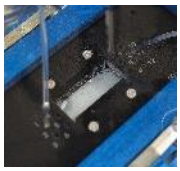

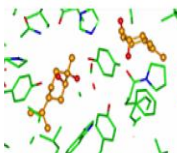
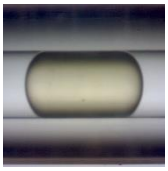

ESAB Webinar

NOVEL ENZYMES

5th March 2021 at 14:00 CET

Chairs: Uwe Bornscheuer, Universität Greifswald, Germany
Jennifer Littlechild, ESAB Vice-Chairman, University of Exeter, UK
Moderator: Willi Meier, DECHEMA, Frankfurt, Germany

PROGRAMME

14.00 **Florian Hollfelder, Dept. of Biochemistry, University of Cambridge, Cambridge CB2 1GA, UK.** (fh111@cam.ac.uk)

How Do We Find New Functional Proteins in Sequence Space?

Functional proteins for a variety of useful applications, as binders and catalysts, are required, but currently not known. Functional metagenomics and directed evolution promise access to such new proteins, but the chances of finding them are low. Therefore high-throughput technologies are crucial to beat the odds: screening in picoliter water-in–oil emulsion droplets produced in microfluidic devices allow screening of >10e7 clones and permit successful selections While potentially faster, the vastness of sequence space (and the scarcity of 'solutions' in it) require strategies for the identification and interconversion of enzymes. In this context the role of 'promiscuous' enzymes, sequencing of full length of genes at high throughput (UMIC-seq) and insertion/deletion mutagenesis (using the transposon-based method TRIAD) will be discussed. Together with a molecular and mechanistic understanding new routes to functional can be charted.

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Fischlechner, M.; Schaerli, Y.; Mohamed, M. F.; Patil, S.; Abell, C.; Hollfelder, F., Evolution of enzyme catalysts caged in biomimetic gel-shell beads. *Nat Chem* **2014,** *6* (9), 791-6.

14.30 **David Bednar**, **Loschmidt Laboratories, Dept. of Experimental Biology & RECETOX, Masaryk University, Brno, Czech Republic.** (davidbednar1208@gmail.com)

EnzymeMiner and SoluProt: searching for novel and soluble enzymes in sequence databases

Databases are an inexhaustible source of biocatalysts which are growing at an incredible pace. On the other hand, classical biochemical characterization techniques are time-demanding, cost-ineffective, and low-throughput. Computational methods are able to explore the unmapped sequence space efficiently. However, the selection of putative enzymes for biochemical characterization based on careful, rational, and robust analysis of all available enzymes remains unsolved. To address this challenge, we have developed EnzymeMiner – a web server for automated screening of diverse enzyme family members and their prioritization for experimental characterization while preserving the catalytic function. EnzymeMiner reduces

the user time for data gathering, multi-step analysis, sequence prioritization, and selection from days to hours. EnzymeMiner is applicable to any enzyme family and provides a highly interactive and easy-to-use web interface freely available at https://loschmidt.chemi.muni.cz/enzymeminer/. Protein solubility is one of the crucial properties that hinders the production of many useful proteins. Computational prediction of protein expressibility and solubility may reduce the cost of experimental studies. Therefore, a new tool for sequence-based prediction of soluble protein expression, SoluProt, was developed using machine learning with the TargetTrack database as a training set and NESG database as a testing set. The tool predicts expressibility in *Escherichia coli* and enables prioritisation of highly soluble proteins. SoluProt analysis is a part of the EnzymeMiner tool or can be used separately in a user-friendly webserver at https://loschmidt.chemi.muni.cz/soluprot/.

15:00 **Anthony Green, University of Manchester, UK** (anthony.green@manchester.ac.uk)

Design and Evolution of Enzymes with non-canonical catalytic mechanisms

Enzyme design and evolution strategies rely exclusively on Nature's standard amino acid alphabet of twenty canonical residues which contain limited functionality. Here we demonstrate that incorporation of non-canonical amino acids into enzyme active sites provides a fruitful avenue to probe complex biological mechanisms and can lead to the creation of designed enzymes with wholly new catalytic functions. Significantly, optimization of enzyme activity can be achieved using directed evolution workflows adapted to an expanded genetic code. We are optimistic that this integration of enzyme design, genetic code expansion and laboratory evolution can provide a versatile strategy for creating enzymes with catalytic functions not accessible to Nature.

15.30 **Pere Clapes, Biotransformation and Bioactive Molecules Group, Catalonia Institute for Advanced Chemistry-CSIC, Barcelona, Spain** (pere.clapes@iqac.csic.es)

Advances in synthetic applications of novel 2-oxoacid metallocarboligases

Here we present two metal cofactor dependent 2-oxoacid carboligases, YfaU and KPHMT, as catalysts for the synthesis of chiral 2-substituted 3-hydroxycarboxylic acid derivatives[1] and for the construction of quaternary centers.[2] Both types of molecules are valuable precursors and intermediates for the preparation of biologically active molecules and APIs. The key step to achieve chiral 2-substituted 3-hydroxycarboxylic acids was the aldol addition of 2-oxoacids to methanal catalyzed by the two enantiocomplementary 2-oxoacid carboligases. The aldol adduct intermediates produced were submitted to oxidative decarboxylation, and esterification affording the target products in 57−88% isolated yields and 88−99% ee, using a substrate concentration range of 0.1−1.0 M. The construction of quaternary carbons is difficult mainly due to the steric hindrance between the carbon substituents. The enzymatic methodology presented features an enantioselective aldol addition of 3,3-disubstituted 2-oxoacids to aldehydes. The chiral 3,3,3-trisubstituted 2-oxoacids thus produced were converted into 2-oxolactones and 3-hydroxy acids and directly to ulosonic acid derivatives, all bearing *gem*-dialkyl, *gem*-cycloalkyl, and spirocyclic quaternary centers. Substrate tolerance, stereoselectivity and examples of products obtained will be discussed.

[1] R. Marín-Valls, K. Hernández, M. Bolte, J. Joglar, J. Bujons, P. Clapés, *ACS Catal.* **2019**, *9*, 7568-7577.
[2] R. Marín-Valls, K. Hernández, M. Bolte, T. Parella, J. Joglar, J. Bujons, P. Clapés, *J. Am. Chem. Soc.* **2020**, *142*, 19754-19762

ABOUT THE SPEAKERS

Florian Hollfelder is Professor for Chemical and Synthetic Biology at the Biochemistry Department of the University of Cambridge/UK. His group employs a broad multi-disciplinary approach that combines methods and ideas ranging from physical-organic chemistry to biophysics, molecular biology and directed evolution. High- and low-throughput approaches are combined with classical kinetic and thermodynamic analysis. For directed evolution, the group has developed microfluidic devices to carry our screening of up to 10^8 clones via assays in emulsion droplets at a picolitre scale. Such high throughput experiments are used to gain insight into the process of protein evolution for binders and catalysts, into strategies to identify new enzymes from metagenomic sources and, on a fundamental level, to investigate the origins of enzymatic rate accelerations. The mechanistic principles that emerge from this work will form a basis for development of transferable, general rules to guide future enzyme evolution.
Website: http://www2.bio.cam.ac.uk/~fhlab//

David Bednar is a leader of a group focused on molecular modelling and bioinformatics within Loschmidt's laboratories. He received his doctoral degree in 2017 from the same university, studying the field of Molecular and Celluar Biology. His expertise was further extended during internships abroad at Rutgers University (USA), University of North Carolina (USA), and Adam Mickiewicz University (Poland). David is a co-author of 33 publications in impacted scientific journals (*h*-index 11), a book chapter and an international patent. He participated in the development of 9 bioinformatics tools for enzyme analysis and protein engineering and one bioinformatics database. In his work, he applies *in silico* approaches to understand basic principles of enzymology and design of mutations to improve protein properties. In recent years, he has been involved also in medical projects covering the study and treatment of Alzheimer's disease, stroke or cancer. The knowledge obtained in both enzymological and medical projects is further utilized in the development of the bioinformatics tools, which bring these analyzes to the broader scientific community. ORCID: https://orcid.org/0000-0002-6803-034

Anthony Green, University of Manchester, UK
Following his PhD in synthetic organic chemistry (total synthesis) under the supervision of Prof. E. J. Thomas, Anthony began postdoctoral research with Prof. Nicholas Turner and Prof. Sabine Flitsch based in the Manchester Institute of Biotechnology (MIB). Following a postdoctoral research position with Prof. Donald Hilvert at ETH (Zurich), Anthony started his independent research career in 2016 based in the Manchester Institute of Biotechnology (School of Chemistry) at the University of Manchester, where he is a professor of organic and biological chemistry, a BBSRC David Phillips research fellow and holder of an ERC starter grant. His research interests lie in the design and evolution of enzymes with new function.

Pere Clapes, Instituto de Química Avanzada, Spain
Prof. Dr. Pere Clapes obtained his B.S. and PhD. in Chemistry from the University of Barcelona (1988) and continued as a post-doctoral fellow (1989-1991) at the Center for Chemistry and Chemical Engineering, Lund University (Sweden). He started his independent scientific career in the Spanish Research Council (CSIC) (1993) at the Catalonia Institute for Advanced Chemistry (IQAC) in Barcelona. He is currently a full professor and head of the biotransformation and bioactive molecules group. He has published more than 180 research works including scientific papers, patents and book chapters in the field of biocatalysis. His research interest is focused on the exploitation of enzymes in asymmetric C–X (X: C, N, S, O) bond formation for the synthesis of natural products analogues and as a means to create novel compound families for bioactivity testing.

NEXT ESAB WEBINARS

ESAB aims to promote the development of Applied Biocatalysis and takes initiatives in areas of growing scientific and industrial interest in the field. Schedule and Topics of next ESAB webinars:

26 March 2021 Amine Biocatalysis 4.5
29 April 2021 Joint ESAB-SusChem Webinar: Broadening the Scope of Biocatalysis in Sustainable Chemistry
May 2021 Biocatalysts from Extremophiles

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