MASARYKOVA UNIVERZITA

Habilitation thesis reviewer's report

Masaryk University **Faculty of Science**	
Field of habilitation	Biomolecular chemistry
Applicant	**RNDr. Radka Svobodová Vařeková, Ph.D.**
Workplace	National Centre for Biomolecular Research Faculty of Science Masaryk University
Habilitation thesis	Analysis of biomacromolecular structural fragments
Reviewer	**Prof.Dr. Dušanka Janežič**
Workplace of reviewer	University of Primorska, FAMNIT, Koper, Slovenia

Reviewer's report:

RE: RNDr. Radka Svobodova Varekova, Habilitation in "Biomolecular chemistry"

Dear Professor Ludek Matyska,

I was happy to get your email requesting my assistance in evaluating application for RNDr. Radka Svobodova Varekova, Ph.D. Habilitation in "Biomolecular chemistry". It is my pleasure to fully endorse her application.

I consider myself well positioned to evaluate Dr. Svobodova Varekova's scientific credentials and her past and potential contributions. I am currently a full professor of the Mathematics in Natural Sciences at the Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Slovenia. I have published more than 100 original research papers and review articles in top-ranked journals such as Nucleic Acids Research, Journal of Medicinal Chemistry, Bioinformatics, and Current Opinion in Structural Biology. I was for over 14 years an Associate Editor of the Journal of Chemical Information and Modeling and I am currently serving as a member of the Editorial Advisory Board for this journal.

I have known the work of Dr. Radka Svobodova Varekova primarily because I have been an Associate Editor for the American Chemical Society's Journal of Chemical Information and Modeling, where Dr. Svobodova Varekova has published several papers. I should emphasize that this leading journal by the American Chemical Society only accepts original papers reporting novel methodology and/or important applications in the fields of chemical informatics and molecular modeling.

Moving beyond my outstanding professional interactions with Dr. Svobodova Varekova, evaluating her Habilitation Thesis leaves me equally impressed. Between 2005 and 2016 she has published as the first, the corresponding or as the co-author16 publications in renowned scientific journals, which received significant visibility. A common topic of these publications is the analysis of biomacromolecular structural fragments such as ligand and metal binding sites, channels and pores, and supersecondary structure elements. Specifically, the articles focus on the development of approaches for the validation, detection, extraction, comparison

and characterization of fragments, and also on the application of these approaches for solving important biological questions. Additionally, the timing of these papers clearly illustrates that her productivity has been consistent and clearly is continuing to increase.

Dr. Radka Svobodova Varekova has submitted for evaluation the Habilitation thesis titled: *Analysis of biomacromolecular structural fragments*.

Dr. Radka Svobodova Varekova's research addresses the two fundamental questions in the field of structural bioinformatics. Her research interest is primarily in studying biomacromolecular structural fragments and approaches for their realization. Her research concentrates on these two interconnected goals. The first was the development of methodologies for the analysis of biomacromolecular structural fragments. The second was their application in resolving important biological and chemical questions. In her habilitation thesis she presented and discussed these topics in sixteen of her publications, related to the above scientific questions.

The validation of ligands, which are frequent components of biomacromolecular fragments and the main sources of their errors, is a complex problem and its methodology is still under development. In articles in the thesis marked as [MV, VDB] she has introduced an extended methodology for the validation of ligand annotation, applicable for any ligands and non-standard residues.

An essential step in biomacromolecular structural fragment analysis is the collection of all fragment samples. Therefore, the fragment should be first described via a proper molecular language, then detected within the structures and then extracted from them. Several molecular languages for describing various molecular structures were introduced as well a methodology for the rapid extraction of fragments described this way from the Protein Data Bank. Important classes of biomacromolecular fragments are their channels and pores, since they provide a substrate with access to an active site. She has developed new methodologies for their extraction MOLEonline and MOLE2.0 that have been published in articles in the thesis marked as [MO] and [MO2].

A substantial task within structural fragment analysis is also fragment comparison. In general, when similar 3D structures are compared, pair corresponding atoms of the compared structures and the structures has to be superimposed based on this pairing. Many state-of-the-art approaches for comparing organic molecules were developed, and she contributed to this field of research by creating an approach employing so-called combinatorial and subgraph matching pairing which is appropriate for fragments, published in the article in the thesis marked as [SB]. The final step in the process of structural fragment analysis is its characterization, i.e. determining the fragment's characteristic properties. Partial atomic charges are one such property, providing information about the electron distribution within a molecule. The most appropriate approach for their calculation is quantum mechanics (QM), which is unfortunately very demanding in terms of time and computational resources. A faster alternative to QM are empirical methods, of which the Electronegativity Equalization Method (EEM) is the most popular and applicable. EEM has been significantly improved in recent years. Specifically, she has developed EEM parameterization for organic molecules, for biomacromolecules and for ligands published in articles in the thesis marked as [EO], [EB] and [EL]. She has also developed a methodology for calculating EEM charges in large biomacromolecules (a parallel approach is published in the article in the thesis marked as

[EPM] and an approximative method in the article in the thesis marked as [EAM]) and in ligands and drug-like molecules published in the article in the thesis marked as [EL].

Other key characteristics of biomacromolecular structural fragments are channel properties. Specifically, she has evaluated the quality of structures for different molecular classes published in the article in the thesis marked as [VDB], she used charges for pKa prediction (introduced in the article in the thesis marked as [PQ,PE,PS]) and for researching apoptotic protein Bax activation (discussed in article in the thesis marked as [BAX]). Based on these results she discovered the anatomy of enzyme channels presented and published in the article in the thesis marked as [AN].

She found that a quality evaluation of individual ligands or biomacromolecules can be straightforward to obtain. She presented these findings in the article in the thesis marked as [MV]. She also performed an analysis on information about the quality of various molecular classes (e.g., drug molecules, organometals, carbohydrates) presented and published in the article in the thesis marked as [VDB]. The best quality proved to be drug molecules, most likely because markedly more effort is expended on determining their structure in biomacromolecular complexes.

Carbohydrates and polycyclic ligands exhibited problems in the chirality of their carbon atoms. The most problematic ligands are organometals, exhibiting clear validation problems in most validation criteria. Partial atomic charges proved to correlate with the acid dissociation constant pKa. This constant measurement is highly demanding and its prediction is still a challenge. She has also focused her research on charge-based approachesto pKa prediction. She demonstrated that QM charges are highly successful descriptors for the prediction of pKa via Quantitative Structure-Property Relationship (QSPR) models, but a proper charge calculation approach must be used which she presented in the articles in the thesis marked as [PQ], [PE] and [PS].

An important biological question is what properties the enzyme channels have. To answer this question she performed analyses of the channels in more than 4,000 enzyme structures presented in the article in the thesis marked as [AN]. She identified that at least 64% of these enzymes contain on average two channels longer than 15 Å leading to the catalytic site. The longest and the most hydrophobic channels were found in oxidoreductases and the shortest and the most hydrophilic channels in ligases. The composition of channel lining residues differed from the average composition of enzyme structures as well as from the composition of the protein surface. Specifically, aromatic, charged and polar amino acids occur more frequently in channel walls. All of these her findings indicate that the active site access channels have a significant biological function, as they are involved in co-determining the enzyme’s substrate preferences.

In closing, I again appreciate the opportunity to evaluate Dr. Radka Svobodova Varekova application for Habilitation in "Pharmacoinformatics" at the Masaryk University. It was a pleasure reading about the numerous successes that Dr. Svobodova Varekova has had since beginning of her scientific carrier.

The methodology of her work is very sound; she is able to provide new scientific results; and most importantly, she has demonstrated mastery of the scientific discipline of Biomolecular chemistry, with special focus on “biomacromolecular structural fragments study and

development of approaches for their realization in conjunction with structure and dynamics of enzyme channels anatomy", and the ability to contribute to its progress.

From this evaluation I again without hesitation recommend to award the Habilitation in "Biomolecular chemistry" at the Masaryk University to Dr. Radka Svobodova Varekova. I am extremely confident that the past success she has demonstrated will lead to even greater future achievements, to become, without doubt, one of the up-and-coming leaders in the field of Biomolecular chemistry.

Sincerely,

Prof. Dušanka Janežič

Reviewer's questions for the habilitation defence:

1. What are potential applications of your current research results, in particular in the field of drug discovery?
2. How do you envisage the further development of your methods?
3. Where do you anticipate their potential uses?

Conclusion

The habilitation thesis submitted by RNDr. Radka Svobodová Vařeková, Ph.D., entitled „Analysis of biomacromolecular structural fragments" ***meets*** the requirements applicable to habilitaion theses in the field of Biomolecular chemistry.

In Koper, on August 31, 2016

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