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Reviewer's report on the dissertation thesis of

Ing. Phuong Ngoc Pham

"Specificity of Protein-Protein Interactions and Their Modulation"

The present dissertation thesis was prepared at the Faculty of Science of Charles University as part of the Biochemistry Ph.D. study program under the supervision of prof. Ing. Bohdan Schneider, CSc., DSc. at the Laboratory of Biomolecular Recognition at the Institute of Biotechnology, Czech Academy of Sciences (BIOCEV). It is written in English on 66 pages of text and 48 pages of appendices, which are the four attached publications forming the professional basis of the thesis. All publications were published by peer-reviewed journals; the Ph.D. candidate is the first author of two of the four articles. For every publication, the thesis also contains a separate summary of the main results achieved and a separate discussion section. The two first-author manuscripts form the essential part of the thesis, with respect to the amount of experimental work performed by the candidate and concerning the space given to this topic within the thesis.

The thesis is structured in a standard subdivision, with the introduction section covering the topics of cytokines of interleukin 10 family, protein scaffold engineering, and photoxenoprotein engineering, followed by the thesis's objectives, summary of results in the form of a commentary on the published research articles, discussion, conclusion, and references sections. I appreciate the relatively detailed commentaries on the published works as well as their individual discussion.

The thesis generally follows common professional standards of its field of research, both formally and graphically. I am confident to say that the results of the present work have advanced this research field by a great deal. The candidate mastered a wide range of molecular biological and biophysical techniques, from heterologous expression of proteins to their functional and structural characterization. This is especially evident from the recently published results on the production and biological characterization of the photo-responsive recombinant proteins utilizing the non-natural

amino acid genetic code expansion methodology, which I consider a highly innovative and promising piece of science. An overall summary of the results obtained is made in the final conclusion section, and their thorough comparison with the present state of knowledge is made in the preceding discussion sections. Comparing the set goals and the achieved results, I cannot but conclude that the candidate has achieved everything she set out to do at the beginning of the thesis.

In conclusion, I am pleased to say that the submitted dissertation thesis is, in my opinion, a very successful work. With its elaboration, Ing. Phuong Ngoc Pham showed distinct creative skills, knowledge, and the ability to work independently as a scientist. The thesis fulfills the requirements of the relevant legal provisions, and I therefore fully recommend it for defense.

Questions for discussion:

1) Would it be possible to create a randomized library consisting of mutants of both 4PSF PatchN and PatchC residues simultaneously? It could increase the chances of selecting high-affinity binders, and the scaffold seems to tolerate a substantial amount of mutations.

2) To select a high-affinity binder potentially blocking the interaction of IL-10 with its receptor, did you consider a competitive elution/binding step with a soluble receptor subunit during the library panning and/or in the ELISA assay?

3) To follow the previous question, have you considered using the photo-crosslinking approach for epitope panning, i.e., introducing a suitable photo-crosslinkable ncAA within IL-10 near its receptor binding site, to capture the desired binders covalently?

4) Do you have any plans for the commercialization / technology transfer of your novel binders/scaffold?

5) The stable recombinant IL-24B4 designed using the PROSS algorithm was extensively characterized for binding to various receptor subunits (Zahradník *et al.*, FEBS J, 2019). In your present work, you used the same approach to develop a stable form of IL-20R2D; however, did you characterize it concerning its binding to IL-24 or other related ILs? How well does it perform compared to the wild-type IL-20R2?

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