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EVALUATION OF DOCTORAL THESIS

Thesis Title: Metal-Ion Selectivity from Quantum-Chemical Perspective
Author: Mgr. Ondrej Gutten
Supervisor: RNDr. Lubomir Rulisek
Reviewer: Prof. Todor Dudev, Sofia University, Bulgaria

I was delighted to read and evaluate the Ph.D. thesis of Mgr. Ondrej Gutten (also referred to as the candidate) dedicated to the application of theoretical methods in studying the metal-ion selectivity in metalloproteins. The topic is of high importance for biochemical/biophysical science as uncovering the factors that govern the metal selectivity in proteins would not only deepen one's understanding about the intimate mechanisms of metal-protein recognition, but also could help engineering novel metalloproteins and designing new drugs. The high citation rate of the four recently published original papers, the thesis is based on (28 independent citations according to the Web of Science), imply significant recognition and appreciation of the conducted research.

The thesis is very well written (in good English) and logically structured. It is a pleasure to read it. Dissertation work comprises 66 pages of written texts including 9 figures and 6 tables. The candidate has cited 68 literature sources. Full-text copies of the four papers are attached as well. The first two chapters of the thesis are dedicated to describing the basic biophysics behind the metal binding and selectivity in proteins. The effects of various internal and external factors influencing the process of metal-protein recognition are systematically assessed in a good story-telling manner. Describing the thermodynamic principles and quantum-chemical approaches

employed in modeling biochemical/biophysical processes is the subject of the next two chapters of the dissertation work. The candidate is well acquainted with the literature on the subject and demonstrates deep understanding of the "philosophy" of theoretical modeling of chemical/biological processes. There are typographical errors here and there but these do not affect the good impression from the narrative presented.

Chapter 5 is the core of the dissertation work where the scientific results of the candidate are summarized. These are presented in a bit economical manner and one has to read the attached original papers in order to get better appreciation of the merits of the candidate's findings. In the first two papers, an exhaustive search for the most suitable methods warranting reliable evaluation of the binding properties (in both the gas-phase and condensed media) of a large series of divalent transition metals (Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Hg^{2+}) has been performed. Various computational methods and model systems have been considered. Contributions from different sources to the accuracy of the computations have been meticulously analyzed. As a result, the BP86 method (with TZ basis set) in combination with COSMO-RS solvation model have been found to be the best suited for the purpose. The calibrated protocol has been then applied in studying the processes of transition metal binding/selectivity in cyclam derivatives (paper III) and metallopeptides (paper IV). The calculations provide new insights about the mechanism of metal complexation in these systems and the role of various factors (e.g. structure and composition of the binding site, chemical character of the first and second coordination shell ligands, and properties of the metal itself) in the process of metal competition. All the papers are published in international reputable journals. The candidate is the first author of three of them and, I assume, has significant contribution to both carrying out the projects and writing the manuscripts.

In conclusion, the thesis of Mgr. Ondrej Gutten, represents a multifaceted, well-planned and thoroughly executed scientific endeavor. The candidate is a promising young researcher possessing knowledge and skills to conduct a high-level research. He has applied a state-of-the-art techniques/methodology to solve non-trivial scientific problems. The results obtained could be classified as novelties in the scientific research. They set the foundations for future investigations of the processes of metal binding and selectivity in biological systems and the application of computer modeling in rational design of metalloproteins with pre-programmed properties. My opinion on the scientific merits of the dissertation work is highly positive and, thus, I recommend

that the thesis be accepted as a basis for the Ph.D. defense and ultimately for awarding the Ph.D. title to Mgr. Ondrej Gutten.

Questions

1. Paper II: Were the calculated vibrational frequencies and the ZPE/thermal energies scaled and, if so, by what factor? Were the calculated energies/free energies corrected for the BSSE contributions?
2. Papers II and IV: The contributions from ZPE energy and molecular entropy to the free energy have been neglected (eqs. 1b and 1, respectively). Is there any numerical data justifying this approximation?
3. Paper III: In the first stage of the complex formation, the host cyclam molecule forms an out-of-cage intermediate with the Cu^{2+} cation bound to the phosphorus-containing arm. Then, very interestingly, the system quickly rearranges itself into an in-cage structure with the metal predominantly coordinated to the nitrogen-containing ring. What is the driving force for such a rearrangement?
4. Paper III: The cyclam derivatives exhibit higher selectivity for Cu^{2+} over Ni^{2+} and Zn^{2+}. What factors do make the Cu^{2+} the species of choice for the host system?

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