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BioTechMed GRAZ

Symposium on "Frontiers in Integrative Structural Biology and Biophysics"

Wednesday, 11th December 2024, 13:00-20:00
HS H "Ulrich Santner", Kopernikusgasse 24, Graz University of Technology

13:00 - 13:10	Opening remarks - Gustav Oberdorfer and Andreas Winkler (TUG)

Session I	Chair:
13:10 – 13:50 (10 min disc.)	Plenary lecture I - **Klara Hlouchova** (Charles University) Title: Towards artificial proteins and alternative alphabets
14:00 – 14:15 (5 min disc.)	selected PhD / Post-Doc Talk Title t.b.a
14:20 – 14:35 (5 min disc.)	selected PhD / Post-Doc Talk Title t.b.a
14:40 – 14:55 (5 min disc.)	selected PhD / Post-Doc Talk Title t.b.a

15:00 – 16:00	Coffee break / Poster session

Session II	Chair:
16:00 – 16:40 (10 min disc.)	Plenary lecture II – **Florian Praetorius** (IST Austria) Titel: Biomolecular design in four dimensions
16:50 – 17:05 (5 min disc.)	selected PhD / Post-Doc Talk Title t.b.a
17:10 – 17:25 (5 min disc.)	selected PhD / Post-Doc Talk Title t.b.a
17:30 – 18:10 (10 min disc.)	Plenary lecture III – **Andrea Hupfeld** (University of Regensburg) Titel: Engineering of photoxenases: Photocontrol of enzymes with unnatural amino acids

18:20 – 20:00	Beer & pretzels / Poster session / Get-together
19:45	Award Ceremony – Poster and Talk awards

Abstracts

Towards artificial proteins and alternative alphabets

Klara Hlouchova

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All extant cells known to humankind build proteins from the same 20 coded amino acids. Although more than 10,000 distinct αβ-folds have been implied possible using our protein alphabet, our biology seems to use a very restricted subset of the protein fold space [1]. While several different explanations to this paradox may exist, it seems probable that protein evolution has been heavily biased by ancestral relationships, at least for most proteins that we see today. While language models and AI-based predictors are heavily exploiting this charted space, the theoretical sequence space of our protein alphabet may provide far more options for protein design that have not yet been heavily explored. Moreover, synthetic biology and the study of origins of life imply that functional proteins can be built using smaller and energetically less costly alphabets and that inclusion of non-canonical amino acids can escape the Central Dogma of all life and be of great potential in biotechnology and therapeutic strategies.

To search for structure and function outside the biological protein space, we study highly combinatorial peptide and protein libraries composed of diverse amino acids [2,3]. Based in the canonical alphabet, we have developed pipelines to search properties of random protein sequence space and select for soluble and compact protein variants and potentially new protein folds [2,4]. While the secondary structure potential is innate to sequences formed from the 20 proteinaceous amino acids and compact/folded variants can be encountered, the solubility of such proteins often presents an experimental challenge. We show that this can be significantly improved by using a smaller version of the canonical alphabet and that the physicochemical properties of such alphabet can guide design of novel xeno-alphabets for building artificial proteins [3,4].

The data presented can teach future versions of de novo protein generators and escape the restricted learning area provided by biological proteins.

References:

[1] Minami S, Kobayashi N, Sugiki T, Nagashima T, Fujiwara T, Tatsumi-Koga R, Chikenji G, Koga N. (2023) Nature Structural & Molecular Biology 30(8):1132-40.

[2] Heames B, Buchel F, Aubel M, Tretyachenko V, Lange A, Bornberg- Bauer E, Hlouchova K. (2023) Nature Ecology & Evolution 10.1038/s41559-023-02010-2

[3] Makarov M, Sanchez Rocha AC, Krystufek R, Cherepashuk I, Dzmitruk V, Charnavets T, Faustino AM, Lebl M, Fujishima K, Fried SD, Hlouchova K. (2023): J. Am. Chem. Soc. 145, 9, 5320–5329.

[4] Tretyachenko V, Vymetal J, Neuwirthova T, Vondrasek J, Fujishima K, Hlouchova K. (2022) Open Biology 12: 220040.

Engineering of photoxenases:

Photocontrol of enzymes with unnatural amino acids

Andrea Hupfeld,

University of Regensburg and Regensburg Center for Biochemistry, Germany

The reversible control of enzyme activity with light is a current research objective that is of high interest for various applications throughout the life science sector. Photoreceptors, which change their conformation upon the light-induced isomerization of a photoswitchable cofactor, are the natural paradigm for photocontrol and are used in the field of optogenetics. Our group instead aims to engineer photocontrol in specific target enzymes with synthetic photoswitches that are incorporated as unnatural amino acids. Previous studies have shown that the incorporation of azobenzene-4'-phenylalanine (AzoF) close to crucial conformational transitions can facilitate the light induced reversible (de)activation of enzyme activity. In order to further develop and understand the engineering of such photoxenases, we work towards five subgoals: i) photoswitch customization, ii) adjustment of the (de)activation strength, iii) mechanism of photocontrol, iv) requirements on the position of incorporation, and v) requirements on the enzyme target. For the last objective, we have selected the chemotherapeutic asparaginase type-II from Escherichia coli (EcAII). The incorporation of AzoF within an active site flexible loop identified two light-sensitive EcAII variants. Interestingly, while their asparaginase activity was unaffected by light, the promiscuous glutaminase activity could be reversibly photocontrolled by up to ~7-fold. Free energy landscapes calculated from Molecular Dynamics simulations correlated the photocontrol of the glutaminase activity with high conformational heterogeneity of the substrate bound enzyme. These findings provide crucial information for the future engineering of photoxenases. Moreover, we envision the photocontrol of EcAII to be a viable solution for the reduction of glutaminase related toxicities in chemotherapy.

Biomolecular design in four dimensions

Florian Praetorius

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Abstract: Wouldn't it be great if we could control the spatial arrangement of molecules on the nanometer- to micrometer scale, building molecular assemblies with arbitrary shapes in which the chemical properties can be tailored with molecular resolution? And wouldn't it be even greater if we could reconfigure these assemblies in response to external stimuli? The Praetorius lab for biomolecular design at ISTA works towards these goals using de novo protein design with a few sprinkles of DNA nanotechnology. We use computational tools to design new proteins from scratch, with functions and properties that can not be found in natural proteins. One of our main interests is to use DNA to introduce addressability into otherwise symmetric protein assemblies. Besides that, we are interested in designing stimulus-responsie proteins and assemblies such as switchable binders or assemblies that can be triggered to assemble or disassemble. In this presentation I will gie an overvew of our research interests and explain some of the strategies we employ. Since our lab just started, I will also include some results from my time in the Baker lab in Seattle that form the foundation for some of our future work at ISTA