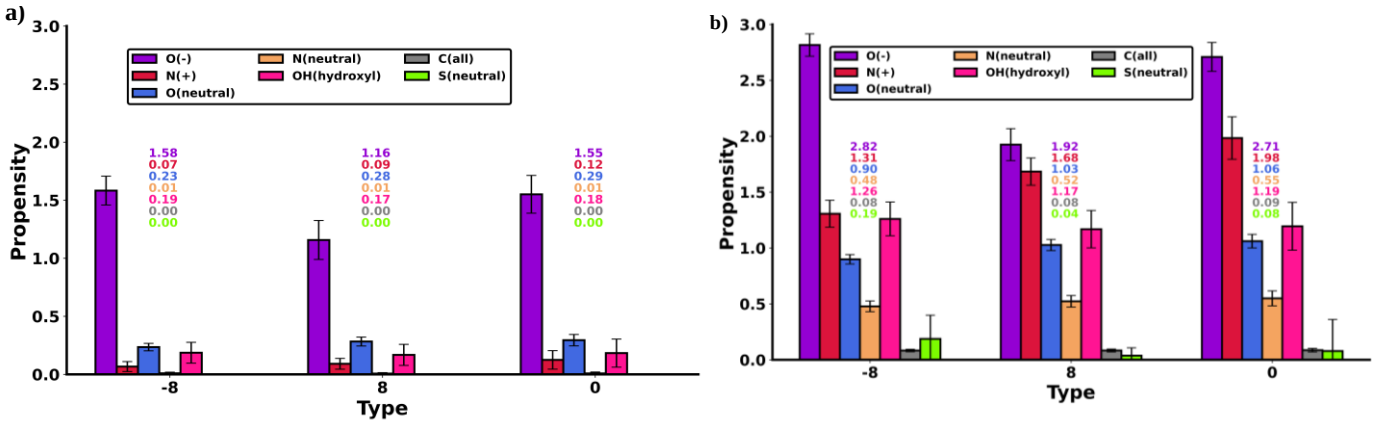

Figure. S1:(a) Propensity value of water molecules towards different types of surface amino acid atoms for all the three investigated systems at a protein-water distance of 2.7 Å in neutralizing condition (b) Propensity value of water molecules towards different types of surface amino acid atoms for all the three investigated systems at a protein-water distance of 3.5 Å in neutralizing condition.

SR3: Probability Distribution of the water orientation cos(θ) for three investigated systems in the 2nd hydration shell

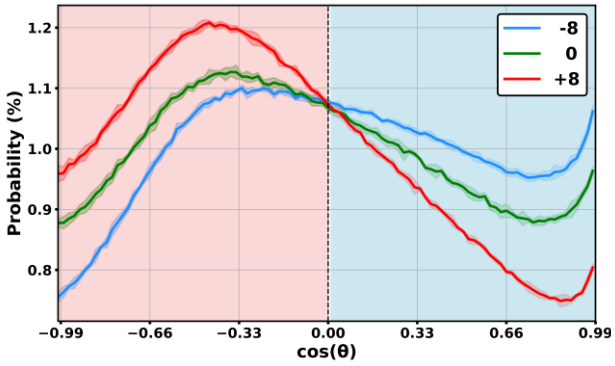

Figure. S2: Comparison of the probability distribution of the water orientation cos(θ) for three investigated systems in the 2nd hydration shell in neutralising condition, all the error bars stand for the SDs from 5 parallel 20ns simulations.

SR4: Relationship between total charge, salt effect, and water molecule orientation

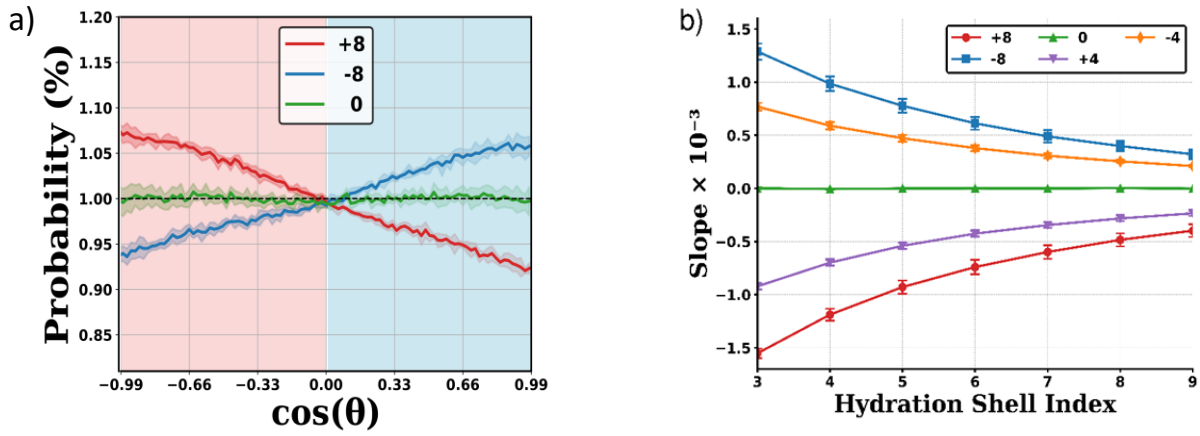

Figure. S3: (a) Comparison of the probability distribution of the water orientation cos(θ) for three investigated systems in the 3rd hydration shell in physiological salt concentration (b) Charge dependency of Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) plot in neutralizing condition, all the error bars stand for the SDs from 5 parallel 20ns simulations.

SR5: Distance-dependent water orientation around specific surface atom types of Ubiquitin in physiological concentration

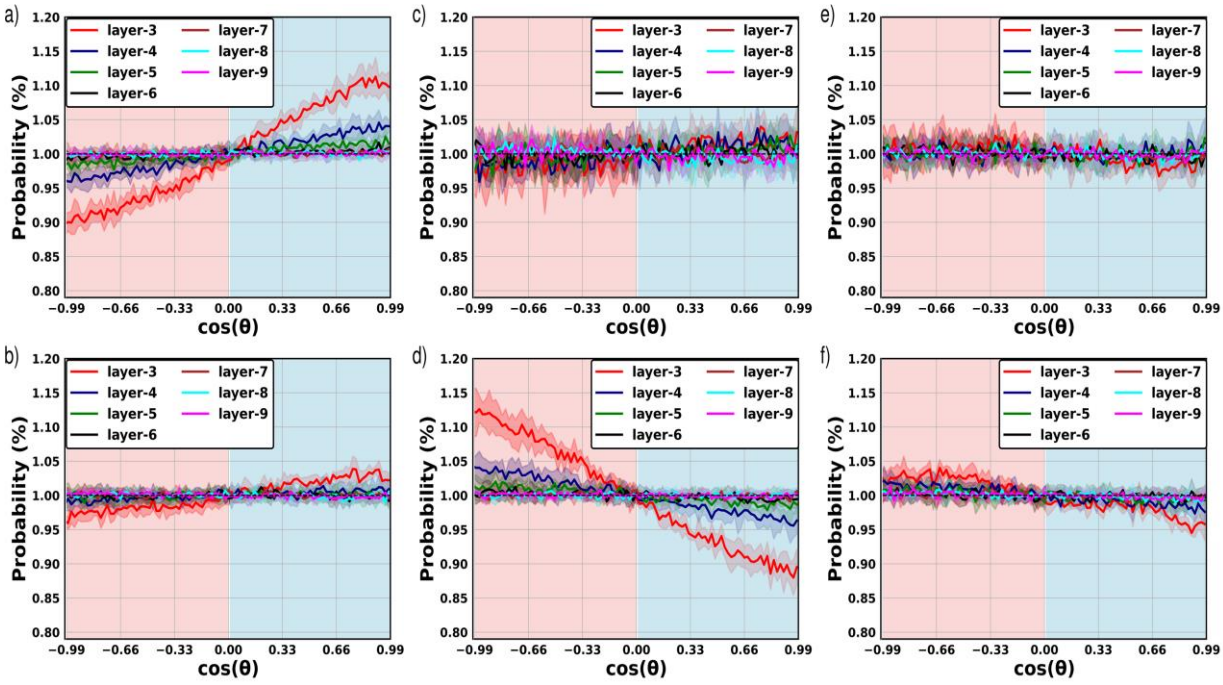

Figure. S4: (a)Normalized distribution of the orientational probabilities of these water molecules in the different hydration shells that choose O (-) atom of Ubiquitin as their nearest neighbour (b), (c), (d), (e), (f) representing the same scenario for the water molecules that choose O(neutral), OH (hydroxyl), N(+), N (neutral)), C(all) as their nearest neighbour respectively in physiological concentration

SR6: A tug-of-war between the atomic influence of two different groups that are members of the N (+) and the total charge of the protein (-8)

Figure. S5 demonstrates the bulk-like behaviour of the water molecules in the 3rd and 4th hydration shell for Lys and Arg respectively and then overall charges takes control and the overall charge effect fades away with increasing the Hydration shell index. In the presence of salt due to screening of total charge of the protein, atomic influence of Lys and Arg persists up to the 3rd and 4th hydration shell respectively.

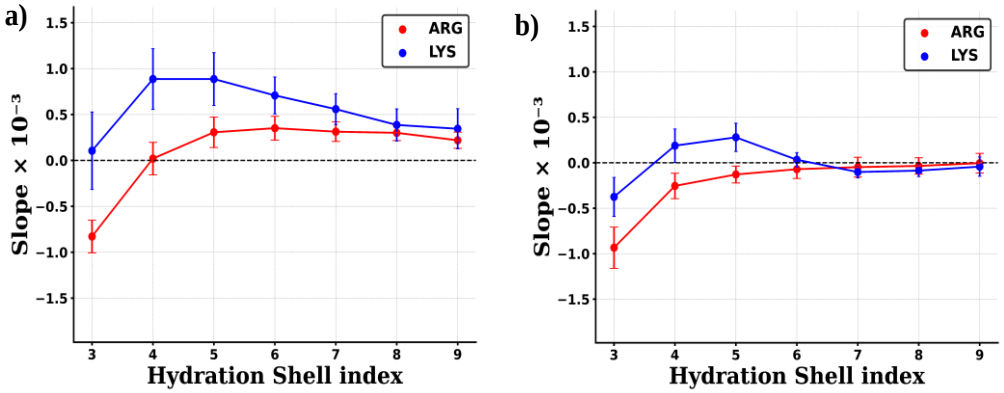

Figure. S5: a) Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to the 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) of these water that choose N (+) of Arg and Lys of a protein with total charge -8 as their nearest neighbours in neutral condition b) Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) of these water that choose N (+) of Arg and Lys of protein with total charge -8 as their nearest neighbours in physiological salt concentration, all the error bars stand for the SDs from 5 parallel 20ns simulation

SR7: A tug-of-war between the atomic influence of two different groups that are members of the O (-) and the total charge of the protein (+8)

In the absence of salt both Asp and Glu exhibit similar behaviour (See Figure S6(a)) as we see for the overall O (-) case (Figure 4(d)). Whereas in the presence of salt, Asp shows the shift in the slope value by holding its influence up to 3rd hydration shell whereas Glu doesn't (Figure S6(b))

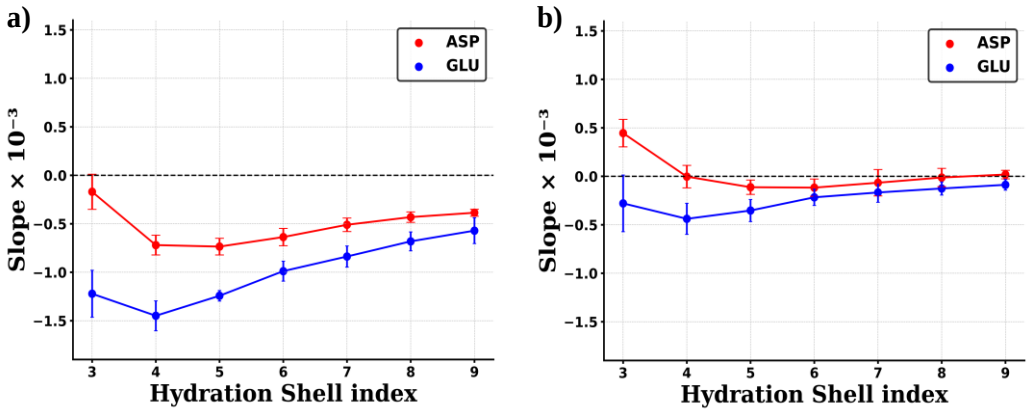

Figure. S6: a) Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) of these water that choose O (-) of Asp and Glu of a protein with total charge +8 as their nearest neighbours in neutral condition b) Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) of these water that choose O (-) of Asp and Glu of a protein with total charge +8 as their nearest neighbours in physiological salt concentration, all the error bars stand for the SDs from 5 parallel 20ns simulation.

SR8: Investigation of the atomic influence of O (-) and N (+) groups in proteins with total charges of -8 and +8 both in neutral and physiological salt concentration

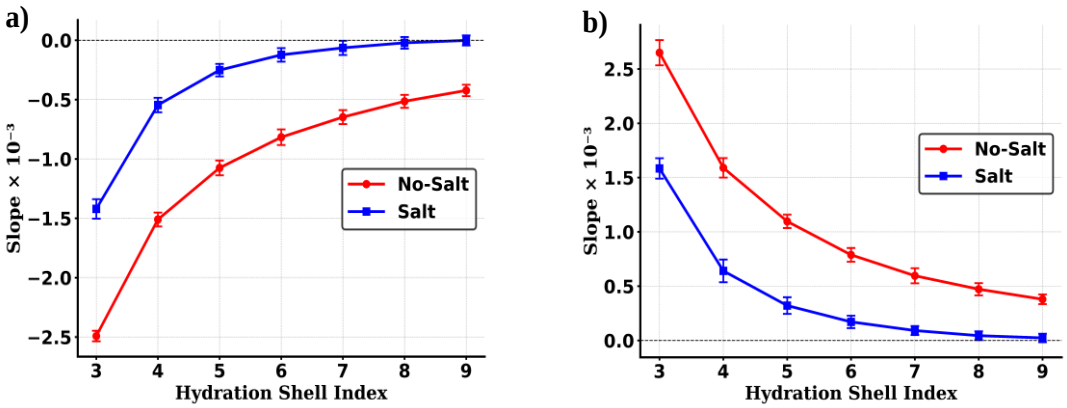

Fig. S7: a) Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) of these water that choose N (+) group of a protein with total charge +8 as their nearest neighbours in neutral condition b) Slope (from the Normalized probability distribution of water orientation cos(θ) in each hydration shell up to 9th hydration shell) vs Hydration Shell index (i.e. Distance from protein surface) of these water that choose O (-) of Asp and Glu of protein with total charge +8 as their nearest neighbours in physiological salt concentration, all the error bars stand for the SDs from 5 parallel 20ns simulation.