Contents

INTRODUCTION

2010 has been an eventful year for the Centre of Protein Engineering (CIP).

Firstly, the writing of the five-year report 2004-2009 led us to redefine the various research themes of the Centre and highlighted the senior researchers responsible for their success. This synthesis served as a blueprint for the development of our new website that was launched in May 2011.

Secondly, the group of Professor Patrick Motte joined the Centre and allowed us to expand our expertise in functional imaging and eukaryotes, especially plants. This unit has brought two new research fields: the study of the spliceosome and the molecular adaptation of plants to high concentrations of metals in the soil. This expansion of the CIP resulted in a reorganization of the Centre and an update of our statutes. Marie Schloesser (technician) who had been working for several years on bacterial proteins belonging to the divisome obtained a tenured position and joined this team of botanists.

During the same year, two new Research Associates (FRS-FNRS) were appointed: the physicist Frédéric Kerff working in the crystallography unit and the botanist Marc Hanikenne investigating metal homeostasis in plants.

Additionally, the Management Committe decided to develop and host the collection of polar cyanobacteria that is under way to become integrated into the Belgian Co-ordinated Collections of Micro-organisms (BCCM™).

Dr Claudine Fraipont retired in December 2010 after more than twenty years of research at the CIP. Claudine initially worked on the cloning of the gene encoding the *Streptomyces* R61 PBP and then on the proteins of the *E.coli* divisome. Despite very difficult weather conditions, with abundant snowfall, the CIP hosted a party at the botanical institute to warmly thank Claudine for her dedication to the community and to wish her the best for life after-the-lab.

Finally, our scientific production during the year 2010 resulted in fifty-six publications in international peer reviewed journals and four book chapters and reviews.

I am thankful to the CIP community for their outstanding work highlighted in this report.

Bernard Joris

RESEARCH GROUPS

APPLIED QUANTUM CHEMISTRY AND MODELLING

Group leader: **Dr Georges Dive**

Associate member: **Dr Dominique Dehareng**

BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS

Group leader: **Prof. Bernard Joris**

Permanent scientists:
Dr Colette Duez
Dr Colette Goffin
Dr Sébastien Rigali
Dr Mohammed Terrak
Dr Annick Wilmotte

Associate members:
Dr Ana Amoroso
Dr Alain Brans
Dr Michaël Delmarcelle
Dr Claudine Fraipont
Dr Zorigto Namsaraev

BIOLOGICAL MACROMOLECULES AND BIOCHEMISTRY

Group leader: **Prof. Moreno Galleni**

Permanent scientists: **Dr Georges Feller**

Associate members:
Dr Etienne Baise
Dr Carine Bebrone
Dr Renaud Berlemont
Dr Jean-Marie François
Dr Patricia Lassaux
Dr Paola Mercuri
Dr Noureddine Rhazi
Dr Frédéric Sapunaric
Dr Brahim Semane

RESEARCH GROUPS

BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY

Group leader: **Prof. Paulette Charlier**

Permanent scientist: **Dr Frédéric Kerff**

Associate member: **Dr Eric Sauvage**

ENZYMOLOGY AND PROTEIN FOLDING

Group leader: **Prof. André Matagne**

Permanent scientist: **Dr Mireille Dumoulin**

Associate members: **Dr Alexandre Di Paolo**
Dr Michaël Nigen
Dr Julie Vandenameele

FUNCTIONAL GENOMICS AND PLANT MOLECULAR IMAGING

Group leader: **Prof. Patrick Motte**

Permanent scientist: **Dr Marc Hanikenne**

Associate members: **Dr Cécile Nouet**
Dr Vinciane Tillemans

EXPERTISES

MOLECULAR BIOLOGY

- [X] Activity screening
- [X] Gene cloning in *E. coli, Bacillus, Streptomyces* and *P. pastoris*
- [X] Site-directed mutagenesis
- [X] Phage display
- [X] Metagenomics
- [X] Protein engineering (random mutagenesis, protein design)

PROTEIN PRODUCTION

- [X] In *E. coli, Bacillus, Streptomyces, P. pastoris* or in environmental strains
- [X] From mL to 60 L
- [X] In flasks or fermentors
- [X] Optimisation of industrial processes
- [X] ^{2}H, ^{13}C, ^{15}N enrichment for NMR studies
- [X] Selenomethionyl enrichment for crystallography studies

PROTEIN PURIFICATION

- [X] Classical purification techniques (ion exchange, affinity, hydroxyapatite…)
- [X] From mg to g
- [X] HLPC, FPLC, Åkta prime, Åkta explorer, Profinia, Biopilot…

MACROMOLECULE CHARACTERISATION

Biochemical characterisation

- [X] Cellular localization of proteins:
  - Fluorescence microscopy
- [X] 2D-DIGE
- [X] DGGE
- [X] ELISA
- [X] EMSA
- [X] Enzymology :
  - Steady- and transient-states kinetics
  - Stopped-flow & quenched-flow
- [X] N-terminal sequencing
- [X] Protein-protein interactions:
  - Bacterial two hybrids, surface plasmon resonance (BIAcore) and immunoprecipitation
- [X] Proteomics
- [X] Western blot

Biophysical characterisation

- [X] Microcalorimetry
- [X] Analysis of peptidoglycan by HPLC (DSC and ITC)
- [X] Protein stability, folding & aggregation:
  - Spectroscopy: UV-Vis, fluorescence and circular dichroism
  - Time-resolved spectroscopy
- [X] X-ray crystallography:
  - Crystallogenesis
  - de novo structure determination
  - Studies of ligand-protein complexes
  - 3D structure determination

PLANT MOLECULAR IMAGING

- [X] Plant physiology
- [X] Plant genetic transformation
- [X] Molecular imaging

In Silico Studies

- [X] **Molecular modelling and applied quantum chemistry**
- [X] **16S rRNA phylogenetic analysis**
- [X] **Prokaryotic regulon predictions: Web tool PREDetector (Prokaryotic Regulatory Elements Detector)**

Major Equipments

Genetic engineering and molecular biology

1 Bio-robot model 9600 (Qiagen)
2 DNA sequencers: 1 ALF model (Pharmacia) and 1 Li-Cor Gene readir 4200 (NEN)
1 Gene Pulser electroporator (Biorad)
Several PCR apparatus including: 1 MJ Mini Real Time Quantitative PCR PTC0148 (Biorad)
1 Nanovue (GE Healthcare)

Green algal cultures

1 Versatile environment test chamber (Sanyo)

Microbial cultures

2 Controlled environment incubator shakers (New Brunswick Scientific)
11 Incubator shakers: five G-25 (New Brunswick Scientific), one 25D (New Brunswick Scientific), one Excella E24 (New Brunswick Scientific), two Innova 44 (New Brunswick Scientific) and two Innova 4330 (New Brunswick Scientific) incubators
1 Gradient Table for crossed gradients of Temperature and Light (Labio chromatography)

Plant cultures

4 Climate-controlled chambers (Binder) for plant growth and cell cultures

Production of proteins

Nine fermentors including: two 2 L (M254, Biolafite and LH 210, Inceltech), one 5 L (Biostat, B. Braun Biotech International), one 8 L (LH 2000, Inceltech), two 10 L (Bioflow 3000 and Bioflow IV, New Brunswick scientific), two 20 L (Bioflow 4500, New Brunswick scientific) and one 80 L (Bioflow 5000, New Brunswick scientific).
1 123 L cooled incubator MIR-154 (Sanyo)
1 Turbidimeter FSC402 (Mettler Toledo)

Purification of proteins

1 Centrifugation system (SA 1-02-175 model, Westfalia)
2 Homogenizers: one Panda (GEA Process Technology) and one Emulsiflex-C3 (Avestin, Inc)
2 Sonicators: one MSE and one Sonifer B-12 (Branson Sonic Power Company)
A range of instruments to perform protein purification at low or high pressure. The most remarkable include: 2 Åkta-explorer (10S 2D-LC and 100-Air), 1 Åkta- purifier, 2 Åkta prime and 2 Åkta prime plus (GE Healthcare)
3 LC210 purification systems (Isco)
1 P-6000 Bio-Pilot autosampler with a Unicorn controller (GE Healthcare)
2 Profinia purification systems (Bio-Rad)
1 Tangential filtration system (Sartoflow Alpha, Sartorius)

Analytical studies

1 BIAcoreX for interaction analysis by surface plasmon resonance
1 Circular Dichroism spectrophotometer J-810 equipped with a Peltier and a 6 cell holder (Jasco)
2 2D-electrophoresis GE Ettan IPGphor3 and Ettan DALTsix apparatus (GE Healthcare)
2 DGGE electrophoresis apparatus (Dcode, Biorad)

1 DynaPro NanoStar DLS/SLS recorder for Dynamic/Static Light Scattering (Wyatt Technology Corporation)
3 Fluorimeters: one SLM-Aminco 8100 (Spectrometric Instruments), one Carry Eclipse (Varian) and one LS50B (Perkin-Elmer)
1 HPLC system (Kontron) and 1 HPLC with an auto-injection system, a diode array and a fluorescence detector (Waters)
2 Microcalorimeters: MCS ITC/DSC and VP-DSC (Microcal)
2 Microplate readers: one Labsystems Multiskan Multisoft (TechGen International) and one PowerwaveX (Bio-Tek instruments, Inc)
1 Microplate Strip Washer EL X 50 (Bio-Tek Instruments, Inc)
1 Procise 492 N-terminus amino acid sequencer (Applied Biosystems, Perkin Elmer)
1 Quenched-flow QFM-5 (Bio-Logic) and 1 Quenched-Flow SFM 400 (Bio-Logic)
1 Rapid filtration system (Bio-Logic)
Several spectrophotometers Uvikon (Bio-Tek Instruments, Inc,), one spectrophotometer Carry 100 Biomelt (Varian), two UV/Vis spectrophotometers: Specord 50 and 200 (Analytik Jena)
2 Stopped-flow apparatus: MOS 450 with UV/visible light, fluorescence and circular dichroism detection and MPS-51 with UV/visible light and fluorescence (Bio-Logic).

Crystallography

1 Cryogenic AD41 cryosystem (Oxford)
4 Graphic-PC stations (Linux)
1 Imaging Plate Marresearch IPmar345 equipped with a RU200B rotating anode (Rigaku)
1 TTP Labtech Mosquito Crystallization robot (compact bench-top instrument for nanolitre liquid handling) (Cambridge UK)

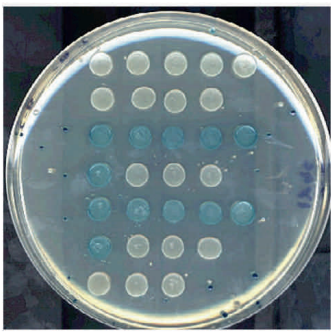
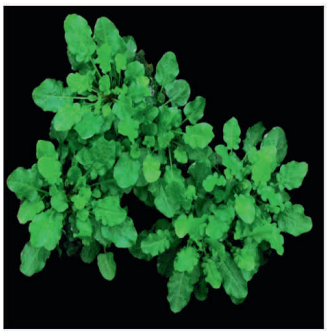
Imaging

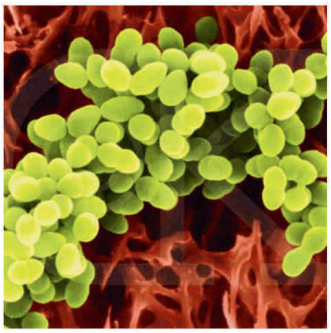
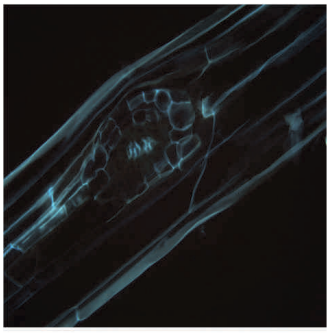
1 Axio Imager Z1 fluorescent microscope (Zeiss)
1 Camera for digitalisation of images and analytical analyses (Deltapix Scandinavia)
1 CKX 31 inversed microscope (Olympus)
1 DMLB2 microscope (Leica)
1 Molecular Imager FX system (Biorad)
1 Phase contrast microscope (Reichert)
1 Binocular microscope (model SZ-6 PHOTO Bauch & Lomb)
1 Binocular microscope with a digital camera (SMZ1500, Nikon)
1 Microscope equipped for epifluorescence (Zeiss)
1 Confocal inverted microscope (Leica TCS SP2 with Argon et 2 Helium/Neon lasers, AOTF, 3 PMTs + transmitted light and MicroLab software) for FRAP and FRET.
1 State-of-the-art Leica TCS SP5 II multiphoton confocal microscope: this microscope is equipped with an inverted electrophysiology microscope, full set of UV (diode laser with 405 nm excitation) and visible lasers (argon laser with 458-476-488-496-514 nm excitation and Helium Neon lasers with 561-594-633 nm), coherent 2-photon infrared, tandem scanner with a resonant scanner (8000Hz). The system has 5 spectral internal detectors two of which for FLIM (Fluorescence Lifetime Imaging) measurements, 1 transmitted light detector, 2 NDD detectors, a Single Molecule Detection (SMD) platform for molecular dynamic analysis, FCS (Fluorescence Correlation Spectroscopy), FCCS (Fluorescence Cross-Correlation Spectroscopy) and FLCS (Fluorescence Lifetime Correlation Spectroscopy) measurements + high resolution and sensitivity digital cameras.
1 Stereomicroscope Stemi 2000C, 10*/23 BR FOC ocular (Zeiss)
1 Typhoon Trio + scanner (GE Healthcare)

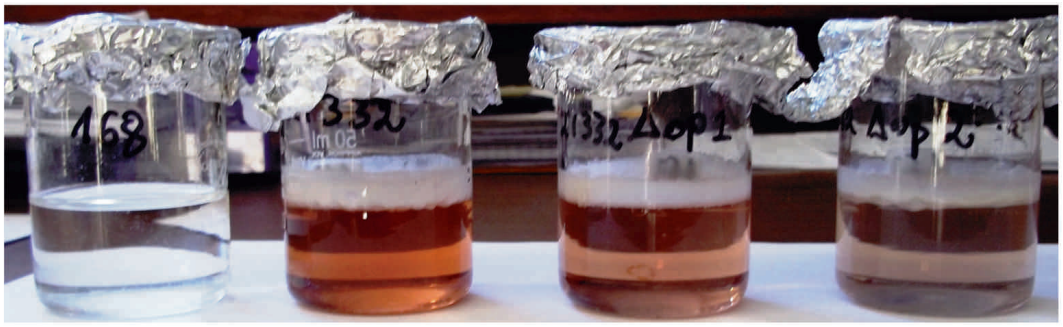
Miscellaneous

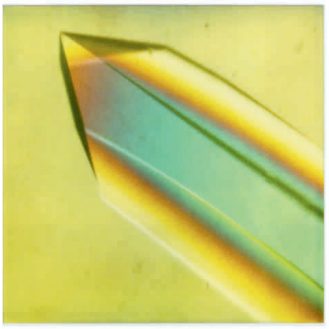
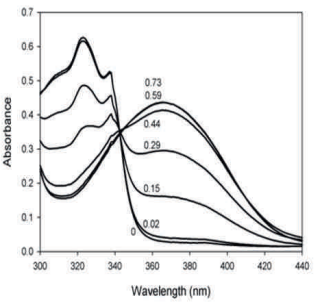
1 Freeze-dryer (Christ)

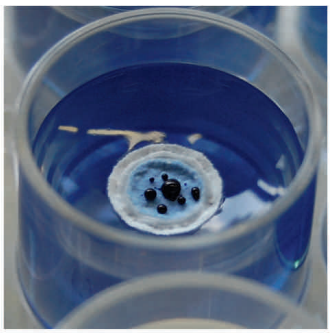
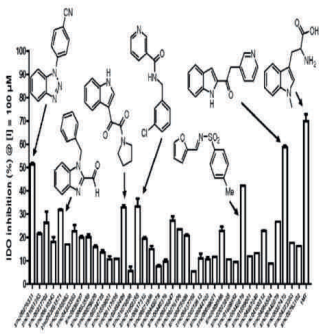
THEMES OF RESEARCH

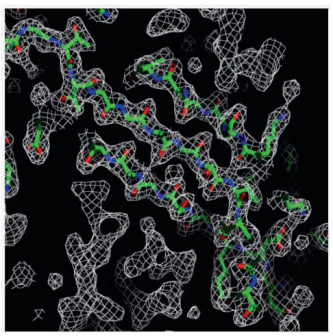
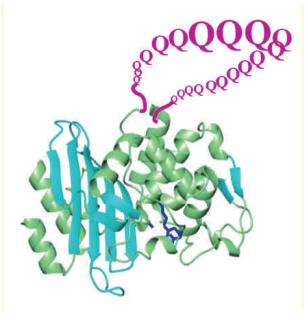



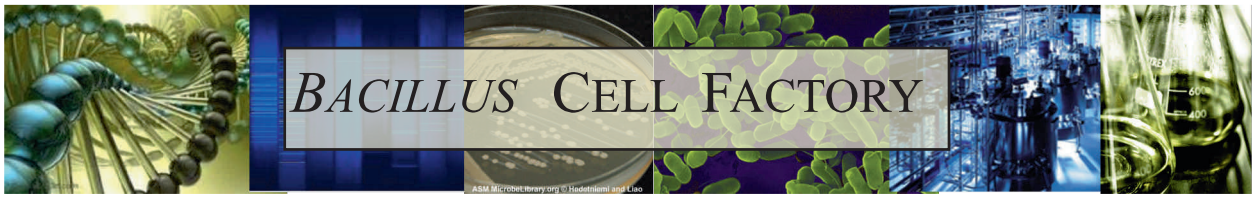







***Bacillus* is a genus of Gram-positive soil bacteria that secretes numerous enzymes and secondary metabolites. The advantage of *Bacilli* as production hosts for protein is essentially due to their efficient secretion machinery. Production by *Bacillus* represents about 60% of the industrial-enzyme market. Moreover, *Bacillus* species produce secondary metabolites such as ribosomal and non ribosomal peptides.**

Bacillus optimization (industrial application)

The CIP is continuously developing original molecular biology methods for *Bacillus* to:

• Improve the transformability of resistant strains e.g. rustic undomesticated *Bacillus* sp.,
• Rapidly generate punctual insertion and deletion mutants free of selection marker,
• Develop GRAS (Generally Recognized As Safe) strains producing high levels of recombinant proteins,
• Carry out directed and random genomic optimization.

Ribosomal peptide or lantibiotics

An unknown lantibiotic is produced by *B. amyloliquefaciens GA1*. Genetic sequence of the gene cluster and structural studies of the compound were carried out. This novel bacteriocin appears to be similar to mersacidin and is active against the Gram-positive bacteria at nanomolar concentrations.

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Associate researchers : Dr A. Brans
Dr M. Delmarcelle

PhD students : A. Argüelles Arias

Bacterial Cell Wall: Biosynthesis and Inhibition

Family 51 bacterial peptidoglycan glycosyltransferases (GTs) catalyze the polymerization of the lipid II precursor into linear peptidoglycan strands (Fig. 1). This activity is essential for the bacteria and represents a validated target for the development of new antibiotics. Peptidoglycan inhibition can be achieved by a compound binding either to the enzyme (moenomycin) or to the lipid II substrate (vancomycin, nisin).

Our objectives are:

- To understand the mechanism of action and the physiological function of the GTs.
- To develop new antibacterial agents against the validated glycosyltransferase target.

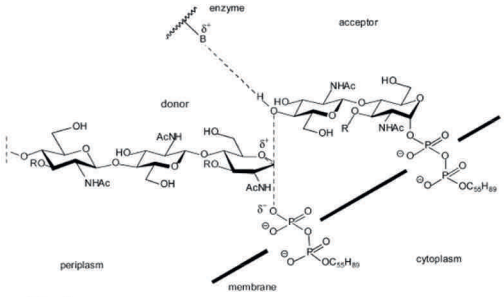

Fig.1

The GT activity of PBP1a of *Thermotoga maritima* has been characterized and the distribution of the glycan chain lengths synthesized *in vitro* has been determined in collaboration with T. Vernet [41].

Small molecules selected using structure-based high throughput virtual screening were found to inhibit the activity of five GTs in the micromolar range and to exhibit antibacterial activity against several Gram-positive bacteria. Investigation of the mechanism of action shows that the compounds specifically target peptidoglycan synthesis. Unexpectedly, despite the fact that the compounds were predicted to bind to the GT active site (Fig. 2), compound 5b was found to interact with the lipid II substrate via the pyrophosphate motif (Fig.3). In addition, this compound showed a negatively charged phospholipid-dependent membrane depolarization and disruption activities. These small molecules are promising leads for the development of more active and specific compounds to target the essential GT step in the cell wall synthesis.

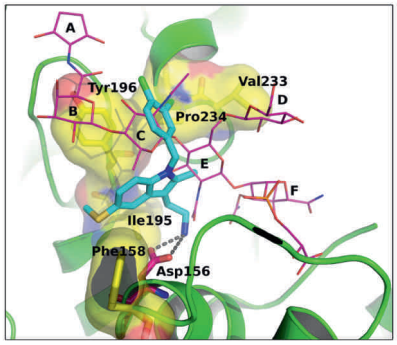

Figure 2: Predicted binding model of compound 5b (cyan) to the *S. aureus* PBP2 active site. Co-crystallised moenomycin is shown in magenta.

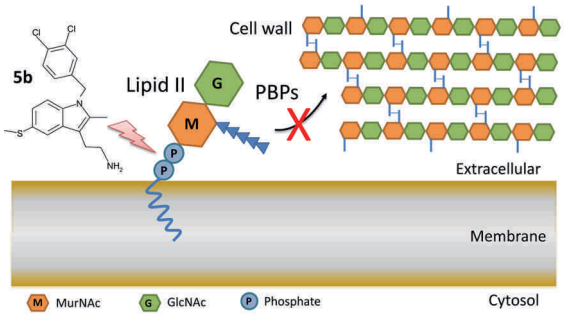

Figure 3: Compound 5b binds to the pyrophosphate group of lipid II and inhibits the polymerization of the peptidoglycan.

This work was done in collaboration with S. Gobec (Ljubljana), E. Breukink, (Utrecht), T. Vernet (Grenoble), I. Chopra (Leeds) and A. Zervosen (ULg) in the frame of the European project EUR-INTAFAR (LSHM-CT-2004-512138).

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Technical assistance: M. Schloesser
Scientific adviser : Dr M. Nguyen-Distèche

Bacterial Cell Wall: Division

During cell division, the peptidoglycan synthesis is performed by a dynamic protein complex, the divisome (Fig. 1). In *Escherichia coli*, this machinery includes more than fifteen proteins which are implicated in a protein-protein interaction network and assemble in two steps (den Blaauwen *et al.*,2008).

Figure 1

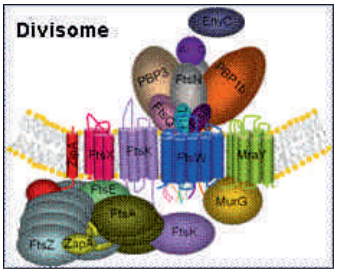
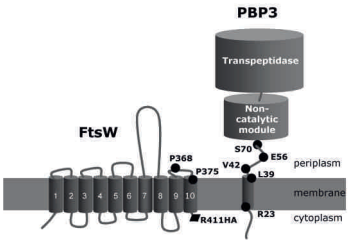


Figure 2



Our objectives are to understand:
- how the cell division proteins interact with each other and synthesize the septal peptidoglycan,
- how the divisome machinery is regulated,
- how the proteins of the divisome and elongasome interact.

The peptidoglycan synthase PBP3 requires the flippase FtsW for its localization and is necessary for the midcell localization of PBP1b, the main peptidoglycan synthase. We have shown direct interactions between FtsW and PBP3 by FRET (Förster Resonance Energy Transfer) and co-immunoprecipitation experiments. These proteins are able to form a discrete complex independently of the other cell division proteins. The K2-V42 peptide of PBP3 containing the membrane spanning segment is a structural determinant sufficient for the interaction with FtsW and PBP3 dimerization (Fig 2). By using a two-hybrid assay, PBP1b was shown to interact with FtsW. The periplasmic loop 9/10 of FtsW appears to be involved in the interaction with both PBP1b and PBP3. It may play an important role in the positioning of these proteins within the divisome.

We have contributed to the enzymatic characterisation of PBP5, the major DD-carboxypeptidase in *E. coli*. The distribution of PBP5 was mapped by immunolabelling and by visualization of GFP fusion proteins. In addition to being scattered around the lateral envelope, PBP5 was also concentrated at nascent division sites prior to visible constriction. Inhibition of PBP2 activity shifted PBP5 to midcell, whereas inhibition of PBP3 led to the creation of PBP5 rings at positions of preseptal wall formation, implying that PBP5 localizes to areas of ongoing peptidoglycan synthesis. A PBP5(S44G) active site mutant was more evenly dispersed, indicating that localization required enzyme activity. Both the membrane bound and soluble forms of PBP5 converted pentapeptides to tetrapeptides *in vitro* and *in vivo*, and the enzymes accepted the same range of substrates, including sacculi, Lipid II, muropeptides and artificial substrates. However, only the membrane-bound form localized to the developing septum and restored wild-type rod morphology to shape defective mutants, suggesting that the two events are related. The results indicate that PBP5 localization to sites of ongoing peptidoglycan synthesis is substrate dependent and requires membrane attachment.

This work was realized in collaboration with T. den Blaauwen (University of Amsterdam), W. Vollmer (University of Newcastle), E. Breukink (University of Utrecht) and was supported by the European project EUR-INTAFAR (LSHM-CT-2004-512138).

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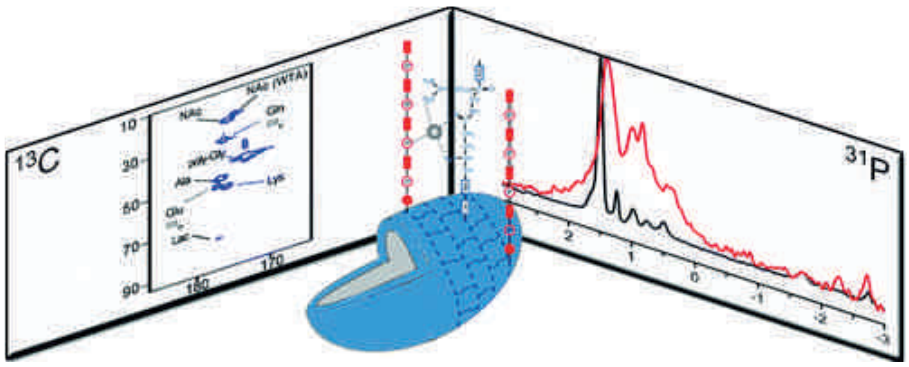
Bacterial Cell Wall: Remodelling

The bacterial cell wall maintains the cell integrity while allowing growth and division. It is made up of peptidoglycan (PG), a biopolymer forming a multigigadalton bag-like structure and, additionally in Gram-positive bacteria, of covalently linked anionic polymers collectively called teichoic acids. These anionic polymers are thought to play important roles in host-cell adhesion, inflammation, and immune activation.

During this year, we have compared the flexibility and the organization of peptidoglycans from Gram-negative bacteria (*E. coli*) with its counterpart from different Gram-positive bacteria using solid-state nuclear magnetic resonance spectroscopy (NMR) under magic-angle sample spinning. The NMR fingerprints suggest an identical local conformation of the PG in all of these bacterial species. Dynamics in the peptidoglycan network decreases from *E. coli* to *B. subtilis* and from *B. subtilis* to *S. aureus* and correlates mainly with the degree of peptide cross-linkage. For intact bacterial cells and isolated cell walls, we have shown that ^{31}P solid-state NMR is particularly well adapted to characterize and differentiate wall teichoic acids of different species. We have further observed complexation with divalent ions, highlighting an important structural aspect of Gram-positive cell wall architecture.



^{13}C and ^{31}P Solid state NMR fingerprints of intact Gram-positive bacteria.

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Collaborators : Dr J.P. Simorre, Institut de Biologie Structurale, Grenoble

Bacterial Resistance: Beta-Lactamase Induction

In several bacteria, the synthesis of one or several β-lactamase(s) is the main factor of β-lactam antibiotic resistance and is induced by the presence of the antibiotic. As this type of antibacterial agent does not significantly cross the cytoplasmic membrane, bacteria in which the β- lactamase is inducible have a mechanism to detect the presence of the antibiotic outside the cell. In the literature, four different mechanisms are reported. During this year, we have focused our effort on those of *Bacillus licheniformis* and *Streptomyces cacaoi*.

Our objective is to understand the mechanism of bacterial resistance, and the coupling between signal transduction and regulation of gene expression.

Three gene products, BlaI, BlaR1 and BlaR2 affect the expression of the inducible BlaP β- lactamase in *B. licheniformis*.
BlaI is a DNA-binding protein acting as a repressor, BlaR1 is a membrane protein that plays the role of a penicillin-receptor (see Fig.1) and BlaR2 is not identified yet. In our working model, BlaI is inactivated by a coactivator generated by the BlaR1 receptor activated by penicillin. During this year we have focused our research on the identification of this coactivator and on how the signal is transduced by BlaR1 acylated by penicillin. For this latter point, we have carried out site-directed mutagenesis experiments to highlight important residues for signal transduction of receptor L2 and L3 loops.

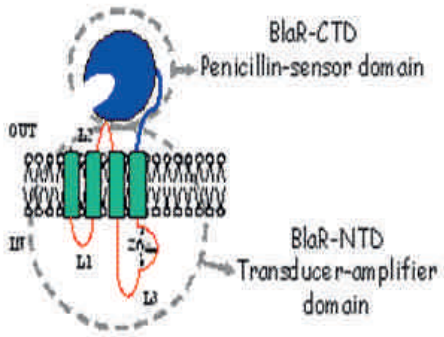

Figure 1: The *B. licheniformis* BlaR1 penicillin receptor topology.

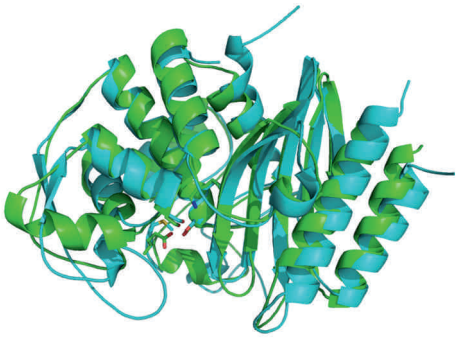

Figure 2: Ribbon superposition of the structures of BlaB(cyan) and the β-lactamase SME1 from *S. marescens* (green).

Two class A β-lactamase genes called *blaL* and *blaU* have been identified in *Streptomyces cacaoi* and are inducible by β-lactam compounds. The two β-lactamase genes are controlled by two cytoplasmic regulators named BlaA and BlaB. Whereas BlaA has been identified as a DNA-binding protein related to the LysR repressor/activator family, the function of BlaB is still unknown. Its primary structure is similar to those of the serine penicillin-recognizing enzymes. BlaB has been overexpressed, purified and its 3D- structure determined by X-ray diffraction.

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BACTERIAL RESISTANCE: BETA-LACTAMASE INHIBITION

The introduction of β-lactam antibiotics like penicillins in medical practice is one of the biggest clinical successes. Unfortunately, bacteria rapidly evolved to develop resistances. The most common resistance mechanism is the bacterial production of β-lactamases which cleave the β-lactam ring. Besides serine β-lactamases of classes A, C and D, metallo-β-lactamases (MβL) of class B are emerging as a problematic group of enzymes challenging the efficiency of modern antibacterial therapy. Notably, MβLs of the VIM family can be observed during nosocomial infections caused by multiresistant pathogens and hydrolyse a broad range of β-lactam substrates including the last generation carbapenems. A successful strategy to counteract β-lactamase mediated resistance is the use of β-lactamase inhibitors which restore bacterial susceptibility to the antibiotic. To date, there is no clinically useful inhibitor of class B MβL.

In the field of drug discovery, camel heavy chain antibodies are of particular interest. These antibodies are devoid of light chains so that their heavy chain variable domains (V_HH or nanobody) are actually the smallest intact antigen-binding fragments derived from functional immunoglobulins. In collaboration with the Structural Biology Brussels group (VUB, Prof. Steyaert), we selected by phage display a dromedary V_HH (CA1838) which inhibits the MβL VIM-4. Its µM level inhibitory ability spans substrates of different types. Using cephalotin as substrate, the inhibition mechanism was shown to be non-competitive with a strong uncompetitive component which promotes substrate inhibition. This highlights the flexibility of MβLs active sites.

Figure 1: Obtention of dromedary V_HHs.

The epitope of CA1838 has been identified by peptide-array in collaboration with the CRP-santé of Luxembourg (retrovirology lab). The epitope corresponds to the loop L7 of VIM-4 which carries amino acids coordinating zinc ions. In an effort to identify binding "hot spots" of this V_HH, alanine scanning mutagenesis has been realized on 17 positions in the CDR2 and CDR3 of CA1838. Measurements of the binding potencies and inhibition percentages of those mutants reveal a mainly hydrophobic interaction.

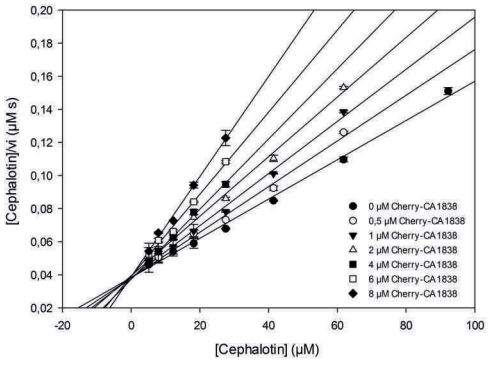

Figure 2: Hanes-Woolf linearization of inhibition curves.

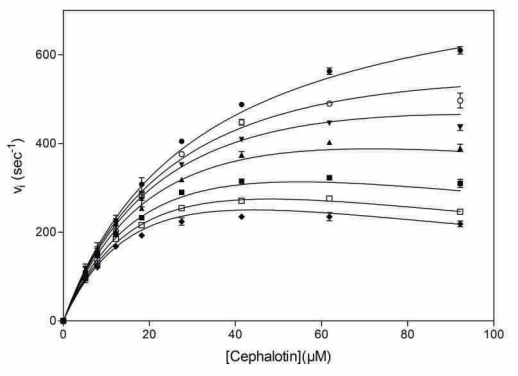

Figure 3: Michaelis-Menten curves versus cephalotin concentrations using various Cherry-CA1838 concentrations.

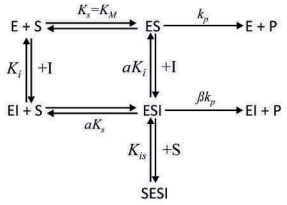

Figure 4: Scheme representing the inhibition model.

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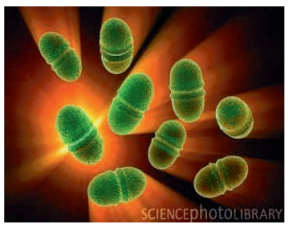
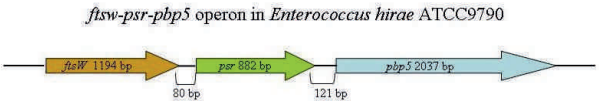
Bacterial Resistance: Low Affinity PBPs

Enterococci have an intrinsic low susceptibility to β-lactams due to the presence of PBP5, a high molecular mass class B PBP. Indeed, PBP5 takes over the DD-transpeptidase function of the other PBPs when they are inhibited by antibiotics. The affinity of PBP5 for β-lactams is extremely low, and thus this PBP is the most resistant class B PBP ever characterized.

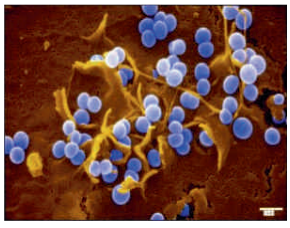


Expression of the *pbp5* gene (encoding the low affinity PBP5) in *Enterococcus hirae* was proposed to be under the control of the Psr protein. Re-examination of the role of Psr by specific gene disruption led to the conclusion that it does neither influence the PBP5 synthesis and consequently the β-lactam resistance nor the cell wall-related properties. The *psr* and *pbp5* genes are preceded by the *ftsW* gene. All three genes form an operon.



Our main objectives are:
- to determine the role of the Psr protein and elucidate how it contributes to the activity of the PBP5 and other proteins related to the peptidoglycan metabolism.
- to find new inhibitors of the PBP5 by chemical or peptide-mimetic approaches .

Staphylococci have acquired methicillin resistance and resistance to all β-lactams and their derivatives by taking up the *mecA* gene, which codes for a foreign PBP named PBP2a. This new foreign PBP2a has a lower affinity for methicillin and other β-lactam derivatives than the endogenous PBPs.



Methicillin Resistant *Staphylococcus aureus* (MRSA) is especially difficult to treat because of its intrinsic resistance to virtually all β-lactams and its tendency to accumulate resistance determinants when exposed to antibiotics, hence its prevalence in hospitals. Resistance depends on the ability of the PBP2a to be rapidly produced upon induction preventing the lethal action of the β-lactams and also it depends on as yet poorly characterized genomic factors that modulate the final resistance levels. The regulation of the PBP2a production is complex, the *mecA* gene is controlled both by its own regulatory element and by the regulatory elements of staphylococcal penicillinases, which are harboured by most MRSA strains. Therefore the kinetics of *mecA* induction, resulting in PBP2a synthesis, differs depending on the genetic elements contained within a specific strain.

Our main objectives are:
- to identify the mechanism regulating the production of PBP2a.
- to use different approaches to discover new PBP2a inhibitors.

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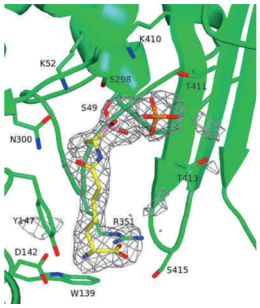
Bacterial Resistance: PBP Inhibition

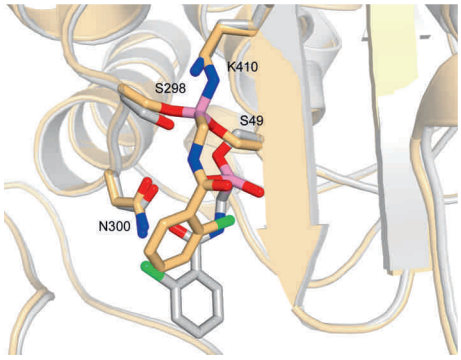
Bacteria exhibit a remarkable capacity to become resistant to commonly used antibacterial compounds. In our efforts to design strategies which will allow to counteract them, we have focused our research to protein targets that are involved in the last stages of peptidoglycan biosynthesis, the DD-peptidases. Boronic acid derivatives are new antibacterial compounds capable of inhibiting β-lactam resistant transpeptidases. For boronic acid synthesis, we collaborate with the groups of Rex Pratt from the Wesleyan University, André Luxen from the University of Liege, Chris Schofield from the University of Oxford and William Gutheil from the University of Missouri-Kansas City. This work was partly conducted within the framework of the FP6 European Integrated Project EUR-INTAFAR (LSHM-CT-2004-512138).

The boronic acid derivatives

Boronic acids bearing appropriate side chains are good inhibitors of serine amidohydrolases. The boron atom usually adopts a tetrahedral conformation, bound to the nucleophilic serine of the active site and mimicking the transition state of the enzymatic reaction. A specific peptidoglycan mimetic boronate inhibitor of the *Actinomadura* R39 DD-peptidase, with a thermodynamic inhibition constant of 32 nM was synthesized and the structure of the complex represents a good transition state analogue for this enzyme [16].



Crystal structure of the R39 DD-peptidase in complex with the specific boronate.



Overlap of monocovalent adduct (white) and tricovalent adduct (gold) of 2-chlorobenzamidomethylboronic acid with R39

We have also solved the structures of R39 with four amidomethylboronic acids. We found that, in each case, the boron forms a tricovalent adduct with three key residues involved in the catalytic mechanism of penicillin-binding proteins. This represents the first tricovalent enzyme-inhibitor adducts observed by crystallography. Formation of the tricovalent complex from a classical monocovalent complex may involve rotation around the Ser49 Cα-Cβ bond to place the boron in a position to interact with Ser298 and Lys410.

Finally, a methodology for the synthesis of a range of boronic acids, both aryl and alkyl types, has been developed. Alkyl boronic acids were shown to be active against PBPs from a number of strains as well as against β-lactamases. As a tool for the conception of new generations of boronate ligands, several crystal structures of complexes between boronic acid analogs DD-peptidases were solved to high resolution.

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BIOCHEMISTRY OF EXTREMOPHILES

Cold-adapted microorganisms, or psychrophiles, thrive efficiently at temperatures as low as -20°C, a temperature recorded for instance in the liquid brine veins between sea ice crystals. At the other extremity of the biological temperature scale, hyperthermophilic Archaea grow up to 122°C in hydrothermal vents, solfataras or hot springs. Life at these extreme biological temperatures obviously requires a vast array of adaptations.

The Laboratory of Biochemistry focuses its research activities on the molecular adaptations displayed by extremophilic biomolecules, with special reference to proteins and enzymes. The scientific staff has produced pioneering contributions in the field of cold-adapted proteins [43]. The three main research topics currently developed by the Laboratory are summarized below.

Protein stability and enzyme activity at extreme biological temperatures

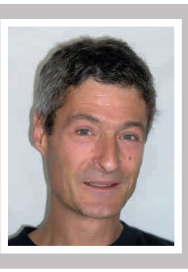
Series of homologous psychrophilic, mesophilic and thermophilic proteins are produced and characterized for specific aspects of protein stability or enzyme activity, involving structure energetics by differential scanning calorimetry, kinetics of unfolding/refolding by chemical denaturants recorded by spectroscopic techniques or newly developed methods for enzyme kinetics recorded by isothermal titration calorimetry.

Proteomics of microbial cold adaptation

The genome of an Antarctic bacterium from our collection has been sequenced, allowing proteomic studies of microbial growth at low temperatures. The main goal is to identify cold acclimation proteins (CAPs), cold repressed proteins (CRPs) and cold shock proteins (CSPs) and to draw a complete metabolic pattern. Cellular proteins are analyzed by 2-dimensional differential in-gel electrophoresis (2D-DIGE) and identified by mass spectrometry.

Biotechnological applications of psychrophilic enzymes

Cold-adapted enzymes are highly active at low and moderate temperatures allowing the use of lower enzyme amounts and the setup of non-heated processes. These enzymes are also heat-labile and easily inactivated. Relevant examples are the industrial production of an Antarctic xylanase by Puratos for baking applications or the Antarctic phosphatase sold by New England Biolabs: both have been isolated from our Antarctic culture collection [32].



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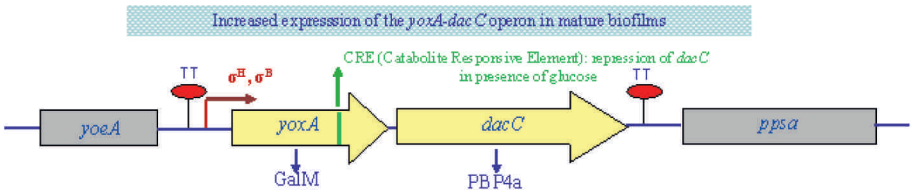
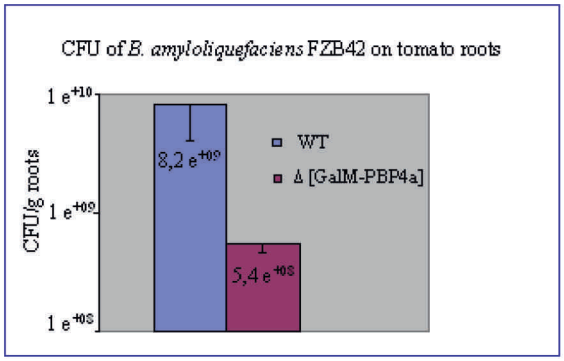
BIOFILM FORMATION BY *BACILLUS*

***Bacillus subtilis* and *B. amyloliquefaciens* are PGPR bacteria (Plant Growth Promoting Rhizobacteria) producing very resistant endospores used as biocontrol agents. They also induce a systemic resistance against various phytopathogens. Much fundamental research remains to be performed to understand and favour the biofilm formation on plant roots, the first step of colonization by the PGPR bacteria.**

In *B. subtilis*, the expression of *dacC* (encoding the PBP4a) is increased in cells isolated from mature biofilms. The *B. subtilis dacC* gene is preceded by *yoxA* in a small two-gene operon.



The *yoxA* gene codes for an aldose-1-epimerase or a galactose-mutarotase that we renamed *GalM* by analogy with its counterpart in *E. coli. GalM* is possibly involved in the scavenging and modification of sugars present in the root exsudates. The *yoxA-dacC* operon has been deleted in *B. amyloliquefaciens* FZB42, an undomesticated strain able to form robust biofilms. The ability of the mutant to swarm on plates with soft agar and its capacity to form biofilm *in vitro* or *in vivo* on tomato roots are analyzed.



A PBP-C1 which seems non essential in laboratory culture conditions likely plays a role in predation in the ecological niche or in fine remodelling of peptidoglycan to allow for example the translocation of polysaccharide components of the extracellular matrix characteristic of biofilms. In association with some uncharacterized cell wall hydrolases, the PBP4a and GalM enzymes could also form a survival system in providing cell wall constituents to starved bacteria.

Another aspect of this research aims at localizing the GalM and PBP4a proteins in the bacterial cell. For this purpose, polyclonal antibodies directed against each purified protein have been obtained.

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Cyanobacterial Blooms: Toxicity and Diversity

Mass proliferation of cyanobacteria is a nuisance that plagues worldwide fresh waterbodies. This nuisance (so-called 'bloom') represents a major potential hazard for human and animal health. It interferes negatively with the sustainable use of surface waters, for e.g. drinking water treatment, recreation, irrigation, and fisheries. Indeed, a number of cyanobacterial genera are considered to be harmful because of their capacity to produce cyanotoxins.

The subject of BELSPO project **BBLOOMS2** (www.bblooms.be) consists in a study of cyanobacterial blooms in Belgium, based on the sampling of reference lakes in Flanders, Brussels and Wallonia (Fig.1). Different approaches are used to determine the environmental conditions, the cyanobacterial diversity and potential toxicity through the detection of *mcy* (microcystin synthase) genes, and the toxin measurements using different techniques.

Figure 1. Lake Féronval (Wallonia) affected by a bloom of *Woronichinia*

Our research group is involved in the development of molecular tools to monitor the dynamics of cyanobacterial diversity and the genotypic composition of cyanotoxin-producing cyanobacteria. We have observed that most of the Belgian cyanobacterial blooms consisted of potentially toxic taxa of the genera *Aphanizomenon, Microcystis, Planktothrix* and *Anabaena*. The assessment of bloom diversity was greatly improved by the molecular approaches. For example, the cyanobacterial blooms in Lake Falemprise (Eau d'Heure) in the period 2002-2009 were dominated by *Aphanizomenon flos-aquae*, but other potentially toxic taxa occur, including *Microcystis aeruginosa* and *Planktothrix agardhii*. The presence and the potential toxicity of these taxa has been confirmed by the genetic analyses.

Based on a culture independent approach, we already showed the presence of sequences that encode the potent toxin cyanopeptolin inside individual colonies of the genus *Woronichinia* (Fig.2).
We are currently developing a real time PCR assay to demonstrate the presence of ecotypes in the genus *Microcystis*.

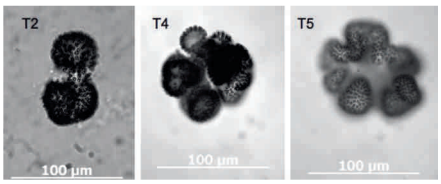

Figure 2. Colonies from the genus *Woronichinia*

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Cyanobacterial Diversity and Biogeography in Antarctica

The cyanobacteria are the major photosynthetic microorganisms in polar (Antarctic and Arctic) terrestrial and limnetic biotopes. Their diversity and biogeography are still largely unknown.

The subject of the **AMBIO** project (www.ambio.ulg.ac.be) is the biogeography of cyanobacteria in microbial mats from Antarctic lakes. On the basis of 58 samples analyzed by Denaturating Gradient Gel Electrophoresis (DGGE), the cyanobacterial OTUs (Operational Taxonomic Units; groups of 16S rRNA sequences sharing more than 98.5% similarity) seem to have a geographical structure on a global scale. The majority (59%) has a global distribution (cosmopolites), whereas the rest seems to be restricted to the "cold biosphere" (polar and alpine habitats). Among the latter ones, 4 are potentially endemic to a particular region.

The new Antarctic Belgian station "Princess Elisabeth" has been built on the Utsteinen granite ridge (71° 57'S, 23° 20'E) in the Sör Rondane Mountains.
The BelSPO project **ANTAR-IMPACT** (http://www.antar-impact.ulg.ac.be/PageFR.htm) aims to study the diversity of the cyanobacteria to assess the initial biological state of the station site. Samples were collected before the construction of the station in 2007. Nineteen OTUs were found in the area of the station. Thirteen of them had a cosmopolitan distribution and 6 were only found in Antarctica.

The BelSPO project **BELDIVA** aims to explore the terrestrial biodiversity in the 50 km zone around the Belgian station. Sixteen different cyanobacteria morphotypes were identified by microscopy. Thirty-one OTUs were found, ranging from 0 to 18 OTUs per sampling area. Fifteen OTUs were endemic to Antarctica and 16 OTUs had a cosmopolitan distribution. In 2010, 8 Open Top Chambers were installed in 4 locations for a long-term experiment to estimate the responses of the cyanobacterial terrestrial communities to a climate change simulation.

In the **HOLANT** project (www.holant.ugent.be), two sediment cores from two lakes in Beak Island (Antarctic Peninsula) were analyzed to observe the changes in cyanobacterial communities over the past ca. 4000 years. The paleodiversity study was based on 16S rRNA genes from ancient or fossil DNA. The shallower of the two lakes showed a higher OTU richness than the deeper lake. *Synechococcus*-related sequences were extensively found in both sediment cores. Cyanobacterial assemblages remained relatively stable through periods of environmental change but distinct changes were observed in the upper layers of the sediment cores, corresponding to the last 4 or 5 decades. This could be related to a response to past climate warming.

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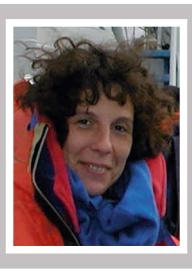


House Dust Mite Allergy

More than 80% of patients suffering of allergic asthma are positive to mite extracts and have large amounts of IgE specific for mite allergens. The proteolytic activity of the Der p 1, Der p 3, Der p 6 and Der p 9 allergens which are secreted by the house dust mite *Dermatophagoides pteronyssinus* contributes to their allergenicity. Indeed, after their inhalation, these enzymes can disrupt the human epithelial barrier increasing the permeability of the bronchial mucosa and then can target cells of the innate and adaptive immune systems, favouring hyper-IgE production and allergy. Like most proteases, the secreted proteolytic allergens are synthesized as inactive precursors termed proDer p 1, proDer p 3, proDer p 6 and proDer p 9 and should be involved in the mite digestion after their activation. These zymogens are formed of an N-terminal propeptide ranging from 6 to 80 amino acids and a protease domain of 220-232 residues.

Our objectives are :

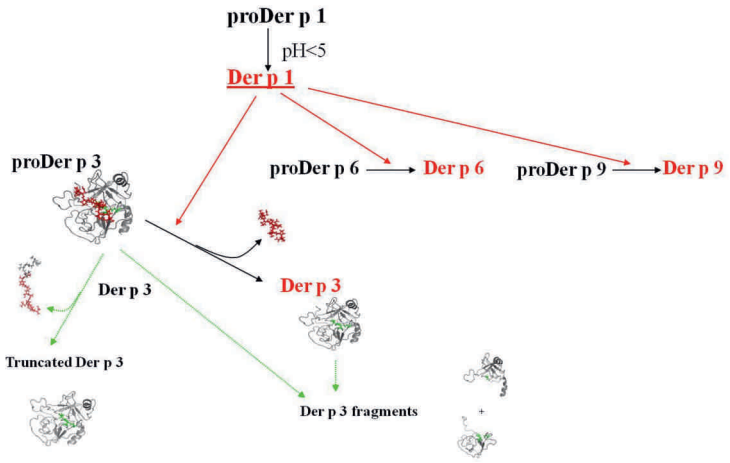
-To understand the activation mechanism of these zymogens, consisting in the cleavage and release of the propeptides and the formation of the allergenic mature proteases.

-To determine the roles of the propeptides in the inhibition of the allergens but also in their folding, stability and allergenicity.

-To study the natural substrates of the allergens in the aim to highlight new sensitive biomarkers and to progress in the development of highly specific protease inhibitors.

Activation mechanisms of the allergens

By using recombinant allergens, we have demonstrated that the cysteine protease proDer p 1 can auto-activate in a multistep mechanism at acidic pH, due to the unfolding of its propeptide. The proteolytic neo-formed enzyme could then process proDer p 3 in its mature form Der p 3. Moreover, Der p 1 could be the major activator of the cysteine (proDer p 1) and serine (proDer p 3, proDer p 6 and proDer p 9) protease zymogens as we demonstrated by using the FRET technique with fluorescent peptide probes. We employ a large panel of techniques like *Pichia pastoris* expression, ELISA, fluorescence and circular dichroism spectroscopies, enzymology, solid phase synthesis of peptides, mass spectrometry,... Currently, we are investigating the allergen reactivity and more particularly the role of their propeptides in the allergic response [10].



Identification of natural substrates

The allergenic proteases are known to cleave proteins and receptors at the membrane surface of different cellular types. We have chosen a proteomic approach (2D LC-MS) to identify the natural substrates of the cysteine and serine proteases located at the bronchial and basophile cell surfaces. We are also searching for highly specific protease inhibitors.

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METAGENOMICS

Metagenomics implies a series of laboratory manipulations leading to the isolation of DNA originating from (ideally) the entire diversity of microorganisms found in a specific sample (soil, water, tissues and so on), bypassing the necessity of dealing with culturing techniques. Moreover, despite the enormous diversity of microorganisms that inhabit our planet, it is estimated that more than 99% of them are not growing in standard culture conditions, and thus await the development of new strategies that can unmask them, along with their wealth of resources.
The metagenomic offers a powerful tool for accessing a range of almost unlimited possibilities for screening new activities from many different environments.

Our objectives are :
• To characterize extreme metagenomes
• To isolate new catalysts by performing activity driven screening
• To elucidate the physiological function of metagenome derived enzymes
• To test the possibility to transfer metagenomes derived enzymes in existing industrial processes (GeneHunt Project)

We are focused on carbohydrate modifying enzymes involved in both carbohydrate synthesis and degradation. We are involved in the accurate characterization of cellulases (Fig.1).

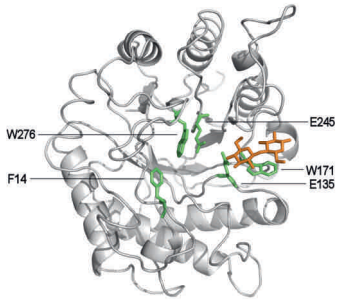

Figure 1: Tridimensional structure of the cellulase RBcel1 in complex with cellobiose (orange). The catalytic residues and the substrates binding sites are shown (green).

These enzymes are thought to be efficient catalysts for plant cellulose breakdown; nevertheless the major part of cellulases are unable to degrade natural polymers. Their physiological function is under characterization and should be associated to cellulose production in bacterial biofilms (Figs 2-3).

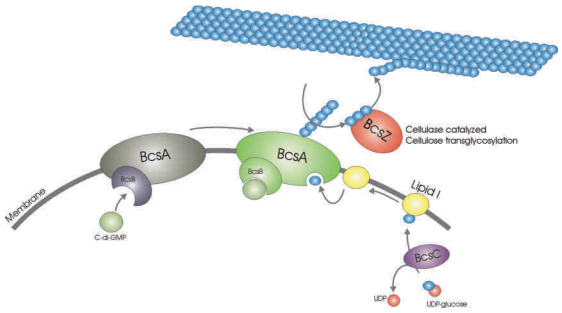

Figure 2: Proposed model for bacterial cellulose synthesis, BcsZ is an endoglucanase requested for the efficient cellulose production.

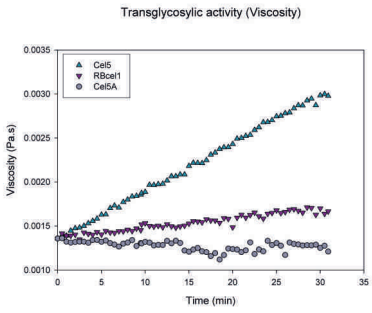

Figure 3: Following the polymerization reaction by viscosimetry, using cellobiose as substrate: RBcel1 and Cel5 are two cellulases able to catalyze the polymerization of cellobiose, leading to the production of longer oligosaccharides.

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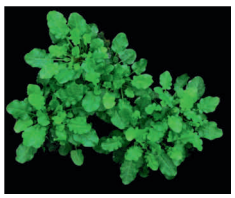
Metal Tolerance and Hyperaccumulation in Plants

A small number of plant species, the so-called hyperaccumulators, can live on soils heavily polluted by metals and possess the ability to accumulate extraordinarily high concentrations of these metals in above-ground tissues (e.g., >1% zinc or 0.01% cadmium in dry leaf biomass). The Brassicaceae *Arabidopsis halleri* exhibits hypertolerance to zinc and cadmium and accumulates high zinc concentrations of up to 2.2% leaf dry biomass.

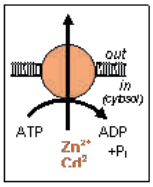
A. halleri is closely related to the model plant *Arabidopsis thaliana* and to *Arabidopsis lyrata*, which are non-accumulating and non-tolerant species. With their contrasted physiologies, *A. thaliana, A. lyrata* and *A. halleri* constitute powerful models to study the mechanisms of speciation, adaptation to an extreme environment and evolution of a complex naturally-selected extreme trait.

Comparative transcriptomic and genetic analyses identified candidate genes which are constitutively over-expressed in *A. halleri* compared to *A. thaliana* and which may have a role in metal tolerance or accumulation. Combining genetic and functional genomic approaches, our project is aiming at identifying the molecular determinants of the differential expression of selected candidate genes in *A. halleri*, to analyse their function in metal tolerance and hyperaccumulation and to study how these traits evolved.

A key candidate gene is *HMA4*. The *A. halleri HMA4* gene is respectively 7-fold and 30-fold more expressed in root and shoot of *A. halleri* compared to *A. thaliana*. The *HMA4* gene encodes a P-type ATPase that acts as a plasmamembrane-located zinc/cadmium pump.



Arabidopsis halleri



The *HMA4* metal pump

High expression of *HMA4* is required for full zinc and cadmium tolerance and zinc hyperaccumulation. Evolution of a higher expression of *HMA4* occurred through a combination of cis-regulatory changes and a tandem triplication of the gene, leading to increased expression of all three gene copies. The expression of *AhHMA4* under the control of its *A. halleri* endogenous promoter in *A. thaliana* transgenic lines recapitulated several typical hyperaccumulator phenotypes, but resulted in increased sensitivity to excess zinc and cadmium, which suggests that metal detoxification requires the products of additional genes to accommodate the high *HMA4*-dependent metal flux into the shoots of *A. halleri*. Similarly, expression of this construct in tobacco caused transgenic plants to be more sensitive than the wild type to high concentrations of both zinc and cadmium [3].

Research objectives:

1. To identify potential cis elements and trans-binding factors that activate the *HMA4* promoters in *A. halleri* through detailed promoter deletion analysis.
2. To determine the localization and interaction partners of the *A. halleri* HMA4 protein in plants.
3. To characterize the metal affinity of cytoplasmic metal binding domains of the *A. halleri* HMA4, and through structure/function analysis, study the role of these domains in the HMA4 activity in plants.
4. To determine if and how selection acts on the *HMA4* locus in *A. halleri*.

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Microbial Production of Hydrogen

Molecular hydrogen is a key intermediate in metabolic interactions of a wide range of microorganisms. In the light of global political instability and increased fossil fuel consumption, hydrogen is also regarded as a key component in future energy systems as it is a sustainable, clean, and transportable energy carrier. It has the highest energy content (143 GJ.Ton-^{1}) per unit weight of any known fuel and it is not bound to a carbon molecule, thus its burning does not produce carbon–based contaminants. The problem is to extract molecular hydrogen from sources such as water or organic matter.

In this project, named Micro–H2, we have focused our research on the biohydrogen production by the fermentative, anaerobic species of the genus *Clostridium*. Clostridia are the dominant microorganisms in mixed microaerophilic communities capable of hydrogen production from biomass waste treatment. They can produce hydrogen by butyric and mixed-acid fermentations (Fig. 1) at optimal pH values ranging from 4.5 to 5.5. The process of biohydrogen production fundamentally depends on the presence of hydrogen producing enzymes, called hydrogenases. Two groups have been described in clostridia, [FeFe] and [NiFe] hydrogenases [8].

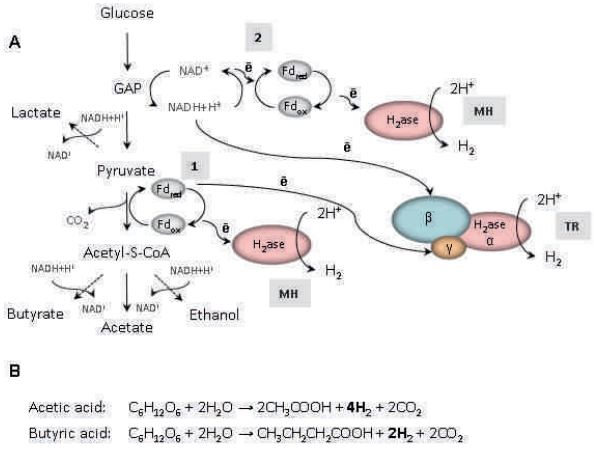

Figure 1: Fermentative hydrogen production in a *Clostridium sp*.

There are two possible pathways for hydrogen production in *Clostridium sp*. One is linked to the oxidation of reduced ferredoxin catalyzed by the enzyme complex pyruvate:Fd oxidoreductase (pathway 1) The second involves ferredoxin-mediated NADH reoxidation catalyzed by NADH:Fd oxidoreductase (pathway 2).
MH – monomeric [FeFe] hydrogenase, **TR** – trimeric [FeFe] hydrogenase, Fd – ferredoxin.

Aims of the project:
1. Molecular analysis of the evolution of the consortium by qPCR and FISH
2. Characterization of the hydrogenases content and the hydrogen metabolism in *Clostridium sp*.; omics (proteomic, metabolomic and transcriptomic) approach.

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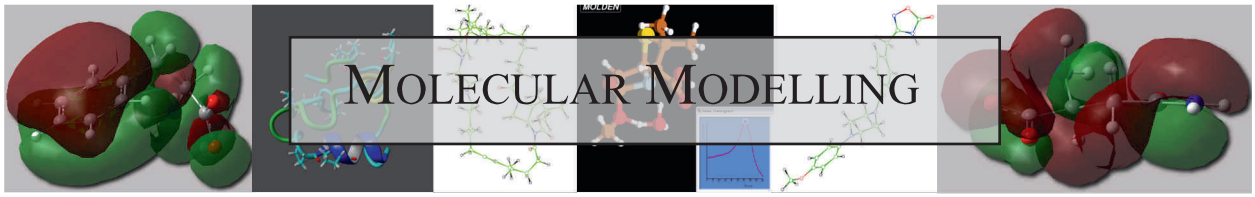
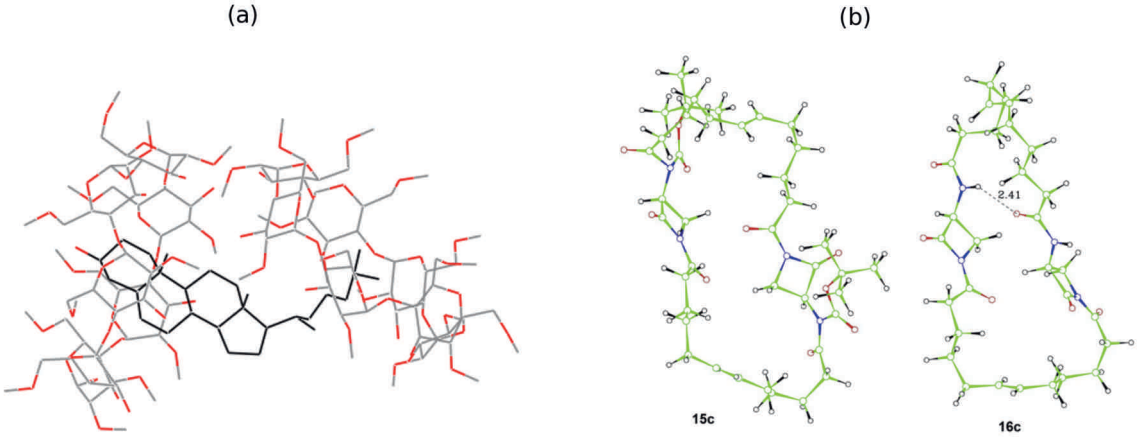


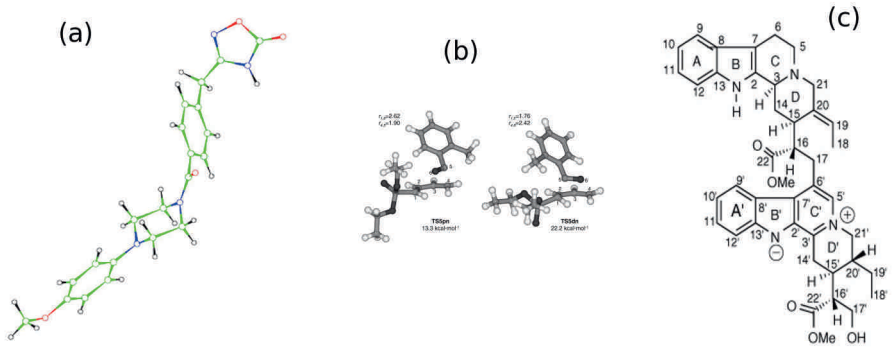
MOLECULAR MODELLING

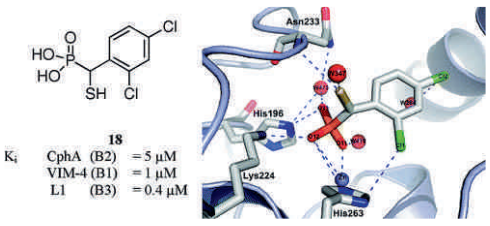
The objective is to understand, at the atomic level, the reactivity and properties of biological macromolecules and their interactions either with drugs or with other macromolecules by using the theoretical tools of quantum chemistry and molecular mechanics.

At the quantum chemistry level, our application studies still concern interactions involving cyclodextrins [9] (a) and serine enzyme inhibitors [47] (b).



Moreover, several other collaborations were fruitful. These were about phospholipase A(2) inhibitors [33] (a) , about a study of cycloaddition [37] (b) and about moandaensine, a dimeric indole [54] (c) .





Molecular Mechanics (MM) and Molecular Dynamics (MD) were applied to study complexes between the beta-lactamase VIM-4 and mercaptophosphate compounds [27].

The collaborations with the Mass Spectrometry Laboratory about the fragmentation of dinucleotide ions [1] and with the Molecular Dynamics Laboratory about the spectroscopy of halogenated compounds [28-30] are continued.

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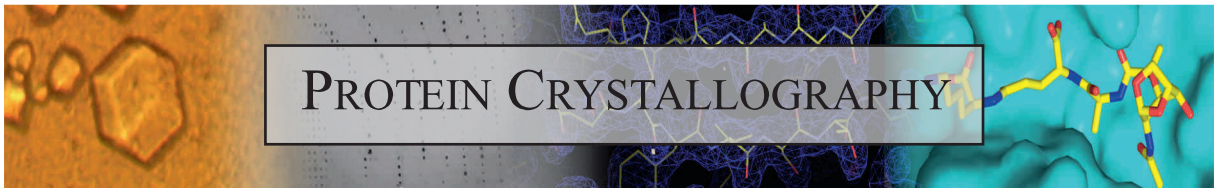
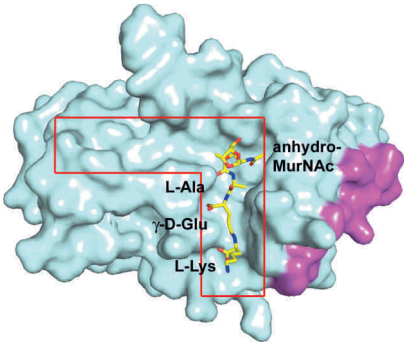


X-ray crystallography is one of the most used techniques for the determination of protein structures at the atomic level. It is particularly adapted for the exploration of protein-protein and protein-ligand interactions and for understanding the catalytic mechanism of enzymes.

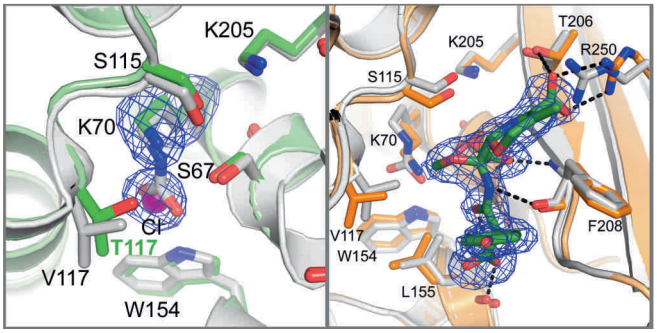
All the steps needed for a protein structure determination are handled within the CIP infrastructures: protein production, purification and crystallization, data collection in house and at the FIP-BM30a beamline of ESRF synchrotron, different phasing techniques including production of seleno-methionine substituted proteins, and detailed analysis of structures. The laboratory of protein crystallography collaborates with the other research groups on the study of β-lactamases, peptidoglycan (PG) related proteins as well as other subjects with external laboratories. Two examples of our recent results are highlighted below.

AmiD, a N-acetylmuramoyl-L-alanine zinc amidase of *E. coli* (in collaboration with the Bacterial Diversity, Physiology and Genetics group)



This periplasmic lipoprotein is anchored in the outer membrane and is capable of cleaving the intact PG as well as soluble fragments containing N-acetylmuramic acid. AmiD could be part of the enzymatic machinery involved in the PG turnover.
The structures of AmiD with and without a substrate in its active site show a relatively flexible N-terminal extension that allows an easy reach of the PG by the enzyme inserted into the outer membrane and a C-terminal domain providing a geometrical complementarity to the substrate. These structures reveal the similarity between the catalytic mechanism of zinc amidases of the AmiD family and the thermolysin-related zinc peptidases [23].

Carboxylation of the class D β-lactamase OXA-10 (in collaboration with the Biological Macromolecules and Biochemistry group)
The activity of class D β-lactamases is dependent on Lys70 carboxylation in the active site. Structural, kinetic and affinity studies show that this post-translational modification can be affected by the presence of a poor substrate such as moxalactam but also by the V117T substitution. Val117 is a strictly conserved hydrophobic residue located in the active site. In addition, inhibition of class D β-lactamases by chloride ions is due to a competition between the side chain carboxylate of the modified Lys70 and chloride ions [52].



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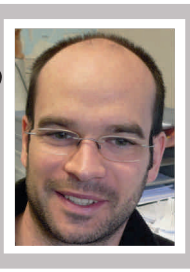
PhD Student: M. Rocaboy



Folding of serine β-lactamases

We analyzed [50] how conformational folding, reactivation, and *cis/trans* peptide bond isomerizations are interrelated in the folding kinetics of β-lactamases that differ in the nature of the Glu166-Xaa167 *cis* peptide bond. The analysis of folding by spectroscopic probes and by the regain of enzymatic activity in combination with double mixing procedures indicates that conformational folding can proceed when the 166-167 bond is still in the incorrect *trans* form. The very slow *trans* → *cis* isomerization of that peptide bond, however, controls the final step of folding and is required for the regain of the enzymatic activity. A second slow kinetic phase is caused by the *cis/trans* isomerization of prolines that are *trans* in the folded proteins. The folding of β-lactamases is best described by a model that involves parallel pathways. It highlights the role of peptide bond *cis/trans* isomerization as a kinetic determinant of folding. Our next goal is to use pulse-labelling H/D exchange (HEX) experiments, in combination with 2D-NMR measurements, to monitor the time-course of formation and stabilization of secondary structure elements during the folding of the BS3 β-lactamase. With this aim, we assigned the backbone amide resonances in the NMR spectrum of the enzyme [51].

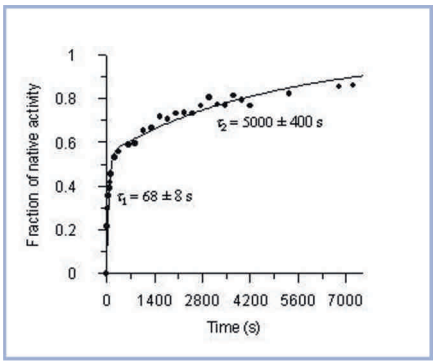


Figure 1: Refolding kinetics of the BS3 ß-lactamase at 10°C, followed by recovery of enzymatic activity.

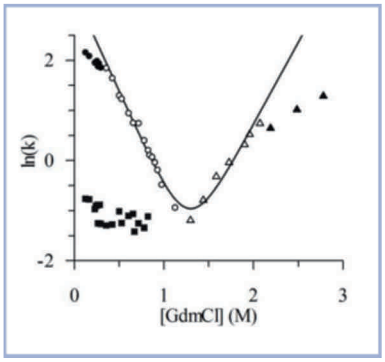


Figure 2: Chevron plot for λ lysozyme folding at pH 5.6, 20°C.

Folding of λ lysozyme

We assigned the backbone resonances in the NMR spectrum of the enzyme [14]. Stopped-flow fluorescence and CD spectroscopy were used in combination with quenched-flow HEX labelling, monitored by 2D-NMR and ESI-MS, to investigate the folding kinetics of λ lysozyme [13]. The first step occurs very rapidly ($\tau < 1$ ms) after refolding is initiated and involves both hydrophobic collapse and formation of a high content of secondary structure, but only weak protection from HEX and no fixed tertiary structure organization. Following accumulation of this kinetic molten globule species, the secondary structural elements are stabilized and the majority (ca. 88%) of refolding molecules acquire native-like properties, including substantial native-like protection from hydrogen exchange, in a highly cooperative two-state process (τ ~0.15 s). Folding of the remaining (ca. 12%) molecules is rate limited by isomerization of prolines that are *trans* in the folded enzyme. In addition, native state HEX and classical denaturant unfolding experiments have been used to characterize the thermodynamic properties of the enzyme. In good agreement with previous crystallographic evidence, our results show that λ lysozyme is a highly dynamic protein, with relatively low conformational stability.

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Protein Misfolding and Aggregation

The deposition of proteins in the form of amyloid fibrils, in the nervous system or in certain peripheral tissues, is associated with more than 40 degenerative diseases, referred to as amyloidoses. Using a multidisciplinary approach, our laboratory seeks to elucidate the mechanisms leading to protein misfolding and aggregation.

Our work is focused mainly on two types of proteins: human lysozyme [11,12,18,39,59] and chimeric proteins made of the β-lactamase BlaP and polyglutamine (polyQ) stretches of different lengths. In collaboration with the group of Chris Dobson (Cambridge) and Jan Steyaert (Bruxelles), our particular approach consists in generating conformational camelid antibody fragments (or V_HHs) against the different species formed on the pathway of aggregation and to use them as i) structural probes to elucidate the mechanism of fibril formation and ii) potential inhibitors of the aggregation process [Dumoulin & Dobson, 2004, Biochimie 86, 589-600].

Here, we highlight our work on the polyQ chimeras.

Nine progressive neurodegenerative disorders, including Huntington's disease, are associated with anomalous expansion of a polyQ tract above a threshold length into nine different proteins. These proteins with expanded polyQ repeats form intranuclear amyloid-like aggregates which are believed along with their precursor species to play an important role in the pathogenesis. In order to gain further insight into this mechanism, we have inserted polyQ sequences into a well studied protein, the β-lactamase BlaP from *B. licheniformis* 749/C (Fig. 1).

Using a range of biophysical techniques including fluorescence and circular dichroism, we have shown that the insertion of a polyQ tract made of up to 79 residues does not modify the structure of the enzyme; it does however significantly destabilize it. The aggregating properties of the chimeras recapitulate the two phenomena characteristics of protein naturally containing polyQ expansions. Indeed, we observed a threshold above which the chimeric β-lactamase aggregates into fibrils, and above this threshold, the kinetics of aggregation is faster with longer repeats. The BlaP chimeras are, therefore, a good model to investigate the mechanism of aggregation of polyQ proteins. Very interestingly, we could clearly show that the threshold number of glutamines above which the chimeric β-lactamases aggregate into amyloid-like fibrils depends on the structural conformation of the β-lactamase moiety (Fig. 2).

Moreover, we have immunised a llama with the soluble form of $BlaP(Gln)_{55}$. From the lymphocytes isolated from the llama's blood, a library has been constructed by cloning all the V_HH genes into the pMES4 vector. This library was then expressed at the surface of phages which were used to carry out the panning in three different ways. Sixty-eight different V_HHs have been obtained and their ability to interfere with the process of aggregation is under investigation.

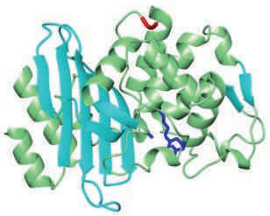

Figure 1: Structure of the BlaP β-lactamase. The active site is coloured in dark blue, and the insertion site in red.

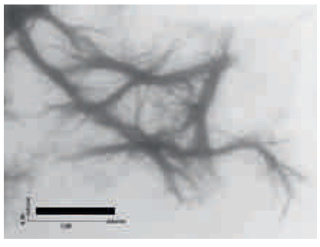

Figure 2: TEM image of aggregates formed from BlaP containing 79Q. Bar =100 nm.

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Streptomyces Genetics and Development

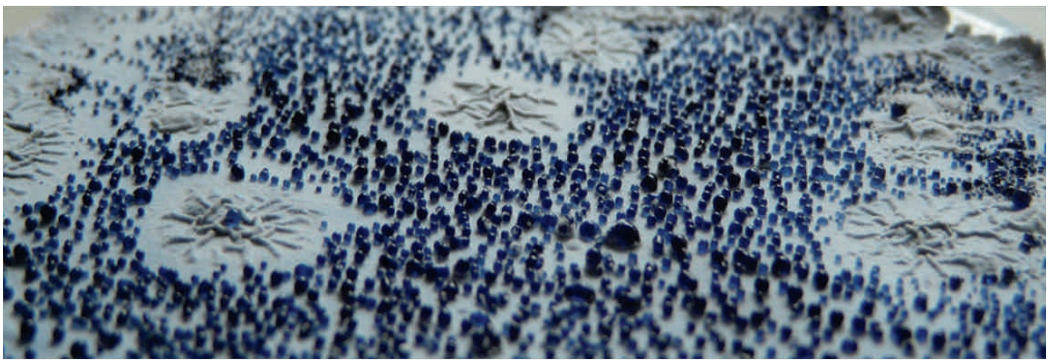
What are the mechanisms that control *Streptomyces* development? [40]

Streptomycetes are filamentous, sporulating, Gram-positive soil bacteria. As producers of some two-thirds of known antibiotics, anti-cancer agents, enzymatic inhibitors, or herbicides, streptomycetes are also considered as a paradigm of secondary metabolite-producing microorganisms. Analyses of *Streptomyces* genome sequences revealed that their "antibiotic"-producing potential had been grossly underestimated: each genome contains more than 20 sets of putative biosynthetic genes for secondary metabolites, but many of them are "cryptic" and not or weakly expressed under typical laboratory conditions of rapid growth on nutrient-rich media. Considering the huge potential impact of the cryptic clusters recently discovered in many Streptomyces genomes, awakening them has become a major challenge for molecular biologists today. The most straightforward approach would be to learn from the organism itself, and since morphological differentiation is intimately coupled to the switch to chemical differentiation, the general purpose of our project aims to understand what the environmental signals that control streptomycetes development are and how they control it.

The availability of nutrients is a major determinant for the timing of morphogenesis and antibiotic production in the soil-dwelling bacterium *Streptomyces coelicolor*. This year we showed that N-acetylglucosamine transport, the first step of an important nutrient signalling cascade, is mediated by the NagE2 permease of the phosphotransferase system, and that the activity of this permease is linked to nutritional control of sporulation and antibiotic production.

What are the true roles played by secondary metabolites in nature?

If secondary metabolites produced by microorganisms possess interesting biological activities from a therapeutic point of view, the role exerted by these molecules in their natural environment is still intensively debated. The triggering of antibiotic production by various environmental stress molecules could be interpreted as bacteria's response to obtain increased fitness to putative danger, whereas the opposite situation - inhibition of antibiotic production - is more complicated to understand. Together with Dr Genevieve Girard (Leiden University, NL) we used the phenazine-1-carboxamide (PCN) production by *Pseudomonas chlororaphis* as a system to investigate the adaptation of secondary metabolite production when bacteria face environmental stresses. We recently demonstrated that switching off PCN synthesis by attenuating expression of the *pip* regulator would favor processes required for survival.



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The SR Protein Family of Splicing Factors

In eukaryotes most nuclear genes are interrupted by non-coding sequences (introns) that must be accurately excised from precursor messenger RNA (pre-mRNA) molecules to give rise to functional mature protein-coding mRNAs. Pre-mRNA splicing is catalyzed by a dynamic macromolecular complex known as the spliceosome. The spliceosome is one of the most elaborate edifices in the cell whose precise assembly at each intron involves five small nuclear ribonucleoprotein particles (snRNPs), comprised of U-rich small nuclear RNAs (snRNAs; U1, U2, U4, U5 and U6) associated with snRNP-specific proteins.

Pre-mRNA alternative splicing (AS) is a highly regulated mechanism that allows the synthesis of multiple mRNAs from a single gene. AS is widespread in eukaryotes and has a significant role in expanding transcriptome and proteome diversity. In mammals, AS has been shown to be prevalent in distinct tissues and a relation between disease and changes in alternative splice forms is established. Recent estimates indicated that ~95% of multiexon human genes undergo AS and that most AS events are regulated between tissues.

Global AS has been investigated in plants. More than 50% of rice AS-related genes undergo multiple AS events producing a variety of transcripts from a single gene highlighting the extremely high complexity of transcriptome regulation. AS has been linked to plant development and response to environment. In addition to spliceosomal snRNPs, constitutive and alternative splicing requires a large number of non-snRNP-associated proteins. The serine/arginine-rich (SR) splicing factors dynamically participate in spliceosome assembly. SR proteins associate on nascent pre-mRNAs for co-transcriptional splicing. In human, at least nine SR proteins have been described with sizes ranging from 20 to 75 kDa. Prototypical SR proteins have a modular architecture consisting of one or two N-terminal RNA recognition motifs (RRM) and a C-terminal SR domain of low complexity enriched in Arg-Ser (or Ser-Arg) repeats. Some SR proteins contain an RNA-binding CCHC Zn-knuckle (ZnK) motif located between the RRM and SR domains.

Analyses of *Arabidopsis thaliana* and rice genomes yielded at least 19 and 24 SR protein-encoding genes, respectively. Some SR proteins are homologous to human prototypes SRSF2 (1 RRM), SRSF1 (2 RRMs) and SRSF7 (1 RRM and 1 ZnK), while others are reported to be plant-specific. For example, members of the RS2Z subfamily are characterized by the presence of two adjacent ZnKs. The complexity of the SR protein family thus appears higher in green plants than in mammals. The functions of plant SR proteins have still to be determined in a physiological context. Much remains unknown with regard to their possible non-splicing activity [44]. Using a multidisciplinary approach, we aim to provide fundamental insights into the functions of *Arabidopsis* SR splicing factors during growth and development

Research objectives:

1. To determine the SR protein determinants controlling their *in vivo* dynamic distribution, interactions and nucleocytoplasmic shuttling
2. To investigate the genetic functions of SR splicing factors during *Arabidopsis* development.
3. To investigate the protein-protein and RNA-protein interaction network orchestrated by *Arabidopsis* SR proteins by using *in vivo* and biochemical approaches.
4. To study the structural features and RNA binding properties of distinct *Arabidopsis* SR proteins.

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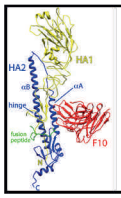
Applied Projects

- Aptarray
- Cantol
- Comparative Study of Enzymatic Production of Tagatose
- GPCR-like
- MED-ATR
- RAIDGBS
- RAPARRAY
- SCALAB

The projects mentionned in coloured boxes are described in the following pages.



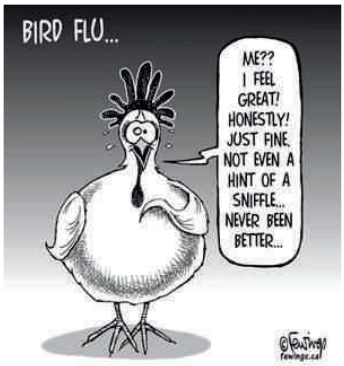
APTARRAY

RÉGION WALLONNE

Characterization of avian influenza hemagglutinin H7HA2 domain produced in *Escherichia coli*

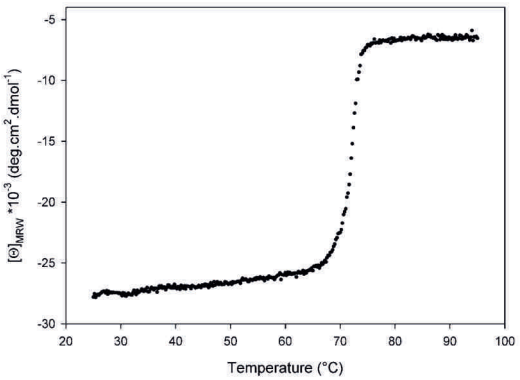
Partner 1 (Coordinator): Prof. Daniel DESMECHT (URPA) -ULg
Partner 2 - Prof. Moreno GALLENI (CIP) – ULg
Partner 3 - Prof. José REMACLE (URBC) – FUNDP

Industrial collaborator: EAT- eppendorf Array Technologies



H7 Influenza A subtype virus was associated with highly pathogenic (HP) Avian Influenza (AI) virus outbreaks in poultry in Europe, Asia, North and South America, and Australia. In 2003, the highly pathogenic H7N7 virus caused 89 human infections and 1 death in the Netherlands. Human infections by IPAI H7N3 virus occurred during the Canadian outbreak in 2004. Serum samples collected from individuals exposed to the virus suggest bird-to-human transmissions. Therefore, the development of rapid and simple diagnostic techniques for AI virus is important. Accordingly, we studied the immunogenic power of H7HA2 antigen in rabbit and its use as a specific biomarker of H7 influenza A viruses.

The HA2 domain of the hemagglutinin of Influenza A virus H7N1 (A/Chicken/Italy/1067/99) was produced in a soluble form in *Escherichia coli* (*E.coli*). A simple chart flow of purification was developed by adding a poly-histidine tag to the C-terminal of the protein. In solution, H7HA2 was mainly found as a trimer. The polypeptide exhibited a high thermostability (apparent Tm=72°C). The thermal denaturation was irreversible. Far UV circular dichroism and fluorescence experiments indicated the absence of an intermediate between the native and unfolded forms of H7HA2. A similar mechanism of denaturation was observed in presence of guanidinium chloride. Finally, we raised rabbit anti-H7HA2 polyclonal antibodies. We observed that our protein preparation was highly immunogenic. This result indicated that H7HA2 is a potential specific biomarker for the detection of viral infection mediated by H7Nx Influenza A viruses.



Thermal unfolding curve of H7HA2 monitored by CD at 224nm.

Project leader: **Dr P. Mercuri**
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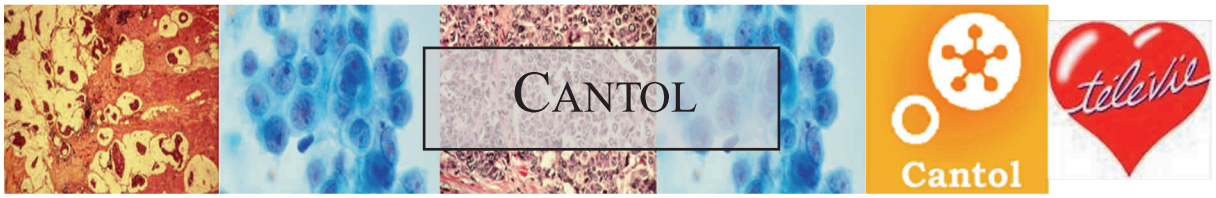


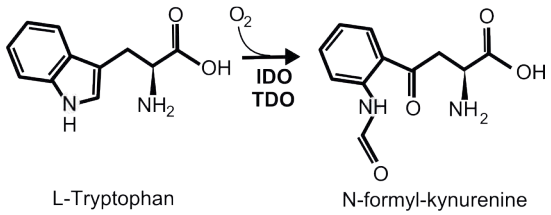
This project aims to discover and validate innovating candidate drugs and additives to inhibit the immune suppression due to the expression of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO), in tumors.

Partners involved in the CANTOL project:

• Euroscreen – Dr T. Ferain,
• GSK – Dr C. Gérard,
• Université Catholique de Louvain UCL-LICR – Prof. B. Van den Eynde*,
• Facultés Universitaires Notre Dame de la Paix Namur (FUNDP) – Prof. B. Masereel and J. Wouters*,
• Université Libre de Bruxelles, Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM) – Prof. J-M. Boeynaems,
• Université Libre de Bruxelles, Institut de Biologie et de Médecine Moléculaire (IBMM) – Prof. M. Moser.

* *Laboratories involved in the Télévie project*

Indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) are the two fundamental heme-containing dioxygenases, which are involved in the rate-limiting step of kynurenine pathway.



L-Tryptophan N-formyl-kynurenine

Both enzymes catalyze the catabolism of the essential amino acid tryptophan to form N-formyl-kynurenine by oxidative cleavage of the 2,3 double bond in the indole moiety of L-tryptophan.
Recently, both enzymes have been found to play an important role in the process of immune evasion by tumors. IDO and TDO dependent T-cell suppression suggests that L-Trp catabolism has profound effects on T-cell proliferation and differentiation. This observation is important for the design of immunotherapies for patients with cancer and chronic infectious diseases.
In collaboration with the partners, several series of innovating inhibitor candidates have been chemically generated and are under investigation for their inhibition efficiency.

Commitments to the Cantol project, a BIOWIN health cluster from the Walloon region and to the Télévie project

- The Centre for Protein Engineering is responsible for producing and purifying both enzymes in adequate quantity and quality for the high throughput screening conducted by Euroscreen IDO and to characterize and understand the biochemical mechanism of TDO,
- Secondly, the CIP is in charge of crystallization trials of the enzymes with and without inhibitors,
- Thirdly, the mechanism of inhibition is studied in details by kinetic measurements using different detection methods.

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PhD student: S. Laurent

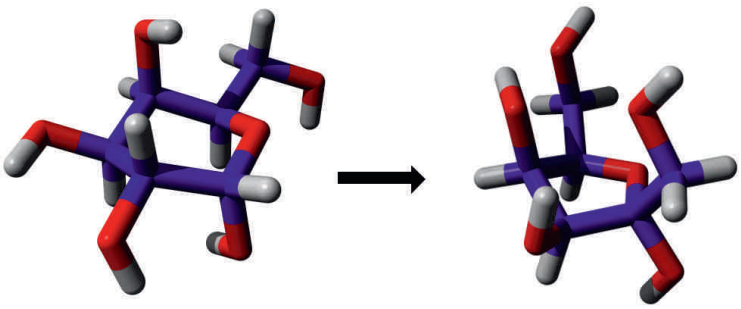
COMPARATIVE STUDY OF ENZYMATIC PRODUCTION OF TAGATOSE

Sugar is one of the major factors causing various adult diseases, such as obesity. Nowadays these problems, due to bad nutrition habits and little physical activity, start to occur more readily at ages below 14. With 2-8% of health costs and 10-13% of deaths, obesity is one of the greatest public health challenges of the 21st century.

Alternative sweeteners have become extremely important in terms of well-being or healthy life. D-tagatose is a sweet-tasting, natural monosaccharide with interesting nutritional and physiological properties. It has 92% of the sweetness of sucrose and its reduced absorption and special fermentation result in a low caloric value of maximum 1.5 kcal/g compared to 4 kcal/g for sucrose. D-tagatose consumption does not induce an increase of blood glucose or insulin levels which makes D-tagatose a desirable sugar substitute for diabetics. In addition, it does not cause dental caries. The aforementioned advantages make D-tagatose an appropriate ingredient in products for people with diabetes, in prebiotic foods, noncariogenic confections and low-calorie foods.

The main activities of Damhert NV [http://www.damhert.be/index.cfm] and Nutrilab NV [http://www.nutrilab.be/index.php] are focused on the development, production and commercialisation of functional food ingredients. During the last few years, these companies were able to perform preliminary experiments showing the feasibility to develop functional food products with tagatose as a sweetener, bringing it to the market (trademark Tagatesse) and compiling market information on its acceptability and impact on production, taste and distribution costs.

To develop a cost effective industrial production process of tagatose, a research proposal has been funded by the Flemish government agency for Innovation by Science and Technology (IWT) [http://www.iwt.be/] where Nutrilab is associated to two research partners (Laboratory of Enzyme, Fermentation and Brewing Technology of KaHo Sint-Lieven [http://www.kahosl.be/] and the CIP). The CIP group is responsible for two distinct work packages aiming at improving the stability of the L-arabinose isomerase used to convert galactose into tagatose by genetic engineering and the *in vivo* biosynthesis of tagatose from glucose through metabolic engineering
of relevant micro-organism strains.



Project leaders: Prof. M. Galleni, mgalleni@ulg.ac.be
Prof. B. Joris, bjoris@ulg.ac.be

Associated Researchers: **Dr J.-M. François**, Dr A. Brans, Dr M. Delmarcelle, M. Counson

Collaborators: Dr G. Aerts (KaHoSL R&D), Dr G. Schoofs (Damhert), Dr I. Van de Voorde (KaHoSL R&D), Dr C. Vastenavond (Nutrilab), Dr A. Verplaetse (KaHoSL R&D)

This project aims at setting up a new strategy for the development of allosteric modulator monoclonal antibodies against G Protein-Coupled Receptors (GPCRs).

Partners involved in the GPCR-Like project:
Partner 1 (coordinator): Prof. Moreno Galleni (CIP) – ULg, Partner 2: Ing. Alfred Collard (CER / Biotech), Partner 3: Prof. Muriel Moser (Laboratoire de Physiologie Animale) – ULB.
Industrial collaborator: ProGenosis

Membrane proteins such as receptors and ion channels are key regulators of cellular function and account for up to two thirds of known "drugable" targets, highlighting their critical pharmaceutical importance. GPCRs are the largest, most versatile, group of membrane receptors and also the most pharmaceutically important, accounting for over 50% of all human drug targets and acting as therapeutic targets for a wide range of disease conditions including cancer, cardiovascular, metabolic, Central Nervous System (CNS) and inflammatory diseases. GPCRs span the plasmic membrane seven times in a serpentine manner (Fig. 1).

These receptors consist of a single polypeptide chain with an extracellular amino-terminal domain and three extracellular loops that participate in receptor-ligand interactions. A cytoplasmic carboxy-terminal domain and three intracellular loops cooperate to the binding and activation of G proteins and other signaling molecules.

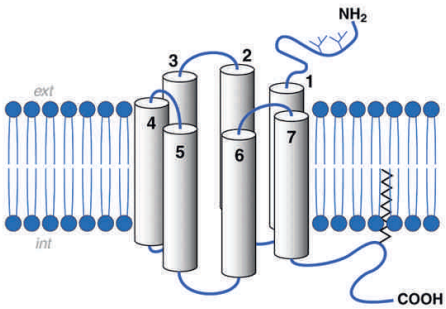


Figure 1

GPCRs are commonly viewed as targets for small molecules. However, small molecule lead discovery has not always been successful. They do not always show the desired specificity due to a high degree of structural similarity among different receptor subtypes within the ligand binding site. It is widely recognized that many GPCRs would make excellent therapeutic targets for antibodies. Raising antibodies to GPCRs has been difficult due to problems in obtaining suitable antigens since GPCRs are often expressed at low levels in cells and are very unstable when purified.
Peptides corresponding to the extra-cellular structures of GPCRs have been used to raise or select antibodies, but since they lack native conformations, the resulting antibodies did not show adequate affinities or specificities for therapeutic use.
Using protein engineering, we developed a strategy that opens new perspectives in the biochemical study of GPCRs and the development of compounds that target their extracellular domains. We have developed a method for stabilizing extracellular structures of receptors in native-like conformations by scaffolding them onto carrier proteins. As models, we studied the CCR5 and CXCR4 transmembrane receptors involved in HIV infection. Our technology is based upon the premise that correctly-folded extracellular domains of membrane receptors in the hybrid protein, when used as immunogens, will enable the development of antibodies that bind to functional extracellular domains, and that such antibodies will thus act as agonists or, more likely, antagonists for that receptor.

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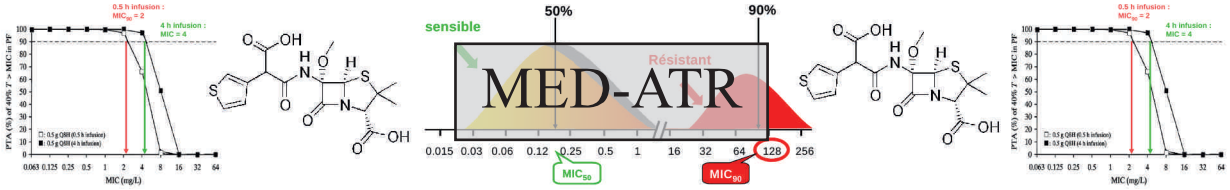
Technical assistance: A. Freichels



Real-Time quantitative monitoring of pharmaceutical substances in physiological fluids (MED-ATR)

β-lactam antibiotics and vancomycin are the most widely used group of antibiotics in the treatment of infections caused by both Gram-positive and Gram-negative bacteria. Antibiotherapies with β-lactam antibiotics and vancomycin via the so-called "continuous infusion" method are more and more required in hospitals due to the increasing occurrence of complicated infections and bacterial resistances. This is particularly true for patients in services of intensive cares, orthopedic surgery and pediatry. The delivery of these antibiotics during antibiotherapy requires a continuous monitoring and adjustment of the doses delivered to the patient. Therefore, it is of paramount importance to be able to rapidly and continuously quantify the free concentration of these antibiotics circulating in the patient's blood during antibiotherapy.
In the medical field, the quantitative analysis of β-lactam antibiotics and vancomycin is performed in clinical laboratories, using mainly microbiological assays. These analyses have the disadvantage that they last a few days and thus rapid follow up of the delivered drug doses is impossible.

Unfortunately, the concentration of free β-lactam antibiotics in serum cannot be predicted from the drug doses delivered to the patient. Indeed, serum concentrations of free β-lactam antibiotics exhibit great differences and variations from one patient to another, since serious infections are characterized by important disorders in drug distribution and elimination parameters, and in serum protein levels. Consequently, the delivery of β-lactam antibiotics via continuous infusion remains restricted to a few advanced hospital services because of the difficulty to get rapid measurements of free β-lactam antibiotics in serum.
Five laboratories have developed a method that allows repeated blood level monitoring and rapid delivery of the results to the clinician in order to ensure optimal treatment of a subject. These laboratories are : Chimie organique et médicinale (UCL, Prof. J. Marchand-Brynaert), Pharmacologie cellulaire et moléculaire (UCL, Prof. P. Tulkens) Centre for Research in Molecular Modeling (UMH, Prof. J. De Coninck), Structure and Function of Biological Membranes (ULB, Prof. E. Goormaghtigh), and The Center for Protein Engineering (ULg, Prof. B. Joris)

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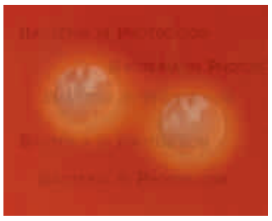
Associated researchers: **Dr A. Brans**, Dr M. Delmarcelle
Technical assistance: R. Barry, P. Simon

RAIDGBS

***Streptococcus agalactiae* or Lancefield group B streptococci (GBS) are the leading cause of morbidity and mortality in newborns. Resulting in pneumonia, sepsis and meningitis, GBS infections affect 0.5-3 ‰ of live births. They are a public health problem because of the risk of lightning deaths and the possibility of neurological or pulmonary sequels.**



GBS colonies on blood agar (10X).

In the vast majority of cases, the origin of these infections is the maternal vaginal carriage of GBS at the time of delivery. In Belgium, despite a prevention strategy based on prenatal testing performed between 35 and 37 weeks of gestation, and intravenous administration of intra-partum antibiotics to women who were positive for GBS at the time of testing, 200 to 300 newborns annually present a serious infection with GBS and more than 10% die. Among the reasons proposed to explain these cases of infection despite routine screening, the great variability of vaginal colonization by GBS and the lack of a sensitive and fast screening test that could highlight the state of colonization at delivery are evoked. Thus, most of these infections could be prevented by intra-partum screening with a sensitive and fast test conducted on genital samples of pregnant women admitted for delivery. In addition, the use of such test would reduce the number of unnecessary antibiotic treatments.

So far, no commercial test exhibits low cost, easiness, sensitivity and specificity required to prevent and/or reduce the incidence of these infections.

The purpose of the project is to develop a test for a rapid detection of GBS on vaginal smears to perform intra-partum identification.

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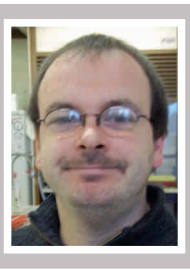
Associated researchers: **O. Verlaine**, Dr A. Amoroso
Partners: Prof. P. Melin CHU-ULg, Prof. E. De Pauw LSM-ULg, Prof. R. Brasseur CBMN-FUSAGx

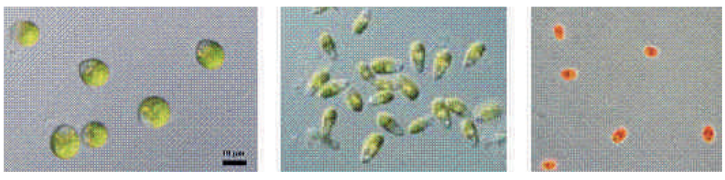


Scalab: A micro-algal bioreactor development. Pharmaceutical recombinant proteins are now widely used in human healthcare. Several expression systems are available but these traditional approaches have all their particular disadvantages in terms of protein yield. Difficulties of manipulation, time required from gene modification and transformation to protein purification, cost of production and scaling-up, risk of incorrect folding or instability of the recombinant protein, inherent bacterial inability to introduce post-translational modifications… are the main obstacles to the development of this promising field. Scalab, a spin-off project granted by the Walloon Region, aims at developing a new process for the production of high-value proteins e.g. growth factors, antibodies, receptors, cytokines….

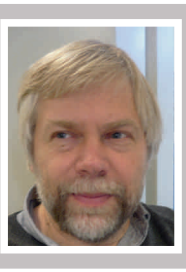
The engineering of bioreactors using eukaryote microalgae is a high potential technology for producing large amounts of these molecules. Indeed, these approaches allow a rapid scaling-up of the production when compared to the more traditional systems such as bio-farming. The biomass necessary to satisfy the market requirements is a real problem in the animal cell cultures, the currently most convenient way to produce therapeutics proteins. By applying recombinant techniques to photosynthetic micro-organisms which are now readily produced in large amounts (ton scale) we hope to circumvent this major obstacle in the production of biopharmaceutics.

The milestones of this approach are well-defined. The first objective consists in finding efficient and non patented algal promoters to drive a high expression level of selected genes. The following steps include, besides the purification process, the full characterisation of the product: a convenient biological activity is the main aspect but we must also consider the pharmaceutical requirements for an injectable drug or the desired posttranslational modifications such as glycosylation pattern or lipoylation.

Different species of micro-algae have been selected by scientists as draft horses for the purpose of transgenic studies: *Chlamydomonas reinhardtii, Chlorella sp., Haematococcus pluvialis, Dunaliella sp*… Different techniques are used to transform these micro-algae: electroporation, glass beads or microparticle bombardment… Up to now we have modified canonical transformation protocols with the aim to get the highest stable insertion rate of exogenous DNA in the selected micro-algae. Another aspect under study is the discovery of very efficient untranslated sequences as promoters and regulatory regions to flank the coding sequences we are dealing with.



Micro-algae such as these *Dunaliella* species are considered as promising eukaryote bioreactors for the mass-production of therapeutic proteins.



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Associated researcher: Prof. M. Galleni

SCIENTIFIC SERVICES

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Technical assistance: Marine Renard

- <u>Protein production et purification facilities</u>:

Manager: Dr Alain Brans
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Tel : +32 (0)4 366 34 50

Collaborators:
Iris Thamm, Fabrice Bouillenne
& Anne-Marie Matton

- <u>Training: "Techniques for protein production and purification"</u>:

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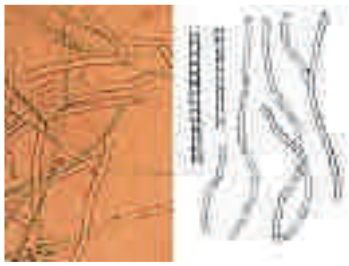
TOWARDS BCCM/ULC : A CULTURE COLLECTION OF (SUB)POLAR CYANOBACTERIA

Since 2005, the BCCM (Belgian Co-ordinated Collections of Microorganisms) has supported the elaboration of a collection of (sub)polar cyanobacteria. The integration towards an official public collection, called BCCM/ULC, has continued in 2010.

In 2010, the implementation of a Quality Management System was started. The aim is to officially set up a public culture collection with a catalogue on the BCCM website in February 2011. This is a part of the consolidation of the Belgian « Biological Resource Centre (BRC) ».

At present, the research collection includes 125 polar unicyanobacterial strains coming from various regions of the Antarctic (South Victoria Land, East Antarctica, Transantarctic Mountains, James Ross Island) and the Arctic (North Canada, Arctic Ocean, Alaska), and different biotopes (microbial mats, lakes, ice shelves, dry valleys, cryptoendoliths, oceans). Twelve strains were isolated in the Sub-Arctic, in Siberian lakes. The most important cyanobacterial orders are represented: Chroococcales, Oscillatoriales, and Nostocales. Moreover, our laboratory is involved in projects for which new isolates are being purified, and will extend the geographic coverage of the collection. The majority of the strains are psychrotolerant and can be cultivated at 18-20°C. They are available as living cultures, and the majority (115) can be cryopreserved (-70°C). Some isolates were screened in a previous project and bioactivities were observed against several pathogens.

Exploration of the biodiversity



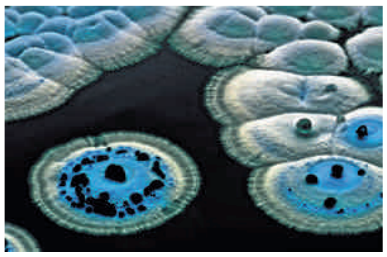
Picture and drawing of the strain ANT.LPR3, type species of the new genus *Plectolyngbya*.

A group of Antarctic strains that is clearly distinct on the basis of its 16S rRNA sequence shows a morphology (see Fig.) with characters corresponding to the traditional genera *Leptolyngbya* (morphology of trichomes), *Pseudophormidium* (type of false branching) or *Schizothrix* (occasional multiple arrangement of trichomes in the sheaths). The cytomorphology of this generic entity is also characteristic. Therefore, a new genus (*Plectolyngbya* with the type species *P. hodgsonii*) is described.

Protein Production and Purification Facilities

An effective research requires access to a broad range of technologies, some of which require expertise and specific equipments. The "Protein Production and Purification Platform" is open to academic laboratories as well as to private companies. One of the objectives of the platform is to provide laboratory and pilot-scale equipment for on- and off-campus users. Services include protein production in bacterial, yeast and filamentous fungal strains, followed by their purification.

The platform can provide many services including:

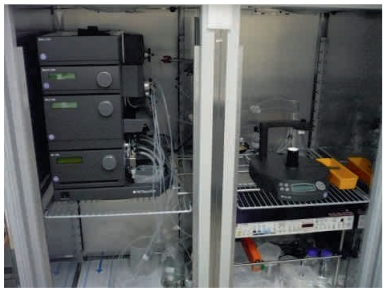


• The genetic engineering and cloning in bacterial strains such as *E. coli, Bacillus subtilis, Bacillus amyloliquefaciens, Streptomyces lividans* or in yeast such as *Saccharomyces cerevisiae, Pichia pastoris* and the filamentous fungi like *Aspergillus niger*
• The analytical scale or pilot-scale recombinant protein production from these organisms
• The cell harvesting or supernatant cleaning using continuous centrifugation or hollow fiber filtration
• The cell disintegration to recover proteins produced in the intracellular compartment
• The protein purification at the analytical and pilot scales

For these purposes the platform is equipped with:

• Several shaking incubators for flasks from 1 ml to 2 L
• Wide range of computer controlled fermentors with working volumes from 1 to 60 L for batch and fed-batch cultures. Dissolved oxygen, pH, temperature, agitation and turbidity are controlled in all fermentors

• 2 continuous centrifuges
• 1 crossflow filtration unit
• Several systems for semi- or totally automated purification of proteins including: ÅKTA prime, ÅKTA explorer, ÅKTA explorer (2D system), ÅKTA purifier, Biopilot, Profinia Protein Purification System.



The Protein Production and Purification Platform has provided proteins for : kinetic studies, protein structure determination by NMR or crystallography, protein-protein or protein-ligand interaction studies, secondary metabolite productions, enzyme-inhibitor studies, amyloid fibril formation and protein folding studies, immunoassay developments, vaccination studies and immuno test kit manufacturing.

Techniques for protein production and purification

Since 2006, the CIP works in close collaboration with the Biotechnology Training Centre Forem-GIGA to develop and organize biotechnology training for jobseekers in the field of protein
production and purification.

At the CIP, the training includes the following technological modules:

• Bacterial transformation (*Escherichia coli* and *Bacillus subtilis*)

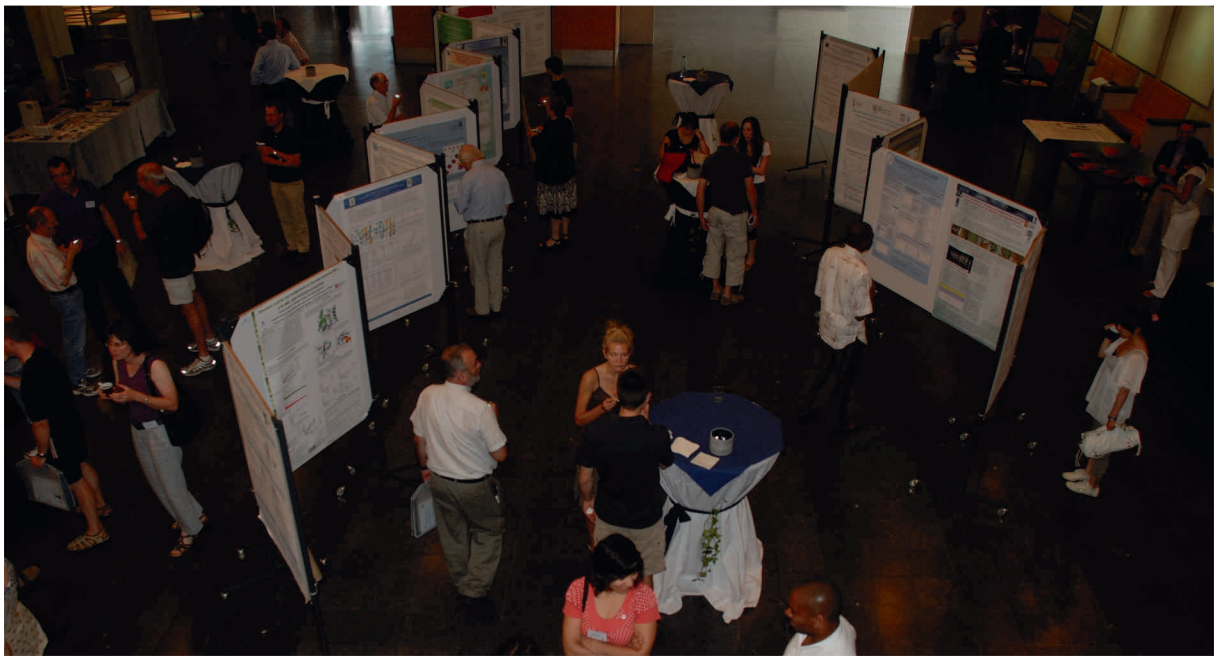
• Protein production in flasks and in 20 L fermentors (batch and fed-batch cultures) with *E. coli, B. subtilis* and *Pichia pastoris*

• Cell harvesting and cell disruption

• Protein purification by different chromatography technologies including: ion exchange, molecular sieve, hydrophobic and affinity chromatography

• Protein identification by SDS-PAGE, enzymatic testing and Western blotting.

SCIENTIFIC PRODUCTION



AWARDS

Julie Herman

Best poster (Group 1: Molecular Chemistry of novel allergens), European Academy of Allergy and Clinical Immunology, London, UK, June 5-9, 2010

Prolines in Der p 3 zymogen propeptide : implication for folding, enzymatic stability, maturation and activity of the allergen

Herman J.[1], Campisi V.[1], Pain C.[1], Chevigné A.[1,3], Devreese B.[4], Luxen A.[5], Matagne A.[2], Galleni M.[1] and Dumez M.-E.[1]

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I. INTRODUCTION

The enzymatic activity of the trypsin-like protease Der p 3, a major allergen of the house dust mite *Dermatophagoides pteronyssinus*, is directly implicated in allergy and inflammation. Der p 3 is synthesized as an inactive zymogen (proDer p 3), which consists of an 11 amino acid N-terminal propeptide of and a protease domain of 232 amino acids. The short propeptide is characterized by the presence of a polyproline motif (**$NP_2ILP_5ASP_8NAT_{11}$**) which could be involved in the inhibition of the protease, in its enzymatic stability and in its specific activation mechanism by the major allergen Der p 1.

II. METHODS

The unglycosylated zymogens proDer p 3 N9Q, N9QP2A, N9QP5A, N9QP8A and N9QP-A mutants (Table 1) were produced in *Pichia pastoris*. A structural analysis of the zymogens was performed by intrinsic fluorescence and far UV circular dichroism. The time-course of their activation in the presence of Der p 1 was monitored with the help of SDS-PAGE and enzymatic activity measurements. Finally, free synthetic propeptides $NPILPASPNAR_{11}$, P2AT11R, P5AT11R, P8AT11R and P-AT11R were tested as potential inhibitors of the recombinant Der p 3 (rDer p 3).

III. RESULTS

III.a. ProDer p 3 mutants are correctly folded

Mutants	Proteins	N-terminal sequencing
proDer p 3 N9Q	$NP_2ILP_5ASP_8$*QATIVGG-*	NPILPASPQ
proDer p 3 N9Q/P2A	$NA_2ILP_5ASP_8$*QATIVGG-*	NAILPASPQ
proDer p 3 N9Q/P5A	$NP_2ILA_5ASP_8$*QATIVGG-*	NPILAASPQ
proDer p 3 N9Q/P8A	$NP_2ILP_5ASA_8$*QATIVGG-*	NPILPASAQ/SAQATIVVG/ATIVGG
proDer p 3 N9Q/P-A	$NA_2ILA_5ASA_8$*QATIVGG-*	NAILAASAQ/ILAAS/ASAQA/ SAQAT/AQATIVG/ATIVGG

Table 1. N-terminal sequencing of proDer p 3 mutants. The mature protease sequence is in italic.

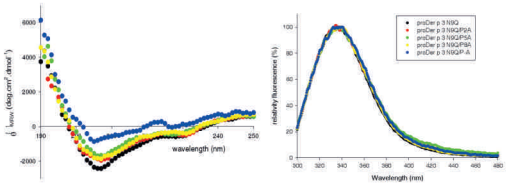


Figure 1. Structural analysis by far UV circular dichroism and intrinsic fluorescence. Mesured in phosphate buffer (pH 7.4).

Mutants produced in ***Pichia pastoris*** exhibited similar far UV CD and fluorescence spectra suggesting that they possess a similar fold than the proDer p 3 N9Q zymogen. However, the N9QP8A and N9QP-A were truncated, suggesting the implication of the proline 8 in the stability of proteins. Moreover, their specific activity was increased (data not shown).

III. b. The presence of Pro 5 and 8 are important for the activation process by rDer p 1

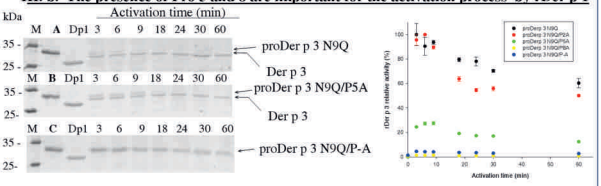
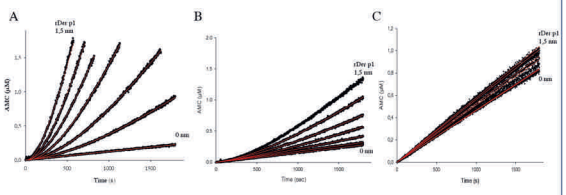


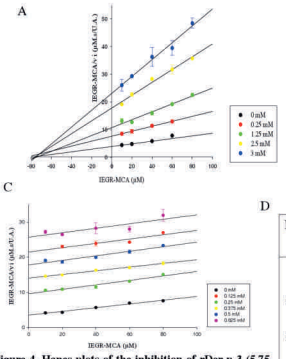
Figure 2. Inter-molecular activation of mutants by rDer p 1. 2.5 µM of proDer p 3 N9Q (A), N9QP2A (data not shown), N9QP5A (B), N9QP8A (data not shown) and N9QP-A (C) were incubated at 37 C in 20 mM Na-citrate buffer (pH 6.5) with 30 nM of rDer p 1 (second lane). Aliquots were taken during time and analysed by SDS-PAGE. The rDer p 3 enzymatic activity was followed using Boc-IEGR-MCA (10 µM) as substrate. M, Standard Weight Marker.

The lower Der p 3 activity measured for the activation of N9QP5A and N9QP8A zymogens indicated that Pro 5 and 8 are important for the specific cleavage by rDer p 1.

III.c. The P5A and P8A mutations decrease the activation rate of proDer p 3



III.d. Prolines 5 and 8 influence the inhibition mechanism of rDer p 3



Peptides	Inhibition mechanism	K_I (mM)		K'_I (mM)	
T1IR	Uncompetitive	1.2	0.2	1.4	0.2
P2AT11R	Uncompetitive	0.8	0.2	0.7	0.2
P5AT11R	Mixed	0.5	0.1	1.5	0.1
P8AT11R	Competitive	0.09	0.02	ND	
P-AT11R	Competitive	1.7	0.3	ND	

Figure 4. Hanes plots of the inhibition of rDer p 3 (5.75 nM) by the $NPILPASPNAR_{11}$ (A), P2AT11R (data not shown), P5AT11R (B), P8AT11R (C) and P-AT11R peptides. Substrate concentration (IEGR-MCA)/initial hydrolysis rate *versus* substrate concentration (10-80 µM) in the presence of increasing concentrations of peptides in 50 mM polybuffer (pH 8.5) at 37 C. (D) Table of inhibition constants. ND *, not determined.

All free prosequences inhibited rDer p 3. However, the inhibition mechanisms varied for the P5AT11R, P8AT11R and P-AT11R.

$$P = v_{ss} \times t \times \frac{v_{ss} - v_0}{k_{obs}} \times \left(\exp\left(-k_{obs} \times t\right) - 1\right)$$

Equation 1

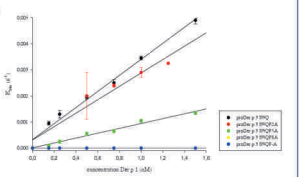


Figure 3. Continuous assay for mutants maturation by rDer p 1. Substrate hydrolysis (10 µM) *versus* time curves related to the activation of proDer p 3 N9Q (A), N9QP5A (B) and N9QP-A (C) (1.25 µM) by increasing concentrations of rDer p 1 (0, 0.15, 0.25, 0.5, 0.75, 1, 1.5 nM) in 50 mM phosphate buffer (pH 7.4) containing 150 mM NaCl, 1 mM DTT and 1 mM EDTA at 37 C. Curves were fitted to equation 1. (D) Secondary data analysis of first pseudo order constants k_{obs} obtained from fits of the five mutants processing in function of the rDer p 1 concentration (nM).

The activation rate measured for the N9QP5A was strongly decreased and the N9QP8A and N9QP-A were not actived in these conditions.

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IV. CONCLUSION

The prolines of the propeptide were found not to be essential for correct folding of the zymogen. Truncation of the N9QP8A and N9QP-A indicated, however, that proline 8 is critical for enzymatic stability. Furthermore, enzymatic experiments suggested that prolines 5 and 8 are important for the specific maturation mechanism of proDer p 3 by rDer p 1. Finally, we found that, in the absence of proline 8, the propeptide behaved as a competitive inhibitor.

Invited Speakers

Dr Mohamed Azarkan, Unité de Chimie des Protéines, ULB, Bruxelles, Belgique, "PPI, un inhibiteur de sérine protéases doté d'une grande stabilité, en tant que molécule modèle pour l'étude de protéines tout beta", January 15

Dr Jacques Georis, Beldem, Andenne, Belgium, "R&D enzymes, a link between innovation process and business", March 5

Prof. Manuel Dauchez, Université de Reims Champagne-Ardenne, France, "Simulations numériques de protéines élastomères: le cas de l'élastine", April 2

Prof. Jean-François Collet, de Duve Institute, UCL, Belgium, "The name's bond, disulfide bond…", April 23

Dr Caroline Montagner, Biozentrum, Université de Bâle, Suisse, "Etude d'interactions protéine-membrane par échanges Hydrogène/Deuterium couplés à la spectrométrie de masse", May 18

Dr Tina Tin, Antarctic Southern Ocean Coalition (ASOC), France, "Impacts of local human activities on the Antarctic environment: review and management implications", September 15

Dr Kerensa Broersen, SWITCH Laboratory, VIB-VUB, Brussels, Belgium, "How a multidisciplinary approach can lead to rapid understanding of molecular mechanisms in a complex protein aggregation disease", September 24

Dr Jean-Michel Brunel, Université de la Méditerranée, Marseille, France, "New insights on the mechanism of action of squalamine against Gram positive and Gram negative bacteria", October 16

Dr Raphaël Robiette, UCL, "Synthèse et évaluation biologique de macrocycles contenant un motif imidazole", November 13

Dr Haywood Dail Laughinghouse, College of Chemical and Life Sciences, University of Maryland, USA, "Algal turf scrubber (ATS): cleaning rivers while producing biofuels and agricultural products", December 2

Prof. Louis Droogmans, Laboratoire de Microbiologie, ULB, Bruxelles, Belgique, "Identification et caractérisation d'enzymes de modification des nucléosides des Arn de transfert", December 3

Oral Presentations

R. Berlemont, "Insights into the metagenomic approach: identification and characterization of cellulases involved in bacterial cellulose synthesis", 239th American Chemical Society National Meeting & Exposition, San Francisco, CA, USA, March 21-25

M. Dumoulin, "Fibril formation by human lysozyme", C. Dobson 60th Birthday Symposium "From Neuroscience to Nanoscience", Cambridge, UK, April 8-9

C. Bebrone, "Interactions mediating the positioning of mercapto-phosphonate inhibitors in the active site of metallo-beta-lactamase, 20th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Vienna, Austria, April 10-13

F. Kerff, "OXA carbapenemases and their inhibition", 20th European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, April 13

A. Wilmotte, "Antarctic microbial biodiversity: the importance of geographical and ecological factors", IPY Symposium, the contribution of Belgian research to the achievements of the International Polar Year 2007-2008, Brussels, Belgium, May 26

M. Craig, "Identification d'une nouvelle cascade signalétique aboutissant à la biosynthèse de sidérophores chez Streptomyces coelicolor" Journées Streptomyces, Institut Pasteur, Paris, France, May 27

S. Rigali, "Identification d'un nouveau transporteur de sucre essentiel à la production d'antibiotiques chez Streptomyces coelicolor" Journées Streptomyces, Institut Pasteur, Paris, France, May 27

M.E. Dumez, "Could the major allergen Der p 1 be the primary activator of the cysteine and serine protease zymogens of the house dust mite *Dermatophagoides pteronyssinus*", European Academy of Allergy and Clinical Immunology, London, UK, June 5-9

S. Rigali, "Streptomyces secrete stories: from in silico predictions to in vivo validations" Microbiology/Biotechnology, Institute of Microbiology and Infection Medicine, Tübingen University, Germany, June 7

A. Wilmotte, "B-BLOOMS 2: Importance and diversity of cyanobacterial blooms in Belgium", INRA – Station d'hydrobiologie lacustre, station de Thonon-les-Bains, France, June 17

M. Delsaute, "Cellulases catalysed cellulose polymerisation", 35th FEBS Congress "Molecules of Life", Göteborg, Sweden, June 26 – July 1

JS Sohier, "cAbVIM4, a nanobody inhibiting the metallo-beta-lactamase VIM-4", FEBS Congress "Molecules of Life", Göteborg, Sweden, June 26 – July 1

P. de Carvalho Maalouf, "Cyanobacterial diversity and distribution in microbial mats from Antarctic and Sub-Antarctic aquatic environments", 18th Symposium of the International Association for Cyanophyte Research (IAC), Ceske Budejovice, Czech Republic, August 16-20

Y. Lara, "Genetic and biochemical characterisations of environmental single colonies of *Microcystis* and *Woronichinia*", 18th Symposium of the International Association for Cyanophyte Research (IAC), Ceske Budejovice, Czech Republic, August 16-20

Z. Namsaraev, "Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas", 18th Symposium of the International Association for Cyanophyte Research (IAC), Ceske Budejovice, Czech Republic, August 16-20

A. Matagne, "A young person's guide to protein folding", Summer School on Single-Molecule Biophysics, University of Leuven, Belgium, August 19

B. Joris, "La résistance bactérienne aux antibiotiques", 48ème Congrés pluraliste des Sciences, FUNDP, Namur, Belgium, August 24-26

M. Hanikenne, "Metal hyperaccumulation in *Arabidopsis halleri*: how is Zinc accumulating in the leaves?" CNRS, Gif/Yvette, France, September 17

M. Dumoulin, "Engineering nanobodies that bind to human lysozyme and inhibit its conversion to amyloid fibrils: prospects in systemic and neurodegenerative protein aggregation diseases", Single domain antibodies come of age, Ghent, Belgium, October 14-15

M. Hanikenne, "Metal hyperaccumulation in *Arabidopsis halleri*: towards tools for bioremediation?" Biolière, Liège, Belgium, October 7

P. de Carvalho Maalouf, "Cyanobacterial diversity and distribution in microbial mats from Antarctic and Sub-Antarctic aquatic environments", Young Botanists's Forum 2010 Cryptogamy in Belgium, National Botanic Garden, Meise, Belgium, November 19

Y. Lara, "Single colony approach applied to *Microcystis* and *Woronichinia*. What single colonies can tell us?", Young Botanists's Forum 2010 Cryptogamy in Belgium, National Botanic Garden, Meise, Belgium, November 19

R. Fernandez Carazo, "Cyanobacterial diversity close to the South Pole (Antarctica), Meeting "Astrobiology: from stars and planets to extreme life", Brussels, Belgium, December 8

C. Chavignon, "V_HHs as model proteins to investigate amyloid fibril formation: effect of seeding and cross-seeding on aggregation kinetics and stability of fibrils". Journée doctorale SFMBBM, Liège, Belgium, December 10

S. Rigali, "L'immobilité au service de l'évolution : adaptations génétiques et physiologiques des Streptomyces", Unité de Recherche en Biologie Moléculaire, FUNDP, Namur, Belgium, December 13

M. Dumoulin, "Interplay between the effects of the length of the polyglutamine tract and its structural context on the aggregation of polyglutamine (polyQ) proteins", Institute of Biomedical Research, Scientific Park of Barcelona, Barcelona, Spain, December 16

PATENTS

A recombinant alpha-hemolysin polypeptide of *Staphylococcus aureus*, having a deletion in the stem domain and heterologous sequences inserted

Filée Patrice (BE), Rhazi Noureddine (BE), Galleni Moreno (BE), Taminiau Bernard (BE), Jolois Olivier (BE), Collard Alfred (BE), Jacquet Alain (BE)

Pub. N°: WO/2010/081875, International Application N°: PCT/EP2010/050453
Publication Date: 22.07.2010, International Filing Date: 15.01.2010

PhD Theses

21/01/2010 **Lionel Vercheval** (Biochemistry)
Etude des facteurs structuraux influençant la carbonation de la lysine 70 chez la β-lactamase OXA-10 de *Pseudomonas aeruginosa*

04/06/2010 **Patricia Lassaux** (Biochemistry)
Caractérisation biochimique et structurale de la métallo-ß-lactamase VIM-4. Sélection de nouveaux inhibiteurs de métallo-ß-lactamases

21/06/2010 **Xavier Henry** (Biology)
Influence de la structure 3D du site catalytique de la protéine PBP5 d'*Enterococcus faecium* sur son affinité vis-à-vis des ß-lactamines

02/07/2010 **Alexandre Di Paolo** (Biochemistry)
Equilibrium and kinetic folding properties of lysozyme from bacteriophage lambda

08/09/2010 **Aimé Christian Kayath** (Sciences)
Contribution à l'étude de l'assemblage de la structure basale du système de sécrétion de type III de *Shigella flexneri* et caractérisation du rôle de la protéine IcsB dans l'autophagie

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Dynamic nucleocytoplasmic shuttling of an Arabidopsis SR splicing factor: role of the RNA-binding domains
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BOOK CHAPTERS AND REVIEWS

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Carbohydrate active enzymes derived from metagenomes: from microbial ecology to enzymology (chapter 10)
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Traduction de "Prescott, Harley and Klein's Microbiology" (2008 -7th edition), J.M. Willey, L.M. Sherwood and C.J. Woolverton

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In: Protein Misfolding Diseases: Basis of protein misfolding, pathophysiology, current and emerging therapies
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J. Phys.: Condens. Matter, 22, 1-17

Protein Structures deposited within the Protein Data Bank

PDB ID	Structure Title	Rel.Date	Resol	Authors
2WKX	CRYSTAL STRUCTURE OF THE NATIVE E. COLI ZINC AMIDASE AMID	2010-01-12	1.80	Petrella, S., Kerff, F., Herman, R., Genereux, C., Pennartz, A., Sauvage, E., Joris, B., Charlier, P.
2WGV	CRYSTAL STRUCTURE OF THE OXA-10 V117T MUTANT AT PH 6.5 INHIBITED BY A CHLORIDE ION	2010-05-19	1.80	Vercheval, L., Kerff, F., Bauvois, C., Sauvage, E., Guiet, R., Charlier, P., Galleni, M.
2WGW	CRYSTAL STRUCTURE OF THE OXA-10 V117T MUTANT AT PH 8.0	2010-05-19	1.80	Vercheval, L., Kerff, F., Bauvois, C., Sauvage, E., Guiet, R., Charlier, P., Galleni, M.
2X71	STRUCTURAL BASIS FOR THE INTERACTION OF LACTIVICINS WITH SERINE BETA-LACTAMASES	2010-07-14	2.10	Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P.
2XDM	CRYSTAL STRUCTURE OF A COMPLEX BETWEEN ACTINOMADURA R39 DD PEPTIDASE AND A PEPTIDOGLYCAN MIMETIC BORONATE INHIBITOR	2010-07-21	2.40	Rocaboy, M., Sauvage, E., Herman, R., Kerff, F., Charlier, P.
2WKH	CRYSTAL STRUCTURE OF THE ACYL-ENZYME OXA-10 K70C-AMPICILLIN AT PH 7	2010-08-25	1.79	Vercheval, L., Bauvois, C., Kerff, F., Sauvage, E., Guiet, R., Charlier, P., Galleni, M.
2WKI	CRYSTAL STRUCTURE OF THE OXA-10 K70C MUTANT AT PH 7.0	2010-08-25	2.10	Vercheval, L., Bauvois, C., Kerff, F., Sauvage, E., Guiet, R., Charlier, P., Galleni, M.
2WUQ	CRYSTAL STRUCTURE OF BLAB PROTEIN FROM STREPTOMYCES CACAOI	2010-10-13	1.80	Dandois, S., Herman, R., Sauvage, E., Charlier, P., Joris, B., Kerff, F.
2X01	CRYSTAL STRUCTURE OF THE OXA-10 S67A MUTANT AT PH 7	2010-12-08	1.90	Vercheval, L., Kerff, F., Bauvois, C., Sauvage, E., Guiet, R., Galleni, M., Charlier, P.
2X02	CRYSTAL STRUCTURE OF THE CLASS D BETA-LACTAMASE OXA-10 AT 1.35 A RESOLUTION	2010-12-08	1.35	Vercheval, L., Kerff, F., Sauvage, E., Herman, R., Galleni, M., Charlier, P.

SYMPOSIA

Joint meeting of the National Committee for Biophysics and the Belgian Biophysical Society on "Bionanotechnology", Palais des Académies, Brussels, Belgium, January 8
Co-Organizer and Chairman: Prof. A. Matagne

Eighth Meeting of the Belgian Biophysical Society on "Protein Folding and Stability", University of Liege, Belgium, September 3
Main Organizer: Prof. A. Matagne

EDUCATION

Academic Courses

Bachelor and Preparation to Masters

Biochimie, 30 h + 30 h Pr - BIOC0002-1 - **P. Charlier**
Bac 2 Sciences de l'ingénieur, orientation ingénieur civil, option génie biomédical.

Biochimie, 30 h - CHIM0678-1 - **A. Matagne**
Bac 3 Sciences chimiques et année préparatoire aux sciences chimiques.

Biochimie et thermodynamique des systèmes biologiques, 40h + 20h Pr - BIOC0204-1.
M. Galleni. Bac 2 Sciences biologiques.

Biologie, 30h + 30h Pr - BIOL2008-1 J.-M Bouquegneau, P. Dauby, A. Hambuckers, **B. Joris**, C. Remacle, C. Sadzot, M. Thiry. Bac 1 Biologie.

Biologie et introduction à la biochimie, 30h + 30h Pr - BIOL2009-1 - **B. Joris.** Bac 2 Chimie.

Chimie des macromolécules biologiques, 60h + 40h Pr + 4h de visite d'usine - BIOC0209-3/4/6
M. Galleni et A. Matagne. Bac 3 Sciences biologiques et année préparatoire en sciences biologiques et en Biochimie et Biologie Moléculaire et Cellulaire (BBMC).

Chimie des macromolécules biologiques et thermodynamique des systèmes biologiques, 70h + 40h Pr + 4h de visite d'usine - BIOC0209-4 - **M. Galleni et A. Matagne**.
Année préparatoire au master en Sciences biologiques.

Compléments de microbiologie (y compris physiologie bactérienne), 30h - MICR0001-1
V. Demoulin, **B. Joris**, J. Piette. Suppléants: A. Goffart, O. Guillitte. Bac 3 BBMC.

Documentation, stages et séminaires (étudiants), 50h St. - STRA0008-1 – J. Dommes et
P. Motte. Bac 3 sciences biologiques et année préparatoire aux masters.

Génétique, biologie moléculaire et chimie des macromolécules, 30h + 30h Pr - BIOC0001-1
J. Dommes et **M. Galleni**. Bac 3 Sciences biologiques. Années préparatoires aux masters en Biologie des Organismes et Ecologie (BOE) et sciences biologiques. Master générique en sciences biologiques.

Microbiologie - MICR0711-1 Partim 2 : Bactériologie : 20h + 10h Pr – **B. Joris**. Bac 3 et années préparatoires aux masters BBMC, BOE et sciences biologiques.

Physiologie cellulaire et histologie végétales, 30h Th + 20h Pr - BIOL0214-1 – **P. Motte**, C. Périlleux. Bac 2 Sciences biologiques.

Physiologie végétale, 40h Th + 25h Pr -BIOL0217-1 – **P. Motte**, C. Périlleux. Bac 3 Sciences biologiques et année préparatoire au master en sciences biologiques.

Principes généraux de la biologie et de la biochimie, 15 h - CHIM0063-1 - **P. Charlier**
3e année Ingénieur civil chimiste.

Masters

Analyse des séquences des gènes et des protéines : partim a, 10h, 10h Pr - GBIO0007-1 - **B. Joris.** Master 2 en Bioinformatique et modélisation, finalité approfondie et master 2 en Ingénieur civil biomédical, finalité approfondie.

Approches moléculaires de la diversité des microorganismes marins, 15h + 15h Pr - BOTA0401- **A. Wilmotte**. Master 2 en Océanographie, finalité approfondie.

Aspects génétiques et biochimiques de l'évolution, 25h + 20h Pr - GENE0432-3 - V. Demoulin, **M. Galleni** et C. Remacle, suppl.: D. Baurain. Masters 1 BBMC et sciences biologiques.

Biochimie, 30 h + 30 h Pr - BIOC0002-1 - **P. Charlier**. Master 1 en Ingénieur civil biomédical, finalité approfondie.

Biochimie, 30 h + 40 h Pr - BIOC0002-2 - **P. Charlier**. Master 1 en Bioinformatique et modélisation, finalité approfondie.

Biochimie et physiologie des microorganismes, 20h + 20h Pr - BIOC0003-2 - **B. Joris** . Masters 1 BBMC et sciences biologiques.
Invited speakers: **C. Duez**, 2h. Les biofilms bactériens. **G. Feller**, 2h. Microorganismes extremophiles. **S. Rigali**. 2h. L'immobilité au service de l'évolution: adaptations génétiques et physiologiques des Streptomyces. **M. Terrak**, 2h. La biosynthèse du peptidoglycane: rôle des Penicillin-Binding Proteins et des glycosyltransférases. **A. Wilmotte**, 2h. Les Cyanobactéries.

Biochimie macromoléculaire, 30h + 30h Pr - BIOC0232-1 - **M. Galleni.** Master 1 Sciences chimiques.

Bioinformatique appliquée, 36h.- BIOC0717-1 - M. Georges et **B. Joris.** Master 2 BBMC, finalité biochimie Industrielle.

Chimie des macromolécules biologiques, 60h + 40h Pr + 4h de visite d'usine - BIOC0209-3/4. **M. Galleni** et **A. Matagne**. Master générique en Sciences biologiques.

Compléments de physiologie moléculaire et cellulaire, 40h Th + 20h Pr - BIOL0803-2. **P. Motte**, M. Muller et M. Thiry. Master 1 BBMC.

Compléments de physiologie cellulaire végétale, 30h Th. - BIOL0827-1- **P. Motte**. Master 2 en Biologie des Organismes et Ecologie.

Enzymologie, 15h - BIOC0719-1 - **A. Matagne**. Master 1 Sciences chimiques.

Enzymologie, 15h + 20h Pr - BIOC0719-2 - **A. Matagne**. Master 1 Bioinformatique et modélisation.

Génomique et bioinformatique, 25h - GENE0208-2 - M. Georges, **B. Joris.** Master 2 BBMC.

Interactions dans les macromolécules biologiques, 20h + 20h Pr - BIOC0712-1 - **M. Galleni.** Master 1 Bioinformatique et modélisation, finalité approfondie.

Mécanique et dynamique moléculaire, 10h + 10h TD - CHIM0625-1 - **D. Dehareng.** Master 2 Bioinformatique et modélisation.

Méthodes de visualisation et de quantification en biologie cellulaire, 30h Th - BIOL0824-1 - **P. Motte**. Master 2 en Biologie des Organismes et Ecologie.

Principes généraux de la biologie et de la biochimie, 15h - CHIM0063-1- **P. Charlier**. Master 2 Ingénieur civil en chimie et sciences des matériaux, finalité approfondie.

Propriétés fonctionnelles des macromolécules biologiques, 30h+15h TD+ 20h Pr - BIOC0210-4/2 E. De Pauw et **A. Matagne**. Master 1 BBMC.

Relations structure-fonction dans les biomolécules, 15h + 25h Pr - BIOC0718-2 – **M. Dumoulin**. Master 2 Ingénieur civil biomédical, finalité approfondie.

Structure et dynamique des macromolécules biologiques, 20h + 20h TD + 20h Pr - BIOC9233-1 **P. Charlier** et **A. Matagne**. Master 1 BBMC, finalités didactique, approfondie et industrielle.

Structure et fonction des protéines, 25h + 25h Pr - BIOC0715-1 - **P. Charlier** et **M. Dumoulin**. Master 2 BBMC, finalités approfondie, didactique et industrielle.

Structure des macromolécules biologiques, 20h + 10h Pr - CHIM0624-1 - **P. Charlier**. Master 2 Bioinformatique et modélisation, finalité approfondie.

Structure des macromolécules biologiques (RX, RMN), 15h + 10h Pr - CHIM0627-1 - **P. Charlier**. Master 2 Bioinformatique et modélisation, finalité approfondie.

Voies de signalisation chez les végétaux, 25h Th + 25h Pr. - BOTA0403-1 – J. Dommes, **P. Motte** et C. Périlleux. Master 2 BBMC.

Inter University Thematic Weeks

Antibiotic resistance. 25h + 25 h Pr – BIOC0716-1 - **J.-M. Frère, M. Galleni et B. Joris**. Master 2 Biochimie et Biologie Moléculaire et Cellulaire (BBMC). **Invited speakers**: J.-D. Docquier, University of Siena, Italy. **S. Rigali**. Bacterial resistance to antibiotics: lessons from the soil.

Biologie cellulaire et méthodes de visualisation. 25 + 25h Pr - BIOL0806-1 - **P. Motte** & M. Thiry. Master 2 BBMC.

Microorganismes extrémophiles. 25 + 25h Pr - MICR0713-1 - **M. Galleni, G. Feller** & **A. Wilmotte**. Master 2 BBMC. Invited speaker: partim "Métagénomique" **R. Berlemont**. 2h.

Structure et fonction des protéines. 25h + 25h Pr - BIOC0715-1 - **P. Charlier** et **M. Dumoulin**. Master 2 BBMC. Invited speaker: partim "NMR" **C. Damblon**

Voies de signalisation chez les végétaux. 25h + 25h Pr - BOTA0403-1 - J. Dommes, **P. Motte** et C. Périlleux. Master 2 BBMC.

Complementary Masters

Biochimie, 30h + 30h Pr - BIOC0002-1 - **P. Charlier**. Master complémentaire en Nanotechnologie.

Chimie des macromolécules biologiques, 20h - BIOC0209-3/4/6 - **M. Galleni** et **A. Matagne**. Master complémentaire en Nanotechnologie.

Génie génétique des bactéries, 15h – GENE2000-1 - **A. Brans** Master complémentaire en Biotechnologie et Biologie appliquée.

Microbiologie - MICR0711-1 Partim 2 : Bactériologie : 20h + 10h Pr – **B. Joris** Master complémentaire en Biotechnologie et Biologie appliquée.

Propriétés fonctionnelles des macromolécules biologiques, 30h +15h TD+20h Pr - BIOC0210-4/2 E. De Pauw et **A. Matagne**. Master complémentaire en Nanotechnologie.

Structure et dynamique des macromolécules biologiques, 20h + 20h TD + 20h Pr - BIOC9233-1 - **P. Charlier** et **A. Matagne**. Master complémentaire en Nanotechnologie.

Third Cycle

Approches Moléculaires de la diversité des microorganismes marins, 15h + 15h Pr - BOTA0401 **- A. Wilmotte**

Advanced course on “Protein Purification: What to do and how” (Coordinated by **Prof. J.-M. Frère**), 15h. Prof. E. Depauw, **J.-M. Frère, M. Galleni, B. Joris** et **A. Matagne**. 10-12 May 2010, Liège.

Production de protéines recombinantes en systèmes procaryotes, 15h - SDOC0004-1- **C. Duez**

Courses given abroad

Bioinformatique, 35 h - **A. Brans**. Bac 3 DUT Génie biologique. IUT de Mont de Marsan, Université de Pau et des Pays de l’Adour, France. 15-22 November 2010.

Bioinformatics tools for the prediction of regulatory networks in recently sequenced actinomycete genomes. **S. Rigali**. 6h. University of Leiden, The Netherlands. 7 and 21 May 2010.

Chimie des Protéines, 20 h - **M. Galleni.** Licence en sciences Alimentaires, IUT Chimie Biologique, Mont de Marsan- Université de Pau et des Pays de l'Adour, France, 15-16 June 2010.

Enzyme kinetics, Protein folding and Protein Purification. **A. Matagne, J.-M. Frère** et **M. Galleni.** Masters en Biotechnologie et Microbiologie. Cycle de 3 ans, 15h/an. Università degli Studi di Siena, Siena, Italy.

Nanobodies or camelid Antibody Fragments: Properties and Applications, 7 h – **M. Dumoulin** Department of Pharmaceutical Sciences, University of Padova, Italy, 22-25 November 2010.

Production de protéines recombinantes, 8 h - **A. Brans**. Bac 3 DUT Génie biologique. IUT de Mont de Marsan. Université de Pau et des Pays de l'Adour, France. 22-23 November 2010.

Statistique multivariée, 30 h - **G. Dive**. Masters 1 et 2 en Chimie et thérapeutique. Université Paris VII Denis-Diderot, France.

Courses given in another Belgian university

Biologie végétale, 30h + 30 Pr **P. Motte**. Bac 2 Pharmacie et Bac 3 Biologie. Université de Mons.

L'immobilité au service de l'évolution: adaptations génétiques et physiologiques des Streptomyces. University of Namur – FUNDP, **S. Rigali**, 3h. 13 December 2010.

Courses given in Technical High Schools

Bioinformatique appliquée, 30h. – **S. Colson**. Spécialisation en biotechnologies médicales et pharmaceutiques. Haute Ecole André Vésale, Quai du Barbou, 2 4020 Liège.

Génie génétique et enzymatique, 30h – Partim 1 - **A. Brans**. Master 1 en Sciences de l'ingénieur industriel, finalité biochimie. Haute Ecole de la Province de Liège, Quai Gloesener 6, 4020 Liège.

Génie génétique et enzymatique, 30h – Partim 2 - **A. Brans**. Master 2 en Sciences de l'ingénieur industriel, finalité biochimie. Haute Ecole de la Province de Liège, Quai Gloesener, 6 4020 Liège.

Bachelor III and Master I Students

Bachelor III

Massonnet Philippe

Master I trainees

André Julie
Bouazza Naïm
Boulanger Benoît
Crasson Oscar
Dauvin Marjorie
de Gonzague Sebarèze Louis
Delbrassine François
Derochette Sandrine
Dettife Cécile
Deward Adeline
Fettweiss Grégory
Gillet Eric
Gillet Grégory
Hansen Sylvain
Huynen Céline
Jeridi Souraya
Jourdan Samuel
Kubica Annick
Lambert Stéphany
Latiri Fériel
Lekeux Gilles
Lorant Anne
Maruccia Catherine
Muller Joséphine
Rigaux Maïlys
Schils Nicolas
Schwartz Pierre
Stéveny Nicolas
Thiry Laura
Widouw Gwennaëlle

MASTER II AND 'GRADUAT' FINAL YEAR STUDENTS

Master II

Campisi Vincent Master II en Biochimie et Biologie Moléculaire et Cellulaire (BBMC) à finalité approfondie, ULg.
Caractérisation fonctionnelle du propeptide de l'allergène proDer p 3 de *Dermatophagoides pteronyssinus* : implication dans le repliement, la stabilité et l'activation du zymogène.

Depouhon Thomas
Master II BBMC à finalité approfondie, ULg.
Caractérisation de mutants multiples d'une α-amylase psychrophile.

Deremiens Léo Master II BBMC à finalité approfondie, ULg.
Etude de la diversité fossile des cyanobactéries de deux lacs Antarctiques.

Freichels Régine Master en sciences de l'ingénieur industriel, finalité biochimie.
Mise au point d'un biosenseur potentiométrique permettant de détecter des bactéries via les ß-lactamases qu'elles produisent.
HEPL, ISIL quai Gloesener 6, Liège.

Huynen Céline Master II BBMC à finalité approfondie, ULg.
Caractérisation des propriétés amyloïdogènes du peptide $A\beta_{42}$ (associé à la maladie d'Alzheimer) replacé dans un environnement protéique.

Joris Marine Master II BBMC à finalité approfondie, ULg.
Etude génétique et fonctionnelle du facteur essentiel d'épissage SRp30 au cours du développement embryonnaire de *Danio rerio*.

Laurent Clémentine Master II BBMC à finalité approfondie, ULg.
Caractérisation de l'inhibition de métallo-β-lactamases de sous-classe B1 par les V_HHs cAbVIM4 et cAbBcII10.

Lebrun Sarah Master II en Sciences de l'ingénieur industriel, finalité biochimie. Recherche de promoteurs forts dans *Bacillus subtilis* en vue de la production à l'échelle industrielle d'une enzyme recombinante. HEPL, ISIL, Quai Gloesener, 6 Liège.

Maréchal Maxime Master II BBMC à finalité approfondie, ULg.
Etude de l'opéron *ftsW-psr-pbp5* et sa régulation chez *Enterococcus hirae*.

Polese Catherine Master II BBMC à finalité approfondie, ULg.
Etude de l'expression de *FRD3*, un gène de l'homéostasie des métaux, chez deux espèces d'*Arabidopsis*.

Rosu Adeline — Master II BBMC à finalité approfondie. ULg Identification de PitE, une nouvelle protéine essentielle à la production des prodiginines chez *Streptomyces coelicolor.*

Spee Olivier — Master II BBMC. Université Catholique de Louvain. Construction et criblage d'une banque metagénomique à partir d'extraits épuisés d'acariens. Purification et caractérisation de l'estérase RBest1 isolée par métagénomique. Séjour au CIP de février à juillet 2010.

Wauthy Maxime — Master II. Biologie des Organismes et Environnement (BOE). Université Catholique de Louvain. Diversité et toxicité potentielle des cyanobactéries dans des plans d'eau wallons. Séjour de 3 mois au CIP

Erasmus Students - Master II

Brissoto Giulia — Master II. Faculty of Pharmacy, University of Padova, Italy. Production and characterization of chimeric proteins made of the *Bacillus licheniformis* β-lactamase 749/C and polyglutamine stretches of different lengths. February- September 2010.

Palladino Chiara — Master II. Microbiology. University of Napoli « Federico II ». Contribution à l'étude des voies d'induction des métabolites secondaires ches *Streptomyces*. 22/03/10-30/04/10

Technical High Schools - Bachelor III

Beckers Baptiste — Bachelier en chimie, finalité biotechnologie.
Nouvelle méthode pour l'insertion aléatoire d'ADN dans le génome de *Bacillus subtilis*. HEPL Rennequin Sualem, Quai Gloesener, 6 Liège.

Cherkaoui Majid — Bachelier en chimie, finalité biotechnologie.
Mise au point d'une technique permettant d'isoler le peptidoglycane septal d'*E. coli.* HEPL Rennequin Sualem, Quai Gloesener, 6 Liège.

Counson Mélody — Bachelier en chimie, finalité biochimie.
Mise au point d'une méthode de mutagenèse dirigée à haut débit dans le cas de la production d'une β-galactosidase psychrophile. HEPL Rennequin Sualem, Quai Gloesener, 6 Liège.

Dignef Mélissa Bachelier en Biologie Médicale, option chimie clinique.
Mise au point d'une méthode de mutagenèse de la β-galactosidase de *Pseudomonas haloplanktis*. Haute Ecole Charlemagne, campus du Sart Tilman, CHU Tour 4 Liège.

Gillard Benjamin Bachelier en Biologie Médicale, option chimie clinique.
Diversité moléculaire des cyanobactéries de tapis microbiens de l'île Macquarie (Sub-Antarctique). HEPL Charlemagne campus du Sart Tilman, CHU Tour 4 Liège.

General Public Activities

Activities for students of secondary schools

- 'Hands on' workshop on pigment extraction by **Annick Wilmotte** and **Patricia Simon** on 8th January 2010 for a 'Retraite scientifique' of a class from the school Saint Roch, Theux.

- Participation to the Printemps des Sciences. 22-28 March 2010. **Annick Wilmotte** and **Marine Renard**: 'Hands on' workshop on pigment extraction from cyanobacteria.

- 'Journée des Rhétos' (organized by the 'Administration de l'Enseignement et des Etudiants'). 9 March 2010. Participation of **Eric Sauvage, Paulette Charlier, Frédéric Sapunaric, Renaud Berlémont, Jean-Marie François, Patricia Lassaux, Sébastien Rigali** and **Annick Wilmotte**.

- 'Portes ouvertes Parents-Rhétos-Passerelles' (organized by the 'Administration de l'Enseignement et des Etudiants'). 24 April 2010. Participation of **Dominique Dehareng**.

Dream Day (www.dreamday.be)

'La vie de chercheur'. 18 March 2010. Participation of **Georges Dive**. Athénée Bomal, site de Marche.

Newspaper articles and press conferences

- 'Des cyanobactéries découvertes près du Pôle sud' (interview **A. Wilmotte**). Article in 'Reflexions', le site de vulgarisation scientifique de l'ULg. 13 August 2010 http://reflexions.ulg.ac.be/cms/c_27953/des-cyanobacteries-decouvertes-pres-du-pole-sud

- 'Baignades interdites pour cause de cyanobactéries'. (interview **A. Wilmotte**). 16 August 2010: http://www.lalibre.be/actu/gazette-de-liege/article/602772/baignade-interdite-pour-cause-de-cyanobacteries.html

- 'De l'Antarctique à l'espace'. 16 August 2010 (interview **A. Wilmotte**). : http://www.lalibre.be/actu/gazette-de-liege/article/602773/de-l-antarctiq-ue-a-l-espace.html

- 'Les algues bleues prolifèrent'. 29 December 2010. Press conference of the B-BLOOMS2 project, with **A. Wilmotte** http://www.lalibre.be/archives/divers/article/632748/lesalguesbleues proliferent.html

Scientific blog

BELDIVA expedition: www.antarcticabelgium.blogspot.com

Wide audience articles

'Eau douce... mais toxique ?!' **Annick Wilmotte** et **Yannick Lara**. Article in the Bulletin of the asbl 'Science et Culture' June 2010 :
http://www.sci-cult.ulg.ac.be/Bulletins/Bulletin426.pdf

'Une superbactérie asiatique détruit tous les antibiotiques'. **Jean-Marie Frère**. Article in 'Réflexions, le site de vulgarisation scientifique de l'Ulg'. 16 November 2010. http://reflexions.ulg.ac.be/

'L'enzyme qui produit du sucre'. **Jean-Marie François**. Article in 'Réflexions, le site de vulgarisation scientifique de l'Ulg'. 14 December 2010. http://reflexions.ulg.ac.be/

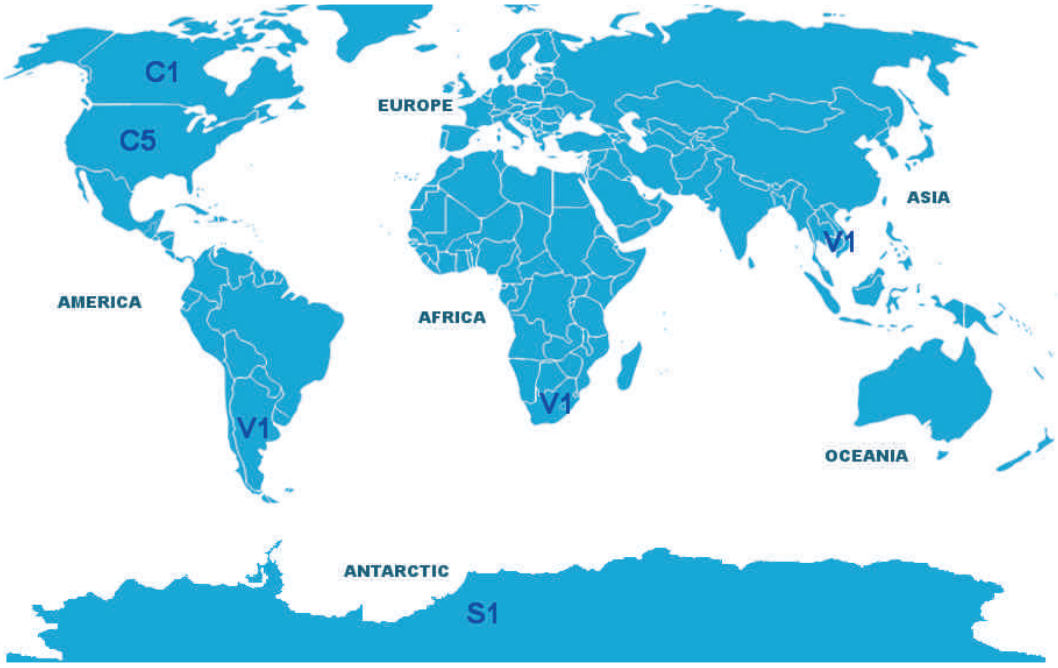
Wide audience conference

'La guerre des étoiles ou la résistance bactérienne à la pénicilline'. **Bernard Joris**. 5 February 2010. Séminaire de l'Institut des Sciences de la vie.

Wide audience debate

'Le bio est dans le pré.....peut-il nourrir la planète ?' **Yannick Lara**, F. Vancutsem, N. Delacollette. 8 June 2010.
http://www2.ulg.ac.be/sciences/doccafe/
Oraganized by the 'Réseau des Doctorants':
http://www.red.ulg.ac.be/
Logistic coordinator : **Jessica Guillerm**

INTERNATIONAL EXCHANGES



C Collaborations
V Visitors
S Stays abroad

COLLABORATIONS

ARGENTINA

University of Buenos Aires - Laboratorio de Resistencia Bacteriana - G. Gutkind & P. Power

AUSTRIA

Institute of Microbiology - University of Innsbruck – Austria - **R. Margesin**

BELGIUM

Beldem-Puratos Group – Andenne - **T. Dauvrin** & **J. Georis**

FUNDP – Laboratoire d'écologie des eaux douces - Namur – **J.-P. Descy**

Progenosis - Liège - **F. Giannotta** & **P. Filée**

KUL – Laboratory for Medicinal Chemistry – Leuven – **P. Herdewijn**

KUL - Division of Molecular and nanomaterials - Department of Chemistry - Leuven - **F. Meersman**

KUL- Functional Genomics and Proteomics Research Unit - Faculty of Sciences – Leuven - **L. Schoofs**

National Botanical Garden of Belgium – Meise – **D. Ertz** & **B. Van de Vyver**

RUG - Department of Organic Chemistry, Organic and Bioorganic synthesis - Gent - **J. Van der Eycken**

RUG – Laboratory for Protistology and Aquatic Ecology – Ghent – **W. Vyverman** & **K. Sabbe**

RUG – Laboratory for Microbiology – Ghent – **A. Willems** & **P. De Vos**

SCK-CEN – Unit of Microbiology – Mol – **M. Mergeay** & **N. Leys**

UCL - Unité de Chimie Organique et Médicinale - Département de Chimie - Louvain-la-Neuve - **J. Marchand-Brynaert**

ULB – Laboratoire de Bactériologie Moléculaire (MBL) - Bruxelles – **A. Allaoui**

ULB – Unité de Chimie des Protéines – Bruxelles – **M. Azarkan**

ULB – Institut de Recherches Microbiologiques Jean-Marie Wiame – Anderlecht – **C. Bauvois**

ULB – Unité Matière et Matériaux – Bruxelles – **K. Batik** & **G. Brylants**

ULB – Structure et Fonction des Membranes Biologiques (SFMB) - Bruxelles – **E. Goormaghtigh**

ULg – Département de Chimie – Liège – **C. Damblon**

ULg – Département de Chimie – Laboratoire de Spectométrie de Masse – Liège – **E. De Pauw**

ULg – Département des Sciences de la Vie – Photobiologie – Liège – **F. Franck**

ULg – Département de Géologie – Paléobotanique, Paléopalynologie, Micropaléontologie – Liège – **E. Javaux**

ULg – Département des Sciences de la Vie – Biologie et génétique moléculaire – Liège – **A. Lejeune**

ULg **-** Département des Sciences de la Vie – Biologie et génétique moléculaire – Liège – **C. Van de Weerdt**

ULg – Département des Sciences et Gestion de l'Environnement – Liège – **M. Poulicek**

ULg - Département des Sciences de la Vie – Génétique des algues – Liège – **C. Remacle**

ULg - Département des Sciences de la Vie – Centre Wallon de Bioindustries – Liège – **P. Thonart**

VUB –MINT Microbial Interactions – Rhode-Ste-Genèse – **P. Cornelis**

VUB - Ultrastructure Research - Faculty of Sciences - Brussels – **E. Pardon, L. Wyns** & **J. Steyaert**

VUB – Plant Science and Nature Management – Brussels – **L. Triest**

CANADA

Université Laval – Département de Biologie – Québec – **W. Vincent**

CZECH REPUBLIC

Academy of Sciences of the Czech Republic – Institute of Botany – Trebon – **J. Elster**

University of South Bohemia – Faculty of Biological Sciences – Ceske Budejovice – **J. Komarek**

FRANCE

CNRS - Populations, Génétique et Evolution - Gif-sur-Yvette - **J.L. Da Lage**

Laboratoire des amino acides, peptides et protéines - Faculté de Pharmacie, Montpellier - **J.-F. Hernandez**

Laboratoire de Bio-cristallographie - Institut de Biologie et Chimie des Protéines – Lyon - **N. Aghajari & R. Haser**

Institut Pasteur – Génétique des Génomes bactériens – Paris et Amabiotics - Evry - **A. Danchin**

Université de Caen, - Laboratoire de Chimie Moléculaire et Thio-organique - Ensicaen - Caen - **M. Gulea**

Université Joseph Fourier – Institut de Biologie Structurale – Grenoble – **T. Vernet**

Université Paris Sud – Institut de Biochimie et Biophysique Moléculaire et Cellulaire - Orsay - **M. Desmadril**

Université Paris Sud – Laboratoire Ecologie, Systématique et Evolution– Orsay – **J. Kroymann**

Université Paris Sud – Laboratoire des enveloppes bactériennes – Orsay – **D. Mengin Lecreulx** & **D. Blanot**

Université Paul Cézanne – Laboratoire de Synthèse et Etude de Substances Naturelles à Activité Biologique (SESNAB) – Marseille – **J.-M. Brunel**

GERMANY

Institute of Marine Biotechnology – Greifswald- **T. Schweder**

RWTH-Aachen - Bioanalytics - Institut für Molekulare Biotechnologie - Aachen - **K. Hoffmann**

University of Bayreuth – Laboratory of Biochemistry and Bayreuth Centre for Molecular Biological Sciences - Bayreuth - **F. X. Schmid**

University of Bochum- Departement for Biology and Biotechnology– Bochum – **U. Krämer**

University of Applied Sciences Münster - Department Oecotrophology - Münster - **F. Titgemeyer**

Martin-Luther University - Halle-Wittenberg - **J. Balbach**

GREECE

University of Crete- Department of Biology – Heraklion - **V. Bouriotis**

ITALY

University of L'Aquila – Department of Sciences and Biochemical Technologies – **M.G. Perilli**

University of Naples Federico II - Department of Organic Chemistry and Biochemistry - **G. Marino & L. Tutino**

University of Padua - CRIBI Biotechnology Centre - Padua - **P. Polverino de Laureto, E. Frare & A. Fontana**

University of Rome Tor Vergata – Department of Biology – Roma – **P. Albertano & D. Bili**

University of Siena – Laboratory of Physiology and Biotechnology of Microorganisms - Department of Molecular Biology - Siena - **J.-D. Docquier**

POLAND

Gdansk University – Department of Biotechnology – Gdansk – **M. & K. Waleron**

PORTUGAL

Institute of Chemical and Biological Technology - Laboratory of Bacterial Cell Biology - Oeiras - **M. G. Pinho**

SLOVENIA

Jozef Stefan Institute - Department of Biochemistry and Molecular Biology - Ljubljana - **R. H. Pain**

University of Ljubljana – Department of Pharmaceutical Chemistry – Ljubljana – **S. Gobec**

SWEDEN

University of Linköping - IFM - Department of Physics, Chemistry and Biology – Linköping **A. C. Brorsson**

THE NETHERLANDS

University of Amsterdam – Swammerdam Institute for Life Sciences – Amsterdam – **T. Den Blaauwen**

Leiden University - Microbial Development - Leiden Institute of Chemistry - Leiden - **G. van Wezel**

Utrecht University – Biochemistry of Membranes – Bijvoet Center – Utrecht – **E. Breukink**

UNITED KINGDOM

British Antarctic Survey – Cambridge – **D. Hodgson, P. Convey & D. Pearce**

Genzyme Diagnostics Ltd - West Malling – Kent - **E. Asilonu**

The James Hutton Institute - Dundee – **J. Brown**

University of Cambridge - Structural Chemistry and Spectroscopy - Department of Chemistry - Cambridge – **A. Duhlesia, J.R. Kumita, E. de Genst & C. M. Dobson**

University of Dundee – Department of Biology - Dundee – **G. Codd**

University of Leeds - Division of Microbiology, School of Biochemistry and Molecular Biology Faculty of Biological Sciences - Leeds - **I. Chopra**

University of Leeds – Ashbury Centre for Structural Molecular Biology – Faculty of Sciences – Leeds – **K.J. McDowall**

University of Newcastle - The Centre for Bacterial Cell Biology – Newcastle – **W. Vollmer**

University of Oxford - Department of Biochemistry - Oxford - **C. Redfield**

USA

Ohio State University – Plant Cellular and Molecular Biology – Columbus – **P. Hamel**

The Scripps Research Institute, Scripps Florida, Lead Identification, Translational Research Institute - Jupiter (Floride) - **P. Hodder**

University of California – Chemistry and Biochemistry - Los Angeles – **S. Merchant**

University of Oregon – Department of Biology – Eugene – **R. W. Castenholz**

Wesleyan University - Department of Chemistry - Middletown - **R.F. Pratt**

Visitors

Baudelet Paul-Hubert, ENSAIA Nancy, France, 4 October 2010 – 15 February 2011

Maciejewska Marta, West Pomeranian University of Technology, Szczecin, Poland, 8 November – 24 December

Manca Marco, University of Pavia, Italy, 8-29 September

Perilli Mariagrazia, University of L'Aquila, Italy, 10 January – 8 February

Raji Moughit K., Université Libre de Bruxelles, Belgique, 18-19 January, 31 March-2 April, 20-21 September

Rezende Roberta, Hôpital UCL Mont-Godinne, Laboratoire de Microbiologie, Yvoir, Belgique, 27-30 October

Ruggiero Melina, University of Buenos Aires, Argentina, 18 August 2010 - 18 February 2011

Schuler Kathrin, University of Münster, Germany, 25 May 2010 – 1 August

Strunecki Otar, University of South Bohemia and Institute of Botany of CAS, Trebon, Czech Republic, 24 May-6 June

Tea Channy, Institut de Technologie du Cambodge, Pnom Penh, 1-28 February

Vukea Phillia, Department of Biochemistry, UKZN, Scottville, 3209, South Africa, 26 October – 10 November

Waleron Krzysztof and **Malgorzata**, University of Gdansk, Poland, 16-17 December

Stays Abroad

Amoroso Ana, NMR Facility, IBS Grenoble, France, September 5-7

Calusinska Magdalena, University of Bochum, Bochum, Germany, February 1 – March 15

Craig Matthias, John Innes/Rudjer Boškovic Summer School in Applied Molecular Microbiology: Signal to drugs, Dubrovnik, Croatia, August 21-29

Dumont Janice, Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université Paris-Sud, Orsay, December 06-17

Mercuri Paola, University of L'Aquila, Department of Sciences and Biochemical Technologies, L'Aquila, Italy, April 12-19

Namsaraev Zorigto, Belgian "Princess Elisabeth" research station in Antarctica, January 3 – February 14

Namsaraev Zorigto, University of South Bohemia, Ceske Budejovice and Institute of Botany of CAS, Trebon, Czech Republic, August 15-29

Sohier Jean-Sébastien, CRP-Santé, Laboratoire de rétrovirologie, Luxembourg, Grand-Duché de Luxembourg, December 6-24

Tenconi Elodie, Leiden Institute of Chemistry, The Netherlands, June 2010

Vandenameele Julie, "Institut für Physik - Fachgruppe Biophysik", Martin-Luther-Universität Halle-Wittenberg, Germany, May 25-28

Wilmotte Annick, University of South Bohemia, Ceske Budejovice and Institute of Botany of CAS, Trebon, Czech Republic, August 15-29

FUNDING

FUNDING

Les Pôles d'Attraction Interuniversitaires

PAI P6/19 (2007-2011) - Proteins: interactions involved in folding, function and supramolecular assemblages PROFUSA (the CIP was the Coordinator)

Politique Scientifique Fédérale Belge

BCCM C3/00/14 (2005-2010) - Stimulation Action of the use and collaboration with the Coordinated Belgian Collections of Microorganisms: Elaborating public culture collections of diatoms, polar cyanobacteria and mycobacteria in Belgium
BELSPO SD/TE/01A (2006-2011) - 'B-BLOOMS2 : proliférations cyanobactériennes : toxicité, diversité, modélisation et gestion'
BELSPO SD/BA/01A (2006-2011) - 'AMBIO: biodiversité microbienne antarctique: l'importance des facteurs géographiques et écologiques'
BELSPO SD/BA/853 (2008-2010) - ANTAR-IMPACT: evaluation of the microbial diversity on the site of the future Belgian Antarctic station in Utsteinen
BELSPO EA/00/05A (2009-2013) - 'BELDIVA : Exploration belge de la diversité microbienne dans la région de la Station Princesse Elisabeth, Antarctique'

Les Actions de Recherche Concertées

ARC 07/12-04 (2007-2012) – MICRO-H2: Microbiological production of hydrogen: study of microalgal and bacterial processes

Fonds de la Recherche Scientifique - FNRS

Projets de recherche
FNRS-IISN 4.4.505.00.F (2007-2010) - 'Utilisation du rayonnement synchrotron : développement de la recherche en biologie structurale par l'étude des macromolécules via la cristallographie'.
FNRS 1670 (2008-2011) 'Fibres amyloïdes : fonctions, structure et mécanisme de formation'.
FNRS 4.4509.090.F (2009-2012) - 'Etude structurale des protéines impliquées dans le métabolisme et la dynamique de la paroi bactérienne'.

Mandats de recherche
Mandat FNRS de Chercheur Temporaire Postdoctoral: Dr Michaël Nigen (1/01/2009 au 30/06/2010), avenant à la Convention F.R.F.C. n° 2.4530.09
Mandat FNRS de Chercheur Temporaire Postdoctoral: Dr Zorigto Namsaraev (1/11/2008 au 31/10/2010), Convention F.R.F.C. n° 2.4570.09
Mandat FNRS de Chercheur Temporaire Postdoctoral: Dr Cécile Nouet (01/10/2009 au 30/09/2011), Convention F.R.F.C. n° 2.4583.08
Mandats de Chargé de Recherche : Bebrone C. Fickers P., M. Hanikenne, Kerff F et V. Tillemans.

Fonds de la Recherche Fondamentale Collective

FRFC 2.4543.05 (2006-2010) - 'Les machineries d'assemblage et de remodelage du peptidoglycane'
FRFC 2.4506.08 (2008-2011) - Study of the glycosyltransferase enzymes catalyzing the glycan chain polymerization of the bacterial wall peptidoglycan
FRFC 2.4535.08 (2008-2011) - 'Exploration du génome d'une bactérie de l'Antarctique : approches protéomiques et biophysiques de la psychrophilie et de l'adaptation aux basses températures'
FRFC 2.4.583.08 (2008-2011) Heavy metal tolerance in zinc and cadmium hyperaccumulators: analysis of overexpression mechanisms of metal transport genes and functional characterization of the corresponding proteins.
FRFC 2.4558.09 (2009-2011) Fossilisation of cyanobacteria : phylogenetic, micropaleontological and sedimentological approaches, and implications for the evolution of the primitive biosphere.
Cooperation to the project of Prof. Emmanuelle Javaux (Geology, ULg, BE)
FRFC 2.4.642.09 (2009-2012) Regulation of gene expression at the post-transcriptional level in photosynthetic eukaryotes: study of a family of conserved SR splicing factors and functional organization of the nucleus
FRFC 2.4530.09 (2009-2012) - 'Etude des cinétiques de folding de trois protéines modèles, à l'aide des méthodes d'échange hydrogène/deutérium, couplées à la spectrométrie de masse et à la RMN
FRFC 2.4548.10 (2009-2013) - 'Caractérisation des interactions entre biomolécules par résonance plasmonique de surface'.
FRFC 2.4570.09 (2009-2012) - 'BIPOLES: Distribution géographique et écologique des cyanobactéries antarctiques et arctiques'.

Région Wallonne

RW 415896 (2005-2011) - 'APTARRAY: Elaboration de biopuces aptamériques destinées à l'identification et au typage des virus animaux (Réseau II)'
RW 516265 (2006-2012) - 'MED-ATR : Dosage en temps réel de substances pharmacologiques dans des fluides physiologiques'
RW BIOWIN 5678 (2008-2011) – CANTOL: Discovery and validation of indoleamine 2,3-dioxygenase inhibitors to overcome tolerance in cancer immunotherapy
RW 816890 (2009-2013) - 'RAPARRAY : Conception d'un support de biopuce à protéines adapté à une méthode de détection sans marquage par spectroscopie non linéaire de génération de fréquence-somme (SFG)'
RW 816889 (2009-2013) - 'RAIDGBS : Développement d'un test pour l'identification rapide et facile de la colonisation vaginale par les streptocoques du groupe B'
SPW-GD06 Economie, Emploi, Formation, Recherche n° 816873 (2009-2013) - GPCR-LIKE : 'Dévelopment de GPCR-like comme source antigénique d'anticorps monoclonaux agissant comme modulateurs allostériques'
Subvention First-Spin off n° 816811 (2008-2011) 'SCALAB : systèmes de production d'anticorps à grande échelle'.

Bilateral Cooperation Wallonie/Bruxelles

International-Czech Republic. (2008-2010) Molecular and phenotypic analysis of the cyanobacterial diversity
International-Italia. (2009-2010) Evolution of the tolerance to dessication in cyanobacteria.
International-Slovenia. (2010-2012) CMP-2009/22676 : Recherche d'inhibiteurs de glycosyltransférases à potentiel antibactérien.

Union Européenne

LSHM-CT-2004-512138 (2005-2010) - EUR-INTAFAR: Inhibition of new targets for fighting antibiotic resistance (CIP: coordinator)
LSHM-CT-2006 (2009-2010) - EURAMY: Systemic Amyloidosis in Europe
PRODEX - European Spatial Agency (2010-2011) – EXANAM: Antarctic base Concordia

Université de Liège

Crédits classiques
Projet C-09/20 (2009-2010) - Role of bacteria in the coral bleaching. Cooperation with Prof. Mathieu Poulicek (Ulg, BE)
Projet C-09/75 (2009-2010) - 'Etude structurale des protéines impliquées dans le métabolisme et la dynamique de la paroi bactérienne'

Crédits de démarrage
Projet D-08/20 (2008-2010) - 'Etude des machineries d'assemblage du peptidoglycane de la paroi bactérienne'
Projet D-09/01 (2009-2010) - 'Etude de la stabilité et des mécanismes de repliement des protéines : le cas des ß-lactamases à sérine et à zinc'

Missions of Expertise

Member of Research Councils

Prof. Moreno Galleni
Membre du Conseil sectoriel de la Recherche "Science et Technique" (2009-)
Membre du Conseil Universitaire de la Recherche (2009-)
Dr. Annick Wilmotte
Membre du Conseil scientifique du Jardin botanique national de Belgique (2002-)

Member of Editorial Board

Dr Georges Feller, Extremophiles (2004-)
Prof. Moreno Galleni, Antimicrobial Agents and Chemotherapy (2001-)
Prof. Jean-Marie Frère, Antimicrobial Agents and Chemotherapy (2001-)

Member of the Editorial Advisory Panel

Prof. André Matagne, The Biochemical Journal (2004-)
Dr Georges Feller, Biologia (Bratislava) (2002-)

Commitees and Societies

Charlier Paulette
Comité National Belge de Cristallographie (représentant ULg) (Vice-president)
Groupe de contact F.R.S.-FNRS « Rayonnement Synchrotron » (Secretary)

Galleni Moreno
BioLiège (Vice-president)

Matagne André
Belgian Biophysical Society (President)
Liège Royal Society of Sciences (Vice President)
National Committee of Biophysics (President)

Wilmotte Annick
Scientific expert of the Belgian Delegation to the Committee of Environmental Protection of the Antarctic Treaty (since 2008)
Belgian National Committee on Antartic Research of the Academies of Sciences (Secretary)
Subcommittee for the Taxonomy of Phototrophic Prokaryotes of the International Committee on Systematic Bacteriology (ICSB) (Secretary)
International Organization for Systematic and Evolutionary Biology

Composition of the Center

Managing Committee

Director
Bernard Joris

Current Executive Committee:
Paulette Charlier, Mireille Dumoulin, Moreno Galleni, André Matagne, Patrick Motte, Annick Wilmotte

Current Managing Committee:
Carine Bebrone, Alain Brans, Paulette Charlier, Dominique Dehareng, Georges Dive, Colette Duez, Mireille Dumoulin, Georges Feller, Claudine Fraipont, Moreno Galleni, Colette Goffin, Marc Hanikenne, Frédéric Kerff, André Matagne, Paola Mercuri, Patrick Motte, Noureddine Rhazi, Sébastien Rigali, Eric Sauvage, Mohammed Terrak, Annick Wilmotte

Current Scientific Advisors :
Jacques Coyette, Martine Distèche, Jean-Marie Frère

Administrative Staff

Paola Catanzaro (Executive secretary)
Fabienne Julémont (Administrative secretary)
Geneviève Lefébure (Administrative secretary)

Technical Assistance

Anne-Marie Matton
Marie Schloesser
Iris Thamm

Temporary Members

Associate researchers

Dr Ana Amoroso
Dr Etienne Baise
Dr Carine Bebrone
Dr Renaud Berlemont
M. Fabrice Bouillenne
Dr Alain Brans
Dr Dominique Dehareng
Dr Michael Delmarcelle
Dr Alexandre Di Paolo
Dr Claudine Fraipont
Dr Jean-Marie François
Dr J. Gangoué-Pieboji
Dr Xavier Henry
Dr Patricia Lassaux
Dr Paola Mercuri
Dr Zorigto Namsaraev
Dr M. Nigen
Dr Cécile Nouet
Dr Noureddine Rhazi
Dr Frédéric Sapunaric
Dr Eric Sauvage
Dr Brahim Semane
Dr Vinciane Tillemans
Dr Julie Vandenameele
Dr Lionel Vercheval

PhD students

Anthony Argüelles Arias (FRIA)
Roya Barumandzadeh (FRIA, ULg)
Stéphane Baurin
Stéphanie Berzigotti (FRIA, ULg)
Ahlem Bouaziz (CGRI)
Sophie Califice
Magdalena Calusinska
Vincent Campisi (FRIA)
Jean-Benoît Charlier (FRIA)
Chloé Chavignon (FRIA)
Alexandre Cipolla (FRIA)
Séverine Colson (FRIA, RW)
Matthias Craig (FRIA)
Sébastien Dandois
Pedro De Carvalho Maalouf (SSTC AMBIO)
Morgane Dehousse (FRIA)
Maud Delsaute (FRIA)
Nicolas Dony (FRIA)
Marie-Eve Dumez (Assistante)
Janice Dumont (FRIA)
Anne Famerie
Rafael Fernandez Carazo (SSTC)
Adriana Fernea
Amandine Godin (FRIA)
Jessica Guillerm (FRIA)
Badre-Eddine Halimi
Julie Herman (FRIA)
Séverine Hubert
Olivier Jacquin (RW ALLERVAC)
Adrien Jehaes (FRIA)
Marine Joris (FRIA)
Yannick Lara (FRIA)
Clémentine Laurent (FRIA)
Sophie Laurent (FNRS Télévie)
Sarah Lebrun (FRIA)
Isabelle Leponce (Assistante)
Marie-José Mano (FRIA)
Maxime Maréchal (FRIA)
Georgios Moutzourelis
Coralie Pain (FRIA)
Anne Pennartz
André Piette
Florence Piette (FRIA)
Delphine Pipers
Mathieu Rocaboy (FRIA)
Frédéric Roulling (FRIA)
Melina Ruggiero (CONICET)
Natacha Scarafone (FRIA, ULg)
Jean-Sébastien Sohier (FRIA, ULg)
Nancy Stankovic (FRIA)
Caroline Struvay (FRIA)
Elodie Tenconi (FRIA)
Edwige Van der Heiden (FRIA)

Technical assistance

Caroline Bortuzzo
Melody Counson
Astrid Freichels
Nicole Gérardin-Otthiers
Raphaël Herman
Alexandre Lambion
Marine Renard
Patricia Simon
Olivier Verlaine