GT ENZYMES 2017

(9 – 12 mai 2017)

- LE CROISIC -

NOS PARTENAIRES

Nous tenons à remercier chaleureusement tous nos partenaires et sponsors pour leur soutien financier et matériel, sans qui l'organisation de ce congrès n'aurait pas été possible. Merci pour votre soutien !

BIENVENUE !

Avant-propos

Les organisateurs du 5éme congrès du groupe thématique « Enzymes » de la SFBBM sont heureux de vous accueillir au Croisic (44).

Après le Congrès de Sète en 2015, qui avait réuni la communauté des enzymologistes (GT enzymes) et celle des modélisateurs (GGMM), nous avons souhaité pour ce congrès rapprocher la communauté du GT enzymes et celle du Groupe Biocatalyse en Synthèse Organique (CBSO).
En effet, pour ces deux communautés scientifiques françaises, les enzymes constituent un objet d'intérêt commun, portant sur la compréhension de leur fonctionnement et de leur rôle biologique pour les uns, et l'utilisation en chimie pour les autres.
Les sujets abordés au cours de ce congrès couvrent donc un domaine très large de l'enzymologie fondamentale et de ses applications : mécanismes enzymatiques, structure et dynamique, ingénierie et criblage, complexes multienzymatiques et cascades enzymatiques, enzymes et métabolisme cellulaire, biocatalyse, enzymes et biologie synthétique.
Nous avons souhaité ouvrir ce congrès à la fois à l'international en invitant plusieurs conférenciers étrangers, et aussi au monde industriel par la participation scientifique de plusieurs entreprises.

Nous espérons que ce congrès sera propice aux échanges entre nos différentes communautés et sera l'occasion d'initier de nouvelles collaborations scientifiques à l'interface chimie-biologie.

Charles Tellier
Pour les membres du comité scientifique

GT Enzymes - SFBBM

En complément des différentes activités de la SFBBM au niveau national et international, la vie de la Société est animée par des réunions régulières de ses Groupes Thématiques (GT). Le but de ces réunions est de rassembler la communauté des scientifiques français, autour d'une thématique ciblée, dans le domaine de la Biochimie, Biologie moléculaire. Il est en effet mentionné dans le règlement intérieur de la Société, que les Groupes Thématiques se réunissent en général tous les 2 ans pour faire le point sur les avancées de leur domaine. Cette réunion est également, l'occasion de nommer le nouveau président du GT.

Le GT "Enzymes" comprend une communauté scientifique, composée de 100 à 150 personnes, présente depuis le premier congrès du GT à Nancy (2009) le troisième à Ax-les-Thermes (2011) et les deux derniers à Paris (2013) et à Sète (2015).

CBSO

Le Club Biocatalyse en Synthèse Organique (CBSO), association loi 1901 crée en 1994, est le réseau français en Biocatalyse fédèrant des laboratoires académiques et des centres de recherche industriels aux compétences pluridisciplinaires (chimie organique et analytique, microbiologie, ingénierie protéique et métabolique, génie des procédés, bio-informatique, modélisation moléculaire).

Les recherches développées par les laboratoires du CBSO s'inscrivent dans le domaine de la chimie verte et visent à concevoir de nouvelles stratégies de synthèse de molécules organiques grâce aux enzymes utilisées comme biocatalyseurs. Ces approches sont basées sur des technologies innovantes qui intègrent toutes les étapes nécessaires pour aller de la recherche de nouveaux biocatalyseurs jusqu'à l'optimisation du procédé (métagénomique, techniques d'ingénierie, tests de criblage, caractérisation de l'enzyme, optimisation du biocatalyseur, mise en œuvre du procédé).

Le CBSO vise à promouvoir les collaborations entre les secteurs privés et publics par l'organisation de colloques dont le but est d'échanger les avancées récentes dans le domaine de la biocatalyse et de favoriser l'interdisciplinarité.

Contact : laurence.hecquet@uca.fr (présidente CBSO)

Colloque Enzymes et Biocatalyse – du 9 au 12 mai 2017 – Le Croisic (France)

Liste des sessions & conférenciers invités

Session 1 : Mécanismes enzymatiques / Enzyme mechanisms
François Talfournier – Université de Lorraine
« Local dynamics play key roles in the catalytic mechanism of non-phosphorylating Aldehyde dehydrogenases »

Session 2 : Criblage et ingénierie d'enzymes / Enzyme screening and engineering
Marco Fraaije - *University of Groningen - Pays-Bas*
« Engineering robust flavoenzymes for industrial processes »

Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems
Murielle Gondry - *CEA, Paris*
« Aminoacyl-tRNA-utilizing Enzymes involved in Natural Product Biosynthesis »

Session 4 : Enzymes : structure et dynamique / Enzymes : structure and dynamic
Christine Orengo - *University College, London, Grande Bretagne*

Session 5 : Enzymes cellulaires et métabolisme / Cellular enzymes and metabolism
Raphael Margueron - *Institut Curie, Paris*

Session 6 : Biocatalyse / Biocatalysis
Frank Hollman – *TU Delft, Pays-Bas*
« Oxyfunctionalization – the next frontier of biocatalysis for organic synthesis »

Session 7 : Enzymes et biologie synthétique / Enzymes and synthetic biology
Macha Anissimova - *Global Bioenergies, Evry*
« Artificial Metabolic pathways for bio-based Isobutene »

Session 8 : Glyco-enzymologie / Glycoenzymology
Tom Desmet - *Gand, Belgique*
« Enzyme engineering for the conversion of sucrose into value-added products »

https://sfbbm-enzybio.sciencesconf.org/

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PROGRAMME

- CONFERENCES ORALES -

Jour	Début	Fin	N°	Session	Titre	Orateur	Modérateur
					09-mai		**Modérateur**
09-mai	17h45	18h00			**ACCUEIL COLLOQUE**	Charles Tellier	Sandrine Boshi
09-mai	**18h00**	**18h45**	**L1**	**Session 1 : Mécanismes enzymatiques / Enzyme mechanisms**	**Local dynamics play key roles in the catalytic mechanism of non-phosphorylating Aldehyde dehydrogenases**	**François TALFOURNIER**	Sandrine Boshi
09-mai	18h45	19h05	O1	Session 1 : Mécanismes enzymatiques / Enzyme mechanisms	New insights into the nucleotide specificity of APH(2")-IVa, an enzyme involved in antibiotic resistance	Corinne Lionne	Sandrine Boshi
09-mai	19h05	19h25	O2	Session 1 : Mécanismes enzymatiques / Enzyme mechanisms	New insights of the mechanisms of bilirubin oxidases	Claire Stines-Chaumeil	Sandrine Boshi
					FIN DE JOURNEE		
					10-mai		
10-mai	8h55	9h00			**ACCUEIL DEBUT DE JOURNEE**		
10-mai	9h00	9h20	O3	Session 1 : Mécanismes enzymatiques / Enzyme mechanisms	Non redox thiolation in transfer RNA occuring via sulfur activation by a [4Fe-4S] cluster	Béatrice Golinelli-Pimpaneau	Corinne Lionne
10-mai	9h20	9h40	O4	Session 1 : Mécanismes enzymatiques / Enzyme mechanisms	Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycatedadducts	Julien Dairou	Corinne Lionne
10-mai	9h40	10h00	O5	Session 1 : Mécanismes enzymatiques / Enzyme mechanisms	The carbon monoxide dehydrogenase from D. vulgaris	Sébastien Dementin	Corinne Lionne
10-mai	10h00	10h20	O6	Session 1 : Mécanismes enzymatiques / Enzyme mechanisms	Redox signaling: interactions of sulfiredoxin with decameric peroxiredoxi	Hortense Mazon	Corinne Lionne
10-mai	10h20	10h40			**PAUSE**		
10-mai	**10h40**	**11h25**	**L2**	**Session 2 : Criblage et ingénierie d'enzymes / Enzyme screening and engineering**	**Engineering robust flavoenzymes for industrial processes**	**Marco Fraaje**	Véronique de Berardinis
10-mai	11h25	11h45	O7	Session 2 : Criblage et ingénierie d'enzymes / Enzyme screening and engineering	Discovery of P1B-TYPE-ATPASE inhibitors by high throughput screening	Xavier Maréchal	Véronique de Berardinis
10-mai	11h45	12h05	O8	Session 2 : Criblage et ingénierie d'enzymes / Enzyme screening and engineering	Turning a β-agarase into a β-transagarase: a theoretical and experimental study	Benoît David	Véronique de Berardinis
10-mai	12h05	12h25	O9	Session 2 : Criblage et ingénierie d'enzymes / Enzyme screening and engineering	Modelling of the glucosylated aspartyl residue in sucrose phosphorylases: parameterization and applications	Bernard Offmann	Véronique de Berardinis
10-mai	12h25	12h45	O10	Session 2 : Criblage et ingénierie d'enzymes / Enzyme screening and engineering	Disruptive mixed in vitro-in silico approach fo protein engineering and screening	Frederic Cadet	Véronique de Berardinis
10-mai	12h45	14h30			**REPAS**		
10-mai	14h30	15h			**SPEED POSTER PRESENTATION (x10)**		
10-mai	**15h20**	**16h05**	**L3**	**Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems**	**Aminoacyl-tRNA-utilizing enzymes involved in natural product biosynthesis**	**Muriel Gondry**	Fernando Rodirguez-Lima
10-mai	16h05	16h25	O11	Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems	One-pot enzymatic cascade : from styrene derivatives to enantiopure phenyl alcohols	Caroline E. Paul	Fernando Rodirguez-Lima
10-mai	16h25	16h45	O12	Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems	Design of multidomain biocatalysts by fusing enzymes from the carotenoid pathway	Gilles Truan	Fernando Rodirguez-Lima
10-mai	16h45	17h00			**PAUSE**		
10-mai	17h00	17h20	O13	Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems	Improving molecule synthesis by tuning a multi-step enzymatic cascade	Katia Duquesne	Véronique Alphand
10-mai	17h20	17h40	O14	Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems	One-pot, two-step cascade synthesis of naturally rare L-erythro (3S, 4S) ketoses by coupling a thermostable transaminase and transketolase	Marion Lorillière	Véronique Alphand
10-mai	17h40	18h00	O15	Session 3 : Système multienzymatique – cascade enzymatique / Multi enzymatic systems	Integrative characterization of the alginolytic system of Zobellia Galactanivorans	Gurvan Michel	Véronique Alphand
10-mai	18h00				**POSTER SESSION**		
					FIN DE JOURNEE		

11-mai							
11-mai	8h55	9h00		ACCUEIL DEBUT DE JOURNEE			
	9h00	**9h45**	**L4**	**Session 4 : Enzymes : structure et dynamique / Enzymes : structure and dynamic**	**A Structural Perspective on the Evolution of Protein Functions**	**Christine Orengo**	Bernard Offmann
	9h45	10h05	O16		Jumping between protein conformers using normal modes	Yves-Henri Sanejouand	
	10h05	10h25	O17		Diffusion of polysaccharide-degrading enzymes in complex substrates investigated by Synchrotron Soleil facilities	Estelle Bonnin	
	10h25	10h45	O18		Matching the diversity of sulfated biomolecules : creation of a classification database for sulfatases reflecting their substrate specificity	Tristan BARBEYRON	
	10h45	11h		PAUSE			
	11h	**11h45**	**L5**	**Session 5 : Enzymes cellulaires et métabolisme / Cellular enzymes and metabolism**	**En cours**	**Raphael Margueron**	Michele Reboud-Ravaux
	11h45	12h05	O19		The carbon monoxide dehydrogenase from d esulfovibrio vulgaris	Mériem Merrouch	
	12h05	12h25	O20		Discovery of L-serine succinyl-CoA transferases in L-cysteine biosynthesis	Thomas Bessonnet	
	12h25	14h15		REPAS			
	14h15	14h45		SPEED POSTER PRESENTATION (x10)			
	14h45	**15h30**	**L6**	**Session 6 : Biocatalyse / Biocatalysis**	**Oxyfunctionalization reactions – the next frontier of biocatalysis for organic synthesis**	**Frank Hollmann**	Laurence Hecquet
	15h30	15h50	O21		Caractérisation biochimique et moléculaire d'une lipase de staphylococcus aureus	Fatima Nehal	
	15h50	16h10	O22		Sugar and glycosidic bond effect on tyrosinase and melanin transfert inhibition	Cédric Peyrot	
	16h10	16h30	O23		Lipase-catalyzed production of lysophospholipids	Gaëlle Pencreac'h	
	16h30	16h50		PAUSE			
	16h50	17h10	O24	**Session 6 : Biocatalyse / Biocatalysis**	Breaking the dogma of aldolase specificity: Simple aliphatic ketones and aldehyde are nucleophiles for fructose-6-phosphate aldolase	Christine HELAINE	Béatrice Golinelli-Pimpaneau
	17h10	17h30	O25		Enzymes to rethink the lifecycle of plastics	Sophie Duquesne	
	17h30	**17h45**	**O26**	**Prix Nicloux**	Functional investigation of the MexA / MexB / OprM multidrug efflux pump from Pseudomonas aeruginosa	**Martin PICARD**	
	17h45			POSTER SESSION			
	20h00			SOIREE DE GALA			
				FIN DE JOURNEE			

12-mai							
12-mai	8h55	9h00		ACCUEIL DEBUT DE JOURNEE			
	9h00	**9h45**	**L7**	**Session 7 : Enzymes et biologie synthétique / Enzymes and synthetic biology**	**Artificial metabolic pathways for bio-based**	**François Stricher**	Gilles Truan
	9h45	10h05	O27		Fungal cell wall degrading enzymes diversity and adaptability to different biomasses	Vincent Phalip	
	10h05	10h25	O28		Triazolo- and azaGly-peptidomimetics: a new generation of potent reversible substrate-like	Mylène Wartenberg	
	10h25	**11h10**	**L8**	**Session 8 : Glyco-enzymologie / Glycoenzymology**	**Enzyme Engineering for the conversion of sucrose into value-aded products**	**Tom Desmet**	Gurvan Michel
	11h10	11h30	O29		Improvement of the versatility of an Arabinofuranosidase against galactofuranose, for	Quentin Pavic	
	11h30	11h50	O30		Galactofuranosyltransferases of Leishmania?	Jihen ATI	
				FIN DE COLOQUE			

- POSTERS-

		10-mai	
10-mai	P1	trifluorosubstrates asmecanistic probes for a family of flavodehydrogenases	Florence Lederer
	P2	new enzymatic synthesis of methylmercaptan from dimethyldisulfide	Hugo Brasselet
	P3	engineering a thermostable transketolase from geobacillus stearothermophilus by site-saturation mutagenesis to broaden its substrates scope	Romain Dumoulin
	P4	the catalytic mechanism of aminopeptidase b: role of tyrosine residues conserved within the m1 family of zn2+ metallopeptidases.	Sandrine Cadel
	P5	inhibiting preferentially caspase-2 with a new irreversible pentapeptide derivative	Elodie Bosc
	P6	unravelling the mechanism of sulfur transfer catalyzed by the 3-mercaptopyruvate sulfurtransferases	Sandrine Boschi
	P7	transketolase - aldolase symbiosis for the stereoselective preparation of aldoses and ketoses of biological interest	Virgil HELAINE
	P8	redox signaling : insights into the interaction mechanism of sulfiredoxin with peroxiredoxin and atp	Florent CANONICO
	P9	mechanism of o2 diffusion and reduction in fefe hydrogenases	Christophe Léger
	P10	biochemical and electrochemical characterization of a new multicopper oxidase	Elodie Roussarie

		11-mai	
11-mai	P11	fully enzymatic synthesis of mono- and diacyl trehalose conjugates: from simple aliphatic to mycolic deriv a tives	Laurent Legentil
	P12	new cellulases cloned from alkalophile b. pumilus strains isolated from lake bogoria	Lydia OGONDA
	P13	dsr-dp: a dextransucrase with catalytic efficiency boosted by polar organic solvents	Manon Molina
	P14	subunit selective noncovalent inhibition of immuno- and constitutive proteasomes	Michèle Reboud-Ravaux
	P15	benzene-induced leukemogenesis: the human h3k36 histone methyltransferase setd2 as a molecular target of benzoquinone, the hematotoxic metabolite of benzene.	Fernando Rodrigues Lima
	P16	active site loop dynamics controls cofactor flip during the catalytic cycle in hydrolytic aldehyde dehydrogenases	Sophie RAHUEL
	P17	automatic reconstruction and modelling of biocatalyst systems for the production of specific biochemical compounds	Mathieu NG FUK CHONG
	P18	biophysical characterization of human mitochondrial binary complexes formed between ethe1 and different sulfurtransferases	Sandrine Boschi
	P19	hidden phage vp16 peptide deformylase (pdf) features are essential for deformylase activity	Freanceco LAVECCHIA

Comité d'organisation

Nom	Fonction
Charles Tellier	Professeur
Bernard Offmann	Professeur
Corinne Miral	Maitre de Conférences
Franck Daligault	Maitre de Conférences
Andy Chauveau	Animateur du réseau GlycoOuest

Composition du comité scientifique

Nom	Organisation
Fernando Rodrigues-Lima	Université Diderot, Paris 7
Christophe Leger	Institut de Microbiologie de la Méditérannée, Marseille
Corinne Lionne	Centre de Biochimie Structurale, Montpellier
Magali Remaud-Simeon	INSA, Toulouse
Sandrine Boschi	Université de Lorraine, Metz
Charles Tellier	UFIP, Nantes
Laurence Hecquet	Institut de Chimie, Clermont Ferrand
Véronique de Berardinis	Genoscope, Evry
Véronique Alphand	Université Aix-Marseille
Didier Buisson	Muséum National d'histoire naturelle, Paris

Session 1 : Mécanismes enzymatiques / Enzyme mechanisms

New insights into the nucleotide specificity of APH(2'')-IVa, an enzyme involved in antibiotic resistance

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Aminoglycosides are broad-spectrum antibiotics used for the treatment of severe bacterial infections. However, their use has been compromised by the spreading of enzymes capable of modifying them, such as Aminoglycosides PHosphotransferases (APH), thereby inducing a loss of their clinical efficacy. Among these enzymes, APH(2")-IVa has the peculiarity of using ATP or GTPwith comparable efficiencies as a phosphate donor[1]. Validated withdifferent enzymes of the same family [2,3], transient kinetic methods were used to reveal the details of the enzymatic mechanism and to identify the predominant intermediates of the reaction. Our studies reveal that the rate-limiting steps of the reactions are different depending on the type of nucleotides used: ADP release in one hand and GTP binding and/or phosphotransfer in the other one (Figure 1).

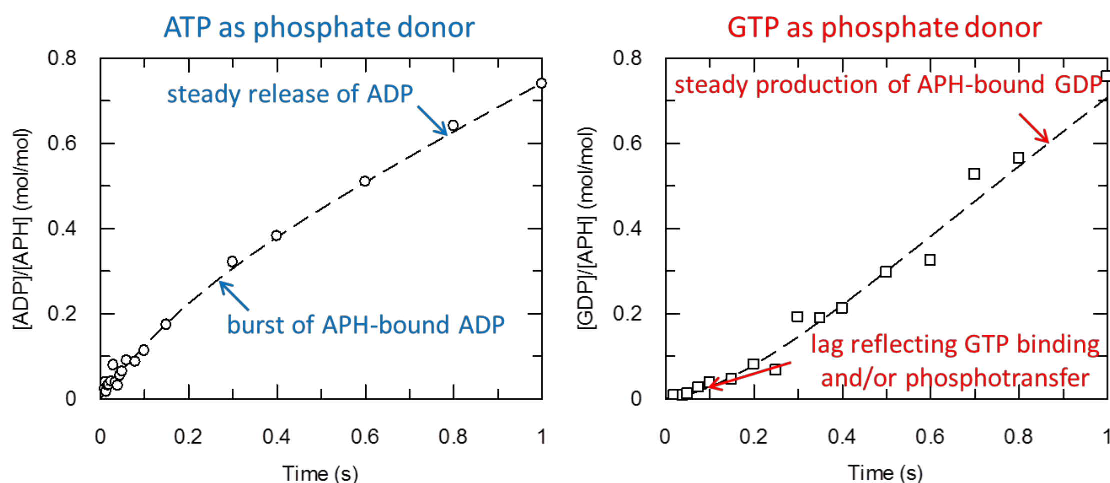

Figure 1. Transient kinetics of phosphotransfer from ATP or GTP to kanamycin A catalysed by APH(2")-IVa.Reaction was triggered at 25°Cby mixing a solution of APH(2")-IVa pre-incubated with kanamycin A with an equal volume of MgATP or MgGTP. Final concentrations were 5 µM APH(2")-IVa, 100 µM kanamycin A and 500 µM NTP. Reaction was stopped with 10% perchloric acid at times indicated and the nucleotide concentrations were assayed by HPLC analysis.

Beside kinetic studies, calorimetry(ITC) experimentswere carried out in order to obtain thermodynamic parameters for the binding of the two nucleotides to APH. To better understand the structure-function relationships, the first structures of the APH(2")-IVa in complex with ADP (4N57) or GDP (publication in preparation) were solved. Analysis of the interactions responsible for nucleotides bindingallowedexplaining the non-specificity of the enzyme for the nucleotides, as well as the observed kinetic differences.

[1] Shi K.;Berghuis A.M.Structural basis for dual nucleotide selectivity of aminoglycoside 2"-phosphotransferase IVa provides insight on determinants of nucleotide specificity of aminoglycoside kinases. J. Biol. Chem.**2012**, *287*, 13094-13102.
[2] Lallemand P.; Leban N.;Kunzelmann S.; Chaloin L.; Serpersu E.H.; Webb M.R.; Barman T.; Lionne C.Transient kinetics of aminoglycoside phosphotransferase(3')-IIIa reveals a potential drug target in the antibiotic resistance mechanism. FEBS Lett.**2012**, *586*, 4223-4227.
[3] Kaplan E.; Guichou J.F.; Chaloin L.; Kunzelmann S.; Leban N.; Serpersu E.H.; Lionne C. Aminoglycoside binding and catalysis specificity of aminoglycoside 2"-phosphotransferase IVa: A thermodynamic, structural and kinetic study.BiochimBiophysActa.**2016**, *1860*, 802-813.

NEW INSIGHTS OF THE MECHANISMS OF BILIRUBIN OXIDASES

Yousefian Narek[1,2,3], Exiga-Huet Stéphanie[1,2], Gounel Sébastien[1,2], Louerat Frédéric[1,2], Durola Fabien[1,2], Mano Nicolas[1,2], Stines-Chaumeil Claire[1,2]

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Enzymatic miniature membrane-less glucose/O_2 Biofuel Cells are of particular interest because they may be used, in the near future, as *in-vivo* power sources for implantable medical devices requiring low-power density [1]. At the cathode, bilirubin oxidases (BODs) are preferred because unlike laccases they show high activity and stability in physiological conditions.

Our work consist in characterizing in solution and immobilized at the electrode surfaces two new BODs recently discovered [2,3,4] and determine the rate-limiting step of the reactions, which are so far unknown for bilirubin oxidases.

Here, we have highlighted in solution several original points: i/ first, we identified the kinetic mechanism at steady state as a Ping-Pong mechanism ; ii/ we elucidate the reaction mechanism at pH7 and iii/ we determined the pre-steady state kinetic parameters of each half reaction with a stopped-flow which allow us to decipher the nature of the rate limiting step of the BODs.

Keywords: bilirubin oxidase – kinetic mechanism – rate-limiting step – steady-state and pre-steady-state studies

References:

(1) Mano *et al.*, *J Am Chem Soc*. **2003**, 125(21): 6588-94.

(2) Durand *et al.*, *Appl. Microbiol. Biotechnol.* **2012**, 96(6): 1489-98.

(3) Durand *et al.*, *Biosens. Bioelectron.* **2012**, 35(1): 140-146.

(4) Gounel, S.; Rouhana, J.; Stines-Chaumeil, C.; Cadet, M.; Mano, N. *Journal of biotechnology*. **2016**, 230: 19-25.

NON REDOX THIOLATION IN TRANSFER RNA OCCURING VIA SULFUR ACTIVATION BY A [4Fe-4S] CLUSTER

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Post-transcriptional modifications of transfer RNA (tRNA) are essential for translational fidelity. Sulfur is present in several nucleosides within tRNAs. The thiolation of the universally conserve dmethyl-uridineat position 54, catalyzed by the enzyme called TtuA, stabilizes tRNAs from thermophilicbacteria and hyperthermophilicarchaea and is required for growth at high temperature. Moreover, the thiolation of uridine 34 in the anticodonloop of tRNAs, whichis required for normal growth and stress resistance in yeast, is carried out by two completely differents ystems: thewell-studied MnmA protein (present in bacteria and in the eukaryoticmitochondrion) and the Nsc6/NcsA/Ctu1 proteins in all other organisms, including the eukaryoticcytoplasm.
Spectroscopic, enzymatic and structural studies demonstrate that the tRNA thiouridine synthetasesTtuA and NcsAcatalyze the simple non-redox substitution of the C2-uridine carbonyloxygenby sulfur using a [4Fe-4S] clúster and thus are representatives of a new enzymes superfamily. A series of crystalstructures show that : (i) the [4Fe-4S] cluster is ligated by only three cysteines that are fully conserved, allowing the fourth unique iron to bindanexogenoussulfide, which likely acts as the sulfurating agent; (ii) the ATP-bindingsite, localized thanks to a protein-bound AMP molecule, a reaction product, is adjacent to the cluster. A new mechanism for tRNA sulfuration is proposed, in which the unique iron of the catalytic cluster functions as a sulfur carrier. Our results open new perspectives regarding functions of iron-sulfur proteins in biology as well as chemical reactions catalyzed by iron-sulfur clusters.

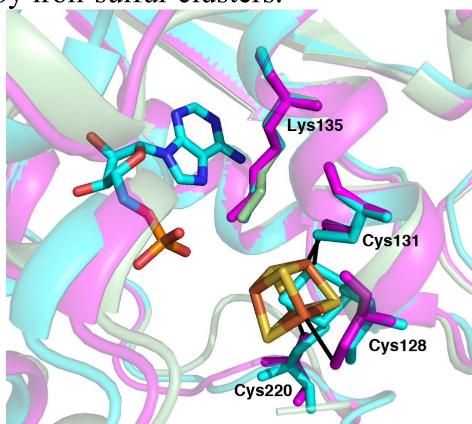

Figure 1. Structure of the active site of TtuA showing the proximity between AMP and the [4Fe-4S] cluster.

Parkinsonism-associated protein DJ-1/Park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycatedadducts.

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Glycation is an inevitable nonenzymatic covalent reaction between proteins and endogenous reducing sugars or dicarbonyls (methylglyoxal, glyoxal) that results in protein inactivation. DJ-1 was reported to be a multifunctional oxidative stress response protein with poorly defined function. Here, we show that human DJ-1 is a protein deglycase that repairs methylglyoxal- and glyoxal-glycated amino acids and proteins by acting on early glycation intermediates and releases repaired proteins and lactate or glycolate, respectively. DJ-1 deglycatescysteines, arginines, and lysines (the three major glycated amino acids) of serum albumin, glyceraldehyde-3-phosphate dehydrogenase, aldolase, and aspartate aminotransferase and thus reactivates these proteins. DJ-1 prevented protein glycation in an Escherichia coli mutant deficient in the DJ-1 homolog YajL and restored cell viability in glucose-containing media. These results suggest that DJ-1-associated Parkinsonism results from excessive protein glycation and establishes DJ-1 as a major anti-glycation and anti-aging protein.

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THE CARBON MONOXIDE DEHYDROGENASE FROM *D. vulgaris*

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Ni-containing Carbon Monoxide Dehydrogenases (Ni-CODHs) catalyze the reversible conversion between CO and CO_2 in some anaerobic bacteria and archaea. In some organisms, they play a key role in energy conservation and/or CO_2 fixation. The active site of Ni-CODHs, called C-cluster, is a distorted Ni–3Fe–4S cubane coordinated to a unique fourth iron [1,2]. Although the enzymes from *Rhodospirilum rubrum*, *Carboxydothermushydrogenoformans* and *Moorellathermoacetica* have been extensively characterized, there are still many open questions regarding the mechanism, the maturation of the complex C-cluster and the cellular functions of Ni-CODHs. Moreover, the role of accessory proteins, CooC, CooF, CooJ and CooT, in the maturation of the C-cluster is unclear because the corresponding genes are not always present in the CODH operons. However, CooC is undoubtedly the most important of the accessory proteins of CODH because it is present in all the Ni-CODH producing organisms; its putative role is to favor the insertion of nickel into the protein.

Desulfovibrio vulgaris (Dv) contains an annotated Ni-CODH operon containing two genes that encode for the CODH enzyme and only the maturase CooC but these two enzymes have not been characterized so far. In the laboratory, we use a combination of biochemical, kinetic, spectroscopic and structural techniques to learn about this complex enzyme and its maturase.

To understand the precise role of CooC, we have compared the kinetic, spectroscopic and structural properties of the Dv CODH produced in the presence or in the absence of CooC. We have confirmed that CooC is essential to fold the active site so that it can bind Ni [3].

Moreover, Ni-CODHs are deemedto be extremely sensitive to O_2, but very little is known about their reactivity to this gas. By using an electrochemical method, we have compared the reaction with O_2 of the CODHs from Dv and from*Carboxydothermushydrogenoformans*. Contrary to expectations, we have shown that the two enzymes react differently with O_2: remarkably, the Dv CODH is much more O_2-resistant [4].

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REDOX SIGNALING: INTERACTIONS OF SULFIREDOXIN WITH DECAMERIC PEROXIREDOXIN

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The typical 2-Cys-peroxiredoxins (Prx) are thiol peroxidases involved in cell physiology of hydrogen peroxide (H_2O_2), an oxidant with concentration-dependent properties ranging from cell signaling messenger to toxic. The established activity of Prx is to degrade H_2O_2 by use of a reactive peroxidatic Cys, which becomes selectively oxidized into sulfenic acid (-SOH). Furthermore, Prx can be inactivated by hyperoxidation into sulfinic acid ($-SO_2H$), turning it into a redox-regulated chaperone holdase [1]. This hyperoxidized Prx is reduced and reactivated by Sulfiredoxin (Srx) by an ATP-dependent reaction. Prxs relay the H_2O_2 redox signal by switching between hyperoxidized and lower oxidation states. The Prx/Srx axis is involved in circadian rhythms regulation [2], but also in cancer and aging processes [3,4].

The crystal structure of a Prx/Srx covalent complex showed that two Srx monomers interact with each subunit within the Prx obligate dimer through two interfaces, one between the Prx and Srx active sites that also binds ATP, and one that involves the C-terminal region of Prx embracing Srx backside [5]. In addition, Prx is organized as a decamer comprising five dimers with a ring-shaped structure, which adds to the complexity of the double-interface interaction and raises the question of the recognition mode of Srx by the Prx decamer. We therefore investigated this interactions using complementary approaches: at the macroscopic level with titration microcalorimetry, anisotropy and activity measurements and at the microscopic level with supramolecular mass spectrometry. Analysis of the non-covalent interaction of Srx monomer (14 kDa) with Prx homodecamer (214 kDa) from *S. cerevisiae* showed a very heterogeneous mixture of Srx bound to Prx, which can reach 10 Srx bound per decamer. Analysis of these data provides a quantitative picture of the mechanism of interaction between Srx and decameric Prx, which could play a role in the regulation of Prx functions.

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Session 2 : Criblage et ingénierie d’enzymes / Enzyme screening and engineering

Engineering robust flavoenzymes for industrial processes

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Enzymes containing flavin cofactors have been shown to be incredibly flexible in catalytic properties. They fulfill numerous crucial roles in biology, e.g. light sensing, electron transfer, oxidations, reductions, (de)halogenations [1]. This makes them a highly interesting enzyme class for enzyme redesign studies.
Part of the work in my group has focused on elucidating the role of covalent flavin-protein linkages, which are present in a defined set of natural flavoproteins, the so-called covalent flavoproteins [2]. Based on the obtained insights into this self-catalytic flavinylation process, we have recently engineered non-covalent flavoproteins into covalent flavoproteins, and monocovalent flavoproteins in to biocovalent flavoproteins [3,4]. Examples of these flavoenzyme engineering studies will be presented.
Other efforts have targeted the redesign of oxidative flavoproteins towards industrially relevant enzymes. One approach for that has been the redesign of the flavin cofactor. By flavin redesign an artifical flavoprotein peroxygenase has been created [5]. Such a flavoenzyme has not been encountered in nature so far. The catalytic features of this created flavoenzyme and the potential of flavin cofactor redesign will be discussed. Also some other recent examples of flavoenzyme discovery and engineering will be presented [6,7,8].

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DISCOVERY OF P_{1B}-TYPE-ATPASE INHIBITORS BY HIGH THROUGHPUT SCREENING

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P-type ATPases are membraneproteins that use the energy of ATP hydrolysis for the active transport of many cations. They are therefore key components of cellular ion homeostasis. CadAfrom *Listeria monocytogenes* belongs to the large family of P_{1B}-type ATPases that gathers Zn^{2+}, $Cu^{+/2+}$, Cd^{2+}, Pb^{2+}-ATPases.In *L. monocytogenes*, CadAwas found responsible for the resistance to cadmium but its physiological role very likely relies on zinc homeostasis[1]. In that context, why do we need inhibitors of P_{1B}-type ATPases and why is CadA a good model?

First, little is known about P_{1B}-type ATPasestructure. The only two available structures of the Cu^{+}-ATPase CopA from *Legionnellapneumophila* and the Zn^{2+}-ATPase ZntA from *Shigellasonnei* have been obtained without the resolution of their N-terminal metal binding domain, in an unmetalled conformation and complexed with phosphate mimics.By stabilizing different conformations of P_{1B}-type ATPases, inhibitors (or ligands in general) could be helpful in the acquisition of new crystallographic structures and thus in a better understanding of the mechanism of metal transfer by these transporters.

Second, P_{1B}-type ATPases could be relevant as therapeutic target. Indeed, in *L. monocytogenes* and in other intracellular pathogens like *Mycobacterium tuberculosis*, this kind of transporters are involved in the resistance of metal based poisoning triggered by macrophages in response to infection [2].Inhibition of these transporters could somehow decrease bacterialvirulence and then help in the treatment of various diseases.

CadA is a transporter we study for years and we know in detail its enzymatic characteristics [3].We chose it as model to develop and perform the first High Throughput Screening (HTS) assay on P_{1B}-type ATPase. This HTS assayis based on the determination of CadA ATPase activity by measuring the quantity of inorganic phosphate formed during the ATP hydrolysis, using the Fiske-Subbarow method.All kinetic and enzymatic conditionswere optimized to 384 wells plate. As reference inhibitor, we used orthovanadate, an analogue of inorganic phosphate that non-specifically blocks all the P-typeATPases. The first screening, using Prestwick chemical library ® (1280 molecules), lead to the identification of 11 compounds with IC_{50} value below 50 µM. In parallel to the study of their mechanisms of inhibition, we are currently evaluating the activity of these hits on a cellular model. Screening of other chemical libraries are also in progress. Due to the similarity of their enzymatic cycle, this assay could easily be adapted to other members of the P_{1B}-type ATPase family.

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Turning a β-agarase into a β-transagarase: a theoretical and experimental study

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Known for their ability to hydrolyse glycosidic linkages, numerous retaining glycoside hydrolases (GH) are also able to catalyse transglycosylation reaction, which can be harnessed for the synthesis of complex oligosaccharides. Although in the vast majority of cases hydrolysis prevails over transglycosylation reaction, this latter has already been increased through mutagenesis and directed evolution experiments [1]. However, little is known about the regulation of the balance between both activities.

We discovered, via crystallographic data and molecular dynamics (MD) simulations, a potential intermittent water channel connecting the bulk to the active site in the endo-β-agarase of *Zobellia galactanivorans* (AgaD) [2]. This observation supports the hypothesis that water channels could be involved in hydrolytic activity. Three amino acid residues located in the vicinity of this water channel were suspected to control water access from the bulk to the channel interior and the active site. Mutagenesis of these three amino acids was performed in order to attempt to increase the transglycosylation/hydrolysis ratio balance.

Biochemical characterization of the best mutant showed a 50-fold decrease of hydrolysis compared to the wild type, while the transglycosylase activity was maintained and even slightly improved. MD simulations revealed that these modifications are correlated with greater water dynamics in the channel, marked by a dramatic reduction of the water survival time as well as the purge time of water in the channel ending close to the active site. These results highlight the importance of internal water dynamics in hydrolases catalysis and suggest that modifying the protein internal water dynamics could serve as a new rational approach to engineer transglycosylase activity in GH.

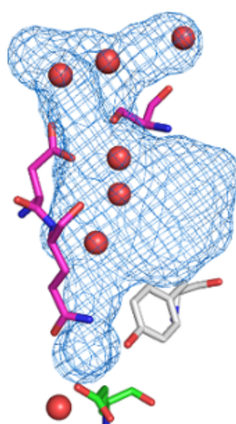

Figure 1. Putative water channel in AgaD wild-type. In red, crystallographic water oxygens positions. In blue mesh, water oxygens simulated trajectory.

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Modelling of the glucosylated aspartyl residue in sucrose phosphorylases: parameterization and applications

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The process of setting up a simulation system for non-standard residue is often one of the major hurdles in computational modeling and simulations. Thus, the ability to add a non-standard residue type for force field parameters is undoubtedly useful to study their structure, dynamics and interactions. One such residue is the glucosylated aspartyl as observed in the experimentally derived covalent intermediate of the sucrose phosphorylase (EC 2.4.1.7) enzyme from *Bifidobacterium adolescentis* (PDB: 2GDV). This enzyme is a member of the glycoside hydrolase GH family 13 and is known to catalyze the reversible phosphorolysis of sucrose into α-D-glucose-1-phosphate and D-fructose [1]. It can also importantly be applied as a transglucosylase *in vitro* when presented with alternative acceptor substrates like glucose (Figure 1) to regio-selectively form products like maltose, kojibiose, trehalose and nigeriose.

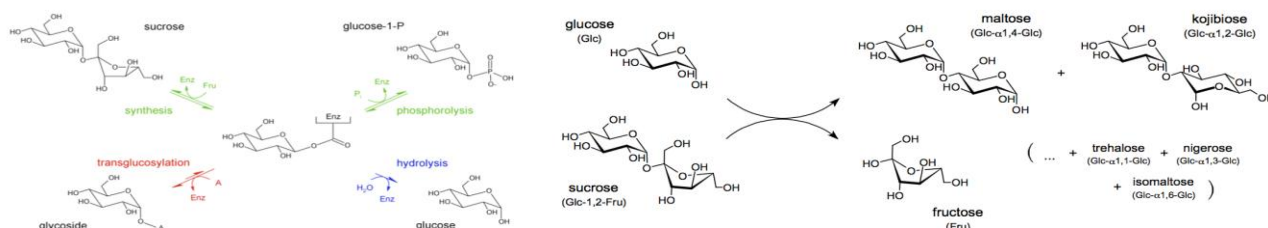

Figure 1. Reactions catalyzed by sucrose phosphorylase (adapted from [2]).

We were motivated to build theoretical models of some variants of sucrose phosphorylase from *Bifidobacterium adolescentis* [3] so as to provide a rational explanation for their observed switch in their transglucosylation regio-selectivity when compared to the wild type enzyme. Towards this end, we parameterized the residue topology for glucosylated aspartyl residues using both Charmm and Amber99sb-ILDN forcefields and introduced this new residue type within Modeller [4] and Gromacs [5] residue topology and parameter libraries. We then successfully used this new residue type to build homology models of covalent intermediates of variants of sucrose phosphorylase that were shown to change the regio-selectivity of the enzyme [3]. Docking studies were conducted on these variants and on the wild type enzyme using ß-D-glucose to simulate the entry of this acceptor in the +1 site. The preferred orientations of the ß-D-glucose in this site were compared and showed that the studied variants indeed displayed a preferred binding mode for the acceptor that could explain selectivity for kojibiose production. This work opens new perspectives for homology modelling of glucosylated forms of sucrose phosphorylase homologue's and of other GH families.

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DISRUPTIVE MIXED *IN VITRO-IN SILICO* APPROACH FOR PROTEIN ENGINEERING AND SCREENING*.

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* Patented by Peaccel (FTO)

We present a strategy that combines wet-lab experimentation and computational protein design for engineering polypeptide chains. The protein sequences were numerically coded and then processed using Fourier Transform (FT). Fourier coefficients were used to calculate the energy spectra called "protein spectrum". We use the protein spectrum to model the biological activity/fitness of protein from sequence data. We assume that the protein fitness (catalytic efficacy, thermostabilty, binding affinity, aggregation, stability…) is not purely local, but globally distributed over the linear sequence of the protein. Our patented method does not require any protein 3D structure information and find patterns that correlate with changes in protein activity (or fitness) upon amino acids residue substitutions. A minimal wet lab data sampled from mutation libraries (single or multiple points mutations) were used as learning data sets in heuristic approaches that were applied to build predictive models. We show the performance of the approach on designed libraries for 3 examples: enantioselectivity, thermostability and binding affinity. We can screen up to 1 billion (10^9) protein variants.

Session 3 : Système multienzymatique – cascade enzymatique /
Multi enzymatic systems

AMINOACYL-tRNA-UTILIZING ENZYMES INVOLVED IN NATURAL PRODUCT BIOSYNTHESIS

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Aminoacyl-tRNAs were long thought to be involved solely in ribosome-dependent protein synthesis and essential primary metabolism processes, such as targeted protein degradation and peptidoglycan synthesis. About 10 years ago, an aminoacyl-tRNA-dependent enzyme involved in the biosynthesis of the antibiotic valanimycin was discovered in a *Streptomyces* strain [1]. Far from being an isolated case, this discovery has been followed by the description of an increasing number of aminoacyl-tRNA-dependent enzymes involved in secondary metabolism.
I will describe the three groups of aminoacyl-tRNA-dependent enzymes involved in the synthesis of natural products [2]. Each group is characterized by a particular chemical reaction and its members are predicted to share a specific fold. The three groups are cyclodipeptide synthases involved in diketopiperazine synthesis[3-5], LanB-like dehydratases involved in the posttranslational modification of ribosomal peptides [6-9] and transferases from various biosynthesis pathways [1, 10-11].

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ONE-POT ENZYMATIC CASCADE: FROM STYRENE DERIVATIVES TO ENANTIOPURE PHENYL ALCOHOLS

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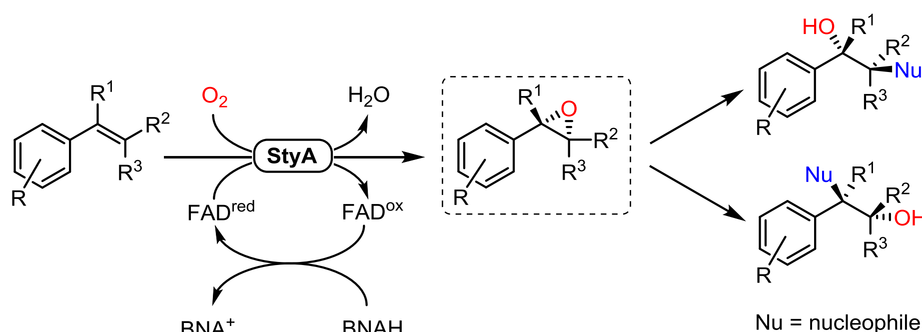
The flavoprotein styrene monooxygenase StyA can catalyse epoxidation and sulfoxidation reactions using molecular oxygen with excellent chemo- and enantioselectivity.[1-3] However, StyA uses the nicotinamide cofactor NADH as an electron donor to reduce the flavin (FAD) through a reductase (StyB). Herein we present the use of a synthetic nicotinamide cofactor, 1-benzyl-1,4-dihydronicotinamide (BNAH), to replace the native NADH/reductase system to reduce the FAD in solution. The StyA/BNAH system gave the same enzyme activity as with the natural StyA/NADH/reductase system, thus demonstrating a highly simplified electron transport to the enzyme.[4] A chemo-enzymatic cascade was also developed for the preparation of a variety of enantiopure phenyl alcohol derivatives. Thus, starting from styrene and employing different nucleophiles, the enantiomerically pure epoxide intermediate was transformed into the desired products (Scheme 1).



Scheme 1. Chemo-enzymatic cascade to produce enantiopure phenyl alcohols.

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Design of multidomain biocatalysts by fusing enzymes from the carotenoid pathway

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Metabolic engineering aims to modify or even design and implement metabolic pathways in living organisms to produce large quantities of high value compounds. Unfortunately, biotechnological processes are often limited by the low yields of product formed and there is a general need to improve, if possible in a predictable way, the efficiency of synthetic metabolic pathways. Amongst various possibilities, synthetic biology methods applied to enzyme engineering are particularly appealing (1). Spatial proximity promoted by various molecular organizations (proteins scaffolds recruiting catalytic domains or multidomain enzymes between enzymes)couldbe critical for metabolic efficiency. Several examples of strategies using fusion proteins or scaffolding methods have been reported in the literature (2,3).

Terpenoids are biological molecules that possess many industrial applications ranging from in medicine, food, feed etc. Carotene synthesis begins with the condensation of two molecules of Geranyl Geranyl di phosphate (GGPP) to give phytoene (C40) by an enzyme called phytoene synthase (CrtB). The four successive desaturation steps of phytoene performed by lycopene synthase (CrtI) produce lycopene. Finally, lycopene cyclase (CrtY) performs the cyclisation on both sides of lycopene to produceβ-carotene. Contrarily to most existing systems, the yeast *Xantophyllomyces dendrorhous* contains only two carotenoid enzymes: the phytoene synthase and the lycopene cyclase activities are carried by a bifunctional enzyme named CrtYB(4). The use of these *X. dendrorhous* enzyme have been described *S. cerevisiae*(5,6). However, despite overexpression of CrtI over CrtYB, there is a visible accumulation of phytoene and other intermediates(5). In order to further understand the efficiency of the CrtYB/CrtI function compared to the three enzyme system, we designed a study to address the role of spatial clustering in carotenogenic enzymes. We designed and created various multidomain proteins using all three carotenogenic enzymes, using linkers bearing different physico-chemical properties (length and dynamics). We tested these fusion enzymes in metabolic engineering situations, for the production of the various molecules present between GGPP and β-carotene.

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IMPROVING MOLECULE SYNTHESIS BY TUNING A MULTI-STEP ENZYMATIC CASCADE

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Enzymes can compensate for their lack of genericity by an extraordinary selectivity. This makes them specific tools for carrying out synthesis reactions under conditions particularly compatible with the environment preservation. However, the expression of enzymes in a heterologous host suffers from a flux imbalance due to the lack of natural regulatory mechanisms. One of the alternatives to circumvent this bias is to construct innovative biocatalysts capable of programming the stoichiometric equilibrium of these enzymes and thus optimize their synthesis and thus the production of the product of interest.

Baeyer-Villiger MonoOxygenases (BVMOs) are well known flavoenzymes able to transform efficiently ketone into ester or lactone with high regio-and stereoselectivities [1]. Nevertheless, BVMOs are strictly NADPH-dependent, and therefore require a stoichiometric amount of the expensive nicotinamide cofactor. To address this issue, the multi-enzyme syntheses provide the opportunity to generate efficient auto-sufficient systems and only a limited number of such systems involving BVMOs has been reported to date [2].
We will present here a new efficient access to molecule of interest like Dihydrocoumarin or Caprolactone, *via* a two-enzyme mediated oxidation of Indanol or Cyclohexanol, involving Alcohol Dehydrogenase (ADH) and BVMO (**Figure 1**). The originality of our approach comes from the features of ADH, an enzyme discovered *via* a dedicated High Throughput Screening. The ADH is a NADP-dependent and non-enantioselective enzyme which enables on the one hand cofactor recycling and on the other hand a complete transformation of the racemic alcohol. Moreover, it presents a quite large scope of substrates.

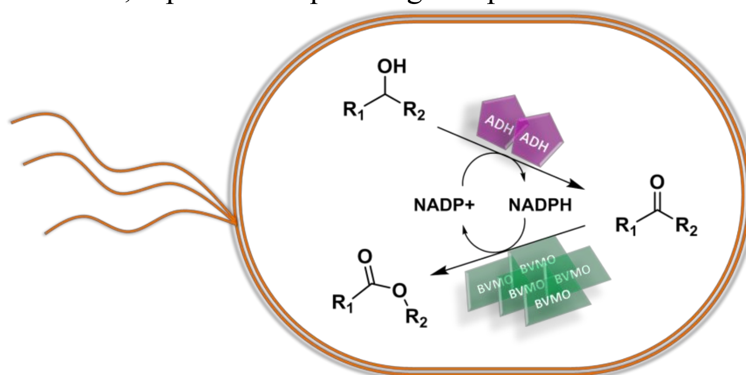

Figure 1. *In cellulo* enzymatic cascade, tuning stoichiometric equilibrium of enzymes.

Several plasmid constructions and combinations have been tested and compared in order to elaborate a versatile platform. For proof of concept we first use ADH and different BVMOs to optimize the formation of Dihydrocoumarin, and limit its hydrolysis. We investigated the biotransformation medium in whole-cell process as well as in purified enzyme system.

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ONE-POT, TWO-STEP CASCADE SYNTHESIS OF NATURALLY RARE L-*erythro* (3*S*, 4*S*) KETOSES BY COUPLING A THERMOSTABLE TRANSAMINASE AND TRANSKETOLASE

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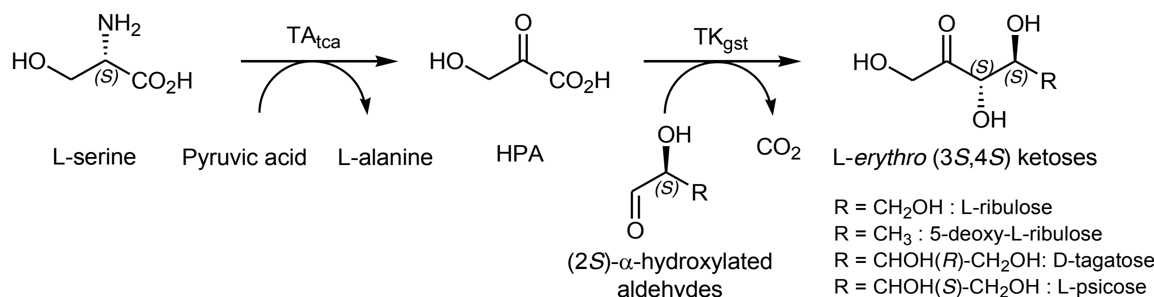
Rare monosaccharides display many applications in pharmaceutical, cosmetic, food and flavor industries. [1] The thermostable transketolase from *Geobacillus stearothermophilus* (TK_{gst}) is able to accept (2*S*)-α-hydroxylated aldehydes as acceptor substrate, unlike other reported mesophilic TKs, yielding naturally rare L-*erythro* (3*S*,4*S*) ketoses by stereospecifically C-C bond formation, in the presence of Li-HPA as donor substrate. [2] For synthetic purposes, the main problem of TK_{gst}-catalyzed reactions at high temperature is the limited stability of the artificial donor substrate Li-HPA. [3-4] We report on the identification and characterization of a novel thermostable serine-glyoxylic acid L-α-TA expressed from the thermophilic bacterium *Thermosinus carboxydivorans* DSM 14886 (TA_{tca}) and its use for the *in situ* biocatalyzed synthesis of HPA at high temperature, from natural L-serine and pyruvic acid. TA_{tca}-catalyzed reaction is shifted towards HPA synthesis by coupling to the irreversible TK_{gst}-catalyzed reaction in an efficient one-pot, two-step simultaneous cascade at high temperature [4] (Scheme 1).



Scheme 1. One-pot, two-step synthesis of L-*erythro* (3*S*,4*S*) ketoses catalyzed by TA_{tca} and TK_{gst} at 60°C from L-serine, pyruvic acid and (2*S*)-α-hydroxylated aldehydes

This biocatalytic approach is applied to the synthesis of highly valuable and naturally rare L-*erythro* (3*S*,4*S*)-ketoses, *i.e.* L-ribulose, 5-deoxy-L-ribulose, D-tagatose and L-psicose, obtained with excellent stereoselectivities and good yields, at 60°C. TK_{gst} activities towards (2*S*)-α-hydroxylated aldehydes, which are poor TK_{gst} substrates, were greatly enhanced by performing the reactions at high temperature, leading to excellent conversion rates within a reasonable time (24 h to 96 h). [4]

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INTEGRATIVE CHARACTERIZATION OF THE ALGINOLYTIC SYSTEM OF *ZOBELLIA GALACTANIVORANS*

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The marine flavobacterium *Zobellia galactanivorans* is a model organism for the bioconversion of algal polysaccharides [1]. Here, we focus on the degradation of alginate, the main cell wall polysaccharide from brown algae, which is widely used as gelling agent in industrial applications. *Z. galactanivorans* possesses seven putative alginate lyases (2 PL6, 3 PL7, 1 PL14 and 1 PL17). These genes are organized into two clusters, together with putative actors of alginate degradation. We have shown that these gene clusters can be transcribed within polycistronic mRNAs and thus form the first described genuine alginolytic operons. Several candidate enzymes were successfully overexpressed in *E. coli*, purified and their activity tested. Particularly, AlyA1, AlyA4, AlyA5 and AlyA7 are confirmed as active alginate lyases. Zg2622 and Zg2614 are a dehydrogenase and a kinase, respectively, further converting the terminal unsaturated monosaccharides released by alginate lyases into 2-keto-3-deoxy-6-phosphogluconate[2]. Furthermore we have determined the crystal structures of the catalytic module of AlyA1 ($AlyA1_{PL7}$) and of AlyA5 and characterized in depth their activity. $AlyA1_{PL7}$ is an endolytic guluronatelyase, and AlyA5 cleaves unsaturated units, α-L-guluronate orβ-D-manuronate residues, at the nonreducing end of oligo-alginates in an exolytic fashion. Despite a common jelly roll-fold, these striking differences of the mode of action are explained by a distinct active site topology, an open cleft in AlyA1PL7, whereas AlyA5 displays a pocket topology due to the presence of additional loops partially obstructing the catalytic groove[3]. Finally, we have recently developed genetic tools for *Z. galactanivorans*. These tools have been used to investigate the biological role of AlyA1, the only Z. galactanivorans alginate lyase known to be secreted in soluble form and to have a recognizable carbohydrate-binding domain. A deletion mutant, Δ*aly*A1, have grown as well as the wild type on soluble alginate but was deficient in soluble secreted alginate lyase activity and in digestion of and growth on alginate gels and algal tissues. Thus, AlyA1 appears to be essential for optimal attack of alginate in intact cell walls. *alyA*1 has been recently acquired via horizontal transfer from marine Actinobacteria, conferring an adaptive advantage that might benefit other algae associated bacteria by exposing new substrate niches[4].

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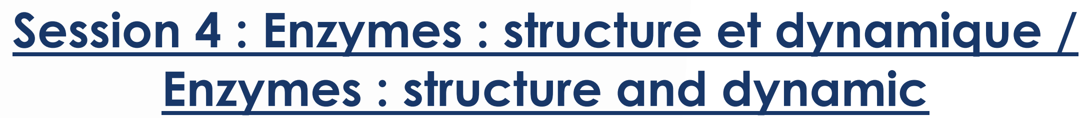

Session 4 : Enzymes : structure et dynamique / Enzymes : structure and dynamic

A Structural Perspective on the Evolution of Protein Functions

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Powerful tools for comparing protein structures and protein sequences have allowed us to analyse proteins from nearly 20,000 completed genomes and identify 2700 evolutionary domain superfamilies. These superfamilies cover nearly 70% of domains from all kingdoms of life and are captured in our resource (CATH-Gene3D). More detailed phylogenetic analyses of the highly populated superfamilies, accounting for nearly two thirds of all known domains, identified some particularly promiscuous superfamilies that can be traced back to the last universal common ancestor (LUCA) and in which relatives can diverge considerably to acquire modified structures and functions. Some structural frameworks seem particularly suited to supporting diverse residue arrangements in the active sites, and considerable structural variations on the surfaces of the domains. We also find a surprising number of examples of convergent evolution within a superfamily where very different catalytic machineries are associated with similar enzymatic chemistries, showing that these scaffolds enable multiple routes to the same function. Phylogenetic analyses of protein families can also yield insights into evolution of novel chemistries or substrate specificities and functional analyses can be combined with thermodynamic analyses to reveal the energetic considerations associated with functional divergence.

Jumping between protein conformers using normal modes

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The relationship between the normal modes of a protein and its functional conformational change has been studied for decades. However, using this relationship in a predictive context remains a challenge. In this work, we demonstrate that, starting from a given protein conformer, it is possible to generate in a single step model conformers that are less than one Angstrom away (RMSD) from the conformer which is the known endpoint of the conformational change, particularly when the conformational change is collective in nature. Such accurate model conformers can be generated by following either the so-called robust or the 50 lowest-frequency modes obtained with various Elastic Network Models (ENMs). Interestingly, the quality of many of these models compares well with actual crystal structures, as assessed by the ROSETTA scoring function and PROCHECK. The most accurate and best quality conformers obtained in the present study were generated by using the 50 lowest-frequency modes of an all-atom ENM. However, with less than ten robust modes, which are identified without any prior knowledge of the nature of the conformational change, nearly 90% of the motion described by the 50 lowest-frequency modes of a protein can be captured. Such results strongly suggest that exploring the robust modes of ENMs may prove efficient for sampling the functionally relevant conformational repertoire of many proteins [1].

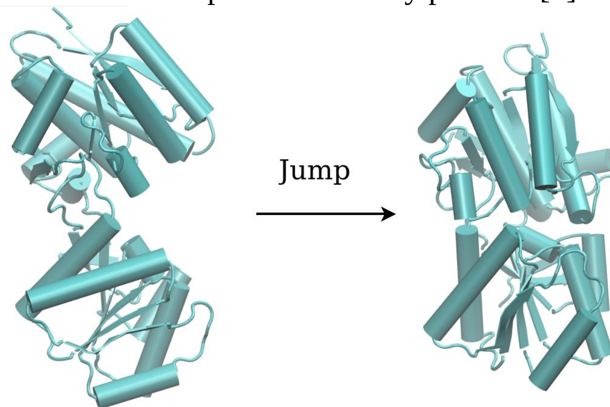

Figure 1. A single-step jump between two conformations of a ligand-binding protein.

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Diffusion of polysaccharide-degrading enzymes in complex substrates investigated by Synchrotron Soleil facilities

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The enzymatic conversion of polysaccharides in plant cell wall biomass is more and more investigated for the production of fine and bulk chemicals in place of the petroleum feedstock. The number of enzymes required for this degradation is huge due to the complexity of the polysaccharide structures and the high specificity of enzymes. The degradability of biomass is limited by the complex architecture of cell walls that is moreover very variable according to cell types and tissues.
In order to identify the bottlenecks limiting the enzyme action, the aim of this study was to investigate the affinity and the binding of the enzymes in relation to cell wall composition and therefore to cell types. For this purpose, methods are searched to observe together enzymes and cell wall composition before and during the enzymatic degradation.
At the Synchrotron SOLEIL DISCO beamline, the autoflorescence of tryptophan in the deep UV makes enzyme visible without any labelling. Using time lapse imaging, we investigated the diffusion of two different pectin methylesterases in a cell wall-mimicking matrix made of various proportions of cellulose and pectin. The mobility of the enzymes was recorded to understand how they diffuse with respect to the matrix structure. The matrix (micro) structure and the physical constraints entailed by its solid state were shown to impact the efficiency and the mode of action of the enzymes.
In the same conditions of excitation, phenolic compounds impart autofluorescence to cell walls, at a different emission range. Multispectral images can be recorded to observe both enzymes and cell walls during degradation. A microfluidic cell that enables localised Mid-Infrared spectra acquisition in a liquid medium was developed at the Synchrotron SOLEIL SMIS beamline. Using appropriate sample preparation, the biochemical evolution of polysaccharides in cell walls could be followed by recording time-lapse FT-IR spectra.
We took advantage of the two beamlines facilities to reveal enzyme localisation and to measure modifications in cell wall composition in different cell types in maize stem during degradation by a cellulase cocktail. Differences between cell types were evidenced. Enzymes were excluded from sclerenchyma cell walls that were consequently not degraded. In degraded parenchyma, enzymes were concentrated on the cell walls. A separation between adjacent cells followed by a complete degradation of cell walls was observed. A huge heterogeneity was observed within cell types. This heterogeneity could explain the global recalcitrance of maize stem cell walls to enzymatic degradation.

MATCHING THE DIVERSITY OF SULFATED BIOMOLECULES: CREATION OF A CLASSIFICATION DATABASE FOR SULFATASES REFLECTING THEIR SUBSTRATE SPECIFICITY

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Widespread in nature, sulfated biomolecules are highly diverse in chemical structure and biological function. Notably numerous sulfated polysaccharides and glycoconjugates occur in animals, plants, algae and bacteria. Sulfatases cleave sulfate groups from such sulfated molecules and constitute a biologically and industrially important group of enzymes. However, the number of sulfatases whose substrate has been characterized is limited in comparison to the huge diversity of sulfated compounds, yielding functional annotations of sulfatases particularly prone to flaws and misinterpretations. In the context of the explosion of genomic data, a classification system allowing a better prediction of substrate specificity and for setting the limit of functional annotations is urgently needed for sulfatases. Here, after an overview on the diversity of sulfated compounds and on the known sulfatases, we propose a classification database, SulfAtlas (http://abims.sb-roscoff.fr/sulfatlas/), based on sequence homology and composed of four families of sulfatases [1]. The formylglycine-dependent sulfatases, which constitute the largest family, are also divided by phylogenetic approach into 73 subfamilies, each subfamily corresponding to either a known specificity or to an uncharacterized substrate. SulfAtlas summarizes information about the different families of sulfatases. Within a family a web page displays the list of its subfamilies (when they exist) and the list of EC numbers. The family or subfamily page shows some descriptors and a table with all the UniProt accession numbers linked to the databases UniProt, ExplorEnz, and PDB.

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Session 5 : Enzymes cellulaires et métabolisme / Cellular enzymes and metabolism

THE CARBON MONOXIDE DEHYDROGENASE FROM *DESULFOVIBRIO VULGARIS* IS REQUIRED FOR ENERGY METABOLISM

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Carbon Monoxide dehydrogenases (CODH) are metalloenzymes that catalyze the reversible oxidation of CO to CO_2at a $NiFe_4S_4$ active site (Fig1). Some organisms use these enzymes to grow on carbon monoxide (CO) as their sole source of carbon and energy[1]. Usually, when CODH are used for energy conversion, they are in complex with a hydrogenase to form a proton motive force, necessary for ATP synthesis.
The anaerobic sulfur-reducing bacterium *Desulfovibrio vulgaris* (*Dv*)[2] lives by oxidizing organics acids, alcohols or hydrogen. This bacterium does not use CO as source of carbon nor energy but contains an operon encoding a CODH[3]. In this operon, this CODH is not associated genetically with a hydrogenase.Moreover, the gene is isolated genetically,thus its function remains unknown. Rajeev et al.,[4] have postulated that the CODH from *Dv* has a detoxifying role by removing exogenous CO from the growth medium. However, the fact that *Dv* is able to grow in presence of CO, even when the CODH gene has been deleted, means that CO is not a general metabolic inhibitor and that *Dv* CODH has another physiological function than detoxifying the environment.
We have investigated the physiological role of the CODH from *Dv* by studying different deletion mutants, and by transcriptomic and proteomic approaches. We have shown that *Dv* CODH has a different physiological function compared to the previously characterized CODH from other bacteria. *Dv* CODH confers physiological advantages in the absence of CO, it is required for energy metabolism and biomass production under certain conditions.

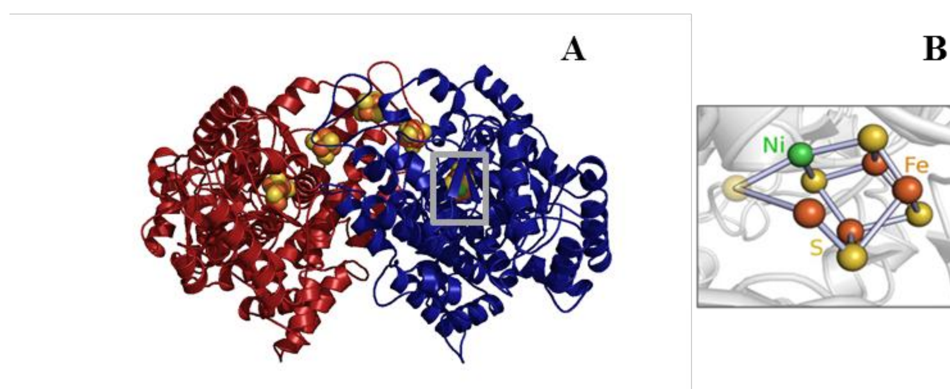

Figure 1.(A) Representation of CODH II from *Carboxydothermus hydrogenoformans*. (B) Structure of the active site of CODH II from *Carboxydothermus hydrogenoformans*.

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Discovery of L-serine succinyl-CoA transferases in L-cysteine biosynthesis

BessonnetT, MariageA, Petit JL, Dairii K, Bazire P, Salanoubat M, Bastard K, de Berardinis V, Perret A.

Until very recently, acetylation was the only known way for activating L-serine in the penultimate step of L-cysteine biosynthesis, in bacteria and fungi. This reaction is catalyzed by L-serine *O*-acetyl transferases (SAT), encoded by *cysE* and *srpH.* These enzymes belong to the transferase hexapeptide repeat family.Nevertheless, we demonstrated that L-serine is succinylated in *Schizosaccharomycespombe* by an enzyme unrelated to the classical SAT (Bastard and Perret et al., Nat Chem Bio, in press). This unique class of L-serine succinyl-CoA transferases (SST), called Cys2, is encoded by *metX* homologs whereas these latter are known to onlybeinvolvedin L-methionine biosynthesis. The conservation of Cys2 in fungi suggeststhat the biosynthesis of L-cysteine *via* the novel metabolite*O*-succinyl L-serine is a common treat in this kingdom. In contrast, few Cys2 homologs have been identified in bacteria,particularly in Xanthomonadales species. The phylogenetic proximity of these enzymes, along withthestructuralsimilarity of theiractive site suggest they share the same function.To support the *in vivo* role for *O*-succinyl-L-serine in Xanthomonadales, we kinetically characterized Cys2 from both*Xanthomonascampestris*and*Frateuriaaurantia*. Their catalytic efficiency (*i.e.*k_{cat}/K_m) is in favor of the formation of *O*-succinyl-L-serine. We also report that their L-cysteine synthase (the next enzyme in L-cysteine biosynthesis pathway) are actually *O*-succinyl-L-serine sulfhydrylases. This new metabolite was detected in the metabolome of *X. campestris*. Together, these results demonstratethe role for this novel metabolite in L-cysteine biosynthesis in bacteria too.

Session 6 : Biocatalyse / Biocatalysis

Oxyfunctionalization reactions – the next frontier of biocatalysis for organic synthesis

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Oxidoreductases are very promising catalysts for synthetic oxyfunctionalization chemistry as they often combine reactivity with selectivity to an extent often unachievable for 'traditional' chemical catalysts (Scheme 1).

Scheme 1. Selection of products obtainable through oxidoreductase-catalyzed redox biotransformations.

Nevertheless, compared to simple hydrolytic enzymes, oxidoreductases are fairly underrepresented as catalysts in organic synthesis. Possible reasons for this imbalance comprise among others (1) their **cofactor dependence** requiring additional efforts for *in situ* cofactor regeneration, (2) their dependence on **aqueous reaction media** and the resulting incompatibility with many hydrophobic reagents.

During this presentation I will discuss the challenges mentioned above and present some possible solutions, which eventually will make oxidoreductases truly practical catalysts for organic synthesis.

RESUMÉ: Les lipides constituent une grande partie de la biomasse de la terre et les enzymes lipolytiques jouent un rôle important dans la transformation de ces composés insolubles dans l'eau. Les lipases microbiennes ne sont pas comparables aux lipases d'origine végétale et animale en termes d'activité, rendement, facilité de purification, modifications moléculaires et stabilité. L'environnement où ces micro-organismes sont isolés confère certaines caractéristiques à ces enzymes. *Staphylococcus aureus* isolée à partir d'un biotope algérien sécrète dans le milieu de culture une lipase non-induite. La Lipase de *S. aureus* nommée (SAL) a été purifiée à homogénéité. La SAL pure est une protéine monomérique possèdant une masse moléculaire apparente d'environ 43 kDa. Le séquençage de 20 résidus d'acides amines de l'extrémité NH_2-terminale a révélé un degré d'homologie élévé comparée aux autres lipases de Satphylocoques. La SAL présente des activités spécifiques d'environ 1600 U/mg ou de 555 U/mg en utilisant la tributyrine et l'émulsion d'huile d'olive comme substrats, respectivement. Contrairement à d'autres lipases Staphylococciques précédemment caractérisées, la SAL est stable à une gamme de pH de 6 à 9 après 1h d' incubation et maintient 50% de son activité après 10 minutes d'incubation à 50°C. L'enzyme purifiée a été également caractérisée en utilisant la technique de couche monomoléculaire. L'activité de lipase peut être mesurée seulement quand la pression de surface atteint 15 mN/m. Les résultats obtenus montrent que la SAL hydrolyse préférentiellement les diacylglycérols à groupements esters adjacents à hautes pressions et les diacylglycérols à groupements esters distals à faible pression et une préférence pour la position sn-3 du *2,3-sn*-dicaprine. Le pouvoir de pénétration (π_c) de la SAL mesuré sur film monomléculaire d'Egg-PC est de 33mN/m. La séquence NH2-terminale de la SAL présente une forte homologie avec une lipase de la même souche nommée SAL 3 .Une délétion de 3 résidus (LKA) du côté NH2-terminale est observée dans la séquence de la SAL avec substitution de la glycine 208 et l'isoleucine 226 par une arginine et une leucine respectivement.

Mots clés: Lipases de *Staphylococcus aureus*, pH-stat, Baro-stat, Régioséléctivité, stéréosélectivité.

SUGAR AND GLYCOSIDIC BOND EFFECT ON TYROSINASE AND MELANIN TRANSFERT INHIBITION

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The overproduction of melanin causes many skin disorders. Tyrosinase is responsible for melanin production and the inhibition of its activity is still the most rational way of reducing these troubles. A large range of small molecules has been tested including Kojic acid which is a hydroquinone derivative, and arbutin, the carbohydrate reference compound [1].

One of our challenge is to produce arbutin analogues using environmentally benign methods and especially to study some structure function relationships. Therefore both *O*-glyconjugate and *S*-glycoconjugate series were produced to study the influence of glycosidic linkage and carbohydrate scaffold on tyrosinase inhibition. For this purpose we firstly developed a new and efficient method for nitroarene reduction, assisted by microwave irradiation, using thiophenol catalysis and dithiothreitol (DTT) [2].

Secondly, thanks to protein engineering we produced new biocatalysts designed for the synthesis of thioglycosides [3]. Directed mutagenesis on the acid/base residue of the wild type glycosidase DtGly [4], from *Dictyoglomus thermophilum*, permitted to reduce hydrolysis properties favoring thioglycosylation reaction. Thanks to the versatility of the enzyme different thioglycosides were prepared i.e. glucose, galactose and fucose series.

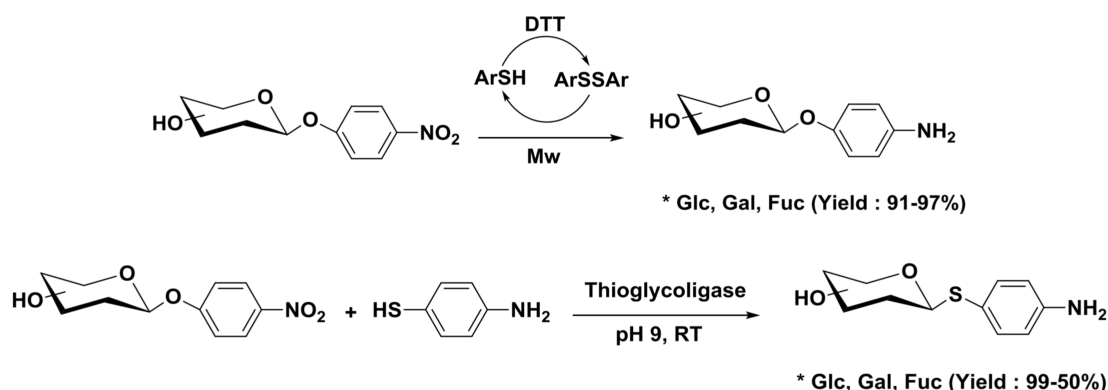

Figure 1: Synthetic strategies of arbutin analogue

All compounds were tested for lectine recognition, tyrosinase and melanogenesis inhibitions and toxicity. Our results in this particular field will be presented and compared to commercial references.

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LIPASE-CATALYZED PRODUCTION OF LYSOPHOSPHOLIPIDS

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Lysoglycerophospholipids (LPLs) are glycerol-based lipids containing one fatty acyl moiety at either the *sn*1 or *sn*2 position and a phosphate group at the *sn*3 position. Except for lysophosphatidic acid (LPA), the phosphate group is esterified to an alcohol or amino-alcohol (mainly choline, ethanolamine, inositol and serine) leading to various LPL species, such as lysophosphatidylcholine (LPC). LPC isomer structures are shown in Figure 1.

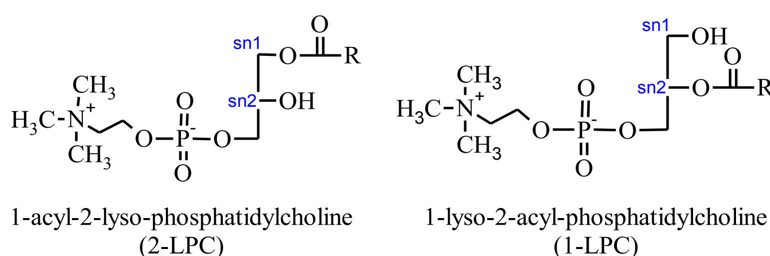

Figure 1. *Structures of lysophosphatidylcholine isomers. (R = acyl chain)*

LPLs are essential bioactive lipids involved in a large variety of both normal and pathological processes such as carcinogenesis, neurogenesis, immunity, vascular development or regulation of metabolic diseases. They also have industrial and pharmaceutical uses such as emulsifiers or components of drug delivery systems.

Lipases (triacylglycerol acyl hydrolases, E.C.3.1.1.3) are ubiquitous enzymes widely distributed among animal, plant and microbial kingdoms. Their natural role is to catalyze the hydrolysis of ester bonds in long chain triacylglycerols. Interestingly, in low aqueous conditions, such as in organic solvent, microbial lipases also efficiently catalyze the reverse reaction, *i.e*. ester bond synthesis. Moreover they display broad substrate specificity. All these reasons make lipases one of the most widespread biocatalysts used in biotechnological applications. Particularly, lipases are widely used in the field of enzymatic modifications of phospholipids.

Thispresentation will describe the various lipase-catalyzed reactions implemented for the production of lysophospholipids. They include hydrolysis or alcoholysis of phospholipids and acylation of the glycerophosphoryl moiety. Special emphasis will be made on our work dealing with the production of lysophospholipids rich in dososahexaenoic acid (DHA, C22:6 ω3), an important dietary polyunsaturated fatty acid *via*the selective hydrolysis of phospholipids extracted from the microalgae *Isochrysisgalbana*[1,2].

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BREAKING THE DOGMA OF ALDOLASE SPECIFICITY: SIMPLE ALIPHATIC KETONES AND ALDEHYDE ARE NUCLEOPHILES FOR FRUCTOSE-6-PHOSPHATE ALDOLASE

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Depuis sa découverte en 2001 par l'équipe allemande du professeur Sprenger[1], la fructose-6-phosphate aldolase (FSA) ne cesse de surprendre de par sa grande tolérance vis-à-vis de son substrat donneur. En effet, les aldolases sont des enzymes qui permettent la formation stéréosélective d'une liaison C-C entre un aldéhyde qualifié de substrat accepteur et une cétone qualifiée de donneur conduisant à des cétoses. Or ces biocatalyseurs sont connus pour être totalement spécifiques de leur substrat donneur. Cette spécificité limite souvent leur application en synthèse, inconvénient tout de même compensé par leur grande tolérance vis-à-vis de l'accepteur. La FSA dans ce contexte trouve toute son originalité. En effet, à l'heure actuelle quatre substrats donneurs sont décrits[2],[3] dans la littérature illustrant la grande capacité catalytique de cette enzyme. De façon exceptionnelle chez les aldolases, cette enzyme accepte parmi ses donneurs un aldéhyde, le glycolaldéhyde[4], permettant ainsi l'accès également à des aldoses. Ces donneurs ont tous en commun la présence d'un groupement hydroxyle sur le carbone adjacent à la fonction cétone.

Les travaux présentés ici concernent une exploration approfondie du potentiel synthétique de cette aldolase en testant de nouveaux donneurs non hydroxylés tels que l'acétone. Tout le travail analytique réalisé ainsi que les synthèses et les déterminations structurales des nouveaux composés obtenus seront détaillés. Ils illustrent une avancée majeure dans le domaine des aldolisations catalysées par des enzymes.

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ENZYMES TO RETHINK THE LIFECYCLE OF PLASTICS

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Representative of our consumer society, plastics have gradually invaded our everyday life, becoming progressively unavoidable in many industrial fields. After several decades of intensive exploitation and accumulation of plastic in our environment, a collective consciousness has finally emerged as to the need to eco-friendly produce and consume.

One of the objectives of the Thanaplast™ project, launched in 2012 and led by CARBIOS, a firm specialized in enzymatic bioprocesses applied to polymers in the fields of plastics and textiles, is to trigger the biodegradation of plastics and more particularly of PLA (Poly Lactic Acid, [1]), depending on the lifetime required for their use and with respect of the environment. The particularly innovative concept of this project relies on the inclusion in the plastic material of enzymes capable of degrading this polymer into organic compounds that can be further assimilated by microorganisms present in nature.

The Catalysis and Molecular Enzymatic Engineering team from the LISBP (INSA Toulouse) identified an enzyme with remarkable catalytic properties enabling to degrade PLA very efficiently. The main challenge was to keep the catalytic activity after its inclusion into the PLA during the extrusion phase at very high temperature (~ 170 ° C). This enzyme was produced recombinantly, and then optimized for activity and thermostability by enzymatic engineering. In parallel, its formulation has also been optimized, all of which making it possible, *in fine*, to take on the challenge: validate the biodegradation of this enzymated plastic, which is complete within a few weeks or a few months according to the time period desired. Furthermore, the enzymated plastic retains the properties of polymers, and do not require any modification of industrial tools for plastic processing.

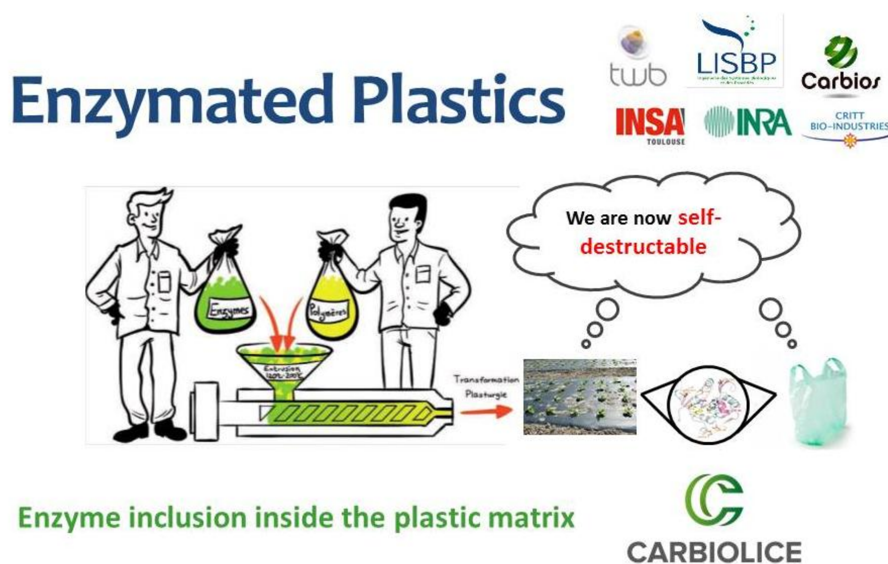

Figure 1. The innovative concept of this project relies on the inclusion in the plastic material of enzymes capable of degrading this polymer

This innovative enzymatic process led to the creation, on the 1st of September 2016, of the Joint Venture CARBIOLICE between CARBIOS, LimagrainCéréales Ingredients (subsidiary of Limagrain, the 3rd largest seed group in the world) and the SPI funds operated by Bpi-France.

Two markets subject to new regulatory laws in France and Europe will be covered in priority by CARBIOLICE: the market for bags, and particularly fruit and vegetable bags, which reaches almost 20 million tons in 2012, and that of agricultural mulching films, estimated at 2 million tons in 2013and with an average growth rate of 10% in Europe in the biodegradable segment.

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Prix Nicloux : Martin Picard

Functional investigation of the MexA / MexB / OprM multidrug efflux pump from *Pseudomonas aeruginosa*

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Among the various mechanisms developed by Gram-negative bacteria to counter to the effect of antibiotics, active efflux is on the front line. In *Pseudomonas aeruginosa*, the constitutive efflux pump is organized as a multicomponent system where MexB, located in the inner membrane, works in conjunction with MexA, a periplasmic protein, and OprM, an outer membrane protein. MexB acts as a proton motive force-dependent pump with broad substrate specificity. We have developed innovative *in vitro* procedures allowing to monitor the transport and the assembly of the efflux pump.

We first present a procedure where the protein partners are reconstituted into respective proteoliposomes that allow mimicking the two-membrane architecture of a Gram negative bacteria. By use of relevant fluorescent probes, we monitor transport of ethidium bromide through the reconstituted pump. We show that OprM needs to interact with MexA and MexB in order to open, a result in accordance with previous models, and that MexB activity is accelerated when the pump is assembled.

We have also designed a new test that allows investigating the assembly state of the pump[2]. The method relies on the streptavidin-mediated pull-down of OprM proteoliposomes upon interaction with MexAB proteoliposomes containing a biotin function carried by lipids. We give clear evidence for the importance of MexA in promoting and stabilizing the assembly of the MexAB-OprM complex. In addition, we have investigated the effect of the role of the lipid anchor of MexA as well as the role of the proton motive force on the assembly and disassembly of the efflux pump.

Finally, we present a single-particle analysis by electron microscopy of the native efflux pump after its reconstitution in a lipid nanodisc system[3].

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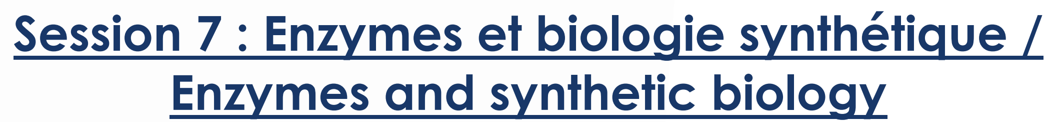

Session 7 : Enzymes et biologie synthétique / Enzymes and synthetic biology

ARTIFICIAL METABOLIC PATHWAYS FOR BIO-BASED ISOBUTENE

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As of today, most industrial bio-processes are based on naturally occurring metabolic pathways, preventing the access to many of the chemistry's largest market. For example, the development of bio-processes for the efficient production of light olefins remains a technological challenge. Since these molecules are not synthesized by natural microorganisms, the design of a complete metabolic pathway for their production is hampered by the lack of identified enzymes able to perform the crucial reaction step.

The purpose of Global Bioenergies is to develop innovative metabolic routes for the bio-production of light olefins, with isobutene being the first target. In order to bridge the gap between natural metabolites and the final product, Global Bioenergies has engineered artificial biocatalysts, and combined them with natural enzymes into metabolic pathways. Thus, the new metabolic routes leading to isobutene involve non-naturally occurring reactions and non-canonical metabolic intermediates.

The scale-up of this bio-based isobutene production process is currently ongoing. Our first pilot plant is running since 2014 in Pomacle (France), with a capacity of 10 tons/year of oxidation-grade isobutene. Several samples of bio-based isobutene or isooctane derived from bio-isobutene have been delivered to Arkema, Arlanxeo, Audi and to CFBP (a French organization representing gas supply companies), and the process yield has now reached more than 70% of target commercial yield at pilot scale. The construction of the Leuna demo plant (Germany) with a capacity of 100 tons/year of polymer-grade isobutene is now completed.

FUNGAL CELL WALL DEGRADING ENZYMES DIVERSITY AND ADAPTABILITY TO DIFFERENT BIOMASSES

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We are currently livinga societal transition started since the Earth Summit of Rio in 1992.The economy is relatively slow but inevitably changing, with entire sectors moving from petrobased to biobased. In this context, the concepts of sustainable development, bioeconomy and bioraffineries are promoted and supported by intergovernmental organizations, states, enterprises, scientists and populations.
One of the challenge to be raised is the efficient use of the plant biomass to yield products for health, chemistry and energy able to compete with petrobased ones. Indeed, plant biomass i.e. plant cell wall is a complex structure of polymers synthetized to be resistant.Actually, plant cell wall is recalcitrant to physical, chemical and biological treatments.
Filamentous fungi especially pathogens and saprophytes have evolved in close contact with plants and have developed powerful mechanisms to digest plant cell wall. The goal of this talk is to discuss the diversity of the cell wall degrading enzymes secreted by filamentous fungi with different approaches: genomics, transcriptomics and proteomics. Majors finding are the following. (i) The genome of these fungi possess many gene encoding Cell Wall Degrading Enzymes (CWDE) putatively able to degrade many fractions of plant cell wall. (ii) The fungi react at the presence of plant cell wall by expressing these genes differentially. (iii) The cocktails of CWDE secreted by a given fungi depend greatly of the biomass they are in contact with.
Taken together, these facts indicated that a given couple fungus/biomass produce a specific cocktail, different from another one.
The efficiency of biomass digestion and valorization could then been optimized by the screening of the more efficient cocktails for a given application.

Triazolo- and azaGly-peptidomimetics: a new generation of potent reversible substrate-like inhibitors of cysteine proteases, cathepsins K and S

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Human cathepsins (Cat) K and S are cysteine proteases (C1 family) that are primarily involved in the degradation of endocytosed proteins [1]. They participate also in specialized processes including processing of thyroid hormones and neuropeptides, maturation of MHC class II molecules or matrix remodeling. Their overexpression and/or dysregulation contribute to a broad spectrum of disorders (e.g. carcinogenesis, osteoporosis, atherosclerosis, emphysema, asthma) making them attractive targets for the development of new therapies.

A valuable strategy for developing reversible and competitive inhibitors is to insert non-cleavable bonds at the scissile P1-P1' position of selective substrates of Cat S and Cat K respectively. Accordingly we introduced a 1,2,3-triazole bond that mimics most of features of the *trans*-amide bond, and can likewise participate in hydrogen bonding and dipole–dipole interactions [2]. Alternatively, we incorporated an azaGly bond by replacing the alpha carbon of the glycyl residue at P1 by a nitrogen atom.

AzaGly-containing peptides inhibited powerfully their respective target proteases in the nanomolar range, while triazolo substrate-derived peptides were weaker inhibitors (Ki in the micromolar range). Their inhibitory potency was further evaluated on selected experimental models, and their selectivity was examined using cell lysates from wild-type mice *vs* deficient-Cat S and deficient-Cat K mice. Bioinformatics studies supported that the AzaGly surrogate is more favorable than the 1,2,3-triazole bond to sustain both a suitable selectivity and an effective affinity between substrate-derived inhibitors and protease targets.

Taken together with a previous report [3], present data suggest that the incorporation of an aza bond at P1-P1' could be extended to design potent and reversible inhibitors of other classes of proteases.

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Session 8 : Glyco-enzymologie / Glycoenzymology

Enzyme engineering for the conversion of sucrose into products with high added value

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Abstract

Sucrose is a very attractive resource for biotechnological transformations, since it is cheap, abundant, reactive and easy to use. Although it primarily serves as feedstock for fermentation, several options also exist for its biocatalytic conversion into products with high added value. Indeed, through the design of a dedicated sucrose transglycosidase, we were able to develop a cost-efficient production process for the rare sugar kojibiose (1). This alpha-1,2-glucobiose shows potential as low-caloric and non-cariogenic sweetener but its large-scale application has previously been hampered by an exuberant price. In turn, by exploiting an optimized sucrose phosphorylase in a two-step process, a whole range of novel anti-oxidant glycosides could be synthesized in gram amounts (2-3). These products display improved solubility and stability, which should be beneficial for use in food or cosmetic formulations. As a third option, an engineered sucrose synthase was combined with a glycosyltransferase for the selective glucosylation of various small molecules in very high yields (4-5). The glycosyltransferase from stevia (*Stevia rebaudiana*) has attracted a lot of attention recently but was now found to be much more promiscuous in acceptor specificity than generally assumed. All of these examples will be discussed with respect to scientific challenges, technological solutions and potential applications.

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Improvement of the versatility of an Arabinofuranosidase against galactofuranose, for the synthesis of galactofuranoconjugates

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Galactofuranoconjugates are xenobiotic in mammals but particularly abundant in the glycocalyx of many microorganisms like mycobacteria or *Leishmania* responsible of deadly infections like tuberculosis or leishmaniosis. Such scaffolds constitute an original feature for the development of anti-parasitic compounds which may have a specific action in their biosynthesis, catabolism or degradation. Already some alkyl *O*- and *S*-galactofuranosides have exhibited good bacteriostatic activities against strains of mycobacteria and *Leishmania* as well as for the activation of the immune system. [1][2]
Given the difficulty of the synthesis of *O*- and *S*-galactofuranoconjugates by glyco-chemistry, we have explored an alternative pathway of *O*- and *S*-glycosylation biocatalyzed by a glycosylhydrolase. The α-L-arabinofuranosidase of *Clostridium thermocellum* (*Ct*Araf51) was first selected as the native hydrolase because the β-D-galactofuranosyl (D-Gal*f*) motif presents some promiscuity with the α-L-arabinofuranosyl motif (L-Ara*f*). However native *Ct*Araf51 showed a very low affinity for β-D-galactofuranosyl donor. The aims of this study were then to tailor its affinity for D-Gal*f* thanks to rationalized mutations (Figure 1).

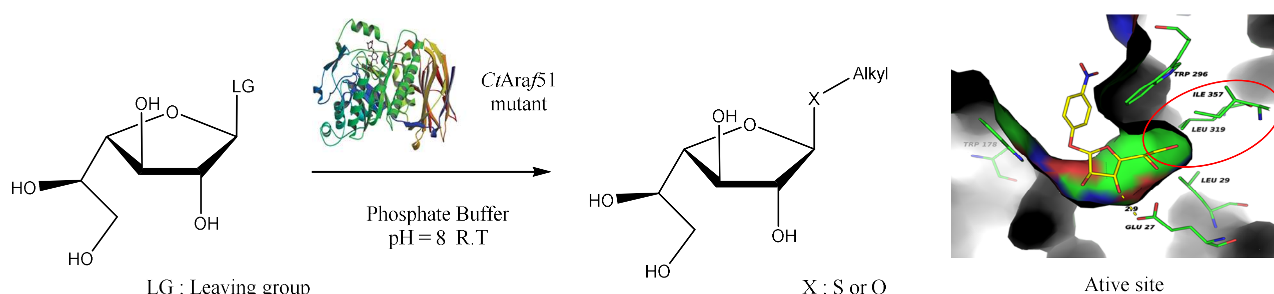

Figure 1. Optimization of the activity and the affinity of *Ct*Ara*f*51 against furanosyl donors

Docking studies highlighted the existence of a hydrophobic pocket surrounding the 5-hydroxymethyl substituent of aryl galactofuranoside. Two amino-acids in this pocket were identified as steric hindrance for the hydroxymethyl and were subsequently mutated. The resulting mutants showed an increased affinity with an improvement in the Km value. The mutant with the lower Km value for aryl galactofuranoside will then be further evolved for *O*- and *S*-glycosylation.

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Galactofuranosyltransferases of Leishmania?

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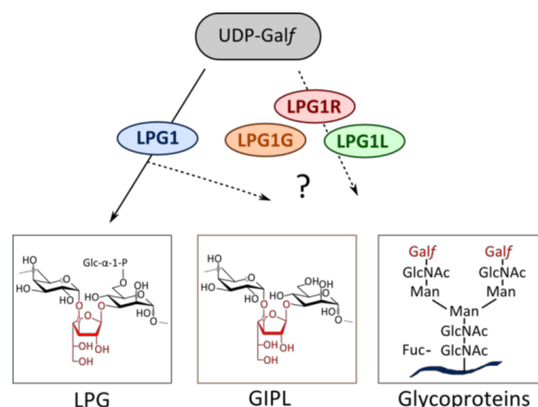
Glycoconjugates at the surface of leishmanian cells play a crucial role in cell recognition, infection and survival of parasite within insect and mammalian host.[1] Interestingly, these structures contain in their core a rare five-membered ring form sugar, galactofuranose (Gal*f*), which is involved in parasite virulence.[2]

Galactofuranosyltransferases (Gal*f*T), enzymes which are responsible for Gal*f* incorporation in glycoconjugates such as lipophosphoglycans (LPGs) and glycosylinositolphospholipids (GIPLs) raised our interest. Four putative genes encoding for Gal*f*T namely *lpg1, lpg1L, lpg1R and lpg1G* were identified on L. *major* genome.[3] Thus, the main goal of this research is to express and characterize these enzymes, to gain knowledge about their biological function and implication in leishmaniasis.

Eukaryotic and prokaryotic expression systems were used to produce our proteins of interest. Then different purification procedures were used to get soluble recombinant enzymes. Kinetic characterization indicates that these enzymes are active and recognize different sugar donors. These encouraging results will be presented and described.



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Posters

TRIFLUOROSUBSTRATES ASMECANISTIC PROBES FOR A FAMILY OF FLAVODEHYDROGENASES

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The family of FMN-dependentL-2-hydroxyacid-oxidizing enzymes comprises several well characterized members, such as L-lactate oxidase, glycolate oxidase (GOX), long chain hydroxy acid oxidase, mandelate dehydrogenase, as well as flavocytochrome b_2 (Fcb2), an L-lactate dehydrogenase with an additional heme domain attached to the flavodehydrogenase domain (FDH). Their crystal structures show a conserved TIM barrel with a highly conserved active site and a mobile loop which appears to play a role in catsalysis. Thus, family members must use the same chemical mechanism for substrate oxidation. Substrate modelling in the Fcb2 active site suggests that the active-site base, a histidine, could either abstract the hydroxyl proton, thus leading to a hydride transfer, or abstract the C2 proton, with formation of an intermediate carbanion, which would yield electrons to the flavin.Nevertheless, neither the 3D structures, nor site-directed mutagenesis, nor theoretical calculations allow an unambiguous mechanistic conclusion. We have published incontrovertible evidence against the hydride transfer mechanism [1]. Nevertheless some authors keep advocating this mechanism [2]. We report here experiments with fluorinated substrate analogues, namely a study of the kinetics of trifluorolactate (F3Lac) oxidation and of trifluoropyruvate (F3Pyr) reduction by the Fcb2 FDH domain and by GOX, as well as with anNAD^+-linked LDH. The fluorine atoms should stabilize a carbanion and make a hydride transfer difficult. Indeed, it has been shown before that F3Lac is an inhibitor and not a substrate for an NAD-linked LDH [3]. We compare the reactivity changes between normal and trifluoro substrates for Fcb2, GOX and an NAD^+-linked LDH. Altogether, our results cannot be rationalized by a hydride transfer mechanism for the FMN-dependent enzymes.

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NEW ENZYMATIC SYNTHESIS OF METHYLMERCAPTAN FROM DIMETHYLDISULFIDE

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Methionine is a sulfur-containing amino acid and used in agri-food industry asdietary complement in animal feeds. Studies showed that the use of this molecule allowed a better animals growth as for exampleschicken, pork or shrimps [1]. Industrially, the mainly way to produce methionine is a chemical multistep synthesis, non-stereoselective and using toxic and corrosive intermediates likecyanhydric acid [2]. Recently, CheilJedang (CJ) and Arkema developed the first enantioselective synthesis of L-methionine at industrial scale using only three steps [3][4]. Two steps are come from the biological world: one step is a fermentation, the other is a biocatalytic step. The third one is a chemical synthesis of methylmercaptan which is ahazardous chemical with highly controlled transport.

Regarding to the coming years, the market of methionine will increase and new methionine plants will be built. The chemo-enzymatic synthesis developed by CJ and Arkema must not be a goal by itself and efforts must be done to go further in the green chemistry processes. Trying to follow this way, Arkema set up and developed a biocatalytic process performing the reduction of dimethyldisulfide (DMDS) to two MeSH molecules [5][6]. DMDS is a stable molecule, transportable and its synthesis could be done in the existing chemical plant. The new reduction way of DMDS could be an interesting alternative to produce MeSH, which could be used in the methionine synthesis but although as an intermediate or substrate in many processes with different final applications [7][8].

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ENGINEERING A THERMOSTABLE TRANSKETOLASE FROM *GEOBACILLUS STEAROTHERMOPHILUS* BY SITE-SATURATION MUTAGENESIS TO BROADEN ITS SUBSTRATES SCOPE

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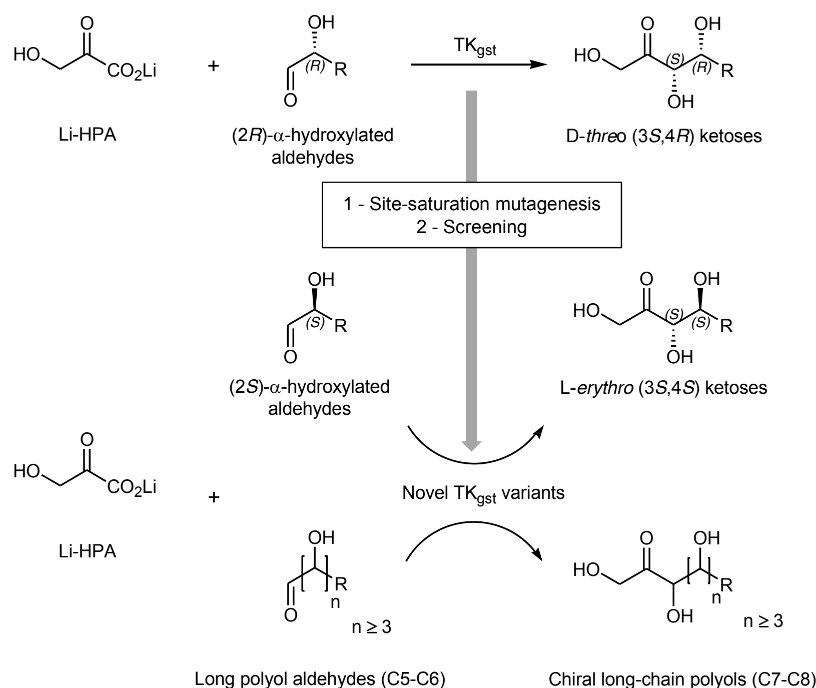
Enzymes must demonstrate enhanced stability and high specific activity towards substrates of interest to achieve industrial feasibility in large-scale processes. With a view to enlarge the substrate scope of the thermostable transketolase from *Geobacillus stearothermophilus* (TK_{gst}), site-saturation mutagenesis is used as a tool to generate new TK_{gst} variants libraries. Key amino acids of TK_{gst} active site are modified and the libraries are screened with a specific pH-based high-throughput assay. [1] The improvement of TK_{gst} activity towards (2*S*)-α-hydroxylated aldehydes [2] and nonphosphorylated long polyol aldehydes (C5-C6), which are TK_{gst} poor acceptor substrates, are current challenges, in order to prepare efficiently, in the presence of Li-HPA as donor substrate, (3*S*, 4*S*) ketoses and chiral long-chain polyols (C7-C8), respectively, highly valuable compounds as low-calorie sweetener, active pharmaceutical ingredients or chiral building blocks [3] (Scheme 1). The construction and the screening of TK_{gst} variants as well as the characterization of the most performing TK_{gst} variants will be presented.



Scheme 1. Enhancement of TK_{gst} activity towards (2*S*)-α-hydroxylated and long polyol aldehydes by site-saturation mutagenesis

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The catalytic mechanism of aminopeptidase B: role of Tyrosine residues conserved within the M1 family of Zn^{2+} metallopeptidases.

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Aminopeptidase B (Ap-B) is an enzyme of the M1 family of Zn^{2+} aminopeptidases that hydrolyzes the peptide bond on the carbonyl side of the basic residues present at the NH_2-terminal end of peptides. The overall physiological functions of this enzyme remain to be discovered. *In vivo*, Ap-B is involved in the proteolytic processing of glucagon and cholecystokinin-9 to give miniglucagon that participates in glucose homeostasis, and cholecystokinin 8, a pleiotropic neurotransmitter. Members of the M1 family are involved in many important physiological functions and hydrolyze many different substrates. Since these enzymes have similar catalytic mechanisms, their substrate specificity and/or catalytic efficiency should be based on subtle structural differences at or near the catalytic site. This leads to the hypothesis that these proteins possess a common structural skeleton, strictly necessary for aminopeptidase activity. A specific amino acids environment located in/or outside the catalytic pocket would determine the substrate specificity and the catalytic efficiency of each enzyme.

A multiple alignment of M1 family aminopeptidase sequences from vertebrate expressed proteins allowed the identification of conserved tyrosines that participate to this catalytic skeleton. Molecular modeling and site-directed mutagenesis approaches were used to specify the role of five partially (Y_{409}) or fully conserved tyrosine residues (Y_{281}, Y_{229}, Y_{414} and Y_{441}) in the Ap-B catalytic mechanism. Tyrosine mutations into phenylalanine confirmed the influence of hydroxyl groups for the enzymatic activity. These groups are involved in the catalytic mechanism (Y_{414}), the substrate specificity and/or the catalytic efficiency (Y_{409}), in the stabilization of amino acids of the active site (Y_{229}, Y_{409}) and potentially in the maintenance of the structural integrity (Y_{281}, Y_{441}). The importance of hydrogen bonds is confirmed by the $Y_{229}H$ mutation, which preserves the enzymatic activity. These data provide new information on the catalytic mechanism of Ap-B within the M1 family of aminopeptidases.

UNRAVELLING THE MECHANISM OF SULFUR TRANSFER CATALYZED BY THE 3-MERCAPTOPYRUVATE SULFURTRANSFERASES

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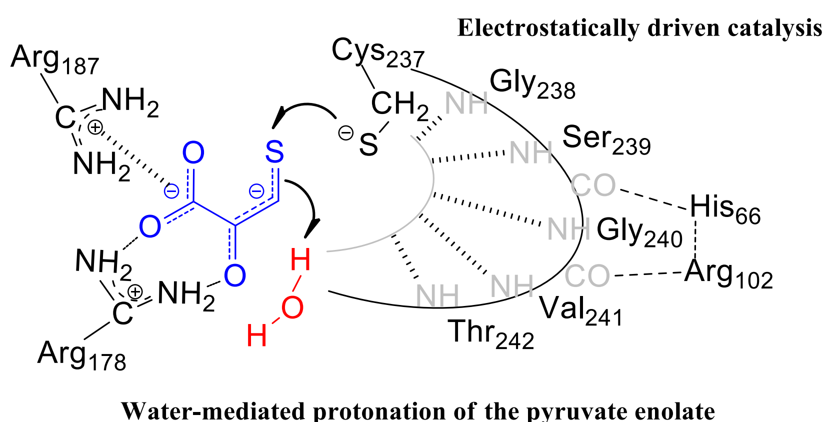
Over the past decade, increasing attention has been paid to cysteine persulfides formed in proteins due to the role ascribed to these entities in hydrogen sulfide (H_2S) signaling pathways [1]. *In vivo*, their formation is mainly achieved by two enzymes, among which the 3-mercaptopyruvate sulfurtransferases (3-MSTs). The ubiquitous 3-MSTs belong to the rhodanese family and catalyze the transfer of a sulfur atom from 3-mercaptopyruvate to a thiol acceptor leading to the formation of H_2S. In the X-ray structures available to date, the catalytic cysteine is mainly present at the persulfide state that is thought to be a key intermediate in the 3-MST-catalyzed reaction [2, 3]. Nonetheless, the catalytic relevance of the persulfide intermediate in the 3-MST catalytic pathway remains to be clearly established and the way by which its formation is catalyzed remains to be elucidated.

To address this gap in knowledge, kinetic approaches permitting the study of each step of the reaction (fluorescence, stopped-flow, quenched-flow, H_2S specific probe) were developed. The results show that: 1) the so-called Ser239/His66/Asp53 triad is not directly involved in catalysis, in contrast to what is proposed in the literature [2,3], 2) the formation of the persulfide intermediate is very rapid and is mainly driven by electrostatic contributions provided by the catalytic loop, 3) a water molecule is required to protonate the pyruvate enolate.



Water-mediated protonation of the pyruvate enolate

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REDOX SIGNALING : INSIGHTS INTO THE INTERACTION MECHANISM OF SULFIREDOXIN WITH PEROXIREDOXIN AND ATP

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Peroxiredoxins (Prx) are ubiquitous enzymes family that reduce H_2O_2 or other hydroperoxides to H_2O. In addition to their peroxidase activity, eukaryotic 2-Cys Prx have moonlighting activities depending on the redox state of their catalytic Cys, that can be hyperoxidized into sulfinic acid ($-SO_2H$), turning it into a redox-regulated chaperone holdase [1]. Hyperoxidized Prxs ($PrxSO_2$) are reduced by ATP-dependent sulfiredoxin (Srx), which thus acts as a redox switch of Prxs. The Prx/Srx system is involved in circadian rhythms maintenance, aging and associated diseases. The first step of Srx catalytic mechanism, which is rate-limiting, consists in chemical activation of $PrxSO_2$ by transfer of the ATP γ-phosphate, and depends on Srx/$PrxSO_2$/ATP ternary complex formation.

We address the mechanisms of interaction between *S. cerevisiae* Srx, $PrxSO_2$ and ATP at the binary and ternary complex levels (figure 1), combining enzyme activity, microcalorimetry and fluorescence anisotropy approaches. Our results suggest that ATP is directly recognized by the Srx/$PrxSO_2$ complex. Furthermore, active site residues such as His85 appear to be involved in ATP recognition rather than Prx-Srx complex stabilization.

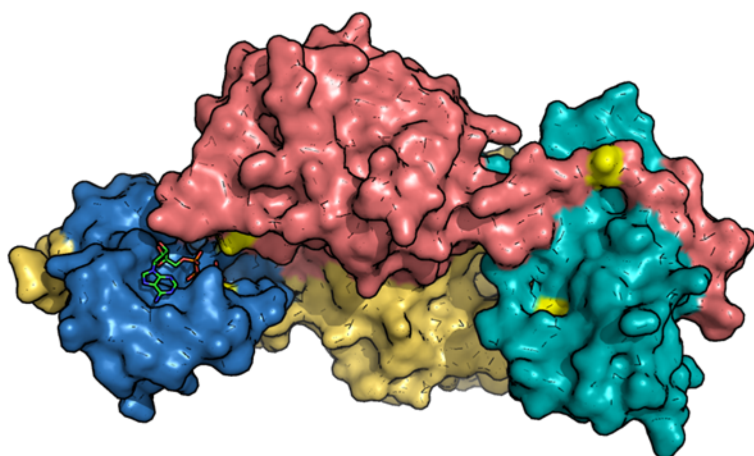

Figure 1: 3D structure of the human dimeric Prx1 (red/yellow) in interaction with two Srx molecules (blue/cyan), showing ATP (green) binding site [2].

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Transketolase - Aldolase Symbiosis for the Stereoselective Preparation of Aldoses and Ketoses of Biological Interest

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Transketolase[1] (TK) was used as biocatalyst for the production of useful aldoses as electrophiles for aldolases[2] to prepare monosaccharides of biological interest.[3] Unconventionally, we operated TK for organic synthesis in the same manner than in the metabolism *ie* without the use of a specially designed nucleophile to shift the equilibrium such as hydroxypyruvate. In addition, we used uncommon formaldehyde as the general TK acceptor substrate to generate aldehydes (figure 1). We demonstrated that the methodology can be generalized by successfully varying the substrates for both enzymes, the aldolase itself and by coupling with other enzymes till a one-pot one-step green cascade reaction process involving four enzymes with atom economy. Finally, new assays have been developed for both monitoring the progress of the reactions involved in the cascades and assessing the purity of the synthetized products.

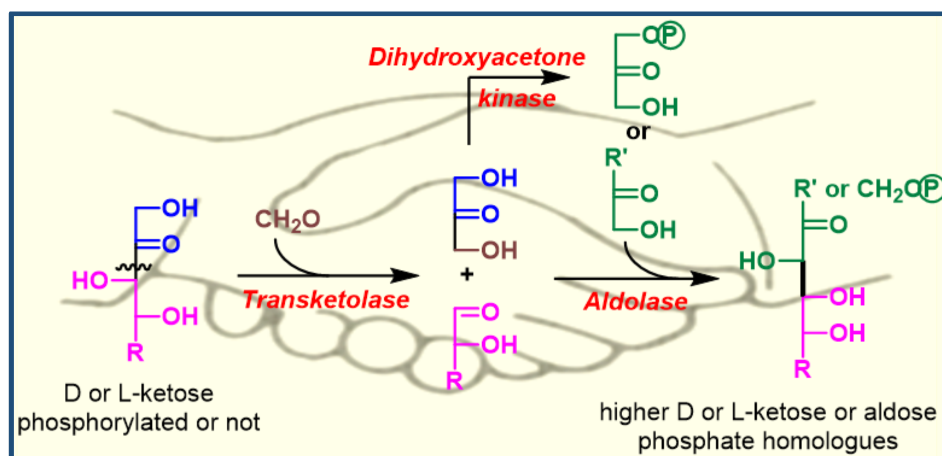

Figure 1. Strategy involving TK and aldolase as complementary tools for stereoselective C-C bond formation via tandem reactions.

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Mechanism of O_2 diffusion and reduction in FeFe hydrogenases

Adam Kubas, Christophe Orain, David De Sancho, Laure Saujet, Matteo Sensi, Charles Gauquelin, Isabelle Meynial-Salles, Philippe Soucaille, Hervé Bottin, Carole Baffert, Vincent Fourmond, Robert B. Best, Jochen Blumberger & Christophe Léger*

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FeFe hydrogenases are the most efficient H_2-producing enzymes. However, inactivation by O_2 remains an obstacle that prevents them being used in many biotechnological devices. Here, we combine electrochemistry,[1] site-directed mutagenesis, molecular dynamics and quantum chemical calculations to uncover the molecular mechanism of O_2 diffusion within the enzyme and its reactions at the active site.[2,3] We propose that the partial reversibility of the reaction with O_2 results from the four-electron reduction of O_2 to water. The third electron/proton transfer step is the bottleneck for water production, competing with formation of a highly reactive OH radical and hydroxylated cysteine. The rapid delivery of electrons and protons to the active site is therefore crucial to prevent the accumulation of these aggressive species during prolonged O_2 exposure. These findings should provide important clues for the design of hydrogenase mutants with increased resistance to oxidative damage.

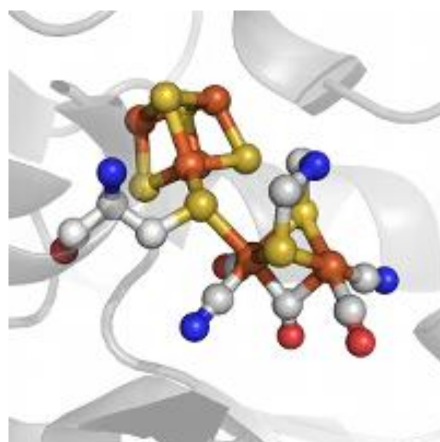

Figure 1. The $[Fe_2(CO)_3(CN)_2$(dithiomethylamine)] active site of FeFe hydrogenase.

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Biochemical and electrochemical characterization of a new multicopper oxidase

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Glucose/oxygen enzymatic biofuel cells may be used to power implanted medical devices. They will rely on the oxidation of glucose at the anode and O_2 reduction at the cathode, both substrates being found in human blood. At the anode, glucose oxidizing enzymes are used. At the cathode, oxygen reducing enzymes such as bilirubin oxidases (BODs) or laccases are used, both belonging to the Multicopper oxidases family (MCOs). In contrast to laccases, BODs display a high activity and stability at physiological conditions (neutral pH and NaCl) which makes them good candidates for the elaboration of cathode in glucose/oxygen enzymatic biofuel cells. [1]

Only few BODs have been identified nowadays [1] In this study we isolated, expressed and purified a new putative BOD from a halothermophile species. We expected this enzyme to be more stable and tolerant to salts and temperature, two important characteristics for implantable biofuel cells. Our first assays with this enzyme, showed very interesting features compared for example to BOD from *Bacillus pumilus* [2].

In solution, the new identified BOD was more stable at 37°C than BOD from *Bacillus pumilus* but its catalytic activity was 30 times lower. Once immobilized onto an electrode surface, this enzyme showed a higher resistance to chloride and a current density only 6 times lower than BOD from *B.pumilus* .

In this context, we started directed evolution studies to increase the activity of this new BOD. In the same time, to characterize in depth this new BOD, we have combined different analytical tools such as electrochemistry, circular dichroism, electron paramagnetic resonance spectroscopy or cristallographic studies to identify the reasons of the moderate activity of this new BOD.

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FULLY ENZYMATIC SYNTHESIS OF MONO- AND DIACYL TREHALOSE CONJUGATES: FROM SIMPLE ALIPHATIC TO MYCOLIC DERIVATIVES

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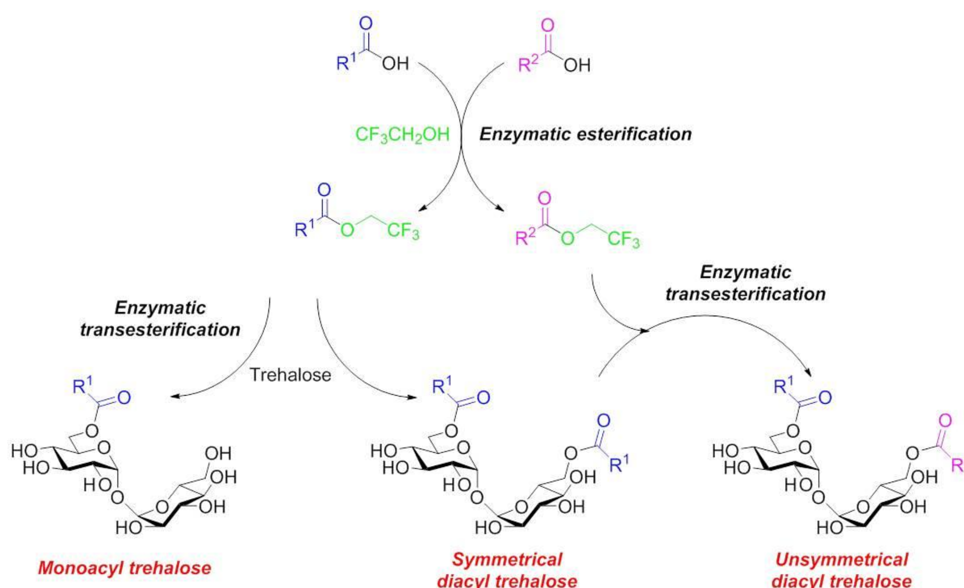
Trehalose-lipid conjugates have gained increased interest for their potential applications: in bioremediation technologies and oil/petroleum industry, as biosurfactants; in biomedical/healthcare industry, as antimicrobial and therapeutic agents [1]. However, their preparation still requires multi-step synthesis [2-3] since lipase-mediated esterifications are not efficient enough, affording only mono-[4] or diester derivatives but in a long reaction time [5].
The issues we have addressed in this study are: i) the development of an efficient enzymatic access to 2,2,2-trifluoroethyl esters, and ii) the control of the transesterification process towards the selective preparation of diacyl and/or monoacyltrehalose, thus opening access to unsymmetrical derivatives. This approach was applied to a set of linear or branched, saturated or unsaturated acids, as well as to extracted membrane mycolic acids. Microwave assistance as well as a well-defined amount of acyl donor were required to efficiently perform the desired synthesis [6].



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DSR-DP: A DEXTRANSUCRASE WITH CATALYTIC EFFICIENCY BOOSTED BY POLAR ORGANIC SOLVENTS

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For numerous applications, solubilizing low-polar or hydrophobic molecules is still a challenge. Now, increasing water solubility can sometimes be achieved by glucosylation. For that purpose, the α-transglucosylases from the family 70 of the Glycoside-Hydrolases [1] are efficient catalysts for the glucosylation of exogenous acceptor molecules, using sucrose as a donor substrate, an abundant and low cost agro-resource. However, glucosylation of a very hydrophobic molecule often needs a prior solubilization in an organic solvent to increase its bioavaibility whereas the enzyme catalytic activity is generally far lower in organic solvent than in water [2]. Here, we describe the effect of dimethyl sulfoxide (DMSO) and tert-butanol on the activity, stability and reaction products of DSR-DP, a new dextransucrase originated from a phage recently discovered in *L. citreum* NRRL B-1299 [3]. Surprisingly, DSR-DP initial activity is impressively enhanced in presence of DMSO and tert-butanol, with a 10-fold improvement of activity in 40% DMSO (w/v). Differential Scanning Fluorimetry experiments showed that DSR-DP melting temperature was affected by DMSO. Indeed, two melting temperatures are observed in the presence of the solvent, indicating that one domain is destabilized faster than the other one and is strongly impacted by the solvent. The dextran size is also affected by the presence of DMSO, with a synthesis mostly directed towards oligosaccharides instead of polymers. In contrast, the α-1,6 linkage specificity is retained. DSR-DP is currently tested as a tool for facilitated glucosylation of hydrophobic molecules (*e.g.*polyphenols) in DMSO.

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β-SUBUNIT SELECTIVE NONCOVALENT INHIBITION OF IMMUNO- AND CONSTITUTIVE PROTEASOMES

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The proteasomes play a central role in nonlysosomal cellular proteolysis and are implicated a wide variety of basic processes. Considerable efforts to develop proteasome inhibitors have been made and led to three approveddrugs in the treatment of hematologic malignancies. All three are covalent inhibitors inhibiting mainly the *β*5 activity of the catalytic core of the constitutive proteasome (cCP) but also that of the immunoproteasome (iCP). Side-effects (partly explained by the lack of selectivity cCP *vs* iCP) and resistance have been observed. Since toxicity will always be an issue because of the myriad of functions of the proteasomes, we pursued noncovalent strategies to obtain inhibitors devoid of the drawbacks associated to the presence of a reactive group. We obtained several inhibitors of the cCP by rational drugdesign (TMC-95A analogs[1]) whereas others were identified using hierarchical structure-based virtual computations (sulfonamide[2, 3]and 1,2,4-oxadiazolederivatives [4]). Our subsequentaim was to identify noncovalent inhibitors of iCP since few (and essentially covalent)iCP inhibitors have been described. Targeting selectively the iCP could be critical in several disease indications (cancers, immunological disorders, graft rejection). We present here new piperazinyl sulfonamide inhibitors obtained by virtual screening [5].Some of them act selectively on the *β*2c and *β*2i subunits (with a preference for the *β*2i) or, simultaneously on the *β*5 and *β*1 subunits of both the iCP and cCP.The subunit preference was supported by computational analysis of selected compounds. Their cytotoxicity, anti-proliferative and anti-invasive effects on two cancer cell lines was also evaluated.

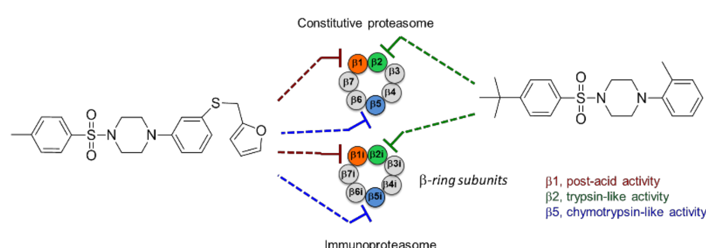

Figure 1. Schematic representation of the *β*-ring subunit of the cCP and iCP and their inhibition byrepresentative sulfonyl piperazines.

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Benzene-induced leukemogenesis: The human H3K36 histone methyltransferase SETD2 as a molecular target of benzoquinone, the hematotoxic metabolite of benzene.

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Benzene (BZ) is a chemical of great industrial interest used to make numerous products including detergents, insecticides and motor fuels. BZ is also known to be a major hazardous air pollutant originating from anthropogenic (road transport, tobacco smoke, etc) and natural sources (forest fires). BZ is classified as a class I human carcinogen. Environmental and occupational exposure to BZ lead to bone marrow malignancies such as leukemia. The leukemogenic effects of BZ relies on its metabolization in bone marrow cells into reactive metabolites, in particular benzoquinone (BQ). However, most of the key molecular and cellular mechanisms underlying its hematotoxicity are not fully understood.
SETD2 is a key histone modifying enzyme that is responsible for catalyzing trimethylation at the lysine 36 of histone H3 (H3K36me3) in mammals. During transcriptional elongation in normal cells, H3K36me3 marks the zone on exons for accurate gene transcription and plays an important role in the regulation of alternative splicing. In addition, SETD2-mediated H3K36me3 is required for DNA repair. Several studies have shown that loss of function mutations in SETD2 are involved in cancer development. In particular, SETD2 inactivating mutations have been shown to be critical drivers in leukemia development.
Here we provide evidence that human SETD2 enzyme is a target of BQ, the prime hematotoxic metabolite of BZ. Kinetic and biochemical analyses indicate that the methyltransferase activity of SETD2 is readily inhibited by BQ in an irreversible manner (k_i=300 $M^{-1}.s^{-1}$). This inhibition is due to the covalent modification (arylation) of cysteine residues present in Zn^{2+} finger motifs (AWS and postSET domains) by BQ of the enzyme with concomitant release of Zn2+. Accordingly, exposure of cells (Hela or HL-60) to BQ leads to global loss of H3K36me3.
The H3K36 histone methyltransferase SETD2 is increasingly recognized as a tumor suppressor gene, which can facilitate the initiation and maintenance of leukemia. Our data suggest that BQ, the major hematoxic metabolite of benzene, can impair the functions of SETD2 which may contribute to benzene-dependent leukemogenesis.

ACTIVE SITE LOOP DYNAMICS CONTROLS COFACTOR FLIP DURING THE CATALYTIC CYCLE IN HYDROLYTIC ALDEHYDE DEHYDROGENASES

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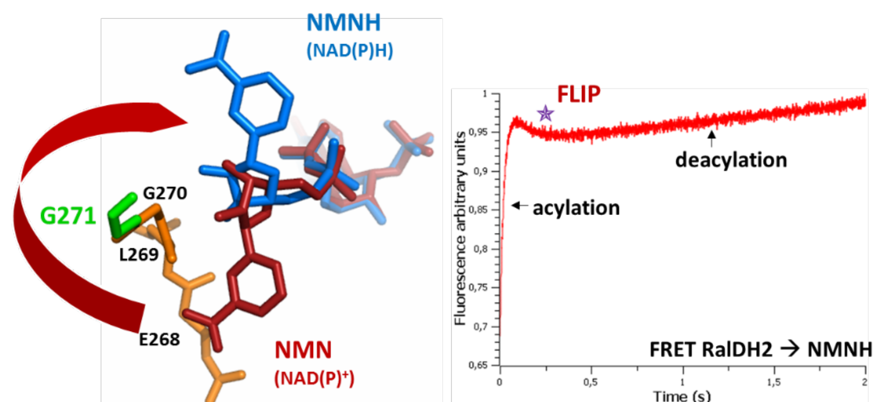
Structural dynamics associated with cofactor binding play key roles in the catalytic mechanism of hydrolytic NAD(P)-dependent aldehyde dehydrogenases (ALDH). The crystal structure of a thioacylenzyme intermediate-NADPH complex provided the first structural evidence for a conformational change of the NMNH [1]. Specifically, this structure revealed after the oxidoreduction step, the reduced cofactor adopts a new conformation with a flip of the NMNH moiety, which positions the reduced nicotinamide in a conserved cavity that might constitute the exit door for NAD(P)H. However the molecular bases that allow the flip to occur are unknown. Based on the known pre- and post-flip X-ray structures, targeted molecular dynamics identified a short conserved loop that may act as a gate for cofactor flipping. To monitor cofactor movements within the active site, we used a FRET signal between Trp177 and the NMNH moiety that permitted to kinetically track the flip during the catalytic cycle of retinal dehydrogenase 2 (RalDH2). As expected, decreasing loop flexibility by substituting an Ala for Gly271 drastically reduced the rate constant associated with this movement that became rate-limiting, thus validating our hypothesis.



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AUTOMATIC RECONSTRUCTION AND MODELLING OF BIOCATALYST SYSTEMS FOR THE PRODUCTION OF SPECIFIC BIOCHEMICAL COMPOUNDS

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Abstract

The knowledge of organisms and their metabolic pathways allowed to construct biological systems for the production of chemicals and pharmaceuticals such as antibiotics and biofuels. Synthetic biology expands the number of these biological systems by the assembly of artificial metabolic pathways, called synthetic pathways, not present in natural organisms. Synthetic pathways could be integrated in modified micro-organisms or in biocatalyst systems. A biocatalyst system is an in vitro assembly composed only of purified enzymes and metabolites that are useful for the production of a desired metabolic compound through a biochemical reaction network. This in vitro assembly, as compared to cellular system, has several advantages, such as the production of only desired metabolites and a great engineering flexibility. We explored an in silico approach to identify and analyse new biocatalyst systems for the production of target metabolic compounds. This approach proceeds in several steps. The first step is the enumeration of several biocatalyst systems that could synthesize a target product from a desired starting substrate. Next, a selection based on several criteria is applied to choose a biocatalyst system among the group of biocatalyst systems identified in the enumeration step. The last step is the modelling of the selected biocatalyst system to evaluate the production rate and the yield of the target product. This communication explains in more detail the modus operandi for the different steps of our in silico approach.

BIOPHYSICAL CHARACTERIZATION OF HUMAN MITOCHONDRIAL BINARY COMPLEXES FORMED BETWEEN ETHE1 AND DIFFERENT SULFURTRANSFERASES

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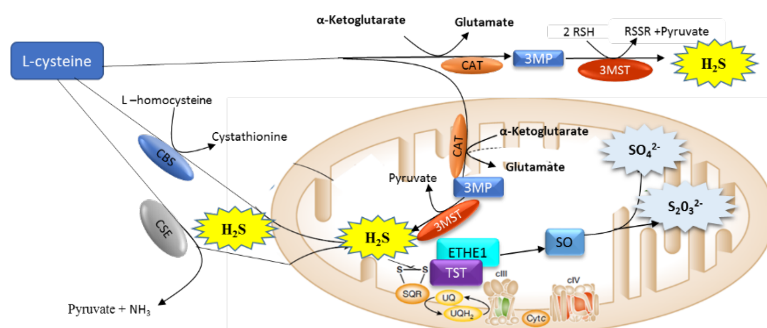
Human persulfide dioxygenase (ETHE1) is an iron-containing protein from the metallo-beta-lactamase superfamily involved in the mitochondrial catabolism of the Janus faced hydrogen sulfide [1]. Loss-of-function mutations in ETHE1 result in sulfide toxicity and are associated with the Ethylmalonic Encephalopathy, a devastating infantile metabolic disorder [2]. ETHE1 catalyzes the oxygen dependent oxidation of glutathione persulfide to sulfite and glutathione, and is reported to be part of a ternary complex, named sulfide oxidation unit (SOU). Although it is accepted that the SOU comprises the sulfide quinone oxidoreductase and ETHE1, the identity of the third partner, a rhodanese-type sulfurtransferase, and the sulfide oxidation pathway remain to be clarified.



In this context, in vitro characterization of binary complexes formed between ETHE1 and one of the three human mitochondrial sulfurtransferases, i.e. Rhodanese, TSTD1 and 3-MST, has been undertaken using different biophysical methods (ITC, fluorescence quenching, fluorescence anisotropy, SPR). Preliminary results will be presented and discussed in relation with the proposed physiological function of the different sulfurtransferases.

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Hidden phage *Vp*16 peptide deformylase (PDF) features are essential for deformylase activity

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Prokaryotic proteins must be deformylated before the removal of their first methionine. Peptide deformylase (PDF) is indispensable and guarantees this mechanism. Recent genome sequencing studies highlighted over $2x10^4$ putative peptide deformylase sequences. Furthermore, unpredicted modified bacterial PDF genes have been retrieved from many viruses [1]. Sequence comparisons with other known PDFs reveal that viral PDFs are devoid of the key ribosome-interacting C-terminal region [2]. Little is known regarding these viral PDFs, including the capacity of the corresponding encoded proteins to ensure deformylase activity. We provide here the first evidence that viral PDFs, including the shortest PDF identified to date, *Vp*16 PDF, display deformylase activity *in vivo.* Large scale N-terminomics characterization reveals that *Vp*16 PDF has substrate specificity similar to that of other bacterial PDFs. However, our integrated biophysical and biochemical approaches also reveal hidden and unique functions of the unusual C-terminus. The high-resolution crystal structures of *Vp*16 PDF, free or bound to the potent inhibitor actinonin, reveal a classical PDF fold and also an unexpected crucial role for the ultimate residue tethering the active site. Our study underscores the structural and molecular characteristics of the unusual C-terminal Ile residue that sustains deformylase activity in the absence of the otherwise indispensable C-terminal domain.

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