

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

Simone Ciofi Baffoni, PhD

Reviewer's home unit, institution

Department of Chemistry "Ugo Schiff", University of Florence

The applicant has an outstanding experience in solution-state NMR spectroscopy, showing a remarkable ability to design and implement advanced NMR approaches for the investigation of complex molecular systems. Throughout his scientific career, the applicant has developed a strong command of both experimental methodologies and data analysis strategies, contributing to the advancement of NMR-based structural and dynamic characterization. The applicant's expertise combines methodological rigor, technical competence, and deep understanding of molecular-level phenomena. This level of proficiency places the applicant among the leading experts in the field of solution NMR.

Throughout his career, the applicant has successfully conducted numerous research projects. He has determined twenty-five NMR-derived structures using classical NOE-based approaches, many of which involved challenging systems. He developed an automated NMR resonance assignment approach using a minimal set of 4D spectra for structure determination of proteins ranging in size from 15.5 to 27.3 kDa. He also studied post-translational modifications by solution NMR, developing a combination of the various labelling protocols with individually adapted NMR experiments to circumvent the restrictions for the unlabelled ligand. This allow him to obtain complete resonance assignments for both protein and the attached lipid and to determine the first NMR structure of a farnesylated protein. The use of NMR spectroscopy and complementary techniques to capture synergies between ordered and disordered regions, both at the intramolecular and intermolecular levels, and to decipher the underlying molecular mechanisms has also been a recent scientific focus of the applicant, resulting in three high-impact publications.

Overall, the achievements of the applicant demonstrate a high level of competence, accuracy, scientific rigor, and the ability to provide structural insights that have significantly contributed to addressing important biological questions.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

The applicant raises a very stimulating and relevant question in his habilitation document: "It is true that lengthy experimental procedures have been substituted by a few mouse clicks by AlphaFold, but do researchers know how to evaluate these structures?" This is, in my opinion, a highly important issue for the future development of structural biology and NMR spectroscopy. I would be very interested in hearing the applicant's perspective on this topic, particularly regarding how experimental NMR approaches can continue to provide critical validation, interpretation, and biological insights in the era of AI-based structure prediction.

The second question is partially connected to the previous one. The applicant has demonstrated outstanding expertise in NMR spectroscopy and has worked throughout his career in leading European NMR laboratories, which has provided him with a broad and comprehensive view of the field. Therefore, I would be interested in hearing how he would envision the organization of his future NMR laboratory and research activities in order to maximize their impact in the coming years, particularly considering the rapid developments in AI-based structure prediction and the increasing competition from cryo-EM developments. Which will be the most promising biological field for NMR to pursue among those mentioned by the applicant, such as IDPs, PTMs, large MW proteins and biomolecular interactions? Or better to switch to other more promising areas?

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: 13/7/2026

Signature: