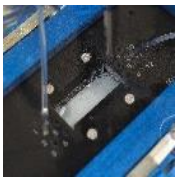
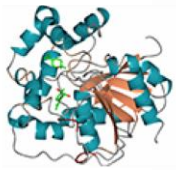


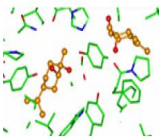
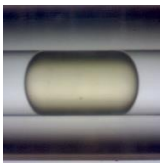

ESAB Webinar

Advances in the Analysis of Enzymatic Reactions and Finding New Enzymes

April 22nd 2022 ***14.00-16.00 Central European Time (CET)***
08.00-10.00 a.m. Eastern Daylight Time (EDT)

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

PROGRAMME

14.00 CET **Prof. Dr. Robert T. Kennedy, Emory Payne, Dr. Dan Steyer, Prof. Dr. Corey Stephenson, Dr. Alex Sun, Prof. Dr. Alison Narayan, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055, USA**

Droplet Microfluidics with Mass Spectrometry for High Throughput Experimentation

Manipulating samples as droplets within microfluidic devices has emerged as an interesting approach for chemical analysis and screening. In segmented flow, one embodiment of this technology, nanoliter samples are manipulated in microfluidic channels as plugs separated by an immiscible fluid, such as air or fluorinated oil. These plugs serve as miniature test-tubes in which reactions can be performed at high throughput. Microfluidic tools have been developed to split, dilute, extract, and filter such plugs at rates >10 samples/s. We have developed methods to analyze plug content by mass spectrometry (MS) at comparable rates. A natural application of this technology is for high throughput experimentation. By coupling droplet manipulation with MS detection, it is possible to greatly reduce reagent consumption and eliminate the need for fluorescent labels or coupled reactions in screens. One area where we have applied this technology is for catalyst discovery. Biocatalysts can be developed by screening enzyme variants to identify active enzymes for a given reaction. Similarly, traditional organic catalysts require extensive exploration of reaction conditions and substrates to develop. We have developed droplet based approaches to decrease the time required to develop such catalysts using droplet-MS. Droplet technology can also be used for screening potential inhibitors of enzymes and protein-ligand interactions. Such measurements reduce the scale of experiments by 100-fold over well-plate systems to enable screens of difficult to produce proteins. The use of unlabeled reactions also has the potential to reduce false positives.

14.30 CET **Dr. Fabrice Gielen, Department of Physics, Living Systems Institute, University of Exeter, UK**

Droplets-on-demand platforms for the high-throughput characterisation of functional enzymes

Progress in the access to protein sequence information - especially through large-scale metagenomic sequencing projects - has so far not been matched by our ability to thoroughly characterise these sequences. In our lab, we engineer new tools that enable rapid characterisation and functional annotation of (putative) enzymes. We combine such platforms with a variety of detection schemes that enable screens on a wide range of molecular targets with the potential to screen reactions without the need for fluorescent surrogates. We show how such droplet-on-demand systems provide a route towards highly quantitative enzyme fingerprinting at the nanoliter scale to deliver detailed kinetic data and thus structure-activity relationships.

PROGRAMME

15.00 CET Prof. Dr. Anthony Mittermaier, Department of Chemistry, McGill University, Montreal, QC, Canada

Measuring Enzyme Kinetics, Inhibition, and Allostery using Isothermal Titration Calorimetry

Isothermal titration calorimetry (ITC) was originally designed for studying host/guest binding interactions but is gaining popularity as general enzyme assay. To characterize enzyme activity, ITC measures the heat released or absorbed by catalysis in real time, following the rapid mixing of enzyme and substrate solutions. Since most chemical reactions are either exothermic or endothermic, ITC can be applied to virtually any enzyme/substrate pair, without the need to design customized reporter molecules, to couple the reaction to additional enzymes, or to perform any post-reaction separation. ITC experiments can be performed under dilute, physiological solution conditions, even with opaque samples and require far less enzyme than traditional ITC binding experiments. Our lab has developed an approach for quantitatively modelling ITC peak shapes in order to apply this technique to rapid reactions that take place on the seconds or tens of seconds timescales. Building on this advance, we have developed a suite of new ITC-based methods that rapidly yield the affinity and the mode of inhibitor binding, the rates of association and dissociation of inhibitors and allosteric modulators, and the full kinetic profiles of Bi-substrate enzymes. We have validated these approaches using trypsin/benzamidine, a panel of covalent and non-covalent inhibitors of prolyl oligopeptidase (POP), the SARS-CoV-2 main protease, and M-type pyruvate kinase, illustrating the versatility of ITC-based enzyme kinetic assays.

15.30 CET Dr. Carine Vergne-Vaxelaire, Génomique Métabolique UMR8030, Genoscope, CEA, Univ Evry, Université Paris-Saclay, France

Identification of diverse enzymes by screening biodiversity using innovative in silico approaches: example with the Amine Dehydrogenase family (MODAMDH Project)

Biodiversity screening aims to provide various templates in terms of sequences and structures, which are essential for biocatalysis to be a more applied alternative to conventional chemistry. Indeed, mutants alone, arising from protein engineering, do not afford the diversity required to reach the full potential of enzymes in biocatalysis. The current boom in the use of genomic data from the exploration of microbial communities provides a gigantic resource of new sequences of potential biocatalysts. Hence, developing bioinformatics approaches to efficiently identify the targeted enzymatic activity from large metagenomic resources is a current major challenge. Through the MODAMDH project, we focus on one of the key biocatalysts named amine dehydrogenases (AmDHs) which enable the access to amines that are important entities in the chemical industry. MODAMDH is an innovative project combining bio-informatics, chemo-informatics and biocatalysis to identify diverse AmDHs among biodiversity. It aims to combine methodologies only scarcely used for biocatalytic purposes. Native AmDHs are searched in very large genomic and metagenomic public databanks using both sequence-driven approaches based on distant homology and three-dimensional topology comparisons of active sites. This will expand the landscape of protein sequences catalyzing reductive amination by finding enzymes with diverse structures and broad characteristics particularly in terms of substrate spectrum or complementary stereoselectivities. This presentation will describe the different steps currently undertaken to create these collections of diverse AmDH candidates. This work is supported by the Agence Nationale de la Recherche (ANR-19-CE07-0007).

ABOUT THE SPEAKERS

Professor Dr. Robert T. Kennedy is the Hobart H. Willard Distinguished University Professor of Chemistry and Professor of Pharmacology at the University of Michigan. His research has combined his interest in biology with chemical analysis and separations. A theme of his group has been development of new chemical analysis tools that can be used at the nanoscale for several applications including screening of drugs, enginee- enzymes, monitoring neurotransmitters in the brain, and studying secretion of insulin and other hormones. He has published over 300 papers on these topics. His work has been recognized by several awards including the American Chemical Society Award in Chromatography, the Ralph Adams Award in Bioanalytical Chemistry, NIH MERIT awards, and several teaching awards. He has mentored over 90 PhD and post-doctoral students since he began his career in 1991. He has held several service posts and is presently Associate Editor of Analytical Chemistry and Chair of the Chemistry Department at University of Michigan.

Dr. Fabrice Gielen received his PhD in Chemistry in 2012 at Imperial College London and conducted postdoctoral research at the University of Cambridge with Professor Hollfelder between 2011 and 2016, developing high-throughput screening tools for the directed evolution of enzymes and drug screening. Since 2017, he is a Lecturer at the University of Exeter, performing research activities at the multidisciplinary Living Systems Institute. His research focuses on the development of novel microfluidic platforms for the accelerated isolation and characterisation of rare biomolecules. He was recently featured as a Lab-on-a-Chip Emerging Investigator for his work on single-cell assays. He is also co-founder of Drop-Tech Ltd, which licenses technology to commercialise droplets-on-demand products.

Prof. Dr. Anthony K. Mittermaier obtained his B.Sc. in biophysics at the University of Guelph in 1996 and his Ph.D. in biochemistry at the University of Toronto under the supervision of Lewis Kay and Julie Forman-Kay in 2003. After a short post-doc under the supervision of Lewis Kay and Regis Pomes at the Hospital for Sick Children in Toronto, he joined the Department of Chemistry at McGill University in 2005, and was promoted to Associate Professor in 2011. He has been the Associate Dean (Student Affairs) in the Faculty of Science since September 2020. His research employs a combination of biophysical techniques, particularly calorimetry and NMR spectroscopy, to address fundamental questions regarding the molecular basis of protein and nucleic acid function and drug design. In 2012 he was awarded the Agilent Early Career Professor Award for his advances in combining NMR with calorimetric techniques. More recently, his laboratory has begun to focus on using ITC to measure enzyme kinetics and thermal hysteresis experiments to measure supramolecular assembly. He was the 2021 winner of the Chemical Biology/Medicinal Chemistry Lectureship award from the Canadian Society for Chemistry.

ABOUT THE SPEAKERS

Dr. Carine Vergne-Vaxelaire obtained her Ph. D in 2006 at the University of Paris-Saclay (France) under the supervision of Dr. Ali Al-Mourabit working on the isolation of key nitrogen-containing natural products from marine sponges and their biomimetic synthesis. After one year as medicinal chemist at Servier (France), she joined the French Alternative Energies and Atomic Energy Commission (CEA) in the newly funded Laboratory of Biocatalysis, Bioremediation and Synthetic Metabolism of the Genoscope Unit UMR 8030 Genomics Metabolics. Her research interests are in the area of biocatalysis with particular emphasis on the discovery of new biocatalysts among biodiversity by genome-mining approaches mastered in the unit, and their applications as green tools for the synthesis of key building blocks. Her current main project is focused upon discovering native amine dehydrogenases for the production of amines and undertaking structural and biochemical studies thereof.

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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 the next ESAB webinar:

27th May 2022 10.00-12.00 CET	Biocatalytic Total Synthesis organized by Roland Wohlgemuth and Jennifer Littlechild
24th June 2022 14.00-16.00 CET	Biocatalysis and Sustainable Chemistry, joint ESAB-SusChem Webinar, organized by Andrés R. Alcántara and Pablo Domínguez de María, ESAB Working Group Sustainable Chemistry

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Personal membership is free.
Institutional membership is welcome and is currently being established as new membership category.
ESAB has been founded in 1980 andd has the mission of promoting the develop-ment 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
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