

The review of the diploma thesis of Bc. Petr Eminger by Martin Srnec; May 27th 2024 in Prague

The diploma thesis of Bc. Petr Eminger is focused on computer modeling of the catalytic activity of the selected coupled binuclear copper (CBC) enzymes. The thesis has a more or less classical structure. It first provides a brief overview of metalloenzymes and their chemistry, and further narrows the focus to CBC enzymes. This is followed by a section introducing basics of computational chemistry. After formulating the aims of the project and describing specifics of the applied methods, the major part of thesis brings the results and discussion on two CBC enzymes - their structures and catalytic properties. Specifically, the candidate presents a mechanistic study of two enzymes whose structures were generated using AlphaFold2 (not otherwise available experimentally) and whose catalytic activities were analyzed through the prism of a prototypical CBC enzyme – tyrosinase Ty – that was recently described in detail in the same group to which the candidate belongs. The project suggests that the enzymes differ in the energetics of the Ty-like reaction coordinate (which they should, since hydroxylation of monophenols has not been observed experimentally for either enzyme). In this respect, the thesis provides a good seed for future and more detailed and thorough studies. **Nevertheless, I have to criticize a few points that prevent me from rating the diploma with the highest mark. Considering the following facts, I recommend to grade the thesis with 2.**

From the formal point of view, I must state that there is a relatively large number of typographic errors. Also, the size of numbers and text in all tables is not uniform, which looks unprofessional. Some figures and tables provide the same information (eg. Table8/Figure21 or Table9/Figure24) and therefore one of them (Table or Figure) seems to be redundant. Similarly, Figure 12-2 is not very informative and may be incorporated into some later figures. This is also the case of some other figures. For example, the importance of the Figure 8 is really questionable. Why is it more important to show the LJ potential than the other parts of the force field? I also think that Figure 10 is badly placed. Also, some references to Figures that are not correct (eg. Page 38, reference to Fig. 16 seems to be fishy). Remember, perfection is in the details, but perfection is not a detail.

Second, I have to say that the thesis contains statements that are not correct, or well explained. To exemplify, I can mention (i) the presentation of the QM/MM subtractive scheme in eq 2.13 – the third term is not well described, (ii) the presentation of the S^2 operator (equation 2.4) – s_1 and s_2 operators are not explained (+ what if we have more than 2 electrons?), (iii) the separation of time and space from time-dependent Schrodinger equation is possible if the Hamiltonian (and therefore energy) is time-independent – the author claims the opposite. Also, the super-exchange mechanism for the coupling between two magnetic centers was invoked to lead to both ferro and antiferromagnetic arrangement, which is not true as the super-exchange is always antiferromagnetic. As for section 2.1, I think that it could be structured in a better way – now the intro on Schroedinger equation is followed by the short presentation of

the spin operators, which is followed by BO approximation. I don't think this is the best logic flow. Later, the relativistic $x2c$ is introduced – but the sentence is really taken from the air, as no single word in the sentence “explaining” what $x2c$ means is really explanatory.

Finally, I also have to admit (but this may be my fault) that it was not always easy to follow the structural changes (and their relation to energetics) along the respective reaction coordinates discussed in the text as they were not super well highlighted in the corresponding figures. In addition to that, some sentences are not clear/explicit. For example, page 42, the sentence „Therefore, instead of previously mentioned proton transfer, the 1O and 2O conversion should be referred to as hydrogen transfer instead.” – from this sentence and from the context it is not really clear what “previously mentioned proton transfer” is – is it in Ty? Or on page 51: “Hydrogen of these water molecules point to nearby atoms (in this case oxygens) with partial negative charges (Figure 23)” – this sentence is used to provide a possible factor making difference in 3O of two enzymes but I do not really follow as I see H-bonding pattern in Figure 23 that is similar in both enzymes.

In Table 4, I would recommend to do a finer scan for obtaining a better TS guess. Especially, the jump from 1.75 Ang to 1.9 Ang seems to me pretty big.

Here, let me stop to criticize and ask several questions that the candidate could try to address:

- 1) In Table 2, I found a very interesting difference in energetics for P/O conversion between NspF and PPO6. Do you have a plausible explanation for such a big change in energetics? Also, is there a difference in spin density on the bridging O (in the O intermediate)? Does it have any implication for different reactivity?
- 2) The value of $\langle S^2 \rangle$ deviating from the pure spin-state value reflects a so-called spin-contamination. Although this is definitely artificial feature of the unrestricted formalism, it is also sometimes claimed in the literature to “mimic” multireference character of the wavefunction. Based on the literature and your $\langle S^2 \rangle$, could you comment/discuss how much of the multireference character would have Cu2O2 active site in P vs. O? Does it correlate with some radical character on the bridging O?
- 3) Although it seems that reaction energetics will be specified in future studies, could it be better presented/reiterated/explained at the diploma defense why the NspF is expected to be so different from the other CBC enzymes (see Table 9)?

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