Congrès 2013 du Groupe Thématique (GT) « Enzymes : Structure/Fonction/Catalyse/Ingénierie/Régulation »
de la Société Française de Biochimie et de Biologie Moléculaire (SFBBM)

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Enzymes :

Des modèles fondamentaux aux applications

Paris, 4-6 septembre 2013

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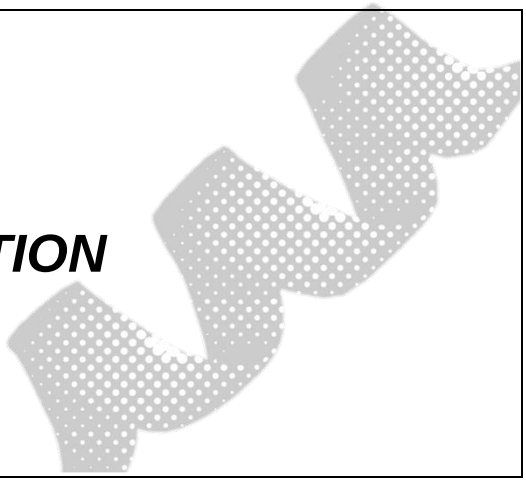
INTRODUCTION



Le congrès 2013 du Groupe Thématique Enzymes de la SFBBM célèbrera en particulier deux anniversaires : les 100 ans du modèle michaëlien et les 50 ans du concept de régulation allostérique. Seront abordées au cours de ce congrès plusieurs thématiques susceptibles d'intéresser les chercheurs des secteurs académique et industriel intéressés par les enzymes.

Des conférences plénières, des présentations orales et par affiches, sélectionnées à partir de résumés, permettront d'aborder les enzymes dans leurs dimensions moléculaires et intégratives au travers de 5 sessions. Une remise de prix viendra récompenser de jeunes chercheurs ayant exposé leurs travaux. Ces récompenses s'inscrivent dans l'objectif principal de ce congrès, celui de susciter l'intérêt pour l'étude des enzymes auprès de la communauté scientifique, et plus particulièrement au sein des jeunes chercheurs.

Comité d'organisation :

Jean-Marie Dupret	**BFA, Paris**
Fernando Rodrigues-Lima	**BFA, Paris**
Giuseppe Baldacci	IJM, Paris
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Avec le concours de :

Agilent Technologies Genomics
Dominique Dutscher
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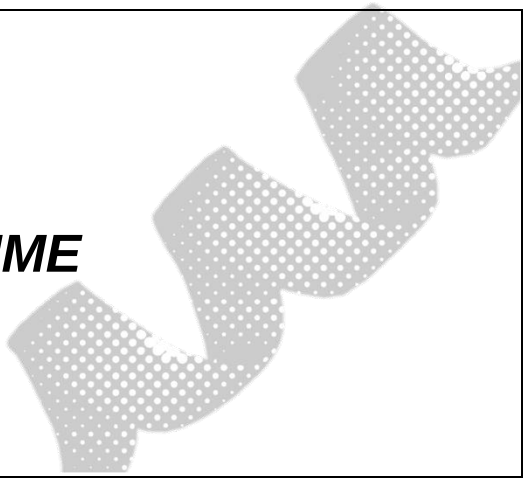
l'unité de Biologie Fonctionnelle et Adaptative (BFA)
l'Institut Jacques Monod (IJM)
l'UFR des sciences du vivant (UFR SdV)
le CNRS

Conférenciers invités :

Athel Cornish-Bowden	BIP, Marseille
Eileen K. Jaffe	Fox Chase Cancer Center, USA
Anja Krieger-Liszkay	CEA, Saclay
Daniel Mansuy	LCBPT, Paris
Dan Tawfik	Weizmann Institute, Israël
Julien Valton	Cellectis, Paris

PROGRAMME



MERCREDI 4 SEPTEMBRE 2013

Après-midi

13h30	Accueil
14h00	Introduction **Session 1 : Un siècle de recherche en enzymologie**
14h30	**Conférence plénière 01 - A. Cornish-Bowden :** 100 ans de la cinétique Michaelis-Menten
15h15	**Communication orale 01 - B. Baudin :** Enseignement de l'enzymologie élémentaire : place d'un logiciel de simulation de cinétiques enzymatiques
15h30	Pause café / Session posters
16h30	**Conférence plénière 02 - D. Tawfik :** The moderately efficient enzyme
17h15	**Communication orale 02 - S. Emond :** Identifying Adaptive Indels using Directed Enzyme Evolution
17h30	**Communication orale 03 - Y. Malbert :** Enzymatic engineering through both random and semi-rational directed evolution to improve an α-1,2 branching glucansucrase
17h45	**Communication orale 04 - C. Miton :** Exploring catalytic promiscuity in the alkaline phosphatase superfamily by directed evolution
18h00	Session posters
19h00	Cocktail dinatoire / Session posters

JEUDI 5 SEPTEMBRE 2013

Matin

	Session 2 : Structures et mécanismes
09h00	**Conférence plénière 03 - D. Mansuy :** Bases structurales et mécanistiques de l'extrême diversité de substrats et de réactions des cytochromes P450
09h45	**Communication orale 05 - P. Belin :** Substrate and reaction specificity of the cytochrome P450 CYP121 from *Mycobacterium tuberculosis*
10h00	**Communication orale 06 - E. Cambon :** One enzyme – Many activities : *Use of multiple enzyme engineering approaches to create novel functions of a carbohydrate-active enzyme*
10h15	**Communication orale 07 - S. Dementin :** Mechanism of two nickel enzymes: Hydrogenase and Carbon Monoxide Dehydrogenase
10h30	Pause café / Session posters
11h30	**Communication orale 08 - E. Kaplan :** Cibler les aminoglycosides phosphotransférases, un moyen de lutter contre les résistances bactériennes
11h45	**Communication orale 09 - P. Lafite :** Caractérisation des galactofuranosyltransférases de *Leishmania*
12h00	**Communication orale 10 - F. Talfournier :** An extensive conformational sampling of the Rossman fold domain is integral to the catalytic mechanism of a bacterial acylating acetaldehyde dehydrogenase
12h15	Déjeuner / Session posters

Après-midi

	Session 3 : Régulations des enzymes
14h00	**Conférence plénière 04 - E.K. Jaffe :** The morpheein model of protein allostery - an anomaly or an overlooked generalization?
14h45	**Communication orale 11** - **L. Chaloin :** Allosteric inhibitors against the cytosolic 5'-nucleotidase II for overcoming resistance to anticancerous treatments
15h00	**Communication orale 12 - B. Golinelli-Pimpaneau :** Morpheein-type allosteric regulation of *E. coli* glucosamine-6-phosphate synthase
15h15	**Communication orale 13 - F. Lecaille :** Modulation de l'activité de la cathepsine S par le chondroïtine 4-sulfate
15h30	**Communication orale 14 - M. Reboud-Ravaux :** Le principe de bivalence pour obtenir de puissants inhibiteurs non covalents du protéasome humain
15h45	Pause café / Session posters
	Session 4 : Approches biophysiques
16h45	**Conférence plénière 05 - A. Krieger-Liszkay :** Generation of singlet oxygen in photosystem II
17h30	**Communication orale 15** - **M. Moussaoui :** Relationship between different flavohemoglobin structural states and its enzymatic activity
17h45	**Communication orale 16 - H. Munier-Lehmann :** CBS domains control allostery behavior and fibre formation in IMPDHs through a common octameric architecture
18h00	**Communication orale 17 - Y-H. Sanejouand :** Dynamic of conserved water molecules in family 1 glycoside hydrolases: an X-ray, DXMS and Molecular Dynamics study
18h15	**Communication orale 18 - G. Truan :** Echanges conformationnels rapides dans une protéine multidomaine : la NADPH cytochrome P450 réductase

	Remise des Prix
18h30	**Prix SFBBM, article de l'année :** **A. Fleurie** : Fine tuning of bacterial cell division and elongation by the StkP kinase
18h45	**Prix du meilleur poster**
19h00	Session posters
19h30	Cocktail dinatoire / Session posters

VENDREDI 6 SEPTEMBRE 2013

Matin

	Session 5 : Applications et interfaces
09h00	**Conférence plénière 06 - J. Valton :** XPC Gene Therapy Using TALEN™
09h45	**Communication orale 19 - A. Frelet-Barrand :** *Lactococcus lactis*, recent developments in functional heterologous expression of membrane proteins
10h00	**Communication orale 20 - C. Lionne :** Nouveaux dérivés de néamine insensibles à l'inactivation par une enzyme de résistance bactérienne
10h15	**Communication orale 21 - X. Maréchal :** Inhibition de la différenciation des myoblastes par des inhibiteurs de l'enzyme insulysine développés par click chemistry *in situ*
10h30	**Communication orale 22 - F. Mavré :** Electrochemical characterization of the soluble PQQ-dependent glucose dehydrogenase. Effect of directed mutagenesis on selectivity and cooperativity
10h45	Pause café / Session posters
11h15	**Communication orale 23 - Hervé Chaulet, Société Agilent Technologies Genomics :** Herculase II & Applications
11h30	**Communication orale 24 - A. Vergès :** Diversification of *Neisseria polysaccharea* amylosucrase activity through a semi-rational engineering approach
11h45	**Communication orale 25 - M. Vuillemin :** Search for new GH-70 α-transglucosylases in lactic acid bacteria diversity dedicated to the synthesis of tailor-made α-glucans
12h00	**Communication orale 26 - J. Dairou :** Mise au point d'un dosage de l'activité kinase de la protéine DYRK1A par HPLC
12h15	Conclusions

MERCREDI 4 SEPTEMBRE 2013

Accueil : 13h30 à 14h00

PROGRAMME DE L'APRÈS-MIDI : 14h00 à 19h00

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18h00	Session posters
19h00	Cocktail dinatoire / Session posters

SESSION 1

Un siècle de recherche en enzymologie

Conférence plénière 01 :

100 ans de la cinétique Michaelis-Menten

Athel Cornish-Bowden
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One hundred years ago Leonor Michaelis and Maud Menten published a paper that has become one of the most highly cited in biochemistry: more than that, it is cited much more often in the 21st century than it was in preceding decades. It laid the foundation of kinetic studies of enzymes, introducing the initial-rate protocol that has remained standard in steady-state studies ever since. Previous workers had attempted to analyse complete time courses, which was impractical with the techniques available. In addition, Michaelis and Menten were almost the first to make control of pH an essential component of kinetic investigations. Although they were not the first to write the equation usually called by their name, as it had been given earlier by Victor Henri, but they were the first to place it on a solid basis. The major increase in citation rate in recent years can be explained in part by a general revival of interest in enzymes, but also by the growth of a number of special disciplines, including systems biology and single-molecule experiments.

Communication orale 01.

Enseignement de l'enzymologie élémentaire : place d'un logiciel de simulation de cinétiques enzymatiques

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Ce logiciel a été conçu pour répondre à un appel d'offre de l'UNF3S et aux besoins de l'enseignement universitaire dans le premier cycle des études scientifiques et médicales, en particulier pour la Première Année Commune des Etudes de Santé (PACES). Vingt-deux simulations permettent de comprendre le fonctionnement des enzymes ($v_o = f([S])$, $v_o = f([E])$, effet de la température et du pH), de définir le type et les constantes de l'enzyme (graphes de Michaelis-Menten et de Cornish-Bowden, Vm et Km, allostérie), de caractériser des inhibiteurs enzymatiques (graphes de Lineweaver-Burk et d'Eadie-Hofstee, Ki), ainsi que de mesurer des vitesses de catalyse. Une autoévaluation progressive est proposée, ainsi que la réalisation d'exercices d'application. Des renvois vers du cours ou des informations complémentaires sont proposés tout au long de ces ressources numériques (rappels de thermodynamique, de cinétique chimique…). Le scénario pédagogique se déroule en 3 parties orientées vers tout ou partie des programmes de la PACES, du L2 en Sciences ou Pharmacie ou encore de la préparation au concours de l'Internat en Pharmacie. Après une introduction sur les enzymes et la catalyse enzymatique, la première partie concerne la simulation du comportement d'une enzyme michaelienne, la seconde est consacrée aux simulations des effets des inhibiteurs enzymatiques, et la troisième montre le comportement des enzymes allostériques et des effecteurs allostériques.

Mots clés : Enzymologie, Cinétiques, Enseignement, Ressources Numériques, Michaelis-Menten, Inhibiteurs Enzymatiques, Allostérie.

Conférence plénière 02 :

The moderately efficient enzyme

Dan S. Tawfik
Department of Biological Chemistry, The Weizmann institute of Science, Rehovot 76100, Israel
dan.tawfik@weizmann.ac.il

I will argue that, in contrast to the textbook superstars, the vast majority of enzymes are only moderately efficient, with the average enzyme exhibiting a k_{cat}/K_M value of 10^5 M^{-1} s^{-1}. The implication is that, in the average enzyme, less than one in 10^4 substrate-enzyme encounters results in product formation. I will address the evolutionary origins of moderate enzyme efficiency. I will also discuss the molecular basis of this phenomenon – the dominance of futile encounters, in relation to enzyme promiscuity and evolvability.

Communication orale 02.

Identifying Adaptive Indels using Directed Enzyme Evolution

Stephane Emond[1], Nobuhiko Tokuriki[2] et Florian Hollfelder[1]

[1] Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, United Kingdom, [2] Michael Smith Laboratories, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

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Proteins evolve and acquire new functions in Nature by accumulating mutations. Several mechanisms, such as insertions, deletions, substitutions, circular permutations and rearrangement of domains, account for the majority of evolutionary changes. While the accumulation and effects of substitutions have been extensively studied, understanding the structural and functional effects of insertions and deletions (*indels*) remains a challenge. Indels are allegedly highly deleterious mutations because they are more likely to disrupt the structural integrity of proteins than substitutions. On the other hand, they may induce significant structural changes that substitutions only cannot cause and thus are believed to be key players in many natural evolutionary processes, such as the modification of active site loops to generate new enzyme functions[1] or the emergence of new protein structures[2].

We aimed at performing laboratory evolution experiments incorporating indels to investigate whether backbone modifications can be selected for functional effects. Starting from a previously reported methodology based on transposition mutagenesis[3], we have developed a new approach to generate libraries of protein variants by randomly introducing short indels inside a gene of interest. We have applied this technique to identify adaptive indels (*i.e.*, conferring new functions) in two promiscuous enzymes (*P. diminuta* phosphotriesterase and *P. aeruginosa* arylsulfatase). Our results indicate that, while being generally more deleterious than substitutions, indels can also be selected for functional effect and may allow access to alternative mutational trajectories in laboratory evolution of proteins.

[1] Afriat-Jurnou L, Jackson CJ, Tawfik DS. 2012. Reconstructing a missing link in the evolution of a recently diverged phosphotriesterase by active-site loop remodeling. Biochemistry 51:6047–6055. [2] Grishin NV. 2001. Fold change in evolution of protein structures. J Struct Biol. 134:167–185. [3] Jones DD. 2005. Triplet nucleotide removal at random positions in a target gene: the tolerance of TEM-1 β-lactamase to an amino acid deletion. Nucleic Acids Res. 33(9):e80.

Mots clés : Directed Evolution, Indels, Promiscuity

Communication orale 03.

Enzymatic engineering through both random and semi-rational directed evolution to improve an α-1,2 branching glucansucrase.

Yannick Malbert[1], Sandra Pizzut-Serin[1], Virginie Rivière[1], Marlène Vuillemin[1], Sandrine Morel[1], Isabelle André[1], Claire Moulis[1] and Magali Remaud-Siméon[1]

[1]Université de Toulouse, France, INSA, UPS, INP, LISBP, 135 Avenue de Rangueil, F-31077 Toulouse, France, CNRS, UMR5504, F-31400 Toulouse, France, INRA, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, F-31400 Toulouse.

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Glucansucrases from Glycoside-Hydrolase family 70 (GH70) are transglucosylases produced by lactic acid bacteria and naturally catalyze the polymerization of glucosyl residues with concomitant fructose release from sucrose, a low-cost and abundant agroresource. Depending on the enzyme specificity, a large variety of glucans containing all types of glucosidic bonds, namely α-(1→2), α-(1→3), α-(1→4) or α-(1→6) and varying in terms of size, structure, degree of branching, and spatial arrangement are synthesized. Among them, GBD-CD2, a truncated form of DsrE isolated from *Leuconostoc citreum* B-1299, exclusively catalyzes α-(1→2) branching onto glucans [1,2]. This linkage type confers attractive prebiotic properties to oligosaccharides [3]. Engineering of this original enzyme is currently investigated to i) improve the understanding of GBD-CD2 mode of action, ii) extend the ability of the enzyme to recognize unnatural acceptors, and iii) improve the enzyme thermostability and resistance to organic solvents.

Two strategies of enzyme evolution were conducted in parallel to take advantage of both rational knowledge and power of combinatorial engineering. To this purpose,

- an original epPCR based strategy was developed to achieve four turns of random mutagenesis. Screening of third and fourth rounds of evolution allowed isolating more than 500 novel enzymes, still active while containing an average of 15 mutations per kb for the last turn of evolution.
- docking analyzes in the recently solved structure of the α-(1→2) branching enzyme were also performed to target amino-acids more specifically involved in unnatural acceptor recognition. Saturation mutagenesis using NDT codon was used to generate four semi-rationally designed libraries.

Characterization of the isolated mutants will be described and discussed with regard to the evolution strategy.

[1] Brison Y, Fabre E, Moulis C, Portais JC, Monsan P, Remaud-Siméon M. 2010. Synthesis of dextrans with controlled amounts of alpha-1,2 linkages using the transglucosidase GBD-CD2. Appl Microbiol Biotechnol, 86(2), pp 545-54.

[2] Fabre E, Bozonnet S, Arcache A, Willemot RM, Vignon M, Monsan P, Remaud-Simeon M. 2005. Role of the two catalytic domains of DSR-E dextransucrase and their involvement in the formation of highly alpha-1,2 branched dextran. J Bacteriol, 187(1), pp 296-303.

[3] Brison Y, Pijning T, Malbert Y, Fabre É, Mourey L, Morel S, Potocki-Véronèse G, Monsan P, Tranier S, Remaud-Siméon M, Dijkstra BW. 2012. Functional and structural characterization of α-(1->2) branching sucrase derived from DSR-E glucansucrase. J Biol Chem 287(11), pp 7915-24.

Mots clés : Glucansucrase, branching enzyme, enzyme engineering, directed evolution, high throughput screening, prebiotics.

Communication orale 04.

Exploring catalytic promiscuity in the alkaline phosphatase superfamily by directed evolution

***Charlotte M. Miton*[1], *Stefanie Jonas*[1,2], *Mark F. Mohamed*[1], *Marko Hyvönen*[1], *Nobuhiko Tokuriki*[1,3], *Florian Hollfelder*[1]**

[1] *Department of Biochemistry, University of Cambridge, Cambridge - United Kingdom*
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Catalytic promiscuity is defined as the ability of an enzyme to catalyse several chemically distinct reactions besides its native activity. This latent promiscuity appears to be more widespread than originally thought and may have played an important role in adaptive evolution by providing possible evolutionary starting points for the emergence of new functions [1-2]. The Alkaline Phosphatase (AP) superfamily provides an ideal framework to explore this hypothesis, as it encompasses a large set of evolutionary related metalloenzymes frequently exhibiting crosswise catalytic promiscuity [3-4]. Based on these observations, our work aims at addressing the following question: which mechanistic and structural features determine specificity or promiscuity among AP superfamily members?

We performed directed evolution to investigate how *P. aeruginosa* arylsulfatase (PAS) can be turned into a phosphonate monoester hydrolase (PMH), one of its secondary activities. Three rounds of neutral drift [5], followed by six rounds of selection for improved promiscuous activity resulted in a highly generalist enzyme with a k_{cat}/K_M of 10^3-10^4 M^{-1} s^{-1} for four chemically distinct substrates: phosphate diesters, phosphate, phosphonate, and sulfate monoesters. Furthermore, the analysis of evolutionary intermediates revealed the clustering of substitutions in loops that are absent in PMH structures. Semi-rationally designed deletion libraries of these regions produced mutants tolerating up to 18 AA deletions and displaying compelling changes in specificity (>10^3-fold). Detailed kinetics, LFER, mutational and structural analysis of evolutionary intermediates shed new light on the fine molecular changes accompanying the respecialization of a promiscuous scaffold. Taken together, our results provide new insights into the respective contribution of binding and catalysis, as well as multi-functional trade-offs in the evolution of new functions. It also emphasizes the role of deletions in opening alternative evolutionary trajectories.

*[1]. Jensen, R. A., Annual Reviews in Microbiology **1976**.*
*[2]. O'Brien, P. J.; Herschlag, D., Chem Biol **1999,** 6 (4), R91-R105.*
*[3]. Mohamed, M. F.; Hollfelder, F., Biochim Biophys Acta **2013,** 1834 (1), 417-24.*
*[4]. van Loo, B.; Jonas, S.; Babtie, A. C.; Benjdia, A.; Berteau, O.; Hyvonen, M.; Hollfelder, F., Proc Natl Acad Sci U S A **2010,** 107 (7), 2740-5.*
*[5]. Gupta, R. D.; Tawfik, D. S., Nat Methods **2008,** 5 (11), 939-42.*

Mots-clés : catalytic promiscuity, directed evolution, neutral drift, design, indels, superfamily

JEUDI 5 SEPTEMBRE 2013

PROGRAMME DU MATIN : 09h00 à 12h15

	Session 2 : Structures et mécanismes
09h00	**Conférence plénière 03 - D. Mansuy :** Bases structurales et mécanistiques de l'extrême diversité de substrats et de réactions des cytochromes P450
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SESSION 2

Structures et mécanismes

Conférence plénière 03 :

Bases structurales et mécanistiques de l'extrême diversité de substrats et de réactions des cytochromes P450

Daniel Mansuy
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Diversité et adaptabilité sont les deux mots-clés qui caractérisent le mieux la superfamille des cytochromes P450. Ces hémoprotéines ubiquitaires chez les êtres vivants ont des rôles biologiques multiples avec un impact dans les domaines de la physiologie, la pharmacologie, la toxicologie, les sciences des plantes et de l'environnement, et la microbiologie.

La très grande diversité de substrats des P450s responsables du métabolisme des xénobiotiques est un élément clé de l'adaptation des êtres vivants à leur environnement chimique tout le temps changeant. Les études structurales réalisées ces dix dernières années ont montré que cette extrême diversité de substrat est basée sur une grande diversité et une grande adaptabilité structurales de ces P450s.

La superfamille des P450s se caractérise aussi par l'étonnante diversité des réactions qu'ils sont capables de catalyser. Des résultats récents montrent que certains P450s catalysent des réactions très différentes des réactions de momooxygénations classiques pour cette classe d'enzymes, comme des hydrolyses, des additions d'H_2O, des décarboxylations, ou des cycloadditions 2+2. Les bases moléculaires de cette grande diversité de réactions seront discutées à partir de résultats récents concernant le mécanisme de ces réactions.

Communication orale 05.

Substrate and reaction specificity of the cytochrome P450 CYP121 from *Mycobacterium tuberculosis*

Pascal Belin[1], Marie-Hélène Le Du[2], Matthieu Fonvielle[1], Olivier Lequin[3], Alain Lecoq[1], Mickaël Jacquet[1], Robert Thai[1], Steven Dubois[1], Guillaume Grach[1], Muriel Gondry[1]

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CYP121 from the human pathogen *Mycobacterium tuberculosis* is one of the mycobacterial P450s that have been shown to be essential for viability of bacteria (1). It is capable of converting the cyclodipeptide cyclo(L-Tyr-L-Tyr) to mycocyclosin with formation of a C-C bond between the aryl side chains (2). Interestingly, it is chromosomally associated with a cyclodipeptide synthase, Rv2275, that has been shown to use charged tRNAs to synthesize cyclo(L-Tyr-L-Tyr), among other cyclodipeptides (3). Nevertheless, apart from azoles, no other CYP121 ligands have been described (4). Here we present our recent investigation on the substrate and reaction specificity of CYP121. The relationship between CYP121 and substrate analogues has been explored using UV-Vis spectrophotometry, enzyme assays, mass spectrometry and crystal structure determination. Results are discussed in terms of catalytic mechanism, biological role of CYP121 and drug design against an essential protein from *M. tuberculosis*.

[1] McLean KJ, et al. (2008) J. Biol. Chem. 283, 33406-33416
[2] Belin P, et al. (2009) Proc. Natl. Acad. Sci. USA 106, 7426-7431
[3] Gondry M, et al. (2009) Nat. Chem. Biol. 5, 414-420
[4] McLean KJ, et al. (2002) J. Inorg. Biochem. 91, 527-541

Mots clés : P450, Mycobacterium tuberculosis, specificity

Communication orale 06.

One enzyme – Many activities: *Use of multiple enzyme engineering approaches to create novel functions of a carbohydrate-active enzyme*

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Glucansucrases are transglycosylases that use sucrose, an abundant agro-resource, as glucosyl donor for the synthesis of α-glucans. Unlike enzymes belonging to the glycosyltransferase family, these enzymes do not require any expensive nucleotide-activated sugar as glucosyl donor to proceed to the reaction and thus, they can be seen as attractive biocatalysts for industrial synthesis and modification of polysaccharides as well as production of added value carbohydrate-based molecules (Champion *et al.*, 2009; Champion *et al.*, 2012; Daudé, *et al.*, 2013). Among glucansucrases, the amylosucrase from *Neisseria polysaccharea*, which naturally synthesizes α-1,4 glucans, has received particular attention. However, the natural efficiency of this enzyme can be limited in terms of productivity or substrate and product specificities. To overcome these limits, we applied several molecular engineering strategies to access optimized biocatalysts for different applications.

First, a random strategy based on the neutral drift theory was applied to the amylosucrase. After 4 rounds of evolution, a library presenting an average of 2.8 mutations per gene and still active on the original donor substrate was constructed and screened towards many different potential acceptor molecules. The isolated enzymatic variants were shown to display altered substrate promiscuity and remarkably, one of them exhibited a novel acceptor specificity.

Next, innovative molecular modeling techniques were used to identify a key amino acid residue involved both in the active site topology, in the substrate recognition and in the glucosylated product release. To investigate further its role, the residue was systematically mutated by the 19 possible amino acids. Screening of the library led to the isolation of one single-variant showing a 10-fold increase in catalytic efficiency and a different distribution of synthesized products compared to the parental wild-type enzyme. This variant appears particularly attractive for the high production of insoluble polymer from sucrose as sole substrate.

Lastly, an original and ambitious semi-rational strategy combining the use of both computational protein design and molecular protein engineering tools led to the construction of a 50,000 variant library with a controlled diversity, each variant containing between 9 and 25 mutations. Upon high-throughput screening of the library, variants showing hyperactivities towards sucrose and original product profiles were isolated and are currently under characterization.

Altogether, these results highlight the high malleability and plasticity of amylosucrase from *N. polysaccharea* to accommodate a large range of different acceptor molecules, making it a multi-purpose biocatalyst of great potential for many biotechnological applications.

[1] E Champion, I André, C Moulis, J Boutet, K Descroix, S Morel, P Monsan, L.A Mulard, M Remaud-Siméon. 2009. Design of alpha-transglucosidases of controlled specificity for programmed chemoenzymatic synthesis of antigenic oligosaccharides. J. Am. Chem. Soc., 131: 7379-7389

.[2] E Champion, F. Guérin, C. Moulis, S. Barbe, T.H. Tran, S. Morel, K. Descroix, P. Monsan, L. Mourey, L.A. Mulard, S. Tranier, M. Remaud-Siméon, I. André. 2012. Applying pairwise combinations of amino acids mutations for sorting out highly efficient glucosylation tools for chemo-enzymatic synthesis of bacterial oligosaccharides. J. Am. Chem. Soc., 134: 18677-18688.

[3] D. Daudé, S. Morel, D. Guieysse, M. Remaud-Siméon, I. André. 2013. Probing substrate promiscuity of amylosucrase from Neisseria polysaccharea. ChemCatChem. In press

Mots clés : Enzyme engineering, neutral drift, semi-rational strategy, carbohydrate-active enzyme, novel functions.

Communication orale 07.

Mechanism of two nickel enzymes: Hydrogenase and Carbon Monoxide Dehydrogenase

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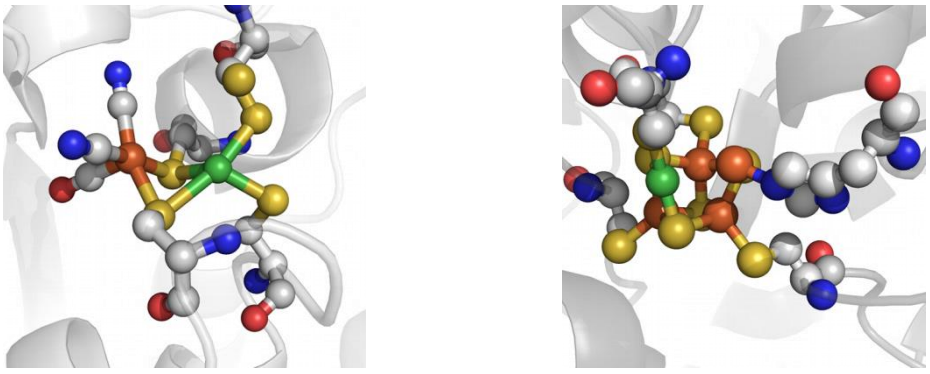
Only eight enzymes are known to contain nickel in their active site, most of them catalyze the use and/or production of small molecules that are central to the global hydrogen, carbon, nitrogen, and oxygen cycles[1]. They include hydrogenases (H2ases) and carbon monoxide dehydrogenases (CODH), the biological catalysts of H^+/H_2 and CO_2/CO conversion, respectively. Their active sites are inorganic clusters made of Ni, Fe and sulfur (see figure). The mechanisms of these enzymes also involve the transport of small molecules from the solvent to the buried active sites, the transfer of protons and the transfer of electrons along chain of FeS clusters.

In our laboratory, we study all the aspects of the mechanism of a H2ase and a CODH purified from *Desulfovibrio* species using a combination of biochemical, spectroscopic, crystallographic and electrochemical approaches. I will compare the two enzymes and present a selection of our latest results [2-6].

Beyond this fundamental aspect, these enzymes are described as potential catalysts or inspiration sources for the design of synthetic compounds to use in H_2 oxidation/production and carbon fixation systems, and their precise characterization is a prerequisite to their biotechnological exploitation.



Active site of hydrogenase (left) carbon monoxide dehydrogenase (right). Atom colors : green (Ni), orange (Fe), yellow (S), white (C), red (O) et blue (N).

[1]Ragsdale SW 2009, J. Biol. Chem. 284:18571-5; [2]Abou-Hamdan A et al. 2013, Nat. Chem. Biol. 9:15-7; [3]Abou-Hamdan A et al. 2012, PNAS USA 109: 19916-21; [4]Dementin et al. 2011, JACS 133:10211-21; [5]Liebgott et al. 2010, Nat. Chem. Biol. 6:63-70; [6]Dementin et al. 2004 J. Biol. Chem 279:10508-13

Mots clés : hydrogenase, carbon monoxide dehydrogenase, mechanism, nickel

Communication orale 08.

Cibler les aminoglycosides phosphotransférases, un moyen de lutter contre les résistances bactériennes

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Les aminoglycosides sont des antibiotiques à large spectre utilisés pour le traitement des infections bactériennes sévères. Cependant, leur utilisation a été compromise par l'apparition d'enzymes capables de les modifier comme les Aminoglycosides PHosphotransférases (APH), induisant alors une perte de leur efficacité clinique. Parmi ces enzymes, l'APH(2'')-IVa possède la particularité d'utiliser indifféremment l'ATP ou le GTP comme donneur de phosphate. Des études de cinétiques à l'état transitoire révèlent que les étapes limitantes de la réaction sont différentes en fonction du type de nucléotide utilisé. Validé avec une enzyme de la même famille[1], ce type d'approche permet de révéler les détails du mécanisme enzymatique et d'identifier les intermédiaires prépondérants de la réaction.

Ces premiers résultats sont complétés par des techniques de biophysique d'étude de stabilité thermique (thermofluor) et de calorimétrie (ITC) de façon à obtenir les paramètres thermodynamiques de liaison des interactions protéine-ligand. Pour mieux comprendre les relations structure-fonction, des essais cristallographiques ont permis de déterminer, à une résolution de 2.35 Å, la première structure du complexe APH(2'')-IVa·ADP. Les interactions responsables de la fixation du nucléotide ont été mises en évidence et permettent d'établir des similitudes avec des protéines kinases. La comparaison avec la structure du complexe APH(2'')-IVa·GDP devrait pouvoir expliquer la non-spécificité de l'enzyme pour le nucléotide ainsi que les différences cinétiques observées.

En parallèle et dans l'optique de développer de nouvelles molécules thérapeutiques capables d'inhiber ces enzymes, nous avons recherché des composés capables d'interagir avec les intermédiaires prépondérants par des approches de criblage virtuel (docking). Ainsi, parmi les 12 000 composés criblés, 14 molécules prédites comme étant les plus affines ont été testés *in vitro*, ce qui a permis d'identifier un puissant inhibiteur de l'APH(2'')-IVa. Sa caractérisation cinétique suggère une inhibition non-compétitive vis-à-vis de l'aminoglycoside. Dans le but de confirmer son mécanisme d'inhibition et les interactions clés gouvernant sa fixation, des essais cristallographiques sont en cours. Les études structure-fonction permettront d'optimiser la molécule pour améliorer son activité mais également de mieux comprendre le mécanisme général ces enzymes.

[1] Lallemand et al. (2012) FEBS Lett. 586(23):4223-7

Mots clés : Aminoglycoside, Résistance bactérienne, Inhibiteur, Docking

Communication orale 09.

Caractérisation des galactofuranosyltransférases de *Leishmania*.

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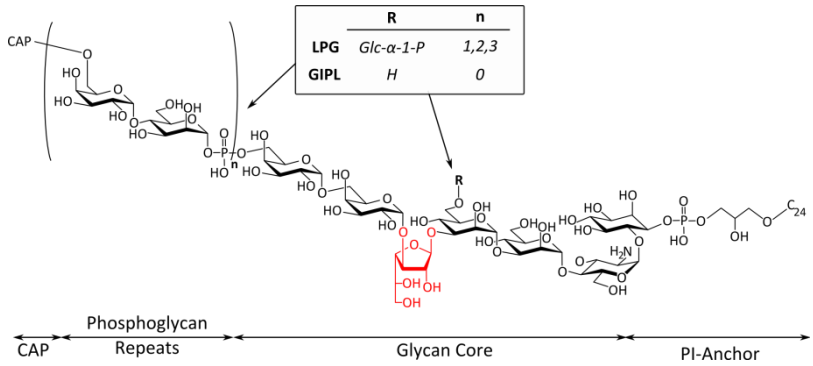
La leishmaniose est considérée comme l'une des Maladies Tropicales Négligées les plus fatales, touchant particulièrement les populations les plus pauvres[1]. A ce jour, aucun vaccin ni traitements médicamenteux efficaces ne sont accessibles à ces populations. La surface cellulaire du parasite *Leishmania* est recouverte de glycanes complexes, dont le rôle lors de l'infection et la survie du microorganisme dans l'environnement hostile de l'hôte infecté a été démontrée[2]. Ces glycanes sont particuliers, car ils contiennent notamment une unité monosaccharidique unique (le **Galactofuranose** Gal*f*), que l'on ne retrouve pas chez les mammifères (voir figure)[3]. La présence de ce sucre inhabituel représente donc un moyen de cibler spécifiquement les voies de biosynthèse de l'enveloppe polysaccharidique des leishmanies.



Dans le génôme de *Leishmania*, 4 **galactofuranosyltransférases (Gal*f*T)** potentiellement responsable de l'incorporation de ce monosaccharide ont été identifiées (LPG1, LPG1G, LPG1L et LPG1G) [4].

- Une quantification des ARNm par RT-qPCR à partir d'isolats cliniques de leishmanies pathogènes a permis de caractériser leur niveau d'expression lors du cycle de vie du parasite.
- Le clonage, la surexpression chez *E. coli* et la purification de ces 4 Gal*f*T (les seules identifiées à ce jour provenant d'un organisme eucaryote) permettent d'envisager à court terme la caractérisation biochimique de ces enzymes (études structurales et mécanistiques).

L'étude au niveau moléculaire de ces enzymes pourra mener à la conception de composés inhibiteurs ayant potentiellement des activités anti-parasitaires.[5]

[1] W.H.O. report, Vol. 949, 2010. [2] Franco et al, J. Parasitol. Res. 2012 (2012) 165126 [3] Peltier et al, Carb Res 343 (2008) 1897-923 ; Chublunova et al 356 (2012) 344-51 [4] Zhang et al, Mol. Biochem. Parasitol. 136 (2004) 11-23. [5] Dureau et al, Carb Res, 345 (2010) 1299-305.

Mots clés : Galactofuranosyltransférases, *Leishmania*, Galactofuranose, synthèse de la paroi cellulaire

Communication orale 10.

An extensive conformational sampling of the Rossman fold domain is integral to the catalytic mechanism of a bacterial acylating acetaldehyde dehydrogenase

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JEUDI 5 SEPTEMBRE 2013

PROGRAMME DE L'APRES-MIDI : 14h00 à 19h30

	Session 3 : Régulations des enzymes
14h00	**Conférence plénière 04 - E.K. Jaffe :** The morpheein model of protein allostery - an anomaly or an overlooked generalization?
14h45	**Communication orale 11** - **L. Chaloin :** Allosteric inhibitors against the cytosolic 5'-nucleotidase II for overcoming resistance to anticancerous treatments
15h00	**Communication orale 12 - B. Golinelli-Pimpaneau :** Morpheein-type allosteric regulation of *E. coli* glucosamine-6-phosphate synthase
15h15	**Communication orale 13 - F. Lecaille :** Modulation de l'activité de la cathepsine S par le chondroïtine 4-sulfate
15h30	**Communication orale 14 - M. Reboud-Ravaux :** Le principe de bivalence pour obtenir de puissants inhibiteurs non covalents du protéasome humain
15h45	Pause café / Session posters
	Session 4 : Approches biophysiques
16h45	**Conférence plénière 05 - A. Krieger-Liszkay :** Generation of singlet oxygen in photosystem II
17h30	**Communication orale 15** - **M. Moussaoui :** Relationship between different flavohemoglobin structural states and its enzymatic activity
17h45	**Communication orale 16 - H. Munier-Lehmann :** CBS domains control allostery behavior and fibre formation in IMPDHs through a common octameric architecture
18h00	**Communication orale 17 - Y-H. Sanejouand :** Dynamic of conserved water molecules in family 1 glycoside hydrolases: an X-ray, DXMS and Molecular Dynamics study
18h15	**Communication orale 18 - G. Truan :** Echanges conformationnels rapides dans une protéine multidomaine: la NADPH cytochrome P450 réductase

	Remise des Prix
18h30	**Prix SFBBM, article de l'année :** **A. Fleurie** : Fine tuning of bacterial cell division and elongation by the StkP kinase
18h45	Prix du meilleur poster
19h00	Session posters
19h30	Cocktail dinatoire / Session posters

SESSION 3

Régulations des enzymes

Conférence plénière 04 :

The morpheein model of protein allostery - an anomaly or an overlooked generalization?

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The unexpected behavior of the enzyme porphobilinogen synthase sets a precedent we have generalized as the morpheein model of protein allostery wherein trapping alternate architecturally distinct multimers serves as a new approach to allosteric drug discovery. By definition, porphobilinogen synthase is the prototype morpheein. Using largely behavioral criteria, ~40 proteins have been identified as putative morpheeins, some of which are drug targets for treatment of inborn errors of metabolism and/or cancer. We will present data addressing the quaternary structure dynamics of two putative morpheeins, phenylalanine hydroxylase and adenylosuccinate lyase and relate these results to the identification of new therapies for phenylketonuria and adenylosuccinate lyase deficiency.

Communication orale 11.

Allosteric inhibitors against the cytosolic 5'-nucleotidase II for overcoming resistance to anticancerous treatments

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Enzyme function can be blocked by small inhibitors through a competitive mechanism (the inhibitor binds into the substrate site). However, this kind of inhibition may turn out fully inefficient at high concentrations of substrate. A way to overcome this major drawback is to design inhibitors that target allosteric or regulatory sites of the enzyme. These inhibitors which bind to another site cannot be ruled out by a high amount of substrate. In this study, we focused on the cytosolic 5'-nucleotidase II (cN-II) that possesses one active and two regulatory sites. The function of cN-II is to maintain the intracellular level of nucleosides by dephosphorylating their monophosphate counterparts (IMP or GMP). However, several clinical evidences have reported a correlation between cN-II expression in cancer cells and patients' outcome treated with cytotoxic nucleoside analogues. For instance, the treatment of acute myeloid leukemia leads to a worse patient outcome when cN-II is over-expressed. One way to define the exact role of cN-II in the phenomenon of cancer therapy resistance is to develop new inhibitors[1,2]. Virtual screening of chemical libraries has been used to find allosteric inhibitors by targeting the cavity formed by the second regulatory site of this enzyme. Conventional strategies make use of a single crystal structure for screening the target protein of interest. In our study, we explored a larger conformational space by including several protein conformers issued from molecular dynamics. Most interesting compounds according to their ranking places were tested with the recombinant enzyme in order to evaluate their inhibitory activity. Three hit compounds were able to block the nucleotidase activity and were further characterized by kinetics. Interestingly, one of them showed an uncompetitive inhibition while other similar compounds exhibited either competitive or mixed inhibition. Moreover, *in vitro* validated hit compounds were further evaluated in cancer cell lines to determine their potential effect in sensitization to anticancer drugs. Interestingly, they were shown to increase the tumoral cell toxicity and current experiments are in progress to define the synergic effect with anticancerous drugs. This work raises the importance in the selection of the targeted cavity for enzyme inhibition that, in fine, may lead to more accurately defined inhibitor type. In addition, this study will provide new small candidate molecules to be used in combination with anticancer drugs for overcoming resistance phenomena encountered in cancer treatment.

[1] Gallier F. et al. PLoS Comp. Biol. ***(2011)*** *7(12): e1002295.doi:10.1371*
[2] Jordheim L.P.et al. Biochemical Pharmacology, ***(2013)*** *85, 497-506*

Mots clés : Enzyme inhibition / allostery / resistance / virtual screening

Communication orale 12.

Morpheein-type allosteric regulation of *E. coli* glucosamine-6-phosphate synthase

Stéphane Mouilleron[1], Marie-Ange Badet-Denisot[2], Ludovic Pecqueur[1,3], Karine Madiona[1], Nadine Assrir[2], Bernard Badet[2], and Béatrice Golinelli-Pimpaneau[1,3]

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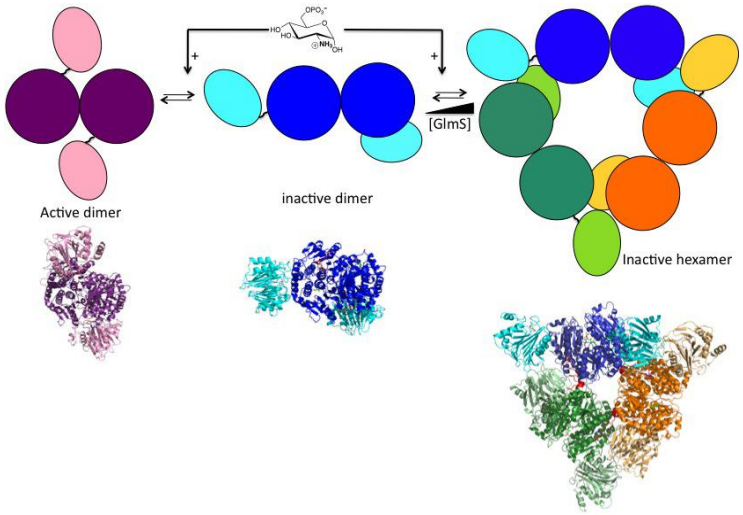
Glucosamine-6P synthase (GlmS) catalyzes glucosamine-6P synthesis from fructose-6P and glutamine. The amino-terminal cysteine acts as a nucleophile to release ammonia from glutamine, which is then transferred to fructose-6-P through an 18 Å-long channel. Although wild-type GlmS is active as a dimer, it is in equilibrium with a hexameric state, in vitro and in vivo. The hexameric state has also been visualized in the inactive C1A mutant crystal structure. The shift of the equilibrium toward the hexameric form in the presence of cyclic glucosamine 6-phosphate, together with the decrease of the specific activity with increasing enzyme concentration, strongly supports product inhibition through hexamer stabilization. Altogether, our data allow us to propose a morpheein model, in which the active dimer can rearrange into a transiently stable form, which has the propensity to form an inactive hexamer (1). This would account for a physiologically relevant allosteric regulation of *E. coli* GlmS.



[1] J. Biol. Chem., 2012, 287, 34533

Key words: morpheein-type allosteric regulation, glutamine-dependent amidotransferase, ammonia channeling

Communication orale 13.

Modulation de l'activité de la cathepsine S par le chondroïtine 4-sulfate

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La cathepsine S est une protéase à cystéine humaine qui participe à de nombreux processus physiologiques et pathologiques tels que l'asthme, l'arthrite rhumatoïde, le psoriasis, l'athérosclérose ou encore l'obésité [1]. Différentes études ont montré que dans ces pathologies, la cathepsine S est fortement exprimée et participe à la dégradation de différents constituants de la matrice extracellulaire et de la membrane basale (collagène IV, nidogène) [2]. Ainsi, la cathepsine S a été validée comme cible thérapeutique dans le traitement de certaines de ces maladies.

Les glycosaminoglycanes (GAG) sont des polysaccharides linéaires chargés négativement présents dans les lysosomes, à la surface des cellules et au niveau de la matrice extracellulaire, qui interagissent et régulent différentes molécules, comme les facteurs de croissance. Les GAG sont aussi capables de réguler l'activité de certaines protéases et faciliter leur maturation.

Dans ces conditions, l'objectif de notre étude était de savoir si les GAG sulfatés (héparine, héparane sulfate, chondroïtines 4- et 6-sulfate, dermatane sulfate) et non sulfaté (acide hyaluronique) participent à la modulation de l'activité protéolytique de la cathepsine S [3].

L'activité protéolytique de la cathepsine S vis-à-vis du collagène IV et d'un substrat fluorogénique, Z-Phe-Arg-AMC est significativement réduite *in vitro* par le chondroïtine 4-sulfate (C4-S) de façon dose-dépendante, selon un mécanisme d'inhibition mixte (Ki ≈16.5 µM). L'activité de la cathepsine S est rétablie en présence de NaCl, ce qui suggère que le C4-S perturbe l'activité de cette enzyme via des interactions électrostatiques. D'autre part, des doses croissantes en C4-S ont pour effet de ralentir la maturation de la procathepsine S à pH acide. Un complexe entre le C4-S et la cathepsine S a été mis en évidence en solution par gel filtration et l'affinité a été mesurée par résonance plasmonique de surface (Kd = 210 ± 40 nM). Trois sites de fixation potentiels du C4-S ont été identifiés (dont un à proximité du site actif de la cathepsine S) par modélisation moléculaire.

[1] F. Lecaille, J. Kaleta & D. Brömme. (2002). ***Chem. Rev.*** 102, 4459-4488. *[2] J. Sage, E. Noblesse, C. Nizard, T. Sasaki, S. Schnebert, E. Perrier, R. Kurfurst, D. Brömme, G. Lalmanach & F. Lecaille. (2012)* ***PLoS ONE*** *7, e43494. [3] J. Sage et al.,* ***J. Mol. Biol.*** *(en révision).*

Mots clés : protéases, glycosaminoglycanes, inhibition, modélisation moléculaire

Communication orale 14.

Le principe de bivalence pour obtenir de puissants inhibiteurs non covalents du protéasome humain

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La multivalence est utilisée par la nature dans beaucoup d'interactions biologiques simultanées entre ligands et récepteurs. Nous avons utilisé ce principe pour augmenter l'affinité et la biodisponibilité d'inhibiteurs non covalents du protéasome que nous avions développés précédemment.[1,2] Ces inhibiteurs sont des mimes linéaires d'un inhibiteur naturel peptidique cyclique, le TMC-95A. La topographie unique des six sites actifs de cette enzyme multicatalytique a été exploitée pour concevoir et synthétiser des inhibiteurs puissants et potentiellement très sélectifs possédant deux têtes inhibitrices reliées par des espaceurs organiques de longueur contrôlée. Leur fixation simultanée dans un site chymotryptique et un site tryptique a été mise en évidence par les études cinétiques et la cristallographie aux rayons X de complexes protéasome/inhibiteurs. L'utilisation du concept de la multivalence a ainsi conduit à des inhibiteurs efficaces (K_i = 6-11 nM) et sélectifs du protéasome.[3,4] Doués d'efficacité cellulaire, ces inhibiteurs sont de bons candidats à des études plus poussées en vue d'applications thérapeutiques. L'inhibition de la dégradation intracellulaire des protéines catalysée par le protéasome fait en effet de celui-ci une cible thérapeutique actuelle,[5] notamment pour les inhibiteurs non covalents, très peu représentés.(5,6)

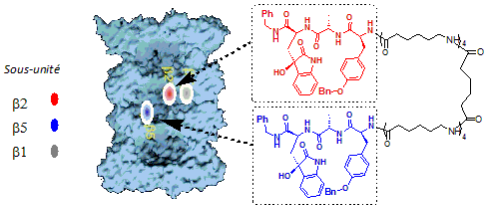

Figure 1. Ciblage simultané des sites actifs de type chymotryptique (ChT-L) et tryptique (T-L) du protéasome 20S humain par les nouveaux inhibiteurs bivalents.

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Mots clés : protéasome ; inhibiteurs non covalents ; inhibiteurs bivalents ; conception rationnelle; mimes du TMC-95A.

SESSION 4

Approches biophysiques

Conférence plénière 05 :

Generation of singlet oxygen in photosystem II

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Photosynthetic organisms are subjected to photo-oxidative stress when more light energy is absorbed than used in photosynthesis. In the light, highly reactive singlet oxygen can be produced via triplet chlorophyll formation in the reaction centre of photosystem II (PSII). Changes in the midpoint potential of the primary quinone acceptor Q_A in PSII modulate the pathway of charge recombination in PSII, the formation of excited chlorophyll in its triplet state and influence the yield of singlet oxygen production.
By titrating variable chlorophyll fluorescence, we have determined the midpoint potential of the redox couple Q_A/Q_A^- to be -80 mV in PSII with an active oxygen evolving complex (OEC) and to be +65 mV when Ca^{2+}, an obligatory cofactor of the OEC, is released. Singlet oxygen generation was measured with the $^1O_2$ probe TEMP using EPR spectroscopy. The midpoint potential of Q_A was also modified by using specific herbicides which bind to the Q_B-binding pocket, the site to which the secondary quinone acceptor binds. The effect of herbicides on Q_A was monitored by measuring chlorophyll fluorescence and thermoluminescence. Phenolic herbicides shifted the midpoint potential of Q_A to a lower value while urea-type herbicides had the opposite effect. Furthermore, the midpoint potential of Q_A was affected by the mutation of A249S located at the Q_A-binding site.
The influence of different herbicides on the midpoint potentials of the primary quinone acceptor Q_A can be used as a tool to modify the yield of $^1O_2$ production in PSII. While production of hydroxyl and superoxide anion radicals were herbicide-independent, the yield of $^1O_2$ with a phenolic herbicide was twice that with an urea herbicide. In addition, an upregulation of gene expression in response to $^1O_2$ produced by PSII was observed. This was tested by following the expression of a reporter gene construct and of a glutathione peroxidase homologous gene from *Chlamydomonas*. These genes were specifically up-regulated by $^1O_2$ in the presence of a phenolic herbicide while they were down-regulated in the presence of DCMU.

Communication orale 15.

Relationship between different flavohemoglobin structural states and its enzymatic activity

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Flavohemoglobins (flavoHbs) are widespread only within microorganisms and play a key role in the bacterial resistance to nitrosative stress and NO• signaling modulation. Typically, flavoHbs contains three domains: an N-terminal globin domain which harbors a single heme type b and a C-terminal ferredoxin reductase-like FAD- and NAD-binding module. Since flavoHbs are not present in mammals, flavoHb inhibition may be an efficient approach to decrease pathogen virulence. Several azole-based heme iron ligating compounds, in particular antifungal imidazole derivatives, inhibit the flavoHb-catalyzed NO•-dioxygenase activity. Recently, we have determined the structural basis for binding imidazole derivatives to flavoHbs [1, 2]. We have shown that these enzymes adopt open and closed states dependent on the absence, presence and chemical nature of the heme ligand. We have also determined kinetically and thermodynamically one step of the internal electron transfer [3].To make progress in understanding the structure, function and mechanism of flavoHbs we have cloned and produced the flavoHb from Staphylococcus aureus, responsible for a large number of human infections and investigated the enzyme properties, the effect of azole antibiotics and the structure-function relationship in comparison to the well-known flavoHbs from the non-pathogenic bacteria. The mutation of key residues situated in the flexible region of the protein appeared to modify drastically the enzymatic properties suggesting that peculiar residue might play a crucial role in regulation of the enzyme activity.

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Mots clés : Flavohemoglobins, NO•-dioxygenase, Rigid-body motion, Structure-Function relationship, Bacterial resistance

Communication orale 16.

CBS domains control allostery behavior and fibre formation in IMPDHs through a common octameric architecture

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Inosine-5'-monophosphate dehydrogenase (IMPDH)[1,2] is a major target for antiviral, antiparasitic, antileukemic and immunosuppressive therapies. It is an ubiquitous and essential enzyme for the biosynthesis of guanosine nucleotides. Up to now, IMPDHs have been reported as tetrameric enzymes harbouring a catalytic domain and a tandem of cystathionine-ß-synthase (CBS) modules. The latter have no precise function assigned despite their nearly absolute conservation among IMPDHs and consistent indication of their importance *in vivo*. The aim of our study was to provide evidence for the role of the CBS modules on the quaternary structure and on the regulation of IMPDHs.
A multidisciplinary approach involving enzymology, site-directed mutagenesis, analytical ultracentrifugation, X-ray crystallography, SAXS, cryo-electron microscopy and molecular modelling allowed us to demonstrate that the *Pseudomonas aeruginosa* IMPDH is functionally active as an octamer and allosterically regulated by MgATP via each CBS module. Revisiting deposited structural data we found this newly discovered octameric organization conserved in other IMPDH structures. Furthermore, we demonstrated that the human IMPDH1 formed two distinct octamers that can pile up into isolated fibres in the presence of MgATP while its pathogenic mutant D226N, localised into the CBS domains, appeared to form massively aggregating fibres. The dramatic impact of this mutation could explain the severe retinopathy adRP10.
Our data[3] revealed for the first time that IMPDH has an octameric architecture modulated by MgATP binding to the CBS modules, inducing large structural rearrangements. Thus, the regulatory CBS modules in IMPDHs are functional and they can either modulate catalysis or/and macromolecular assembly. Targeting the conserved effector binding pockets identified within the CBS modules might be promising to develop antibacterial compounds or drugs to counter retinopathy onset.

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Mots clés : nucleotide metabolism, supramolecular assembly, CBS domains, crystal structure, site-directed mutagenesis, effector binding site

Communication orale 17.

Dynamic of conserved water molecules in family 1 glycoside hydrolases: an X-ray, DXMS and Molecular Dynamics study

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Taking advantage of the wealth of structural data available for family 1 glycoside hydrolases (GH1), a study of the conservation of the numerous internal water molecule sites (WMS) found in this family was undertaken1. It appears that more than 70% of these WMS are well conserved among evolution, as they are found in the same position in more than 60% of the 193 molecules from 74 PDB structures that originate from the three kingdoms of life. Interestingly, 75% of these WMS are not isolated within the structures but belong to several clusters of WMS, potentially revealing water molecule paths.

Such a high conservation among evolution substantiates for an important structural or functional role. However, the determination of this role only on the basis of the static X-ray coordinates appears to be puzzling. Then, to assess which among the conserved WMS thus found have significant intrinsic dynamics, molecular dynamics simulations were performed on the β-glycosidase of Thermus thermophilus, the GH1 for which the highest resolution of X-ray data are available (0.99Å, 1ug6.pdb). Inside the protein, global or partial displacements of water molecules observed during MD simulations mainly occur along the suggested paths. Interestingly, several water molecules from these WMS could exchange with either bulk water or water from the active site, suggesting the existence of these “water channels” inside this protein could participate to the functional mechanism. Such a functional water channel has been proposed for the 1,3-α-3,6-anhydro-L-galactosidase of Zobellia galactanivorans (GH117), which may provide the nucleophile water molecule to the active site2.

In order to have an experimental confirmation of these water channels, we performed Deuterium Exchange Mass Spectrometry (DXMS) on the β-glycosidase of Thermus thermophilus to analyze the exchange rates of the protein protons with the solvent protons, i.e. their accessibility to water. DXMS confirmed a high-rate exchange zone inside the protein corresponding to the largest water channel identified by Molecular Dynamics simulations.

These results strongly suggest that there are conserved water channels inside GH1, which might have an important role in the function of the protein.

Mots clés : Glycoside hydrolases, water channel, DXMS, molecular dynamics

Communication orale 18.

Echanges conformationnels rapides dans une protéine multidomaine: la NADPH cytochrome P450 réductase

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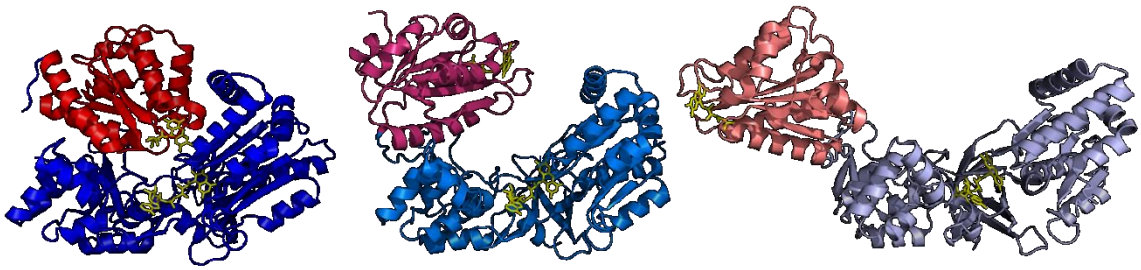
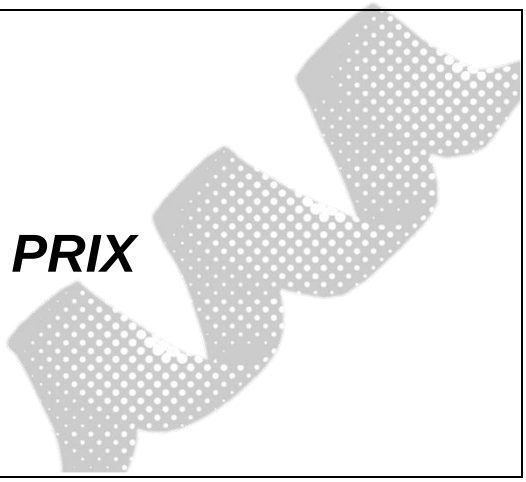
La NADPH-cytochrome P450 réductase (CPR) est une protéine multidomaine composée : d'un domaine contenant un FAD et un domaine contenant un FMN reliés par un domaine contenant un linker flexible et un domaine en hélice alpha. A partir d'analyses à la fois structurales et biochimiques, la communauté scientifique a mis en évidence des mouvements importants des domaines catalytiques (voir figure) entre deux états (fermé et ouverts) représentant chacun une étape du cycle catalytique (état fermé : transfert entre FAD et FMN ; état ouvert : transfert entre FMN et accepteurs). Nos travaux démontrent qu'en en absence de cycle catalytique, la CPR est présente sous forme d'un équilibre dynamique rapide entre la conformation rigide et fermée (également appelé état verrouillé) et une forme nouvellement caractérisée et hautement flexible où les contacts interdomaines sont quasiment absents (état déverrouillé). La force ionique et le pH contrôlent les populations relatives des deux états à l'équilibre. Un modèle cinétique a été établi pour décrire la variation de l'efficacité du flux d'électrons en fonction du changement conformationnel rapide entre les états actifs aux différentes étapes du cycle catalytique. Ce mécanisme de régulation cinétique pourrait constituer un point clé dans la conception de nouvelles enzymes multidomaines synthétiques au sein desquelles des changements conformationnels seraient nécessaires pour réaliser la catalyse.



Mots clés : Protéine multidomaine, transfert d'électrons, dynamique, mouvements de domaines, SAXS, RMN

REMISE DES PRIX



Remise des Prix

Prix SFBBM du concours "l'article du mois" : article de l'année 2012

A. Fleurie : Fine tuning of bacterial cell division and elongation by the StkP kinase

Prix du meilleur Poster

Fine tuning of bacterial cell division and elongation by the StkP kinase

Aurore Fleurie[1], Chao Zhao[1], Sylvie Manuse[1], Nathalie Campo[2], Caroline Cluzel[3], Jean-Pierre Lavergne[1], Céline Freton[1], Christophe Combet[1], Sébastien Guiral[1], Boumediene Soufi[4], Boris Macek[4], Anne-Marie Di Guilmi[5], Jean-Pierre Claverys[2], Anne Galinier[6] et Christophe Grangeasse[1]

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Bacteria possess a versatile repertoire of protein-kinases. With mounting evidence of the role of serine/threonine and tyrosine signaling in the regulation of diverse cellular processes, their critical role is gaining prominence. Recently, we have demonstrated that the membrane Hanks-type serine/threonine kinase StkP plays a central role in regulating cell wall synthesis and controls septum positioning, assembly and closure in *Streptococcus pneumoniae*. However, the underlying regulatory mechanisms remain unknown. In addition, little information is available on the mechanisms responsible for peptidoglycan synthesis and how pneumococcus achieves its characteristic ellipsoid-shape. Thus, investigating StkP-mediated phosphorylation represents a promising avenue to decipher the regulatory mechanisms governing *S. pneumoniae* morphogenesis and division. We have already observed that DivIVA could be phosphorylated on Thr-201 by StkP and that expression of non-phosphorytable DivIVA affects cell shape integrity. Here I will present our most recent results related to the role of StkP in pneumococcal cell morphogenesis. We notably identify a modulator of StkP kinase activity that is required for StkP septal localization and subsequent phosphorylation of DivIVA. We show that the StkP/DivIVA/modulator triad connects the Z-ring to the peptidoglycan biosynthesis machinery. We propose that StkP regulates the dynamics of septal and peripheral peptidoglycan synthesis responsible for the ovoid-shape of pneumococcus cells.

VENDREDI 6 SEPTEMBRE 2013

PROGRAMME DU MATIN : 09h00 à 12h30

	Session 5 : Applications et interfaces
09h00	**Conférence plénière 06 - J. Valton :** XPC Gene Therapy Using TALEN™
09h45	**Communication orale 19 - A. Frelet-Barrand :** *Lactococcus lactis*, recent developments in functional heterologous expression of membrane proteins
10h00	**Communication orale 20 - C. Lionne :** Nouveaux dérivés de néamine insensibles à l'inactivation par une enzyme de résistance bactérienne
10h15	**Communication orale 21 - X. Maréchal :** Inhibition de la différenciation des myoblastes par des inhibiteurs de l'enzyme insulysine développés par click chemistry *in situ*
10h30	**Communication orale 22 - F. Mavré :** Electrochemical characterization of the soluble PQQ-dependent glucose dehydrogenase. Effect of directed mutagenesis on selectivity and cooperativity
10h45	Pause café / Session posters
11h15	**Communication orale 23 - Hervé Chaulet, Société Agilent Technologies Genomics :** Herculase II & Applications
11h30	**Communication orale 24 - A. Vergès :** Diversification of *Neisseria polysaccharea* amylosucrase activity through a semi-rational engineering approach
11h45	**Communication orale 25 - M. Vuillemin :** Search for new GH-70 α-transglucosylases in lactic acid bacteria diversity dedicated to the synthesis of tailor-made α-glucans
12h00	**Communication orale 26 - J. Dairou :** Mise au point d'un dosage de l'activité kinase de la protéine DYRK1A par HPLC
12h15	Conclusions
12h30	Déjeuner et départ

SESSION 5

Applications et interfaces

Conférence plénière 06 :

XPC Gene Therapy Using TALENTM

Julien Valton[1], Fayza Daboussi[1‡], Aurélie Dupuy[1, 2‡], Roman Galetto[1], Agnès Gouble[1], Jacques Armier[2], Anne Stary[2], Alain Sarasin[2], and Philippe Duchateau[1]

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Xeroderma Pigmentosum group C (XPC) is a rare, autosomal, recessive syndrome characterized by hypersensitivity to UVC light associated with a dramatic predisposition to skin neoplasms. XPC cells are deficient in the nucleotide excision repair (NER) pathway, a complex process involved in the recognition and removal of DNA lesions. XP-C mutation corresponds to the deletion of a TG dinucleotide (ΔTG) located in the middle of exon 9 of the human *XPC* gene. This deletion leads to the expression of an inactive truncated XPC protein, normally involved in NER mechanism. The lack XPC activity potentializes UVC dependent DNA damages and is responsible for the development of high number of skin cancers. In the past few years, several studies have demonstrated the tremendous potential of nuclease-based targeted approaches for gene correction. These approaches rely on the ability of engineered nucleases known as Meganucleases, Zinc Finger nucleases or TALENs, to precisely generate a double strand break at a specific locus and promote its correction through the insertion of an exogenous DNA repair matrix. Here, we describe the targeted correction of *XPC* mutation in the XPC deficient cell line using TALEN™. The presence of methylated CpGs in the *XPC* locus, an epigenetic modification known to inhibit TALEN DNA binding capacity, led us to adapt its design and our experimental conditions to optimize its *in vivo* efficacy. Our results show that our approach enabled successful gene correction of the *XPC* locus, re-expression of full-length XPC protein and allow full recovery of wild-type UVC resistance of the XPC deficient cell line. Overall, we demonstrate that TALEN-based targeted approach represents reliable and efficient strategies for gene correction. In addition to these results, the design and specificity of TALENs will be discussed.

Communication orale 19.

Lactococcus lactis, recent developments in functional heterologous expression of membrane proteins

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In spite of the functional and biotechnological importance of membrane proteins, their study remains difficult because of their hydrophobicity and their low abundance in the cells. Moreover, in the well known heterologous systems, these proteins are often produced at very weak rates, toxic and/or not correctly folded up. *Lactococcus lactis*, Gram-positive lactic bacterium, traditionally used in food fermentation, are now largely used in biotechnology for the production on a large scale of prokaryotic and eukaryotic proteins [1,3,4,5,6]. The expression of 20 membrane proteins was tested in this bacterium, proteins which could not be produced in sufficient quantities by using traditional systems of expression. With this intention, a strategy of cloning in the vector of expression of *L. lactis*, pNZ8148, compatible with the Gateway entry vectors has been preliminary established. Thus, *L. lactis* proved to be an alternative system of expression for membrane proteins. First, all the proteins of *Arabidopsis thaliana* tested could be produced on levels compatible with biochemical analyses. Processes of solubilization and purification could also be developed and several proteins were active [2,4,5,6]. Moreover, since last year, some human proteins could be expressed and active in this system [7, 8, unpublished data]. Finally, these data suggest that *L. lactis* would be an attractive system for an effective and functional production of difficult membrane proteins.

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Mots clés : *Lactococcus lactis*, membrane proteins, Gateway

Communication orale 20.

Nouveaux dérivés de néamine insensibles à l'inactivation par une enzyme de résistance bactérienne

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Les aminoglycosides, dont font partie la kanamycine et la streptomycine, sont des antibiotiques à large spectre, principalement actifs sur les bactéries Gram (–), notamment les entérobactéries et *Pseudomonas aeruginosa*, mais aussi sur quelques bactéries Gram (+), en particulier les staphylocoques, et sur certaines mycobactéries. Leur utilisation a cependant été ralentie par des problèmes liés, d'une part à leur néphro ou ototoxicité, résultant d'interactions non spécifiques avec l'ARN, et d'autre part, à la résistance bactérienne causée principalement par l'inactivation de l'antibiotique par des enzymes de modification.
L'analyse des éléments structuraux communs à la famille des aminoglycosides naturels ou semi-synthétiques montre qu'ils contiennent souvent un noyau néamine. Tout en ayant une activité antibiotique modeste, il constitue cependant un motif minimal requis pour la liaison à la cible bactérienne, l'ARN ribosomal 16S. Par conséquent, ce pseudo disaccharide a été utilisé comme élément de base pour générer de nouveaux antibiotiques potentiels dans le but de contourner la résistance microbienne, tout en conservant l'activité et en diminuant la toxicité associée[1].
Nous avons testé la sensibilité de ces nouveaux dérivés de néamine à l'inactivation bactérienne par une des enzymes impliquées dans la résistance, l'Aminoglycoside PHosphotransférase(3')-IIIa, APH (3')-IIIa. Pour cela, nous avons comparé les constantes de vitesse obtenues avec ces dérivés et le composé de départ, la néamine, par une méthode préalablement validée[2]. L'activité de l'enzyme est significativement réduite avec deux d'entre eux, suggérant qu'ils sont moins susceptibles que la néamine à l'inactivation par phosphorylation. Ces molécules ont été testées sur des souches d'*E. coli* sur-exprimant l'APH(3')-IIIa. Nous avons montré que les deux composés qui étaient les moins bons substrats pour l'APH(3')-IIIa *in vitro*, permettaient de restaurer la sensibilité à la kanamycine A à un niveau comparable à celui d'*E. coli* n'exprimant pas d'APH.
Le docking de ces nouveaux dérivés de la néamine dans le site actif de l'APH(3 ')-IIIa donne des explications structurales aux résultats biochimiques et biologiques observés.

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Mots clés : Antibiotiques, enzymes bactériennes, phosphorylations, composés chimiques à visée thérapeutique.

Communication orale 21.

Inhibition de la différenciation des myoblastes par des inhibiteurs de l'enzyme insulysine développés par click chemistry *in situ*

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L'insulysine (ou IDE, insulin degrading enzyme) est une metalloprotéase à zinc (EC 3.4.24.56) hautement conservée et ubiquitaire. En dégradant des substrats amyloïdogéniques comme le peptide amyloïde β et l'amyline, ou l'insuline, IDE pourrait intervenir dans certains processus pathologiques tel que la maladie d'Alzheimer ou le diabète de type 2 respectivement. Des fonctions d'IDE sont également attribuées à son interaction avec d'autres protéines comme le protéasome ou la glycoprotéine E du virus de la varicelle. Epting *et al*. ont démontré que IDE était associée à la protéine Stem Cell Antigen 1 (SCA-1 ou Ly-6A/E) dans les myoblastes C2C12 en différenciation[1]. Un défaut d'expression de cette protéine au sein des cellules C2C12 provoque une absence de différenciation de ces dernières[2]. De plus une diminution de l'expression d'IDE aboutit également à un défaut de différenciation des cellules C2C12[1].

Nous avons testé des inhibiteurs d'IDE développés par click chemistry *in situ* pour leur capacité à inhiber la différenciation des cellules C2C12. Les cellules ont été traitées durant les deux heures précédant leur différenciation et durant toute la durée de la différenciation. En calculant l'index de fusion des myoblastes ainsi qu'en regardant l'expression de gène codant des protéines marqueurs de différenciation (tels que MyoG ou Myh1), nous avons pu montrer qu'une molécule inhibitrice provoquait un défaut dans la différenciation des cellules C2C12 de manière dose dépendante. En revanche un composé analogue 100 fois moins actif sur l'enzyme IDE isolée ne modifie pas la capacité des C2C12 à se différencier.

Par cette étude nous confirmons le rôle d'IDE dans le mécanisme de différenciation de cellules myoblastiques. De plus l'effet de ces inhibiteurs sélectifs sur ce modèle cellulaire et leurs caractéristiques physicochimiques et pharmacodynamiques favorables font de ces produits des outils pharmacologiques de grande utilité pour étudier les rôles d'IDE dans des modèles cellulaires et animaux.

[1]. Epting, CL. et al. 2008, J. Cell. Physiol. 217(1) : 250-60
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Mots clés : Insulysine, inhibiteur, myoblaste, différenciation, expression génique.

Communication orale 22.

Electrochemical characterization of the soluble PQQ-dependent glucose dehydrogenase. Effect of directed mutagenesis on selectivity and cooperativity

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The enzyme PQQ-GDH (pyrroloquinoline quinone dependent glucose dehydrogenase) is of particular interest for the detection of glucose in biosensor format. Indeed, in comparison with the traditionally used Glucose oxidase (GO), PQQ-GDH possesses a high catalytic efficiency towards glucose oxidation (> 10^6 $M^{-1}.s^{-1}$, that is two orders of magnitude higher than GO), a high turnover (> 1500 s^{-1}), insensitivity to dioxygen, as well as a capacity to accept a wide range of artificial electron acceptors. For these reasons, PQQ-GDH is a promising enzyme for several biotechnological applications such as biofuel cells or biosensors. However, the counterpart of its high reactivity is a poor selectivity. For instance, PQQ-GDH catalyzes the oxidation of several mono- or di-saccharides such as galactose or maltose. Some efforts have thus been made to improve the selectivity of this enzyme by directed mutagenesis with the aim to take advantage of improved mutants for the development of more sensitive and specific glucose biosensors.

With the aim to rationally improve the selectivity of PQQ-GDH by directed mutagenesis, we have undertaken a detailed kinetic analysis of its mechanism by redox-mediated electrochemistry. From such investigation, we were able to demonstrate substrate inhibition and cooperativity between the two subunits. This allostery is concomitant to an opening of the catalytic site pocket, leading to a decreased selectivity in cooperative mode. Through a rational approach of directed mutagenesis as well as a detailed study of the influence of each mutation on the mechanism, we were able to improve the selectivity by a factor 10 while conserving a reactivity superior to GO.

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Mots clés : glucose dehydrogenase, selectivity, cooperativity, electrochemistry, directed mutagenesis.

Communication orale 23.

Herculase II & Applications

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Herculase II Fusion DNA Polymerase provides you with superior yields in both routine and challenging PCR applications, with the additional benefit of allowing the use of fast cycling times. Some PCR targets are problematic to amplify due to their content or structure. Our Herculase II Fusion DNA Polymerase helps you overcome these PCR challenges with successful amplification of complex and GC-rich templates. The Herculase II enzyme also provides fidelity comparable to *Pfu* DNA Polymerase, which is 6 times more accurate than *Taq* DNA polymerase.

Applications where Herculase II Fusion DNA Polymerase Excels

Herculase II is an ideal polymerase to choose for applications where fidelity is required but may not be the only factor. (If fidelity is the primary concern, *PfuUltra* II Fusion HS DNA Polymerase would suit the application.) Combine high fidelity with Herculase II's ability to deliver unrivaled yields, extreme sensitivity, and the ability to amplify difficult samples, and you have an ideal polymerase for routine high fidelity use. In addition, Herculase II is economical enough to be used for high throughput applications.

Herculase II in Next-Gen Sequencing

Our data indicates that Herculase II is a superior polymerase for Next-Gen Sequencing. The high yields allow for fewer cycles of amplification thereby reducing PCR bias. In addition, during library preparation, Herculase II delivers the best size distribution of any polymerase with the vast majority of amplicons in the ideal sequencing size range. In conjunction with SureSelect, this leads to the need for fewer sequencing reactions and better sets of sequencing data.

Communication orale 24.

Diversification of *Neisseria polysaccharea* amylosucrase activity through a semi-rational engineering approach

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Chemical synthesis of complex oligosaccharides still remains critical. Enzymes have emerged as powerful tools to circumvent chemical boundaries of glycochemistry. However, natural enzymes do not necessarily display the required properties and need to be optimized by molecular engineering. Chemo-enzymatic approaches may thus be attractive for exploring new synthetic routes.

The amylosucrase from *Neisseria polysaccharea* (*Np*AS) is a transglycosylase that uses sucrose, a widespread and low-cost substrate, as glucosyl donor for the synthesis of α-glucans and glyco-conjugates[1]. A semi-rational engineering approach has been considered to extend the potential of this enzyme towards glycodiversification. Computational protein design methodologies were used to guide the construction of a library of *Np*AS mutants which was subsequently screened for their activity on sucrose utilization and transglucosylation reaction. A pH-based colorimetric screening protocol enabled first to isolate 57 sucrose-utilizing amylosucrase variants[2,3,4]. A secondary screening based on high-performance liquid chromatography was further applied to characterize the reaction products synthesized by these variants. Altogether 4 enzymatic variants displaying original product profiles or an enhanced sucrose activity compared to the parental wild-type enzyme were identified and characterized in more details at both biochemical and structural levels.

X-ray crystallography studies were also undertaken to better understand molecular determinants involved in the novel properties of amylosucrase mutants and improve our comprehension of the sequence-structure-function relationships with the view of providing new insights to guide the design of tailor-made enzyme libraries.

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Mots clés : semi-rational engineering, amylosucrase, glycodiversification, transglucosylation

Communication orale 25.

Search for new GH-70 α-transglucosylases in lactic acid bacteria diversity dedicated to the synthesis of tailor-made α-glucans

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The potential of carbohydrate active-enzymes to synthesize new polysaccharides from renewable resources is immense and far from having been fully explored and exploited. This paradox is due to several factors. Many biopolymers are synthesized from nucleotide activated sugars which are expensive. Their synthesis relies in some cases on enzymes which are difficult to isolate, characterize and produce at prices compatible with their use at the industrial scale. However, there are signs that this is changing. The demand for biopolymers from renewable natural resources often biodegradable and produced by environmental safe processes is increasing. Clearly, polymers produced by biological systems appear to be a promising alternative to petroleum-based polymers. In parallel, the progress of recombinant enzyme over-expression and engineering allows decreasing the enzyme cost, and offers new possibilities to improve the enzyme performances.

Working in the field of glycobiology, our group has a long standing interest in Glucansucrases, bacterial polymerases classified in GH family 70[1]. From sucrose, and without any nucleotide activated sugars, they catalyze the synthesis of high molecular mass homopolymers of glucose. These transglucosylases are also able to catalyze the transfer of glucosyl units onto exogenous hydroxylated acceptors for the production of oligosaccharides and glycoconjugates, thus giving access to a large variety of products[2,3]. This presentation will describe our recent findings on several very original Glucansucrases isolated thanks to a recent campaign of lactic acid bacteria genome sequencing. Distinctive specificities in term of i) glucan molecular masses and/or ii) degree of α-1,2 or α-1,3 branching onto linear α-1,6 backbones will be reported, as their impact on the physico-chemical properties of the final products.

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Mots clés : biopolymers, oligosaccharides, glucansucrases.

Communication orale 26.

Mise au point d'un dosage de l'activité kinase de la protéine DYRK1A par HPLC

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Le Syndrome de Down est une maladie chromosomique congénitale ayant pour cause première la présence d'un chromosome 21 surnuméraire. L'établissement des cartes de corrélation génotypes/phénotypes chez les patients atteints du Syndrome de Down a permis de mettre en évidence le rôle du gène *DYRK1A*. Celui-ci code pour une protéine kinase tyrosine-sérine/thréonine appartenant à la famille des DYRK ou «Dual specificity tyrosine phosphorylation-regulated kinase». Cette enzyme catalyse la phosphorylation sur des résidus serine et/ou thréonine de protéines cibles, en particulier certains facteurs de transcription, des protéines d'épissage et des protéines synaptiques.

Afin de comprendre les implications de DYRK1A dans le développement neuronal normal et pathologique, il est essentiel de disposer d'un test d'activité robuste de l'enzyme. Les méthodes de dosage de l'activité kinase de DYRK1A reposent essentiellement sur des approches utilisant la radioactivité. Cependant, ces méthodes sont très fastidieuses et ne permettent pas de développer un test haut débit pour par exemple le criblage d'inhibiteurs. Nous avons mis au point un dosage basé sur la séparation par chromatographie à haute pression (HPLC) d'un peptide substrat de l'enzyme et de son peptide produit phosphorylé. Afin d'augmenter la sensibilité du dosage, nous avons couplé le peptide à un groupement fluorescent. Cette méthode de dosage s'est révélée très rapide, peu onéreuse et sensible.

Le test a permis de confirmer les propriétés inhibitrices de certaines molécules identifiées comme inhibiteurs de DYRK1A. Finalement, l'utilisation d'extraits de cerveau de souris transgénique du syndrome de down a permis de confirmer que notre dosage peut être utilisé avec des milieux biologiques complexes.


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Poster 01.

Cost Function Network Optimization based Framework for Computational Protein Design

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The engineering of tailored proteins with desired properties holds great interest for applications ranging from medecine, biotechnology and synthetic biology and nanotechnologies. In recent years, computational protein design (CPD) approaches have demonstrated their potential to adequately capture fundamental aspects of molecular recognition and have already enabled the successful (re)design of several enzymes.

In CPD, the protein is often modeled as a fixed backbone with a set of mutable and flexible side chains. Flexible residues explore a discrete set of low-energy conformations (called rotamers) of the native amino acid type while mutable residues explore rotamers of all 20 possible amino acid residues or a reduced subset. The resulting combinatorial problem consists then in finding the combined assignment of amino acid type and rotamer at all variable positions that optimizes a target pairwise score.

The Dead-End Elimination (DEE) is the most commonly used deterministic CPD approach. Besides the benefit of provably finding the Global Minimum Energy Conformation (GMEC), such methods ensure that the source of any discrepancy between experimental results and CPD predictions resides in the inadequacies of the biophysical models and not the algorithm.

In this work, we modeled the CPD problem as a “Cost Function Network” [1] using toulbar2 (a dedicated open source CFN solver) and compared its performance against DEE implemented in Osprey 2.0 (a dedicated open source CPD software [2]) and Integer Linear Programing (ILP) using the IBM ILOG Cplex ILP solver [3]. The three methods were evaluated on a set of 35 benchmark systems showing the remarkable performance of the CFN-based framework that brings CPD beyond the limits of usual dedicated methods.

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Mots clés : Structural biology, combinatorial optimization, computational protein design

Poster 02.

Large-scale exploration of enzyme biodiversity to discover novel biocatalysts

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Some years ago, Genoscope, the French Sequencing Centre, had decided to diversify its activities to include large-scale functional genomics studies and to develop a high throughput platform for cloning genes and biochemical screenings of enzymes from prokaryote genomes and metagenomes. This platform is mainly use in a systematic enzymatic screening of large enzyme families to discover new biocatalysts for synthetic chemistry and metabolic engineering. We combine various bioinformatic genome analysis to select the smallest set of enzymes the most representative of the biodiversity and a systematic biochemical screening to explore the biocatalytic capabilities of a family.

Enzymes could have a large spectrum of activities and exploiting enzyme catalytic promiscuity might lead to new and efficient catalysts with, as yet, unprecedented activity for reactions where no enzyme alternatives exist today. Today, novelty in terms of biocatalysts is one of the most challenging problems. Moreover, a large majority of the known enzymatic reactions were discovered in cultivated bacteria but now we are faced with a new situation in which sequences from metagenomes, which are orphans of organisms, become technically available. These metagenome sequences provide a reservoir of genes and represent an interesting source of novel proteins that will indubitably extend our knowledge of enzymatic reactions and lead to discover novel biocatalysts to fulfill the needs of synthetic biology or industrial processes and to propose alternatives to chemical synthesis. The Genoscope strain collection includes today 750 prokaryote strains and a metagenome from a waste water plant reservoir. A collection of ~10 000 genes from oxidases (Bayer-villigerases, dioxygenases,..), hydrolases (nitrilases, lipases), lyases families (aldolases, tranketolases) and transferases (transaminases) have been cloned and are screened for new biocatalysts and new activities.

Mots clés : Large-scale exploration, biocatalysis, synthetic chemistry , biodiversity, screening, promiscuity, genome analysis, synthetic biology

Poster 03.

La signalisation cellulaire redox par les peroxydases à thiol : Quels sont les mécanismes moléculaires à l'origine de la spécificité du relais redox H_2O_2/Gpx3/Yap1 chez *S. cerevisiae* ?

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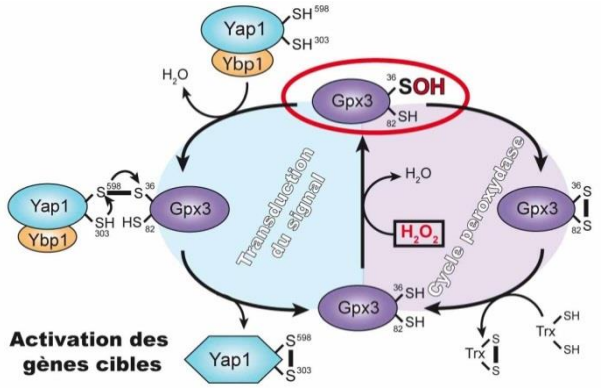
Les peroxydases à thiol, initialement identifiées en tant qu'enzymes antioxydantes, ont récemment été associées à la signalisation cellulaire redox comme détecteur et relais du signal transmis par H_2O_2 (1). Un des exemples les mieux documentés d'un tel mécanisme est l'activation du facteur de transcription Yap1, un régulateur clef de la réponse transcriptionnelle au stress H_2O_2 chez *Saccharomyces cerevisiae,* qui dépend de la formation de ponts disulfure intramoléculaires catalysés par la peroxydase à thiol Gpx3 (2 ; 3). Dans ce mécanisme, il a été proposé que le relais d'oxydation procède *via* un intermédiaire acide sulfénique porté par la Cys36 catalytique C_P, Gpx3-C_P-SOH (figure) (3). D'autre part, la protéine Ybp1 a été identifiée comme partenaire essentiel de l'activation de Yap1 par Gpx3 (4).



La réactivité intrinsèque d'un acide sulfénique implique l'existence de réactions en compétition avec la réaction d'activation de Yap1, en particulier avec la Cys82 de recyclage C_R de Gpx3 ou avec d'autres thiols cellulaires, ce qui soulève la question de la spécificité de l'activation de Yap1. Pour aborder cette problématique, et élucider le rôle de la protéine Ybp1 dans ce mécanisme, nous avons utilisé une approche combinant i) la caractérisation du cycle peroxydasique de Gpx3 dans le but de définir les paramètres cinétiques du relais redox ; ii) l'étude du rôle de Ypb1 dans l'évolution de l'intermédiaire Gpx3-C_P-SOH vers les deux voies en compétition (figure) ; iii) l'analyse des interactions entre les trois partenaires par des méthodes spectroscopiques.

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Mots clés : H_2O_2, peroxydase à thiol, Yap1, signalisation redox, cinétique, *S. cerevisiae*

Poster 04.

La 3-mercaptopyruvate sulfurtransférase : Mécanisme, catalyse et spécificité structurale

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Poster 05.

REDUCTIVE ACTIVATION OF RESPIRATORY NITRATE REDUCTASE FROM *ESCHERICHIA COLI*

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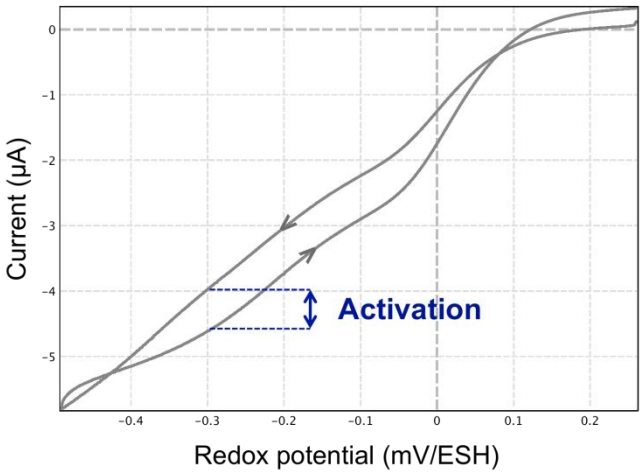
Nitrate reductases (NR) catalyze the 2 e^-/2 H^+ reduction of nitrate to nitrite and water. Most of them are molybdenum enzymes. Three NR are members of the Mo-bisPGD superfamily, and have to be activated to perform catalysis [1, 2]. Respiratory and assimilatory nitrate reductases (NR), respectively from *Paracoccus pantotrophus* and *Synechococcus elongatus*, activate through a complex process involving both chemical and redox steps [1]. In our lab, we have developed chromoamperometric methods to analyse the activation and inactivation of redox enzymes [3]. In the present work, we propose to apply this quantitative approach to the reductive activation of respiratory NR from *Escherichia coli* (Figure 1), for which several aspects of the mechanism have been probed for many years [4, 5, 6, 7, 8].

Figure 1. First cyclic voltammogram of adsorbed wild type *E. coli* NarGH on a PGE electrode. The forward scan shows less reduction current than the backward one, revealing an activation process.

Experimental conditions: 1 mM nitrate, pH 6,0, 37 °C, scan rate : 20 mV/s.



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Mots clés : metalloenzymes, molybdoenzymes, enzyme kinetics, bio-inorganic catalysis, oxidoreductases, electrochemistry

Poster 06.

N-acétylation des amines aromatiques par *Trichoderma* spp.

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Les amines aromatiques (AA) représentent l'une des classes majeures de contaminants retrouvés dans les sols (dérivés de pesticides, composés industriels, etc.). Nous avons récemment rapporté que l'ascomycète *Podospora anserina* pourrait détoxiquer efficacement des composés de type AA grâce à l'activité d'une arylamine *N*-acétyltransférase (NAT) fongique[1,2]. Les NATs, membres de la famille des enzymes du métabolisme des xénobiotiques, catalysent le transfert d'un groupement acétyl de l'acétyl-coenzyme A sur les AA, contribuant ainsi à leur détoxication. Les NATs, encore peu étudiées chez les champignons, ont été caractérisées chez *Trichoderma virens* et *Trichoderma reesei* [3]. Les enzymes recombinantes obtenues présentent une spécificité de substrat similaire à celle de *P. anserina* bien que leur efficacité catalytique soit beaucoup plus faible. Des tests de toxicité ont cependant révélé que *T. reesei* et *T. virens* peuvent croître en présence de fortes concentrations d'AA dont la 3,4-dichloroaniline (3,4-DCA), un résidu toxique de pesticide. Pour déterminer quelle est l'implication des NATs dans cette résistance, nous avons étudié la formation d'acétyl 3,4-DCA dans le milieu de culture ou dans de la terre contaminée par de la 3,4-DCA, en présence de *T. reesei* ou de *T. virens*. Les résultats obtenus, démontrent que ces deux espèces ont la capacité de métaboliser efficacement la 3,4-DCA présente dans leur environnement. De plus, chez *T. reesei*, la voie de *N*-acétylation a été identifiée comme l'un des mécanismes de détoxication des AA. Ces résultats offrent de nouvelles perspectives de bioremédiation pour ces 2 champignons, jusqu'à présent, essentiellement utilisés comme source de cellulases (*T. reesei*) ou comme agent de bio-contrôle (*T. virens*).

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Mots clés : Arylamine *N*-acétyltransférase, amine aromatique, biotransformation, champignons.

Poster 07.

Isolation of promiscuous hydrolases from metagenomic libraries screened in microfluidic droplets

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Functional metagenomics enables the isolation and the study of proteins from microorganisms that cannot be otherwise cultured under laboratory conditions. Indeed, uncultured microorganisms represent 99% of the microorganisms living on earth and, as such, provide an enormous reservoir of new enzymes[1]. However, their accessibility in metagenomic libraries is limited by the rather low throughput of current screening methods. Conversely, microfluidic droplets offer a very high throughput capacity and provide an attractive platform for functional screening of large metagenomic libraries. Through the integration of several microfluidic modules, it is indeed possible to perform numerous successive biological operations (*e.g.* storage, detection, sorting of droplets)[2]. The miniaturization of cell lysate assays to the single cell level in droplets[3] and its optimization for functional metagenomics enabled us to screen more than 10^6 variants in less than 3 hours. Such high throughput functional studies of soil and cow rumen metagenomes allowed us to isolate previously unidentified sulphatases and sulfotransferases demonstrating the power of our system to select new biocatalysts from environmental samples. Preliminary characterization results confirmed the isolation of a new promiscuous[4] sulphatase exhibiting activity toward sulphate monoester (k_{cat}/K_M = $1.14x10^5$ $M^{-1}.s^{-1}$) and phosphonate monoester (k_{cat}/K_M = $2.75x10^1$ $M^{-1}.s^{-1}$). In future works, we plan to use different substrates (including non-natural substrates) during screening campaigns in order to discover new promiscuous catalysts in metagenomic libraries. Through the use of our high throughput functional screening system, we thus aim at testing the hypothesis that promiscuity could be a common trait among natural enzymes.

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Mots clés : Functional Metagenomics, Microfluidic Droplets, High-Throughput Screening, Promiscuity

Poster 08.

The SAXS Structure of a Minimal Module from a Polyketide Synthase

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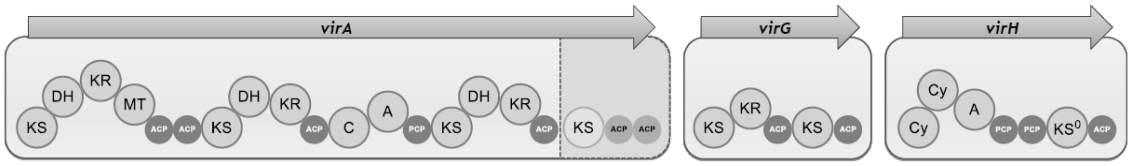

Figure 1 – The subunit organisation of the virginiamycin M1 synthase, consisting of three proteins (rectangles) and multiple catalytic domains (circles).

The modular polyketide synthases (PKS), common to many bacterial antibiotic-producers, are huge multienzyme, multidomain systems which can be likened to molecular assembly lines.[1] An understanding of the structural and mechanistic aspects which underpin interdomain cooperation during biosynthesis by modular PKS is crucial to ongoing efforts to re-engineer these systems for the production of novel compounds of medicinal value.[2]

Previous attempts to resolve the structures of PKS modules by X-ray crystallography have been unsuccessful, likely due to the inherent flexibility of the multienzymes. Here we have employed small-angle X-ray scattering (SAXS), in combination with NMR structure elucidation and homology modelling of individual domains, to obtain the first low-resolution structural data on an entire PKS module.

The model system comprises a multidomain region of the virginiamycin M1 PKS from *Streptomyces virginiae* (Fig. 1, dashed lines), a target of relevance for its involvement in production of the commercial antibiotic dalfopristin. The investigated region, at the C-terminus of the VirA protein, interacts with a wide range of protein partners, including a complex of discrete enzymes responsible for β-methylation of the polyketide, and the N-terminus of the downstream PKS protein VirG.[3] Our studies have shown the region to adopt an unusual extended conformation, which we propose to be related to the necessity of interacting with its many partners.

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Mots clés : polyketide synthase, SAXS, multienzyme, structure, enzyme mechanism

Poster 09.

Proteasome regulation by the circadian clock: implications for the degradation of oxidized proteins

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The circadian clock generates rhythms with a periodicity of 24 hours of various biochemicals and physiological processes. Recent data suggest a mutual influence between the circadian clock and the cell cycle, and provides a functional link between the circadian clock, cancer and ageing (Sahar and Sassone-Corsi et al., 2009).
Circadian rhythmicity of antioxidant mechanisms has also long been reported (Hardeland et al., 2003). The established link between the circadian clock and anti-oxidative defence suggests that elements of the redox homeostasis, especially protein redox homeostasis, including oxidized protein degradation and repair pathways such as the proteasome, could be modulated by the circadian clock. Interestingly, microarray analyses previously revealed that several proteasome subunits and activators, such as PA28αβ, are regulated by the circadian clock at the transcript level in mice tissues (Panda et al., 2002).
Using HEK cells synchronized by a serum shock as an initial cellular model we have shown that the catalytic subunits of the proteasome (β1, β2 and β5) and the immunoproteasome (β1i, β2i and β5i) have a circadian transcriptional expression. In addition, the proteasome peptidase activities are modulated with a circadian rhythmicity.
We have also demonstrated that the level of oxidized (i. e. carbonylated) proteins varies rhythmically following a 24 hours period. Interestingly, the rhythms of the proteasome activities match with the circadian oscillations observed for protein oxidative damage.
To further investigate the modulation of proteasome function for the degradation of carbonylated proteins mediated by the circadian clock, we have monitored the transcript level of some proteasome regulators. In fact, Pa28αβ (11S), a major proteasome and immunoproteasome regulator that has been previously implicated in the oxidative stress response (Pickering et al., 2011) was found to exhibit a circadian rhythmicity.
To continue with the same objective the protein expression levels of the different catalytic subunits and regulators will also be analyzed. In addition, the regulation of the assembly of proteasome subunits and regulators may also have a circadian regulation that will allow us to explain the involvement of the circadian clock in the oxidized proteins degradation.

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Mots clés : Circadian rhythmicity, Protein oxidation, Proteasome, Ageing

Poster 10.

Construction of a highly active xylanase displaying oleaginous yeast: Comparison of anchoring systems

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Nowadays, the use of enzymes as biocatalysts is widespread to perform reactions in eco-friendly conditions compared to chemical processes. As the cost of the enzymatic process can be dramatically affected by the use of free enzymes, many immobilisation techniques were developed. Amongst these techniques, the display of enzymes at the surface of microorganisms is a powerful emerging strategy (1). This technique has several advantages compared to chemical immobilisation techniques: the immobilised biocatalyst can be produced by standard fermentation and evolved by conventional genetic engineering.

Three *Yarrowia lipolytica* cell wall proteins (YlPIR, YlCWP and YlCBM) were evaluated for their ability to display the xylanase TxXYN from *Thermobacillus xylanilyticus* on the cell surface of *Y. lipolytica*. Endoxylanases (EC 3.2.1.8) are naturally involved in the depolymerisation of the complex xylan backbone from plant cell wall. This property is highly valuable as xylan is a complex polymer second to cellulose regarding abundance in nature. Therefore, xylanases could be a low cost means to produce value-added products from renewable carbon sources (2).

The fusion proteins were produced in *Y. lipolytica* JMY1212, a strain engineered for mono-copy chromosomal insertion, and enabling accurate comparison of the displaying systems (3). Cell bound activities reached to 50% for the YlPir fusion (71.6 U/g dried cells). Cell display using YlPir was found three times more efficient than the YlCWP system formerly developed for *Y. lipolytica,* and enabled the anchoring of 1.5 to 40 times the xylanase activity reported until now. Through this major improvement, we thus propose an efficient alternative display system in *Y. lipolytica*.

This work is the first step to the construction of a biotechnologically relevant yeast for the sequential degradation of hemicellulosic materials.

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Mots clés : *Yarrowia lipolytica*, yeast display, xylanase

Poster 11.

Isothiocyanates Alter the Acetylation of Carcinogenic Aromatic Amines by Inhibition of Arylamine N-acetyltransferase

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Isothiocyanates (ITCs), including benzyl ITC (BITC) and phenethyl ITC (PEITC), are compounds that are found in cruceferus vegetables such as brocoli, brussels sprouts or cress. Epidemiological studies have shown that dietary intake of ITCs is associated with reduced risk of certain human cancers.

Arylamine *N*-acetyltransferase (NATs) are xenobiotic metabolizing enzymes that are present in a variety of mammalian tissues. NATs catalyze the acetylation of arylamine compounds such as drugs, carcinogens, and other xenobiotics, leading to their detoxification and/or bioactivation.

We found that both BITC and PEITC inhibit human NATs activity. Kinetic and molecular analysis on recombinant NATs showed that the NATs inactivation by ITCs is fast and irreversible. Moreover, biologically-revelant ITCs concentration impaired NATs activity in HepG2 cells.

Our results suggest a new molecular mechanism of anti-carcinogenic effect of ITCs via the inactivation of NATs bioactivation pathway.

Poster 12.

Thioglycoligases : Voies d'accès innovantes aux *S*-glycoprotéines

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L'intérêt porté aux glycoprotéines s'est accru depuis la mise en évidence du rôle crucial des sucres sur leurs propriétés structurales et fonctionnelles favorisant ainsi le développement d'enzymes recombinantes permettant leur synthèse.[1] Parmi la diversité des types de glycosylation retrouvés dans la Nature, la *S*-glycosylation apparait comme l'une des plus rares.[2] Dans ce contexte, les thioglycoligases, qui catalysent la création de liaison de type *S*-glycosyle (schéma 1), apparaissent comme des biocatalyseurs de choix pour la synthèse de thioglycoprotéines.[3]

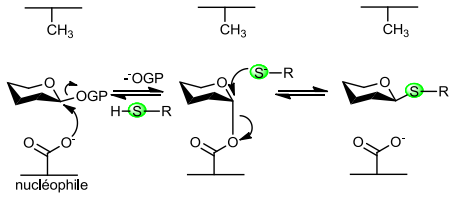

Schéma 1 : Mécanisme des thioglycoligases. GP = groupe partant

L'objectif du projet Glycopeps (financé par la Région Centre) est de générer une banque de thioglycoligases à partir de glycosidases, afin de :

- synthétiser des analogues de sucres non usuels (L-fucose, D-rhamnose,…) pour évaluer biologiquement leurs activités,

- étendre ce processus à la synthèse de *S*-glycoprotéines de sucres rares et avoir une meilleure compréhension de leur rôle biologique.

Cette étude présente la caractérisation enzymatique et structurale d'une glycosidase originale, BT2192 (appartenant à la famille GH29[4]), qui a conduit à l'élucidation de son mécanisme de reconnaissance de substrat, unique au sein de la famille GH29.

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Mots clés : thioglycoligase, α-L-fucosidase, glycoside hydrolase family 29, relation structure-fonction

Poster 13.

Functional characterization of glycosyltransferases involved in glycosaminoglycan biosynthesis – Structure-function study of chondroitin sulfate *N*-acetylgalactosaminyltransferase-1 (CSGalNAcT-1) involved in chondroitin sulfate initiation: Impact of sulfation on activity and specificity

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Our studies are focused on the identification of key-proteins involved in glycosaminoglycan (GAG) biosynthesis in cartilage during normal and pathological conditions. In this tissue, GAGs play a critical role in the biomechanical and functional properties of the joint. However, depletion in GAG content represents a main factor contributing to cartilage matrix degradation and loss of articular function in traumatic or degenerative arthropathies. Thus, a better understanding of GAG chains assembly is of central importance towards the development of pharmacological and engineering strategies aimed to counteract the loss of matrix components during arthropathies.
GAG assembly initiates through the formation of a linkage tetrasaccharide region serving as primer for both chondroitin sulfate (CS) and heparan sulfate (HS) chain polymerization. A possible role for sulfation of the linkage structure and of the constitutive disaccharide unit of CS chains in the regulation of CS-GAG chain synthesis as been suggested. To investigate this, we determined whether sulfate substitution of galactose (Gal) residues of the linkage region or of N-acetylgalactosamine (GalNAc) of the disaccharide unit influences activity and specificity of CSGalNAc-transferase-1 **(**CSGalNAcT-1), a key glycosyltransferase of CS biosynthesis. We synthesized a series of sulfated and unsulfated analogs of the linkage oligosaccharide and of the constitutive unit of CS and tested these molecules as potential acceptor substrates for the recombinant human CSGalNAcT-1. We show here that sulfation at C4 or C6 of the Gal residues markedly influences CSGalNAcT-1 initiation activity and catalytic efficiency. Kinetic analysis indicate that CSGalNAcT-1 exhibited 3.6-, 1.6- and 2.2-fold higher enzymatic efficiency due to lower K_m values towards monosulfated trisaccharides substituted at C4 or C6 position of Gal1, and at C6 of Gal2, respectively, compared to the unsulfated oligosaccharide. This highlights the critical influence of Gal substitution on both CSGalNAcT-1 activity and specifity. No GalNAcT activity was detected towards sulfated and unsulfated analogs of the CS constitutive disaccharide (GlcA-β1,3-GalNAc), indicating that CSGalNAcT-1 was involved in initiation but not in elongation of CS chains. Our results strongly suggest that sulfation of the linkage region acts as a regulatory signal in CS chain initiation.

Gulberti S, Jacquinet JC, Chabel M, Ramalanjaona N, Magdalou J, Netter P, Coughtrie MW, Ouzzine M, Fournel-Gigleux S, Chondroitin sulfate N-acetylgalactosaminyltransferase-1 (CSGalNAcT-1) involved in chondroitin sulfate initiation: Impact of sulfation on activity and specificity, Glycobiology, 2012, 22(4), 561-571

Mots clés : glycosaminoglycan synthesis / linkage region / sulfation / glycosyltransferase / chondroitin sulfate

Poster 14.

Study of the mechanism of the CO dehydrogenase from *Desulfovibrio vulgaris*

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Capture of atmospheric CO_2 and recycling into biofuels by microorganisms is one of the major scientific challenges of the XXIth century. Researches in this field are boosted by rising atmospheric CO_2 levels and associated climate change owing to the ongoing exploitation of remaining fossil fuel reserves. However, the precise knowledge of the mechanism, genetic regulation and metabolism implying enzymes using CO_2 is required. Some microorganisms contain carbon monoxide dehydrogenases (CODHs) which catalyze the reversible reduction of carbon dioxide by reaction with two protons and two electrons to yield carbon monoxide and water according to the reaction:

$$CO_2 + 2H^+ + 2e^- \leftrightarrow CO + H_2O$$

Two types of CODHs are known: the Mo- and Cu-containing CODH found in aerobic bacteria, and the Ni-containing CODH found in some anaerobic organisms. Ni-CODH can be associated with Acetyl-CoA synthase to form a bifunctional complex. Here, we study the monofunctional CODH from *Desulfovibrio vulgaris Hildenborough*, containing a $[NiFe_4S_4]$ cluster. Our goal is to develop structure-function approaches to understand the catalytic mechanism of this enzyme.

Mots clés : CODH, CO_2.

Poster 15.

Oxydation de *L*-2-hydroxy acides par des flavoenzymes: transfert d'hydrure ou mécanisme par carbanion?

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Nous nous intéressons à une famille d'enzymes à FMN qui catalysent l'oxydation de L-2-hydroxy acides en céto acides (*1*). Le domaine à flavine (FDH) du flavocytochrome b_2 (Fcb2, une L-lactate déshydrogénase de levure) en est membre. La flavine réduite par le substrat y est réoxydée par l'hème b_2. Des structures tridimensionnelles de membres de la famille indiquent un repliement et un site actif très conservés, ce qui suggère un mécanisme catalytique identique pour toute la famille. On peut envisager deux mécanismes *a priori*. Dans le premier cas, une base de l'enzyme arrache le proton de l'hydroxyle en favorisant le départ de l'hydrogène du C2 vers le FMN, sous forme d'hydrure, comme pour les enzymes à nicotinamide. Dans le deuxième cas, la base du site actif arrache l'hydrogène du C2 sous forme d'un proton, engendrant un carbanion intermédiaire, qui cède les électrons à la flavine. Ni la structure tridimensionnelle, ni la modélisation du substrat dans le site actif du Fcb2, pas plus que des calculs par DFT ne permettent de conclure quant au mécanisme d'oxydation du lactate. Bien que certains arguments semblent exclure un mécanisme par hydrure (*2*), une controverse existe encore. Le trifluorolactate (F3Lac) est une sonde mécanistique intéressante, car l'effet attracteur des fluors doit stabiliser un carbanion et rendre l'arrachement d'un hydrure difficile. Il a déjà été montré que ce composé est un inhibiteur et pas un substrat pour une LDH à nicotinamide (*3*). Par des études à l'état stationnaire et préstationnaire, nous montrons ici que le F3Lac est un substrat pour la FDH du Fcb2 oxydée et que le trifluoropyruvate (F3Pyr) est un substrat pour la FDH réduite. Nous avons déterminé l'effet isotopique primaire du deutérium dans le sens de l'oxydation du F3Lac et de la réduction du F3Pyr. De plus, nous confirmons que le F3Lac est un inhibiteur pour la LDH à nicotinamide, et qu'elle réduit le F3Pyr avec une extrême lenteur. Enfin, nous montrons que F3Lac et F3Pyr sont des substrats pour un autre membre de la famille de la FDH, la LCHAO (oxydase d'hydroxy acides à longue chaîne). Une interprétation mécanistique des résultats sera présentée.

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Mots clés : Carbanion, Flavoprotéine, Hydrure, Mécanisme chimique, Trifluorosubstrat

Poster 16.

Using electrochemical kinetics, DFT and MD to determine the molecular mechanism of oxidative inactivation of FeFe hydrogenases

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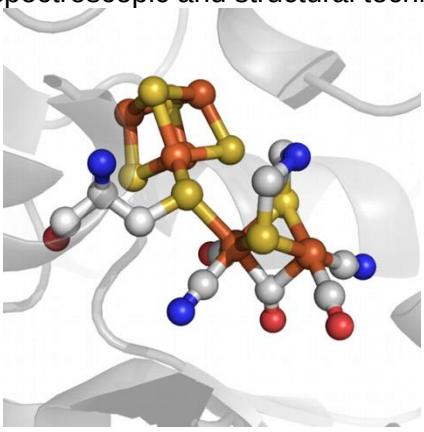
Nature is a valuable source of inspiration for designing catalysts, and various approaches are used to elucidate the mechanism of hydrogenases, the enzymes which oxidize or produce H2. The H-cluster is the place of H2 oxidation in FeFe hydrogenases, and catalysis involves H2 binding on the vacant coordination site of an iron center. Using electrochemistry (1,2), site-directed mutagenesis, DFT calculations (3) and Molecular Dynamics (4), we found that the reversible oxidative inactivation of this enzyme results from the non-productive binding of H2 to coordination sites that are normally blocked by intrinsic CO ligands. This reversible reaction prevents oxidative destruction. The plasticity of the coordination sphere around the reactive iron center therefore confers the enzyme the ability to avoid harmful reactions under oxidizing conditions. Minor conformations may also be important for function in the case of other redox enzymes, but we expect that they should escape detection by spectroscopic and structural techniques.



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Mots clés : Metalloenzymes, Enzyme kinetics, Enzyme mechanism , Electrochemistry , Theoretical chemistry, Density functional Theory, Molecular dynamics, Site-directed mutagenesis

Poster 17.

The effect of the biogenic amines spermidine and putrescine on the gills of the Amazon river shrimp, *Macrobrachium amazonicum* (Decapoda, Palaemonidae): kinetic analysis and phosphorylation by inorganic phosphate

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In the transport ATPases like (Na^+,K^+)-ATPase, ATP is hydrolyzed via formation and hydrolysis of phosphorylated intermediates, where phosphate is bound to an aspartyl residue at the enzyme's substrate site. The resulting phosphorylated enzyme intermediates (EP) play a crucial role in the common reaction scheme for (Na^+,K^+)-ATPase where their formation, catalysed by Mg^{2+} and Na^+, and breakdown, catalysed by K^+, are important steps in the energy transduction and cation transport. The biologically active polyamines putrescine and spermidine are ubiquitous, small, positively charged molecules that are essential for normal cellular function and proliferation. Polyamines inhibit the (Na^+,K^+)-ATPase supposedly by binding to clusters of acidic residues at the enzyme. The goal of this work was to examine the effect of exogenously added polyamines on the activity and phosphorylation levels of the (Na^+,K^+)-ATPase from *M. amazonicum* gills in vitro. Total ATPase activity was notably inhibited (-82%) by 20 mmol L^{-1} spermidine and (-43%) by 100 mmol L^{-1} putrescine. As spermidine concentration increased from 10^{-5} to 2 $\times 10^{-2}$ mol L^{-1}, total ATPase activity decreased in juvenile from 231 U mg^{-1} (1 U mg^{-1}= 1 nmol Pi^{-1} min^{-1} mg) to less than 41 U mg^{-1}. Though, as putrescine concentration increased from 10^{-5} to 10^{-1} mol L^{-1}, total ATPase activity decreased from 231 U mg^{-1} to less than 132 U mg^{-1}. In presence of both polyamines, ouabain insensitive ATPase activity varied little, suggesting that only (Na^+,K^+)-ATPase activity is affected. The K_I's for spermidine and putrescine were 2.69±0.08 and 157.61±4.73 mmol L^{-1}, respectively. In the presence of suboptimal concentrations of both Na^+ and K^+ (5 and 2 mmol L^{-1} for Na^+ and K^+, respectively), maximum rates of 33 U mg^{-1} were estimated for spermidine. There was a increase in the apparent affinity of the enzyme to around 1.5 for spermidine. Modulation of V and K by Na^+, K^+ and Mg^{2+} varied considerably in the presence of 5 mmol L^{-1} spermidine and 25 mmol L^{-1}putrescine. Besides, in the presence of 100 mmol L^{-1} Na^+, both 5 mmol L^{-1} spermidine and 25 mmol L^{-1} putrescine inhibited formation of the phosphoenzyme in 47% and 32%, respectively. Putrescine exhibited a non-competitive inhibition patter for ATP, Mg^{2+}, Na^+ and K^+ stimulation of the enzyme, in contrast to the mixed-partial inhibition pattern to Mg^{2+} and Na^+ in presence of spermidine. These findings suggest that spermidine compete for the cation binding sites on the enzyme molecule, affecting both V and $K_{0.5}$.

Mots clés : biogenic amines, *Macrobrachium amazonicum*, phosphorylation, (Na^+,K^+)-ATPase, inhibition.

Poster 18.

Investigation of enzyme mechanisms by a new rotating droplet electrochemical method.

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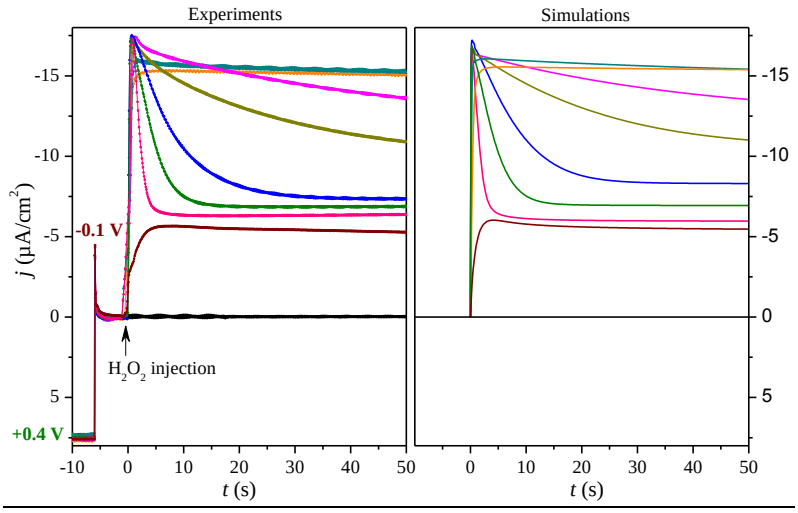
We recently developed a new generic, simple, sensitive and easy-to-implement electrochemical kinetic method for monitoring, in real time, the progress of a biochemical reaction in a microdrop of few tens of microliters, with a kinetic time resolution of less than 1 s. A simple set-up makes it possible to mix and rotate a droplet at a high spin rate and hence to generate a well-defined hydrodynamic steady-state convection layer at an underlying stationary electrode. The features afforded by this new kinetic method allowed us to investigate the reversible inhibition of the redox-mediated catalytic cycle of horseradish peroxidase (HRP) by its substrate H_2O_2. The experimental time-course amperometric kinetic plots were reproduced from numerical simulations of the whole HRP catalytic reaction. From the best fits of simulations to the experimental data, the kinetics rate constants primarily associated to the inactivation/reactivation pathways of the enzyme were retrieved. The ability to perform kinetics with microliter-size samples makes this methodology particularly attractive for reactions involving low-abundant and/or expensive reagents.



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Mots clés : kinetic analysis, inhibition, horseradish peroxidase, rotating droplet electrochemistry

Poster 19.

Relations structure-activité dans la famille des Mur ligases

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L'assemblage de la partie peptidique du peptidoglycane implique une famille d'enzymes, les Mur ligases. Bien que ces enzymes partagent un mécanisme réactionnel similaire et une structure 3D comparable, leur spécificité doit être très stricte puisque l'incorporation d'un « mauvais » acide aminé peut être délétère pour la bactérie. L'élucidation des motifs structuraux responsables de la haute spécificité des Mur ligases pourra aider la recherche d'inhibiteurs agissant comme composés antibactériens. Dans cette communication, nous rapportons la caractérisation biochimique d'enzymes MurD et MurE de différentes espèces bactériennes.

Les orthologues de MurD de cinq espèces (*E. coli*, *S. aureus*, *S. pneumoniae*, *M. tuberculosis* et *B. burgdorferi*), ont été purifiées et caractérisées. Des dérivés de l'acide D-glutamique, qui ont été montrés comme étant d'excellents inhibiteurs de MurD de *E*. *coli*, apparaissent moins efficaces envers les quatre autres orthologues. Cela peut être expliqué par les différences dans les séquences d'acides aminés et dans la topologie des sites actifs.

La spécificité de substrat acide aminé de trois enzymes MurE (*E. coli*, *S. aureus* et *B. burgdorferi*), représentatives des trois types principaux de peptidoglycane (*meso*-DAP, L-Lys et L-Orn) a été élucidée : le substrat préférentiel *in vitro* est le seul retrouvé dans le peptidoglycane final. D'un point de vue structural, cela est corrélé avec l'existence d'une poche de liaison pour le *meso*-DAP chez MurE de *E*. *coli* et de séquences consensus spécifiques de l'acide aminé chez différents orthologues de MurE.

Finalement, les ligases MurE de deux espèces à Gram-négatif phylogénétiquement apparentées, *Chlamydia trachomatis* et *Verrucomicrobium spinosum*, ont été purifiées et caractérisées. Ces deux enzymes se sont révélées être de typiques enzymes d'addition du *meso*-DAP. L'analyse tridimensionnelle de MurE de *V*. *spinosum*, en utilisant la modélisation protéique, a montré que les acides aminés clés composant le site actif des enzymes d'addition du *meso*-DAP sont conservés dans cette protéine.

Mots clés : peptidoglycane, Mur ligases, inhibiteurs, structure tridimensionnelle

Poster 20.

Identification by high resolution mass spectrometry of a new metabolite in the strict aerobic bacterium *Acinetobacter baylyi* ADP1

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We aim to complete the metabolic activities inventory in the soil bacterium *Acinetobacter baylyi* ADP1 (ADP1) by different methods (comparative genomics, high throughput growth phenotyping, RNAseq…), with a special focus on the discovery of new enzymatic activities through metabolomics.

We monitored metabolism adaptation by the use of an alternative carbon source by high-resolution mass spectrometry (LC-ESI-LTQ/Orbitrap). The comparison of a metabolome from cells grown on succinate (reference carbon source) or quinate (alternative carbon source) highlighted the different intermediaries of this well-known catabolic pathway.

Similar studies done on *Pseudomonas* strains showed that the major changes regarding the metabolite composition involve the degradation intermediaries of the carbon sources used [1-2]. However, in ADP1, we did not notice such a 'core metabolome': almost 40% of the detected metabolites in ADP1 present at least a 4 fold variation according to the carbon source used.

But the most important point is that many metabolites, not identified, are only present on quinate. We present here the structural elucidation of one of these metabolites. The database interrogation using accurate mass did not allow us to propose an identification consistent with the CID fragmentations. These fragmentations were analyzed by high resolution and led us to suggest that the compound is most likely a tyrosine substituted in benzylic position by an aminomethyl group. To our knowledge, this metabolite has never been described so far. The experimental approach to elucidate this structure is discussed.

Thus, high-resolution mass spectrometry can be a very powerful tool to elucidate new metabolites structure.

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Mots clés : Key words: microbial metabolomics, LC-MS, structural elucidation

Poster 21.

Identification of key residues of β1,4-galactosyltransferase 7 (β4GalT7) implicated in acceptor binding site

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Glycosyltransferases (GTs) represent an important family of enzymes involved in the biosynthesis of glycosaminoglycan (GAG) chains of proteoglycans (PG), which play crucial roles in numerous physiopathological conditions such as blood coagulation, cancer process, osteoarthritis, as well as genetic disorders. GAG biosynthesis is initiated by the formation of a "tetrasaccharide linker", constituted of a GlcAß1,3Galß1,3Galß1,4Xyl- sequence, which primes heparin/heparan-sulfates and chondroitin-sulfate chains. This initiation step requires the consecutive action of four GTs, among which β1,4-galactosyltransferase 7 (β4GalT7) catalyzes the transfer of galactose from UDP-galactose onto a xylose acceptor residue. Genetic defect of this enzyme causes the progeroid form of Ehlers-Danlos syndrome (EDS), a severe disorder (1). Noticeably, β4GalT7 is able to initiate GAG chain biosynthesis from exogenous xyloside compounds that exhibit anti-cancer and anti-thrombotic properties (2). Therefore, β4GalT7 represents an interesting therapeutic target whose structure-function study is required for the design of xyloside compounds with therapeutic applications. In this work, we aim to identify key amino acid residues of the human recombinant β4GalT7 active site that govern xyloside acceptor substrate binding and recognition. Phylogenetic analyses, sequence alignments and molecular modeling based on the 3D structure of the *Drosophila* β4GalT7 catalytic domain (3), revealed that Y194 and Y196 residues are (i) conserved among species, (ii) predicted to interact with the acceptor substrate, or with the neighboring amino acid residues in the acceptor substrate binding site. To analyze their role, we examined the consequences of conservative and non-conservative mutations on the kinetic properties of the human β4GalT7 purified from recombinant bacteria (4). Results showed a loss of enzymatic activity for Y194A, Y194F and Y196A mutants. Indeed, these mutants present a low V_{max} value (<30 $nmol.min^{-1}.mg^{-1}$) compared to 1450 ± 37 $nmol.min^{-1}.mg^{-1}$ for the wild-type enzyme, while their affinity towards both donor and acceptor substrates is weaker with K_m value > 2 mM, compared to 0.22 ± 0,02 mM for UDP-galactose and 0,35 ± 0,02 mM for 4-methylumbelliferylxylose (4-MOX) for the wild-type β4GalT7. Interestingly, a partial loss of activity was found for the Y196F mutation. Indeed, the V_{max} for this mutant was 68% lower than the wild-type β4GalT7, and the K_m value towards the donor substrate was similar to the wild-type, while it was 3-times higher for 4-MOX (K_m = 1,06 ± 0.06 mM). These results suggest a possible implication of this residue in the interaction with xylose or in the stabilization of the acceptor substrate-binding site. *Ex vivo* assays using 4-MOX were performed to analyze the consequences of these mutations upon GAG chain synthesis in CHO-pgsB618-deficient cells. Cells were transiently transfected with a β4GalT7-expression vector and GAG synthesis rate initiated from 4-MOX was monitored by radiolabelled sulfate incorporation. In agreement with the *in vitro* results, mutations affecting either Y194 or Y196 position led to a drop of GAG chain formation. This confirmed the possible role of these residues in the capacity of β4GalT7 to use xylosides as substrate either by stabilizing the binding site structure and/or by adequately positioning the acceptor substrate. We also investigated the role of R270 whose mutation contributes to the progeroid form of EDS. *In vitro* results showed that both conservative and non-conservative mutations, R270K and R270A respectively, slightly decreased the enzymatic activity without affecting the affinity for UDP-galactose, while significantly increased K_m value towards 4-MOX. These results confirm the possible role of this residue in the binding of the acceptor substrate (Bui et al., 2010). Current investigation is ongoing to solve the 3D-structure of human recombinant β4GalT7 and its genetic mutants to bring further insights into the molecular basis of acceptor substrate recognition, towards the rationale design of xyloside effectors of GAG synthesis.

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Mots clés : Glycosyltransferases, xyloside, glycosaminoglycan , Ehlers-Danlos syndrome, mutations, enzymatic activity

Poster 22.

Kinetics of PQQ reconstitution and activation of soluble glucose dehydrogenase from *Acinetobacter calcoaceticus*

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Soluble glucose dehydrogenase (s-GDH) with a PQQ cofactor catalyzes the oxidation of glucose into gluconolactone. This enzyme is widely used in glucose biosensors or biofuel cells [1].

s-GDH from *Acinetobacter calcoaceticus* is homodimeric with one PQQ and three calcium ions per monomer. Two calcium ions are involved in the enzyme dimerization process and in the stabilization of the dimer, and the third is involved in the PQQ binding in the active site. In *Escherichia coli,* an apoenzyme is produced. A reconstitution step with PQQ is required to have a catalytically active enzyme. Several amino acids from the PQQ binding pocket and one calcium ion allow the PQQ anchoring [2] and the PQQ dissociation constant is lower than the nanomolar one [3]. During the catalysis, PQQ is regenerated inside the active site by an artificial electron acceptor and is not released in solution. Under pre-steady-state conditions, a fast kinetic reconstitution study of the GDH with PQQ allows us to understand, from a fundamental point of view, the reconstitution mechanism. Two techniques were used: 1/ fluorescence quenching of tryptophan during the PQQ binding process and 2/ detection of PQQ reduction in conditions of a first catalytic cycle in presence of glucose. The main result shows that conformational rearrangment around the tryptophan reflects the enzymatic catalysis activation by PQQ and calcium ion.

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Keywords : Cofactor reconstitution, Glucose dehydrogenase, Pyroloquinoline quinone, Rapid Kinetics

Poster 23.

Semi rational approach to design new transglycosidases

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The important role of oligosaccharides and their conjugates in biology has been increasingly recognized in the recent years. Unfortunately, despite the considerable development of efficient synthetic methods in this field, the assembly of oligosaccharides remains a substantial challenge. As an alternative, enzymatic glycosylation has become a powerful tool for stereoselective synthesis of various glycosidic compounds. The approach, which was developed recently in our laboratory, was based on the directed evolution of glycosidases into transglycosidases using an original screening strategy [1]. Mutant enzymes that have almost completely lost their hydrolytic activity while keeping the transglycosidase one can now be obtained. This approach has been validated on two glycosidases, Thermus thermophilus β-D-glycosidase (TtβGly, GH1) [2] and Thermotoga maritima α-L-fucosidase (TmαFuc, GH29) [3]. These new transglycosidases can compete with glycosynthases for oligosaccharide synthesis. However, obtaining useful transglycosidases by directed evolution is time consuming even with efficient screening methods. This limits the number of new enzymes activities that can be obtained rapidly.
Based on the result of our previous random mutagenesis, three TtβGly mutants were kinetically characterized and their structures were determined by X-ray crystallography in order to understand the molecular basis of this improvement.
We propose a working hypothesis about the effect of mutations and find general rules that could speed up the creation of transglycosidases from glycosidases by rational mutagenesis. We identified several amino-acid positions, for which mutations significantly improve the transglycosylation activity of glycosidases. Thus, by single-point mutagenesis on TtβGly we created four new mutants that exhibit improved synthetic activity, and allow the formation of disaccharides with yields of 68 to 90% against only 36% when TtβGly native was used. As all of the chosen positions were well-conserved among GH1 enzymes, our approach is most probably a general route to convert GH1 glycosidases into transglycosidases. We are currently extending this approach to glycosidases from others families, and particularly on α-glycosidases and furanosidases for which the glycosynthase approach is less efficient.
Thus, we demonstrated that the outcome of evolution techniques can provide useful information for subsequent semi-rational design of transglycosidases for oligosaccharide synthesis.

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Mots clés : Glycosidases, transglycosidases, directed evolution, mutagenesis

Poster 24.

Des petits inhibiteurs non covalents du protéasome humain : une fixation originale dans le site actif

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La régulation par des molécules spécifiques de l'activité du protéasome, machinerie essentielle de la dégradation intracellulaire des protéines, est d'intérêt dans le traitement de diverses pathologies.[1] Les inhibiteurs non covalents, très peu représentés, constituent une alternative à l'inhibition covalente réalisée par les deux inhibiteurs utilisés en thérapie anticancéreuse. En parallèle de nos travaux sur des mimes peptidiques d'un inhibiteur tripeptidique cyclique[2-5], nous avons découvert, par criblage *in silico* d'une collection de 430.000 molécules organiques, une nouvelle classe d'inhibiteurs non peptidiques du protéasome humain 20S, les 1,2,4-oxadiazoles (Figure 1). Ces molécules ont été optimisées par synthèse chimique. Il s'agit d'inhibiteurs mixtes de l'activité de type chymotryptique du protéasome 20S (K_i = 26 nM et K'_i = 7 nM pour la meilleure molécule). Ils agissent sélectivement sur cette enzyme. Leur mécanisme d'action a été corroboré par des études de modélisation moléculaire démontrant une fixation inhabituelle de ces petits inhibiteurs dans le sous-site S5. Des effets cytotoxiques ont aussi été observés sur les lignées cellulaires modèles HeLa et HEK-293. Ce travail introduit donc une nouvelle stratégie pour inhiber le protéasome : le ciblage d'un sous-site (ici S5) éloigné du sous-site S1 classiquement ciblé pour développer des inhibiteurs de cette enzyme.

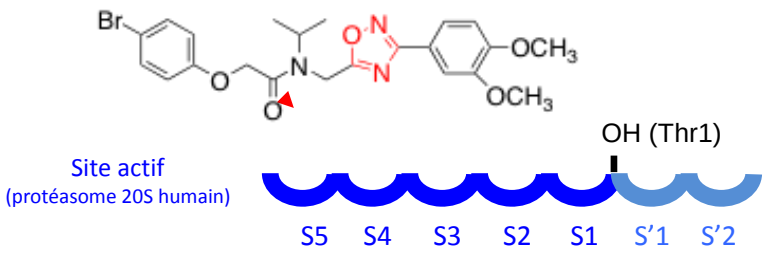

Figure 1. Fixation postulée des oxadiazoles dans le site de type chymotryptique du protéasome humain 20S.

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Mots clés : protéasome ; inhibiteurs non covalents ; criblage *in silico* ; optimisation chimique; 1,2,4-oxadiazoles.

Poster 25.

From Transglutaminases to Arylamine *N*-Acetyltransferases: Evolutionary specialization of a spatially conserved position for transition-state stabilization

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Transglutaminase (TG), papain (PAP) and arylamine *N*-acetyltransferase (NATs) enzymes belong to three different family enzymes which catalyze different reactions. Nonetheless, these enzyme families share somme common catalytic centers such as a Cys-His-Asp/Asn catalytic triad. Although the structures of PAP and TG are different, structural superposition has allowed to identify a unique spatially conserved amino acid position (Gln in PAP and Trp in TG enzymes, respectively) involved in transition-state stabilisation.

Structural analysis allowed us to identify an equivalent amino acid position in the active site of NAT enzymes. Interestingly, in NATs this amino acid position corresponds to an aromatic residue (mainly Phe, but also Tyr and Trp) which is part of a conserved motif (P-[F/Y/W]-EN).

In this study, a structurally and functionally well-characterized NAT (*Mesorhizobium loti* NAT1) was used as a NAT model enzyme to study the structural and functional role of this position (Phe42 in M. loti NAT1). Mutant enzymes harbouring different amino acids at this position (Y, W, H, I, L, A) were expressed and purified. Mutant Phe42Ala was found unstable and very poorly expressed. The other mutants were soluble and correctly folded as analyzed by circular dichroism and x-ray crystallography. Activity assays and kinetics analyses showed that only Phe42Tyr and Phe42Trp mutants were active. Phe42Trp displayed a kinetic behavior similar to the WT enzyme whereas Phe42Tyr was found to be 20 times more active than WT enzyme. As observed with transglutaminases (Lismaa et al., 2003 PNAS), our data suggest that this position contributes to transition state stabilisation through hydrogen-bonding and/or hydrophobic/aromatic interactions.

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