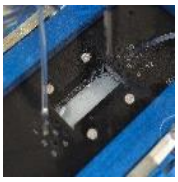
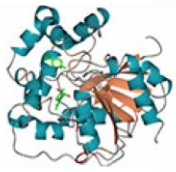


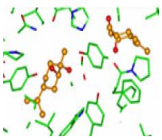
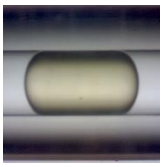

ESAB Webinar

Advances in Experimental Tools and Methodologies for Screening Enzyme Functions

December 10th 2021, 14.00-16.00 CET

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

PROGRAMME

14.00 Prof. Dr. R. Graham Cooks and Nicolás M. Morato
Purdue University, West Lafayette, IN, United States

High Throughput Desorption Electrospray Ionization (DESI) for Reaction Screening, Small-scale Synthesis and Bioanalysis

This talk describes a high throughput automated system that is used for (i) reaction screening (ii) small-scale synthesis, including late-stage functionalization, and (iii) label-free enzyme assays. The system uses low ng amounts of sample, plates densities of up to 6,144 samples, and <0.5 seconds per measurement. The solvent spray method of desorption electrospray ionization (DESI) is used for sampling and mass spectrometry (MS) for analysis. The platform is fully automated, capable of 24/7 continuous operation, and it automatically records MS and MS/MS spectra.

Using this high throughput system several organic reactions have been screened to identify optimal conditions for particular transformations or to study reaction mechanisms. Multiple reaction parameters that can be tested with the platform include stoichiometry, pH, solvent, catalyst and, at lower speed, temperature. Note that this reaction screening capability depends on the fact that reactions in microdroplets have rate constants which are typically 3 - 5 orders of magnitude greater than the corresponding bulk chemical reactions [1,2]. The increase in rate means that the droplets react on the millisecond timescale, allowing one to screen reaction parameters to find optimal conditions at rates of ca. 6,000 unique reactions/hour [3,4]. Importantly, the droplet reactions predict, with reasonable accuracy, the course of scaled-up experiments (e.g., flow or batch chemistry), as exemplified by multiple drug candidate syntheses that will be cited. Note that the system can also be used to perform biological analyses, such as the monitoring of enzymatic activity [4,5], which will be discussed in detail during the next presentation.

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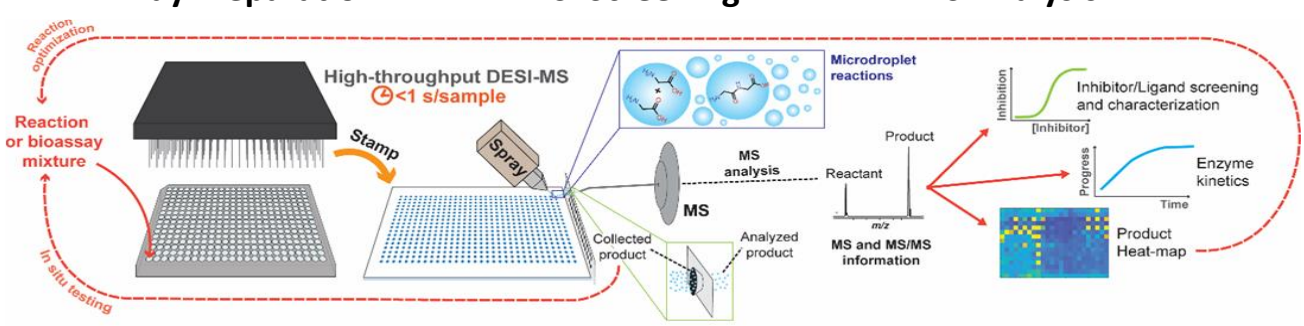

14.30 Nicolás M. Morato and R. Graham Cooks,

High-Throughput Label-Free Enzymatic Assays using Automated Desorption Electrospray Ionization Mass Spectrometry (DESI-MS)

High-throughput (HT) screening is integral to current drug discovery workflows. Both the synthesis of large compound libraries and the bioactivity assessment of those molecules against particular biological targets rely on HT experimentation. With regards to bioassays, traditional screening methods are based almost exclusively on optical or radiometric detection techniques, which are fast and sensitive, but require the use of labels or coupled reactions to generate a detectable output. This alters the native conditions of the system under study, makes the assays susceptible to interferences, slows method development and limits flexibility, increasing at the same time costs and safety concerns. On the other hand, more recent mass spectrometry (MS) based platforms have become a fast alternative to traditional HT bioactivity screening, providing high versatility and molecular specificity for label-free assays. However, in spite of these advantages, some sample work-up is still required prior to analysis in many cases [1,2].

Here we present an automated HT platform based on desorption electrospray ionization (DESI), an ambient ionization technique that allows for direct analysis from complex samples, such as buffers with detergent and high salt concentrations, removing completely the need for any sample work-up [3,4]. This HT DESI-MS system was initially developed for organic reaction screening, and has been recently extended to perform enzymatic assays. Sub-second analysis times (<500 ms), low sample consumption (50 nL), great matrix tolerance and excellent quantitative performance (CVs < 10%), make this system a powerful tool in label-free enzymology and drug discovery, as it has been proven in several enzymatic systems [1,5]. In particular, the cases of acetylcholinesterase (AChE), angiotensin-converting enzyme II (ACE2), and sulfotransferases (SULTs) will be discussed. Diverse applications such as the rapid kinetic study of the enzymatic systems with various native substrates, identification and characterization of inhibitors and reactivators, and enzyme determinations directly from tissue extracts, have been demonstrated and will be presented, focusing on cases where DESI-MS label-free assays outperform traditional approaches.

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15.00 Dr. Martin Held, Technology Development and Discovery Group, Bioprocess Laboratory, Department of Biosystems Science and Engineering, ETHZ, Basel, Switzerland Switzerland

Harnessing the Power of Nature for Enzyme Screenings

High throughput screening is key for innovation, new processes and products in virtually any discipline of biotechnology. Especially discovery and optimization of enzymes for catalysis or for new substances for medicinal applications are screening-heavy activities.

In this talk, we are presenting a number of nature-inspired approaches for activity assessment of whole cell biocatalysts and enzymes meant to supplement the frequently employed microtiter plate and / or classical analytic approaches. More precisely, we introduce high throughput protocols for screening of recombinant enzymes expressed in E. coli of identification of improved industrial production strains catalyzing complex pathways based on fluorogenic indicator strains, intracellular detection of hydrogen peroxide, cell motility, and buoyancy of microcarriers.

Any of these systems allows for rapid sampling of microbial libraries at minimal costs and low liquid volumes thereby satisfying the demand for rapid experimental assessment of a large sequence-space frequently required in order to engineer biocatalysts that display the desired properties.

15.30 Prof. Dr. Dominic J. Campopiano, School of Chemistry, University of Edinburgh, Edinburgh, United Kingdom

Applying Spectroscopic Methods and New Reagents for Biocatalysis Screening

Amino acids are key synthetic building blocks that can be prepared in an enantiopure form by biocatalytic methods. In previous work we developed a synthetic strategy to convert racemic mixtures of N-acetylated amino acids to enantiopure L- or D- amino acids. This dynamic kinetic resolution (DKR) was achieved by combining an engineered N-acetyl amino acid racemase (NAAAR) with an appropriate N-acylase. In more recent work we show that the L-selective ornithine deacetylase ArgE catalyses hydrolysis of a wide-range of N-acyl-amino acids. This activity was revealed by 1H NMR spectroscopy that monitored the appearance of the acetate product. Furthermore, the assay was used to probe the subtle structural selectivity of the biocatalyst using a substrate that could adopt different rotameric conformations. Enantiopure amines are also valuable synthetic targets than can prepared from keto substrates using well known pyridoxal 5'-phosphate (PLP) dependent transaminases (TAs). To overcome the problematic equilibrium of TAs, various smart amino donors have been developed for synthesis and screening. Here we describe a new natural product-inspired amine donor, N-phenylputrescine (NPP), that performs very well with various TAs, over a range of synthetic conditions, with high % conversions.

References

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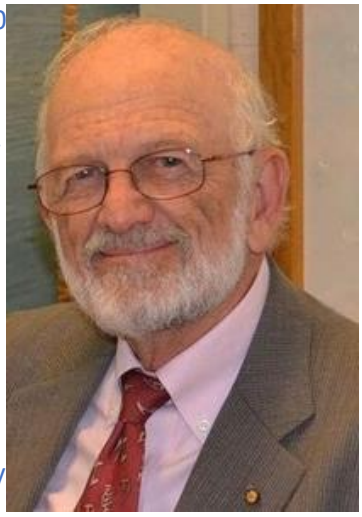
ABOUT THE SPEAKERS

Professor Dr. R. Graham Cooks is the Henry Bohn Hass Distinguished Professor in the Department of Chemistry at Purdue University where he has served as major professor to 150 PhD students. Dr. Cooks' was a pioneer tandem mass spectrometry (MS/MS) and desorption ionization. His work includes the development of miniature mass spectrometers using ambient ionization and application of this combination to problems of trace chemical analysis at point-of-care. His group also studies collisions of ions at surfaces for new methods of molecular surface tailoring and analysis, and nanomaterials preparation by soft-landing of ions and charged droplets. Dr. Cooks has been recognized with the Mass Spectrometry and the Analytical Chemistry awards of the American Chemical Society, the Robert Boyle Medal and the Centennial Prize of the Royal Society of Chemistry, and the Camille & Henry Dreyfus Prize in the Chemical Sciences. He is an elected fellow of the American Academy of Arts and Sciences, the Academy of Inventors and the U.S. National Academy of Sciences.



Nicolas Morato is a Ph.D. candidate in Analytical Chemistry at Purdue University under the mentorship of Prof. R. Graham Cooks. Nicolás attended Universidad de los Andes (Bogotá, Colombia) and obtained bachelor's degrees in Chemistry (*cum laude*, 2017) and Industrial Engineering (*summa cum laude*, 2018). His graduate research has focused on the development of ambient ionization methods for the rapid and simple analysis of complex samples. His initial efforts were oriented towards forensic applications such as in situ drug testing, whereas his current research is mostly related to high-throughput bioanalysis utilizing desorption electrospray ionization (DESI).

Nicolás' graduate work has resulted in several honors including the Charles H. Viol Memorial Fellowship, the Eastman Summer Fellowship in Analytical Chemistry, the ACS Division of Analytical Chemistry Graduate Fellowship, and the Tomas Hirschfeld Scholar Award.

ABOUT THE SPEAKERS

Dr. Martin Held graduated as a technical biologist at the University of Stuttgart and received his PhD for the development of a scaled biocatalytic process for manufacturing of fine chemicals employing hydroxylating whole cell biocatalysts from the Institute of Biotechnology of the Swiss Federal Institute of Technology (ETH). He is currently employed as senior scientist at the ETH's Bioprocess Laboratory at the Department of Biosystems Science and Engineering (D-BSSE) where he runs the Technology Development and Discovery Group. While operating with an interdisciplinary team of chemists, molecular biologists, microbiologists, and engineers, research of his group focuses on the development of novel technologies and platforms for application in life science and industry.
As he likes to work on topics of immediate interest for industrial applications, his research activities provided the basis for the foundation of several startup companies.

Prof. Dominic Campopiano has been an academic at the University of Edinburgh since 1998, beginning as a lecturer and progressing through to Chair of Industrial Biocatalysis.
He is a BSc Chemistry graduate (University of Glasgow, 1984-88) and PhD Chemistry graduate (University of Edinburgh, 1988-92). His research interests span natural product biosynthesis (e. g. sphingolipids, antibiotics vitamins) and he uses natural and evolved enzymes as biocatalysts for synthetic chemistry.
He has served on the Scientific Advisory Board of the Industrial Biotechnology Innovation Centre (IBioIC), is a Fellow of the Royal Society of Chemistry (FRSC) and co-organiser of the upcoming RSC meeting Directing Biosynthesis VI (Edinburgh, June, 2022).

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:
21st January 2022 Advances in Experimental
14.00-16.00 CET Tools and Methodologies for Screening Enzyme Functions, organized by
Jennifer Littlechild and
Roland Wohlgemuth

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