MUNI

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Mgr. David Bednář, Ph.D.
Habilitation thesis	Computational Tools for Analysis and Design of Proteins
Reviewer	Prof. Ing. Vojtěch Spiwok, Ph.D.
Reviewer's home unit, institution	Department of Biochemistry and Microbiology, University of Chemistry and Technology, Prague

The habilitation thesis *Computational Tools for Analysis and Design of Proteins* by David Bednář presents bioinformatics tools and databases for enzyme mining and protein engineering. These services are used by thousands of researchers worldwide and are the flagship services of ELIXIR CZ infrastructure (and of European ELIXIR). David Bednář is the key researcher behind these services.

The thesis presents the topics of enzyme mining, prediction of protein solubility and stability, and analysis of concavities in protein structures. For each topic, the problem is defined, the current state of the art is presented, and contribution of the applicant to the field is clearly described. The thesis covers nine publications. Many of them are published in the special database and tools issues of *Nucleic Acid Research*, which is a prestigious forum of bioinformatic services. Other articles were published in prestigious journals. The applicant is a senior author of many articles presented in the thesis, which indicates his trajectory towards group leadership.

The thesis is clearly written and describes the contribution of the applicant to the presented articles and to the field. David Bednář is an excellent scientist and an important person in enzyme and protein engineering.

Reviewer's questions for the habilitation thesis defence:

1. The topics of protein solubility and stability are strongly interconnected. Some researchers present a protein as soluble when it forms something similar to the true solution, and insoluble when it forms aggregates of native protein globules. Other researchers use the term soluble as an equivalent to properly folded and insoluble as un/misfolded or denaturated. Is there any clear border between these two definitions? Does the applicant use these terms consistently? Is there any evolution in understanding the term solubility in the light of new findings on intrinsically disordered proteins, phase separation, and other hot topics in the field? Does this ambiguous terminology cause any trouble to providers of bioinformatics services oriented to protein solubility and stability?

2. AlphaFold is mentioned several times in the thesis. This is a great tool for protein structure prediction and many researchers are trying to transform it to study the dynamics, affinity, and other characteristics of proteins. How far is the application of AlphaFold or similar methods in the prediction of protein function, solubility or stability?

3. Ancestral protein reconstruction is a very elegant method to address protein stability. Since coevolution of residues in proteins plays an important role in recent success in protein structure prediction, namely AlphaFold, are there any approaches for ancestral protein reconstruction based on coevolution?

4. Many researchers in the drug design field recently focus on the kinetics of drug unbinding from its target (k_{off}) as an alternative to more common affinities (e.g., dissociation constants). I see this as a great opportunity for the tool CaverDock. This tool is discussed in the corresponding article mostly in the context of enzymatic catalysis and substrate prediction. Are you aware of any applications of CaverDock in ligand design based on unbinding kinetics?

5. An associate professor is not only a scientist, but also a lecturer and mentor. In the thesis, I did not find a detailed description of the future plans of the applicant to transfer his expertise to students. I explain this by a different convention of habilitation theses at different universities. The field of enzyme and protein engineering is very interesting and attractive, however, it may be seen as too specialized or narrow-focused. Do you find this field suitable for training new students?

Conclusion

The habilitation thesis entitled "Computational Tools for Analysis and Design of Proteins" by David Bednář **fulfils** requirements expected of a habilitation thesis in the field of Environmental Health Sciences.

Date: 28 February 2023   Signature: