Thesis Summary:

I aimed to provide a more fine-tuned understanding of the charge regulation mechanism by systematically investigating the charge regulation in weak polyampholytes using a simple coarse-grained bead-spring model in Langevin dynamics simulations.

The systematically investigated charge regulation in weak polyampholytes is as follows:

Objective 1: Charge regulation in short peptides[1,2]

I initiated my research by systematically investigating the impact of $\mathrm{p}K_\mathrm{A}$ values on short peptides. I performed CG simulations of various combinations of amino acids and calculated the total charge on various peptides. I also performed CG simulations of two types of peptide sequences: diblock and alternating, each of which consists of an equal number of amino acids with acid and base side chains. The specific choice of amino acids in various peptide sequences was determined based on the difference between the $\mathrm{p}K_\mathrm{A}$ values of the acid and base groups, denoted as $\Delta\mathrm{p}K_\mathrm{A} = \mathrm{p}K_\mathrm{A}^\mathrm{base} - \mathrm{p}K_\mathrm{A}^\mathrm{acid}$. We observed that $\Delta\mathrm{p}K_\mathrm{A}$ plays a key role in the total charge and the degree of peptide ionizable groups in the whole pH range. We observed two inflection points in the $\mathrm{Lys}_5 - \mathrm{Asp}_5$ peptide, at $\Delta\mathrm{p}K_\mathrm{A} \gg 0$. This observation suggests that the ionizable groups experienced separate changes. However, we did not observe any inflection point in the $\mathrm{Glu}_5 - \mathrm{His}_5$ peptide, at $\Delta\mathrm{p}K_\mathrm{A} \approx 0$, indicating that the ionizable groups experienced simultaneous changes. In addition, we observed that these simulation results show significant deviations from the Henderson-Hasselbalch (HH) equation due to electrostatic interactions between the charged groups. Moreover, our simulation results show that altering the sequence of amino acids while preserving the overall composition led to nearly indistinguishable ionization responses in diblock and alternating sequences. Finally, in collaboration with experimental colleagues from our department, Miroslav Štěpánek group, we successfully demonstrated the accuracy of our simulation model in predicting the charges of different peptides. This validation was achieved by comparing the simulation results with experimental findings. The excellent agreement between simulations and experiments confirmed that our simulation could be reliable and useful for predicting peptide charge behavior at given pH.

Objective 2: Charge regulation in peptides interacting with polyelectrolytes[3]

In this study, I aimed to address a perplexing research question: why are proteins attracted to polyelectrolytes on the "wrong side" of their isoelectric point, despite possessing like charges? To understand this phenomenon, I conducted a CG simulation of the same peptide sequences used for Objective 1 interacting with star-like polycations. Our simulation results showed that peptides could adsorb to the polyelectrolytes on the wrong side of their isoelectric point due to charge regulation or charge patchiness

mechanism. We observed that $Glu_5 - His_5$ peptide adsorb to polyelectrolytes due to the charge regulation mechanism, and $Lys_5 - Asp_5$ peptide adsorb due to the charge-patchiness mechanism on the wrong side of the isoelectric point. These observations are further confirmed by looking at charge distribution as a function of distance from center of the star. Furthermore, we compared diblock (patchy) and alternating (non patchy) peptide sequences that maintained the same overall amino acid composition to investigate the specific contributions of the charge patchiness and charge regulation mechanisms. Our observations revealed that the patchy sequences consistently exhibited adsorption on the wrong side of the isoelectric point, regardless of the ΔpK_A value. However, we discovered that the presence of charge regulation significantly enhanced the adsorption process. In contrast, the nonpatchy sequences only demonstrated adsorption on the wrong side of the isolelctric point when the $\Delta pK_A \approx 0$. Therefore, we can conclude that the charge regulation mechanism makes charge inversion during adsorption possible. These findings shed light on the intricate mechanisms that govern protein-polyelectrolytes interactions. In collaboration with Fernando Barroso from the University of Sao Paulo in Brazil, we developed a generic framework which can be used to assess the role of charge regulation and charge patchiness in protein-polyelectrolytes interactions based on the outcomes of our peptide-polyelectrolytes simulations. This research has the potential to inform the development of new strategies for drug delivery and related applications.

Objective 3: Charge regulation in polyzwitterions[4]

In an effort to understand charge regulation in more complex In an effort to understand charge regulation in more complex polyampholytes systems, I extended my research to polyzwitterions. These polymers consist of monomers that each contain both acidic and basic functional groups, making them a challenging system to study. To investigate whether our simple CG model, which we previously used for peptides, could be applied to polyzwitterions, I performed CG simulations on several polyzwitterions, including poly(2-(imidazole-1-yl)acrylic acid) (PImAA), poly(N,N-diallyl glutamate) (PDAGA), and poly(dehydroalanine) (PDha). Consistent with our findings in the peptide simulations, we observed significant deviations from the HH equation in the net charge per monomer group for the different polyzwitterions due to electrostatic interactions. Finally, in collaboration with Felix Schacher's group, who synthesized the polyzwitterions and provided experimental data, we demonstrated that our CG model could reliably predict the charge behavior of certain polyzwitterions, such as PDAGA and PDha. However, our model failed to accurately predict the charge behavior of PImAA, likely due to the contribution of other specific interactions. We have also shown that the equivalence point recognition criteria (ERC), often used in potentiometric titrations, cannot be used to directly get the effective pK_A values for these polyzwitterions. Overall, this research advances our understanding of polyampholytes behavior and has the potential to inform the development of new materials and technologies.

References

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