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Re: A supervisor's assessment of the diploma (MSc.) thesis of Bc. Petr Eminger

The diploma thesis of Bc. Petr Eminger entitled "Computer modeling of the catalytic activity of coupled binuclear copper enzymes" describes candidate's efforts in the field of QM/MM modeling of metalloenzymatic reactions.

Petr joined the group in October 2022. He immediately became involved in the project(s) dealing with the catalytic activity of the coupled binuclear copper (CBC) enzymes. Over the last decade, an intense collaboration between our group and the experimental group of Prof. Solomon (Stanford University, U. S. A.) lead to a deep insight into the reaction mechanism of tyrosinase (Ty), a member of the CBC family of enzymes. This has been accomplished by correlating experimental (mostly kinetic and spectroscopic) and state-of-the-art theoretical (computational) data which nicely complemented each other.

Petr's task was to utilize our knowledge obtained previously for Ty (materialized in the Ph.D. thesis of Mgr. Inž. Agnieszka Stańczak) and compare the other two CBC enzymes, *o*-aminophenol oxidase (specifically NspF) and catechol oxidase (represented by the polyphenol oxidase 6, PPO6), with tyrosinase. The task represented initial steps in the longer-term goal which is to fully understand factors that govern the varying chemoselectivity of the CBC enzymes.

Petr has, to a satisfactory degree, mastered the QM/MM methodology needed to accomplish the goals of his diploma project. He also understood the need to use QM/MM approach instead of 'simpler' QM-only method for studying biomacromolecule, especially metalloenzymes are particularly difficult to model. This is, in my eyes, a solid achievement, *per se*, as this is a non-trivial (computational) methodology, prone to many errors and computational artifacts. At the same time, he understood the role of spin states in catalytic action of metalloenzymes; he was able to use various analyses of the fairly complex molecular electronic wave functions (or Kohn-Sham determinants) and perhaps obtained certain feeling

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for the accuracy of the computed values. The latter is one of the most important qualities that may distinguish experienced computational chemists from the educated users of the quantum chemical programs. Major conclusions of Petr's work are in a qualitative agreement with the previous experimental observations and provide a solid basis for further experimental and computational investigations of structure/function relationship among CBC proteins.

The thesis is well organized, though I have found some sections that remained (after my comments and revisions) somewhat repetitive and at some place, the flow of login might have been clearer. Also, more space in this work could be devoted to discussing structural differences in the internal architecture of the active site of CBC proteins.

As for the candidate, I appreciated that he was working hard on accomplishing some of the goals of the thesis. He made visible methodological improvements throughout the year and half he had been working in the group. He had some gaps when it came to general knowledge of quantum chemistry and in his future career, he has to improve his writing abilities as well as the ability to work independently.

In summary, I consider the thesis of Bc. Petr Eminger as the solid piece of work with a potential impact in the field of CBC enzymes, once the data are correlated with planned experiments. I recommend awarding it with grade **2**.

Yours sincerely,

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