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Review of the Ph.D. Thesis of Pham Ngoc Phuong

The focus of the Ph.D. thesis written by Pham Ngoc Phuong is the specificity of protein-protein interactions and their modulation by protein engineering. The thesis outlines three objectives: (i) identification and development of novel protein scaffolds for high-affinity and specific binders to human interleukin IL-10, (ii) determination of the structure of interferon gamma receptor 2, and (iii) modulation of protein-protein interactions between interleukin IL-24 and interleukin receptor IL-20R2.

The thesis is structured into an Introduction, which covers topics important for understanding the core articles. This Introduction encompasses protein-protein interactions, cytokines with a special focus on the interleukin 10 family, protein scaffold engineering, and photoxenoprotein engineering. Reading this section was a pleasure, and I gained many new insights. The text is complemented with comprehensive figures and self-explanatory figure captions.

The subsequent section clearly defines the general objectives of the thesis. A minor inconsistency arises when three objectives are described in the abstract, while only two are noted in the Goals section. However, this is a minor issue.

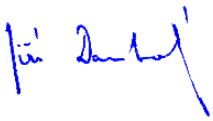
The Results section summarizes key findings covered in detail by the three articles comprising the thesis. The organization is logical and easy to follow, with important findings presented in figures, graphs, and tables. In some instances, experiments are mentioned without including the results in this section, requiring reference to the articles for further information (e.g., two designs by PROSS are described with no information about their properties).

The Discussion section contextualizes the results within a broader framework, providing interpretations and comparisons with previously published studies. Particularly notable is the figure comparing the free structure of interferon gamma receptor 2 obtained by Ing. Phuong and co-workers with the ternary complex obtained by Mendoza and co-workers, published in *Nature*, which underscores the quality and importance of the research.

The Conclusions section carefully outlines the main outcomes of the thesis. I would appreciate a more detailed description of the knowledge obtained by solving the crystal structure of the receptor; only three structurally highly variable regions are described.

In total, 225 research papers were cited in the thesis, including recent papers published in 2022 and 2023. The last three chapters present published articles in the journals *Viruses*, *Journal of Applied Crystallography*, and *Frontiers of Molecular Biosciences*.

Based on the submitted thesis and three scientific publications, I am pleased to confirm that Pham Ngoc Phuong demonstrated the ability to carry out challenging protein engineering projects, collect high-quality data, interpret them, and discuss them in the context of previously published studies. The results obtained within this thesis are novel with potential implications for the development of practically useful products for healthcare. I recommend awarding the academic title of Ph.D. after a successful defense.



Prof. Jiri Damborsky

Questions to be possibly discussed during the defense:

1. Could the author of the thesis briefly define her contribution to the three papers covered by the thesis?

2. Please explain the properties of the two designs obtained by the PROSS computation platform. How did these constructs compare to the wild type in terms of stability and expression level?

3. Full-length proteins were mixed with truncated variants (p. 46, section 3). What was the observed yield/ratio for each fraction?

4. The difference in affinity obtained by MST and yeast display is very large, reaching three orders of magnitude. Please explain this discrepancy. Avidity is only briefly mentioned.

5. Only one out of three constructs showed significantly changed protein-protein interactions upon exposure to UV light. Epistasis is provided as the explanation. Any other hypothesis to explain this observation?

Minor notes of consideration:
"100 uM" should be "100 μM" on page 47.
"One of the two mutable interfaces of 4PSF" — poor formulation on page 54.