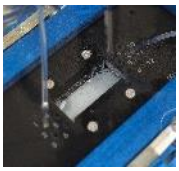
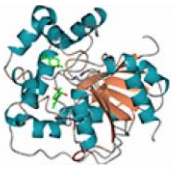
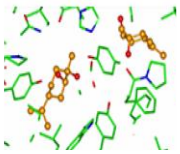
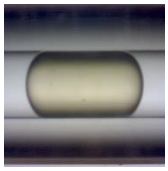

ESAB European Society of Applied Biocatalysis

ESAB Webinar

Synthetic Biology and Metabolic Engineering Tools and Methodologies

August 26th 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)
07.00-09.00 Central Daylight Time (CDT)

Chairs: Frangiskos Kolisis (National Technical University Athens)
Roland Wohlgemuth (Lodz University of Technology)

PROGRAMME

14.00 Prof. Dr. Alfonso Jaramillo, Instituto de Biologia Integrativa de Sistemas (i2SysBio) CSIC, Valencia, Spain

Phage-assisted directed evolution of proteins

In vivo directed evolution techniques allow engineering protein and nucleic acids with targeted functions inside living cells. The efficiency of such techniques is determined by the evolution speed and sampling size. Transducing phage particles are able to support higher mutagenesis rates than any viral system, allowing for a faster evolution, where the host cell is re-engineered according to the desired selection. We will show how phages can be used to accelerate the directed evolution of proteins. We have engineered the genomes and hosts of phages M13, T7 and P2 to evolve proteins and RNA. For this, we have developed phage infection cycles implementing positive and negative selections. The implementation of positive and negative selections allowed the engineering of stronger activity and specificity respectively. We demonstrate the usefulness of our system by engineering the smallest transcription factor activator/repressor, a set of orthogonal transcription factors activator/repressor and a riboswitch in *E. coli*. Our methodology for accelerated directed evolution can be used to evolve any protein or RNA where its activity could be coupled to gene expression.

14.30 Prof. Dr. Georgios Skretas, Director, Institute for Bio-innovation, Biomedical Sciences Research Center "Alexander Fleming", Athens, Greece

Engineered bacteria as an early-stage drug discovery platform for protein misfolding diseases

Protein misfolding and aggregation is a common molecular feature for many devastating human diseases, such as Alzheimer's disease, Parkinson's disease, type 2 diabetes and others. Toward increasing the effectiveness of early-stage drug discovery for these conditions, we have developed a bacterial platform that enables the efficient biosynthesis of molecular libraries with expanded diversities and their direct functional screening for discovering chemical rescuers of disease-related protein misfolding and aggregation. We will describe the development of engineered bacteria with the ability to produce combinatorial libraries of >200 million of short, drug-like, cyclic peptides and to rapidly screen them to discover aggregation inhibitors against the amyloid-β peptide (Aβ) and Cu/Zn superoxide dismutase (SOD1), linked to Alzheimer's disease and amyotrophic lateral sclerosis, respectively. We will describe how this technology has enabled the identification of hundreds of macrocyclic molecules that efficiently reduce disease-related protein aggregation and toxicity both *in vitro* and *in vivo*. Furthermore, we will showcase how a combination of high-throughput sequencing and mutagenesis analyses can enable the rapid determination of structure-activity relationships and define consensus motifs required for bioactivity. Finally, we will discuss the steps that we have taken to pursue the commercialization of our technology and the further preclinical development of the bioactive molecules we have discovered.

15.00 Prof. Dr. Sotirios C. Kampranis, Biochemical Engineering Group, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

Biochemical engineering drives the green transition

Production of many valuable biomaterials today is neither green nor sustainable. Flavours, fragrances, dyes, pesticides, and biopolymers, are currently produced either from petroleum using environmentally damaging catalysts and solvents, or by over-cultivation and over-harvesting of plants. Other biomaterials, such as proteins derived from milk, egg or meat, require animal-farming, considerable amounts of land and energy, and are responsible for a large percentage of total greenhouse gas emissions. The future of our planet depends on our ability to replace current technologies for producing these biomaterials with new, sustainable and, at the same time, economically viable methods.

Engineering organisms to produce the biomaterials we use today by converting simple biomass to valuable products holds the key for the green transition. In this lecture, I will present how our work combines basic research in biochemistry with protein engineering of biosynthetic enzymes and metabolic engineering of industrially amenable microorganisms to provide solutions for a greener planet. Using monoterpenoid production as an example, I will demonstrate the complete process from the identification of a target compound, the elucidation of its biosynthesis, the establishment of laboratory strains that synthesize the desirable product, the upscaling of production to achieve viable scales and cost, and finally, the commercialization process that brings the final product to the consumer.

15.30 Prof. Dr. Ashty Karim, Department of Chemical and Biological Engineering and Director of Research for the Center for Synthetic Biology at Northwestern University, Evanston, IL 60208, USA

Cell-free synthetic biology enables industrial biotechnology

The accelerating climate crisis combined with rapid population growth are posing some of the most urgent economic and social challenges to humankind, all linked to the unabated release and accumulation of CO2 in our atmosphere, intensifying the demand for carbon-negative technologies. Industrial biotechnology is one of the most attractive approaches to address this need; nearly all current investments in biomanufacturing (more than $20B in 2020) are based on production using cells in which engineers seek to design enzymatic reaction schemes to meet manufacturing criteria. Unfortunately, this process is difficult because design-build-test (DBT) cycles—iterations of re-engineering organisms to test new sets of enzymes—remains costly, risky, and slow. This is because cells themselves impose inherent limitations on the effective synthesis of bioproducts. One key limitation is that cellular survival objectives are often diametrically opposed to the objectives of chemical engineers. As a result, a typical project today might only explore dozens of variants of an enzymatic reaction pathway which is often insufficient to identify a commercially relevant solution. It is essential that we speed up the biochemical engineering pipeline to create new sustainable biomanufacturing practices.

Cell-free synthetic biology allows us to rethink how we meet this global challenge by facilitating rapid building of prototypes with biology. Cell-free technologies allow us to conduct precise, complex biomolecular transformations in cell lysates without living, intact organisms. In other words, cellular machinery harvested from cells that are broken open can be used to build biological systems and processes. By harnessing the advantages of cell-free synthetic biology, we've developed an *in vitro* prototyping and rapid optimization of biosynthetic enzymes (iPROBE) approach to inform cellular design. In this talk, I will describe our recent efforts to reimagine R&D for industrial biotechnology. I will first show how we used the iPROBE approach to screen over 760 unique enzymatic pathway combinations to optimize reverse β-oxidation (r-BOX) for enhanced selectivity and synthesis of C4-C6 acids and alcohols. Implementation of these pathways into *Escherichia coli* generated designer strains for the selective production of butanoic acid, hexanoic acid, and 1-hexanol. We also generated *Clostridium autoethanogenum* strains able to produce 3-hydroxybutyrate and 1-hexanol from syngas in continuous fermentation. I will then highlight our efforts to engineer key enzymes for the enzymatic assimilation of CO2 using an *in vitro* evolution approach. Cell-free synthetic biology provides a powerful complement to metabolic engineering approaches enabling new paradigms for industrial biotechnology.

ABOUT THE SPEAKERS

Alfonso Jaramillo has a PhD in particle physics (1999) and after postdocs at the labs of Prof. Wodak (Free University of Brussels) and Prof. Karplus (Strasbourg and Harvard) he joined the Ecole Polytechnique (France) as a tenured lecturer in 2003, tenured senior scientist at CNRS (France) in 2009 and as a full Professor at the University of Warwick (UK) in 2013. Since 2021 he is tenured investigator at CSIC (Spain) and on leave Research Professor at CNRS (France). His research interests involve the accelerated evolution of engineered phages for new antimicrobial development and the engineering of neuromorphic gene circuits in bacteria for new living artificial intelligence development.

Georgios Skretas received his Bachelor's degree from the School of Chemical Engineering, National Technical University of Athens (Greece) in 1998 and his PhD in Chemical and Biological Engineering from Princeton University (USA) in 2006. He then worked as a post-doctoral research associate at the Institute of Cell and Molecular Biology of the University of Texas at Austin (USA) (2006-2009). In 2010, he received a Marie Curie International Reintegration Fellowship to establish his independent research group in Greece. During 2010-2022, he served as Head of the Laboratory of Enzyme and Synthetic Biotechnology and the Institute of Chemical Biology of the National Hellenic Research Foundation (Greece). Since 2022, he is the Director of the Institute for Bio-innovation at the Biomedical Sciences Research Center "Alexander Fleming" (BSRC Fleming). Georgios Skretas has been awarded a Consolidator Grant by the European Research Council (ERC) (2019-2024) and a grant by the European Commission to establish an ERA Chair in Biomolecular Engineering and Synthetic Biology at BSRC Fleming (2023-2027). He is the Founder and Chief Executive Officer of ResQ Biotech, a spin-off company applying innovative biotechnology approaches to advance early-stage drug discovery against diseases caused by protein misfolding and aggregation**.** The main goal of his research activities is the development of engineered microbial cells with the ability to perform novel and complex functions by employing principles of Synthetic Biology. The lab utilizes simple organisms, such as the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae* as "biological chassis" and seeks to evolve them into efficient cell factories for the production of valuable chemical and biological products, and for the performance of industrially important processes, such as drug sensing and discovery, bio-transformations etc. Genetic engineering techniques are applied to redesign and rearrange the genome of the organism of interest, while protein engineering (directed protein evolution) and synthetic biology approaches are utilized so as to introduce novel functions in the cell. A key aspect of the work that is carried out is the design and development of high-throughput screening systems, which are used to isolate the rare biomolecules and microbial strains that execute the desired function among large combinatorial libraries comprising hundreds of millions of variants.

ABOUT THE SPEAKERS

Sotirios C. Kampranis studied Chemistry at the Aristotle University of Thessaloniki Greece, and obtained his PhD from the University of Leicester, UK, in 1998 on DNA enzymology. He continued to study the function of chromatin regulators and histone modifications in cancer as a post doc at the Tufts University School of Medicine in Boston. He was elected Assistant Professor of Biochemistry at the Faculty of Medicine of the University of Crete in 2011. In 2016, he moved to the University of Copenhagen as Associate Professor, where from 2021 he is full Professor of Biochemical Engineering.

My work aspires to develop biological synthesis as the method of choice for the production of complex chemicals, replacing current organic chemistry methods, or extraction from plants, which are inefficient and detrimental to the environment. To achieve this goal, I apply a multi-disciplinary approach that begins with the identification and characterization of biosynthetic enzymes, continues with the optimisation via engineering of the enzymatic activities involved, and concludes with the reconstruction of biosynthetic pathways in biological systems for the sustainable synthesis of valuable compounds. My group has pioneered the development of tools and applications in the following areas of Biochemical Engineering:

- Identification, characterization, and optimization through protein engineering of enzymes involved in biosynthetic pathways of specialized metabolites.
- Metabolic engineering of the yeast *Saccharomyces cerevisiae* for the bioproduction of high-value specialized metabolites, focusing on terpenoids, cannabinoids, and alkaloids.
- Production of non-canonical new-to-nature metabolites by the combination of protein and metabolic engineering.
- Development of whole-cell biosensors for the determination of small chemical molecules, proteins or cells.

Ashty Karim currently serves as a Research Assistant Professor of Chemical and Biological Engineering and the Director of Research for the Center for Synthetic Biology at Northwestern University. With a foundation in synthetic biology, he works at the intersection of biology and chemistry developing powerful, enabling technologies to efficiently harness biological systems to produce valuable chemicals as well as building new educational frameworks for teaching synthetic biology. His work is highly cited across Nature Biotechnology, Nature Chemical Biology, Metabolic Engineering, and ACS Synthetic Biology. He earned his B.S. degrees in Chemical Engineering and in Biology from the University of Texas at Austin in 2013 and received his Ph.D. in Chemical Engineering from North University in 2018. During his graduate studies, Ashty was a National Science Foundation Graduate Research Fellow and a U.S. delegate to the 67th Lindau Nobel Laureate Meeting on Chemistry. He is also the recipient of several awards including the 2018 ACS Best-of-BIOT award, the 2018 first place paper at the ACS I & EC's Graduate Student Award Symposium, and Northwestern's 2017 Department of Chemical and Biological Engineering Distinguished Graduate Researcher Award. In addition, Dr. Karim participated in the University of Washington's 2020 Distinguished Young Scholar Seminar (DYSS) series.

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:

23rd September 2022 14.00-16.00 CET	Advances in the Analysis of Enzymatic Reactions, organized by Jennifer Littlechild and Roland Wohlgemuth
21st October 2022 14.00-16.00 CET	Biocatalytic Process Engineering, organized by Polona Žnidaršič-Plazl and ESAB Working Group Biocatalytic Process Engineering
18th November 2022 10.00-12.00 CET	Enzymatic Reaction Mechanisms and their Biocatalytic Applications, organized by Jennifer Littlechild and Roland Wohlgemuth

HOW TO JOIN ESAB

You are cordially invited to join ESAB by completing the membership application form online *via* https://esabweb.org/Join+us/Application+form.html
Personal membership is free.
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
ESAB - European Society of Applied Biocatalysis (esabweb.org)