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To
Habilitation Committee
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Opponent Review

on the Habilitation Thesis of Dr. Roman Kouřil, Ph.D.

Photosynthesis sustains virtually all life on the planet with energy from the Sun. Research in photosynthesis is driven by the conviction that deeper understanding of its molecular mechanisms can improve the efficiency and resilience of crops and provide the basis of sustainable energy technologies. Plants and algae use photon energy to extract protons and electrons from water and use them to reduce carbon dioxide to carbohydrates. The photooxidation of water is carried out by Photosystem II (PSII) – one of the major membrane protein complexes of the chloroplast thylakoid membranes, dubbed "the engine of life". The structure and function of PSII has thus been a central theme of photosynthesis research since the mid 20th century. However, although the basic reactions associated with PSII had been known, detailed molecular models of these reactions could not be devised without comprehensive knowledge of the molecular structure. Dr. Roman Kouřil has made a significant contribution to the knowledge of the structure and function of PSII through his seminal research done at the University of Groningen and the University of Olomouc. This research is highlighted in his habilitation thesis reviewed here.

The habilitation thesis, entitled *"Structural variability of Photosystem II in land plants"*, presents a comprehensive body of work aimed at elucidating the structural organization, dynamics, and variability of Photosystem II (PSII) across different plant taxa. The thesis is structured as a cumulative dissertation, comprising a series of published peer-reviewed papers complemented by an integrative commentary that contextualizes the candidate's scientific contributions within the broader research field.

1. Scientific contributions and impact

The habilitation thesis consists of 12 publications - 11 peer-reviewed articles in international scientific journals and one book chapter, published between 2009 and 2023. The publications, including one review article in a special issue of the journal BBA, dedicated to PSII, summarize the main contributions of the candidate to the field. The number of publications conforms with the norm for habilitation theses of this kind, considering the historical record of the University of Olomouc. All articles are found in highly reputable biophysics and plant science journals ranked in the first quartile (Q1); most of them (8) are in leading (D1) journals – Nature Plants, Plant Cell, New Phytologist, Plant Journal, EMBO Journal. Dr. Kouřil is listed as the first author in 6 of the 12 publications, the last author in 3 and a corresponding author in 8 publications. This clearly shows that the candidate has the main contribution to the scientific content of the listed publications and thus the habilitation thesis. All publications are closely related to the topic of the habilitation thesis. Additionally, the candidate has a large scientific track record extending beyond the scope of the thesis, which will not be analysed here.

The research of Dr. Kouřil is well known to the international scientific community, specifically in the field of photosynthesis research. His studies combine biochemical fractionation techniques with high-resolution electron microscopy, revealing how the protein composition and supramolecular architecture vary in response to phylogenetic and environmental factors. His work has materially influenced contemporary views on the diversity, structural plasticity and adaptability of the photosynthetic apparatus in land plants. Methodological innovations in native gel electrophoresis (CN-PAGE) and mild solubilization techniques have become standard protocols in the field. Some of the main scientific discoveries highlighted in the habilitation thesis include:

1. *Structural diversity of PSII across light taxa*: the identification of PSII supercomplexes in gymnosperms lacking Lhcb3 and Lhcb6; the discovery of specific gymnosperm-specific forms including the incorporation of Lhcb8 and rearranged antenna topologies (Kouřil et al. 2016, 2020, Ilíková et al. 2021).
2. *High resolution cryo-EM and cryo-ET studies on PSII:* structural characterization of PSII supercomplexes and megacomplexes and elucidation of the organization of PSII in native thylakoid membranes by cryo-ET (Kouřil et al. 2011, Nosek et al. 2017, van Besouwen et al. 2017).
3. *Structural plasticity of PSII under light acclimation*: the discovery and documentation of structural changes of PSII and changes in the antenna composition and oligomerization Kouřil et al. 2013).
4. *Functional role and assembly pathways of PSII components*: functional roles and consequences of the deletion of specific Lhcb subunits and isoforms; conceptual model of assembly and stabilization of PSII supercomplexes (de Bianchi et al. 2011, Kouřil et al. 2018, Ilíková et al. 2021).
5. *Methodological advances in isolation of native membrane protein complexes*: development of optimized solubilization and CN-PAGE methods and reliable isolation of intact PSII supercomplexes and megacomplexes (Caffarri et al. 2009, Kouřil et al. 2012, Kouřil et al. 2020).

The impact of his research is attested by an exceedingly large (for the field) number of citations – more than 1600 citations to the 11 journal articles, with more than 500 citations to Caffarri, Kouřil et al. (2009, EMBO J.) and more than 50 citations to each listed article, bar for three most recent ones.

2. Summary of the thesis text

The complementary text of the thesis adheres to the norms of the University of Olomouc in terms of content, structure, presentation, and length, based on a comparison with representative published habilitation theses. The text is organized in five chapters, spanning 41 pages, including 12 figures. This is followed by an extensive list of cited literature sources. The first chapter briefly introduces PSII and its organization in the thylakoid membranes. Chapters 2 and 3 outline the main methodologies employed for isolating native photosynthetic subcompartments and protein complexes, and for elucidating their structure using electron microscopy, respectively. These chapters not only showcase the candidate's methodological expertise but also represent a useful introductory handbook that is easy to read and understand and references the necessary literature resources for further reading. I found these chapters enjoyable as well as informative. Chapter 4 constitutes the core scientific narrative, tracing the historical and current

understanding of PSII supercomplexes, with detailed attention to their structural diversity across plant taxonomic groups. This includes pioneering work on the PSII organization in gymnosperms, especially the structural peculiarities observed in the Pinaceae family. The chapter represents an integrative summary of the publication record of the thesis that is reprinted afterwards in the appendix. The candidate's personal contribution is highlighted. The chapter also reviews scientific literature outside of the 12 listed publications, including publications with contributions by the candidate (clearly marked) as well as by other researchers in the field. Chapter 5 concludes with future perspectives on PSII structural biology. Overall, the text logically supplements the publication record and combines it into a cohesive, well structured overview.

3. Questions and comments

Questions and comments to the scientific content

1. *Dynamic structural changes of PSII*. The thesis and publications therein mention, on the one hand the ability of EM to study the structural dynamics of complex biological molecules (p.14), and on the other hand, the importance of dynamic structural reorganizations of PSII involved in the acclimation of the photosynthetic apparatus. In this context, has the candidate considered performing time-resolved structural studies, for example using recently developed time-resolved cryo-EM approaches, to study the structural dynamics of PSII during light acclimation?
2. *Ability of single-particle EM to resolve structural heterogeneity*. The thesis acknowledges that the intrinsic heterogeneity of biological macromolecules presents a challenge in structural analysis (p.19). The same can be said for functional analyses, for example, it is well known that heterogeneity, or structural and energetic disorder, of the pigment-protein complexes profoundly affects their photophysical and photochemical function. This is mainly because small changes in the orientation or protein environment of pigments can have large effects on their excitation energies and dynamics. One way to approach this problem is single-molecule spectroscopy – an avenue of intense research. Are there any attempts to resolve heterogeneous conformations (i.e. heterogeneity beyond the composition and subunit organization of supercomplexes) in the same sample using single-particle EM? Conversely, is it feasible with the existing methodologies to resolve subclasses that have only minor structural differences?
3. *The role of Lhcb8 in high-light adaptation*. The presence of the Lhcb4.3 or Lhcb8 isoform in gymnosperms is said to represent an adaptation to high-light exposure. What mechanistic experimental evidence is available of a photoprotective function of Lhcb8?
4. *(related to 3.) High-light adaptation of spruce/pine PSII*. Is the notion that PSII in Pinaceae is adapted to prolonged high-light exposure (p. 25) aligned with its reported unique characteristic, i.e. the capability of binding a much larger antenna (Kouřil et al. 2020)?
5. *The functional roles of different Lhcb subunits in PSII*. The thesis presents a detailed and informative treatment on the roles of the different Lhcb subunits in the assembly and structural stabilization of the PSII supercomplexes in different plants. However, while the text denotes these "functional roles" of the said subunits, it seems to neglect that these are also, and primarily, light-harvesting antenna complexes, and so their functional role is not only to maintain the supercomplex but also to harvest light energy, mediate excitation energy transfer and regulate the excitation energy flow. Undoubtedly, this is implied, however, it could be mentioned explicitly at times for clarification.
6. *Functional differences between angiosperm and gymnosperm PSII*. The thesis illuminates the structural diversity of PSII, especially by revealing the specific composition and

architecture of PSII in the Pinaceae family. Are there any detailed comparative analyses, published or ongoing, on how this structural diversity translates to specific functional traits of PSII, for example with respect to light harvesting or electron transfer?

Critical comments to the habilitation thesis text

1. *Overuse of superlatives*. Chapters 1-5 of the habilitation thesis are informative, logical and written as a typical scientific text, with high information density, complex syntactic structures and domain-specific terminology. The tone is generally formal, objective and consistent with academic writing in the physical and molecular sciences. That said, the text is unusually rich in superlatives and biased qualitative descriptions with the apparent aim to emphasize the significance of the candidate's own results. For example, the word "significant" itself is used 29 times across 41 pages, "important" – 17, "crucial" – 16 times, and so on. This is probably admissible and perhaps even necessary to a certain extent in a habilitation thesis, as its very objective is indeed to demonstrate the significance of the candidate's own scientific contribution. Nevertheless, it is my personal opinion that superlatives could be distracting, and a more neutral tone would be preferable to the accustomed reader of scientific literature. A case in point, it is said that Lhcb6 is "crucial" for PSII, yet conifers seem to function fine without it.
2. *Stylistic editing*. The thesis text is pleasingly free (almost) from grammatical, spelling and typographic errors, for which the author deserves compliments. Nevertheless, some stylistic editing could further enhance readability, avoiding some unwieldy phrases and repetitions, e.g. "structural analysis by EM… has revolutionized the structural analysis of protein complexes" (p.13) or "cryo-EM studies enabled and significantly supported MD simulations, which have contributed significantly to the understanding…".

4. Conclusion

The habilitation thesis represents an outstanding body of research conducted by Dr. Roman Kouřil that exemplifies scientific maturity, methodological rigor, and independent leadership in the fields of structural biology and biophysics. Dr. Kouřil's scientific achievements and contributions to understanding the molecular organization, variability and adaptability of PSII supercomplexes are significant and have opened new research directions in photosynthesis research. It is my considered opinion and recommendation to the Habilitation Committee that the thesis be accepted as a habilitation work of Dr. Roman Kouřil.

Sincerely,

Petar H. Lambrev