Assessment report on dissertation
"Catalytic and Electronic Properties of Redox-Active Metalloenzymes and Transition-Metal Complexes: Insight from Computational Chemistry"
written by RNDr. Martin Srnec

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Reviewer: Dr. Imre Pápai

Owing to successful methodological developments achieved in computational chemistry and also to rapid advance of computer infrastructure, quantum chemical methods became widely applied in various research fields of chemistry. One of the most exciting and challenging fields involves mechanistic studies on enzyme catalysis, which evolved to near perfection in Nature. The work presented in the dissertation written by Martin Srnec aims at providing new insight into the electronic and catalytic properties of metalloenzymes and closely related transition-metal complexes that are involved in redox reactions. Theoretical studies on this type of reactions should go far beyond the routine computational approaches as almost all possible concerns related to reliable model construction have to be taken into consideration (use of extended molecular models, adequate description of open-shell metallic and radical states, consideration of close-lying excited states with usually multiconfigurational character, consideration of relativistic effects). After reading the dissertation I can definitely admit that all these aspects have been carefully taken into account in this PhD work.

The dissertation is structurally devided into two parts. In the first 60 pages or so, the applied methodology is summarized and the main results are presented and discussed in a concise but clearly understandable way. The second half of the dissertation includes copies of the papers published or submitted for publication. Most of the results were published in reputed journals such as JACS, Faraday Discussions, or JPCB. In the majority of the papers the candidate is the first author underlying the importance of his contribution to these studies. It should be noted that in addition to the results discussed in the PhD thesis, Martin Srnec is the co-author of 8 more papers, which is quite remarkable.

The methods applied in the presented work include conventional DFT and TD-DFT techniques combined with accurate multiconfiguration ab initio methods (CASSCF, CASPT2, MRCI). The enzyme environment in mechanistic

studies is modeled in terms of QM/MM methods that allow a quite reasonable approach to explore various reaction pathways in the active sites and also an adequate representation of long-range effects that appear to be essential for the investigated redox reactions. Another important point of the general approach in these studies is that it heavily relies on available experimental findings (X-ray structures, spectroscopic data, kinetic measurements, etc.), which are maximally taken into account in the construction of mechanistic models. Also, the results are always evaluated critically with respect to experiment and previous studies, which enables to assess the reliability of computational predictions. The critical discussion of the results, which point out occurent uncertainties and sometimes discrepancies with previous results, is a very positive feature of this dissertation.

Two major types of theoretical studies are presented in the thesis. In section 3.1., the reactivity of various metalloenzymes in complex redox processes are examined using QM/MM methods. Several reaction routes are explored for the disproportionation of the radical anion O_2^- with manganese superoxide dismutase (MnSOD) and for the reduction of O_2 with stearoyl-ACP Δ^9 desaturase; whereas reorganization energies associated with an electron-transfer process in a multicopper oxidase are discussed, and the key step in the conversion of O_2 to H_2O is analyzed. In section 3.2., the photochemical properties of octahedral Ru and Os complexes are examined focusing on the effect of the inclusion of spin-orbit coupling on computed reduction potentials, and also on the effect of solvation on the electronic structure of $[RuCl_6]^{4-}$. In my view, all these studies provide new insight into the above redox-active systems.

Although I am not an expert in this particular research area, I have a few comments and questions regarding some of the mechanistic details of enzyme catalysis:

1. In the mechanistic study of MnSOD-catalyzed O_2^- dismutation, the associative and dissociative scenarios assume that the O_2^- ion is bound to the Mn^{3+} center. Is any experimental evidence available for this reaction intermediate? Can we exclude the possibility that the electron transfer (from O_2^- to Mn^{3+}) occurs along the approach of O_2^- to the metal center prior to the formation of Mn^{3+}–O_2^- complex?
2. In Figure 4 of Article II, one-dimensional potential energy profiles are shown for the activation of the peroxodiferric (**P**) intermediate in the catalytic cycle of the desaturation process. One of the profiles corresponding to the Fe-O bond cleavage in the hydroperoxo-bridge structure in the Fe^{II}/Fe^{III} state (red curve in Figure 4C) reveals an extremely

low activation barrier. What is the main driving force for this unique reactivity in this particular oxidation state? Most of the figures depicted in the dissertation are clear and illustrative, but this figure does not really help the reader understand the two pathways refered to as α and β in the text.

3. The QM/MM study carried out for the activated peroxi intermediate of the trinuclear copper active site in multicopper oxidases reveal that the exothermicity of the O-O bond cleveage step is significantly reduced (and accordingly the activation barrier is increased) in the presence of enzyme environment. Do we understand the reason for this effect? What types of interactions are responsible for this huge effect?

In summary, I believe that the dissertation submitted by Martin Srnec describes extremely thorough theoretical studies to characterize the photochemical properties and reactivities of redox-active systems. The research has been carried out at high standards and the new results represent valuable contribution to the related research fields. I consider the thesis appropriate for defense and I highly recommend to award the PhD degree to the candidate.

Budapest, 9 July, 2010.

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