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Report on the dissertation thesis by Raju Lunkad, enrolled in the study programme Physical Chemistry at the Faculty of Science, Charles University.

Summary of the thesis:

The thesis is divided into two parts. The first part consists of 7 main chapters that include the introduction/motivation to the work, a brief overview of the theory behind the ionization of weak groups as well as the principles of molecular simulations, a summary of the results of four articles presented in three chapters, and a summary and outlook. The second part of the thesis consists of a collection of four papers published between 2021 and 2022.

The work of the PhD candidate is based on molecular modelling, using coarse-grained models, to study the acid-base ionization equilibrium in peptides and how this equilibrium is affected by the distribution of the ionizable groups within the peptides and by the interaction of these peptides with other molecules. The charge regulation in polyzwitterions was also investigated. The developed coarse-grained models were validated with experimental methods and used to rationalise the titration behaviour of peptides and of polyzwitterions when electrostatic interactions were predominant.

Despite the simplicity of the models, the work by Lunkad could be used to predict peptide charge at a given pH. He has also shown that the equivalence point recognition criteria cannot be applied to extract the effective pK_A values and that these should be extracted using the inflection points of the net charge of the polyzwitterions as a function of pH instead.

In addition, Lunkad very elegantly rationalized why proteins adsorb to polyelectrolytes on the wrong side of the isoelectric point (protein charge distribution vs. protein charge regulation).

Assessment of the scientific value of the thesis and PhD candidate contribution:

The work presented in the thesis is scientifically sound, as demonstrated by the fact that it has been published in well-established journals with a high impact factor within the field, of which I highlight the article in JACS.
The candidate is the first author in all four articles that the thesis is based on, and his role in each of the publications is clearly explained in the thesis.

Assessment of the language and form

The thesis is well structured. It has a very appealing design and a good usage of figures that guide the readers through the concepts that are discussed throughout.

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The aims and objectives of the thesis are clearly described in the "Introduction" and nicely linked to the "Summary". At the same time, I think Lunkad could have been more ambitious in the formulation of his objectives. From the text in pages 9 and 10 one reads that objective 1 was "to understand" and objective 3 was "to study", when the work presented in the respective chapters show a larger impact than just that. Just a small, related comment, the statement of an objective should start with a verb/action.

There are a few mistakes and inconsistencies throughout the thesis, both in the text and in some equations. However, these typos are not present in the published work, so they did not affect the results and conclusions presented on the thesis. Further, I believe that these mistakes do not affect the overall comprehension of the thesis. Nevertheless, here is a list of some of them:

- In Eqs. 2.3, 2.5, 2.7, 2.9, and 2.11 the symbol v_i is used instead of ν_i.
- The $c^{\ominus}$ is missing in the third term of Eq. 2.8 and a minus sign is missing in the superscript of the last term.
- The Velocity-Verlet scheme described in page 25 does not look entirely correct (see question below).
- The first term on the right-hand side of Eq. (3.13) as an extra δt and the second term includes the particle velocity instead of the acceleration. Note that the units do not match.
- The first term on the right-hand side of Eq. (3.14) contains Δ instead of δ, and a δt term is missing in the second term.
- In Section 4.3, there is a typo in the description of the two $\Delta \mathrm{p}K_\mathrm{A}$ cases, where $\Delta \mathrm{p}K_\mathrm{A} \gg 0$ and $\Delta \mathrm{p}K_\mathrm{A} \approx 0$ are swapped.
- In section 5.3.2, the discussion of the two cases of $\Delta \mathrm{p}K_\mathrm{A}$ (similar too or very different than) is not consistent with the results shown in the respective figures 5.9 and 5.10. However, the conclusion in the end of the section is correct.
- In the end of page 59 and beginning of page 61 is mentioned that "This indicates that both these peptides adsorb on the star […] at pH $\geq$ pI […] as well as […] at pH $\lesssim$ pI". This describes the whole pH range, and it is not correct as desorption will occur at pH $\ll$ pI.
- Other small details: the list of symbols should be provided in alphabetic order and figure 5.6 is called after figure 5.7.

Suggested question for the dissertation:

- In the first paragraph of Section 1.1.1, a recent study on the interaction between SARS-CoV-2 and heparin sulfate is mentioned. It is also stated that knowledge about weak ampholytes can contribute to understanding the binding mechanisms of the coronavirus. What is the relevance of the heparin sulfate binding to SARS-CoV-2? Why does the knowledge of weak polyampholytes contribute to this problem?
- Regarding Eq. (3.8) it is mentioned that F_i^D and F_i^R are a dissipative force that represents the effect of the solvent and a random force, respectively. It is inferred from the sentence that F_i^R does not represent the effect of the solvent. Why is it introduced? Could you be more specific about the role of the two terms, without getting into too much detail?
- In the description of the Velocity-Verlet scheme, section 3.2.2 in page 25, why are two velocity steps calculated in sequence and why is there no calculation of the force?
- Regarding the work described in Chapter 4 and the differences between alternating and diblock peptides: It is concluded on the top of page 46 that "the total charge on the alternating peptide

deviates slightly more from the HH equation compared to the diblock peptide because of the weaker effect between acid and base charged groups". Is it counterintuitive to me that a weaker effect will deviate more from an ideal case. Could you expand on this?

- The approach adopted in Chapter 5 to separate the contributions from charge patchiness and charge regulation, using peptides as simple protein models was very elegant. My question goes to the choice of partner: Why did you chose to work with a star-shaped polyelectrolyte?
- It is mentioned in the top of page 85 (chapter 6.4) that the imidazole group of PImAA may exhibit hydrophobic attraction or hydrogen bonding. How do you expect each of these types of interactions to affect the titration curve?

Final evaluation

Based on the considerations written above, I recommend the thesis to be defended for the title of PhD within the study programme Physical Chemistry at the Faculty of Science, Charles University.

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