Electronic Supplementary Material (ESI) for Soft Matter.

Supporting information on Influence of Macromolecular Crowding in the Charge Regulation of Intrinsically Disordered Proteins

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S1 Aminoacid titration curves at $c_s = 0.01$ M without crowding agents

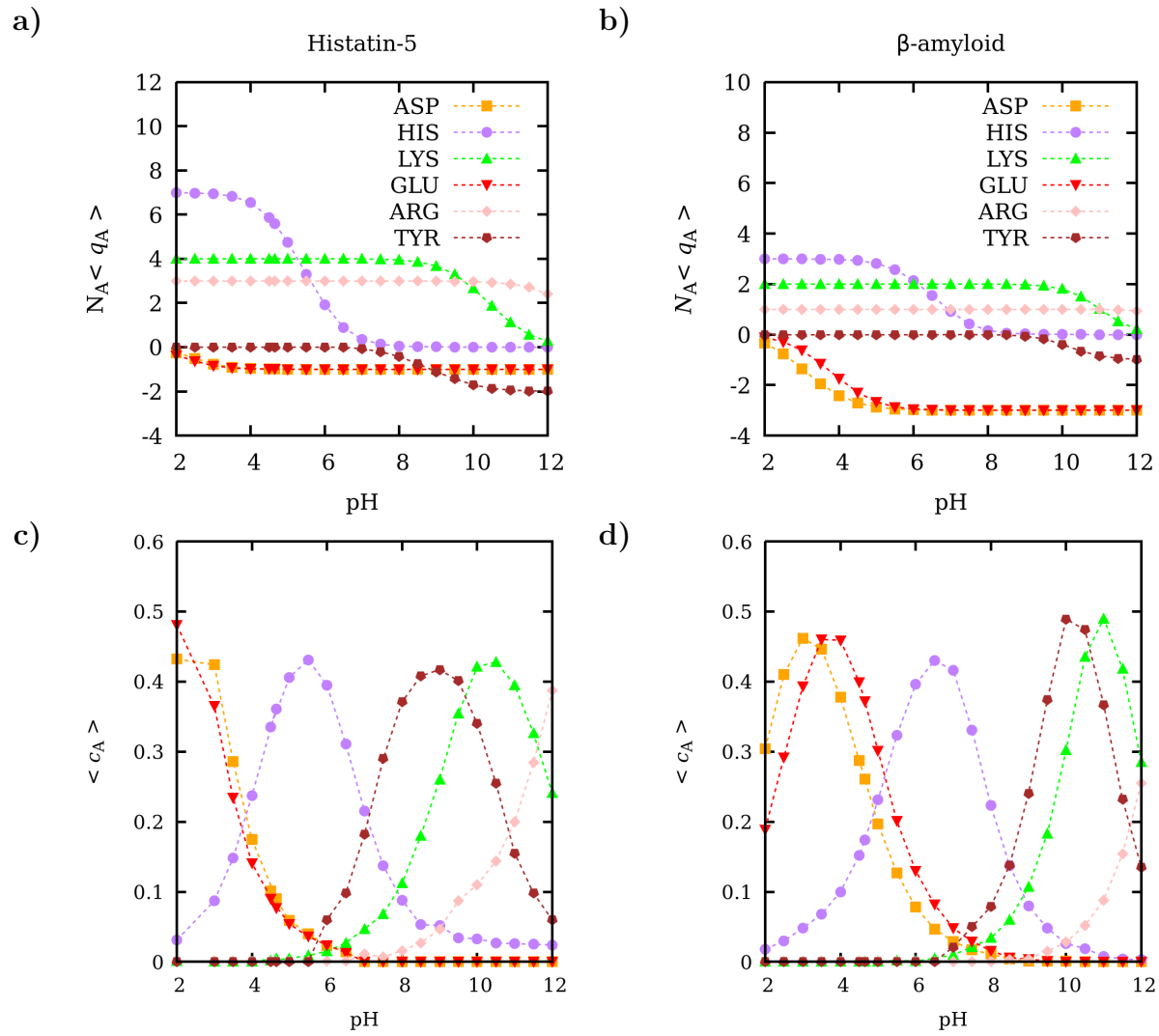


Figure 1: Aminoacid total charge $N_A \langle q_A \rangle$ (panels a and b) and binding capacitance c_A (panels c and d) titration curves for the Intrinsically Disordered Proteins (IDPs) histatin-5 (panels a and c) and β-amyloid 42 (panels b and d). The obtained results for each aminoacid are depicted as follows: aspartic acid (ASP, orange squares), histidine (HIS, purple circles), lysine (LYS, green triangles), glutamic acid (GLU, red inverted triangles), arginine (ARG, pink diamonds) and tyrosine (TYR, pentagons). All data was computed by Semi-Grand Canonical Monte Carlo (SGCMC) simulations of each protein without crowding agents and an added salt concentration $c_s = 0.01$ M of NaCl.

S2 Average charge variation of aspartic acid, histidine and lysine aminoacids for varying salt concentration

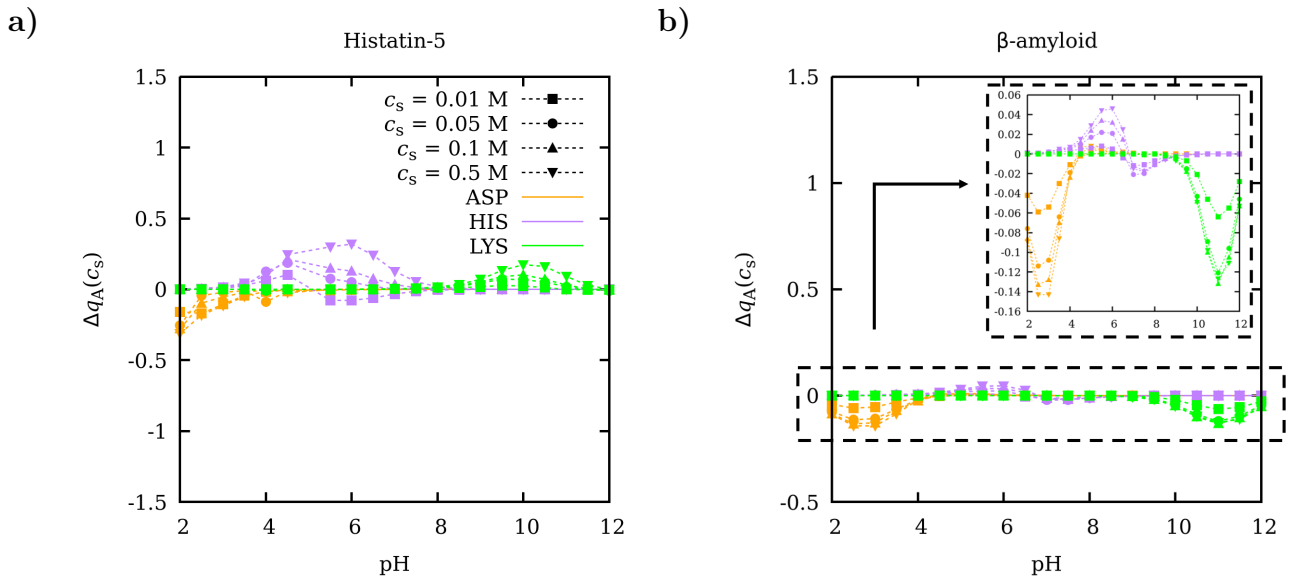


Figure 2: Charge increment due to the added salt in the aminoacid type A $\Delta q_{\mathrm{A}}(c_{\mathrm{s}}) = |q_{\mathrm{A}}(c_{\mathrm{s}})| - |q_{\mathrm{A}}(0)|$ for aspartic acid (orange), histidine (purple) and lysine (green) as a function of the pH-value. The results were obtained with Semi-Grand Canonical Monte Carlo simulations without crowding agents, at constant added salt concentrations of 0.01 M (squares), 0.05 M (circles), 0.1 M (uppwards triangles) and 0.5 M (downwards triangles).

S3 Charge regulation in a medium with neutral crowders and salt concentration $c_s = 0.01$ M

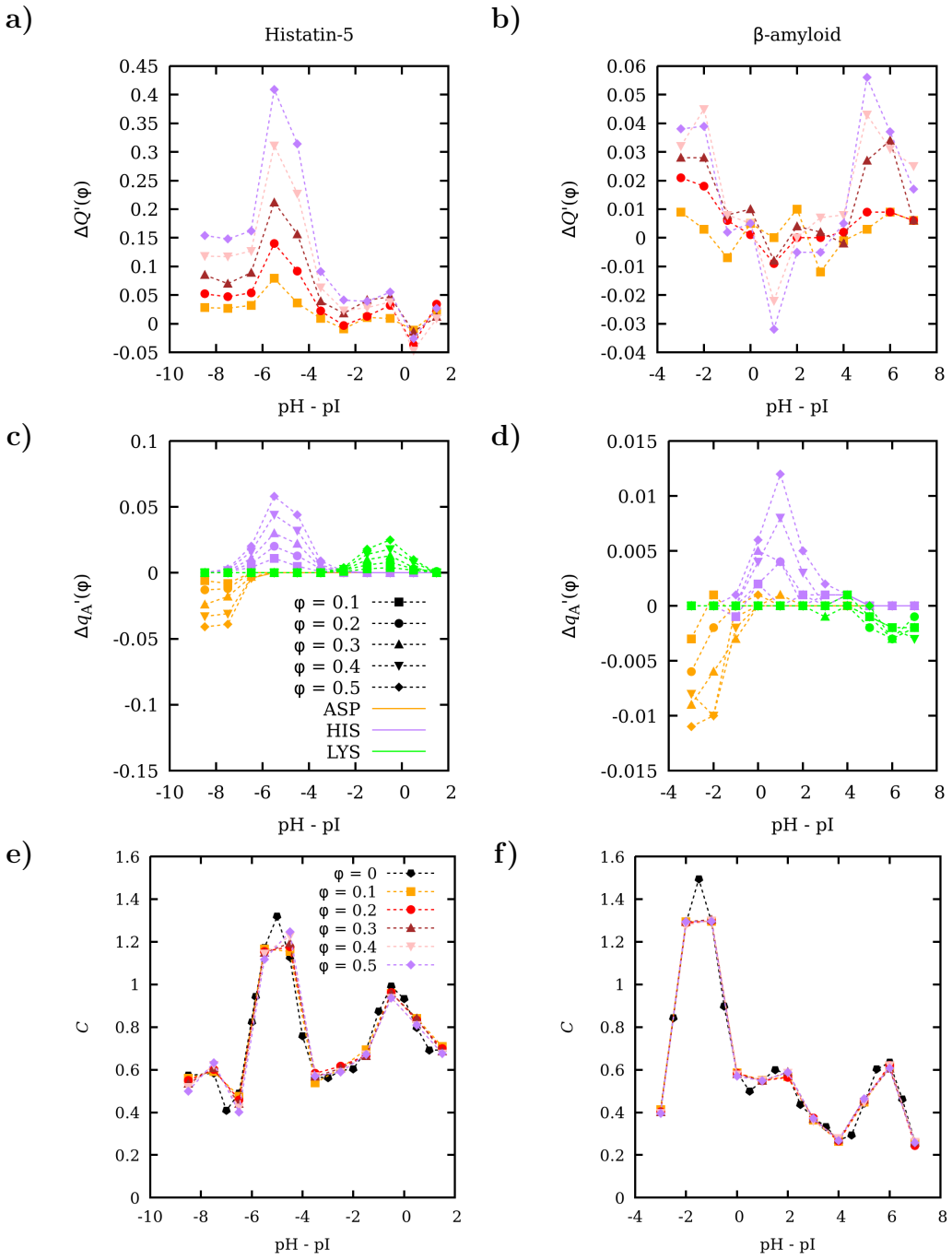


Figure 3: (a and b) Increase in the absolute macromolecular charge due to the presence of neutral crowders, $\Delta Q'(\phi) = |Q(\phi)| - |Q(0)|$; (c and d) increase in the average charge per aminoacid, $\Delta q'_{\rm A}(\phi) = |q_{\rm A}(\phi)| - |q_{\rm A}(0)|$; and (e and f) binding capacitance C. Panels on the left-hand side refer to histatin-5 and the ones on the right-hand side to β-amyloid 42. The added salt concentration is $c_s = 0.01$ M of NaCl in all the simulations, while ϕ ranges from 0.1 to 0.5. The meaning of the markers used can be read inside the panels.

S4 IDP global charge variation *vs.* excluded volume curves

For all the range of pH values, it is found that the curves can be very well fitted by the power law

$$\Delta Q'(\phi) = kC\phi^{\alpha}, \tag{1}$$

where k and the α are empiric parameters (see the dashed lines in Figs. 4 (neutral crowders) and (charged crowders). For neutral crowders, the best fit k and α parameters are reported in Tab. 1. It can be observed that $|k|$ ranges from 0.01 to 1.30 and α from 0.40 to 6.88. Interestingly, for the pH-values where crowding induces a significant charge regulation, α is close to one thus $\Delta Q'(\phi)$ scales almost linearly with ϕ. When charged crowders are considered, the best-fit parameters are listed in Tab. 2. It is observed that k and α values range from 0.08 to 4.60 and from 0.21 to 2.0, respectively. It was not possible to properly fit the data corresponding to β-amyloid 42 at pH 4.65, since $\Delta Q'(\phi)$ was very small.

Table 1: k and α values obtained from the best fit of the $\Delta Q'(\phi)$ *vs.* ϕ curves for systems with neutral crowders (Fig. 4), to the empiric power law (Eq. 1). The standard error is always ~ 0.005 units

pH		2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00	11.00	12.0
Histatin 5	$\boldsymbol{k}$	0.57	0.47	0.63	1.29	1.00	0.89	0.36	0.13	0.34	-0.59	-1.22
	$\boldsymbol{\alpha}$	1.02	1.15	1.06	1.27	1.31	1.41	0.918	0.934	1.41	2.65	7.90
β-amyloid	$\boldsymbol{k}$	0.13	0.07	-1.01	-0.13	-0.31	-0.174	0.82	0.41	0.30	0.05	0.01
	$\boldsymbol{\alpha}$	1.50	0.67	4.48	1.45	1.25	1.14	6.88	1.48	1.06	0.40	1.56

Table 2: k and α values obtained by the best fit of $\Delta Q'(\phi)$ *vs.* ϕ curves (Fig. 5) to the empiric power law (Eq. 1) in the presence of charged crowders. The standard error is ~ 0.005 units.

pH		3.00	3.50	4.00	4.50	4.65	5.00	6.00	7.00	8.00	9.00	10.0	11.0
Histatin 5	$\boldsymbol{k}$	2.24	2.04	3.65	4.60	0.63	1.28	1.01	1.10	0.34	8.28	0.35	0.70
	$\boldsymbol{\alpha}$	0.55	0.52	0.80	1.00	1.00	0.84	0.38	0.31	0.10	12.69	0.22	0.91
β-amyloid	$\boldsymbol{k}$	0.42	0.28	0.32	0.08	-	-0.21	0.04	0.12	0.43	0.63	1.68	1.88
	$\boldsymbol{\alpha}$	0.70	0.56	0.45	0.38	-	2.02	0.09	0.74	0.85	0.41	0.67	0.57

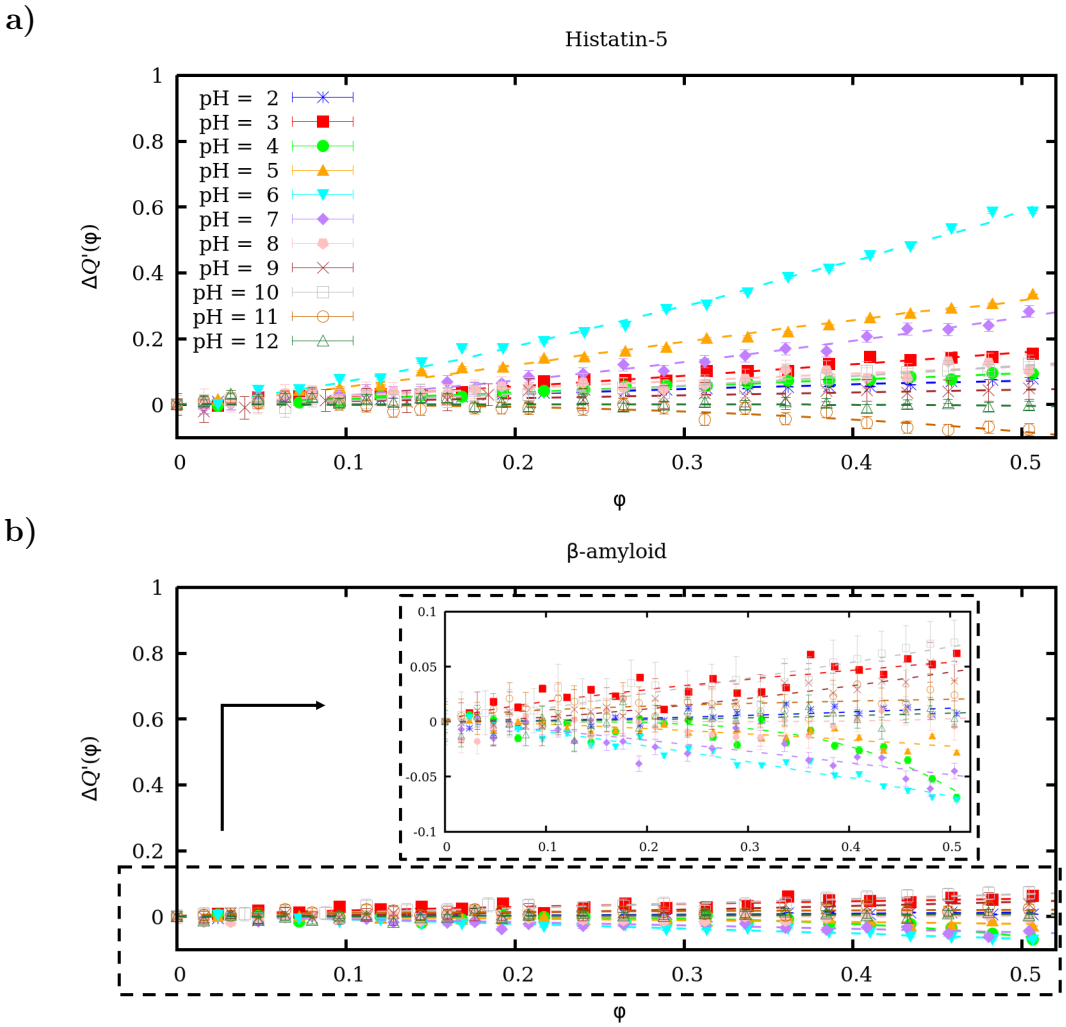


Figure 4: Difference between absolute charges $\Delta Q'(\phi) = |Q(\phi)| - |Q(0)|$ for (a) histatin-5 and (b) β-amyloid 42 as a function of ϕ in the presence of neutral crowders at pH-values ranging from 3 to 11. The meaning of lines and markers is indicated in the top panel. Dashed lines correspond to the best-fit to the empiric power law (Eq. 1). The resulting best-fit parameters are listed in Tab. 1.

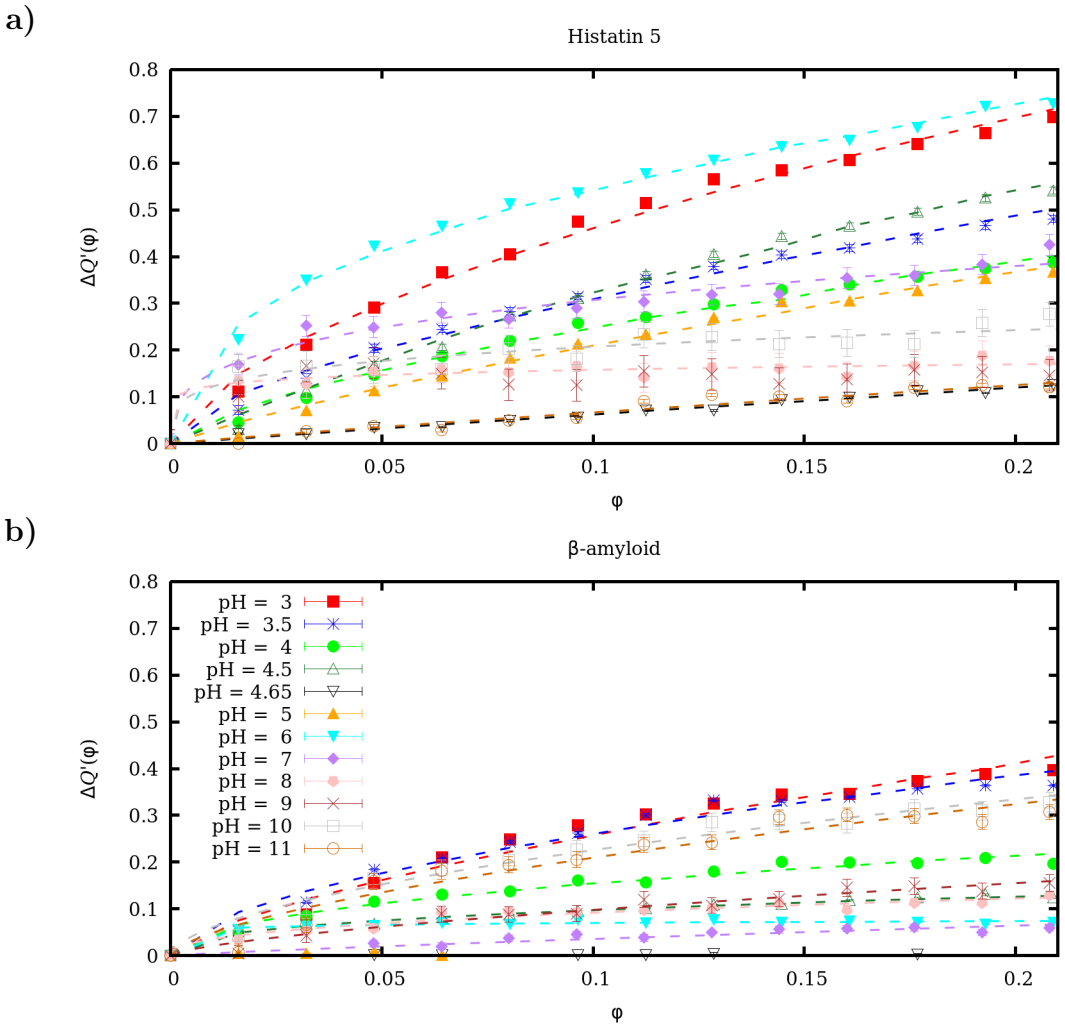


Figure 5: Difference between absolute charges $\Delta Q'(\phi) = |Q(\phi)| - |Q(0)|$ for (a) histatin-5 and (b) β-amyloid 42 as a function of ϕ in the presence of charged crowders at pH-values ranging from 3 to 11. The meaning of the line colors and markers is indicated in the top panel. Dashed lines correspond to the best-fit to the empiric power law (Eq. 1). The resulting best-fit parameters are listed in Tab. 2.

S5 Binding capacitance titrations curves per aminoacid type in a medium crowded with neutral crowders at $c_s = 0.1$ M

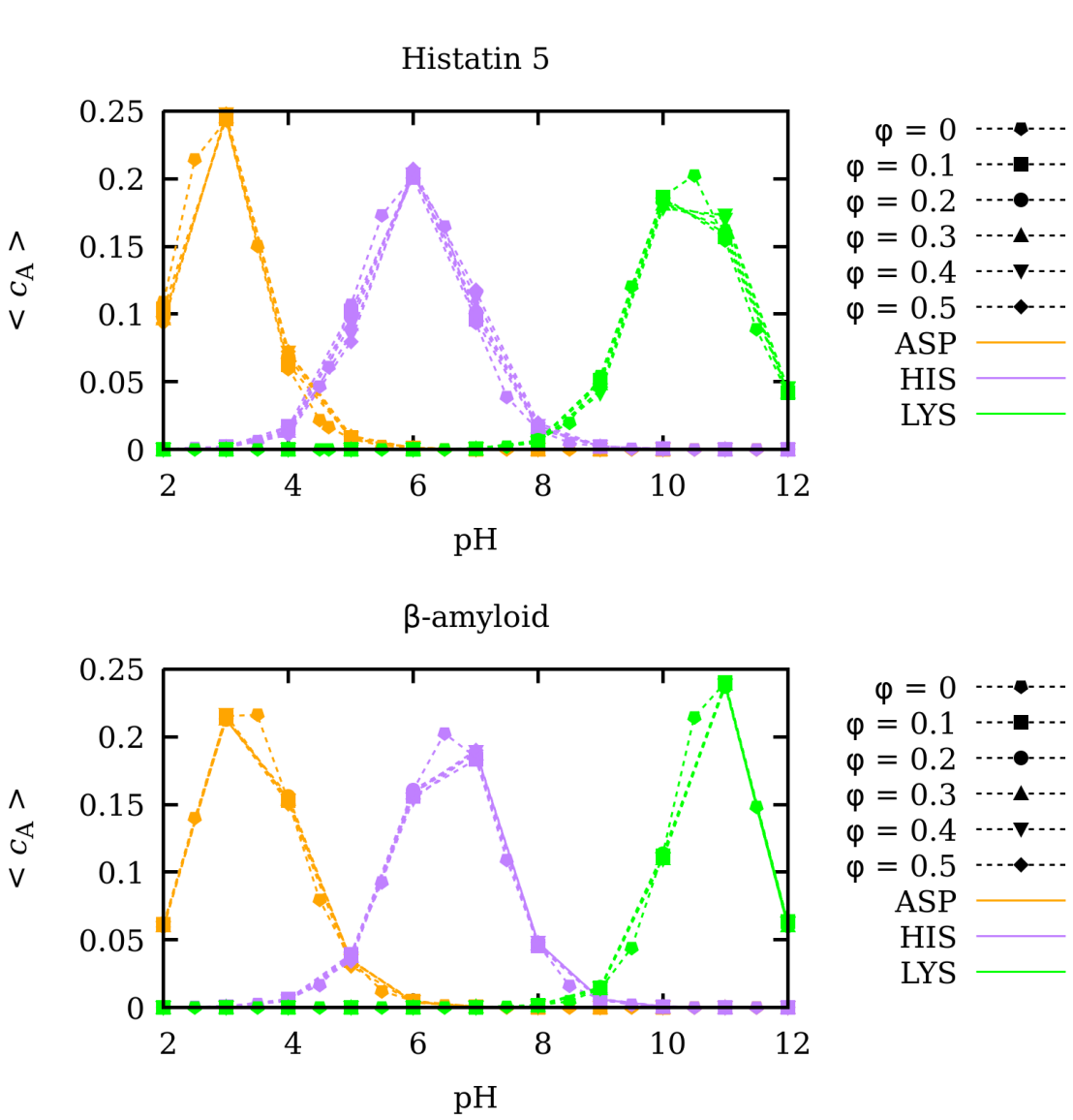


Figure 6: Binding capacitance titration curve per aminoacid type $\langle c_A \rangle$ for (a) histatin 5 and (b) β-amyloid 42. For clarity, only the values of aspartic acid (orange), histidine (purple) and lysine (green) are shown as representatives of aminoacids with acidic, neutral and basic isoeletric points, respectively. The results were obtained by SGCMC simulations with an NaCl concentration $c_s = 0.1$ M and neutral crowder with an excluded volume fraction ϕ of 0 (pentagons), 0.1 (squares), 0.2 (triangles), 0.3 (triangles), 0.4 (inverted triangles) and 0.5 (diamods).

S6 Best fit parameters to the scaling law (Eq. 13 in the main text) for the R_g *vs.* ϕ curves

Table 3: Best fit c-values of the scaling law (Eq. 13 in the main text) to the computed R_g *vs.* ϕ curves for histatin 5 and β-amyloid 42 at different pH values and in presence of neutral crowders. The standard error is ~ 0.005 units

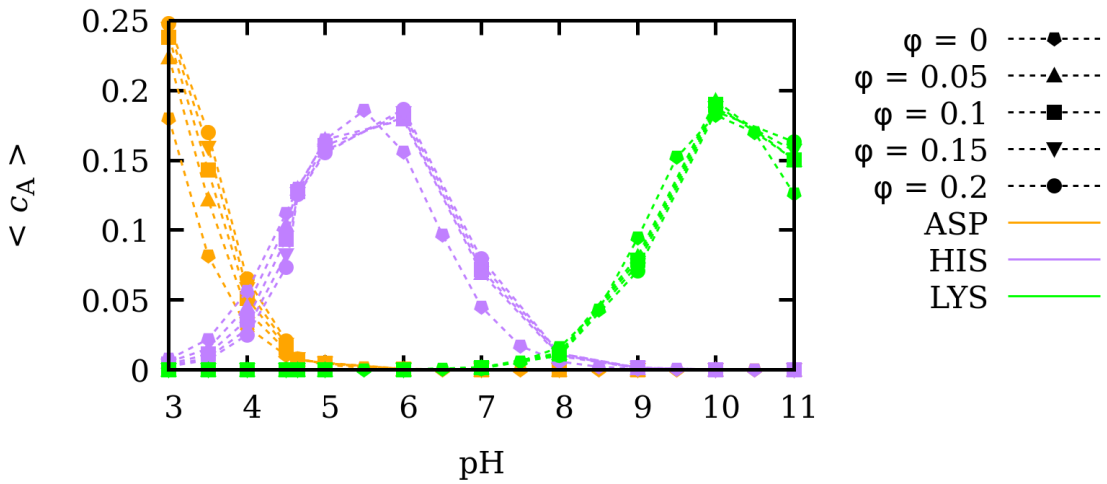
pH	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00	11.00	12.0
Histatin 5	0.11	0.10	0.10	0.10	0.10	0.09	0.09	0.09	0.09	0.08	0.09
***β*-amyloid**	0.09	0.09	0.09	0.08	0.09	0.09	0.09	0.09	0.09	0.09	0.09

Table 4: List of the c-values obtained by the best-fit of the R_g *vs.* ϕ curves to the power law (Eq. 13 in the main text) in presence of charged crowders. The standard error is ~ 0.005 units

pH	3.00	3.50	4.00	4.50	4.65	5.00	6.00	7.00	8.00	9.00	10.0	11.0
Histatin 5	0.33	0.30	0.19	0.14	0.07	0.06	0.03	0.07	0.08	0.14	0.07	0.07
***β*-amyloid**	0.15	0.12	0.10	0.09	0.07	0.08	0.10	0.12	0.13	0.12	0.15	0.17

S7 Binding capacitance per aminoacid titration curves in a media with charged crowders at $c_s = 0.01$ M

a)



b)

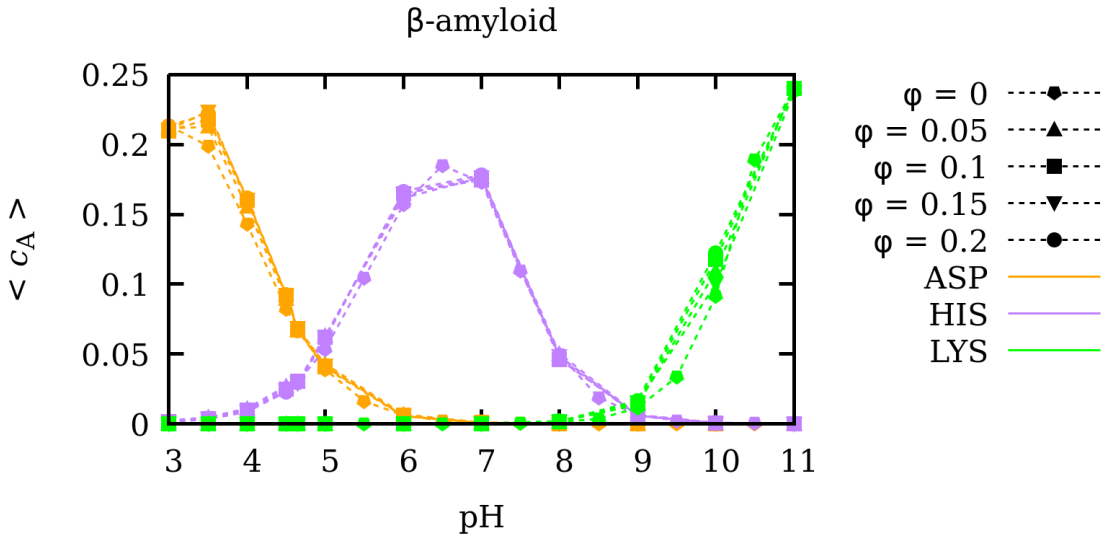


Figure 7: Binding capacitance titration curves per aminoacid type $\langle c_A \rangle$ for histatin-5 (a) and β-amyloid 42 IDPs. For clarity, only the values of aspartic acid (orange), histidine (purple) and lysine (green) are shown as representatives of aminoacids with acidic, neutral and basic isoeletric points, respectively. The results were obtained by SGCMC simulations with a NaCl concentration $c_s = 0.01$ M and charged BSA crowders with excluded volume fraction ϕ of 0 (pentagons), 0.05 (uppwards triangles), 0.1 (squares), 0.15 (downwards triangles) and 0.2 (circles).

S8 Calculation of the effective ionic strength I' for the case with charged crowders

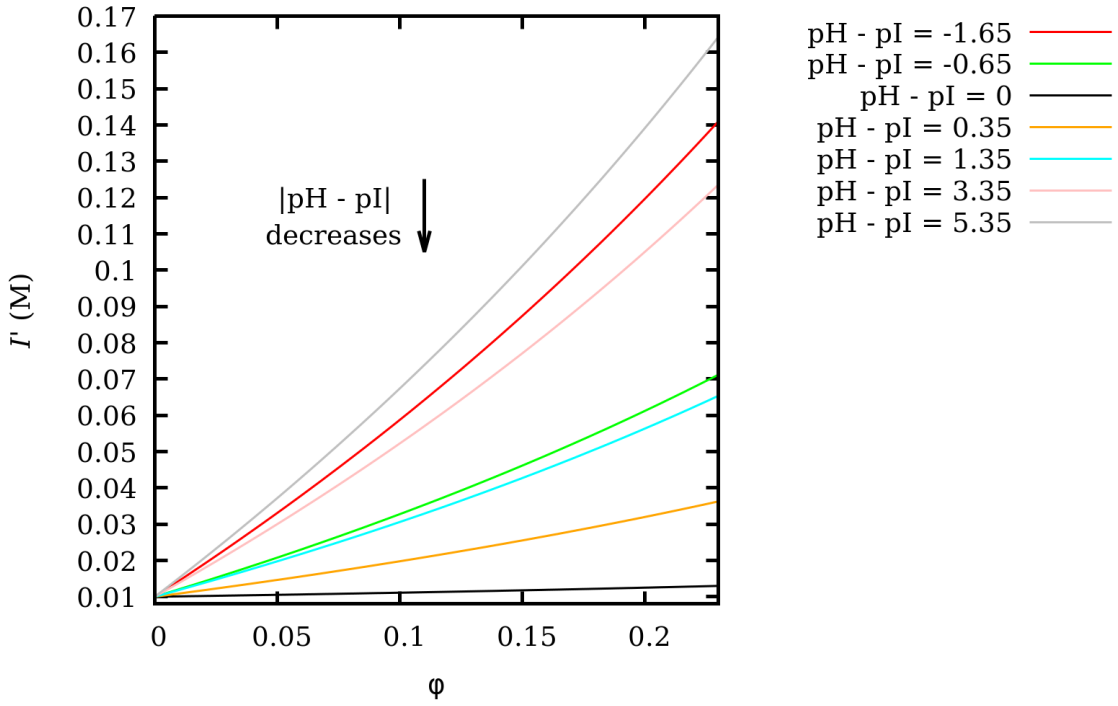


Figure 8: Effective ionic strength I' as a function of the crowder excluded volume fraction ϕ at different pH-values. For clarity, the pH-values are refereed to the Bovin Serum Albumin isoelectric point pI = 4.65.

S9 Comparison of $\Delta Q(I' = 0.05\text{M})$ and $\Delta Q'(\phi)$ *vs.* pH for the case with charged crowders

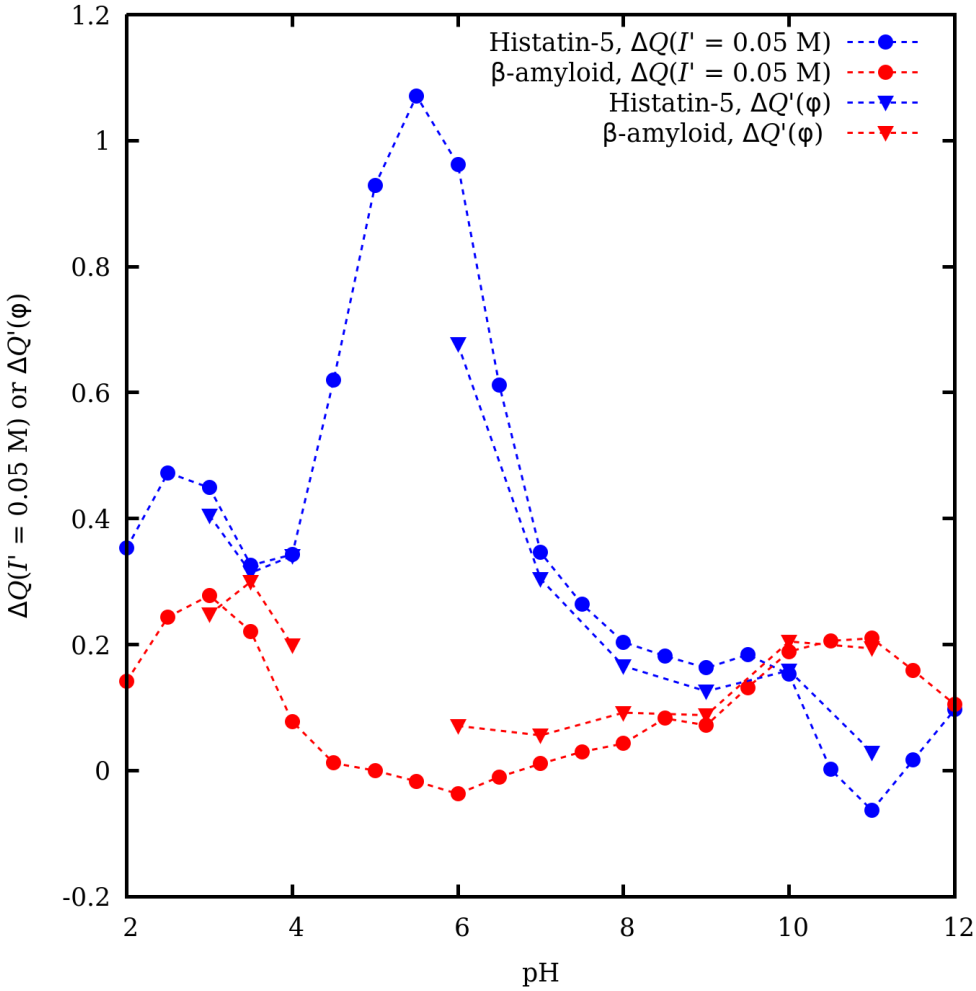


Figure 9: Comparison between i) charge variation produced when the added salt concentration is incremented from $c_s = 0.01$ M to 0.05 M without crowders ($\Delta Q(I' = 0.05\text{M}) = |Q(\phi = 0, c_s = 0.05\text{M})| - |Q(\phi = 0, c_s = 0.01\text{M})|$, circles); and ii) charge variation produced when adding charged crowders $\Delta Q'(\phi)$ (triangles) to a protein solution with added salt concentration $c_s = 0.01$ M. The excluded volume $\phi(\text{pH}, 0.05\text{M})$ is chosen such as the effective ionic strength I' (calculated using Eq.**??**) is always $I' = 0.05$ M (circles). Blue color refers to Histatin-5 while red color refers to β-amyloid.