Evaluation of Doctoral Thesis of RNDr. Martin Srnec:

"Catalytic and Electronic Properties of Redox-Active Metalloenzymes and Transition-Metal Complexes: Insights from Computational Chemistry"

In this thesis, the candidate uses quantum chemical techniques to investigate a number of reactions of both metal-containing enzymes and metallocomplexes of biological and materials science interest. The thesis is based on the results reported in six attached papers, published or submitted for publication.

Both the popular density functional theory (DFT) approach and traditional *ab initio* techniques, such as complete active space self-consistent field (CASSCF) and multireference configuration interaction (MRCI) are used. In the enzyme modeling studies, mainly hybrid quantum mechanics/molecular mechanics (QM/MM) methods are employed.

The enzymatic systems studied are the manganese-dependent superoxide dismutase (MnSOD), stearoyl Δ^9 desaturase (Δ^9D), and multicopper oxidases (MCOs). These systems are admittedly difficult to treat computationally. Several catalytic proposals are considered and potential energy profiles for many spin states are constructed. For MCOs, both the internal electron transfer and the O-O bond cleavage processes are analyzed.
The transition-metal complexes considered involve osmium and ruthenium and are analyzed on terms of physicochemical properties. In particular, the importance of spin-orbit coupling for these properties is discussed.

There is no doubt that the candidate masters both the computational methods and the experimental background excellently. Both the depth and breadth of the thesis are impressive. The candidate has solved important, and in fact quite complicated, problems in chemistry and biochemistry and produced large amounts of data that will be of great value of both the experimental and theoretical communities. One very attractive feature of the thesis is the close collaboration between theory and experiment. For one thing, this is indicative of good communicational skills on the part of the candidate. I believe that this kind of interactive collaborative work, in which theory not only explains experimental observations but also makes predictions, is essential if one wants to achieve fundamental understanding and progress. In my opinion the science presented in this thesis is of the highest class and it will certainly receive wide international recognition.

Nevertheless, there are a few issues concerning the theoretical approaches used in the thesis that I would like the candidate to elaborate a bit further on:

1. In Chapter 2.4 there is a brief discussion about the QM/MM approach. This can be extended to highlight the following questions:

- What are the strengths and weaknesses of the QM/MM approach?

- What are the most common errors made in the QM/MM method?
- What is a good size of the QM part in a QM/MM calculation? Or in other words, how shall one choose the QM part?

2. An alternative approach for studying enzymatic reactions is the so-called Cluster Approach, in which a relatively small model of the active site (typically 100-150 atoms) is cut out and treated with accurate electronic structure methods. The rest of the enzyme is approximated as a homogenous medium with some dielectric constant and some centers are kept fixed to preserve the overall structure of the active site.
What is the candidate's opinion about this? How does this approach compare to QM/MM? When does it fail?

3. The thesis includes studies of both enzymes and metal complexes. From computational/theoretical point of view, what are the main differences between the two fields? Why is QM/MM methodology frequently used in the enzyme context but not for the metal complexes (to treat surrounding solvent molecules, for example)?

4. In several places throughout the work of the thesis, the exact transition states were not optimized. Rather, scans (one- or two-dimensional) were performed to estimate the transition states. For many cases this procedure is sufficient. However, there are cases where it could lead to severe errors? What are these and how can one recognize them?

5. The way entropy effects are estimated from a gas phase cluster model and then included in the QM/MM calculations is quite interesting (e.g. Article IV). Are there other ways to estimate entropy effects in QM/MM? How different are enthalpy *vs* free energy profiles for enzymatic reactions?

To conclude, in my opinion the quality of the presented thesis is very high, certainly sufficient of the degree of PhD. I thus recommend it to be used as the material for obtaining a PhD degree.

Prof. Fahmi Himo
Department of Organic Chemistry
Stockholm University
Sweden