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Supporting information for: Quantifying the influence of the ion cloud on SAXS profiles of charged proteins

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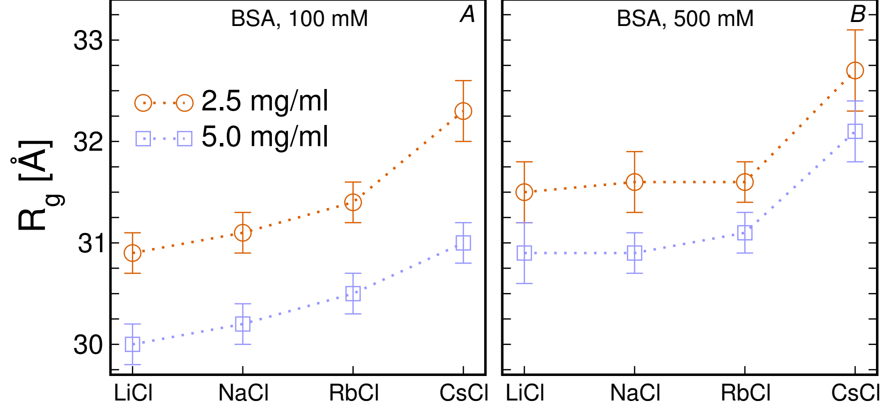

Figure S1: On systematic errors of the R_g estimates from experimental SAXS curves: absolute values of experimental R_g of BSA in different salt solutions from Guinier analysis, for protein concentrations of 2.5 mg/ml (orange circles) and 5.0 mg/ml (blue squares), at 100 mM salt (A) and 500 mM salt (B). R_g estimates from 5.0 mg/ml samples are systematically smaller compared to 2.5 mg/ml samples, presumably due to increased protein-protein interactions at higher protein concentrations. Such systematic errors are reduced at 500 mM salt concentration (B) compared to 100 mM salt concentration (A), reflecting reduced protein-protein interactions in consequence of increased screening of the protein charge. Critically, the *increase* of R_g, ΔR_g upon varying the alkali cation from Li^+ to Cs^+ is similar between the 2.5 mg/ml and 5.0 mg/ml samples, suggesting that ΔR_g is hardly affected by systematic errors due to protein-protein interactions. Hence, we averaged ΔR_g estimates from 2.5 mg/ml and 5.0 mg/ml samples before comparing with the calculated ΔR_g values.

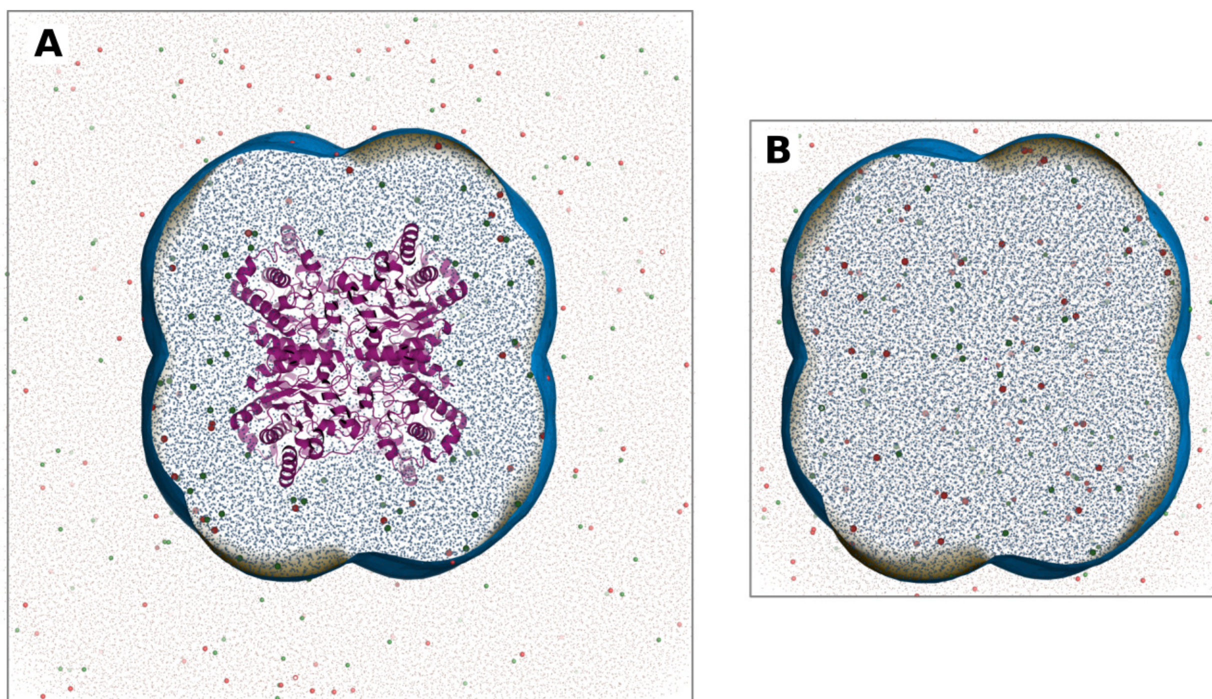

Figure S2: Simulation systems of (A) glucose isomerase (GI) and (B) pure solvent, each containing 100 mM NaCl. GI is shown in purple cartoon representation, Na^+ and Cl^- ions as green and red spheres, respectively, and water as blue spheres inside the envelope and as brown spheres outside of the envelope. The envelope is represented by a blue surface, constructed at a distance of 30 Å from the protein atoms in (A). The same envelope is used in the pure-solvent system (B). To compute the buffer-subtracted SAXS curve, scattering contributions from all atoms inside the envelopes of the protein/solvent (A) and the pure-solvent system (B) were taken into account, averaged over 3000 MD simulation frames. For more details, we refer to previous work (Chen and Hub, Biophys. J., 2014, 107, 435–447).

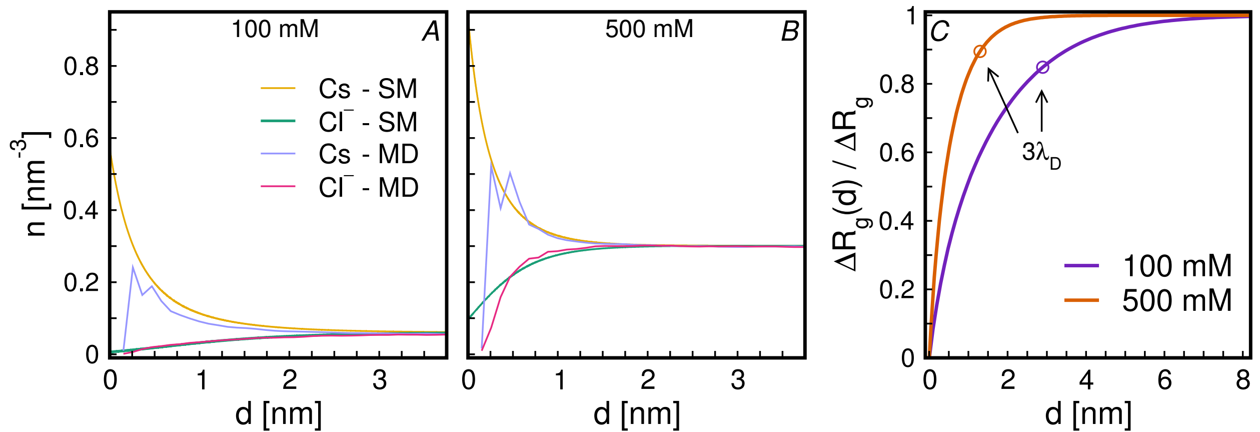

Figure S3: Number density of caesium (Cs) and chloride (Cl) ions as a function of distance d from the protein surface, taken from the spherical model (SM) and atomistic MD simulations (MD) of glucose isomerase at 100 mM CsCl (A) and 500 mM CsCl (B). The ion distributions from the SM and from MD agree at large distances from the protein, following the decay according to Debye-Hückel theory. At smaller distances, in contrast, large deviations between the SM and MD are found; here, specific salt bridges and solvation layers of ions revealed in MD simulations are not captured by the SM. (C) Cumulative contributions to ΔR_g from ions computed from the spherical model, plotted versus the distance d from the sphere surface. The circles indicate the points at $d = 3\lambda_D$, where λ_D is the Debye length.

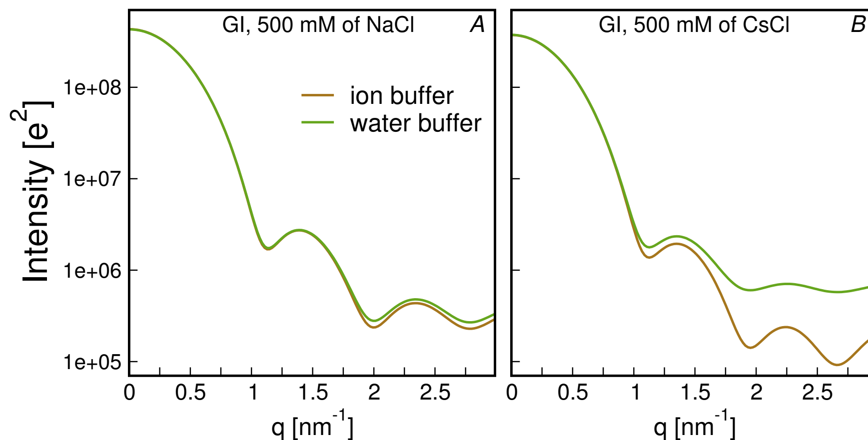

Figure S4: On the importance of buffer matching during explicit-solvent SAXS calculations with ionic buffers. (A) Calculated SAXS curves of GI in 500 mM of NaCl and (B) 500 mM CsCl. SAXS curves were computed for the pure-solvent simulations either with a matching salt buffer (brown lines) or using a non-matching pure-water buffer (green lines). Evidently, if a non-matching buffer is applied, large buffer mismatch artifacts appear with 500 mM CsCl (B), and small artifacts appear with 500 mM of NaCl (A). In these SAXS calculations, the mean buffer density was corrected as described previously (Chen and Hub, Biophys. J., 2014), suggesting that such artifacts do *not* appear owing to a mismatch of the mean density between solute and solvent systems, but instead owing to a mismatch in ion-ion correlations. This analysis highlights the importance of using identical buffers in solute and pure-solvent simulations, as done throughout in this study, in particular in systems with high salt concentration and large electron-rich ions, even when correcting the mean density.

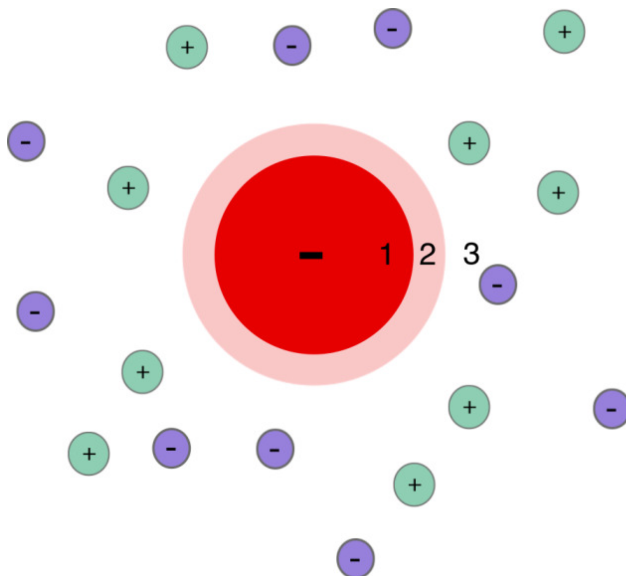

Figure S5: Spherical model of a charged protein in a salt solution, represented by 3 regions: (1) protein (red); (2) exclusion layer (pale red); (3) solvent with salt (green/purple spheres). The ion-exclusion layer accounts for the finite size of the ions, avoiding that the ions overlap with the protein.

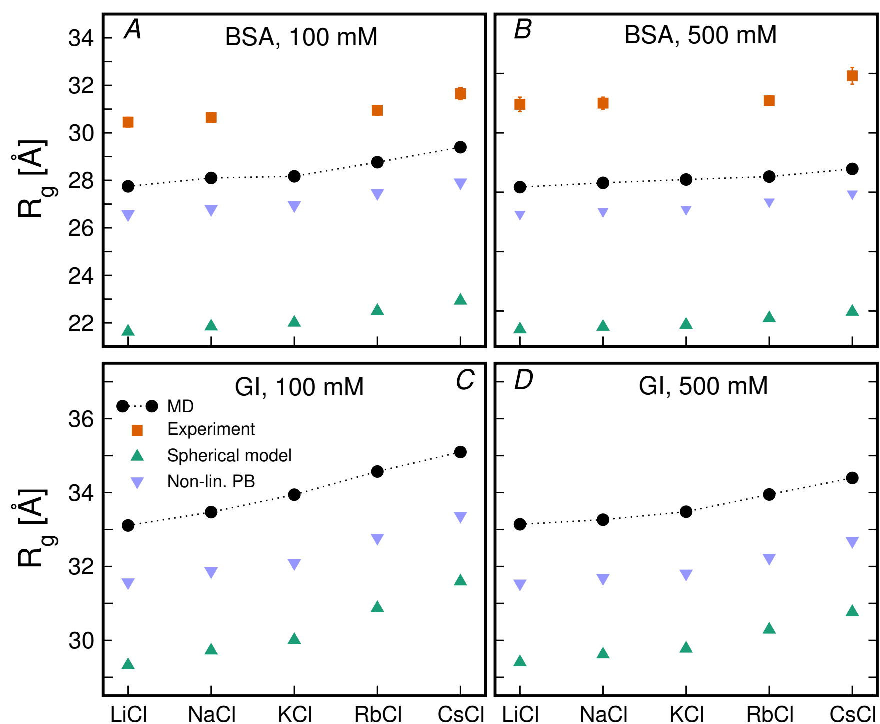

Figure S6: Absolute radii of gyration R_g taken from MD simulations (black circles), experiment (red squares), the spherical model (green triangles down), and non-linear Poisson-Boltzmann calculations (blue triangles right). R_g is shown for bovine serum albumin (BSA, A/B) and glucose isomerase (GI, C/D), at bulk salt concentrations of 100 mM (A/C) and at 500 mM (B/D). For a discussion on the differences, see main text.

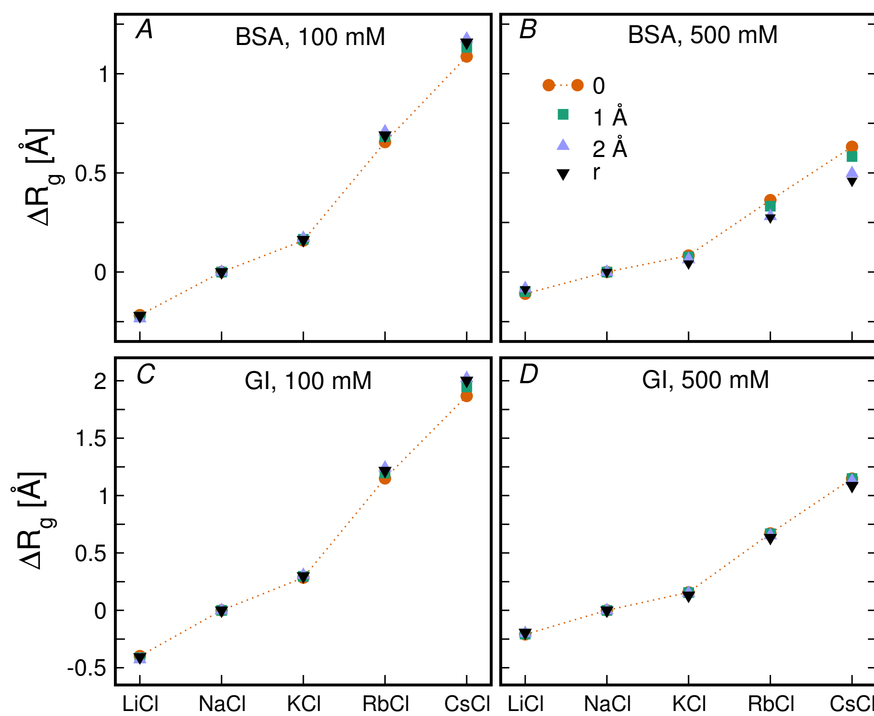

Figure S7: ΔR_g computed with the spherical model, using different values for exclusion layer thicks 0 Å, 1 Å, 2 Å, and ion radius (r), as indicated in the legend.