M U N I

COMMENTARY TO HABILITATION THESIS[1]

Studying biomolecular structures, interactions, and molecular mechanisms using advanced NMR techniques

Konstantinos Tripsianes, Ph.D.

NMR spectroscopy is a powerful tool for biologists interested in the structure, dynamics, and interactions of biological macromolecules. Several developments in isotopic labeling, magnet technology, electronics, and spectroscopy have pushed the boundaries of biomolecular NMR. For many years NMR has followed the footsteps of X-ray crystallography and focused on globular proteins composed of regular secondary structure elements. It is however important to remember that the NMR derived 3D representation of a protein is not an image of the real structure (as for X-ray) but a model of that structure that is compatible with the experimental data. Therefore, NMR 3D structure determination, is a complicated and repetitive procedure, which involves a substantial book-keeping effort. Automated procedures were developed over the years promising to increase the throughput and accessibility of NMR in studying protein structures. In November 2020, the results of the 14th Critical Assessment of Structure Prediction (CASP14) competition revealed that AlphaFold2 (AF2), an artificial intelligence (AI) developed by DeepMind, performed significantly better than all other methods. Through a portal hosted by the European Bioinformatics Institute one can now access predictions of protein structures made using AlphaFold. In an instant, detailed, atomic-level models of protein structures have become available for most of the protein sequences in the human proteome. Many of these proteins, especially those involved in signaling, transcription, and coordinating protein-protein interaction networks, are likely to feature large, disordered regions. A key question is how should one interpret the “unstructured regions”. NMR spectroscopy has been the leading tool in characterizing intrinsic disorder regions (IDRs) of proteins at the atomic level. Thanks to conceptual breakthroughs in the study of IDRs facilitated by NMR and emerging NMR techniques that bridge molecular descriptions to cellular functions, we now have the tools to tackle some of the most challenging multi-domain proteins and can anticipate the emergence of a new, non-reductionist era of structural biology in which the functional synergies between ordered and disordered regions are fully revealed. The 21 research papers presented in this thesis highlight the transformation of my NMR research throughout the years, or in other words my scientific journey involving structures, interactions, dynamics and molecular mechanisms.

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[1] The commentary must correspond to standard expectations in the field and must include a brief characteristic of the investigated matter, objectives of the work, employed methodologies, obtained results and, in case of co-authored works, a passage characterising the applicant’s contribution in terms of both quality and content.

[1] **Tripsianes K.**, Folkers G.E., AB E., Das D., Odijk H., Jaspers N.G., Hoeijmakers J.H., Kaptein R., Boelens R.* (2005). The structure of the human ERCC1/XPF interaction domains reveals a complementary role for the two proteins in nucleotide excision repair. *Structure*, *13*(12), 1849-1858.
Impact factor: 5.543

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	—	70	10

[2] **Tripsianes K.**, Folkers G.E., Zheng C., Das D., Grinstead J.S., Kaptein R., Boelens R.* (2007). Analysis of the XPA and ssDNA-binding surfaces on the central domain of human ERCC1 reveals evidence for subfunctionalization. *Nucleic Acids Res*,*35*(17), 5789-5798.
Impact factor: 6.954

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	—	80	50

[3] Meyer N.H., **Tripsianes K.**, Vincendeau M., Madl T., Kateb F., Brack-Werner R., Sattler M.* (2010). Structural basis for homodimerization of the Src-associated during mitosis, 68-kDa protein (Sam68) Qua1 domain. *J Biol Chem*, *285*(37), 28893-28901.
Impact factor: 5.328

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
25	20	20	—

[4] Kateb F., Perrin H., **Tripsianes K.**, Zou P., Spadaccini R., Bottomley M., Franzmann T.M., Buchner J., Ansieau S., Sattler M.* (2013). Structural and functional analysis of the DEAF-1 and BS69 MYND domains. *PLoS One*, *8*(1), e54715.
Impact factor: 3.534

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	—	10	—

[5] **Tripsianes K.***, Friberg A., Barrandon C., Brooks M., van Tilbeurgh H., Seraphin B., Sattler M.* (2014). A novel protein-protein interaction in the RES (REtention and Splicing) complex. *J Biol Chem, 289*(41), 28640-28650.
Impact factor: 4.573

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
60	10	70	50

[6] Anosova I., Melnik S., **Tripsianes K.**, Kateb F., Grummt I.*, Sattler M.* (2015). A novel RNA binding surface of the TAM domain of TIP5/BAZ2A mediates epigenetic regulation of rRNA genes. *Nucleic Acids Res*, *43*(10), 5208-5220.
Impact factor: 9.202

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	—	10	—

[7] Meyer N.H., Mayerhofer H., **Tripsianes K.**, Blindow S., Barths D., Mewes A., Weimar T., Kohli T., Bade S., Madl T., Frey A., Haas H., Mueller-Dieckmann J.*, Sattler M.*, Schramm G.* (2015). A Crystallin Fold in the Interleukin-4-inducing Principle of Schistosoma mansoni Eggs (IPSE/alpha-1) Mediates IgE Binding for Antigen-independent Basophil Activation. *J Biol Chem*, *290*(36), 22111-22126.
Impact factor: 4.258

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	20	10	—

[8] Zhang Z., Porter J., **Tripsianes K.**, Lange O.F.* (2014). Robust and highly accurate automatic NOESY assignment and structure determination with Rosetta. *J Biomol NMR*, *59*(3), 135-145.
Impact factor: 3.141

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	—	10	10

[9] Evangelidis, T., Nerli, S., Novacek, J., Brereton, A. E., Karplus, P. A., Dotas, R. R., Venditti, V., Sgourakis, N. G.*, & **Tripsianes, K.*** (2018). Automated NMR resonance assignments and structure determination using a minimal set of 4D spectra. *Nat Commun*, *9*(1), 384.
Impact factor: 11.878

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	50	50	80

[10] Marcos, E.*, Chidyausiku, T. M., McShan, A. C., Evangelidis, T., Nerli, S., Carter, L., Nivon, L. G., Davis, A., Oberdorfer, G., **Tripsianes, K.**, Sgourakis, N. G., & Baker, D.* (2018). De novo design of a non-local beta-sheet protein with high stability and accuracy. *Nat Struct Mol Biol*, *25*(11), 1028-1034.
Impact factor: 12.109

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
5	10	5	—

[11] Harnos, J., Canizal, M. C. A., Jurasek, M., Kumar, J., Holler, C., Schambony, A., Hanakova, K., Bernatik, O., Zdrahal, Z., Gomoryova, K., Gybel, T., Radaszkiewicz, T. W., Kravec, M., Trantirek, L., Rynes, J., Dave, Z., Fernandez-Llamazares, A. I., Vacha, R., **Tripsianes, K.**, Hoffmann, C., Bryja, V.* (2019). Dishevelled-3 conformation dynamics analyzed by FRET-based biosensors reveals a key role of casein kinase 1. *Nat Commun*, *10*(1), 1804.
Impact factor: 12.121

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	10	10	—

[12] Yperman, K., Papageorgiou, A. C., Merceron, R., De Munck, S., Bloch, Y., Eeckhout, D., Jiang, Q., Tack, P., Grigoryan, R., Evangelidis, T., Van Leene, J., Vincze, L., Vandenabeele, P., Vanhaecke, F., Potocky, M., De Jaeger, G., Savvides, S. N.*, **Tripsianes, K.***, Pleskot, R.*, & Van Damme, D.* (2021). Distinct EH domains of the endocytic TPLATE complex confer lipid and protein binding. *Nat Commun*, *12*(1), 3050.
Impact factor: 17.694

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
30	30	20	20

[13] **Tripsianes K.**, Madl T., Machyna M., Fessas D., Englbrecht C., Fischer U., Neugebauer K.M., Sattler M.* (2011). Structural basis for dimethylarginine recognition by the Tudor domains of human SMN and SPF30 proteins. *Nat Struct Mol Biol*,*18*(12), 1414-1420.
Impact factor: 12.712

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
70	—	70	80

[14] Rodriguez Camargo D.C., **Tripsianes K.**, Buday K., Franko A., Gobl C., Hartlmuller C., Sarkar R., Aichler M., Mettenleiter G., Schulz M., Boddrich A., Erck C., Martens H., Walch A.K., Madl T., Wanker E.E., Conrad M., de Angelis M.H., Reif B.* (2017). The redox environment triggers conformational changes and aggregation of hIAPP in Type II Diabetes. *Sci Rep*, *7*, 44041.
Impact factor: 4.122

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	10	10	—

[15] Emmanouilidis L., Schütz U., **Tripsianes K.***, Madl T., Radke J., Rucktaschel R., Wilmanns M., Schliebs W., Erdmann R., Sattler M.* (2017). Allosteric modulation of peroxisomal membrane protein recognition by farnesylation of the peroxisomal import receptor PEX19. *Nat Commun*, *8*, 14635.
Impact factor: 12.353

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
50	50	50	40

[16] **Tripsianes, K.***, Schutz, U., Emmanouilidis, L., Gemmecker, G., & Sattler, M.* (2019). Selective isotope labeling for NMR structure determination of proteins in complex with unlabeled ligands. *J Biomol NMR*, *73*(3-4), 183-189.
Impact factor: 2.634

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
80	50	100	50

[17] Hanakova, K., Bernatik, O., Kravec, M., Micka, M., Kumar, J., Harnos, J., Ovesna, P., Paclikova, P., Radsetoulal, M., Potesil, D., **Tripsianes, K.**, Cajanek, L., Zdrahal, Z., & Bryja, V.* (2019). Comparative phosphorylation map of Dishevelled 3 links phospho-signatures to biological outputs. *Cell Commun Signal*, *17*(1), 170.
Impact factor: 4.344

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	10	10	—

[18] Kravec, M., Sedo, O., Nedvedova, J., Micka, M., Sulcova, M., Zezula, N., Gomoryova, K., Potesil, D., Sri Ganji, R., Bologna, S., Cervenka, I., Zdrahal, Z., Harnos, J., **Tripsianes, K.**, Janke, C., Barinka, C., & Bryja, V.* (2024). Carboxy-terminal polyglutamylation regulates signaling and phase separation of the Dishevelled protein. *EMBO J*, *43*(22), 5635-5666.
Impact factor: 8.300

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	10	10	—

[19] Papageorgiou, A. C., Pospisilova, M., Cibulka, J., Ashraf, R., Waudby, C. A., Kaderavek, P., Maroz, V., Kubicek, K., Prokop, Z., Krejci, L.*, & **Tripsianes, K.*** (2023). Recognition and coacervation of G-quadruplexes by a multifunctional disordered region in RECQ4 helicase. *Nat Commun*, *14*(1), 6751.
Impact factor: 14.700

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	50	80	60

[20] Kumar, J., Micka, M., Komarek, J., Klumpler, T., Bystry, V., Sprangers, R., Barinka, C., Bryja, V., & **Tripsianes, K.***(2025). A class III ligand oscillates between internal and terminal binding modes as it engages with the Dishevelled PDZ domain. *Structure*, *33*(8), 1362-1373
Impact factor: 4.300

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
10	80	80	90

[21] Micka, M., Kumar, J., Paclíková, P., Hayek, Z., Hanáková, K., Bechara, C., Plešingerová, H., Šedo, O., Bologna, S., Del Nero, E., Gömöryová, K., Bystrý, V., Gybeľ, T., Číhalová, T., Kravec, M., Potěšil, D., Zdráhal, Z., **Tripsianes, K.*** & Bryja, V.* (2026). A Wnt-induced conformational phospho-switch in DVL3 controls association with Frizzled receptors and Wnt/β-catenin signaling. *Sci Adv* (in press)
Impact factor: 12.400

Experimental work (%)	Supervision (%)	Manuscript (%)	Research direction (%)
20	80	60	80