CSC-RUB PhD Project Proposal

Title: NMR-based structure-activity relationship studies on the FDP protein to decipher its role in the development of the inner ear and hearing loss

Sector of research: Biochemistry and Structural Biology

Degree awarded: Dr. *rer.nat.*

Keywords: Protein, structure-function-relationship, dynamics, NMR spectroscopy

Supervisor of PhD project:

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Research focus of supervisor:

Our research focuses in the main on medically-relevant proteins, specifically those involved in the development of cancerous tumours, to be able to better understand the causes of the condition and propose more effective treatment strategies. Examples include oncogenic proteins, tumour suppressors as well as proteins involved in malignant melanoma, to name but a few. Other current areas of investigation cover the structure, function, dynamics, and ligand interaction of proteins associated with the transduction of physiological signals as well as bioenergetics and infectious diseases, such as tuberculosis. In particular, we would like to understand structure-function-relationships of MDM2, p53, Ras as well as Rheb GTPases, the melanoma inhibitory activity (MIA) protein, NFκB, and HMGA1a. Our additional research efforts in medicinal chemistry include an "SAR by NMR"-like approach in order to develop potential lead structures of small molecular antagonists for these proteins and to combat antimicrobial resistance.

Publications:

1) Structural insights into photosystem II assembly. Zabret J, Bohn S, Schuller SK, Arnolds O, Möller M, Meier-Credo J, Liauw P, Chan A, Tajkhorshid E, Langer JD, **Stoll R**, Krieger-Liszkay A, Engel BD, Rudack T, Schuller JM, Nowaczyk MM. *Nat Plants.* 2021 Apr;7(4):524-538. doi: 10.1038/s41477-021-00895-0.
2) Comparison of backbone dynamics of the p50 dimerization domain of NFκB in the homodimeric transcription factor NFκB1 and in its heterodimeric complex with RelA (p65). Kohl B, Granitzka V, Singh A, Quintas P, Xiromeriti E, Mörtel F, Wright PE, Kroon G, Dyson HJ, **Stoll R.** *Protein Sci.* 2019 Dec;28(12):2064-2072. doi: 10.1002/pro.3736.

3) Phosphorylation orchestrates the structural ensemble of the intrinsically disordered protein HMGA1a and modulates its DNA binding to the NFκB promoter. Kohl B, Zhong X, Herrmann C, **Stoll R.** *Nucleic Acids Res.* 2019 Dec 16;47(22):11906-11920.
4) Molecular Basis of Class III Ligand Recognition by PDZ3 in Murine Protein Tyrosine Phosphatase PTPN13. Kock G, Dicks M, Yip KT, Kohl B, Pütz S, Heumann R, Erdmann KS, **Stoll R.** *J Mol Biol.* 2018 Oct 19;430(21):4275-4292. doi: 10.1016/j.jmb.2018.08.023.
5) Allosteric Activation of GDP-Bound Ras Isoforms by Bisphenol Derivative Plasticisers. Schöpel M, Shkura O, Seidel J, Kock K, Zhong X, Löffek S, Helfrich I, Bachmann HS, Scherkenbeck J, Herrmann C, **Stoll R.** *Int J Mol Sci.* 2018 Apr 10;19(4):1133.

Second Supervisor of PhD project:

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Summary of research plan

Background: The Melanoma Inhibitory Activity (MIA) protein is strongly expressed and secreted by malignant melanoma cells and was shown to promote melanoma development and invasion. The MIA protein was the first extracellular protein shown to adopt an Src homology 3 (SH3) domain-like fold in solution that can bind to fibronectin type III domains. Together with MIA, the homologous proteins FDP, MIA-2, and TANGO constitute a protein family of non-cytosolic and – except for full-length TANGO and TANGO1-like (TALI) – extracellular SH3-domain containing proteins. Members of this protein family modulate collagen maturation and export, cartilage development, cell attachment in the extracellular matrix, and melanoma metastasis. These proteins may thus serve as promising targets for drug development. The FDP protein is homologous to MIA and expressed from embryonic day 10.5 in the mesenchyme surrounding the otic epithelium. During mesenchyme development, the cells differentiate into cartilage cells that form the otic capsule. The expression of FDP protein is absent in the otic capsule, but strongly present in the fibrocytes surrounding the epithelia. Silencing experiments revealed by reduction of the chondrogenesis, the importance of the FDP protein in the initiation of periotic mesenchyme chondrogenesis. Hitherto, the exact function of the FDP protein is unknown but it seems to be related to deafness with malformations of the otic capsule. Here, we propose to carry out NMR-based experiments to correlate the structure-activity relationship of FDP with its physiological function. To this extent we collaborate with the Medical School at our University.

Study objective: MIA is a highly homologous protein to FDP. Like FDP, MIA is an extracellular protein highly expressed by malignant melanoma cells, plays an important functional role in

melanoma development, progression and metastasis. After its secretion, MIA directly interacts with extracellular matrix proteins, such as fibronectin (FN). By this mechanism, MIA actively facilitates focal cell detachment from surrounding structures and strongly promotes tumour cell invasion and migration. Hence, the molecular understanding of MIA's function provides a promising target for the development of new strategies in malignant melanoma therapy. Recently, we have published for the first time the discovery of small molecules that are able to disrupt the MIA-FN complex by selectively binding to a new druggable pocket, which we could identify on MIA by structural analysis and fragment-based screening. Our findings may inspire novel drug discovery efforts not only aiming at a therapeutically effective treatment of melanoma by targeting MIA but also to treat hearing loss by targeting FDP.

Expected Results: We aim to contribute to understanding of FDP's physiological mode of action and, ultimately, pave the way for the treatment of hearing loss, one of the most common sensory defects. To this extent we will carry out NMR-based experiments to elucidate the structure-activity relationship of FDP as well as its interaction with low molecular weight compounds to further our understanding of this protein's mode of action. Our results will be published in numerous prestigious peer-reviewed international journals.

Methods: Biochemistry seeks to understand life at a molecular level by examining the relationship between the structure and function of biomolecules, such as proteins, nucleic acids, and lipids. To achieve this, we use a variety of techniques, particularly biomolecular NMR spectroscopy, in order to determine the three-dimensional structures and dynamics of biomolecules as well as how they interact with each other and other molecules in solution at near-physiological conditions. Cancer cells are hallmarked by the ability to divide unrestrictedly, posing an often-lethal threat to an organism. Thus, the development of selective small antagonistic ligands tailored to bind to crucial protein targets is paramount to be able to treat cancer effectively. In order to achieve this goal, the molecular mechanism of a potential therapeutic effect needs to be elucidated at atomic resolution.

Candidate Requirements: We expect a completed university M.Sc. degree in chemistry or biochemistry, convincing academic performance, above-average motivation and a high level of commitment. Furthermore, basic knowledge of biomolecular NMR spectroscopy as well as molecular dynamics simulations for protein structure determination, good English skills, and experience in working with computers are required.

Motivation for CSC application: The successful applicant will receive comprehensive and in-depth training in the complex methodological skills that are required for NMR-based structural

biology research. The necessary equipment for carrying out preparative and spectroscopic work is readily available (www.rub.de/bionmr). Sufficient access to modern high-field NMR spectrometers operating at field strengths of 14.1 and 16.4 Tesla in RUBiospec|NMR at the Faculty of Chemistry and Biochemistry of the Ruhr-Universität Bochum (RUB) is assured. For the production of the isotope-enriched proteins, a fully-equipped molecular biological and protein chemical S1 laboratory is available to all group members. For mass spectrometric investigations, the equipment of the RUBiospek mass spectrometry service department of the Faculty of Chemistry and Biochemistry at the Ruhr-Universität Bochum can be used, which habours a LTQ-Orbitrap XL from Thermo-Scientific. An isothermal microcalorimeter (ITC) and a CD spectrometer are also available at the faculty. For *in silico* screenings and molecular modelling of protein-ligand complexes, the working group has access to the central computing capacities at the RUB. The PhD project will be closely supervised by Prof. Dr. Raphael Stoll who is internationally recognised in the field of NMR-based structural biology and biochemistry. Further, PhD candidates will be supported by a highly skilled team of fellow scientists and will become members of the RUB Research School, a campus-wide institution of all faculties for the promotion of doctoral and postdoctoral researchers of Ruhr-Universität Bochum. It offers complementary qualification beyond disciplinary borders, dedicated consulting, and a unique *Research Around the World* internationalisation program. It supplements the offers of our 21 faculties to RUB's 4.000+ emerging researchers who can benefit from an extensive range of support and services preparing them in the best possible way for a career in academia and beyond. Finally, RUB is committed to ensuring that work/academics/studies are compatible with family responsibilities. The Family Friendly University Office promotes the strategic development of a family-friendly university culture in science and administration.