Posudek habilitační práce **Mgr. Zdeňka Chvala, Ph.D.**

„Biologically Active Metal Ions and Complexes: Reactivity and Interactions"

v oboru **Biofyzika**, Přírodovědecká fakulta Jihočeské univerzity v Českých Budějovicích

Předložená habilitační práce je psána v anglickém jazyce a má formu komentovaného souboru 12 přiložených publikací. Vybrané publikace pocházejí z let 1998-2016 a prošly náročným oponentním řízením v mezinárodních impaktovaných odborných časopisech. Jejich kvalita je nesporná (jsou hojně citovány) a dokladuje vysokou vědeckou úroveň Dr. Chvala.
Hlavní text komentáře habilitační práce je stručný, avšak jako komentář k souboru publikací je dostačující. Je napsán přehledně a je doplněn 4 obrázky. Uvádí čtenáře do řešené problematiky (zde bych si dovolila poznamenat, že „cisplatina" je účinná především při léčbě nádorů varlat, přibližně 95% remise, příkladem Lance Armstrong), teoretických výpočtů a popisuje významné dosažené výsledky autora. Z přiložených publikací a komentované části habilitační práce vyplývá, že uchazeč využívá metody počítačového modelování ke studiu reakčních mechanismů protinádorově účinných komplexů platiny a ruthenia. Ve dvou pracích se také zabývá úlohou hořečnatých kationtů v metabolismu molekul RNA. Pomocí kvantově mechanických výpočtů se svými spolupracovníky přispěl k objasnění mechanismů vazby klinicky využívaných platinových preparátů a potenciálních protinádorových léčiv, komplexů ruthenia, na DNA a proteiny. Jeho práce tak pomáhají porozumět cytotoxickým účinkům těchto komplexů a možnému vzniku získané rezistence, které limitují jejich využití při léčbě zhoubných nádorů.

Dotazy oponenta
1) Mohl by uchazeč konkrétněji popsat možné katalytické působení komplexů Pt(II) a Ru(II)?
2) Zabýval se autor při studiu vlivu Mg^{2+} na RNA také úlohou Mn^{2+}?

Shrnutí posudku:

Práce Mgr. Zdeňka Chvala, Ph.D. **splňuje** požadavky kladené na habilitační práce v oboru Biofyzika a **doporučuji** ji k obhajobě.

[redacted]

Dne 30. 8. 2017

doc. Mgr. Olga Nováková, Dr.
Biofyzikální ústav, AV ČR, v.v.i.
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IN KRAKÓW

A review of the habilitation thesis entitled "Biologically Active Metal Ions and Complexes: Reactivity and Interactions" by Zdeněk Chval.

General comments

The candidate has earned the PhD degree from the University of South Bohemia in 2001 (Department of Molecular Biology). The post-doctoral and international scientific experience have been gained in France (16 months in Nancy University) and Switzerland (Freiburg University). At present time Dr Chval carries an assistant position in the same place. He has published 18 papers in the international high quality journals (e.g. Inorganic Chemistry, Journal Computational Chemistry, Journal of Physical Chemistr A, B, etc.), the current h-index is h=9 and the overall number of citations is 213 (196 without self-citations). The candidate has participated in grants as both principal investigator and co-investigator as well as has been invited by recognized journals in the field for reviewing papers (e.g. Journal Computational Chemistry, Chemical Physics Letter, etc.). It is noteworthy that the candidate has also acted as the associate editor for the Journal of Applied Biomedicine. Furthermore, Dr Chval has participated in 28 conferences in the field of biochemistry. The candidate has been a teacher of the following subjects at the University of South Bohemia: physics, chemistry, biochemistry and molecular biophysics as well as has been a scientific tutor of 6 students (at both the BSc/MSc and PhD levels). He actively collaborates with the group of Prof. Jaroslav Burda (Charles Univeristy).

Detailed comments on habilitation thesis

The habilitation thesis of Zdeněk Chval is focused on very important aspect of biochemistry – namely, molecular description of reactivity of anticancer drugs (Pt(II) and Ru(II) based complexes), a mechanism of action of Mg^{2+} in human body as well as development of new force field parameters for correct description of nucleic acid bases. These findings are gathered in 12 publications (labelled as P1-P12). Below detailed scientific comments are provided on the content of P1-P12.

In the publication P1 novel force fields parameters (i.e. bending constants in the steric energy) have been estimated to correctly take into account the flexibility of nucleic acid bases. The kinetics of cisplatin hydrolysis have been described in P2 based on ab initio methods – it has been determined that these reactions proceed via pentacoordinated transition states (with the rate determining step by ca. ~26 kcal/mol) and they are endergonic. Reactions of hydrolyzed Pt(II) complexes with the model compounds which represented the most important cellular targets were described in P3 (nucleobases guanine and adenine). The mechanism of substitution water exchange reactions in the square planar trans-$Pt[(NH_3)_2T(H_2O)]^{n+}$ complexes is studied in P4 (T=H_2O, NH_3, OH^-, F^-, Cl^-, Br^-, H_2S, CH_3S^-, SCN^-, CN^-, PH_3, CO, CH_3^-, H^-, C_2H_4) in terms of factors determining the trans effect. An in depth study has revealed σ-donation strength is linearly correlated with the Pt–OH_2 (leaving ligand) bond length (trans influence). I shall comment that the same conclusion has been reached by us a bit later for other planar complexes (Int. J. Q. Chem. 2009, 109, 3379).

Interestingly, the ligands with strong π-backdonation ability such as C_2H_4 and CO appeared to stabilize transition state structures. In the next stage the candidate has focused on cisplatin interactions with sulfur-containing amino acids considering the polarizable continuum model (P5) – it has been explained that at first stage the substitution occurs (aqua ligand is replaced by one of the three active amino acid sites) and subsequently the dissociation process leads to formation of the chelate and water (or chloride anion). The hydration processes for two Ru(II) representative half-sandwich complexes with respect to the cisplatin were studied in P6 – it was determined for the first time that the hydration model CPCM works well for the replacement of the first chloride by water where an acceptable agreement for both Gibbs free energies and rate constants was obtained. However, in the second hydration step worse agreement of the experimental and calculated values was achieved. The publication P7 covers in depth mechanistic investigations of the RNA activation (at 2-OH position) by Mg^{2+} using DFT and MP2 methods (at CPCM level). It has been determined that the cation coordination to 2-OH position leads to facile deprotonation and the developing negative charge on the 2'O center. The change from the inner-sphere to the outer-sphere coordination appeared to be driven by the energy cost of the first coordination shell reorganization rather than by the electrostatic repulsion between the ligands. In the next publication P8 the candidate has provided mechanistic insight into the kento-enol equilibrium within the uracil and Mg^{2+} adduct – the bidentate N3–Mg–O(keto) binding is preferred, whereas the water environment and/or presence of the phosphate group stabilize the diketo tautomer with oxygen atoms as the only metal's binding targets. The publication P9 contains very comprehensive and in depth modelling of the reaction mechanism between DNA model (double-stranded pGpG·CpC dinucleotide) and fully aquated oxaliplatin cis-$[Pt(DACH)(H_2O)2]^{2+}$ (DACH = cyclohexane-1R,2R-diamine) – this work not only allowed for obtaining of the most realistic theoretical description of the course of the DNA platination available up to now (in agreement with the experiment), but also pinpoint the importance of steric effects or formation of hydrogen bonding. In order to get deeper insight into the reactions of hydration process of three representative Ru(II) and Pt(II) complexes the authors have analysed changes in molecular electrostatic potential, averaged local ionization energy, and reaction electronic flux based on post-HF and DFT methods (the paper P10). In the very interesting paper P11 the candidate have demonstrated based on molecular dynamics as well as in depth ETS-NOCV bonding calculations that both the electronic stabilization within the non-classical Pt---H interactions as well as solvent contributions are crucial to correctly describe the solvation shell of cis-platinum complexes. Finally, in the paper P12 hydration processes of another anticancer drug family (i.e. the quinolone Ru(II) half-sandwich complexes) have been described based on DFT methods.

Minor deficiencies:

- At some places English statements in the summary are too long and accordingly, they are hard to correctly understand (e.g. part on the content of P3). The full attached papers lack of such disadvantages.

Recommendation and summary

To summarize, the habilitation thesis by Zdeněk Chval is highly evaluated since it sheds entirely novel light on the molecular mechanisms of actions of biologically active metal ions and their complexes. The results have been published in twelve high quality publications in the world leading journals (the overall impact factor of P1-P12 is ~ 35 what provides ~ 2.9 per one paper). They have been cited more than 100 times, what demonstrates the importance of these achievements. The candidate has participated in grants as both principal investigator and co-investigator as well as has been invited by recognized journals in the field (e.g. Journal

Computational Chemistry, Chemical Physics Letter, etc.) for reviewing papers. It is noteworthy that the candidate has also acted as the associate editor for the Journal of Applied Biomedicine. It demonstrates in my view the maturity and the scientific independence of the candidate. Hereby, I declare that the habilitation of Zdenek Chval meets all the criteria specified in the provided document "Rules for habilitation procedures at the faculty of chemical science, University of South Bohemia, Českiě Budějovice (valid since September 2014)". Accordingly, I would like to express my positive recommendation to the Faculty of Chemistry Board of University of South Bohemia concerning the Dr Chval's admission to further stages of the habilitation (associate professorship – docent) procedures.

Yours Sincerely,

[redacted]

Dr hab. Mariusz Paweł Mitoraj

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08.10.2017

Review of habilitation thesis of Mgr. Zdeněk Chval, Ph.D.

Dear Professor Ettrich,

I have been asked to review the habilitation thesis of Dr. Zdeněk Chval for the field of Biophysics, which I am very happy to provide herewith. I know the candidate in person from discussions at some bioinorganic chemistry meetings, but we are currently not engaged in any formal interaction and therefore I do not consider any conflict of interest to be present.

Dr. Chval studied at the Charles University in Prague, and obtained a Master degree in Physical and Macromolecular Chemistry in 1994. After a short stay at the J. Heyrovsky Institute of Physical Chemistry and the Czech Ecological Institute in Prague, he joined the University of South Bohemia to work towards a doctoral degree, which was awarded in 2001 in the field of Molecular and Cell Biology. After some industrial experience, Dr. Chval then did a 1 1/2 year postdoc at CNRS-UHP in Nancy, France, which contributed a lot to his international experience, and was thereafter appointed as an assistant professor at the Faculty of Health and Social Studies at the University of South Bohemia in 2004, where he has stayed since then.

His scientific work is focused on theoretical studies of the interaction of metals with bio(macro)molecules, with a particular focus on modelling of the speciation and (oligo)nucleotide-binding of platinum- and ruthenium-based anticancer drug candidates in physiological medium. More recently, he has expanded his range of studies and also contributed to the modelling of ionic liquids, which are important materials for future battery applications as well as functional solvents.

So far, he has published 18 papers in peer-reviewed internationally renowned journals, including some of the top specialist journals in his field of research, such as *J. Phys. Chem.*, *Inorg. Chem.*, *J. Chem. Phys.*, and *Phys. Chem. Chem. Phys.* At about 15 years post-Ph.D. and with the restricted ressources apparently available to him, I consider this a very decent publication record, which also shows a notable upward trend during the last couple of years.

The applicant has also been involved in the supervision of young academics, with 5 Bachelor, 1 Master, and 1 Ph.D. thesis carried out under his guidance. As such, he fulfills all the recommended formal criteria for a successful habilitation procedure.

The habilitation thesis itself starts with an compact overview of the properties and behaviour of platinum(II) complexes of biomedical relevancy. Two of the papers listed are focused on a theoretical rationalisation of the *trans* effect, which determines the preference with which ligands in square-planar Pt(II) compounds exchange with solvent molecules and/or constituents of bio(macro)-molecules, which is important for the speciation of platinum-based drug candidates on the way to their main target structure, the nuclear DNA. In addition the the main lead structure, cisplatin, the applicant has also studied other platinum compounds such as oxaliplatin, and looked into the role of secondary interactions such as hydrogen bonding in the ligand periphery, which are difficult to model theoretically, but of vital importance to elucidate the binding preference of metal complexes to oligonucleotides. In addition, soft donor centers in proteins are also considered as alternative binding sites for platinum drugs, and thought to scavenge high amount of the active species. Consequently, another study was directed at the investigation of the interaction of hydrolyzed cisplatin follow-up products with the sulfur amino acids cysteine and methionine.

These results are then contrasted with an investigation of the properties of ruthenium(II) arene organometal compounds now also widely studied in the context of bioorganometallic chemistry and medicinal inorganic chemistry. This is particularly welcome as this class of compounds has received much less attention from theoretical chemists relative to the platinum complexes.

Moving from transition to main group alkaline earth metals, a final group of papers presented then deals with magnesium(II) binding to RNA building blocks as another vital class of biomolecules. For these hard metals, the preferential binding is to the sugar-phosphate backbone, not the nucleobase nitrogen atoms.

As such, the scientific work of the applicant has significantly contributed to a theoretical understanding of the role of metal ions in binding to bio(macro)molecules and shows a progression to more and more detailed insights, for the latest contributions also including molecular dynamics (MD) simulations. Although not directed at program development, they present state-of-the-art applications of theoretical chemistry methods to problems relevant to biology and medicine in a timely manner.

Therefore, in summary, **I am happy to suggest this thesis for acceptance as a habilitation.**

Best regards

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(Ulrich Schatzschneider)