Electronic Supplementary Information (ESI)
Ion-specificity and surface water dynamics in protein solutions

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^{1}H NMR relaxometry of water in protein-buffer-salt solutions.

The effect of the increasing salt concentration on the self-diffusion coefficient of water

Figure 1c of the main paper shows the decrease of the self-diffusion coefficient of water, D, upon the increase in the lysozyme (LZM) concentration in acetate-LZM-salt solutions with the concentration of the salt 0.1 mol dm^{-3}. Here, in Figure ESI-1 we show the changes in D upon increasing the concentration of the salt ($NaNO_3$) at a given concentration of the LZM (50 mg mL^{-1}) in acetate buffer (pH = 4.6). We note that the LZM solution experiences a phase transition when the concentration of the $NaNO_3$ exceeds 0.35 mol dm^{-3} (*cf.* Figure 2 of the main paper). In Figure 1 we see that the addition of $NaNO_3$ decreased the self-diffusion coefficient of water compared to the protein-free acetate buffer $NaNO_3$ solution. However, increasing the concentration of the added $NaNO_3$ had a very small influence on the self-diffusion coefficient of water. D was also quite insensitive on the phase transition and remained practically unaltered even after the protein underwent the phase transition.

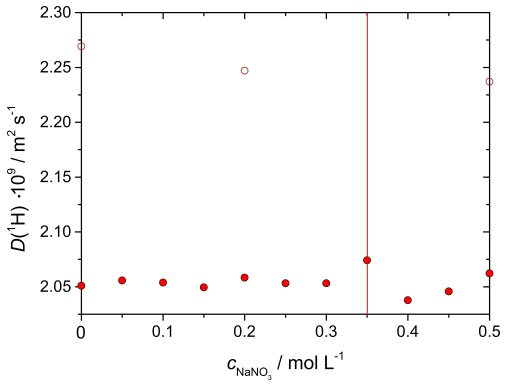

Figure ESI- 1: The self-diffusion coefficient of water, D, as a function of $NaNO_3$ molar concentration in acetate-LZM mixture with LZM concentration 50 mg mL^{-1} and pH = 4.6. Empty symbols apply to aqueous-buffer-$NaNO_3$ solutions without the protein, and filled symbols to the protein-buffer-$NaNO_3$ mixtures. Vertical line denotes the c_{NaNO_3} at which the protein solution undergoes a phase transition. All experiments were performed at T = 25 °C.

Influence of the pH: A comparison between the acetate and phosphate buffer LZM-salt solutions.

In the main paper, results for the water proton longitudinal (spin-lattice) and transversal (spin-spin) relaxation rates, R_1(^{1}H) and R_2(^{1}H), respectively, and the self-diffusion coefficient of water, D (H_2O), were presented for solutions of LZM and bovine serum albumin (BSA) in acetate buffer (pH = 4.6 and 4.0, respectively) in mixture with various low molecular weight salts. Here, we wish to present results for LZM in phosphate buffer (pH = 6.8) and for BSA in HEPES buffer (pH = 7.5).

Since the isoionic points of LZM and BSA are approximately pI = 11.2 and 4.7, respectively, both proteins carry a net positive charge in acetate buffer solutions (pH < pI). The net charge of LZM and BSA was estimated to be approximately $+10e$ under the conditions studied [1, 2]. By increasing the pH of the solution, the net positive charge diminishes, and the protein becomes net negatively charged at pH > pI. To achieve such charge inversion for the LZM, a highly alkaline buffer medium would need to be used, which would denature the protein. Therefore a buffer with somewhat higher pH value from the acetate buffer was instead used, inducing in such a way a decrease in the net positive charge of the LZM. The estimated net positive charge of LZM in phosphate buffer (pH = 6.8) was approximately $+8e$ [1]. In contrast, BSA in HEPES buffer at pH = 7.5 carries a net negative charge, estimated to be approximately $-20e$ [2].

We show here that increasing the solution's pH (different buffer) does not change the order of salt-specific effects seen in R_1, R_2 and D. In the case of LZM solutions, and *inverse* Hofmeister series for the salt anions was obtained also in phosphate buffer, while the order in HEPES-BSA solutions followed the *direct* anion Hofmeister series.

Results of a water proton NMR relaxometry study (R_1, R_2, and D) in aqueous LZM solutions with pH = 6.8 (phosphate buffer) and with added low molecular weight salts (NaCl or NaI) are presented as a function of the LZM mass concentration in Figure ESI-2 (*cf.* Figure 1 in the main paper showing results for LZM in acetate buffer). The concentration dependence of R_1 is shown in panel a, of R_2 in panel b, and of the water self-diffusion coefficient, D, in panel c. In mixtures with added salt the molar concentration of the salt was 0.1 mol dm^{-3}.

Compared to acetate solutions, the values of R_1, R_2, and D at a given LZM concentration were somewhat higher in phosphate buffer. Here, the trend in R_1 was not linear as observed in acetate (Figure 1a of the main paper), and in addition it showed differences with respect to the identity of the added salt: R_1 (without salt) < R_1 (NaCl) < R_1 (NaI). This trend was observed also for R_2 and it was the same as in acetate solutions (*inverse* anion Hofmeister series). The sensitivity of R_2 on the

identity of the added salt was in case of phosphate buffer solutions observed at lower LZM concentrations compared to the acetate cases.

In contrast to the acetate buffer solutions, the self-diffusion coefficient of water in phosphate buffer solutions was lower from the salt-free case both in the case of added NaCl and NaI. At a given LZM concentration the trend was D (without salt) > D (NaI) > D (NaCl). Both kaotropic and cosmotropic salts decreased the self-diffusion coefficient of water compared to the salt-free protein solution, while in the case of the acetate buffer D (NaI) was larger than in the solution without any added salt.

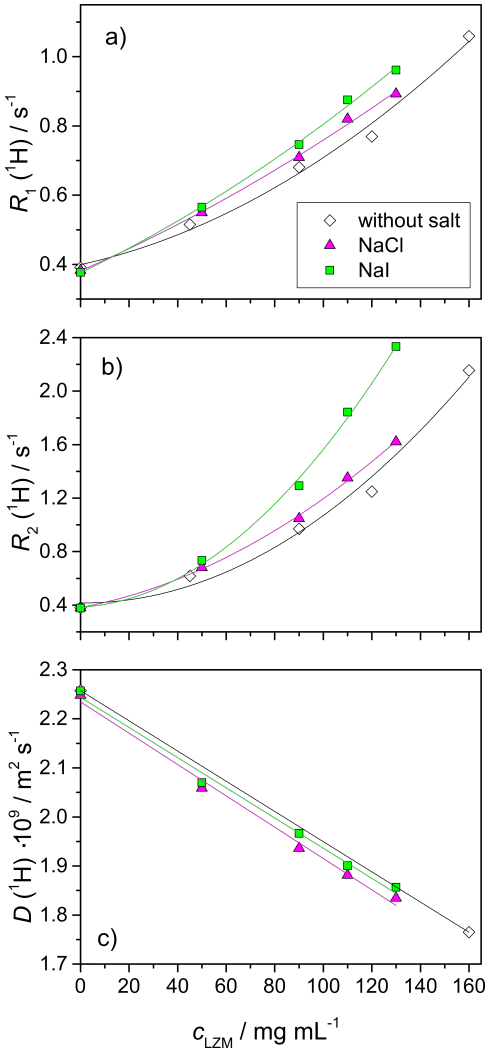

Figure ESI- 2: Dependence of the longitudinal relaxation rate R_1 (panel a), transverse relaxation rate R_2 (panel b), and the self-diffusion coefficient D (panel c) of the water proton as a function of the lysozyme mass concentration, c_{LZM}. All solutions were prepared in phosphate buffer with pH = 6.8. The concentration of the added low molecular weight salt (NaCl or NaI) was 0.1 mol dm^3. All data apply for 25 °C.

In Figure ESI-3 results for R_1 and R_2 in 50 mg mL^{-1} aqueous LZM solutions in phosphate buffer (pH = 6.8), mixed with various salts (NaCl, $NaNO_3$, and NaI), are presented as a function of the increasing salt concentration (*cf.* Figure 2 of the main article). By increasing the salt concentration, the protein solution experienced a phase transition. The order with respect to the salt concentrations needed to achieve the phase transition at 25 °C followed the trend: c (NaCl) > c ($NaNO_3$) > c (NaI). Compared to the acetate buffer solutions, the phase transition in phosphate buffer solutions occurred at lower salt concentrations. Such trend in the stability of the protein solutions was also shown in cloud point temperature measurements [3]. Similar to the acetate buffer solutions, R_1 (panel a) and R_2 (panel b) were practically independent on the increasing salt concentration up to the occurrence of the phase transition (marked with vertical lines in Figure ESI-3), and showed a drastic increase afterwards.

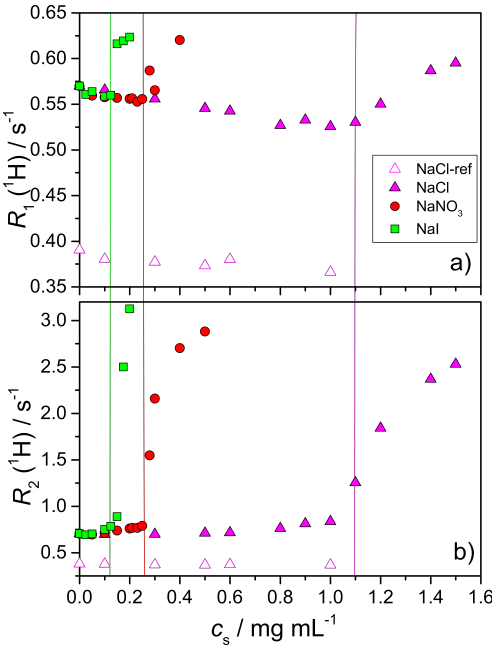

Figure ESI-3: Dependence of the longitudinal relaxation rate R_1 (panel a) and the transverse relaxation rate R_2 (panel b) of the water proton as a function of the low molecular weight salt concentration, c_s. All solutions were prepared in phosphate buffer with pH = 6.8. The mass concentration of the LZM was 50 mg mL^{-1}. Various salts were tested: NaCl, $NaNO_3$ and NaI. Empty symbols apply to aqueous-buffer-NaCl solutions without the protein, and filled symbols to the protein-buffer-salt mixtures. Vertical lines denote the c_s at which the protein solution undergoes a phase transition. All data were performed at T = 25 °C.

Influence of the pH: A comparison between acetate and HEPES buffer BSA-salt solutions

In Figure ESI-4 results for BSA-HEPES-salt mixtures are represented at pH = 7.5 and T = 25 °C. At this pH value BSA carries a net negative charge. Dependence of R_1 and R_2 as a function of the BSA concentration are shown in panels a and b, respectively.

The comparison in magnitude of R_1 and R_2 between solutions in acetate and HEPES buffers shows no significant differences (except for R_1 of the HEPES-NaCl-BSA solution). The longitudinal relaxation rate R_1 is here more sensitive to the chemical nature of the added low molecular weight salt than in the acetate buffer solutions (see Figure 3a of the main paper). Solutions with NaCl show higher R_1 compared to other

cases. At a given (high enough) BSA concentration the order with respect to the added salt is: R_1 (NaCl) > R_1 (NaI) > R_1 (without salt) > R_1 ($NaNO_3$). However, the differences between the salt free solution and solutions containing NaI or $NaNO_3$ are small.

The same salt-specific trend in R_2 was observed for BSA in acetate buffer (pH = 4.0, see Figure 3 of the main article) and HEPES buffers (direct anion Hofmesiter series): R_1 (NaI) < R_1 ($NaNO_3$) < R_1 ($NaNO_3$) < R_1 (without salt). The order of the salt-specific effect is in the case of BSA different from the effect in LZM solutions, as already discussed in the main article.

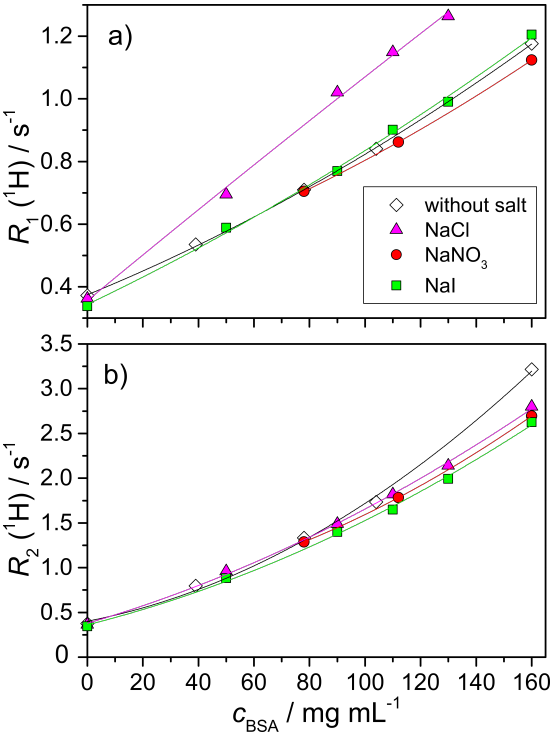

Figure ESI-4: Dependence of the longitudinal relaxation rate R_1 (panel a) and the transverse relaxation rate R_2 (panel b) of the water proton as a function of BSA concentration. All solutions were prepared in HEPES buffer with pH = 7.5. The concentration of the added low molecular weight salt (NaCl, $NaNO_3$, or NaI) was 0.1 mol dm^3. All experiments were performed at T = 25 °C.

NMR relaxation of ^{14}N

In Figure ESI-5 we present dependence of R_2 (^{14}N) on $NaNO_3$ concentration, while concentration of BSA was constant, 50 mg mL^{-1}, and pH value was 4.0 (acetate buffer). In the main paper similar dependence was presented in Figure 5 for ^{35}Cl. R_2 decreases with increasing $NaNO_3$ concentration while R_2 of reference ($NaNO_3$ in buffer without BSA) is not dependent on $NaNO_3$ concentration in this concentration range (from approximately 0.2 to 1.3 mol dm^{-3}). In other words, when we add $NaNO_3$ in BSA-buffer solution the fraction of free salt becomes higher (R_2 decreases), because we are converging to the saturation of ion binding on protein surface.

In Figure ESI-6 dependence of R_2 (^{14}N) on BSA concentration is presented, where $NaNO_3$ concentration is constant and buffer is the same as in upper case. This dependence is linear. That was already noticed from R_2 (^{35}Cl) results presented in the main article (*cf.* Figure 6). R_2 (^{14}N) increases with BSA concentration and the reason of this increase can be explained with same arguments as to explain results in shown Figure ESI-5.

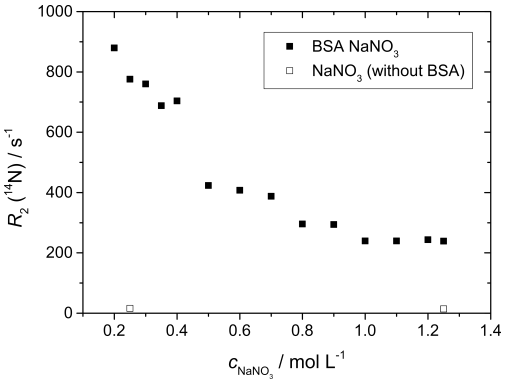

Figure ESI-5: Transverse relaxation rate, R_2, of ^{14}N in mixture of 50 mg mL^{-1} BSA and variable $NaNO_3$ concentration in acetate buffer (pH = 4.0) at T = 25 °C. Filled symbols present samples with BSA, while empty ones present samples with no protein added.

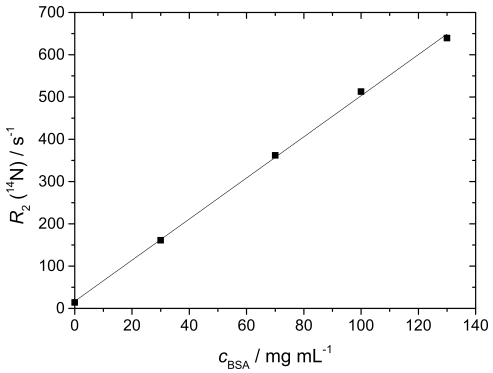

Figure ESI-6: R_2 (^{14}N) dependence on BSA concentration in acetate buffer (pH = 4.0) and at T = 25 °C. $NaNO_3$ concentration was 1.25 mol dm^{-3}.

References

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[3] Janc, T.; Kastelic, M.; Bončina, M.; Vlachy, V. Salt-specific effects in lysozyme solutions. *CondenS. Matter Phys.* **2016**, *19*, 23601: 1–12.