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ARTICLE TYPE

Supplementary Information for: Tuning phase transitions of aqueous protein solutions by multivalent cations[†]

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1 Calculation of effective protein surface charge, $Q(e)$, from ζ potentials

From the ζ potential data obtained from the measurements, the effective protein charge Q was calculated as follows.

First, the Debye screening length, κ^2 was calculated as[1]

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

with the Bjerrum length λ_B[2]

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

with e being the elementary charge, ε_0 the vacuum dielectric permittivity and ε_{H_2O} the total dielectric permittivity of water at the respective temperature[3].

The Henry equation relates the electrophoretic mobility μ of a colloidal particle with a spherical shape, a radius a and a zeta potential ζ in a medium with a viscosity η via[4,5]

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

Here, the Henry function $f(\kappa a)$ as given by Ohshima[5]

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

is used to relate the electrophoretic mobility. The ζ potential is then calculated with the BSA radius a=3.3 nm. The default Zetasizer setting uses a Henry function $f(\kappa a) = 1.5$, which does not consider effects specific to the systems investigated here. Therefore, corrected ζ values ζ_{corr} are obtained by multiplying each ζ potential value by its corresponding $f(\kappa a)$ value. The ζ_{corr} values are furthermore rescaled by multiplying them by e/k_BT. The rescaled values are referred to as ζ^*.

The protein surface charge density, σ (C/m^2), is given by[6]

$$\sigma = \frac{\varepsilon_0\varepsilon_r\kappa k_BT}{e}(2\sinh(\zeta^*/2) + (4/(\kappa a)\tanh(\zeta^*/4))) \tag{5}$$

Finally, the surface charge Q of BSA under the respective sample conditions given by salt concentration and temperature are calculated as

$$Q = 4\pi a^2\sigma \tag{6}$$

2 Fitting model for $Q(e)$ as a function of temperature and salt concentration

We begin with a standard Langmuir-like equation describing the binding of cations to the protein surface:

$$Q = Q_0 + \frac{N\nu c}{c + K} \tag{7}$$

which can be converted into its alternative form

$$N\nu c + K(Q_0 - Q) + c(Q_0 - Q) = 0 \tag{8}$$

Here, Q_0 is the initial protein surface charge in the absence of salt, N is the number of binding sites, ν the valence of the salt (here, it is always $+3$), K the binding constant and c the salt concentration.

Next, we define the point of zero charge:

$$Q(c_0) = 0 \tag{9}$$

and the charge at $c_s = c_1 = 1$ mM (plateau value) as

$$Q(c_1) = Q_1 \tag{10}$$

Substituting expressions 9 and 10 into Eqn. 8, we obtain a system of linear equations:

$$N\nu c_0 + KQ_0 + cQ_0 = 0 \tag{11}$$

$$N\nu c_1 + K(Q_0 - Q_1) + c_1(Q_0 - Q_1) = 0 \tag{12}$$

which allows us to express N and K from Eqn. (7) as

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$$\begin{pmatrix} \nu c_0 & Q_0 \\ \nu c_1 & Q_0 - Q_1 \end{pmatrix} \begin{pmatrix} N \\ K \end{pmatrix} = \begin{pmatrix} -c_0 Q_0 \\ -c_1(Q_0 - Q_1) \end{pmatrix} \qquad (13)$$

Solving this system of equations yields the following expressions for K and N:

$$N = \frac{1}{\nu} \frac{Q_0(Q_0 - Q_1)(c_1 - c_0)}{c_0(Q_0 - Q_1) - c_1 Q_0} \qquad (14)$$

$$K = \frac{c_0 c_1 Q_1}{c_0(Q_0 - Q_1) - c_1 Q_0} \qquad (15)$$

and, finally, the equation used to fit the temperature- and salt concentration-dependent Q values:

$$Q = Q_0 \left(1 + \frac{c(Q_0 - Q_1)(c_1 - c_0)}{c(c_0(Q_0 - Q_1) - c_1 Q_0) + c_0 c_1 Q_1} \right) \qquad (16)$$

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