M U N I

Annex No. 10 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Applicant	Konstantinos Tripsianes, Ph.D.
Habilitation thesis	Studying biomolecular structures, interactions, and molecular mechanisms using advanced NMR techniques
Reviewer	Dr. Janez Plavec
Reviewer's home unit, institution	Slovenian NMR Centre, National Institute of Chemistry

Dear Selection Panel,

I am writing this letter in support of Dr. Konstantinos Tripsianes and the submission of his habilitation thesis for review. I first met Dr. Tripsianes during my visit to CEITEC in 2013, on the occasion of the ceremonial inauguration of the Josef Dadok National NMR Centre. In 2015, I attended his lecture at an international conference, where he presented his latest results on protein structure determination and provided in-depth insights that were highly inspiring and resonated strongly with the participants. Since then, I have followed his work closely, and our paths have regularly crossed at conferences focused on NMR and structural biology.

Dr. Tripsianes received his Ph. D. degree for a thesis entitled Structural and biochemical studies of the human ERCC1/XPF DNA repair complex at Bijvoet Center, Utrecht University, The Netherlands in 2008 under the supervision of Profs. Rolf Boelens and Robert Kaptein.

His work experience includes a five-year (2007-2012) postdoctoral experience at Helmholtz Zentrum and Technische Universitat Munchen, Germany under the supervision of Prof. Michael Sattler. Since 2012, he has been a group leader of research group - Protein-DNA lnteractions at Central European Institute of Technology (CEITEC), Brno, Czechia.

Dr. Tripsianes is a highly trained expert in NMR spectroscopy, a powerful tool for investigating the structure, dynamics, and interactions of biological macromolecules. Advances in isotopic labeling, magnet technology, electronics and spectroscopic methods have substantially expanded the capabilities of biomolecular NMR. Historically, NMR followed in the footsteps of X-ray crystallography, focusing largely on globular proteins with regular secondary structures. However, unlike structures determined by X-ray crystallography, an NMR-derived 3D structure is not a direct image but rather a model consistent with experimental data. Consequently, NMR structure determination is a complex, iterative process requiring considerable data analysis and management. Automated approaches have therefore been developed to improve its throughput and accessibility.
A major change in structural biology came in 2020, when AlphaFold2, developed by DeepMind, dramatically outperformed other methods in the CASP14 structure-prediction competition. AlphaFold predictions have since made atomic-level structural models readily

available for most proteins in the human proteome. However, many proteins, particularly those involved in signaling, transcription and protein-protein interaction networks, contain large intrinsically disordered regions, raising an important question on how to interpret and present unstructured regions.

NMR spectroscopy has been the leading method for characterizing protein disorder at atomic resolution. Conceptual advances in understanding intrinsically disordered regions, together with emerging NMR approaches that link molecular properties to cellular functions, now enable the investigation of increasingly challenging multidomain proteins. These developments point toward a new, non-reductionist era of structural biology in which the functional interplay between ordered and disordered regions can be fully explored.

Dr. Tripsianes has not only actively followed these developments but has also contributed to them through his own ideas, methodological approaches, and research findings. The 21 research papers presented in his habilitation thesis reflect the evolution of his NMR research. The originality and distinctive strength of Dr. Tripsianes' approach lie in integrating protein structure, interactions, and dynamics with the underlying molecular mechanisms.

The publication record of Dr. Tripsianes is substantial for his career stage. His 35 papers have been published in highly regarded journals, including *Nature Structural & Molecular Biology* and *Nature Communications*. His publications have attracted considerable attention from the international scientific community, as reflected in the number of citations his work has received.

Taken together, Dr. Konstantinos Tripsianes has the research record, international standing and teaching experience that demonstrate the excellence, independence, and leadership expected at this stage of his academic career. I therefore strongly support the acceptance of his habilitation thesis and recommend that the habilitation procedure proceed toward its successful completion.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)
No specific questions.

Conclusion

The habilitation thesis entitled *Studying biomolecular structures, interactions, and molecular mechanisms using advanced NMR techniques* by Konstantinos Tripsianes **fulfils** requirements expected of a habilitation thesis in the field of **Biomolecular Chemistry and Structural Biology**.

Date: August 29, 2026 Signature: