

Interdisciplinary PhD positions at the Graz University of Technology

The Institute of Biochemistry at the Graz University of Technology is recruiting highly motivated PhD candidates.

Three open PhD positions are available in the research group of **Gustav Oberdorfer**. The group's primary research interests are designing and engineering of biomolecular structures and their functions - a multi-faceted effort that combines computational biology, structural biology, biochemistry and biophysics approaches. The PhD projects focus on designing and characterizing protein structures with central cavities or pores for passive transport and catalytic functionalization of *de novo* proteins.

Two PhD positions are to be filled in the research group of **Andreas Winkler**. The objectives revolve around an integrative structural biology approach addressing mechanisms of signal transduction in natural light-regulated enzymes. Understanding principles of sensor-effector modularity and how nature adapts them for diverse requirements, is key to improve success rates of artificial designs for the generation of optogenetic tools.

The group of **Peter Macheroux** is looking for one PhD student to work in the field of bacterial bioluminescence. Efficiency of light emission critically depends on the availability of the substrate molecule of the luciferase reaction. The enzymes involved in generating this compound form a large supramolecular complex. A detailed biochemical characterization combined with structural analyses will enable a better understanding of the underlying molecular details and facilitate the engineering of bioluminescent systems for *in vivo* imaging or optimized reporter systems.

All projects provide in-depth, interdisciplinary training in biomedical and biotechnological research in an international and stimulating environment. The thesis projects follow an integrative approach and combine structural biology, enzymology, computational protein design and biophysics to decipher biosynthetic pathways, molecular mechanisms of catalysis and development of biomedical tools, employing a wide range of cutting-edge techniques.

Applicants must hold (or be close to obtaining) an undergraduate degree equivalent to a Master in any discipline of natural, life sciences or computer sciences. The ability to work in a team, initiative, flexibility as well as good organizational and learning skills are required. The selection procedure, all training activities and communications will be in English. Thus, excellent written and spoken English skills are required. Successful applicants will get employed for up to four years (initial contract for one year with the option of prolongation upon successful performance) with a contract that includes social benefits.

Applicants should send a motivation letter, CV, one-page summary of their research experience and contact details of one referee until 9th of February 2019 to office.ibc@tugraz.at. Prospective starting dates throughout 2019. Detailed descriptions of the projects can be found here: ftp.TUGraz.at/outgoing/ibc/announcement.pdf or by scanning the QR-code on the right.



Projects in the computational protein design group of Gustav Oberdorfer

Computational design of novel protein structures and enzymes is a promising tool to create superior biological materials with tailor-made properties, new pharmaceuticals, complex fine chemicals or renewable fuels. Recently we have developed a computational method for the design of helical protein structures that produces helical backbones by varying the parameters in the Crick coiled-coil generating equations [1]. Combinatorial design calculations using the software suite Rosetta [2] identify low energy sequences for alternative helix supercoil arrangements. We applied this approach to monomeric three- and four-helical bundle structures as well as a pentameric five-helix bundle structure using idealized coiled-coil geometries [3]. At the moment we are focusing on functionalizing these helical structures. Specifically, we are interested in designing types of helical proteins with different geometries to harbor binding sites for metal and small molecule binding. We also expanded the computational protocols to be able to design higher complexity backbones, which resulted in the *de novo* design of helical barrel structures with architectures not found in nature. Within the three PhD projects, successful applicants will follow a highly interdisciplinary computational-experimental approach to address these challenges and aim to:

Project 1: Characterize to which extent we can harness the stability of parametrically designed helical bundles to introduce deviations from ideal geometry. Ensembles of idealized *de novo* helix bundle backbones will be generated using our established parametric design code and designed with constraints accounting for an envisioned functional site. This will be followed by detailed computational, biophysical, crystallographic and site-saturation mutagenesis analysis to isolate critical design features.

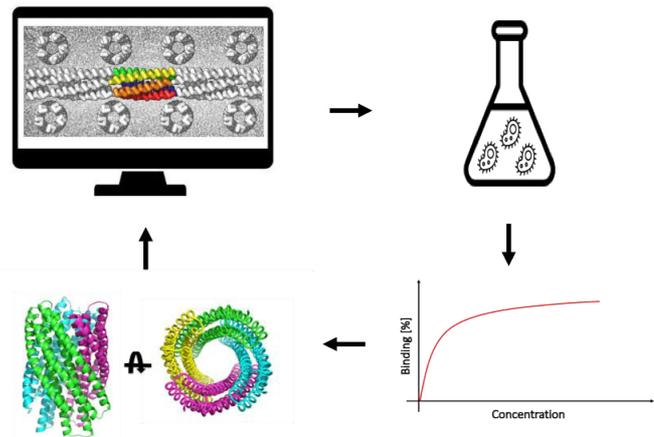


Figure 1. Artistic representation of the combined computational and experimental approach to all advertised research projects

Project 2: Computational design of highly stable helical oligomers with custom geometries. The ultimate goal is to design proteins with ideal geometries for passive transport, suitable to be used in e.g. nanopore sequencing. For pore-like assemblies, *de novo* proteins will be oligomerized through protein-protein docking and interface design. The focus will be on soluble designs first, followed by redesign to make the designs membrane spanning.

Project 3: Develop a caged version of the PD-1/PD-L1 antibodies. This collaborative project with the groups of Julia Kargl at the Medical University of Graz and Andreas Winkler at the Graz University of Technology is a combined protein design, optogenetics and cell biology approach. Light-inducible heterodimerization systems will be redesigned for photo-switchable antigen recognition site caging and dimerization.

[1] F. H. Crick. (1953) The Fourier Transform of a Coiled Coil, *Acta Cryst.*, 6: 685

[2] Leaver-Fay, A. et al (2011) Rosetta 3, *Method. Enzymol.* 487, 545-574

[3] *Huang, P-S., *Oberdorfer, G., *Xu, C., et al. (2014) High thermodynamic stability of parametrically designed helical bundles. *Science*, 346 (6208): 481-485 DOI: [10.1126/science.1257481](https://doi.org/10.1126/science.1257481) *equal contribution

Projects in the photobiochemistry group of Andreas Winkler

During evolution, nature has developed an astonishingly modular architecture of covalently linked individual protein domains. Using an array of building blocks with diverse functionalities enabled organisms to develop complex cellular networks that are critical for cell survival. The frequently observed coupling of sensory modules with enzymatic effectors enables direct allosteric regulation of, for example, second messenger levels in response to diverse stimuli.

The interest in light-regulated systems has recently increased due to the establishment of ‘optogenetics’, which refers to the concept of genetically targeting biological systems to enable optical control of cellular processes. Even though progress in understanding concepts of light activation has been made, the rational design of synthetic tools is still challenging. Since mechanistic descriptions of light-signalling differ even within photoreceptor families, it is obvious that a more detailed understanding of the modularity of sensor-effector couples is required.

To this end, we will perform a detailed study on red-light sensors linked to diguanylate cyclases. The identification of important signalling elements for the sensory domain and regulatory regions of the effector domain will provide insight into the modular coupling frequently observed in nature. We will perform an interdisciplinary approach combining biochemistry with biophysics and structural biology. Atomic models obtained by crystallography will be functionally extended by solution scattering studies, nuclear magnetic resonance spectroscopy and hydrogen-deuterium exchange coupled to mass spectrometry.

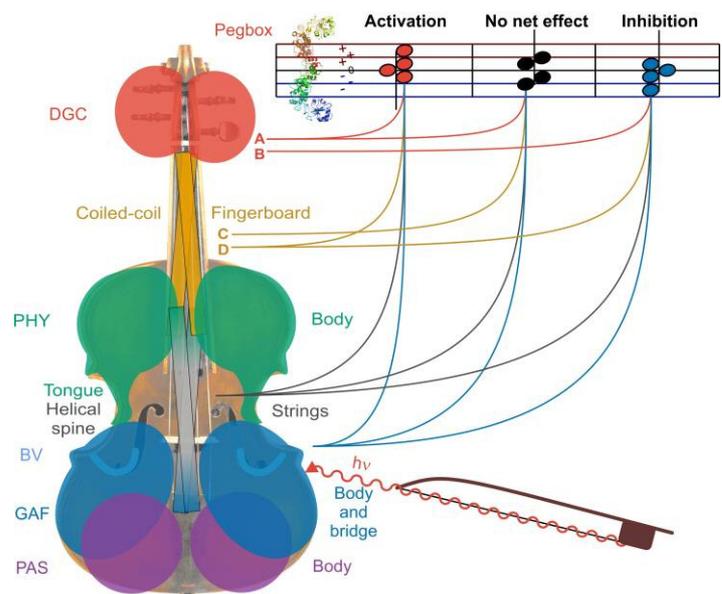


Figure 2. Artistic representation of the complex interplay of structural rearrangements with effector regulation

The combined results will significantly strengthen our understanding of light-signal transduction from the point of the photoreceptor as well as the effector domain. Eventually this will enable a better understanding of the modularity observed in natural light-regulatable systems and support the rational design of artificial optogenetic tools.

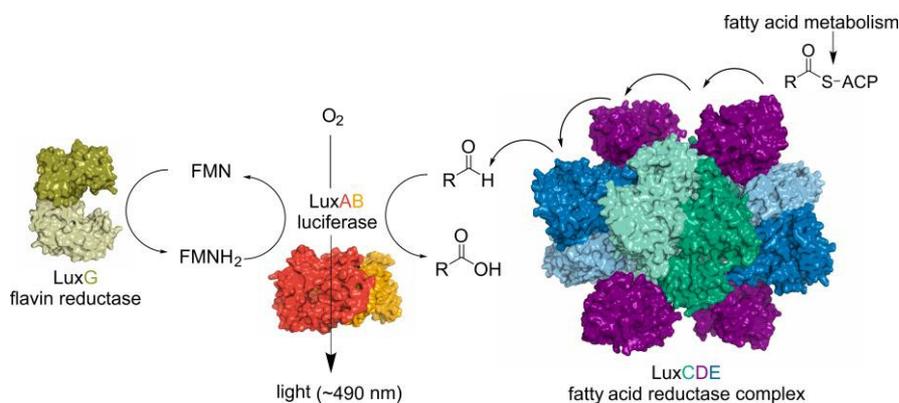
References

- 1) Gourinchas G, et al. (2018) *eLife* 7 e34815, DOI: [10.7554/eLife.34815](https://doi.org/10.7554/eLife.34815).
- 2) Etzl S, et al. (2018) *J Biol Chem* 293 9078-89, DOI: [10.1074/jbc.RA118.003069](https://doi.org/10.1074/jbc.RA118.003069).
- 3) Gourinchas G, et al. (2017) *Sci Adv* 3 e1602498, DOI: [10.1126/sciadv.1602498](https://doi.org/10.1126/sciadv.1602498).

Projects in the biochemistry group of Peter Macheroux

Bioluminescence refers to the formation of light during the course of enzymatic reactions in living organisms. This peculiar phenomenon has been observed in different kingdoms of life and has fascinated scientists throughout the last decades. While significant advances in understanding the underlying processes have been obtained, there are still many open questions that we would like to address in part during the course of this project. Specifically, we are interested in bacterial species that enable bioluminescence using a flavin dependent reaction for the generation of the light-emitting molecule.

The biological light reaction in bacteria is enabled by an enzyme, the so-called luciferase, that catalyzes the oxidation reaction of long-chain aldehydes to their corresponding acids. During the course of the reaction intermediates populate excited states that can spontaneously emit photons with a wavelength of around 490 nm corresponding to the blue-greenish color of bacterial bioluminescence. In addition to the aldehyde substrate, bacteria also require oxygen and reduced flavin mononucleotide for the bioluminescent reaction to take place. In total, bacteria require five enzymes for processing all compounds involved and thereby influencing the intensity of light emission.



Aim of this project is the biochemical and structural characterization of the enzymes and enzyme complexes involved in the bioluminescent reaction. Specifically, the interaction of members of the fatty acid reductase complex and the hypothesized interaction of the luciferase and the flavin reductase will be central targets of the initial characterization. For this purpose, standard techniques of molecular biology, protein expression and purification will precede a more detailed functional characterization using tools of biochemistry and biophysics. As far as structural analyses are concerned, we will employ crystallography and cryo-electron microscopy combined with lower resolution techniques such as hydrogen-deuterium exchange coupled to mass spectrometry.

- 1) Bergner T. *et al.* (2015) *Biochim Biophys Acta* 1854, 1466-1475. DOI: [10.1016/j.bbapap.2015.07.008](https://doi.org/10.1016/j.bbapap.2015.07.008)
- 2) Brodl E. *et al.* (2017) *Bioorg Med Chem* 25, 1487-1495. DOI: [10.1016/j.bmc.2017.01.013](https://doi.org/10.1016/j.bmc.2017.01.013)
- 3) Tabib C., Brodl E. *et al.*, (2017) *Mol Microbiol* 104, 1027-1036. DOI: [10.1111/mmi.13676](https://doi.org/10.1111/mmi.13676)
- 4) Brodl E. *et al.*, (2018) *J Vis Exp* e57881. DOI: [10.3791/57881](https://doi.org/10.3791/57881)
- 5) Brodl E. *et al.*, (2018) *Comp Struct Biol J*, 16, 551-564. DOI: [10.1016/j.csbj.2018.11.003](https://doi.org/10.1016/j.csbj.2018.11.003)