SUPPORTING INFORMATION

Modulating of electrostatic potential around α-lactalbumin using oligoelectrolyte chains, pH and salt concentration

Paola Torres[1], Sofia Baldor[2], Evelina Quiroga[3], Antonio Jose Ramirez-Pastor[3], Dario Spelzini[2], Valeria Boeris[2] and Claudio Narambuena[1].

[1] Grupo Bionanotecnología y Sistemas Complejos. (UTN-CONICET), Facultad Regional San Rafael, Argentina. Av. General Urquiza 314 C.P.:5600, San Rafael, Mendoza, Argentina.

[2] Facultad de Ciencias Bioquímicas y Farmacéuticas, Área Fisicoquímica, Universidad Nacional de Rosario, Rosario C.P: 2000, Argentina.

[3] Instituto de Fisica Aplicada (INFAP), Universidad Nacional de San Luis – CONICET, San Luis C.P: 5700, Argentina.

S1. Additional computational details

S1.1. Coarse-grained model details

Figure S1 shows a sequence of three aminoacids GLU-LYS-LEU from α-lactoalbumin. Figure S1A depicts the model obtain from the PDB structure corresponding to the protein (1F6S), the alpha carbon (CA) is shown as yellow beads, the carbon from the peptide bond is shown in gold and then oxygen, nitrogen and other carbons are depicted as red, blue and aquamarine beads.

Figure S1B shows the coarse-grained model used to represent the protein in the simulation. In this model, the alpha carbon (yellow bead) has the same position than in the PDB structure. The side chain position is calculated computing the center of mass of all the atoms involve and the radius corresponds to its radius of gyration.

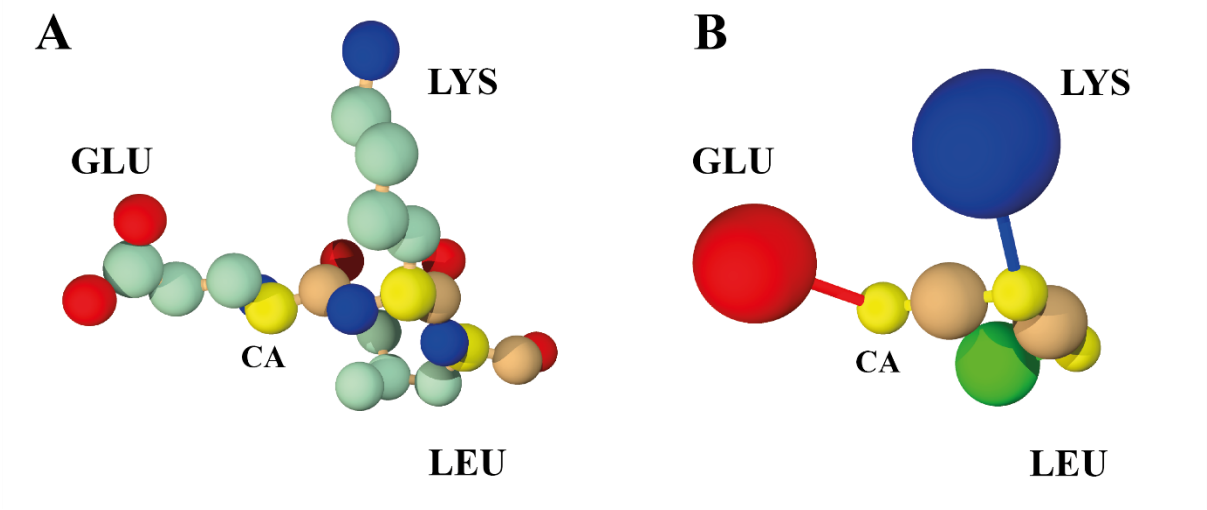

Figure S1. Model representation of a three aminoacid GLU-LYS-LEU sequence from α-lactalbumin (A) Model representation of the structure obtained from the PDB and (B) Coarse-grained model.

S1.2. Interaction energy

The total energy of the system is given by pair-wise electrostatic interactions:

$$\frac{U}{k_bT} = \sum_{i=1}^{N} \sum_{j>i}^{N-1} u_{ij}(r_{ij})$$

(S1)

where N is the total number of particles: $N = N_{ALac} + N_{SC} + N_{SA} + N_{PE}$; N_{ALac} is the number of protein beads, N_{PE} is the number of monomer of the polyelectrolyte and N_{SC} and N_{SA} the number of small cations and anions, respectively. The interaction between two particles is given by the Coulomb potential corrected with a hard sphere core:

$$u_{ij}(r_{ij}) = l_b \frac{z_i z_j}{r_{ij}} \text{ if } r_{ij} > rad_i + rad_j$$

$$u_{ij}(r_{ij}) = \infty \text{ if } r_{ij} \leq rad_i + rad_j$$

(S2)

where $r_{ij} = |r_i - r_j|$ is the distance between particles *i* and *j*, $l_B = e^2/4\pi\varepsilon_r\varepsilon_0 k_b T$ is the Bjerrum length, k_bT is the thermal energy and $\varepsilon = \varepsilon_r\varepsilon_0$ is the dielectric constant of the solvent. The charge of a particle q_i can be calculated as the product of the elementary charge (e) times its valence (z_i), i.e. $q_i = ez_i$. We consider $l_B \approx 0.71$ nm corresponding to water at room temperature as solvent.

Ewald sums were used to calculate the electrostatic energy.

S.1.3. Monte Carlo simulation

S1.3.1 General description

The Monte Carlo (MC) simulations were performed using the Metropolis algorithm[1,2]. In each MC simulation, we performed 2×10^6 MC steps, where the first 10^6 steps were discarded as part of the equilibration and the remaining 10^6 MC steps were used to calculate the ensemble averages. To sample the system, we used three types of trial moves: i) translational motion of small ions, ii) protonation/deprotonation of the acidic and basic groups of the protein, and iii) creation/destruction of neutral pairs of small ions.

S1.3.2 Trial move: titration of the acid and basic groups

The ionization state of the titratable groups is sampled in the Semi-Grand Canonical ensemble, which is described in detail found in references[3,4].

In this procedure, the charge of one of the acid or basic groups is changed in mimicking the protonation or deprotonation processes described in Eqs. S5 and S6. The electroneutrality of the system is preserved by creating or destroying one small ion with the opposite charge matching the new ionization state of the acidic/basic group. These protonation or deprotonation trial moves were accepted with probability

$$min\left(1, exp\left(-\frac{\Delta U}{k_B T} \pm (pH - pK_a)\ln 10\right)\right)$$

(S12)

Where the sign − or + corresponds to the protonation or deprotonation, respectively.

This equation is an adaptation obtained from the work of Reed and Reed, which studied the average fractional ionization $\overline{\alpha}$ as a function of the pH using Metropolis Monte Carlo algorithm for a linear polyelectrolyte. The PE chain is treated as a threefold rotational isomeric state model polymer, where each unit can have two states: negatively charged or neutral. They assume a Debye-Hückel screening between charges and that the polymer has the same dielectric constant than the solvent. The number of units in the polyelectrolyte was given by N. For their system they write the equation for the average Gibbs free energy F per individual polymer chain as:

$$F = \overline{U} - TS - \ln(10)\, k_{\mathrm{B}} T\, N\, \overline{\alpha}(\mathrm{p}H - \mathrm{p}K_{\mathrm{a}})$$

(S13)

where $\overline{U}$ is the average electrostatic energy, S is the configurational entropy, $\overline{\alpha}$ the average dissociation degree and N the number units of the polyelectrolyte chain. Then, defining:

$$X \equiv \ln 10 \cdot k_{\mathrm{B}} T\, N(\mathrm{p}H - \mathrm{p}K_{\mathrm{a}})$$

(S14)

$$F = \overline{U} - TS - X\,\overline{\alpha}$$

(S15)

Next, Reed and Reed calculate the probability of a state J :

$$p(J) \propto \exp\left[-\frac{(\mathrm{U}(J) - \mathrm{X}\,\alpha_J)}{k_\mathrm{B}T}\right]$$

(S16)

This probability takes as a reference state a completely deionized chain, where all its groups are neutral. This state is called N and has $\mathrm{U}(J) = 0$ and $\alpha_J = 0$.

In our system, we simulate a protein as a rigid body that has 8 types of titratable groups (given in Table 1 of the main text). Each titratable group type of protein has a number total of elements ω_i, which is equivalent to the variable N used for Reed and Reed. For example, for the aspartic acid $\omega_i = 13$ and the $pK_{\mathrm{ai}} = 4$.

Then the probability p for our system is:

$$p(J) \propto \exp\left[-\frac{\mathrm{U}(J)}{k_\mathrm{B}T} + n_{A_i^-}^J \ln 10 \cdot (\mathrm{p}H - \mathrm{p}K_\mathrm{a})\right]$$

(S17)

Where the original term $\alpha_J N$ is replacement for:

$$\omega_i \alpha_J = n_{A_i^-}^J$$

with $\alpha_J = \frac{n_{A_i^-}^J}{\omega_i}$, where $n_{A_i^-}^J$ is the number of groups i that are ionized in the estate J.

To accurately execute the Monte Carlo simulation, the transition matrix $\pi_{O\to N}$ from an old state (O) to a new state (N) , wherein a deprotonation or protonation assay is conducted, must adhere to the principle of detailed balance:

$$p(O)\pi_{O\to N} = p(N)\pi_{N\to O}$$

(S17)

Or

$$\frac{\pi_{O\to N}}{\pi_{N\to O}} = \frac{p(N)}{p(O)} = \exp\left[-\frac{\Delta U^{\mathrm{O}\to\mathrm{N}}}{k_{\mathrm{B}}T} + \left(n_{A_i^-}^{N} - n_{A_i^-}^{O}\right)\ln 10 \cdot N(\mathrm{p}H - \mathrm{p}K_{\mathrm{a}})\right]$$

(S18)

Where $\Delta U^{\mathrm{O}\to\mathrm{N}} = \mathrm{U(N)} - \mathrm{U(O)}$ and $\left(n_{A_i^-}^{N} - n_{A_i^-}^{O}\right)$ is the change in the number of dissociate groups. For the dissociation/deprotonation trail this is $\left(n_{A_i^-}^{N} - n_{A_i^-}^{O}\right) = 1$ and for the protonation trail this value is -1, which explains the term $\pm$ in equation S12.

S1.3.3 Trial move: translation of small ions

In each MC step, a new configuration is generated which consists of a translational move per small ion[5] . The translational trial move is accepted according to the probability

$$min\left(1, exp\left(-\frac{\Delta U}{k_B T}\right)\right)$$

(S19)

where ΔU is the difference in the energy between the new and old configurations. Note that we approximate the protein as a rigid body, thus configurational movements were not performed to the protein beads.

S1.3.4 Trial move: rotation of the oligoelectrolyte chain

The rotation of the oligoelectrolyte chain requires as a first step to choose a rotation axis given by the unit vector $u = (a, b, c)$ [6]. For practical purposes, the initial point of this vector is in the centre of mass of the oligoelectrolyte chain:

$$r_{\mathrm{cm}} = \frac{1}{N}\sum_{i=1}^{N} r(i)$$

(S20)

The rotation is applied to the relative position of the monomers with respect to the center of mass $(r_{\mathrm{old}}(i) - r_{\mathrm{cm}})$. Then, the expression for the new position is:

$$r_{\mathrm{new}}(i) = A_{\theta,\mathrm{u}}(r_{\mathrm{old}}(i) - r_{\mathrm{cm}}) + r_{\mathrm{cm}}$$

(S21)

where $A_{\theta,\mathrm{u}}$ is the standard matrix for a counterclockwise rotation through a random angle θ about u

$$A_{\theta,\mathrm{u}} = \begin{bmatrix} a^2(1-\cos\theta)+\cos\theta & ac(1-\cos\theta)-c\sin\theta & ac(1-\cos\theta)+b\sin\theta \\ ab(1-\cos\theta)+c\sin\theta & b^2(1-\cos\theta)+\cos\theta & bc(1-\cos\theta)+a\sin\theta \\ ac(1-\cos\theta)+b\sin\theta & bc(1-\cos\theta)+a\sin\theta & c^2(1-\cos\theta)+\cos\theta \end{bmatrix}$$

(S22)

This trial move is accepted according to the probability of Equation S13[6].

S1.3.5 Trial move: pivot and bending movement of an oligoelectrolyte chain segment

The pivot and bending movement consist on the rotation of a oligoelectrolyte chain segment[6]**.** For the pivot movement, the unit vector $u = (a, b, c)$ determines the rotation axis. Then, we choose a random monomer j, and the initial point of this vector is the position r_{j}. The segment from monomer j to the end of the chain is rotated following the choise of a random angle θ about u:

$$\boldsymbol{r_{\mathrm{new}}(i) = A_{\theta,\mathrm{u}}\left(r_{\mathrm{old}}(i) - r_{\mathrm{j}}\right) + r_{\mathrm{j}}}$$

(S23)

where $A_{\theta,\mathrm{u}}$ is the standard matrix given in Equation S15.

In the case of the bending move, the rotation of an oligoelectrolyte chain segmen is between two monomeros j and k. The rotation axis is defined by the directation that connect the two monomers:

$$u = \frac{r_{\mathrm{k}} - r_{\mathrm{j}}}{\left|r_{\mathrm{k}} - r_{\mathrm{j}}\right|}$$

(S24)

The starting point of this vector is the position r_{j}. The pivot and bending trial moves are accepted according to Equation S13[6].

S1.3.6 Trial move: creation and destruction of neutral pairs of small ions

The values of $\gamma_{\pm}$ and ρ for the salt concentrations considered in this work for Equation 12 on the main text are given in Tab. S1:

c_{salt}[mM]	$ln\ \gamma_{\pm}$	ρ [particles/ nm^3]
1	0.0122	$6.022\ 10^{-4}$
10	-0.087	$6.022\ 10^{-3}$
100	-0.232	$6.022\ 10^{-2}$

Table S1. Mean activity coefficient $\gamma_{\pm}$ and density of small ions ρ at the three salt concentrations of the reservoir c_{salt} of study.

S2. Average electrostatic potential: different reference point

Figure S1 shows the average electrostatic potential as a function of the protein centre of mass. In this case, the value of $\bar{\psi} = 0$ was located on the protein centre of mass.

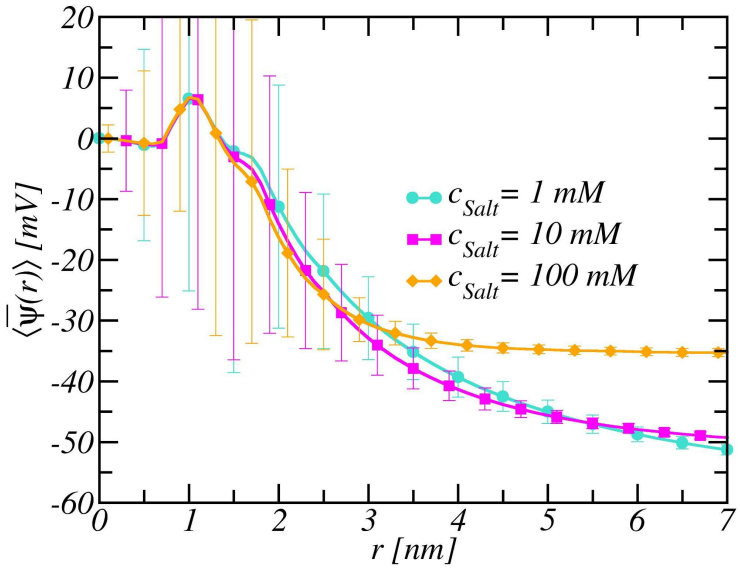

Figure S1. Average electrostatic potential as a function of the distance r to the protein centre of mass.

S3. Electrostatic potential of α-lactalbumin – oligoelectrolyte complex for Nm = 7 y Nm = 8.

Figure S2 shows the average electrostatic potential as a function of the distance r.

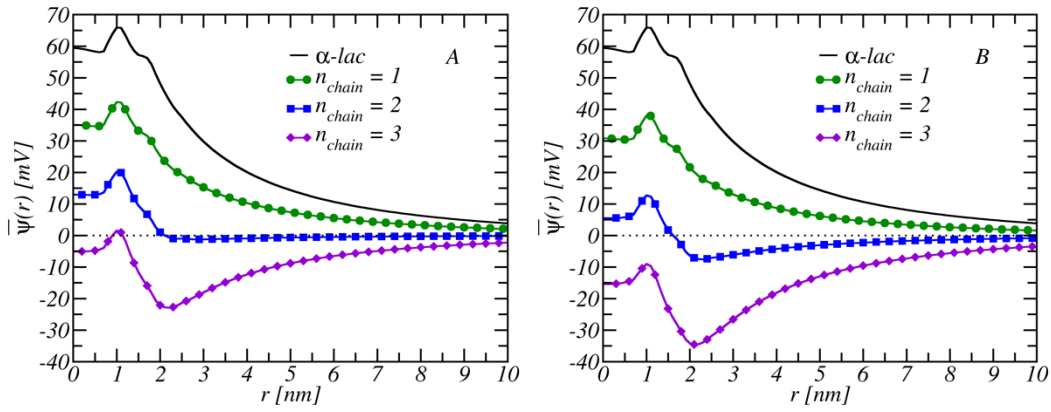

Figure S2. Average electrostatic potential as a function of the distance r for (A) $N_m = 7$ and (B) $N_m = 8$. In both cases pH = 3.

S4. Surface electrostatic potential of α-lactalbumin – oligoelectrolyte complex for $N_m = 7$ y $N_m = 8$.

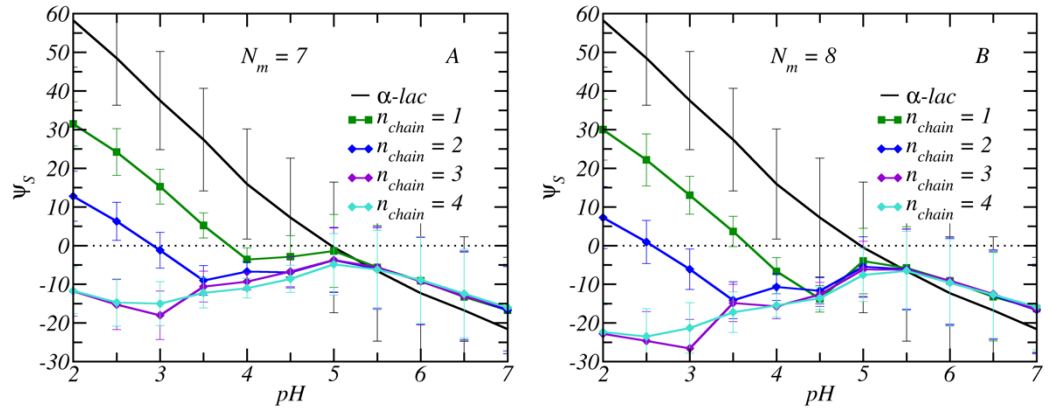

Figure S3. Surface electrostatic potential as a function of the pH for (A) $N_m = 7$ and (B) $N_m = 8$

References

1. Metropolis, N., Rosenbluth, A. W., Rosenbluth, M. N., Teller, A. H. & Teller, E. Equation of State Calculations by Fast Computing Machines. *J Chem Phys* **21**, 1087–1092 (1953).
2. Frenkel, D. & Smit, B. *Understanding molecular simulation : from algorithms to applications*. *Computational science series* (Academic Press, 2002).

3. Lund, M. & Jönsson, B. On the Charge Regulation of Proteins. *Biochemistry* **44**, 5722–5727 (2005).
4. Lund, M. & Jönsson, B. Charge regulation in biomolecular solution. *Q Rev Biophys* **46**, 265–281 (2013).
5. Narambuena, C. F. & Leiva, E. P. M. Monte Carlo Studies in Polyelectrolyte Solutions: Structure and Thermodynamics BT - Polyelectrolytes: Thermodynamics and Rheology. in (eds. P. M., V., Bayraktar, O. & Picó, G. A.) 349–379 (Springer International Publishing, 2014). doi:10.1007/978-3-319-01680-1_10.
6. Narambuena, C. F. & Leiva, E. P. M. Monte Carlo Studies in Polyelectrolyte Solutions: Structure and Thermodynamics BT. in *Polyelectrolytes:Thermodynamics and Rheology* (ed. Visakh P. M. Oguz Bayraktar, G. A. P.) 349–379 (Springer International Publishing, 2014). doi:10.1007/978-3-319-01680-1_10.
7. Valleau, J. P. & Cohen, L. K. Primitive model electrolytes. I. Grand canonical Monte Carlo computations. *J Chem Phys* **72**, 5935–5941 (1980).
8. Torrie, G. M. & Valleau, J. P. Electrical double layers. I. Monte Carlo study of a uniformly charged surface. *J Chem Phys* **73**, 5807–5816 (1980).
9. Torres, P. B., Blanco, P. M., Garcés, J. L. & Narambuena, C. F. The electrostatic potential inside and around α-lactalbumin: Fluctuations and mean-field models. *Journal of Chemical Physics* **157**, (2022).