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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	Prof. Anastassis Perrakis
Reviewer's home unit, institution	The Netherlands Cancer Institute Oncode Institute University of Utrecht, Netherlands

The habilitation of Dr. Konstantinos Tripsianes is a commented collection of 21 peer-reviewed papers published between 2005 and 2026. They are introduced by a ~20-page narrative that places the work in the wider context of structural biology. The thesis has four thematic parts discussed seperately.

Structure determination of proteins and complexes (papers 1–7). The work is solid structural biology. The structures of the ERCC1/XPF interaction domains and the ERCC1 central domain gave insight into nucleotide excision repair, and the work on Sam68, the RES complex and others followed. The work clearly shows real experimental skill. The candidate's own account of how much expert human intervention reliable NMR structures need is candid and informative.

Automation of NMR structure determination (papers 8–12). To me this is the most original methodological part of the thesis. It builds on the chemical-shift-guided Rosetta approaches. While I am not an expert in NMR methods it is clear that the work had measurable impact and was original. The candidate frankly reports that a follow-up deep-learning was stopped when the AlphaFold database appeared. This shows good scientific judgement about where NMR adds value after AlphaFold, and later work in the thesis follows from that decision.

Post-translational modifications (papers 13–18). Two papers stand out. The structures of the SMN and SPF30 Tudor domains bound to symmetric and asymmetric dimethylarginine (paper 13, NSMB 2011, first author) explain how the aromatic cage reads the methylation state. The farnesylated PEX19 structure (paper 15, Nature Communications 2017, joint corresponding author) is, to my knowledge, the first structure of a farnesylated protein. The lipid allosterically remodelling the cargo-binding surface was an original mechanism validated in patient fibroblasts. The related methods paper (paper 16) described isotope-labelling schemes, including perdeuteration combined with selective and reverse labelling was an excellent complement. Contributions to Dishevelled phosphorylation and polyglutamylation (papers 17, 18) show the candidate as a valued partner in cell-biology-led projects.

Order–disorder interplay (papers 19–21). This is where the thesis is most forward-looking and where NMR is truly irreplaceable. Paper 19 (Nature Communications 2023, joint corresponding author) shows that the disordered N-terminal region of RECQ4 binds nucleic acids while staying disordered. Paper 20 (Structure 2025, sole corresponding author) combines a 1.36 Å crystal structure with NMR relaxation dispersion to show that the C-terminal class III ligand of Dishevelled-3 switches between "terminal" and "internal" carboxylate binding modes in the PDZ groove. I appreciate here the strong move towards an

integrative study. Paper 21 (Science Advances 2026, joint corresponding author) describes a phosphorylation-driven conformational switch in DVL3. These studies form a coherent, well-defined research direction set out explicitly in the thesis. The programme is well placed and timely.

Applicant's contribution. The commentary gives a quantitative breakdown of the candidate's contribution to each paper. I applaud it for its honesty: small contributions to several collaborative papers are declared as such (5–20%). The key papers in each section (1, 2, 5, 9, 13, 15, 16, 19, 20, 21) show leading contributions to experiments, writing and research direction. Seven of them carry corresponding authorship, and the most recent work comes from his own group with his students and postdocs as first authors. The candidate has moved convincingly from a skilled spectroscopist to independent principal investigator. There is sustained publication record in strong journals combined to productive local and international collaborations.

Presentation. The narrative is clear, well structured and readable for a non-NMR specialist. Its introductory and methodological sections would serve well as teaching material.

The thesis shows a broad and deep command of solution NMR as a tool for biological questions. It includes original methods work with a lasting conceptual message. Combinaton with high-impact structural findings, and a clear independent research direction in an area where experiment is still indispensable is a good basis for the future. The entire body of work fully meets, and in several respects exceeds, the standard I would expect of a habilitation thesis in structural biology.

Reviewer's questions for the habilitation thesis defence

1. The commentary says an NMR structure is "a model compatible with the experimental data" rather than "an image of the real structure (as for X-ray)". Would argue that a crystal structure is also a model fitted to data, the dat abeign a 3d image. How do you see the real differences in how much NMR and crystallographic models can be trusted? Which validation metrics should a non-specialist look at when judging an NMR ensemble, the way R-free and electron density are used for crystal structures?
2. You stopped 4D-GRAPHS when the AlphaFold database appeared. Looking back, where do you now see the most productive interface between NMR and structure prediction? Could NMR data to guide or score ensemble-generating methods (AF-Cluster, AlphaFold3 sampling, generative ensemble models) some kind of AI-enables HADDOCK?
3. Is there an experimental strategy do you favor for full-length proteins in a more native context (segmental labelling, methyl-TROSY, in-cell NMR or condensate NMR)? How do you see integration with cryo-EM, crosslinking-MS or HDX-MS? Where do you expect the main bottleneck to be?
4. The thesis stresses that attracting young scientists to NMR is a serious challenge. How would you teach NMR to biology students who take predicted structures for granted, have a poor physics and mathematics background and what concrete steps do you take in your own group?

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**.

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