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Review of the Ph.D. thesis of Fatemeh Fadaei

The candidate, Fatemeh Fadaei, has submitted a cumulative Ph.D. thesis comprising three scientific publications in peer-reviewed journals with impact factors of five and above, where she was the first author of two of them.

Chapter 1

The candidate starts with the motivation of her thesis. The behavior and properties of biomolecules in solutions are affected by solvation, particularly by water, which surrounds and interacts with biomolecules. Adding salts and co-solvents, such as ionic liquids, can alter the interaction of water with biomolecules. A so-called Hofmeister series characterize their effects. Although many publications have already reported on several aspects of the Hofmeister series, the respective findings are mostly restricted to proteins and not DNA and common ions and ionic liquids but not so much on deep eutectic solvents, which are the topics of the present thesis.

The methodology used in the thesis involves classical molecular dynamics simulations within the GROMACS program package. The chapter describes the fundamentals of the force field, the propagation of molecules under periodic boundary conditions, and the statistical ensembles used to relate the microscopic qualities of particles to macroscopic parameters such as temperature, pressure, and volume. The candidate demonstrates a significant understanding of molecular dynamics simulations, their possibilities, and their limitations. For the trajectory data analysis, the candidate explains standard properties such as the radial distribution function, the root-mean-square deviation, and the root-mean-square fluctuation. The radial distribution function characterizes the structure of the salts, water, and co-solvent at the protein's surface or in the bulk phase. In contrast, the root-mean-square deviation and the root-mean-square fluctuation characterize the stability of the protein or DNA strand.

In section 3, she describes the biomolecules under investigation. DNA has been recognized as a versatile molecule in nanotechnology due to its unique properties, such as miniature scale, sequence programmability, and highly specific molecular recognition. DNA can be used to construct novel nanomaterials, including drug delivery systems, and enable single-molecule observations in biology. The right-handed double helical structure of DNA is the most commonly known, and the stability of DNA is dependent on the hydration shells around the DNA. It can be affected by solution conditions

such as pH, temperature, and ionic strength. The DNA melting temperature value can be used to investigate the impact of solution conditions on the stability of the double-helical structure of DNA. To understand the function of proteins, we need to understand their molecular structure. The secondary structure is formed by the local packing of the polypeptide chain into α-helices and *beta* sheets, while the tertiary structure is formed by the interactions between these domains, including H-bonds, disulfide bridges, ionic bonds, and hydrophobic interactions. The quaternary structure is defined by how multiple polypeptides interact to form a single, larger, biologically active protein.

The thesis then explores the effect of ionic liquids and deep eutectic solvents on the properties of biomolecules, as co-solvents play a crucial role in the functioning of biological systems. While several studies have been conducted to understand the effect of salts on biomolecules in aqueous solutions, further research is needed to fully understand the molecular-level effect of solvents on biomolecule behavior in mixed aqueous solutions. In particular, ionic liquids and deep eutectic solvents are very complex co-solvents in this context, as they comprise several molecular species. The candidate also summarizes type I- III deep eutectic solvents and their properties. Here, an overview figure describing the differences between the types of deep eutectic solvents would have been helpful. The student also explicitly mentions amino acid-based and magnetic ionic liquids, which are less common than the classic combination of imidazolium-based cations and inorganic anions.

The candidate concludes with a literature summary on the Hofmeister series of simple ions on the biomolecules of interest. However, I would have appreciated extending this summary to ionic liquids, e.g., Haberler et al. [JCTC 2012, 8, 3911], Klähn et al. [PCCP 2011,13, 1649 or PCCP 2011, 13, 18647] or Schwierz and co-workers [Langmuir, 2020, 36, 5979] for RNA interactions.

Overall, the introduction is well-structured, logically presented, and clearly written. The candidate demonstrates a sound research methodology and appropriate data analysis techniques. She has a significant understanding of molecular dynamics simulations, their possibilities, and their limitations and has contributed new knowledge to the field.

Chapter 2-4

Due to the numerous open questions that persist within this research field, the present thesis is a valuable contribution to the ongoing discourse. The thesis showcases original research, and I particularly appreciated the collaboration with experimental groups.

The first paper examines the capacity of imidazolium-based ionic liquids to stabilize the native structure of nucleic acids, specifically for DNA storage and handling in biotechnology. The researchers employ synchrotron-UV Resonance Raman spectroscopy and molecular dynamics simulations to investigate the binding interactions between ionic liquids and DNA. Their findings reveal that ionic liquids preferentially bind to the DNA grooves, with a preference for guanine residues and shorter alkyl-chain length on the imidazolium cation. Raman experiments demonstrate cooperative transitions and reversible pre-melting structural transformations involving specific DNA tracts, with adenine-rich tracts exhibiting a strong interaction with chloride anions. Molecular dynamics simulations suggest that electrostatic

and hydrophobic interactions of short alkyl-chain imidazolium cations have a stronger interaction with the DNA major groove.

The second study investigates the effects of imidazolium chloride on a synthetic DNA oligonucleotide composed entirely of T/A bases using fluorescence emission of the groove-binding fluorophore 4,6-Diamidin-2-phenylindol (DAPI) and molecular dynamics simulations. The results show that the ionic liquid primarily binds to the DNA minor groove at low concentrations, while the phosphate regions and major groove binding sites become essential contributors to the complete set of ionic liquid-DNA duplex interactions at higher concentrations. The study also reports on changes in spectral shifts, broadening, and fluorescence lifetimes for DAPI ionic liquid solutions and MD radial distribution functions, which reveal dominant π-π stacking interactions between the imidazolium ring at lower ionic liquid concentrations and electrostatic and hydrophobic interactions at higher ionic liquid concentrations.

The third publication utilized molecular dynamics simulations to investigate the effect of aqueous solutions of selected ionic liquids on the structure and dynamics of Ideonella sakaiensis PETase with bis(2-hydroxyethyl) terephthalate substrate. The objective was to identify the potential use of specific ionic liquids to enhance the enzymatic hydrolysis of polyethylene terephthalate, as these ionic liquids are known to dissolve PET partially. The results showed that the aqueous solution of cholinium phosphate had the slightest effect on the structure of PETase, and its interaction with terephthalate as a substrate was comparable to that with pure water. Therefore, cholinium phosphate was identified as a possible candidate for use as an ionic liquid co-solvent in studying the enzymatic hydrolysis of PET. In particular, the last topic is of current general interest because it addresses the problem of plastic pollution, which is a global environmental issue. PET is an ordinary plastic used in bottles, clothing, and packaging materials, and it can take hundreds of years to decompose in the environment. The candidate presented a promising avenue for developing more sustainable solutions to plastic waste management.

Chapter 5

The concluding chapter provides a comprehensive overview of the primary outcomes of the three scientific publications. The employed theoretical methods are deemed appropriate for addressing the corresponding scientific inquiries. The outcomes have been meticulously scrutinized, and the author's lucid and astute elucidations have offered a valuable contribution to the ongoing dialogue. The candidate has demonstrated a profound comprehension of pertinent literature and theories in the research domain and has exhibited the ability to amalgamate this knowledge into the research project. This thesis fulfills the quality standards of the University of Vienna for doctoral theses. The candidate has demonstrated remarkable proficiency in simulation design, execution, data analysis, and deducing meaningful inferences. Consequently, I highly recommend this Ph.D. thesis for defense.

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Reviewer's report on doctoral thesis of Fatemeh Fadaei

The doctoral thesis of Ms. Fatemeh Fadaei entitled "Biomolecular simulations to non-aqueous media" is devoted to molecular simulations of DNA and enzymes in various solvents. It addresses how the components of solutions affect the structure and functioning of investigated biomolecules. The thesis is based on three publications in international peer-reviewed journals which have already resulted from this work. The candidate is the first author of two papers, her substantial contribution to all of them is clearly stated.

The introduction starts with a brief summary of studies of the effect of inorganic salts or ionic liquids on properties of biomolecules. This shows that the candidate has a good knowledge about the state-of-the-art in this field. The section about classical molecular dynamics describes the methodology at the level of usual tutorials, but it does not provides specific details or perhaps issues that the candidate had to face during the work on her projects. However, the details can be found in the attached publications. I appreciate the text about potential technological applications of DNA. The general paragraphs about biomolecules are followed by an overview of ionic liquids and ion-specific effect on proteins. I would appreciate more details about the motivation of the choice of the model systems. The last part of the "Hofmeister series" is a bit confusing, because it contains description of recent results obtained by the candidate (references [22, 23]) which might better fit in the following chapters.

Chapters 2 - 4 seem to me like attachments of the three publications. Chapter 5 provides summary of the most important findings. It is not clear from the text how are individual chapters connected, whether there are any general conclusions and what are the future directions. It is a pity that the candidate missed the opportunity to show her ability to write independently and to formulate her opinion. However, I am aware of the fact that this is the accepted format of a Ph.D. thesis. Ms. Fatemeh Fadaei will for sure express her own ideas during the defense.

Questions:

1. What are the most common ionic liquids used for dissolving proteins? How is their enzymatic activity then affected? What are the advantages of hydrated ionic liquids with respect to pure ionic liquids or water?
2. It was not easy for me to find how you obtained the initial configuration of DNA for the simulations in Chapter 2.
3. RMSD of 0.6 nm in Figure 8 seems large in comparison with values around 0.1 nm that are not uncommon for proteins. What is the reason?
4. Can you comment the asymptotic behaviour of the RDFs in Figure 12? Is the solution homogeneous?
5. How reliable is the empirical force field for the description of the π–π interactions in Chapter 3?

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6. Do you expect that the results obtained for the A/T DNA in Chapter 3 will be applicable for DNA of any composition? What is the advantage of A/T in comparison with the Dickerson dodecamer?
7. Can you formulate general conclusions connection all three publications?

I read the thesis and I recommend it for the defense.

Sincerely,

[redacted]

Dr. Eva Krupičková Pluhařová
J. Heyrovský Young Scientist