Opponent's Review on the Habilitation Thesis of RNDr. Roman Kouřil, Ph.D.

Field: Biophysics, Faculty of Science, Palacký University Olomouc
Title of the Habilitation Thesis: Structural Variability of Photosystem II in Land Plants

The habilitation thesis of RNDr. Roman Kouřil consists of a collection of 12 publications from 2009 to 2023, including 11 peer-reviewed articles published in impact-factor journals and one peer-reviewed book chapter in *Subcellular Biochemistry* (Springer). This collection is accompanied by a 53-page introduction written in English, which presents the research topic—focused on the detailed study of the organization and variability of Photosystem II (PSII)—and the methods employed for its investigation.

The introductory part of the thesis is very well written and provides an appropriate and clear entry point into the topic of PSII structure and function within the context of photosynthesis in land plants. The author clearly and accessibly outlines the biological background, the significance of PSII in the thylakoid membrane, and its spatial organization in chloroplasts. This is followed by a discussion of the complexity and variability of PSII supercomplex organization, their role in plant adaptation to varying stress conditions, and their relation to physiology and evolution. The technical section describing sample preparation and electron microscopy methods provides precise and comprehensive information that demonstrates the author's deep understanding of the modern techniques that form the basis of his research. The explanation of single-particle EM principles, negative staining, cryo-EM, and cryo-electron tomography is both scientifically accurate and pedagogically effective, reflecting the author's experience in scientific communication.

All publications included in the second part of the thesis focus on the study and organization of the PSII system. The choice and application of methods for membrane protein isolation, their identification, and structural analysis via electron microscopy play a key role in these studies. The author demonstrates both a deep understanding of methodological aspects and the ability to interpret structural data in a biological context. This research has led to significant findings, including:

- Detailed visualization of the supramolecular structure of PSII supercomplexes and megacomplexes *in situ*, i.e., within the physiological context of native thylakoid membranes (e.g., *Biochim Biophys Acta* 2011; *Plant J* 2017; *Nature Plants* 2023).
- Characterization of fine grana architecture through 3D reconstructions (cryo-ET), contributing to the understanding of the spatial arrangement of photosystems within membranes (*Biochim Biophys Acta* 2011).
- Documentation of the functional plasticity and structural variability of photosynthetic machinery in response to light conditions, genetic mutations, and interspecies differences (*EMBO J* 2009; *Plant Cell* 2011; *Plant J* 2020).

A notable feature of the habilitation thesis is the clear development in the application of electron microscopy methods, which has had a substantial impact on the quality of scientific results. In earlier works, the author used conventional transmission electron microscopy at room temperature and negative staining techniques, which enabled basic morphological resolution of photosystem complexes. With the advancement of cryo-methods in recent years, he adopted more sophisticated techniques, especially cryo-electron tomography, which enabled three-dimensional visualization of thylakoid membrane organization in its native state. The latest studies employ state-of-the-art methodological advances in

biomolecular structure detection, including single-particle cryo-EM with high resolution, for which the Nobel Prize was awarded in 2018. This technique enabled the author to describe in detail the spatial arrangement of individual subunits, pigments, and cofactors within Photosystem II. This technological shift was not just a methodological improvement—it significantly enhanced the quality, depth, and interpretive power of the data obtained. Each step in the methodological progression opened new questions and enabled their resolution at higher levels of structural and functional precision. This shows the candidate's high level of expertise and ability to apply cutting-edge tools to biologically significant problems.

All listed publications appeared in prestigious, high-impact journals such as *EMBO Journal*, *Nature Plants*, *Plant Cell*, and *Plant Physiology*, proving their scientific quality. In half of these works, RNDr. Kouřil is listed as the first author, in seven of them as the corresponding author. It indicates his leading role in experimental design, data collection, and interpretation. The author teams in these works also demonstrate long-term international collaboration, particularly with the laboratory of Prof. E.J. Boekema, a world-renowned expert in structural biology of photosynthesis. The publication overview reveals long-term thematic consistency, methodological precision, and originality in addressing specific research aspects—demonstrating the candidate's scientific maturity and quality.

Suggested Question for the Habilitation Defense:

In several publications, you employ cryo-electron microscopy and tomography. What are the main challenges and limitations of these methods when studying native membrane structures *in situ*, and to what extent did these limitations influence your results? Can further technological advances in this field bring any significant improvements?

Conclusion:

The submitted collection of publications constitutes a coherent and high-quality scientific work that has significantly contributed to the advancement of knowledge in the structural biology of photosynthesis. The candidate demonstrates professional competence, independence, and sustained research engagement. Given the high quality of the results, international recognition, originality of approach, and methodological sophistication of the habilitation thesis, I recommend that RNDr. Roman Kouřil, Ph.D., be appointed Associate Professor in the field of Biophysics at the Faculty of Science, Palacký University Olomouc.

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