Poster **#1**

Dynamic freeze-drying

Jean DELAVEAU Lyophitech

J.Delaveau, J.Bomparet, X.Galland

Lyophitech has developed and patented a new freeze-drying technology with the support of the University of Lyon. This technology can work in a batch or continuous mode. Lyophitech has invested in an industrial site located in Vénissieux near Lyon. The site shows a demonstrator of this technology. Using this technology, numerous trials have been successfully completed on different products : pharmaceutical, biochemical, cosmetics and food. Lyophitech can produce pilot and industrial equipments. The company has started sales in 2023.

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Poster **#2**

A new metagenome mining strategy unlocks Glycoside Phosphorylases discovery

Simon LADEVEZE TBI-INSA Toulouse

Ladeveze S., Cooper N., Cioci G., Terrapon N., Lombard V. Henrissat B., Li A., Laville E., Esque J. & Potocki-Veronese G.

Among carbohydrate active enzymes of interest for industrial biocatalysis, Glycoside Phosphorylases (GPs) are valuable catalysts for white biotechnologies as the reversibility of the phosphorolysis reaction combines two reactions into one single catalyst without the need of costly nucleotide-activated sugars as substrates. The identification of new GPs in sequences database is however complex and requires to handle large amounts of data and align thousands of sequences. Sequence similarity networks (SSNs) allow simple and easy visualization of key information (single catalytic residue for inverting GPs, no signal peptides or CBMs). SSNs constitute an unbiased and quantitative manner to predict isofunctional protein groups or subfamilies and allow highlighting unexplored sequence spaces, helping to discover novel functions. The SSN-based exploration of the GH130 family generated 15 robust sequence clusters and identified new functions (e.g. GH130 Man-GlcA phosphorylase). The RadicalZ project explored 6 GP-containing inverting-GH families with SSNs: from 19 selected candidates, 11 could be expressed, and 9 new GPs could be identified in a single campaign. An unusual GH161 b-1,3-glucan phosphorylase could be identified and a first 2.7 Å 3D structure for a GH161 family member could be obtained by cryo-EM. These examples show the power of the SSN-based approach to identifying new GP targets and expand the knowledge on a so far elusive class of enzymes.

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Poster **#3**

Tapping into the rich reservoir of transglycosylases to access rare and designer oligosaccharides

Naim STITI Biotechnology Institute

Naim Stiti, Etienne Severac, David Guieysse, Claire Moulis, Magali Remaud-Simeon

Aiming at the expansion of the realm of oligosaccharides through the discovery and rational engineering of efficient biocatalysts, we performed a comprehensive activity screening of our collection of transglycosylases (TGs) from the Glycoside Hydrolase families GH13 and GH70, which exhibit distinct linkage specificities. Activities were inspected using a wide range of bio-sourced potential glycosyl acceptors. The screening campaign led to the identification of 'promiscuous' TGs that glucosylate di- and oligosaccharides from sucrose, (a cheap and abundant agro-resource) as glucosyl donor. Each enzyme has preferred acceptor(s). A given acceptor gives rise to a single or a panel of oligosaccharides with variable proportions, depending on the used TG. These products differ in size, sugar units, linkage type, and degree of branching, thereby display distinctive physicochemical properties. By doing so, we are enriching the portfolio of 'the glycosides engineering machine' by making rare and 'designer' molecules which could have a far-reaching impact as prebiotics, in cosmetics and/or food. Interestingly, the enzyme GS-D emerges as one of the most versatile enzymes. We target GS-D for rational engineering, with reshaping the subsite +1, to improve catalysis with compatible acceptors, besides broadening the acceptor scope to include arrays of galactooligosaccharides.

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Poster **#4**

Lipase catalyzed selective production of isopropyl esters and lignin derived lipophilic antioxidant esters

Winnie DEJONGHE Flemish Institute for Technological Research

Yamini Satyawali, Marta Martinez-Garcia, Wouter Van Hecke, Winnie Dejonghe

Esters are used in cosmetic industries for all classes of products such as skin, hair care, toiletries, colour cosmetics and fragrances. Esters are currently synthesized chemically using metal oxides, acids, or bases as catalyst at high temperature. Enzyme (e.g., lipase) catalyzed esterification reactions present a greener route and have several advantages especially in terms of milder reaction temperature, better product quality etc. In a first study, we demonstrated at kg scale the lipase catalyzed solvent free synthesis of isopropyl esters with coupled pervaporation for water removal. In a second case, we conducted selective esterification of a fatty acid to the aliphatic OH of the monolignol dihydroconiferyl alcohol using lipases, without modifying the phenolic group which is responsible for the antioxidant activity of this compound. The esterified monolignol showed relevant long-term radical scavenging activity, comparable to other petroleum-based antioxidants.

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Poster **#5**

Deconvoluting the effect of binding density and duration on protease activity by single molecule studies

Emily SOERENSEN University of Copenhagen

Emily Winther Sørensen, Freya Björk Reinhold, Andreas Faber, Steen Bender, Per Hedegaard, Sune M. Christensen and Nikos S. Hatzakis

The impressive specificity and efficiency of proteases sustain a plethora of functions in life and have demonstrated great industrial value in a wide variety of biotechnological industries. In light of the growing concerns regarding climate change, many companies are making active efforts to become more environmentally friendly and sustainable, and here proteases can play a pivotal role by offering innovative and eco-friendly solutions. However, the precise steps and chemical interactions involved in the binding of proteases to substrate are still the subject of ongoing research. Here, current knowledge about protease performance is primarily based on ensemble-type measurements, which are good at revealing average functionalities of a population of proteases but fail to inform us of underlying mechanistic behaviours relating to function. In this work, a fluorescence based single particle tracking microscopy assay was employed to study the behaviour of individual protease molecules in order to extract the underlying functional parameters of protease activity. Establishing a correlation between single molecule mechanistic behaviour and enzymatic activity of proteases will institute new tools for investigating future protease candidates.

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Poster **#6**

Ionic liquid and hemicellulolytic enzymes for fractioning and valorization of horse manure

Lindsay DORSCHNER PELCOQ Unité Génie Enzymatique et Cellulaire (GEC UMR CNRS 7025) - Université de Picardie Jules Verne

Lindsay Dorschner Pelcoq, Bruno Battistini, Catherine Sarazin, Éric Husson

The production of horse manure (HM) exceeds annual requirements and leads to environmental issues, but its seasonality independence makes it an adequate raw material for biogas production by methanization. However, the biochemical methanogenic potential (BMP) of this contaminated lignocellulosic biomass is limited in some extent by the high lignin content inducing deleterious effects on anaerobic microorganisms during fermentation. To overcome this constraint, a step with an ionic liquid (1-ethyl-3-methylimidazolium acetate) is followed by enzymatic hydrolysis catalyzed by hemicellulolytic enzymes (Cellic®CTec 2) prior to methanization. This sequential strategy generates a liquid sugars-enriched fraction (56,37 ± 0,10 % of D-glucose and 52,78 ± 0,16 % of D-xylose) while isolating a valuable solid lignin-enriched fraction named hydrolysis lignin. Each solid fraction generated throughout the strategy were finely characterized by spectroscopic (FTIR, UV, NMR) and microscopic (SEM) techniques. The sugar-enriched liquid fraction will provide the carbon source for methanization and isolated hydrolysis lignin will be chemically or enzymatically functionalized for the conception of biomaterials.

Poster **#7**

Enzymes for seaweed processing

Guillaume MANAT **AberActives SAS**

Manat G., Kloareg B., Michel G., Larocque R.

We are a start up coming from the Station Biologique de Roscoff that challenge the algal polysaccharides extraction and oligosaccharides production using specific enzymes. When one looks at the various methods which are currently used for their preparation, there are three types of technologies, which we refer to as first, second and third generation. All of these processes present several drawbacks, in that they generate the bioactive molecules in low concentrations, they are polluant and request a lot of energy. This is why we propose to implement a mild technology, based on the use of enzymes which specifically modify the algal polysaccharides. We refer to it as a 4th generation technology. This technology is derived from a very large number of cumulated years of research at the Station biologique de Roscoff, on both marine algae and the marine bacteria which are associated with the algal biomass. To feed on the algal sugar chains, those marine bacteria are equiped with a vast arsenal on enzymes which recycle the carbon biomass. Hence, we can rely on an exhaustive catalogue of enzymes to design our processes. Our processes consist in de-structuring the algal matrix polysaccharides. This allows for the preparation of a variety of ingredients, that either stay solid or are solubilized.

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Poster **#8**

Combinatory library of microorganisms in the selection of active strain in biotransformation

Didier BUISSON CNRS-MNHN

Stéphane Mann and Didier Buisson

Bioconversion is used in chemistry with two purposes, either for the preparation of a target product in a synthetic scheme or for the generation of molecular diversity from an active molecule. The biocatalytic toolbox can be enriched by new enzyme discovery or protein engineering. Microorganisms are the main source of enzymes, and screening of active strains involves cell culture followed by incubation with the substrate to be transformed and the monitoring of the reaction. We have developed an efficient screening method where microorganisms are tested in mixtures. In our strategy, a combinatorial approach in the composition of mixtures could reduce the number of screening assays, to divide it by three compared to the conventional method. In addition, the method makes it possible to highlighting unexpected activity and obtain products resulting from successive biotranformations involving two different microorganisms. This possibility is interesting when the objective is to generate molecular diversity. We have recently successfully applied this method to mixtures of substrates. We will present examples of strain selections in the search for targeted activities, in the generation of molecular diversity and in the study of drug metabolism.

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Poster **#9**

Enzymatic epimerization of deoxynivalenol for food and feed decontamination: enzyme discovery and engineering strategies

Valeria DELLA GALA The Novo Nordisk Foundation Center for Biosustainability (Technical University of Denmark)

Valeria Della Gala (1), Jog Raj (2), Hunor Farkaš (2), Marko Vasiljević (2), Ditte Hededam Welner (1) (1) The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kemitorvet 220, Kgs. Lyngby DK-2800, Denmark (2) PATENT CO

Deoxynivalenol (DON) is a mycotoxin produced by the fungus Fusarium graminearum which contaminates many crops, causing diseases and negatively affecting plant growth. Consuming food or feed contaminated with DON can lead to health issues for humans and animals. To tackle this problem, the food and agriculture industries are looking for ways to eliminate mycotoxins such as DON. DON exists in two different forms: the toxic S-conformation (DON) and the non-toxic R-conformation (3-epi-DON). Enzymes such as PQQ-dependent dehydrogenases and NADPH-dependent aldo-keto reductases can convert DON to 3-epi-DON, producing 3-keto-DON as an intermediate. However, these enzymes are not very stable, and more efficient ones are needed, especially ones that can withstand broad industrial and environmental conditions. This study aims to discover and potentially engineer new enzymatic activities that can efficiently convert DON to 3-epi-DON, creating highly efficient biocatalysts that can eliminate mycotoxins from food and feed, promoting food safety and human and animal health.

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Poster **#10**

Enzymes and non-conventional solvents for fractioning and bio-conversion of the main polymers from Miscanthus

María Catalina QUESADA-SALAS **Unité de Génie Enzymatique et Cellulaire GEC, UMR 7025 CNRS, Université de Picardie Jules Verne, France & UMRT BioEcoAgro UMR1158, Institut Charles Viollette, INRAe, équipe Biotransformation/Biocatalyse et Enzymes - Université de Lille, France**

María Catalina Quesada-Salas, Catherine Sarazin, Renato Froidevaux, Eric Husson

As part of the biorefinery concept, these research activities propose innovative and sustainable strategies, combining a rational choice of solvents and biocatalysts, to covalorize the three main constituent polymers (cellulose, hemicelluloses and lignin) from the dedicated crop, Miscanthus x giganteus. The pretreatment with the ionic liquid 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]), followed by two enzymatic hydrolyses catalyzed by the hemicellulolytic cocktail Cellic® Ctec2, allowed the production of platform sugars (D-glucose and D-xylose) with competitive yields (greater than 90%), and the isolation of a lignin-enriched fraction (50% w/w), namely hydrolysis lignin. Two distinct ways of valorization of this fraction were subsequently explored. First, oxidative depolymerization of hydrolysis lignin, catalyzed by laccase mediator system (LMS) in aqueous buffer supplemented or no with [Emim][OAc], led to the selective production of p-hydroxybenzaldehyde, as a promising phenolic intermediate. Secondly, Novozym435®-catalyzed esterification of hydrolysis lignin with various acylating agents was succeeded in different non-conventional solvents (ionic liquids, single or binary, and the biodegradable organic MeTHF-3-one solvent), opening a promising route towards biomaterials conception.

Poster **#11**

Photoactive Proteins: Light for Life

Pavel MÜLLER I2BC

(Sun)light is not merely a source of heat and energy for photosynthesis. Earth-dwelling organisms have been using light in multifarious other ways than photosynthesis: to reverse UV-induced DNA damage, to convert one organic compound into another, to regulate their growth and activity, to communicate, to attract a prey to eat or a partner to mate with and most likely also to perceive the Earth's magnetic field and to use it for orientation on the long journeys around the globe. Behind all these processes are photoenzymes and photoactive proteins – fascinating light-driven molecular machines that have become increasingly sophisticated over the long course of evolution of life on our planet and without which life as we know it could never exist. Out of the plethora of natural (and bio-inspired) photoactive systems, our team is particularly interested in the following: photosystems I & II, the superfamily of DNA-repairing enzymes photolyases and photoreceptors cryptochromes, the recently discovered fatty acid photodecarboxylase and fluorescent proteins. We are also beginning to explore the possibility of making non-photoactive proteins photoactivatable. This approach holds enormous potential, as it allows reactions normally occuring in the dark to be triggered in a synchronous/controlled manner and studied using time-resolved spectroscopic techniques.

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Poster **#12**

Biological scaffolding around a self- assembling protein ring

Alexis BOUTILLIAT Catsalyze

Alexis Boutilliat, Marc Quinternet, Alexandre Kriznik, Marie-Eve Chagot, Louis Bézert

Peroxiredoxin (PRX) is a ubiquitous protein with physiological involvement in the cell's redox machinery. Many PRX variants naturally form a self-assembled decameric ring structure.In this study, we harnessed this structural property to create a robust scaffold using a non-catalytic variant of PRX sourced from thermophilic organisms, known for their thermal stability. Specifically, the N and C termini of the PRX monomer were employed as anchor points to scaffold proteins, such as enzymes, onto a resilient framework resistant to thermal denaturation. This scaffold was constructed either through genetic fusion of protein sequences or by utilizing specific protein adapter pairs. The scaffolded enzymes were expressed and purified in the E. coli system, employing straightforward chromatographic techniques like metal affinity and gel filtration. This innovative scaffold technology facilitates the development of soluble and sizable catalytic structures, offering potential benefits such as improved stabilization, solubilization, production efficiency, and enzyme activity. The diversity within the PRX family provides a wide range of biophysical properties, enabling the customization of scaffolds to suit specific needs. Furthermore, this approach allows for the co-localization of two or more complementary enzymatic activities on the same ring structure.

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Poster **#13**

Diverse Amine dehydrogenases for the biocatalytic synthesis of amines

Carine VERGNE-VAXELAIRE **Génomique Métabolique, Genoscope, Institut François Jacob, CEA, CNRS, Univ Evry, Université Paris- Saclay**

Carine Vergne-Vaxelaire1, Eddy Elisée1* ,Laurine Ducrot1, Ombeline Mayol1, Raphaël Méheust1, Mark Stam1, Eric Pelletier1, Karine Bastard1, Jean-Louis Petit1, Aurélie Fossey-Jouenne1, Véronique de Berardinis1, Anne Zaparucha1, Gideon Grogan2, David Vallen*

Amines are found in many active compounds and in the most frequently used chemical intermediates for the production of pharmaceuticals and fine chemicals. In the course of the discovery of biocatalysts for amine production, interest for enzymes catalyzing the NAD(P)H-reductive amination of ketones with ammonia to primary chiral amines has grown. Previously restricted to engineered α-aminoacid dehydrogenases, this enzymatic toolbox has been recently extended by the discovery of genes coding for native Amine Dehydrogenases (nat-AmDHs) by our group, in addition to reductive aminases and engineered ε-deaminating L-lysine dehydrogenase. The diversity of AmDHs must be as large as possible in terms of sequences and active sites to be able to provide numerous enzymatic tools for amine synthesis, and thus contribute to the transformation of our amine industry towards more sustainable technologies. An extensive exploration of (meta)genomic data enable us to identify such diverse nat-AmDHs. We will present the (meta)genome mining approach which led to the discovery of 17k homologs with key structural variations, the enzyme characterization of some representants, and the potentiality of these enzymes for amine synthesis via some example of biocatalytic synthesis at laboratory scale. This work is supported by the Agence Nationale de la Recherche through the MODAMDH (ANR-19-CE07-0007) and ALADIN (ANR-21-ESRE-0021) projects.

Poster **#14**

The INCITE project - INnovative Chemoenzymatic InTEgrated processes

Clara GUILLERMOU OLEON

Due to the increasing concern on climate change and environmental issues, the focus of chemicals manufacturing industries has been gradually shifting towards the development of alternative greener, safer and sustainable processes. Chemo-enzymatic conversion therefore presents immense opportunities for developing more sustainable processes. Enzymatic processes are also particularly interesting to produce chiral molecules because of their high regio- and enantio-selectivity, which greatly simplifies downstream processing compared to traditional chemical synthesis. Additionally, their compatibility with ambient pressures and temperatures conditions is on the plus side for reducing the environmental footprint of the produced molecules. In that context, the INCITE project aims to demonstrate novel integrated upstream and downstream processing paths involving flow chemistry and membrane technology in chemo-enzymatic processes. The modularity and flexibility of the developed processes is showcased through two demonstration cases in real industrial settings and using hydrolases for the sustainable, safe and energy-efficient production of commodity and fine chiral chemicals. Their development to TRL7 will open up the way to their industrialization and thus lead to major positive economic and environmental impacts.

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Poster **#15**

Applying ligninases to resolve end-of-life issues of thermoset composite plastics

Beatrice MONGILI Biosphere

Beatrice Mongili, Mariella Sacco, Claudia Sabia, Erwin S.M. Swennen and Marco Pistocchi

Thermoset composite materials are polymer resins that, once cured, cannot be remoulded. This kind of materials, represented by epoxy-based resins, polyester, or vinyl-ester resins, finds a principal application in aircraft, wind turbines, and as insulating material in construction. Due to their resistance to physical stresses, management of their end-of-life destiny is a challenge. Thermoset resins are currently not recycled but incinerated or stored in landfills until further processing will be possible. BIZENTE, a BBI-funded project under the support of the EU Horizon 2020 programme, proposes an innovative recycling process based on applying ligninolytic enzymes in the degradation of thermoset resins. To make this real, BIZENTE is working on the selection of candidate enzymes from a group of hydrolases and peroxidases with putative catalytic activities against thermoset resins, as well as by developing a new class of thermoset resins containing more biodegradable moieties and characterized by faster enzymatic processing at the end-of-life. In this frame, this work shows the road map and the results reached in developing a fermentation process for the production of ligninolytic enzymes with engineered Pichia pastoris, and their application in the set-up of an enzymatic degradation process. More in detail, the production of a selected esterase up to a pilot environment and its application with a pre-treated polyester-based resin are illustrated.

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Poster **#16**

Amulett: Super-Fast Targeted Multi-Site Mutagenesis based on Uracilated Single-Stranded Transient Template

Adèle DRAMÉ-MAIGNÉ CEA Saclay / Purpl Research

Rocio Espada, Rémi Sieskind, Yannick Rondelez, Adèle Dramé-Maigné

Tailor-made libraries based on structural information, consensus or phylogenetic analysis, as well as outputs of algorithms trained on directed evolution deep sequencing data, all benefit from Targeted Multi-Site Mutagenesis methods to introduce either pre-defined or random mutations at identified distal sites. This step can be challenging and time-consuming, limiting the speed of projects and sometimes their feasibility. Several methods exist with various efficiency and complexity but rely mostly on the use of mutagenic primers. Annealing of these primers on ssDNA templates have shown some of the best performances. Importantly, the removal of this template strand drastically decreases the number of wild-type contaminants. Here we propose a fully in vitro multi-site targeted mutagenesis method based on PCR-generated uracilated ssDNA transient templates. The output of the Annealing of Mutagenic oligonucleotides on Uracilated λ-exonuclease generated Transient Template (Amulett) is a fully mutated dsDNA. The preparation of the transient template takes only one day and getting the mutated dsDNA, less than 4 hours. Contrary to pre-existing methods, the process does not require any nicking site or phage production and uses only regular T4 Polynucleotide Kinase phosphorylated primers. The Amulett thus provides a fast, cheap and efficient alternative to existing methods.

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Poster **#17**

PolDENZ, a glycosyl hydrolases collection for the degradation of natural polysaccharides

Gwennaëlle LOUIS Université de Namur

Gwennaëlle Louis, Jérôme Coppine, Philippe Gabant and Francesco Renzi

Polysaccharides are carbohydrate chains bound by glycosidic linkages. They are widely distributed in the environment and can be extracted from plants or microorganisms. Various polysaccharides are involved in a broad variety of industrial processes (pharmaceutical, food, textiles, …). We aim at breaking down natural polysaccharides in smaller fragments, which are widely used for many industrial applications, by using synthetic biology. Extensive bibliographic and bioinformatics research allowed us to identify and select a wide range of enzymes involved in this process among microorganisms specialized in the degradation of different polysaccharides. We generated a collection PolDENZ (Polysaccharide Degradation ENZymes) of more than 150 enzymes, naturally found in the environment, able to degrade 10 different types of polysaccharides (alginate, β-mannan, α-mannan, xyloglucan, arabinogalactan, galactan, xylan, iota-carrageenan, laminarin and arabinan). These enzymes can be used alone to degrade a specific polysaccharide or also in combination with other enzymes to degrade more complex polysaccharides and to generate different fragments. For instance, we produced an alginate lyase able to degrade five different sources of alginate, a xylanase able to degrade two different sources of xylan, a laminarinase able to degrade laminarin and a iota-carrageenase able to degrade 5 different sources of iota-carrageenan. We characterized, for all these enzymes, their ability to degrade different polysaccharides. Thanks to our new expression system, we are able to produce a large amount of each enzyme in a quick and efficient way. In addition, we are also able to easily modify the enzymes by synthetic biology in order to improve the production or optimize the activity of these enzymes. Our screening method also allows us to find the best candidate(s) for the degradation of a specific polymer.