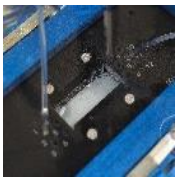
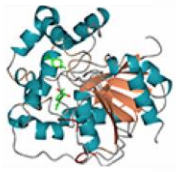
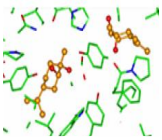
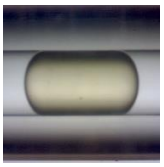





ESAB Webinar

Advances in Experimental Tools and Methodologies for Screening Enzyme Functions

January 21st 2022, 14.00-16.00 CET

Chairs: Jennifer Littlechild (University of Exeter)
Roland Wohlgemuth (Lodz University of Technology)

PROGRAMME

14.00 Prof. Dr. Nigel Scrutton, Manchester Institute of Biotechnology and Department of Chemistry, University of Manchester, Manchester M1 7DN, UK

Enzyme engineering for fuels production

Propane and butane are the main constituents of liquefied petroleum gas and are used extensively for transport and domestic use. They are clean burning fuels, suitable for the development of low carbon footprint fuel and energy policies. In this presentation, I discuss blueprints for the production of bio-alkane gas (propane and butane) through the conversion of waste volatile fatty acids by bacterial culture. I show that bio-propane and bio-butane can be produced photo-catalytically and thermocatalytically by protein engineering and through the use of bioengineered strains of *E. coli* and *Halomonas* (in non-sterile seawater) using fatty acids derived from biomass or industrial waste, and by *Synechocystis* (using carbon dioxide as feedstock). Scaled production using available infrastructure is calculated to be economically feasible using *Halomonas*. These fuel generation routes could be deployed rapidly, in both advanced and developing countries, and contribute to energy security to meet global carbon management targets and clean air directives.

References

[1] Mohamed Amer, Emilia Z. Wojcik, Chenhao Sun, Robin Hoeven, John M. X. Hughes, Matthew Faulkner, Ian Sofian Yunus, Shirley Tait, Linus O. Johannissen, Samantha J. O. Hardman, Derren J. Heyes, Guo-Qiang Chen, Michael H. Smith, Patrik R. Jones, Helen S. Toogood, Nigel S. Scrutton, Low carbon strategies for sustainable bio-alkane gas production and renewable energy. *Energy Environ. Sci.*, 13, 1818-1831 (2020), https://doi.org/10.1039/D0EE00095G

[2] Derren J. Heyes, Balaji Lakavath, Samantha J. O. Hardman, Michiyo Sakuma, Tobias M. Hedison, Nigel S. Scrutton, Photochemical mechanism of light-driven fatty acid photodecarboxylase. *ACS Catalysis* 10, 6691-6696 (2020), https://doi.org/10.1021/acscatal.0c01684

[3] Mohamed Amer, Robin Hoeven, Paul Kelly, Matthew Faulkner, Michael H. Smith, Helen S. Toogood, Nigel S. Scrutton, Renewable and tuneable bio-LPG blends derived from amino acids. *Biotechnol. Biofuels* 13, 125 (2020), https://doi.org/10.1186/s13068-020-01766-0

[4] Mohamed Amer, Helen Toogood, Nigel S. Scrutton, Engineering nature for gaseous hydrocarbon production. *Microbial Cell Factories* 19, 209 (2020), https://doi.org/10.1186/s12934-020-01470-6

[5] Duangthip Trisrivirat, John M. X. Hughes, Robin Hoeven, Matthew Faulkner, Helen Toogood, Pimchai Chaiyen, Nigel S. Scrutton, Promoter engineering for microbial bio-alkane gas production. *Synthetic Biology* 5(1), ysaa022 (2020), https://doi.org/10.1093/synbio/ysaa022

[6] Balaji Lakavath, Tobias M. Hedison, Derren J. Heyes, Muralidharan Shanmugam, Michiyo Sakuma, Robin Hoeven, Viranga Tilakaratna, Nigel S. Scrutton, Radical-based photoinactivation of fatty acid photo-decarboxylases. *Anal. Biochem*. 600, 113749 (2020), https://doi.org/10.1016/j.ab.2020.113749

14.30 Dr. Andreas Vogel, Vice President R&D Enzyme Development, c-LEcta GmbH, Leipzig, Germany

Enzyme engineering as a key step for the development of industrial biotransformations

Enzyme engineering has become an indispensable tool for the development of industrial biotransformation processes. The blueprint for enzyme engineering according to evolutionary principles is identical for all scientific groups, but the implementation differs from laboratory to laboratory. In the talk, I will discuss the key decision points for developing an enzyme engineering strategy, specifically addressing industrial aspects. Insights will be provided on the c-LEctas enzyme engineering strategy as well as biotransformations where enzyme engineering has been used for selective sugar transfer.

15.00 Dr. Xavier Garrabou, CEO, Lock and Key Biosciences, Zurich, Switzerland

Promiscuous artificial enzymes: a platform to explore versatile catalytic motifs

Artificial enzymes harnessing simple catalytic motifs are nowadays accessible by computational design and directed evolution. Although these catalysts are typically no match to their natural counterparts, some of them exhibit a remarkable chemical promiscuity. In addition, the gap to nature-like activity levels can be bridged using ultra-high throughput screening methods. This lecture will disclose the screening strategies developed and applied in the laboratory of Prof. Donald Hilvert (ETH Zürich, Switzerland) to diversify a rudimentary artificial aldolase into a family of proficient and synthetically useful enzymes.

15.30 Assistant Prof. Dr. Fabio Parmeggiani, Politechnico di Milano, Italy

Rapid MS-based screening of diverse biotransformations for enzyme evolution

Fabio Parmeggiani, Cunyu Yan, Emily E. Kempa, Sabine L. Flitsch, Perdita E. Barran, Nicholas. J. Turner, The University of Manchester, Manchester Institute of Biotechnology, Manchester M1 7DN, UK, and Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering “G. Natta”, 20131, Milano, Italy

The need for efficient high-throughput methods for the selection of active colonies often constitutes the major bottleneck in the identification of improved biocatalysts. Colorimetric screening methods have been successful in specific cases, but they are generally hampered by a narrow range of chromophores and high incidence of false positive/negative results. Mass spectrometry (MS), with its high specificity, sensitivity, and speed is an obvious analytical alternative for screening biotransformation reactions. A label-free on-colony screening method based on ambient ionisation MS was developed from previous colorimetric solid-phase assays. Cells harbouring the desired plasmid library are plated onto a nylon membrane laid on an agar plate, induced and then incubated with a substrate solution. Subsequently, in a desorption electrospray ionisation (DESI) platform, the surface of the membrane is swept with a spray of ionised solvent, and the resulting ions are continuously extracted into the mass spectrometer. An ion mobility (IM) separation step was also integrated to minimise the background noise. The correlation of the intensity of the desired m/z to the spatial position on the surface affords a mass-selected image of the areas with higher product concentration, thus identifying the most active colonies. The position of all colonies can be monitored at the same time by tracking a common species. After the analysis, direct liquid culture and DNA isolation (or colony PCR) allow the identification of the active clones.[1] This DESI-IM-MS screening method was demonstrated for the screening of a variety of enzymes, in different formats, achieving sample throughputs equivalent to ~40 s per sample. The heat map output allows rapid selection of active enzymes within 96-well plates facilitating identification of industrially relevant biocatalysts. As a case study, this screening workflow has been applied to the directed evolution of a phenylalanine ammonia lyase (PAL), enhancing its activity toward electron-rich cinnamic acid derivatives. Additional benefits of the screening platform include the discovery of biocatalysts (sugar kinases, imine reductases) with novel activities and the incorporation of ion mobility technology for the identification of product hits with increased confidence.[2]

References

[1] C. Yan, F. Parmeggiani, E. A. Jones, E. Claude, S. A. Hussain, N. J. Turner, S. L. Flitsch, P. E. Barran, *J. Am. Chem. Soc.* 139, 1408-1411 (2017), https://doi.org/10.1021/jacs.6b12165

[2] E. E. Kempa, J. L. Galman, F. Parmeggiani, J. R. Marshall, J. Malassis, C. Q. Fontenelle, J. B. Vendeville, B. Linclau, S. J. Charnock, S. L. Flitsch, N. J. Turner, P. E. Barran, *J. Am. Chem. Soc. Au* 1, 4, 508-516 (2021), https://doi.org/10.1021/jacsau.1c00027

ABOUT THE SPEAKERS

Professor Dr. Nigel Scrutton is Director of UK Future Biomanufacturing Hub (FutureBRH), the Synthetic Biology Research Centre (SYNBIOCHEM) and is former Director of the Manchester Institute of Biotechnology (MIB; 2010-20). He is also co-founder, Director and CSO of C3 BIOTECH Ltd. He is currently Professor in the Department of Chemistry at the University of Manchester/ MIB.

His research interests span enzyme structure, mechanism and engineering, metabolic engineering and the production of chemicals using microbial cell factories. He led the development of SYNBIOCHEM as a UK biofoundry for automated microbial strain engineering for chemicals production. He also has major interests in the physical basis of enzyme catalysis, including mechanisms of hydrogen/electron tunnelling.

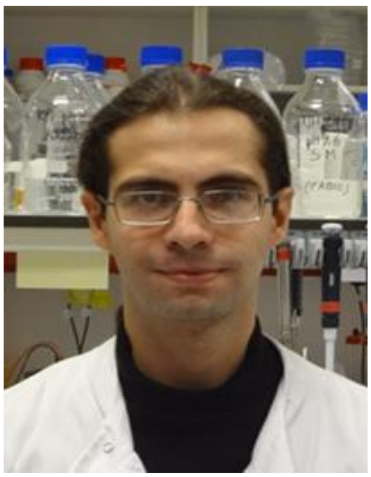
He is a Fellow of the Royal Society (FRS), Fellow of the Royal Society of Chemistry (FRSC) and the Royal Society of Biology (FRSB).

Dr. Andreas Vogel has experience in the field of biocatalysis and enzyme engineering for more than 15 years. He is currently "Vice President R&D Enzyme Development" at c-LEcta. He is originally trained in chemistry (University Munster, Germany) and focused on biochemistry after he had realized how accurate and fast catalysis can be with enzymes. After he obtained his PhD degree in Biochemistry/Chemistry from the University of Munster he moved to a postdoctoral work to the EMBL outstation in Hamburg in 2000, where he focused on structure-function relationships of enzymes. In 2003 he started his second postdoctoral research in Max-Planck-Institute for Coal Research with Prof. M. T. Reetz, where he was involved in the development of methods for enzyme optimization (CASTing, B-Fit), biocatalysis, high-throughput screening and stereoselective biotrans-formations. In 2006 he started to work in the c-LEcta company, first as a Scientist and since 2007 as Head of Biocatalysis & Enzyme Engineering. He is author of more than 40 scientific publications and patents. In 2019 he co-edited the book "Industrial Enzyme Applications" which was published by Wiley-VCH.

ABOUT THE SPEAKERS

Dr. Xavier Garrabou Pi is the founder and CEO of Lock and Key Biosciences, a biotech company dedicated to the tailoring of proteins as innovative catalysts and functional materials. He received a college degree in organic chemistry (IQS, Spain), and obtained a PhD in Biotechnology for his work in the engineering of aldolases with Prof. Pere Clapés (IQAC-CSIC, Spain). He subsequently joined the group of Prof. Donald Hilvert (ETH Zürich, CH), where he used directed evolution to diversify a promiscuous artificial enzyme into a family of synthetically useful catalysts. Following the filing of a patent with ETH, he received financial support of the ETH foundation to pursue his current career as entrepreneur.

Assistant Professor Dr. Fabio Parmeggiani obtained his Ph.D. in Industrial Chemistry and Chemical Engineering in Milano (Italy) in 2013, then moved to the University of Manchester (UK) to work as a research associate with Nicholas J. Turner, Sabine L. Flitsch and Jon R. Lloyd. Since 2019, he is assistant professor of general and organic chemistry at Politecnico di Milano (Italy), working in the field of biocatalysis as part of the BiocatLab research group. His current research interests include the design and exploitation of novel biocatalysts and chemo-enzymatic cascades for industrial synthetic applications.



NEXT ESAB WEBINARS

ESAB aims to promote the development of Applied Biocatalysis and takes initiatives in areas of growing scientific & industrial interest in the field.

Schedule and Topics of next ESAB webinar:

25th February 2022 Enzyme Engineering
10.00-12.00 CET organized by
Jennifer Littlechild and
Roland Wohlgemuth

HOW TO JOIN ESAB

You are cordially invited to join ESAB online *via* https://esabweb.org/Join+us/Application+form.html

Personal membership is free. ESAB, founded in 1980, has the mission of promoting the development of Applied Biocatalysis throughout Europe. The aims of ESAB are to promote initiatives in areas of growing scientific and industrial interest of importance within the field of Applied Biocatalysis. Further information can be found on the ESAB website www.esabweb.org

ESAB - European Society of Applied Biocatalysis (esabweb.org)