Report on SAXS measurements on deca-arginine and its mutants at BM29 during beamtime MX-1908

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Introduction

Due to their long-range nature, electrostatic interactions often dominate both intra- and intermolecular interactions in aqueous biomolecular solution. For instance, protein stability can be controlled either by varying the salt concentration, or via pH, which determines the ionization state of titratable amino acid side-chains. The solution behavior of certain charged globular proteins has been recently shown to be characterized by the interplay of two counteracting electrostatic forces:[1] the generic Coulomb repulsion, and an anisotropic, short-range electrostatic attraction between patches, i.e. localized charge distributions on the protein surface. As a non-trivial consequence, the propensity of these globular proteins to aggregate is heightened at low-to-intermediate ionic strength.

In this project we aim to develop both experimental and theoretical methods for investigating fundamental aspects of intra- and intermolecular interactions in biomolecules. To cleanly decipher the results, and to develop a 1:1 correspondence between experiment and

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theory, we initially focus on simplistic peptide systems where the contributions from active mechanisms can be unambiguously decomposed. In particular we are interested in studying deca-arginine (R10) and deca-lysine (K10). These are short, homogenous peptides that retain random-coil conformations and bear a net positive charge of $10e$ at neutral pH. Despite their similarities, R10 and K10 interact very differently with biological membranes. R10 is a cell-penetrating peptide that traverses biological membranes via a direct mode of entry, while the cellular uptake of K10 is considerably lower.[2]

Previous SAXS measurements, performed at BM29 at ESRF in Grenoble (MX-1763), revealed a striking difference in intermolecular interactions between R10 and K10. R10 self-associates, while interactions between K10 chains are purely repulsive. The propensity of R10 to self-associate is heightened at low-to-intermediate ionic strengths indicating that the attraction has an important electrostatic component. The self-association of R10 may increase the peptide coverage of the lipid bilayer surface, and contribute to explain the superior cellular uptake of R10, in contrast to K10. To find a possible molecular mechanism, we performed all-atom molecular dynamics (MD) simulations to compute the interaction free energy between pairs of R10 and K10 molecules. MD simulations elucidate the origin of the R10-R10 attraction by providing structural information on the dimeric state. The last two C-terminal residues of R10 constitute an adhesive patch achieved by stacking of the side chains of two arginine residues, and by salt bridges formed between the like-charge ion pair and C-terminal carboxyl groups.

Results and Discussion

During beamtime MX-1908, we complemented our studies with additional SAXS experiments that provided a strict test of the mode of interaction between R10 molecules as predicted by MD simulations. We performed SAXS measurements on solutions of R8KR and K8RK at various ionic strengths. The former is an analogue of R10, where the mutation of the ninth

residue to lysine is expected to hamper the attraction, whereas the latter, is a mutant of K10, where the presence of the ninth arginine residue might impart an attractive interaction between the peptides. At physiological ionic strength, Figure 1 shows that the solution behavior of both K8RK (b) and R8KR (c) resembles the one observed for K10 (a), i.e. the scattering intensity at low angles decreases with increasing peptide concentration, c_p. The intensities of the scattering curves for R10 (Figure 1a) are considerably higher for q <2 nm^{-1}, compared to the other peptides. Moreover, for R10 the intensity at $q = 0$, $I(0)$, increases with increasing peptide concentration, when going from 11.8 g/l to 24.6 g/l, whereas $I(0)$ slightly decreases upon a further increase up to c_p = 47.9 g/l. The trends of $I(0)$ *vs.* c_p for the four studied peptides at physiological ionic strength (Figure 1d) indicate that for K10, K8RK and R8KR the net peptide-peptide interaction is repulsive, since the osmotic compressibility decreases with increasing c_p. Conversely, the high $I(0)$ values for R10 in the whole c_p-range and the increasing trend are consistent with net attractive interactions, and the presence of a monomer-dimer equilibrium in solution.

Figure 2 shows the scattering curves measured for peptide solutions at ionic strength 0.025 M. For K10, K8RK and R8KR, the lower salt screening of the repulsive Coulombic interactions between the highly charged peptides results in lower $I(0)$ values. For R10, the scattering intensities at low angles are higher at 0.025 M than at 0.015 M ionic strength, confirming that the driving force for the self-association of R10 has an important electrostatic component. Moreover, comparison of scattering curves for R10 and R8KR indicate that the ninth arginine residue is essential for the net peptide-peptide attraction. However, the fact that the interaction between K8RK molecules is repulsive suggests that the arginine residue in the ninth position is necessary, but not sufficient for the attraction to occur.

The above experiments are complemented with all-atom molecular dynamics simulations performed using the umbrella sampling technique to calculate the potential of mean force (PMF) between pairs of peptides. These simulations elucidate the origin of the peptide-peptide interactions with atomistic detail, and can thus be used to rationalize experimental

observations. Figure 3a shows the PMF between R10 and R8KR molecules as a function of the distance between the guanidino-C atoms of the eighth and tenth residues, $CZ10-CZ8$. The interaction is repulsive at all separations for R8KR, while for R10 it is attractive at $CZ10-CZ8 \approx$0.4 nm and for 1 nm $< CZ10-CZ8 <$ 1.5 nm. The PMF for R10 as a function of the distance between the guanidino-C atoms of the ninth residues, $CZ9-CZ9$ (Figure 3b) displays a deep minimum at $CZ9-CZ9 \approx$0.4 nm and screened electrostatic repulsion for $CZ9-CZ9 >$ 1 nm. Figure 3c shows the average unbiased $CZ9-CZ9$ distances in the simulation trajectories used to calculate the PMFs of Figure 3a, where the $CZ10-CZ8$ was constrained at different separations. The $CZ10-CZ8$ distance at which the PMF for R10 is attractive corresponds to $CZ9-CZ9 <$ 0.5 nm for R10, whereas distances between the ninth lysine residues of R8KR are larger than 1.5 nm. This result indicates that the stacking of the guanidinium moieties of the ninth residues is key for the net attraction. In order to strengthen this conclusion, Figure 3d shows that in simulations where the $CZ9-CZ9$ separation is constrained, $CZ10-CZ8 <$ 0.8 nm only when also $CZ9-CZ9 <$0.8 nm, while $CZ9-CZ9 \approx$ 1 nm corresponds to the onset of the repulsive interaction, as well as to the loss of guanidinium-guanidinium stacking[3] between any pairs of residues.

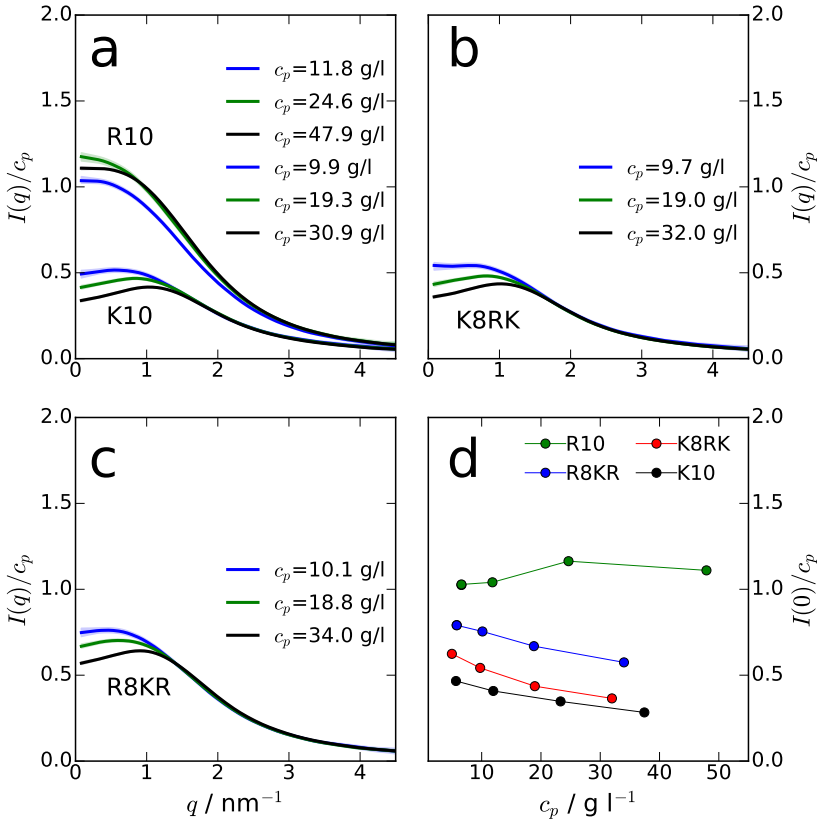

Figure 1: Small-angle X-ray scattering experiments for solutions of various peptide concentrations, c_p, in Tris buffer pH 7.8 and ionic strength 0.15 M at 293.15 K. Concentration normalized scattering curves $I(q)/c_p$ are shown as a function of the scattering vector, q, for solutions of R10 (a), K10 (a), K8RK (b) and R8KR (c). Scattering intensities are extrapolated at zero angle and reported as a function of c_p for R10, K10, K8RK and R8KR in solutions at 0.15 M ionic strength (d). Lines connecting points are guides to the eye.

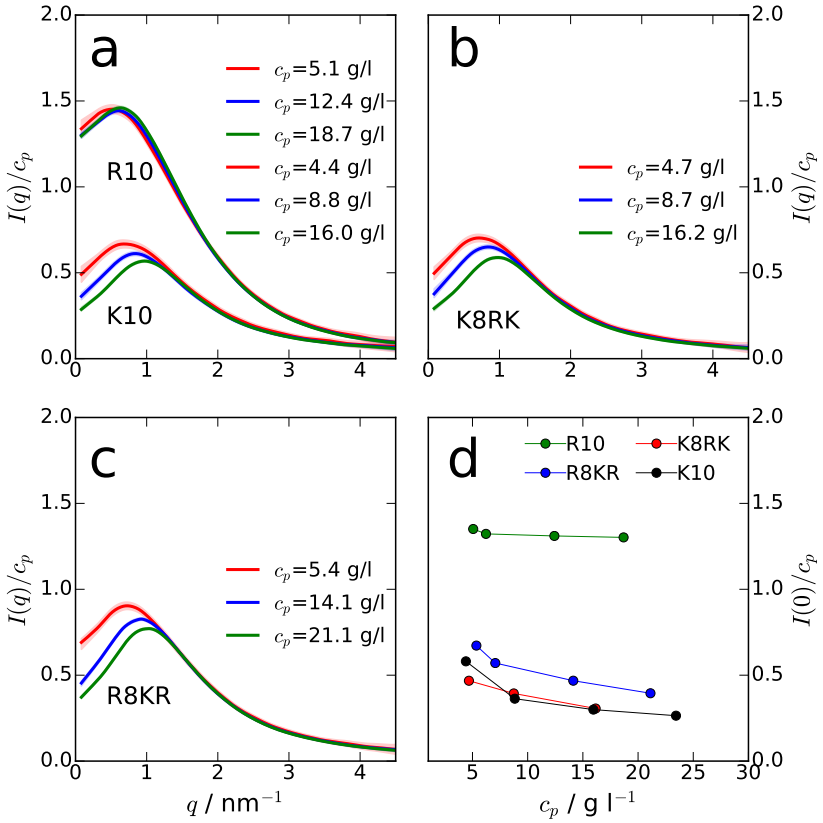

Figure 2: Small-angle X-ray scattering experiments for solutions of various peptide concentrations, c_p, in Tris buffer pH 7.8 and ionic strength 0.025 M at 293.15 K. Concentration normalized scattering curves $I(q)/c_p$ are shown as a function of the scattering vector, q, for solutions of R10 (a), K10 (a), K8RK (b) and R8KR (c). Scattering intensities are extrapolated at zero angle and reported as a function of c_p for R10, K10, K8RK and R8KR in solutions at 0.025 M ionic strength (d). Lines connecting points are guides to the eye.

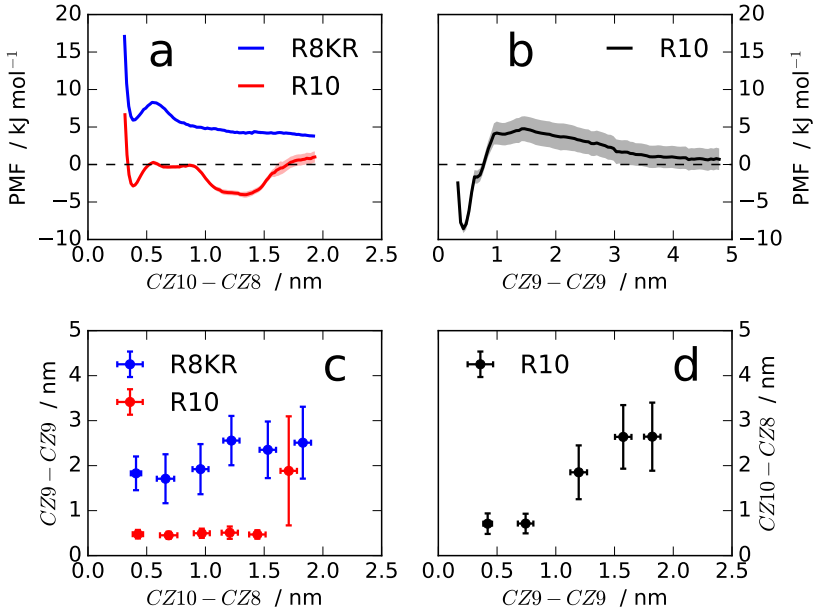

Figure 3: Potentials of mean force calculated from umbrella sampling molecular dynamics simulations for pairs of R10 and R8KR molecules at c_s=58 mM as a function of the separation between the guanidino-C atoms of the eighth and tenth residues (a), and the ninth residues (b). Shaded areas along the PMFs represent standard deviations of bootstrapped free energy profiles. Unbiased averages of $CZ9 - CZ9$ separations are calculated from simulation trajectories where the $CZ10 - CZ8$ distance is constrained (c), and *vice versa* (d).

Outlook

We are planning to complement our SAXS and simulation results with NMR experiments which are going to clarify the details of the interactions between R10 molecules. The SAXS data collected at BM29 at ESRF in Grenoble (MX-1763 and MX-1908) has already been included in a manuscript that will be soon submitted to a high impact journal. To our knowledge, this study has not been published before, and it is a step towards a more thorough understanding of the role of arginine in peptide-peptide and peptide-biomembrane interactions.

Acknowledgements

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References

1. Li, W.; Persson, B. A.; Morin, M.; Behrens, M. A.; Lund, M.; Oskolkova, M. Z. Charge-Induced Patchy Attractions between Proteins. *The Journal of Physical Chemistry B* **2015**, *119*, 503–508.

2. Mitchell, D. J.; Kim, D. T.; Steinman, L.; Fathman, C. G.; Rothbard, J. B. Polyarginine enters cells more efficiently than other polycationic homopolymers. *The journal of peptide research : official journal of the American Peptide Society* **2000**, *56*, 318–325.

3. Mason, P. E.; Brady, J. W.; Neilson, G. W.; Dempsey, C. E. The Interaction of Guanidinium Ions with a Model Peptide. *Biophysical Journal* **2007**, *93*, L04–L06.