

## Electronic Supplementary Information

# The Molecular Motion of Bovine Serum Albumin in Physiological Conditions is Ion Specific

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### Experimental section

*Chemicals.* Bovine Serum Albumin (A7906) was purchased from Sigma-Aldrich and used without further purification. Sodium chloride (> 98%), lithium chloride (99%), cesium chloride (99.8%), potassium chloride (99%), rubidium chloride (>99%), sodium fluoride ( $\geq 99\%$ ), Sodium Bromide (98%), sodium iodide ( $\geq 99.5\%$ ), sodium thiocyanate ( $\geq 98\%$ ), sodium dihydrogen phosphate (98%) and sodium hydrogen phosphate (99%) were purchased from Sigma-Aldrich (Milan, Italy). Standard buffers at pH 4, and 7 were purchased from Hanna Instruments (Szeged, Hungary).

*Sample Preparation.* All salts were dried overnight at 110°C, cooled at room temperature in a desiccator and dissolved into purified water (conductivity  $\leq 0.054 \mu\text{S cm}^{-1}$ ), prepared by means of a Millipore water purification system. Concentrated stock solutions of salts (2 M) were prepared in 10 mM phosphate buffer at pH 7 in order to set the same pH value for all solutions. BSA sample solutions (5, 10, 20, 30, 40 mg/mL) were prepared by dissolving a weighed amount of protein in phosphate buffer solution (10 mM, pH=7) and adding an appropriate volume of salt stock solution to reach the final salt concentration (0, 50, 100, 300 mM).

*Measurement of the Diffusion coefficient ( $D_c$ ) by means of Dynamic Light Scattering (DLS).* Dynamic light scattering (DLS) measurements were carried out through a Zetasizer nano ZSP (Malvern Instruments) and the acquired data were elaborated with the Zetasizer software version 7.03. DLS technique measures the intensity fluctuations of the light scattered by particles subject to Brownian motion. The intensity of the fluctuations is time-dependent and can be related to the diffusion coefficient  $D_c$  of the particles by means of a correlation function  $g(\tau)$  according to the equation:

$$g(\tau) = A \left[ 1 + B \exp(-2D_c q^2 \tau) \right] \quad (\text{S1})$$

where  $\tau$  is the sample time,  $A$  and  $B$  are respectively the background term and the intercept of the correlation function, and  $q$  is the scattering vector which is given by:

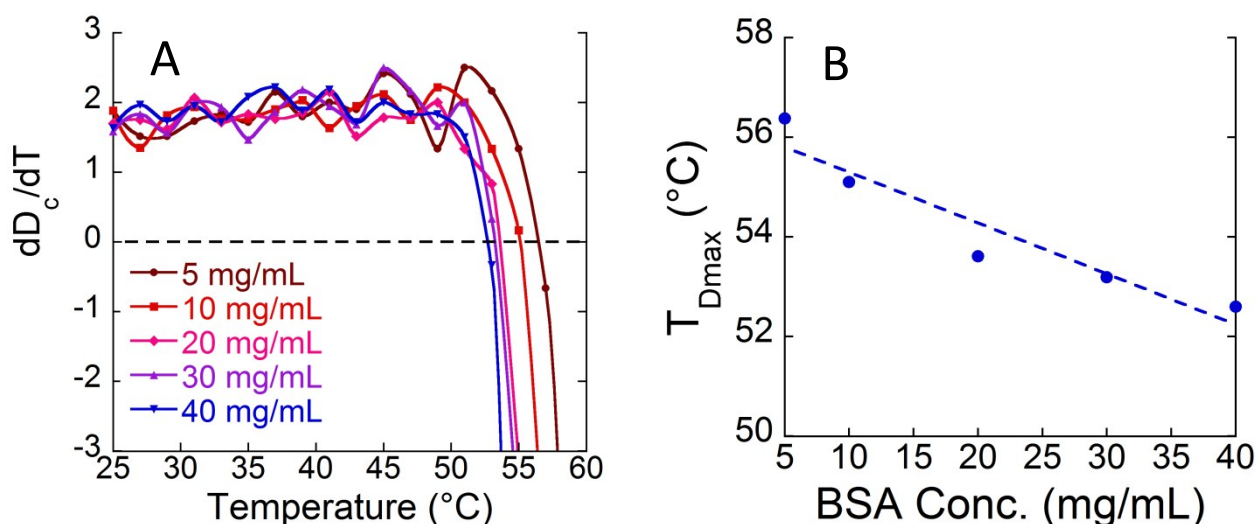
$$q = \left[ \frac{4\pi n}{\lambda_0} \sin\left(\frac{\theta}{2}\right) \right] \quad (\text{S2})$$

where  $n$  is the refractive index of the medium,  $\lambda_0$  is the wavelength of laser, and  $\theta$  is the scattering angle.

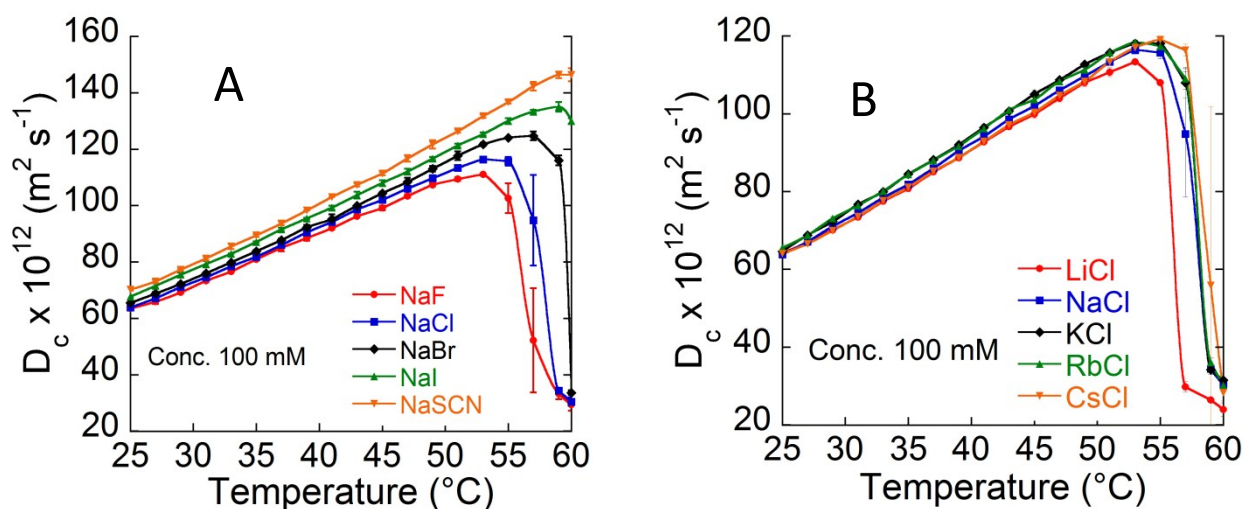
Before to carry out the DLS measurements the BSA/salt solutions were filtered by means of disposable filters (Supelco Iso-disc Nylon 25 mm  $\times$  0.2  $\mu\text{m}$ ) to avoid possible interferences caused by dust impurities.  $D_c$  values were obtained as a function of temperature in the range 25 - 60 °C by increasing the temperature with steps of  $2.0 \pm 0.1$  °C, each step with an equilibration time of 120 s.

All samples were prepared at least in triplicate and at least three different  $D_c$  vs  $T$  curves were obtained for each sample.

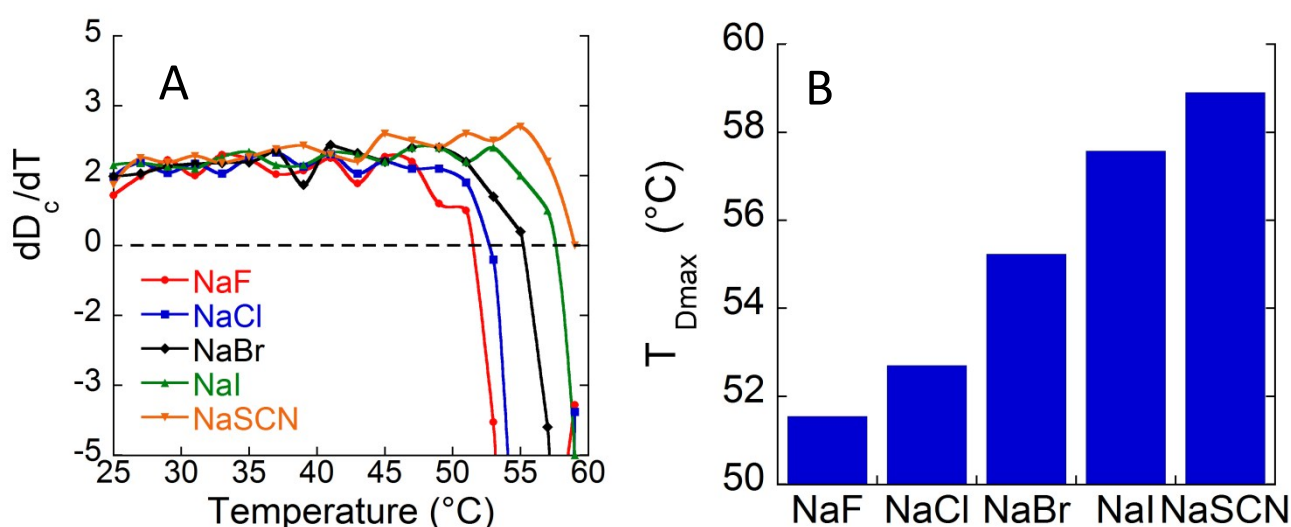
*Determination of the temperature ( $T_{D_{max}}$ ) corresponding at the maximal value of the diffusion coefficient.* Figs 1-3 in the main text show the variation of the diffusion coefficient as a function of temperature.  $D_c$  increases up to a maximal value ( $D_{max}$ ) and then decreases to very low values likely due to protein aggregation.  $T_{D_{max}}$  is defined as the temperature at which  $D_{max}$  is obtained. In order to accurately obtain the values of  $T_{D_{max}}$ , the first derivative of  $D_c$  respect to temperature ( $dD_c/dT$ ) was plotted as a function of temperature (Fig. S1A).  $T_{D_{max}}$  is the temperature at which  $dD_c/dT = 0$ . We experimentally observe that the value of  $T_{D_{max}}$  decreases by increasing BSA concentration (Fig. S1B). A plot of  $dD_c/dT$  versus temperature obtained for BSA samples in the presence of different sodium salts (Fig. S3A) shows that  $T_{D_{max}}$  is anion specific and decreases along the series  $SCN^- > I^- > Br^- > Cl^- > F^-$ . (Fig. S3B).



**Fig. S1** Effect of BSA concentration on  $T_{D_{max}}$  values (see also Figure 1 in the main text). (A) Procedure for the accurate determination of  $T_{D_{max}}$ ; the first derivative of  $D_c$  respect to temperature ( $dD_c/dT$ ) is plotted as a function of temperature.  $T_{D_{max}}$  is the temperature at which  $dD_c/dT = 0$ . (B) Variation of  $T_{D_{max}}$  due to the increase of BSA concentration (0.1 M NaCl; pH = 7).



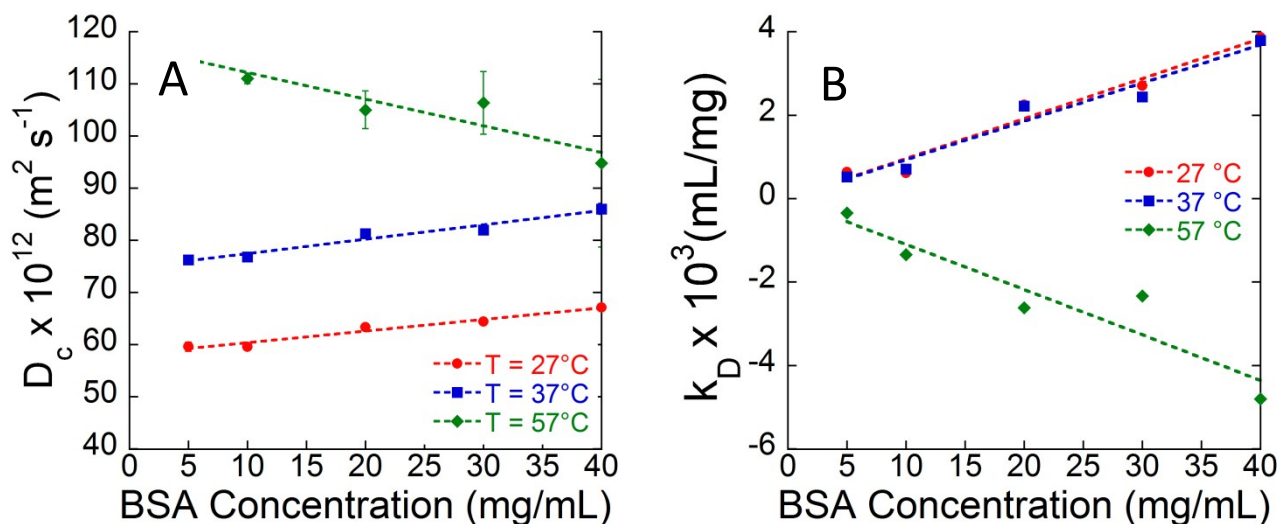
**Fig. S2** Specific ion effects on the diffusion coefficient of BSA (40 mg/mL) in 10 mM phosphate buffer solutions at pH 7, and 100 mM salt concentration. (A) Effect of anions; (B) Effect of cations.



**Fig. S3** Specific anion effects of  $T_{D_{max}}$ . (A) Plot of the first derivative of the  $D_c$  respect to temperature ( $dD_c/dT$ ) for different 0.1 M sodium salts. (B)  $T_{D_{max}}$  follows a clear Hofmeister series for anions.

*Determination of the interaction parameter  $k_D$ .* The interaction parameter  $k_D$  is determined by measuring the diffusion coefficient  $D_c$  at different concentrations of BSA and at a fixed temperature. These data, as shown in Fig. S4A, follow a linear trend according with eq. 1 (see main text). The intercept of the straight lines with y axis is  $D_0$ , that is, the value of the diffusion coefficient for  $c_{BSA} \rightarrow 0$  at a certain temperature (Fig. S4A shows the trends at 3 different temperatures). The interaction parameter  $k_D$  is then calculated by using the suitable values of  $D_c$ ,  $D_0$

and  $c_{protein}$  in eq. 1. Similarly to the second-virial coefficient ( $B_{22}$ ) obtained by static light scattering (SLS) measurements,  $k_D$  can be used to evaluate the stability of protein in solutions.<sup>[1–3]</sup> Positive/negative values of  $k_D$  are associated with a stable/unstable protein solution. Fig. S4B shows that, at a certain protein concentration, the stability is affected by the temperature, since  $k_D$  is positive at low temperatures (i.e. 27°C and 37°C) and negative at high temperatures (i.e. 57°C).



**Figure S4.** Procedure for the calculation of the interaction parameter  $k_D$  according to eq. 1 ( $D_c = D_0[1 + k_D c_{protein}]$ ). (A)  $D_0$  is the value of  $D_c$  for  $c_{protein} \rightarrow 0$  at a fixed temperature (i.e. 27°C; 37°C; or 57°C). (B) The value of  $k_D$  gives a clear sign of the stability of the protein solution. At a fixed protein concentration  $k_D$  is positive (protein solution is stable) at low temperatures and negative (protein solution is unstable) at higher temperatures.

## References

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