REVIEW OF THE OFFICIAL OPPONENT

on the dissertation
"Computer simulations of polymer-enzyme complexes and
chelate structures of heavy metal ions"
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Applied for the degree of Doctor of Philosophy
104 – Physics and Astronomy

The development of technologies for removing toxic substances, such as heavy metals and molecular contaminants, from water is a complex and pressing challenge that requires both experimental and theoretical advances. Achieving significant progress in this area necessitates the creation of new computational methods and models, as well as the integration of simulations across multiple levels of molecular organization, from electronic to atomic and mesoscopic scales. In this context, the research conducted within the framework of the dissertation "Computer simulations of polymer-enzyme complexes and chelate structures of heavy metal ions" by Halyna Butovych represents a highly relevant and timely contribution.

The present dissertation is dedicated to the study of complex formation of polymers with the enzyme and the chelation of heavy metal ions by small polymers and ligands. On the basis of the detailed computational study of the polymer-enzyme systems the binding degree between the polymer and enzyme was determined and the details of molecular mechanisms of complex formation were described. The developed model can be used for the computer simulations studies of biofuel production. The molecular mechanisms of chelation of Hg^{2+}, Cd^{2+} and Pb^{2+} ions by polyethyleneimine (PEI) and ethylenediaminetetraacetic acid (EDTA) were studied and the optimal characteristics for the complex formation were established. Thus, the research, conducted within the framework of the thesis by H.A. Butovych, has clear results, which are important for the understanding of fundamental physical problems, as well as for the development of green technologies. Such a combination is an essential advantage of the present research work.

Halyna Butovych performed here research work at the Institute for Condensed Matter Physics of the National Academy of Sciences of Ukraine and Lappeenranta-Lahti University of Technology, Lappeenranta, Finland. The studies were conducted within the framework of the projects of the NAS of Ukraine in 2021-2024 (project No. 0120U100202), National Research Foundation of Ukraine in 2021-2023 (project No. 2020.02/0317), Finnish National Agency for Education (EDUFI Fellowship for doctoral students from Ukraine, reference number OPH-4602-2022), Lappeenranta-Lahti University of Technology, Scholarship of the President of Ukraine for young scientists (2024-2025, Ukraine). Extensive calculations for the research work were performed using the Finnish IT Centre for Science.

The dissertation consists of introduction, four chapters, conclusions, appendices and bibliography. The bibliography consists of 166 references. The volume of work is 130 pages.

Introduction substantiates the relevance of the chosen research topic, formulates the purpose of the work, the main objectives, the object and subject of the research, lists the research methods, indicates the novelty of the results obtained and their practical significance, indicates the applicant's contribution to the research, provides information on testing and publication on the topic of the dissertation.

The first chapter provides a literature review on molecular complexation, focusing on two main topics: the formation of polymer-enzyme hybrids and the chelation of heavy metal ions by molecules such as polyethyleneimine (PEI) and ethylenediaminetetraacetic acid (EDTA). Through a critical review of the current literature, it was shown that both areas remain underexplored, highlighting the need for further research and model validation.

In the second chapter a simplified model is proposed for the description of the physical mechanism of interaction of a bottlebrush-like polymer scaffold with globular enzymes. Within the framework of the model the case of a single binding site per enzyme was considered. Using the Langevin dynamics simulations and Wertheim first-order thermodynamic perturbation theory, the degree of binding efficiently was studied. The differences between terminal and intermediate polymer monomers were taken into account. The binding is analyzed as a function of the number of polymer functional groups under conditions where the total functional groups equal the number of enzyme molecules and packing fractions are matched. The results demonstrate that the binding degree between polymer and enzymes is highly sensitive to the number and distribution of functional groups on the polymer chains, with steric constraints limiting binding efficiency at high functionalisation levels.

In the third chapter the atomistic molecular dynamics simulations and quantum-chemical calculations for the linear polyethyleneimine (PEI) with mercury ions (Hg^{2+}) in aqueous solution have been carried out. The goal was to determine the molecular mechanism of ion chelation by the polymer. The OPLS/AA force field in atomistic molecular dynamics and density function theory (DFT) in quantum-chemical calculations were used. The microscopic configurations of the complexes formed between PEI chains and Hg^{2+} ions were analyzed, considering different PEI chain lengths. The results show that a single linear PEI chain with ten amino groups can bind up to four Hg^{2+} ions. At the same time, the most stable is the complex of PEI-5 with 1 mercury ion. The stability of these complexes is further supported by density functional theory calculations.

In the fourth chapter the study of interaction of EDTA with divalent heavy metal ions (Hg^{2+}, Cd^{2+}, and Pb^{2+}) has been performed. The atomistic molecular dynamics simulations and DFT calculations were used. The methodology used here was the same as in the study of PEI-Hg^{2+} complexes. The study finds that fully deprotonated EDTA ($EDTA^{4-}$) forms the most stable six-coordinated complexes with Me^{2+}, while increasing protonation weakens binding due to reduced negative charge and hydrogen occupation of coordination sites. It means that the efficiency of chelation of the considered metals by EDTA will be in the conditions of high pH (≈14). Water molecules can enter the coordination shells of partially protonated complexes, especially for Pb^{2+}, affecting stability. Gibbs free energy calculations and charge analysis indicate that Hg^{2+}

complexes are the most stable, followed by Cd^{2+}, with Pb^{2+}. Overall, the combined computational approach highlights the importance of protonation state, metal ion properties, providing insights for designing effective heavy metal removal strategies.

Among the results obtained in the dissertation, the following, in my opinion, have the greatest scientific value:

- The binding degree between polymer and enzymes was found to be highly sensitive to the number and distribution of functional groups on the polymer chains, with steric constraints limiting binding efficiency at high functionalisation levels.
- The molecular mechanisms of Hg^{2+} ion chelation by PEI chains of varying lengths were elucidated, revealing that a PEI-5 provides the highest chelation efficiency for a single ion.
- The role of EDTA protonation for the chelation of Hg^{2+}, Cd^{2+}, and Pb^{2+} metal ions was studied in details, and it was shown that fully deprotonated $EDTA^{4-}$ forms the most stable complexes with metal ions.

All the obtained results have been analyzed in detail. Their authenticity is ensured by the use of well-established methods of theoretical and computational physics. In particular, the methods of atomistic molecular dynamics, coarse-grained models and DFT calculations were used. At the same time, the analysis of the dissertation work gives rise to a number of comments:

1. The simulations of enzyme-polymer complex were performed using the reduced parameters (size of the molecules, temperature and others). This approach allows the results to be transferable to different temperatures with the main governing energy parameter ε_{csw}. However, in practice, when dealing with proteins, the applicable temperature range is rather narrow due to conformational changes and denaturation occurring at temperatures above 42 °C. This point could be discussed in the text by providing a concrete example with a specific temperature, for instance, 300 K.
2. The neutralization of the system in the molecular dynamics simulations of PEI and EDTA with the metal ions was done in a very specific way, where the counterions were fixed during the simulation at the maximal distance from the polymer. This approach to system neutralization is rather artificial. A more natural approach would be to add a certain concentration of salt (for example, 0.1 M NaCl) along with two additional Cl^- ions to neutralize Me^{2+}, and allow all ions to move freely.
3. In the molecular dynamics simulation of PEI and EDTA with Me^{2+} ions, the system was prepared so that the ions were initially placed close to the polymer to allow rapid complex formation. However, this approach raises questions about the efficiency of complex formation in a real system, because ion-polymer interactions require the formation of contacts with specific atomic groups.
4. In the initial stage, the molecular dynamics simulations of PEI and EDTA with Me^{2+} ions were performed in an implicit solvent. At this stage, the metal-polymer complex forms. However, in a real system, complex formation

requires partial dehydration of the ions, an effect that cannot be accounted for in implicit solvent simulations.

5. Some details of the simulation are described too generally. In particular the following.
   - In the Chapter 2, in the model of enzyme-polymer system, the origin of the factor 160.0 in the rigidity constant K, as well as the facto 1.5 in $\varepsilon_{phs} = \varepsilon_{csw} T^*/1.5$, is not clear. In the simulation protocol, it is unclear how the length of the simulation trajectory was determined to be sufficient, particularly during stages 1 and 2.
   - The description of the DFT calculations does not specify whether the basis set superposition error (BSSE) was taken into account.
   - From the text, it appears that the free energy of the complex was calculated in the same way for both atomistic molecular dynamics simulations and density functional theory. However, it is not discussed whether there are any differences in the energy calculation between these two methods.

However, these comments do not call into question the reliability of the main results of the work, as well as the conclusions drawn on their basis, and do not affect the overall essentially positive assessment of the work.

The main results of the work are fully published in 3 articles: 1 article in the Journal of Molecular Liquids (Q1), 1 article in Physical Chemistry Chemical Physics (Q2), and 1 article as a preprint on arXiv. The journal articles are already cited (3 citations of JML and 5 citations of PCCP papers) reflecting the high quality and importance of the published scientific results. H.A. Butovych presented her research work at three conferences: EMLG-JMLG Annual Meeting 2024 in Trieste and two conferences held in Lviv. These meetings had very high scientific level. H.A. Butovych is the first author of all the publications disclosing her key contribution to the published work. The dissertation is written in a competent language. The content of the dissertation corresponds to specialty 104 – Physics and Astronomy.

Taking into account the relevance of the topic, its novelty, scientific and practical significance of the obtained results, I conclude that the dissertation “Computer simulations of polymer-enzyme complexes and chelate structures of heavy metal ions” satisfy the requirements of the “Procedure for Awarding the Degree of Doctor of Philosophy” approved by Resolution No. 44 of the Cabinet of Ministers of Ukraine on January 12, 2022. I have not found any violations of academic integrity in the dissertation and scientific papers in which the results of the dissertation were presented. The presented work deserves a positive assessment, and its author, Halyna Anatoliivna Butovych, deserves to be awarded the degree of Doctor of Philosophy in specialty 104 - Physics and Astronomy.

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