Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting information for:

Trivalent cation-induced phase separation in proteins: ion specific contribution in hydration also counts

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Zeta-potential (ζ) measurements

Zeta-potential (ζ) measurements by Nano S Malvern instrument are mainly based on the basic mean-field approach of the Poisson-Boltzmann (PB) theory. The ion distribution around the dispersed charged object creates an electrostatic double layer (EDL), which is a reason for charge screening in electrolyte solutions, over the Debye screening length.

The Debye Huckel parameter (κ) in m^{-1} is first calculated by,[1]

$$\kappa = \sqrt{4\pi\lambda_B N_A \sum_i n_i Z_i^2} \tag{1}$$

with the Bjerrum length[2] in m,

$$\lambda_B = \frac{e^2}{4\pi\varepsilon_0\varepsilon_{H_2O}k_BT} \tag{2}$$

Here, i-th number of ionic species are accounted with Z_i , valences and n_i, number concentration. e is the elementary charge, k_BT denotes the product of Boltzmann constant and absolute temperature. ε_0 and ε_{H_2O} are the vacuum dielectric permittivity and the total dielectric permittivity of water at the respective temperature.[3] The value of $\varepsilon_0$and ε_{H_2O} are taken $8.85 \times 10^{-12} C^2 N^{-1} m^{-2}$ and 80, respectively.

Electrophoretic mobility μ of the charged colloidal particle with spherical in shape towards the oppositely charged electrodes under an external electric field relates to the measured ZP ζ by Henry's equation,[4]

$$\mu = \frac{2\varepsilon_r\varepsilon_0}{3\ \eta}\zeta f(\kappa a) \tag{3}$$

Where η is the viscosity of the medium and a is the radius of the spherical particle.

Here we have measured the ZP of BSA with a value of 3.3 nm. The default Henry function $f(\kappa a) = 1.5$ used in Zetasizer settings, which does not show the distinct effect on the investigated systems. Therefore, $f(\kappa a)$can be calculated as given by Ohshima,[5]

$$f(\kappa a) = 1 + \frac{1}{2}\left[1 + \left(\frac{2.5}{\kappa a[1 + 2exp(-\kappa a)]}\right)\right]^{-3} \quad (4)$$

To get the corrected ZP (ζ_{corr}), ζ for each system is multiplied by its corresponding calculated $f(\kappa a)$ value. Then ζ_{corr} is multiplying with $\frac{e}{k_B T}$ to rescale it as to ζ^* .[6]

The surface charge density of BSA protein, $\sigma\ (C/m^2)$, is obtained by,[7]

$$\sigma = \frac{\varepsilon_0 \varepsilon_{H_2O} \kappa k_B T}{e}\left(2sinh\left(\frac{\zeta^*}{2}\right) + \left(\frac{4}{(\kappa a)}tanh\left(\frac{\zeta^*}{4}\right)\right)\right) \quad (5)$$

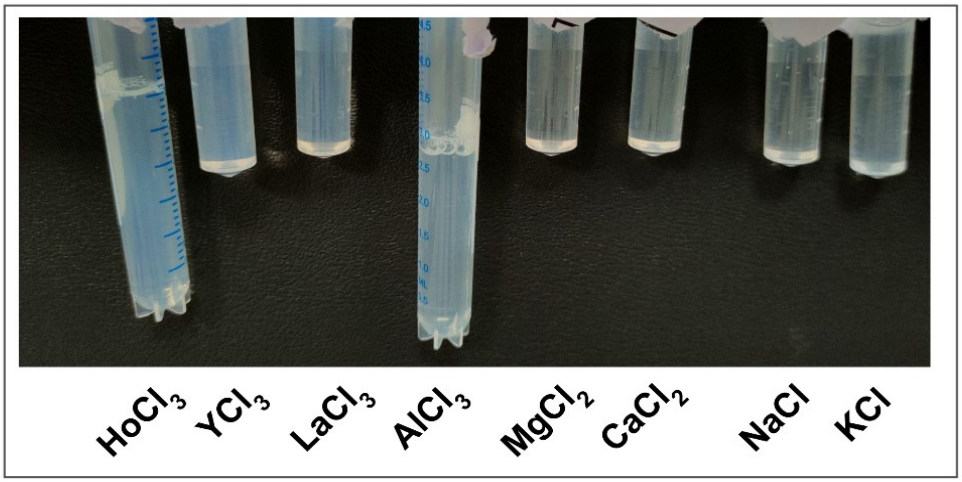

Figure S1. Images of different phase of samples, containing 40 mg ml^{-1} (0.6mM) of BSA and Chloride salts with different valences. In every sample, the protein concentration (C_p) is 0.6mM and the salt concentration (C_s) is 14mM to maintain the C_s : C_p ratio of 23.33. Images show that the protein solutions get turbid in presence of trivalent salts compared to mono and divalent salts at room temperature.

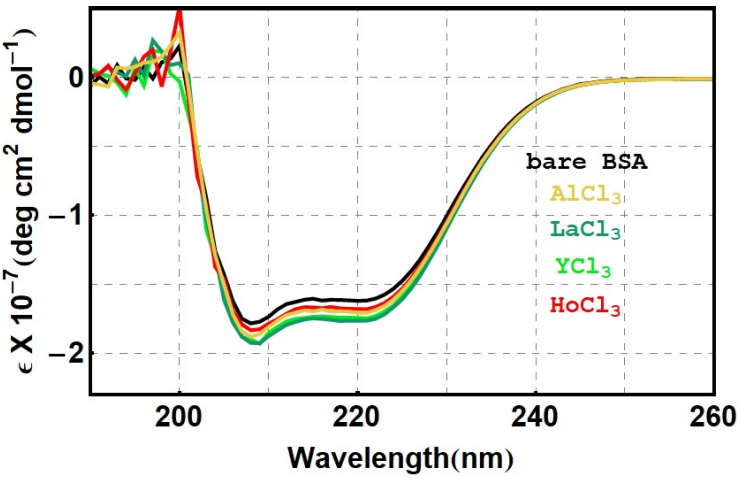

Figure S2. Representative CD signals (analysed in molar ellipticity, ε) of bare BSA protein, and in presence of the mentioned trivalent salts.

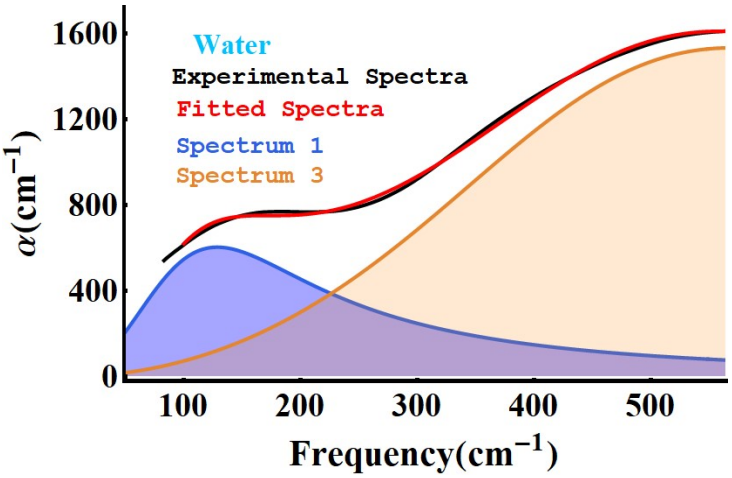

Figure S3. Deconvoluted frequency dependent absorption coefficient, α(ν) spectra of bulk water. Two damped modes are observed: HB stretching mode (~129 cm^{-1}), and librational mode (~569 cm^{-1})

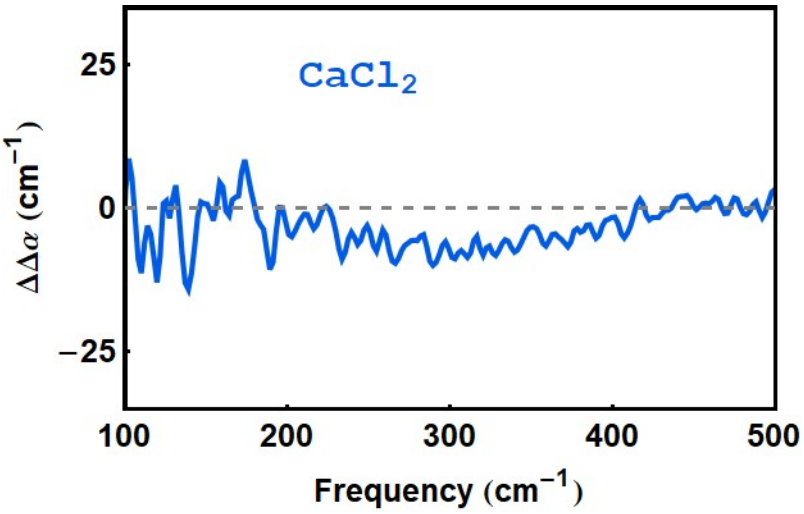

Figure S4. Frequency dependent change in difference absorption coefficients $\Delta\Delta\alpha(\nu)$ (= $\Delta\alpha_{p+s}(\nu)$- $\Delta\alpha_{p}(\nu)$) of $CaCl_2$.

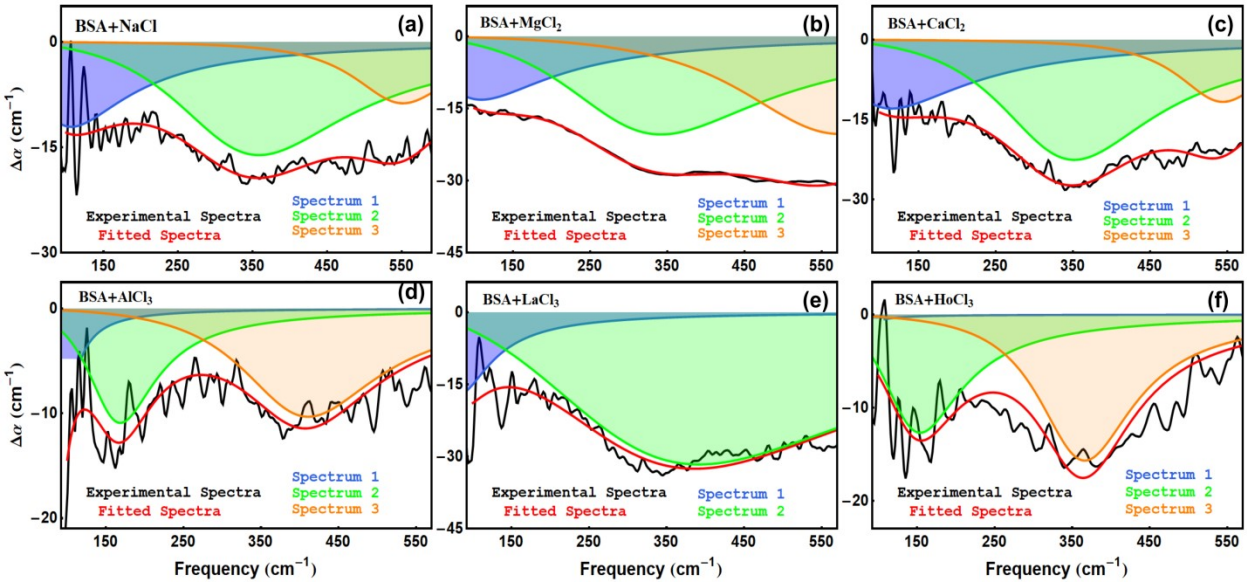

Figure S5. Dissection of experimental Δα(ν) spectra fitted using a damped harmonic oscillator model for (a) BSA-NaCl, (b) BSA-$MgCl_2$, (c) BSA- $CaCl_2$, (d) BSA- $AlCl_3$, (e) BSA-$LaCl_3$, and (f) BSA-$HoCl_3$ solutions. The black solid lines represent experimental data and the red lines stand for the overall fit. Three damped modes are observed: HB stretching mode (spectrum 1), rattling motion of ions (spectrum 2), and librational mode (spectrum 3).

Table S1. The listed values of the fitted parameters; amplitude (a_0), damping width (ω_0), damped frequency (ν_d), and unperturbed center frequency (ν_c) : Respective values for hydrogen bond stretching mode, for rattling motions of ions, and for water librational motion of each protein-salt system and only aqueous solution of BSA protein.

Salt	a_0 ($cm^{-1}dm^3mol^{-1}$)	ω_0 (cm^{-1})	ν_d (cm^{-1})	ν_c (cm^{-1})
HB stretch				
0	-83811.5	444.6(57)	16.7(55)	72.7(56)
NaCl	-77012.7	507.8(111)	69.8(17)	106.8(24)
KCl	-127663.8	720(100)	82(14)	141(22)
$MgCl_2$	-98570	596.5(60)	53.3(17)	108.9(20)
$CaCl_2$	-104690.1	645(132)	51.3(32)	114.8(39)
$AlCl_3$	-27390.8	94.8(12)	82.2(1)	83.5(2)
$LaCl_3$	-58199.7	272.4(36)	70.2(5)	82.5(7)
YCl_3	-21539.6	90.8(15)	91.2(2)	92.4(3)
$HoCl_3$	-19243.8	28.1(4)	52.5(0.4)	52.7(0.8)
Ion rattling				
0	-	-	-	-
NaCl	-182736.1	903.9(183)	328.5(10)	358.6(31)
KCl	-197652.5	942(154)	374(7)	403(26)
$MgCl_2$	-257425.9	1005.5(53)	302.1(4)	341.9(9)
$CaCl_2$	-220001.4	776.6(86)	329.4(6)	351.8(15)
$AlCl_3$	-45848.9	334.4(56)	159.8(6)	168.5(11)
$LaCl_3$	-668883.5	1683(55)	286(11)	391.8(14)
YCl_3	-730030.6	1871.7(72)	244(15)	385 (19)
$HoCl_3$	-59994.3	376.2(71)	142.5(7)	154.5(13)
Water libration				
0	-446439.3	1827.9(100)	374.3(16)	474.1(23)
NaCl	-56641.7	517.1(140)	545.6(10)	551.7(25)
KCl	-68296	541.8(99)	571.3(7)	577.7(17)
$MgCl_2$	-263277.8	1033(27)	550(3)	574.1(5)
$CaCl_2$	-66210.2	452(64)	539.6(5)	544.3(11)
$AlCl_3$	-88089	678.8(65)	396.5(8)	410.9(13)
$LaCl_3$	-	-	-	-
YCl_3	-	-	-	-
$HoCl_3$	-93958.5	474.2(52)	358(6)	365.8(10)

Table S2.

System	$\Delta\Delta\alpha(\nu)$	ΔS_{hyd}	Hydration geometry	Remarks
Ho^{3+}	almost zero or nearly positive	positive	square antiprismatic (8 co-ordinated)	Red shift of the ion rattling mode Weak hydration water exchange
Al^{3+}	Distinctly positive	positive	octahedrally co-ordinated	Red shift of the ion rattling mode. Major desolvation of ocatahedrally co-ordinated ion

References

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