Opponent's Report on the PhD Dissertation
Computer Simulations of Polymer-Enzyme Complexes and Chelate Structures of Heavy Metal Ions
By Halyna Anatoliivna Butovych

The proposed dissertation presents the candidate's results in studying polymer-enzyme complexes and chelate structures of heavy metal ions using computer simulations, and quantum mechanical density functional theory. The results of the two methods are also compared to the results of Wertheim's first order thermodynamic perturbation theory, although the author does not list those calculations amongst her personal contributions of the PhD thesis, and it is from that aspect not clear why the results are described in such detail within the thesis.

In the first part of the thesis that is described in Chapter 2 the candidate used a simplified coarse-grained polymer and enzyme model to study the complexation of the two via Langevin dynamics. The candidate showed 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 the binding efficiency at high functionalisation levels. She further compared the results with those of Wertheim's first order thermodynamic perturbation theory, and its modification. Good agreement was obtained between simulation results and modified TPT.

In the second part of the thesis that is described in Chapter 3 the candidate studied the chelation of mercury ions with polyethyleneimines (PEIs) of different lengths using all-atom computer simulations in the combination with the quantum mechanical density functional (DFT) calculations. Her results show that linear PEI forms stable complexes with mercury ions, and the PEI chain with five nitrogen-containing units demonstrated the highest stability, achieving a balance between nitrogen availability and steric accessibility. The binding strength per ion gradually decreases with an increasing number of coordinated ions which is due to electrostatic repulsion between closely spaced metal ions and the reduced flexibility of the polymer backbone in extended conformations.

In Chapter 4 the candidate extends the chelation study of heavy metal ions (Hg^{2+}, Cd^{2+}, and Pb^{2+}), by EDTA, again, using a combination of all-atom computer simulations and DFT calculations, but with the focus on the role of ligand protonation. She showed that the most stable EDTA complex is formed with fully deprotonated EDTA complexes, particularly with Hg^{2+}, which is due to enhanced charge transfer and complete coordination. The candidate further showed that partial protonation weakens complexation by reducing available donor sites and promoting competition from water molecules.

The key results from the thesis have already been published in two scientific articles, one preprint, and 3 conference abstracts which also reflects the quality of the presented scientific work.

Never-the-less some parts of thesis could profit from additional clarifications.

- In Chapter 2 it is not clear from the TPT theory description (section 2.4) what is the modification, compared to the original TPT theory. In the scientific paper where the results were published (ref. 3) it is stated in the Introduction of the paper that the modification is in analytical description of the reference system which is not clear from the thesis. In section 2.7 the candidate on the other hand summarizes that the modification was in distinguishing between terminal and inner monomers along the polymer chain. Also, when describing the theory, $g^{(ref)}$ is stated to be the monomer-monomer pair correlation function, while it should be stated as a pair distribution function of the reference system.
- In Chapter 2 the candidate justifies the use of implicit solvent by using Langevin dynamics. While LD provides a stability for the system the implicit solvent cannot account for the hydration/dehydration effect that could modify the interaction in the system. The secindary role of solvent should be discussed.
- In Chapter 3 the candidate should discuss the possible effect of the force-field used on the obtained results.
- In Chapter 3, as well as in Chapter 4, the candidate during the simulation prevented the interactions between complexes and counterions, that in real systems do exist. The candidate should justify this methodology.
- There are some minor technical comments: in several places the scale is missing for the g(r) graphs, tMD sholud be just MD, the adsorption energies given in tables should be appropriately rounded, the term „van't Hoff equation“ is wrongly used, „lgβ“ notation should be „log K“ ...

To conclude, despite the above comments, my overall assessment of the work in the thesis, as well as the dissertation text, is fully positive. The candidate explored various theoretical methods, and appropriately applied them to study complex phenomena, contributing to their better understanding. The dissertation presents a new, valuable, contribution to the science of physics, and **its author Halyna Anatoliivna Butovych fully deserves the doctoral phylosophy degree in physics**.

B. Hribar Lee

Dr. Barbara Hribar Lee,
Full professor of physical chemistry,
University of Ljubljana, Faculty of Chemistry
and Chemical Technology, Slovenia

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1