Reviewer's report of the habilitation thesis by

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Modelling Physico-Chemical Properties of Bioinorganic Systems

The submitted habilitation thesis is written as a collection of previously published works accompanied with a short introduction and commentary. It is based on 20 papers published in peer-reviewed journals covering a range of topics, the main unifying element being metal ion chemistry in biological context.

As for the thesis itself, it is well written and serves as an easy-to-read introduction into the author's numerous efforts. Its individual chapters are very condensed, well ordered and aimed at important issues. I have just two minor notes. First, at some places the text is perhaps even too brief as an introduction to the field. The reader could also appreciate more reading about the applied methodologies. Second, I would appreciate if the author's contributions were specified in each chapter. In the most important chapters, the authors contribution is very clear and persuasive, but for instance in Chapter 4. (Computational Electrochemistry and Beyond) it is less clear. Nevertheless, given the high quality of the main body of the work, this is not an important issue. Overall, I appreciate the briefness and clarity of the discussion of the main scientific achievements detailed in the attached publications.

The author's long-term work focuses on some of the most important (and topical) problems of the enzymatic catalysis with metal-containing enzymes. Enzymatic catalysis is a widely studied area, but studies of reaction centers containing multiple metals such as iron or copper are relatively rare. This is due to the difficulty associated with describing the complicated electronic and spin structure of catalytically active metals. In my opinion, the authors works on Manganese superoxide dismutase (mechanism of superoxide anion radical oxidation and reduction, in *J. Phys. Chem. B* 2006 and *J. Phys. Chem. B.* 2010), multicopper oxidase (suggested catalytic cycle of MCOs in *Inorg. Chem.* 2006 and) and also binuclear non-heme active site of Δ^9 desaturase (activation energy of the assumed rate-determining step, in *J. Am. Chem. Soc.* 2014) brought new energetic and structural information on catalytic mechanisms and new insights. They represent important contributions that have greatly helped in developing the field of metalloenzyme catalysis.

Also important and useful are works that attempt to estimate the free energy of metal ion complexation (*J. Phys. Chem. A* 2011) and explore methodological issues of calculating the reduction potentials of Group 8 elements (*J. Phys. Chem. C* 2013). Valuable lessons are learnt on the applicability of current continuum solvation models and overall accuracy of the theoretical predictions of the studied properties.

Lubomír Rulíšek has become a respected scientist over the past ten years. His publication record is very good. During his career he has established many collaborations with several renown scientists in the field of quantum chemistry and enzymatic catalysis both in the Czech Republic and abroad, naming for example Prof. Ulf Ryde from Lund University or Prof. Edward Solomon from Stanford university. His dedication to scientific work is documented not only by his research

achievements, but also by his activity in organizing scientific meetings and as a member of grant and journal boards.

In conclusion, the presented results represent important original contributions to development in the field of computational chemistry. The topical research is conducted at a high level and using high-quality and often state-of-the art methodologies. The relatively long scientific effort summarized in this thesis documents the applicant's ability to formulate interesting scientific questions and to conduct and supervise high-quality research.

The habilitation thesis submitted by Luboš Rulíšek entitled Modelling Physico-Chemical Properties of Bioinorganic Systems fulfills requirements for the habilitation work and I recommend it as a basis for awarding the title docent (associate-professor) in this field.

Questions for the habilitation thesis defense:

1) DMRG based calculations (DMRG-CASSCF and DMRG-CASPT2) were used as a reference for the rate-determining step in Δ^9 desaturase enzyme to establish a reliable theoretical barrier height value. In your opinion, what are the prospects of DMRG based methods for this type of applications? Could you compare computer demands, affordable orbital spaces and reliability of this method with other widely used wave-function methods?

2) When estimating the free energies of complex formation you have come to the conclusion that the accuracy of the solvent model is one of the most limiting factors. However, only continuum solvent models were used. In some cases, solvation energies calculated by explicit molecular dynamics (MD) with thermodynamic integration (TI) can provide more accurate solvation energies than implicit solvent models. I think that this might apply also to the solvation of singly/doubly charged metal aquacomplexes and ligands. Have you considered calculating solvation energies using these approaches? Are you aware of any study where MD/TI has been applied to estimate solvation energies of metal aquacomlexes?

In Olomouc, on 24-th September 2018

doc. RNDr. Petr Jurečka, Ph.D.