Electronic Supplementary Information (ESI)

Ion-specificity and surface water dynamics in protein solutions

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1H 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 ($p$H = 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 acetic acid-LZM mixture with LZM concentration 50 mg mL$^{-1}$ and $p$H = 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 $c_{\text{NaNO}_3}$, at which the protein solution undergoes a phase transition. All experiments were performed at $T = 25$ $^\circ$C.](image)

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, $R_1$(1H) and $R_2$(1H), respectively, and the self-diffusion coefficient of water, $D$ (H$_2$O), were presented for solutions of LZM and bovine serum albumin (BSA) in acetate buffer ($p$H = 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 ($p$H = 6.8) and for BSA in HEPES buffer ($p$H = 7.5).

Since the isionic points of LZM and BSA are approximately $p$I = 11.2 and 4.7, respectively, both proteins carry a net positive charge in acetate buffer solutions ($p$I < $p$I). The net charge of LZM and BSA was estimated to be approximately +10e under the conditions studied [1, 2]. By increasing the $p$I of the solution, the net positive charge diminishes, and the protein becomes net negatively charged at $p$I > $p$I. 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 $p$I 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 ($p$H = 6.8) was approximately +8e [1]. In contrast, BSA in HEPES buffer at $p$I = 7.5 carries a net negative charge, estimated to be approximately −20e [2].

We show here that increasing the solution’s $p$I (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 $p$I = 6.8 (phosphate buffer) and with added low molecular weight salts (NaCl or Na$_2$SO$_4$) 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) < $R_1$ (Na$_2$SO$_4$). 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.

In Figure ESI-2 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 $25 \degree C$ 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.

In Figure ESI-3 results for BSA-HEPES-salt mixtures are represented at $pH = 7.5$ and $T = 25 \degree C$. 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 buffer 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 Hofmeister 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.

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.

**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.

![Figure ESI-4](image-url)

**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 $T = 25^\circ$C.

![Figure ESI-5](image-url)

**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 $T = 25^\circ$C. Filled symbols present samples with BSA, while empty ones present samples with no protein added.

![Figure ESI-6](image-url)

**Figure ESI-6**: $R_2$ ($^{14}$N) dependence on BSA concentration in acetate buffer ($pH = 4.0$) and at $T = 25^\circ$C. NaNO$_3$ concentration was 1.25 mol dm$^{-3}$.

**References**

