



ESAB Webinar

Advances in the Analysis of Enzymatic Reactions

September 23rd 2022 13.00-15.00 British Summer Time (BST)
14.00-16.00 Central European Time (CET)
08.00-10.00 Eastern Daylight Time (EDT)

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

PROGRAMME

14.00 Dr. Jonathan Farjon, Department of Chemistry, Nantes University / CNRS, CEISAM lab, France

Real-time enzymatic monitoring by compact NMR: Methods and applications to foods

Better optimizing biochemical processes induced by enzymes is crucial to improve the production of high added value commercial products in pharmaceuticals, cosmetics, food and energy sciences. NMR is essential to the monitoring and optimization of bioprocesses since it provides both structural and quantitative information in a non-destructive fashion. In this context, emerging benchtop NMR instruments are particularly attractive since they are miniaturized, cheap, mobile and compatible with industrial settings. However, at lower fields, the limits of NMR are worsened: low sensitivity and weak spectral resolution are detrimental to the analytical performance. To overpass such limitations, we implemented NMR techniques using pulse field gradients in diffusion NMR, pure-shift methods, ultrafast 2D NMR and schemes for removing the water signal in biological samples, on a 1 T apparatus equipped with a gradient coil [1].

In the field of food processes, we showed that quantitative WET-180-NOESY [1] is the most suitable tool able to selectively remove the water signal to monitor under flow for key enzyme-catalyzed bioprocesses in food industry: sucrose inversion for enhancing the sweetness [2] and lactase for producing lactose free milk [3] for intolerant persons. NMR developments on mobile apparatus were showing their interest for monitoring enzymatic transformations. These proofs of concept open the avenue to the optimization and control of food bio-transformations.

References

- [1] Jonathan Farjon, Boris Gouilleux, Patrick Giraudeau, Gradient-based pulse sequences for benchtop NMR spectroscopy. *J. Magn. Res.* 319, (2020), 106810-106822. <https://doi.org/10.1016/j.jmr.2020.106810>
- [2] Alper Soyler, Dylan Bouillaud, Jonathan Farjon, Patrick Giraudeau, Mecit H. Oztop, Real-time benchtop NMR spectroscopy for the online monitoring of sucrose hydrolysis. *LWT Food Sci. Tech.* 118, 108832-108839 (2020). <https://doi.org/10.1016/j.lwt.2019.108832>
- [3] Alper Soyler, Sevil Cikrikci, Cagri Cavdaroglu, Dylan Bouillaud, Jonathan Farjon, Patrick Giraudeau, Mecit H. Oztop. Multi-scale benchtop ¹H NMR spectroscopy for milk analysis. *LWT Food Sci. Tech.* 139, 110557-110567 (2021). <https://doi.org/10.1016/j.lwt.2020.110557>

**14.30 Prof. Dr. Petra S. Dittrich, ETH Zurich, Department of Biosystems Science and Engineering
Mattenstrasse 22, CH-4058 Basel, Switzerland**

Drop by drop: Analysis of single cells and biochemical reactions at high throughput

Droplet microfluidics is a particularly powerful method for screening applications e.g. for protein engineering and single-cell studies. Monodisperse aqueous droplets of pico- or nanoliter volume immersed in a hydrophobic fluid are formed in a microfluidic device at kHz frequencies. In recent years, droplet microfluidics has been employed for analysis of bioanalytical assays and chemical synthesis, such as kinetic studies of enzymes, cell-free protein synthesis, protein crystallization, nanoparticle formation and many more. In the field of single-cell analysis including single-cell sequencing, droplet microfluidics is nowadays a well-approved method and alternative to cytometry. In the first part of the presentation, I will show applications of droplet microfluidics, where the compartmentalization is of high importance, e.g., when secreted compounds of cells are analyzed.

Most assays in nL droplets, however, are based on fluorescence spectroscopy, which limits the choice of assays and multiplexing capability. Mass spectrometry, on the other hand, allows for label-free detection and identification of multiple components. Recently, we have interfaced droplet microfluidics with matrix-assisted laser desorption/ionization (MALDI)-MS. Instead of the standard MALDI targets where the sample is pipetted in 384 wells, we have fabricated custom-made, transparent, indium-tin oxide coated targets, on which thousands of aqueous nL-droplets reside on the surface, covered by fluorinated oil. Analysis of this droplet array is performed by optical and fluorescence microscopy as well as by means of a MALDI-MS imaging system (Bruker rapifleX). We employ the method for single-cell studies at high throughput, e.g. analysis of biosynthesized enzymes, which will be discussed in the second part of the presentation.

**15.00 Prof. Dr. Matthew Crump, School of Chemistry, University of Bristol, Cantock's Close,
Bristol BS8 1TS, UK**

Nuclear magnetic resonance, mass spectrometry and chemical probes in natural product biosynthesis.

Polyketide natural products (produced by polyketide synthases (PKSs)) currently serve as a vast source of high value compounds with applications spanning agrochemicals, antibiotics, anti-cancer and human and veterinary medicine as well as tool compounds for use in biotechnological research. Enormous strides have been made in understanding the chemistry, biochemistry and structural biology of the PKSs that are complex collections of enzymes with a diverse array of architectures. Our work focuses on the biosynthesis of key structural features of several natural products including the marine bacterium derived thiomarinol, the anti-MRSA antibiotic kalimantacin and mupirocin (a mixture of pseudomonic acids). We have recently elucidated a number of important mechanistic steps in the assembly of these metabolites and this presentation will focus on the role of structural biology, state-of-the-art NMR, MS and ACP bound chemical probes in achieving this. Ref.: PD Walker, C Williams, ANM Weir, L Wang, PR Race, J Crosby, TJ Simpson, CL Willis, MP Crump, Control of beta-branching in kalimantacin biosynthesis: Application of ¹³C NMR to polyketide programming. *Angew. Chem. Int. Ed.* 58, 12446-12450 (2019). <https://doi.org/10.1002/anie.201905482>.

15.30 Prof. Dr. Loredano Pollegioni, Dipartimento di Biotecnologie e Scienze della Vita, Università degli Studi dell'Insubria, via J. H. Dunant 3, 21100 Varese, Italy

Plastic-eating enzymes: a protein engineering study

Polyethylene terephthalate (PET) is a largely produced/used plastic that can be degraded by enzymes. Enzymatic biodegradation represents an innovative circular economy approach aimed at simultaneously remove this plastic from the environment (bioremediation) and at producing added-value compounds (upcycling). Several enzymes depolymerize PET into its monomers terephthalic acid (TPA) and ethylene glycol (EG). The most promising are the PET hydrolase from *Ideonella sakaiensis* (IsPET) and the leaf-branch compost cutinase (LCC, from an unknown thermostable enzyme). We set up a multidisciplinary approach for the evolution of "plastic-eating enzymes" based on bioinformatics investigation, site-saturation mutagenesis, high-throughput screening, biochemical characterization, and test of biodegradation on different PET materials. This workflow identified several interesting variants of IsPET and LCC with improved performances and different ability to degrade distinctive PET forms. The W159H/F238A/S121E/D186H-ΔIsPET (TS-ΔIsPET) hydrolyzes ~80% of amorphous PET nanoparticles in 1 h at 45 °C and the F243T-ΔLCC almost fully depolymerizes (~96%) an amorphous PET film in two days at ≤60 °C.

ABOUT THE SPEAKERS

Jonathan Farjon received his Ph.D. in 2003 from the University of Paris 11 Orsay. His doctoral research project was supervised by Prof. Jacques Courtieu on new NMR developments in liquid crystalline media. After a two years postdoctoral stay at the Max Planck Institut für Biophysikalische Chemie in Göttingen in the group of Prof. Christian Griesinger where he was implementing NMR methods and gels, he joined in 2008 the team of Dr Bernhard Brutscher in the CEA of Grenoble for developing fast NMR for studying RNAs. In 2009 he was hired as CNRS Associate Scientist in the team of NMR in oriented media at the University of Orsay. Since September 2016, he moved to the CEISAM lab of Nantes in the Magnetic Resonance, Isotopomics, Metabolomics, Monitoring team (MIMM). In 2022, he is promoted to Research Director, with more than 21 years of experience in NMR, his main research are now focused on pushing over the limits of NMR at high and low fields to enhance sensitivity and resolution for studying complex mixtures in the field of forensic, as well as metabolism and metabolomics in foods, plants, cancer cells, ants and microalgae.



Petra Dittrich is Associate Professor for Bioanalytics at the Department of Biosystems Science and Engineering since 2014. Her research in the field of lab-on-chip-technologies focuses on the miniaturization of high-sensitivity devices for chemical and biological analyses, and microfluidic-aided organization of materials.

She studied chemistry at Bielefeld University (Germany) and Universidad de Salamanca (Spain) from 1993 to 1999. She earned her PhD degree at the Max Planck-Institute for Biophysical Chemistry (MPI Göttingen, Germany) in 2003 for her thesis on single fluorescent molecule spectroscopy and fluorescence correlation spectroscopy in microfluidic channels. After another year as post-doctoral fellow at the MPI Göttingen, she had a postdoctoral appointment at the Institute for Analytical Sciences (ISAS Dortmund, Germany) (2004-2008). For research stays, she was at the Cornell University (2002) and the University of Tokyo (2005). In 2008, she became Assistant Professor at the Organic Chemistry Laboratories of the Department of Chemistry and Applied Biosciences (ETH Zurich).

Petra Dittrich was fellow of the Studienstiftung des Deutschen Volkes, the German Exchange Organization DAAD and the Christiane Nüsslein-Vollhard-foundation. Her PhD was awarded by the Westfälisch-Lippische Universitätsgesellschaft. She received the Starting Grant from the European Research Commission (ERC) (2008), the Analytica Forschungspreis of the German Society of Biochemistry and Molecular Biology (GBM), donated by Roche Diagnostics GmbH (2010), the Heinrich Emanuel Merck award (2015) and the ERC Consolidator Grant (2016).



ABOUT THE SPEAKERS

Matthew Crump is Professor of NMR and Structural Biology at the University of Bristol (UoB). He was appointed as a lecturer in NMR at the School of Biological Sciences, University of Southampton (1999–2002) before taking up his current post in biological NMR at the University of Bristol (2003–2020). He is the Director of UoB's biological NMR facility with >28 years' experience working at the interface between the physical and biological sciences. His major focus is on structural and mechanistic aspects of natural product biosynthesis that began with reporting the first ever polyketide protein NMR structure in 1997 and continues with NMR and X-ray crystallography of natural product enzymes and their interactions with intermediates. He has published over 125 papers in leading international journals e.g. Science, Nat. Chem. Biol., JACS and has gained funding for research from the Research Councils, Industry and Charities and has significant contributions to major programmes including the BBSRC BrisSynBio Centre for Synthetic Biology BBSRC- and EPSRC-funded multi-centre research projects including CDTs in Synthetic Biology, Chemical Synthesis and the BBSRC funded SouthWest Biosciences Doctoral Training Partnership (SWBio DTP). He has also served on multiple national steering committees and advisory boards, e.g. the UK CCPN executive committee and the KCL biomolecular NMR centre



Loredano Pollegioni is full professor of Biochemistry at the University of Insubria (Varese, Italy), treasurer of IUBMB, president of Regional Foundation for Biomedical Research (Regione Lombardia, Italy) and director of The Protein Factory 2.0 lab (www.theproteinfactory2.it). He received in 1986 his B.Sc in Biology (with honour) at University of Milan (Italy) and in 2003-2004 carried out his Post-doctoral training in Enzymology at University of Michigan (USA). His scientific career focused, as a general topic, on the structure-function relationships in enzymatic proteins by using a multidisciplinary methodological approach. This experience has been exploited in the field of protein biochemistry and Biotechnology investigating dozens of enzymes. In last years, Prof. Pollegioni specialised in the evolution of enzymatic activities by using rational (site-directed mutagenesis) directed evolution (random and site-saturation mutagenesis) methods. He has published over 250 peer-review papers on these topics.



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 webinars:

21 st October 2022 14.00-16.00 CET	Biocatalytic Process Engineering, organized by Polona Žnidaršič-Plazl and ESAB Working Group Biocatalytic Process Engineering
18 th November 2022 10.00-12.00 CET	Enzymatic Reaction Mechanisms and their Biocatalytic Applications, organized by Jennifer Littlechild and Roland Wohlgemuth
16 th December 2022 14.00-16.00 CET	Biocatalytic Total Synthesis organized by Roland Wohlgemuth and Jennifer Littlechild

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Institutional membership is welcome and is currently being established as new membership category.

ESAB has been founded in 1980 and 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 on ESAB Conferences and other activities can be found on the ESAB website

www.esabweb.org

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