CONTENTS

INTRODUCTION

The CIP was founded in 1990, so this year we celebrate 20 years of excellent work and fruitful collaboration between its members. The CIP was the first such multidisciplinary centre in the field of biology at the University of Liège, covering a large number of domains from molecular biology to theoretical chemistry. In the beginning, the research topics largely concerned the bacterial world but the broad range of expertise of the members resulted in a wide diversification of the problems studied. Projects dealing with bacteriology however have remained a strong and attractive part of the activities. With time the composition of the managing committee has also been significantly modified. Of the eleven initial members, only four remain, although Jacques Coyette, Martine Nguyen-Distèche and Jean-Marie Frère are still active as scientific advisors. However, as shown in the previous reports, a large number of younger scientists have joined the management committee over the years. The 2005-2009 period was particularly successful, with three new Research Associates (F.R.S.-FNRS) being appointed: Mohammed Terrak in 2006, Mireille Dumoulin and Sébastien Rigali in 2008. They are respectively developing research topics focused on the bacterial cell morphogenesis including the bacterial transglycosylase reaction and the search for inhibitors, the mechanism of formation of amyloid fibrils *in vitro* and the control of secondary metabolite production in *Streptomyces*.

Jacques Coyette retired in October 2006 and, on the 25th of January 2007, the CIP organised a celebration which included a symposium in recognition of his excellent teaching career, his important contributions to the understanding of penicillin resistance in *Enterococci*, and to the organisation and administration of the CIP. In November 2007, it was Martine's turn, and this also provided an opportunity for some festivities. On the 21st of May 2008, a second symposium was organised entitled "Peptidoglycan and bacterial shape", during which the invited speakers stressed Martine's strong involvement and interesting discoveries in the area of the relationship between cell wall biosynthesis and morphogenesis. Finally, I retired at the end of September 2008. It took longer to organise the meeting and fiesta which lasted three days (1st-3rd of July, 2009). The topic of the meeting was "Penicillin-recognising enzymes: from enzyme kinetics to protein folding". At these three scientific meetings, several foreign colleagues and former graduate students of the Centre presented excellent talks. The festive parts of these celebrations were also very successful and a lot of former students and collaborators were happy to participate. In addition, the weather was splendid at least during the July 2009 meeting, an experimental observation which is not insignificant in Belgium!

The teaching positions of Jacques and Jean-Marie became vacant. Although there was some restructuring of their lecture programs, the Faculty of Sciences and the University agreed to open vacancies for two full-time professorships. Bernard Joris obtained the position of Jacques' and André Matagne that of Jean-Marie's. The directorship of the CIP was taken over by Bernard Joris. Taking into account the three new

Research Associates, the number of senior, permanent scientists has not decreased despite the departures of Jacques, Martine and Jean-Marie. Moreover, at the end of 2009, Paulette Charlier was promoted to an academic position, increasing the number of professors at the CIP to four and Georges Feller decided to join the CIP with his group devoted to the study of "cold" proteins.
In addition, over the 2005-2009 period, Paola Catanzaro (executive secretary), Iris Thamm (specialised technician) and Anne-Marie Matton (specialised technician, half time) were appointed to permanent staff positions.
In 2007, Nicole Gérardin-Otthiers ("Mrs N-terminus") retired. She deserves our gratitude for her efficient activity in the laboratory.

Fabrizzio Giannotta left the Centre in 2007. But not really, since Progenosis, the spin-off he is managing, is a direct result of one of the activities of the CIP (the design and study of hybrid proteins). He remains in close contact with his former colleagues and the spin-off has already hired some former members of the staff.

Between 2005 and 2009, the CIP coordinated EURINTAFAR, a large European Integrated Project devoted to the discovery of new bacterial targets to fight antibiotic resistance. The project involved 16 teams from 6 countries and was highly successful in terms of published and publishable results, of new insights into the biosynthesis of the bacterial cell wall, and discovery of "lead" compounds which could be further developed to yield efficient antibacterial drugs. As a further example of its activities, the CIP has launched a Protein Production and Purification platform, for mainly external users, which has been very successful. Finally, with the renewal of the IUAP contract in 2007, the CIP is in a strong position to continue producing cutting edge science.

A snapshot at the end of 2009 lists 11 academic and tenured (University or F.R.S.-FNRS) senior researchers, 27 PhD's, 41 graduate students, 11 technicians and 2 secretaries. It should be noted however that, among the academic/scientific staff, the number of new tenured appointees is identical to the number of colleagues who retired, so that the ratio of tenured to non-permanent staff is in fact lower than in the previous period.

Jean-Marie Frère

RESEARCH GROUPS

APPLIED QUANTUM CHEMISTRY AND MODELLING

Group leader: **Dr Georges Dive**

Associate member: **Dr Dominique Dehareng**

BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS

Group leaders: **Dr Martine Nguyen-Distèche (-2007)**
Prof. Jacques Coyette (-2006)
Prof. Bernard Joris

Permanent scientists: **Dr Colette Duez**
Dr Colette Goffin
Dr Sébastien Rigali (2008-)
Dr Mohammed Terrak (2006-)
Dr Annick Wilmotte

Associate members: **Dr Ana Amoroso (2007-)**
Dr Alain Brans
Dr Michaël Delmarcelle
Dr Adeline Derouaux
Dr Valérie Duval (-2006)
Dr Patrick Fickers
Dr Claudine Fraipont
Dr Fabrizio Giannotta (-2006)
Dr Serge Leimanis (2006)
Dr Zorigto Namsaraev (2007-)
Dr Catherine Raskin (-2007)
Dr Sébastien Rigali (2006-2008)
Dr Olga Savichtcheva (2008-2009)
Dr Mohammed Terrak (-2006)
Dr Frédéric Zakhia (2007-2008)
Dr Astrid Zervosen (2005)
Dr Krzysztof Waleron (2007-2008)

RESEARCH GROUPS

BIOLOGICAL MACROMOLECULES AND BIOCHEMISTRY

Group leaders: **Prof. Jean-Marie Frère (-2008)**
Prof. Moreno Galleni

Permanent scientist: **Dr Georges Feller (2008-)**

Associate members:
Dr Etienne Baise (2009 -)
Dr Carine Bebrone
Dr Renaud Berlemont (2009-)
Dr Frédéric De Lemos Esteves
Dr Jean-Denis Docquier (-2007)
Dr Diane Drescher (2007-2008)
Dr Patrice Filée
Dr Jean-Marie François (2006-)
Dr Paola Mercuri
Dr Joseph Gangoué Piéboji (2008-)
Dr Pablo Power (2007-2009)
Dr Noureddine Rhazi
Dr Nadia Ruth (-2008)
Dr Frédéric Sapunaric (2006-)
Dr Brahim Semane (2007-)

BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY

Group leader: **Prof. Paulette Charlier**

Associate members:
Dr Eric Sauvage
Dr Frédéric Kerff

ENZYMOLOGY AND PROTEIN FOLDING

Group leaders: **Prof. Jean-Marie Frère (-2008)**
Prof. André Matagne (2008-)

Permanent scientist**:** **Dr Mireille Dumoulin (2008-)**

Associate members:
Dr Mireille Dumoulin (2006-2008)
Dr Michaël Nigen (2009-)
Dr Julie Vandenameele (2009-)

EXPERTISES

MOLECULAR BIOLOGY

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

PROTEIN PRODUCTION

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

PROTEIN PURIFICATION

- ☒ Classical purification techniques (ion exchange, affinity, hydroxyapatite…)
- ☒ From mL to L
- ☒ HLPC, FPLC, Åkta prime, Åkta explorer, Profinia, Biopilot…

MACROMOLECULE CHARACTERISATION

Biochemical characterisation

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

Biophysical characterisation

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

IN SILICO STUDIES

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

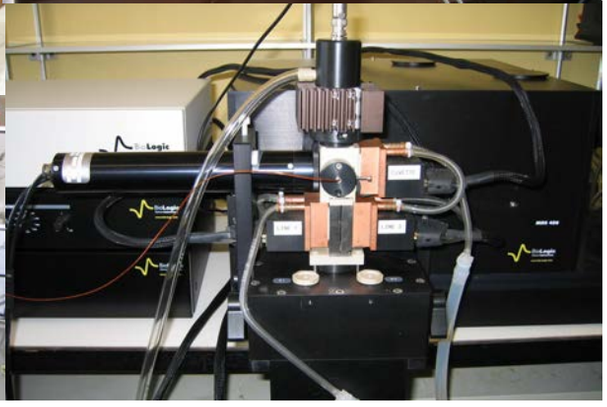



VIP-DISC

W BRUNSWICK SCIENTIFIC





MAJOR EQUIPEMENTS

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 one MJ Mini Real Time Quantitative PCR PTC0148 (Biorad)
1 Nanovue (GE Healthcare)

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)
1 Gradient Table for crossed gradients of temperature and light (Labio chromatography)

Cultures of green algae

1 Versatile environment test chamber (Sanyo)

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 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)
A range of instruments to perform protein purification at low or high pressure including 2 Åkta-explorer (10S 2D-LC and 100-Air), 1 Åkta- purifier, 2 Ákta prime and 2 Ákta 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 (Biacore AB, Uppsala, Sweden)
1 Circular dichroism spectrophotometer J-810 equipped with a Peltier temperature control and a 6 cell holder (Jasco)
2 2D-electrophoresis GE Ettan IPGphor3 and Ettan DALTsix apparatus (GE Healthcare)
2 DGGE electrophoresis apparatus Dcode (Biorad)
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 Microplate readers: one Labsystems Multiskan Multisoft (TechGen International) and one PowerwaveX (Bio-Tek instruments)
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), 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 detection (Bio-Logic)

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 Phase contrast microscope (Reichert)
1 Molecular Imager FX system (Biorad)
1 SZ-6 PHOTO binocular microscope (Bauch & Lomb)
1 Typhoon Trio+ scanner (GE Healthcare)

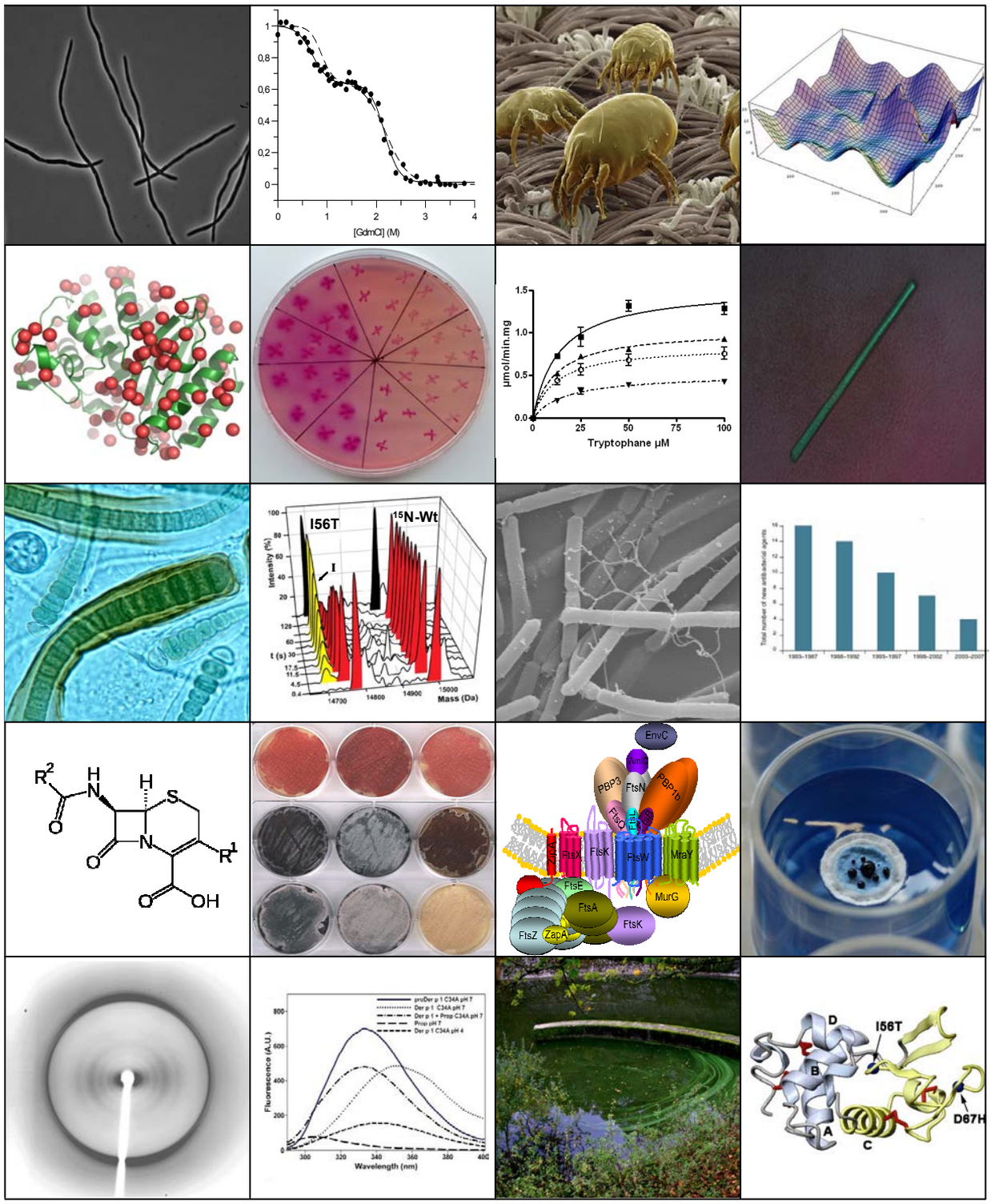
Crystallography

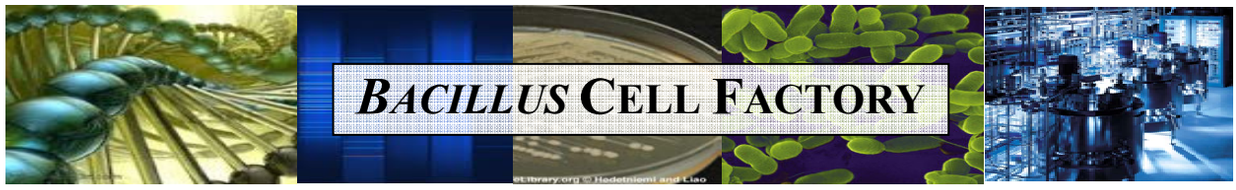
1 Cryogenic AD41 cryosystem (Oxford)
4 Graphic-PC stations (Linux)
1 Imaging Plate Marresearch IPmar345 equipped with a RU200B rotating anode (Rigaku)

Miscellaneous

1 Freeze-dryer (Christ)

THEMES OF RESEARCH





***Bacillus* is a genus of Gram-positive soil bacteria that secrete numerous enzymes and secondary metabolites. The advantage of *Bacillaceae* as protein production hosts 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)

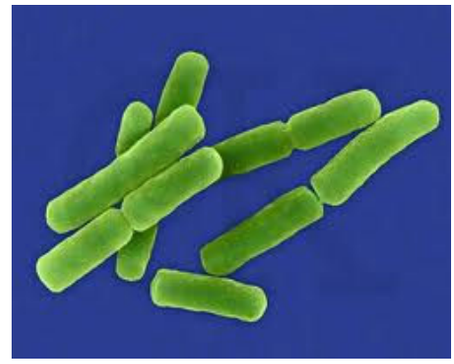
The CIP is continuously developing original molecular biology methods for *Bacillus* to:

- 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 level of recombinant proteins,

- Carry out directed and random genomic optimization.



Non ribosomal peptides

Cyclic lipopeptides and particularly surfactins produced by *Bacillus* species retain antibacterial, antifungal, antiviral and biofilm-forming activities. Multiple strains of *Bacillus* sp. were demonstrated to stimulate plant defense responses; the surfactin and fengycin lipopeptides may be involved in this elicitation process.

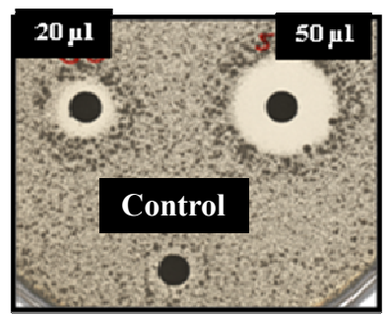
Engineered strains derived from *B. subtilis* 168 are developed for the overproduction of the surfacting and/or fengycin lipopeptides. The level of production is economically viable for an industrial scale production.

The genome of the plant-associated *B. amyloliquefaciens* GA1 was sample sequenced. Several gene clusters involved in the synthesis of biocontrol agents were detected. Four gene clusters were shown to direct the synthesis of the following cyclic lipopeptides: surfactin, iturin A, fengycin and bacillibactin. Beside these non-ribosomaly synthetised peptides, three additional gene clusters directing the synthesis of the antibacterial polyketides macrolactin, bacillaene and difficidin were identified. The production of all these antibiotic compounds highlights *B. amyloliquefaciens* GA1 as a good candidate for the development of biocontrol agents.

High-level production and isolation of the lipopeptide mycosubtilin produced by a genetically engineered *Bacillus subtilis* strain are performed. Antifungal activity suggests its potential therapeutic use as an antifungal agent, in particular against *Candida* sp.

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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PhD students: A. Argüelles-Arias & B. Halimi

BACTERIAL CELL WALL: OVERVIEW

The peptidoglycan is a covalently closed net-like polymer in which glycan chains made of alternating β-1,4-linked *N*-acetylglucosamine and *N*-acetylmuramic acid residues are crosslinked by short peptides. Located outside of the cytoplasmic membrane of most bacteria, it preserves the cell integrity and is responsible for the cell morphology. Because it is exclusively present in bacteria and it is essential for their survival, the peptidoglycan remains a privileged target for the design of antibacterials.

The immediate precursor of the cell wall peptidoglycan is lipid II, a disaccharide peptide linked to an undecaprenyl lipid carrier via a pyrophosphate bridge. This precursor is used by multimodular penicillin-binding proteins (PBPs) to polymerise the peptidoglycan at the outer face of the plasmic membrane. The major part of this synthesis is accomplished by class A PBPs that possess a glycosyl transferase (GT) module catalysing the glycan chain elongation and a penicillin-binding module catalysing the formation of peptide crosslinks. Monofunctional glycosyl transferases (Mtgs) only catalyse the glycan chain elongation [**140**].

In rod-shaped bacteria, two morphogenetic networks channel the assembly of peptidoglycan into wall expansion and septum formation in a cell-cycle dependent fashion in order to allow the bacterial cell to grow and divide [**109**]. These protein complexes include class B PBPs whose modular design is similar to that of class A PBPs and which are monofunctional transpeptidases [**140**].

Currently, three phases of peptidoglycan growth are thought to occur during the cell cycle of rod-shaped bacteria [Vollmer and Bertsche, 2008, Biochim Biophys Acta 1778:1714-1734; **109**]. The elongation phase is performed by a protein complex, the elongase, which consists of RodA, a class A PBP, the class B PBP2, three cytoskeletal proteins (MreB, C and D), RodZ, MurG and MraY. MreB, an actin-like protein, polymerises into large fibrous spirals at the inner face of the plasmic membrane and could operate by exerting spatial control over the cell wall synthesising machinery. Prior to cell division, there is a second, FtsZ-dependent phase of cell elongation (also called preseptal) (Aaron *et al*., 2007 Mol Microbiol 64, 938-952) in which the elongation-specific peptidoglycan synthesis complexes are controlled by FtsZ, a tubulin-like protein, and the early cell division proteins.

During cell division, the class B PBP3 and class A PBP1B are directly involved in the polymerisation of the septal peptidoglycan in conjunction with cell division proteins which assemble at the division site on the FtsZ-ring to form the mature divisome, a supramolecular complex [**109**].

Throughout the bacterial cell cycle, the wall peptidoglycan undergoes constant chemical changes. To enlarge a completely closed bag-like network, strings must be cleaved to allow the insertion of new material between the existing netting and the cleavage of the septum to separate the new daughter cells. Hydrolases are involved in these processes as well as in peptidoglycan recycling and induction of β-lactamases [**141**].

The wall peptidoglycan manufacturing machineries are very complex and are far from being understood. Very likely some of their components have not been identified yet. These machineries are the targets of some of the most extensively used antibiotics and constitute potential targets for the discovery of new antibacterial compounds, a program developed in the EUR-INTAFAR European project (LSHM-CT-2004-512138), IAP (P6/19) and ARC (03/08-297).

Our research project aims at characterizing the GTs and developing new antibacterials against them, at studying the functioning of the divisome and its relation with the elongasome and at analyzing the cell wall remodelling.

BACTERIAL CELL WALL: DIVISION

During cell division, peptidoglycan synthesis is performed by a dynamic protein complex, the divisome [109]. In *Escherichia coli*, this machinery includes more than fifteen proteins which are involved in a protein-protein interaction network and assembles in two stages: 1) the assembly of an intracellular cytoskeleton at the division site comprising FtsZ, FtsA, ZapA, ZipA that form the so-called FtsZ ring and 2) the recruitment of other cell division proteins FtsK, FtsQ, FtsL, FtsB, FtsW, PBP3, FtsN, and AmiC [1].

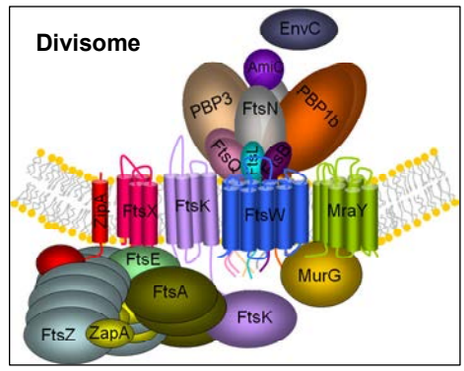
Our objectives are to understand:

- How the cell division proteins interact with each other and synthesise the septal peptidoglycan,

- How the divisome machinery is regulated,

- How the cell division proteins interact with the proteins of the elongasome.

For this purpose, we use site-directed mutagenesis, surface plamon resonance (SPR), structure prediction, fluorescence microscopy, FRET, two-hybrid assays and co-immunoprecipitation.



PBP3 requires the putative flippase FtsW for its correct localisation. We have shown that these proteins are able to form a discrete complex independently of the other cell division proteins. The PBP3-FtsW complex appears to be a broadly conserved unit of the division machinery.

We have also shown that PBP3 interacts directly with the class A PBP1B [33], which localizes at the lateral wall during elongation and at the division site during septation. Its localization at the division site depends on the presence of PBP3 [33]. These proteins might thus act together to form the peptidoglycan of the two new cell poles.

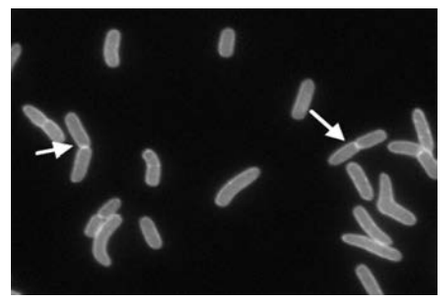
PBP3 also interacts with FtsN, which interacts with PBP1B. FtsN stimulates PBP1B activity [86]. Thus FtsN might coordinate or modulate the activities of a PBP1B-PBP3 complex in the cell.

The monofunctional glycosyltransferase MtgA localizes at the division site of *ponA*(ts), *ponB E. coli* cells and is able to interact with PBP3, FtsW and FtsN, suggesting that it may play a role in peptidoglycan assembly during the cell cycle [110].

The transmembrane segment of PBP3 is involved in the interactions with FtsW and PBP1B. Studies are in progress to characterize these interactions.

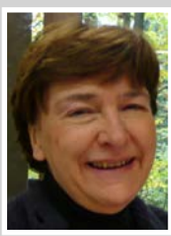
The divisome and the elongasome probably share part of their proteins. We are studying the interaction between proteins of these complexes.

Interaction between PBP1B and FtsN
By a bacterial two-hybrid assay



Localization of GFP-MtgA in *E. coli* EJ801

This work was realized in collaboration with T. den Blaauwen (University of Amsterdam), W. Vollmer (University of Newcastle), D. Mengin-Lecreulx (University of Orsay), E. Breukink (University of Utrecht), R. Brasseur (FUSAGx) and E. Goormaghtigh (ULB).



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PhD students: N. Dony, A. Piette & B. Wolf

Technical assistance: M. Schloesser

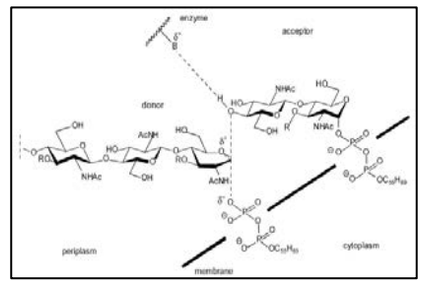
BACTERIAL CELL WALL: BIOSYNTHESIS

The last two steps in peptidoglycan biosynthesis are the assembly of the cell wall polymer from the monomeric intermediate, lipid II [129, 140]. They take place outside the plasma membrane and rely on the activity of the bifunctional (glycosyltransferases/transpeptidases) class A penicillin-binding proteins (PBPs). Peptidoglycan glycosyltransferases (GTs) of family 51 catalyze the polymerization of the lipid II precursor into uncross-linked peptidoglycan strands. This activity is essential to bacteria and represents a validated target for the development of new antibacterials.

Our objectives are:

- To understand the structure-function relationships and the catalytic mechanism of the GTs.

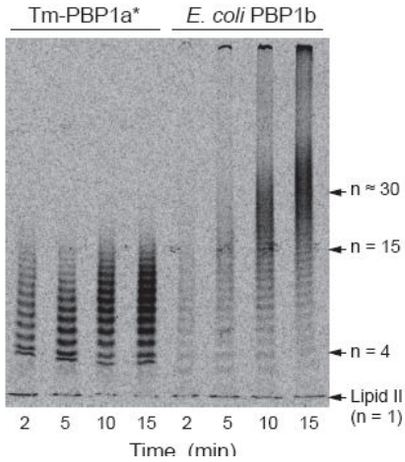
- To develop new antibacterial agents against the validated glycosyltransferase reaction.



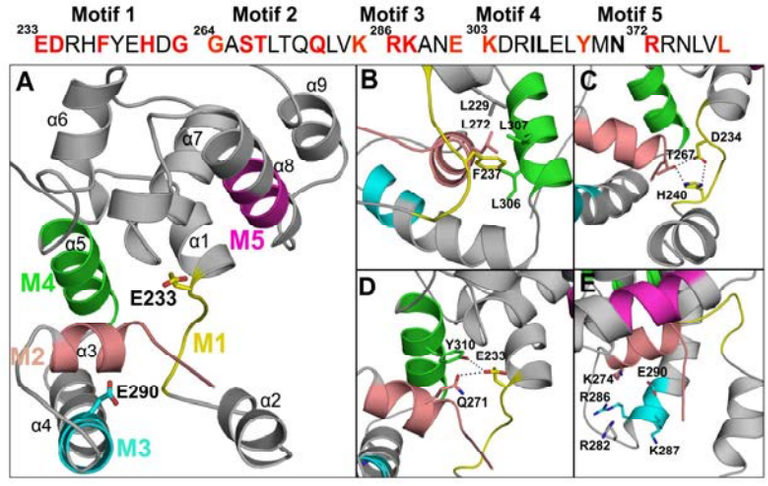
The class A PBP1b is our model enzyme for the study of the GT mechanism. We have determined its substrate specificity [40] and characterized all the residues of the conserved motifs [130]. Several GT proteins have been purified and characterized using lipid II: the two monofunctional GTs of *S. aureus*, the class A PBP4 of *Listeria monocytogenes* and the PBP1a of *Thermotoga maritima* [63, 65].

Analogs of lipid II have been synthesized (P. Herdewijn, KULeuven) and some of them were shown to inhibit the GT activity of PBP1B [18, 40].

Using structure-based virtual screening two small compounds have been discovered and found to inhibit the GT activity and growth of several pathogenic Gram-positive bacteria (in the frame of the Eur-Intafar Project). Their mechanism of action has been characterized.



Comparison of glycan chain size distribution of *T. maritima* PBP1a and *E. coli* PBP1b products



Close view into the active site of *E. coli* PBP1b GT domain

This work has been realized in collaboration with D. Mengin-Lecreulx (University of Orsay), E. Breukink (University of Utrecht), S. Gobec (University of Lubjana), T. Vernet (IBS), I. Chopra (University of Leeds), P. Herdewijn (KULeuven) and A. Zervosen (ULg).

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Technical assistance: M. Schloesser

BACTERIAL CELL WALL: REMODELLING

Peptidoglycan or murein is an essential and specific constituent of the bacterial cell wall. Its main function is to protect bacteria against their internal osmotic pressure and to maintain their shape. It also serves as a platform for the anchoring of specific proteins and other cell wall components such as teichoic acids in Gram positive bacteria. Peptidoglycan is a polymer formed by alternating residues of N-Acetyl glucosamine (GlcNAc) and N-Acetyl-Muramic acid (MurNAc). The carboxyl group of MurNAc is subtituted by peptides that are involved in the cross-linking of the glycan chains. During bacterial cell growth and division, the peptidoglycan mesh is constantly cleaved by a set of highly specific hydrolases that allow remodelling of the cell wall including the insertion of new glycan chains, control of cross-linking, cell separation, etc. The action of these peptidoglycan hydrolases results in cell wall turnover and release of peptidoglycan fragments at a rate of fifty percent by generation in *E. coli*.

Our main objective is to characterize the proteins involved in cell wall remodelling. In Gram-negative bacteria, the degraded fragments are transported into the cytoplasm, degraded and reused by the cell. AmpG is the key permease in this transport and we have contributed to the determination of its membrane topology [7].

We have identified three new proteins: two in *Escherichia coli* (YajG and YjbR) and one in *Bacillus subtilis* (YoaJ). YajG and YjbR are both lipoproteins. YjbR is an N-acetyl muramoyl amidase and is the fourth enzyme with the same activity in the *E. coli* periplasm; it has been renamed AmiD [171]. The function of YajG is still unknown and the determination of its three-dimensional structure by NMR has been initiated [69].

The crystal structure of *B. subtilis* YoaJ has been solved and is remarkably similar to that of plant beta-expansins (group 1 grass pollen allergens) and renamed EXLX1 according to expansin nomenclature [114]. EXLX1 binds to plant cell walls, cellulose, and peptidoglycan, but it lacks lytic activity against peptidoglycan and a variety of plant cell wall polysaccharides. Deletion of the gene encoding EXLX1 does not affect the growth or peptidoglycan composition of *B. subtilis* in liquid medium, but slows the lysis upon osmotic shock and greatly reduces the ability of the bacterium to colonize maize roots.

Superposition of the structures of EXLX1 (cyan and magenta) and the β-expansine EXPB1 (orange)

Finally, we collaborated with Dr J-P. Simorre and Dr W. Vollmer to analyze intact peptidoglycan sacculi by solid state NMR. The high-quality solid-state NMR spectra allow atom-resolved investigation of the peptidoglycan structure and dynamics as well as the study of protein-peptidoglycan interactions [115].

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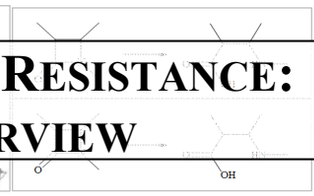
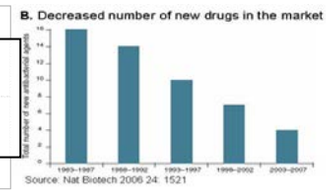
BACTERIAL RESISTANCE: OVERVIEW





Since their discovery, antibiotics - such as β-lactam compounds - have substantially reduced the threat posed by infectious diseases. The use of these "wonder drugs", combined with improvements in sanitation, housing, nutrition and the advent of worldwide immunization programmes, has led to a major drop in deaths from diseases that were previously widespread and frequently fatal. By helping to bring many serious infectious diseases under control, these drugs have also contributed to the major gains in life expectancy experienced during the later part of the last century.

These gains are now seriously jeopardized by another recent development: the emergence and spread of microbes that are resistant to cheap and efficient first-choice, or "first-line" drugs. The bacterial infections contributing to most human diseases are also those in which emerging and microbial resistance is most evident: diarrhoeal diseases, respiratory and urinary tract infections, meningitis, sexually transmitted infections, and hospital-acquired infections. Some important examples include vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus,* multi-resistant *Pseudomonas* and *Klebsiella*, and multi-resistant *Mycobacterium tuberculosis*. The consequences are severe. Infections caused by resistant bacteria fail to respond to treatment, resulting in prolonged illness and high risk of death. Treatment failures also lead to longer periods of infectivity, which increase the numbers of infected people moving in the community and thus expose the general population to the risk of infection by a resistant strain. Most alarming of all are bacteria which are developing resistance to virtually all currently available drugs, thus raising the spectrum of a post-antibiotic era.

Resistance to antimicrobials is a natural biological phenomenon that can be amplified or accelerated by a variety of factors, including human practices. The use of an antimicrobial for any infection, real or feared, in any dosage and over any time period, forces microbes to either adapt or die in a phenomenon known as "selective pressure". From 1930 to 1980, medicine and science were able to stay ahead of this natural phenomenon through the discovery of potent new classes of antimicrobials but this has now slowed to a virtual standstill, partly because of misplaced confidence that infectious diseases had been conquered. In just the past few decades, the development of resistant bacteria has been greatly accelerated by several trends including urbanization, pollution, environmental degradation, changing weather patterns, demographic changes, the resurgence of old foes such as malaria, and the growth of global trade and travel. Furthermore, veterinary prescription of antimicrobials also contributes to the problem of resistance. In North America, Asia and Europe, still in 2005, an estimated 50% in tonnage of all antimicrobial production was used in food-producing animals and poultry as regular supplements for prophylaxis or growth promotion. Such antibiotic uses, irrespective of the animals' health status, have been paralleled by an increase in resistance in bacteria (such as *Salmonella*) that can spread from animals, often through food, to cause infections in humans.

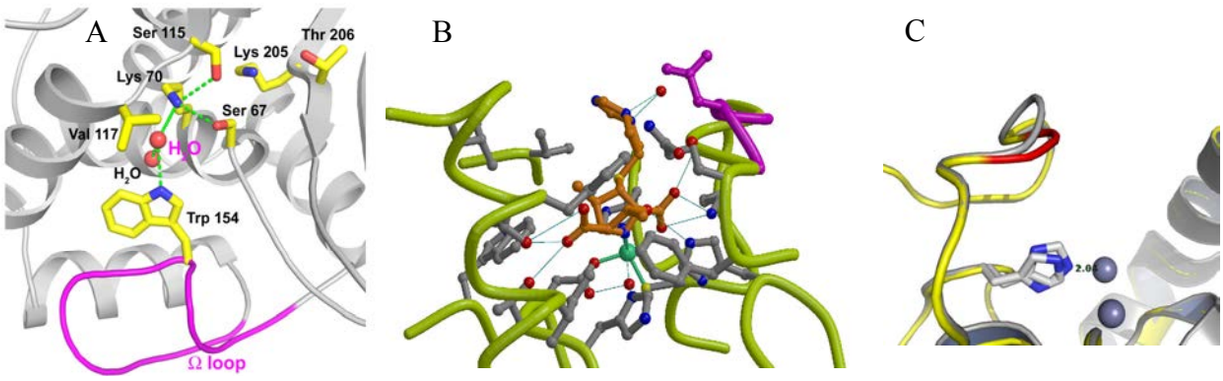
One of the major research topics of the CIP aims at deciphering the mechanism of action of β-lactamases and Penicillin-Binding Proteins within a multidisciplinary approach including molecular biology, protein chemistry, structural biology, organic chemistry and quantum chemistry. This approach should lead to the rational design of new and specific antibiotics.

BACTERIAL RESISTANCE: CHARACTERISATION OF BETA-LACTAMASES

β-lactams are among the most clinically important antimicrobials in both human and veterinary medicine. Bacterial resistance to beta-lactams has been increasingly observed in bacteria, including those of animal origin. The mechanisms of beta-lactam resistance include decreased accessibility of the target, target alterations and/or inactivation of the drugs by β-lactamases. The latter contributes predominantly to beta-lactam resistance in Gram-negative bacteria. A variety of beta-lactamases have been identified in bacteria derived from clinical isolates but also from environment samples, food-producing and companion animals and may further serve as a reservoir for beta-lactamase-producing bacteria in humans. The genes encoding these enzymes often coexist with other antimicrobial resistance determinants and can also be associated with transposons/integrons, increasing the potential selection of multidrug resistant bacteria by multiple antimicrobial agents as well as the dissemination of resistance determinants among bacterial species. In consequence, the antibiotic efficiency decreases due to global spread of drug-resistant bacteria.

β-lactamases are divided into four molecular classes A, B, C and D. Our researches which combine all the different aspects of protein chemistry and structural biology aim to gain insights in the catalytic mechanism of class B and D β-lactamases. Furthermore, we pursue our efforts to select new non β-lactam scaffolds (peptides, nanobodies, natural or synthetic products) as new sources of specific beta-lactamase inhibitors.

Among our major results, we have identified the structural features involved in the carboxylation of class D β-lactamases [**145**]. We have also determined the mechanism of inhibition of class D enzymes by chloride ions. Our work on class B β-lactamases (or metallo β-lactamases (MBL)) has led to the complete characterisation of the structure of the mono and di-zinc forms of CphA, a subclass B2 MBL [**14, 146**]. In addition, we have solved the first structure of a complex between an MBL and a carbapemen [**14**]. Furthermore, the specificity of CphA was modified from a strict carbapenemase to a broad spectrum activity [**78**].We have also selected new specific inhibitors against CphA [**4**]. Finally, we have studied a new MBL (IMP-22) which was transferred from an enviromental bacterium to nosocomial strains [**170**].



A) Class D OXA10 active site and carboxylated Lys70. B) The active site of CphA in complex with biapenem. C) Superposition of the mono- (in grey) and di-zinc (in yellow) forms of CphA

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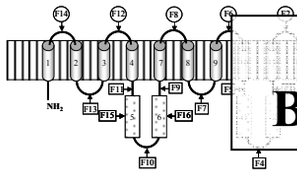
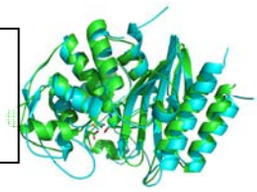


BACTERIAL RESISTANCE: BETA-LACTAMASE INDUCTION

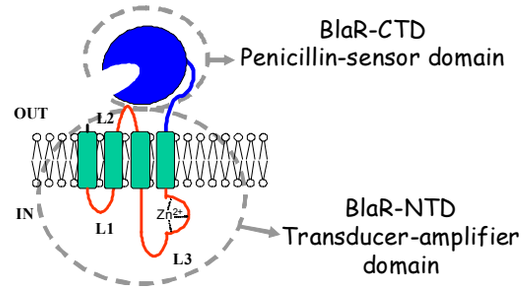


To survive and continuously adapt their cellular machinery to the external environment, living cells have acquired membrane proteins which allow communication between the outside and the inside of the cell. These proteins perform a range of different functions such as metabolite uptake and cell signalling. 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 (Philipon *et al*. Cell Mol Life Sci. (1998) 54: 341-6). We have studied those of *Citrobacter freundii*, *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.

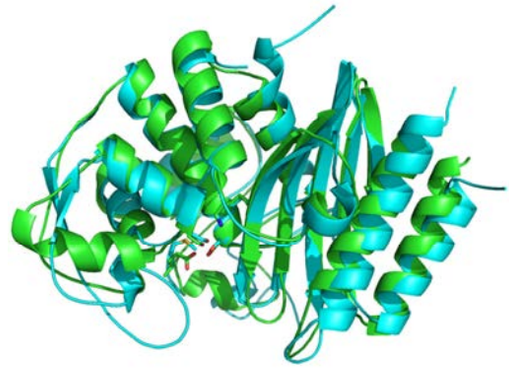
In *C. freundii*, the expression of the gene encoding the AmpC β-lactamase is controlled by the DNA-binding protein AmpR, which acts both as a repressor and a transcriptional activator of *ampC*. Two other genes, *ampD* and *ampG*, are also involved in the induction phenomenon. In the absence of the antibiotic, the cytoplasmic peptidoglycan precursor, the UDP-N-acetyl-muramic acid-pentapeptide (UDP-MurNAc-peptide) is bound to AmpR that acts as a repressor. In the presence of penicillin, the cell wall synthesis is impaired and the resulting penicillin stress allows the accumulation of the anhydro-MurNAc-pentapeptide in the cytoplasm where its concentration becomes sufficient to restore the activating properties of AmpR by displacing the UDP-MurNAc-peptide ligand. To elucidate the transport mechanism of the anhydro-MurNAc-peptide by AmpG and the mode of action of AmpR, we have overproduced and purified the membrane-bound AmpG protein and the AmpR C-terminal domain. The 3D-structure of this latter protein has been solved by X-ray diffraction.

Two gene products, BlaI and BlaR1, are known to affect the expression of the inducible BlaP β-lactamase in *B. licheniformis*. BlaI is a DNA-binding protein and BlaR1 is a membrane protein that plays the role of a penicillin receptor. We have studied: i) the stress generated by penicillin in *Bacillus* sp., ii) the mode of action of the penicillin receptor BlaR1 and iii) the interaction of the repressor BlaI with its DNA operator [70].



The *B. licheniformis* BlaR1 penicillin receptor topology
BlaR-NTD and –CTD are respectively: BlaR1 N-terminal and C-terminal domains

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.



Ribbon superposition of the structures of BlaB (2WUQ) in cyan and the beta-lactamase SME1 from *Serratia marcescens* (1DY6) in green.

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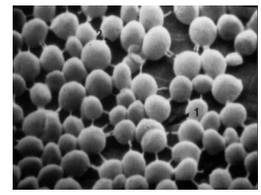
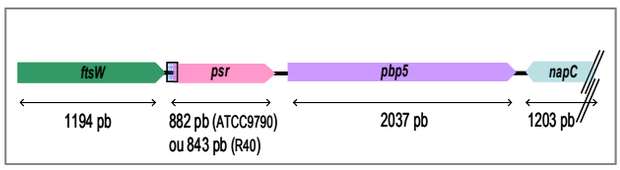
BACTERIAL RESISTANCE: LOW AFFINITY PBPS

Bacteria have evolved a variety of mechanisms to escape the action of antibiotics. Soon after their discovery as the target of β-lactams, the PBPs were identified as playing a key role in β-lactam resistance of some important Gram-positive pathogens such as *Staphylococcus aureus* and enterococci.



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 [48]. 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. Reexamination of the role of Psr by specific gene disruption led to the conclusion that it does neither influence 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 identify the mechanism regulating the expression and the overproduction of PBP5.

- To determine the role of Psr and elucidate how it contributes to the activity of PBP5 and of other proteins related to peptidoglycan metabolism.

- To identify the structural features responsible for the low-affinity of PBP5 towards penicillin.

- To use different approaches to find new inhibitors of PBP5.

Staphylococcus aureus is an opportunistic intracellular organism. Restricted to the hospital setting for many years, the methicillin-resistant *S. aureus* (MRSA) epidemic is now reaching an increasing variety of other environments, such as patients in the community in various parts of the world and animals. Beyond its spectacular ability to adapt and to develop resistance to most antimicrobial agents, including drugs of last resort, such as vancomycin, linezolid, and daptomycin, the capacity of *S. aureus* to invade, sojourn, and thrive intracellularly creates an additional challenge since intracellular forms tend to be poorly susceptible to most available antibiotics. Although they poorly accumulate in eukaryotic cells, β-lactams show activity against intracellular methicillin (methicillin)-susceptible *S. aureus* (MSSA) if the exposure times and the drug concentrations are sufficient. Intraphagocytic methicillin-resistant *S. aureus* (MRSA) strains are susceptible to penicillins and carbapenems because the acidic pH favors the acylation of PBP2a by these β-lactams through pH-induced conformational changes [165].

Our main objectives are:

- To identify the mechanism regulating the expression of PBP2a.

- To use different approaches to discover new PBP2a inhibitors.

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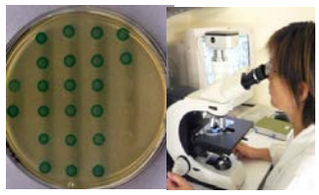
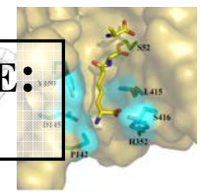
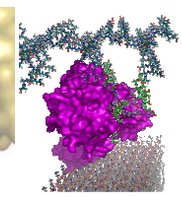


BACTERIAL RESISTANCE: PBP INHIBITION

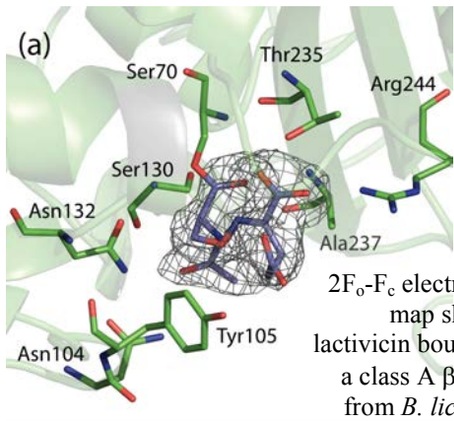




Bacteria exhibit a remarkable capacity to become resistant to commonly used antibacterial compounds. In our efforts to design new strategies to counteract them, we have focused our research on protein targets that are involved in the last stages of peptidoglycan biosynthesis, the DD-peptidases. In particular, our goal was to identify new non-beta-lactam antibacterial compounds, capable of inhibiting β-lactam resistant transpeptidases and active against pathogenic organisms such as streptococci, staphylococci, enterococci or chlamydiae. This work was conducted within the framework of the FP6 European Integrated Project EURINTAFAR (LSHM-CT-2004-512138), the Interuniversity Attraction Pole Project PROFUSA (IAP P6/19) and in collaboration with the groups of André Luxen from the University of Liège and Rex Pratt from the Wesleyan University.

Several enzymes were chosen as model targets for our search: 1) PBP2x, PBP1b and PBP2b from *Streptococcus pneumoniae* and the *Actinomadura* R39 DD-peptidase as penicillin-sensitive enzymes and 2) *S. pneumoniae* PBP2x, *S. aureus* PBP2a and *E. faecium* PBP5 as pencicillin-resistant enzymes. Combining the identification of compounds by computational analysis, the development of new organic chemistry synthetis methodology, the evaluation of MIC values against Gram-positive and Gram-negative species, the development of 96-well microtiter plate assays to test transpeptidase activity and the high resolution crystal structures of complexes between model enzymes and ligand hits, several non-β-lactam inhibitors of penicillin-resistant transpeptidases have been identified. As a snapshot of this work, some results obtained with two classes of inhibitors, the γ–lactam antibiotics and the peptidoglycan mimetics, are highlighted here.

The γ-lactam antibiotics

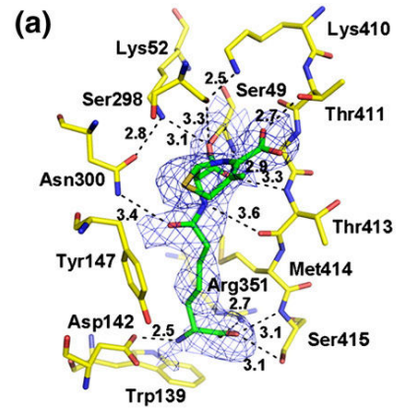
The γ-lactam antibiotics include lactivicin (LTV) and its analogs and were isolated from soil bacteria in Japan in the 1980s. They were identified as PBP and serine-active β-lactamase inhibitors. The kinetic studies with PBPs from both drug-sensitive and –resistant organisms, confirmed their effect and their interest as potential lead molecules for the development of novel inhibitors. Crystal structures of both PBPs and beta-lactamases complexed with γ-lactams revealed that the mechanism of action involves the formation of a covalent bond with the active site serine and that both cycloserine and lactone rings of LTV are opened [84].



$2F_o$-F_c electron density map showing the lactivicin bound to BS3, a class A β-lactamase from *B. licheniformis* (PDB ID 2X71)

The peptidoglycan mimetics

Fragments of peptidoglycan and series of *β*-lactams (penicillins and cephalosporins) containing peptidoglycan mimetic side chain have been synthesized. The kinetics of the inhibition of a panel of penicillin-binding proteins have been studied. The results obtained with the DD-peptidase from *A.* R39 are in complete agreement with the crystal structures of the complexes [45, 89, 125]. The R39 structures reveal the presence of a specific binding site for a D-α-aminopimelyl side chain, characteristic of the stem peptide of *A.* R39.



$2F_o$-F_c electron density map showing a 7-β (D-α-Aminopimelyl)-aminocephalosporanic Acid derivative bound to R39 DD-peptidase (PDB ID 2VGJ)

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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, solfatares or hot springs. Life at these extreme biological temperatures obviously requires a vast array of adaptations.

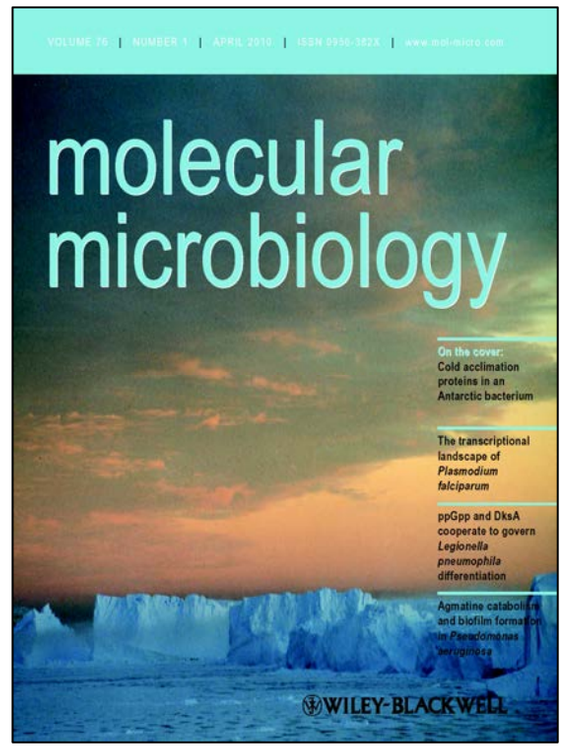
The Laboratory of Biochemistry focuses its research activities on the molecular adaptations displayed by extremophilic biomolecules, with special reference to proteins and enzymes. The scientific staff has produced pioneering contributions in the field of cold-adapted proteins. The three main research topics currently developed are summarized below [**121, 136, 137, 138, 142**].

Protein stability and enzyme activity at extreme biological temperatures

A series of homologous psychrophilic, mesophilic and thermophilic proteins are produced and characterized for analyzing 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 [**152**].

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 [**151, 162**].

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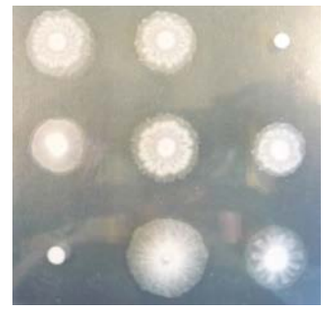
BIOFILM FORMATION BY *BACILLUS*

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



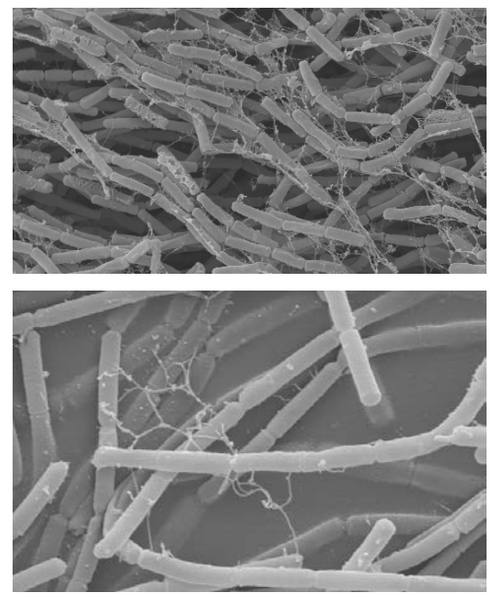
Swarming patterns of *B. subtilis* ATCC21332 or mutants on LB medium supplemented with 0.4% agar

Study of the *B. subtilis* GalM and PBP4a proteins in relation with the process of biofilm formation

B. subtilis PBP4a is a low molecular weight PBP (Penicillin-Binding Protein) belonging to the PBP-C1 family and possessing a DD-endopeptidase activity [156]. This class of PBP is present in almost all bacteria (except in *Listeria* and Gram positive cocci) and could be involved in the formation of biofilm in natural niches: in *B. subtilis*, the expression of *dacC* (encoding 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 galactose-mutarotase that we renamed GalM by analogy with its counterpart in *E. coli* [156]. The GalM enzyme is possibly involved in the recovery and modification of sugars present in the root exsudates.

The *yoxA-dacC* operon will be deleted in *B. subtilis* ATCC21332, 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 on tomato and cucumber roots will be 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 provide nutrients to starved bacteria.



Scanning Electron Microscopy of *B. subtilis* ATCC21332 taken off swarming plates

Superposition of GalM structures:
from *Lactococcus lactis*
from *B. subtilis*

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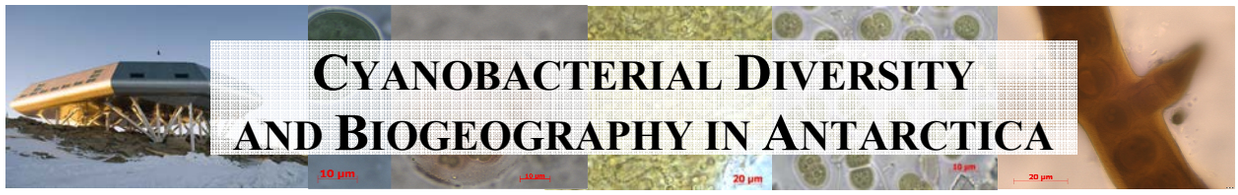
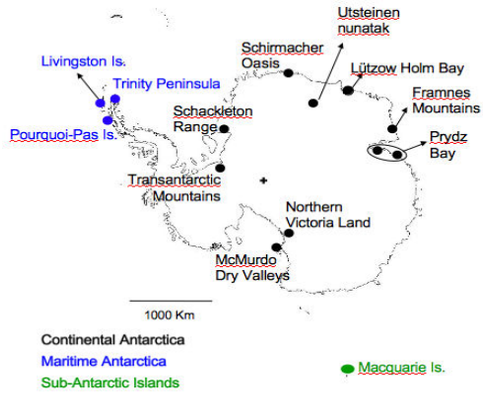


CYANOBACTERIAL DIVERSITY AND BIOGEOGRAPHY IN ANTARCTICA

Antarctica is a microbial continent and cyanobacteria are the major photosynthetic organisms in continental freshwater and terrestrial biotopes. However, their diversity is not well characterized. We use a polyphasic approach, combining the isolation and characterisation of strains and the direct study of environmental samples. The tools are based on classical morphological identifications and molecular taxonomic markers (16S rRNA gene, ITS spacer between the 16S and 23S rRNA genes, and house-keeping genes). We use the DGGE (Denaturating Gradient Gel Electrophoresis) and clone libraries.

In the **AMBIO** BelSPO project, we study the diversity and the biogeography of cyanobacteria in more than 90 samples coming from the three biogeographic regions (Sub-Antarctic islands, maritime Antarctica and continental Antarctica, see map). These samples come from water bodies with a range of limnological features. We seek to investigate the importance of geographical (isolation, obstacles to dissemination...) and ecological (local conditions) factors that govern the distribution of taxa along the eco-climatic gradient.



The question of geographic distribution of taxa is also at the center of the **BIPOLES** FNRS project, aiming to investigate the relationships between Arctic and Antarctic cyanobacteria in similar habitats.

In the **ANTAR-IMPACT** BelSPO project, we study the impact of the new Belgian research station, Princess Elisabeth. It was built in the Sør Rondane Mountains (Dronning Maud Land). This region is largely unexplored, especially from the biological point of view. The construction site is situated close to the Utsteinen Nunatak (71°57'S 23°20'E), on a small granite ridge. In this pristine area, exhaustive baseline data are needed to improve the knowledge of the human impact in the region, with the aid of a chemical and biological monitoring.

The aim of the BelSPO project **BELDIVA** is to realize an inventory of the cyanobacterial diversity in a radius of 50 km around the station. Open Top Chambers were installed to study climate change impacts on the phototrophic communities. OTCs are small plastic structures that simulate climate change effects. Small probes were installed in and out of the OTCs to follow the temperature and humidity variations that are induced. Strains are also isolated from environmental samples and added to the Belgian Culture Collections of polar Cyanobacteria (BCCM BelSPO-project).

In addition to living diversity, we study ancient DNA (aDNA) from cyanobacteria in the frame of the BelSPO **HOLANT** project. This project aims at determining how the climate of coastal Sub-Antarctic regions has varied during the Holocene and especially, how records from these coastal areas and inland locations are interrelated with respect to timing, duration and magnitude of climatic variations. Using lake sediment cores, we recorded the fossil cyanobacterial biodiversity changes during the last ca 4000 years, in 2 coastal Antarctic lakes, BK1 and BK2 in Beak Island, Antarctic Peninsula.

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CYANOBACTERIAL BLOOMS: TOXICITY AND DIVERSITY

Cyanobacterial blooms, mass developments of cyanobacteria floating at the surface of waterbodies, have become a recurrent and increasingly important phenomenon worldwide in freshwater lakes and ponds over the last decades. The formation of such blooms in surface waters is highly linked to human activities (eutrophication, climate changes). These nuisance blooms represent major potential hazards for animal and human health. They negatively impact water treatment systems, irrigation, fisheries and recreation lakes. Between 25 and 70% of the blooms are toxic. The most studied cyanotoxin is a hepatotoxic heptapeptide called microcystin.

Cyanobacterial blooms also plague surface waters in Belgium, particularly in summer and autumn. Eighty percent of the blooms analysed belong to taxa with the genetic potential to produce microcystins, and the presence of these toxins in the algal biomass has been shown in 40% of the bloom samples.

Our objectives:

In the framework of the BBLOOMS2 BelSPo project, we try to identify genetic markers for estimating the microcystin concentration in Belgian surface waters. In particular, we develop molecular tools to characterize the diversity of planktonic cyanobacteria and their potential toxicity.

SINGLE COLONY APPROACH

In collaboration with the group of Geoffrey A. Codd (University of Dundee, UK), we have tested the applicability of a combination of biochemical (ELISA) and genetic (PCR) typing on *Microcystis* and *Woronichinia* environmental colonies.

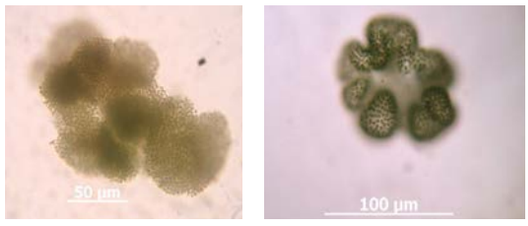
The DNA polymerase Phi29 allows the amplification of genomic DNA from single cells. In order to link the content of microcystin in 'single' colonies with genomic data, we used Phi29 to amplify whole genomes from a few cells of environmental colonies isolated under the binocular.

Our approach has shown the potential production of secondary metabolites (cyanopeptin, microginin) from biosynthesis operons present in the *Woronichinia* genome. Recently, it was shown that cyanopeptolins can be as toxic as some microcystins.

Since the toxigenicity and the cyanotoxin content of *Woronichinia* were not yet characterised in the literature, our approach represents a concrete answer to the lack of data on non-cultivable or difficult-to-isolate cyanobacteria.

BLOOM MONITORINGS BY MOLECULAR TOOLS

In order to characterize the cyanobacterial diversity, the dynamics of microcystin producing or non producing genotypes during blooms, we have monitored the presence of *mcy* genes in Brussels and Wallonia (2007-2009), and we have evaluated the copy numbers of 16S-23S rRNA intergenic spacers (ITS) during a *Microcystis* bloom in 2007 in the Westveld park pond (St Amandsberg). Moreover, we have analysed the 16S rRNA gene sequences in environmental samples (2007-2008) from Brussels and Wallonia.



Single colony of (A) *Microcystis novaceckii,* (B) *Woronichinia.*

Bloom of *Woronichinia* in lake Féronval (Eau d'Heure)

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HOUSE DUST MITE ALLERGY

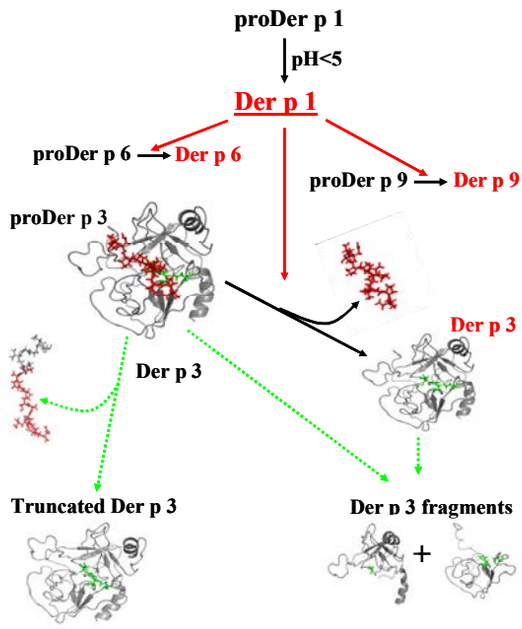
More than 80% of patients suffering from allergic asthma are positive to mite extracts and have large amounts of IgE specific for mite allergens. The proteolytic activity of the Der p 1, Der p 3, Der p 6 and Der p 9 allergens which are secreted by the house dust mite *Dermatophagoides pteronyssinus* contributes to their allergenicity. Indeed, after their inhalation, these enzymes can disrupt the human epithelial barrier increasing the permeability of the bronchial mucosa and can subsequently 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 consist 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, involving cleavage and release of the propeptide 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 to highlight new sensitive biomarkers and to progress in the development of high 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 [71]. The proteolytic neo-formed enzyme could then process proDer p 3 in its mature form Der p 3 [111]. 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) proteases zymogens as we demonstrated by using the FRET technique with fluorescent peptides probes. We are employing a large panel of techniques like expression in *Pichia pastoris*, ELISA, fluorescence, circular dichroism, enzymology, solid phase synthesis of peptides and mass spectrometry.

Currently, we are studying the reactivity of the allergens and more particularly the role of their propeptides in the allergic response.

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

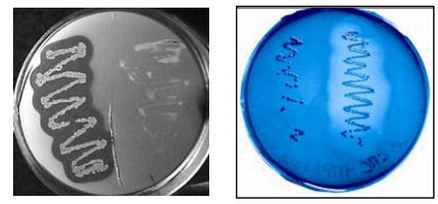
Metagenomics implies a series of laboratory manipulations leading to the isolation of DNA originating from (ideally) the entire diversity of microorganisms found in a specific sample (soil, water, tissues and so on), bypassing the necessity of dealing with culturing techniques. Moreover, despite the 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 disclose them, along with their wealth of resources.

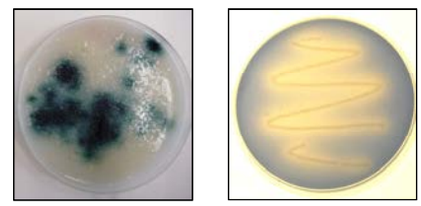
The metagenomic approach requires the cloning of DNA fragments directly extracted from samples; these 'metagenomic libraries' are then screened to isolate phenotypically (by activity-driven screening) or genotypically (by sequence-driven screening) specific clones of interest. Therefore, it offers a powerful tool for accessing a range of almost unlimited possibilities for screening new activities from many different environments, by the isolation and cloning of DNA from microorganisms that have the ability to synthesize useful compounds, degrade waste products or even provide evidence to elucidate biological processes.

Our objectives are:

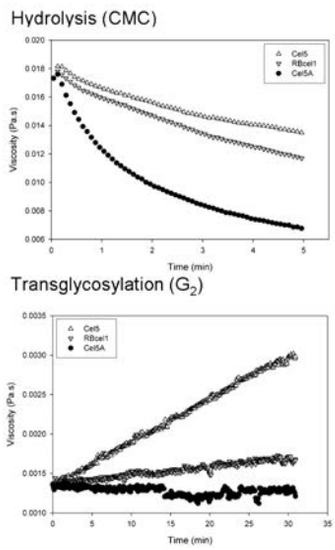
- To characterize extreme metagenomes (e.g. from Antarctica samples),
- To isolate new catalysts by performing activity driven screening,
- To elucidate the physiological function of metagenome derived enzymes,
- To test the possibility to transfer metagenomes derived enzymes in existing industrial processes,

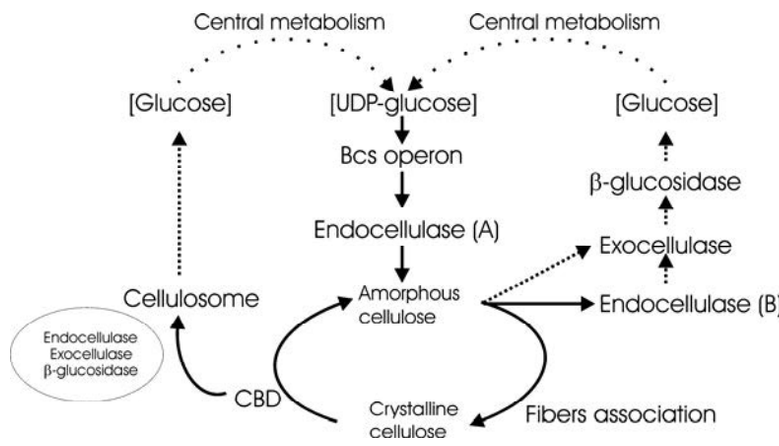
We are focused on carbohydrate modifying enzymes involved in both carbohydrate synthesis and degradation.





In addition, we are involved in the accurate characterisation of cellulases. 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 analysis and should be associated to cellulose production in bacterial biofilms [151].





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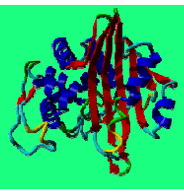
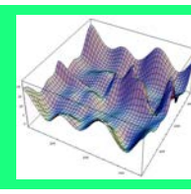
PhD students: M. Delsaute & D. Pipers



MOLECULAR MODELLING



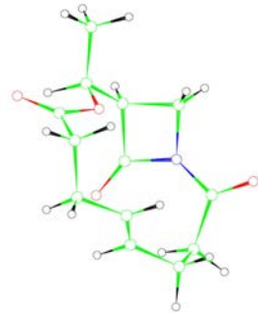
The objective is to understand, at the atomic level, the reactivity and properties of biological macromolecules and their interactions either with drugs or with other macromolecules by using the theoretical tools of quantum chemistry and molecular mechanics. Nowadays, these tools allow studying small molecules (100 atoms) at a high calculation level of quantum chemistry or very large systems (10.000 atoms) at lower calculation levels such as molecular mechanics or molecular dynamics.

Our quantum chemistry expertise includes conformational analysis and the localization of local minima and transition states. This approach also involves many aspects of the molecular properties such as reaction paths following topology of potential energy surfaces, complex formation, vibrational analysis, electronic transfer, excited electronic states study, Hartree-Fock instabilities and mixed QM/MM approach [**41**]. More specifically, for very large molecules, molecular mechanics and dynamics are efficient to study proteins and enzyme-ligand dockings as well as small DNA and RNA fragments.

At the quantum chemistry level, our application studies concern serine enzyme inhibitors and inclusion complexes with cyclodextrins:

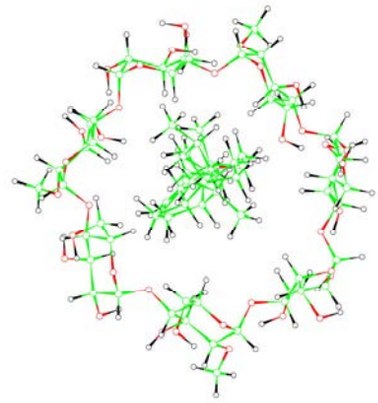
Serine enzyme inhibitors

Models of the acylation reaction have been proposed involving the minimal reactional entities which lead to the breaking of an amide/ester bond of a putative ligand. Along the reaction pathway, conformational deformations occur. Based on this feature, several bridged β-lactam derivatives have been synthetised in collaboration with the organic medicinal group of J. Marchand in Louvain-la-Neuve (UCL) [**174,175**].



Inclusion complexes with cyclodextrin

The inclusion complexes of miconazole have been studied in several cyclodextrins [**5**]. The influence of organic acids on the ternary inclusion complexes is analyzed in terms of interaction and complexation energies. A very good correlation has been derived between experimental and calculated IR spectra.



Molecular mechanics (MM) and molecular dynamics (DM) are applied to study several kinds of biological molecules: the amidase AmpD from *Citrobacter freundii*, the DD-transpeptidase of *Streptomyces* K15, a peroxidase from a *Bjerkandera* strain, the class A Penicillin-binding Protein 1b of *Escherichia coli*, the Crp transcription factor of *Streptomyces coelicolor*, the propeptide ProDer p 1 of *Dermatophagoides pteronyssinus* and dinucleotide negative ions (dAT, dT2) (collaboration with the ULg Mass Spectrometry Laboratory) [**22**].

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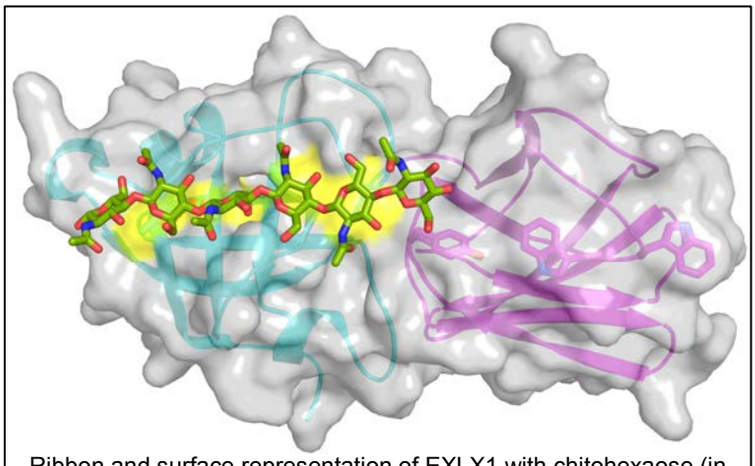
PROTEIN CRYSTALLOGRAPHY

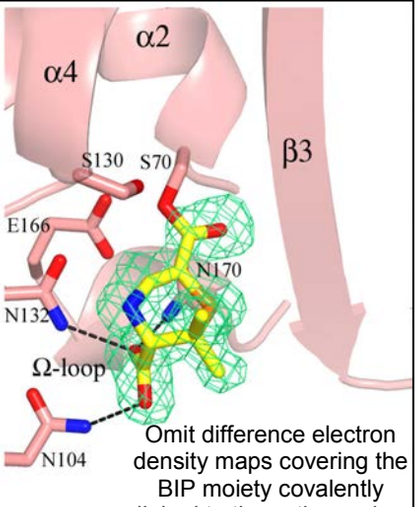
X-ray crystallography is one of the most used techniques for the determination of protein structures at the atomic level and is particularly adapted for the exploration of protein-protein and protein-ligand interactions and for understanding the catalytic mechanism of enzymes. All the steps needed for 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 units on the study of β-lactamases [21, 56, 103, 116, 121, 145, 173] and penicillin binding proteins [30, 45, 89, 125, 140, 173], as well as with external laboratories [29, 114, 141]. Two examples of our recent research are highlighted below.

EXLX1 structure

We have solved the crystal structure of a secreted protein, EXLX1, encoded by the *yoaJ* gene of *Bacillus subtilis*. Its structure is similar to that of plant β-expansins (group 1 grass pollen allergens), consisting of 2 tightly packed domains (D1, D2) with a potential polysaccharide-binding surface spanning the 2 domains. Domain D1 has a double-psi beta-barrel fold with partial conservation of the catalytic site found in family 45 glycosyl hydrolases and in the MltA family of lytic transglycosylases. Domain D2 has an Ig-like fold similar to group 2/3 grass pollen allergens, with structural features similar to a type A carbohydrate-binding domain. We have shown that EXLX1 binds to plant cell walls, cellulose, and peptidoglycan and promotes plant cell wall extension similarly to β-expansins. Deletion of the gene encoding EXLX1 slows cell lysis upon osmotic shock and greatly reduces the ability of the bacterium to colonize maize roots. The presence of EXLX1 homologs in a small but diverse set of plant pathogens further supports a role in plant-bacterial interactions [114].



Ribbon and surface representation of EXLX1 with chitohexaose (in sticks) from the superimposed MltA–chitohexahose structure.



Omit difference electron density maps covering the BIP moiety covalently linked to the active serine

Enzyme-inhibitor complexes

6-β-Iodopenicillanate (BIP) is a powerful, irreversible inhibitor of various β-lactamases and PBPs. Upon acylation of these enzymes, the inhibitor undergoes a structural rearrangement associated with the departure of the iodide and formation of a dihydrothiazine ring. Crystals of BS3 (class A β-lactamase of *Bacillus subtilis*) and of R39 (D,D-peptidase of *Actinomadura* R39) have been soaked with a solution of BIP and their structures solved at 1.65 and 2.2 Å, respectively. These structures provide direct proof of the unique rearrangement involved in inhibition of β-lactamases and PBPs by BIP.
The BS3 structure also reveals a new mode of carboxylate interaction with a class A β-lactamase active site that is of interest in future inhibitor design [173].

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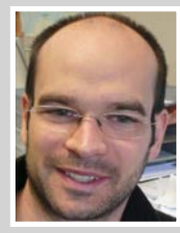
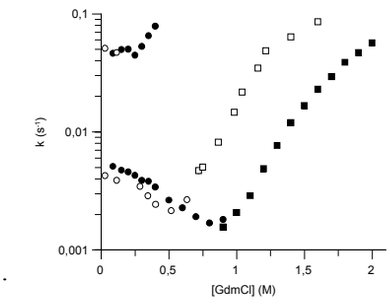


PROTEIN FOLDING *IN VITRO*

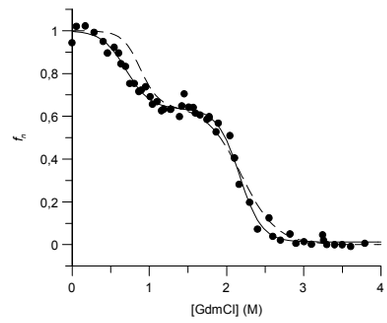
Although major insights into the mechanisms of folding of globular proteins have emerged since Anfinsen's seminal experiments in the 60's, the elucidation of the mechanism by which a disordered polypeptide chain rapidly acquires its complex folded conformation, typically on a second to minute time scale, both *in vitro* and *in vivo*, remains a major issue in modern structural biology. The goal of our research work is to contribute to a better understanding of the protein folding problem. Towards this aim, a few model proteins with essentially different folds have been selected for detailed investigation. These include active-site serine β-lactamases (α + β structure; M_r ~29000), Zn(II) metallo-β-lactamases (α + β structure; M_r ~25000), lysozymes (α + β structure; M_r ~14000-18000), single domain antibody fragments from camelid heavy chain antibodies (V_HH; all-β structure; M_r ~15000) and *Erwinia chrysanthemi* pectine methylesterase (mainly β structure, organized into a right-handed parallel β-helix; M_r = 36953).

Characterisation of the thermodynamic properties of a protein is a prerequisite to detailed kinetic studies. This has been achieved for all model proteins, using chemical agents, heat or extreme pH as denaturant (see e.g. [71, 116, 157]. In the case of the BcII Zn(II) β-lactamase, we showed that chemical unfolding of BcII can be described by a three-state equilibrium model, where a partially folded species is significantly populated. We also demonstrated that binding of the two catalytic zinc ions is a highly cooperative phenomenon, with less than 20% of mono-zinc enzyme populated at any value of the [Zn(II)]/[E] ratio [163].

In order to characterize early collapse and very rapid formation of non-native structures in β-lactamase folding, the conformational stability and kinetics of refolding and unfolding of the W290F mutant of the TEM-1 β-lactamase were determined [116]. The activity and spectroscopic properties of the mutant enzyme did not differ significantly from those of the wild type, indicating that the mutation has only a very limited effect on the structure of the protein. The stability of the folded protein was reduced, however, by 5-10 kJ·mol^{-1} relative to the molten globule intermediate (H), but the values of the folding rate constants were unchanged, suggesting that Trp-290 becomes organized in its native-like environment only after the rate-limiting step, i.e. the C-terminal region of the enzyme folds very late. In contrast to the significant increase in fluorescence intensity seen in the dead-time (3-4 ms) of refolding of the wild type protein, no corresponding change was observed with the mutant enzyme which allowed to specifically attibute the burst phase to the C-terminal Trp-290. This residue is suggested to be buried in a non-polar environment from which it has to escape during subsequent folding steps. With both proteins, fast early collapse leads to a folding intermediate in which the C-terminal region of the polypeptide chain is trapped in a non-native structure, consistent with a non-hierarchical folding process.



Apparent first-order rate constant values for unfolding (squares) and refolding (circles) of the wild type (filled symbols) and the W290F (open symbols) mutant β-lactamases



GdmCl-induced equilibrium unfolding transition of the wild type (dotted line) and W290F (continuous line) TEM-1 enzymes at pH 7, 25 °C, monitored by the change in ellipticity at 220 nm.

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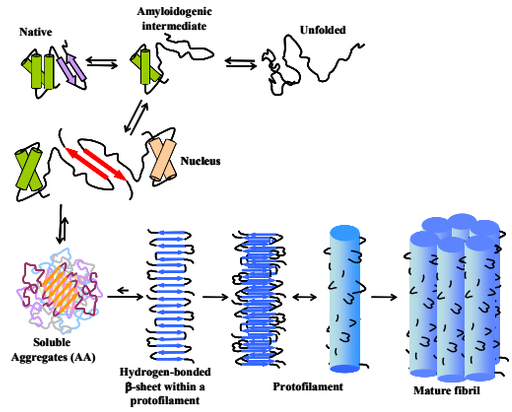
PROTEIN MISFOLDING AND AGGREGATION

The deposition of proteins in the form of amyloid fibrils in the nervous system or in certain peripheral tissues, is associated with more than forty degenerative diseases, also known as amyloidoses, including Alzheimer's disease and spongiform encephalopathies. Amyloidoses are often detected late in life and, given the ageing of the population, they inflict enormous psycho-sociological and economical burdens in western societies.

The process of amyloid fibril formation is complex and involves a series of species including amyloidogenic intermediates, soluble aggregates and mature fibrils.



Our objectives are to understand:

- The mechanisms leading to protein misfolding and aggregation by determining the structural characteristics at the atomic level of the various species formed during the process of fibril formation,

- The molecular mechanism by which some of the species are cytotoxic.

For this purpose, we are using a combination of complementary techniques including fluorescence, CD, mass spectrometry, NMR, H/D exchanges and electron microscopy. Our work is focssed mainly on two types of proteins: human lysozyme and chimeric proteins made of the β-lactamase BlaP and polyglutamine stretches of different lengths.

HUMAN LYSOZYME – This work is done in collaboration with the groups of C. M. Dobson (University of Cambrige, UK), A. Fontana and P. Polverino de Laureto (University of Padova, Italy), and S. Muyldermans, J. Steyaert and L. Wyns (VUB, Belgium) **[11, 107, 159]**.

Six variants of human lysozyme (I56T, F57I, W64R, D67H, F57I/T70N and W112R/T70N) are associated with a hereditary non-neuropathic systemic amyloidosis. We have compared the properties of the I56T and D67H variants to those of the wild-type lysozyme and the results obtained suggest that the loss of global structural cooperativity due to these mutations is at the origin of the amyloidogenicity [**11**]. Moreover, we have generated a series of 19 camelid antibody fragments specific for the native state of lysozyme and we are using them as structural probes to follow the structural characteristics of the different species occurring on the pathway of lysozyme amyloid formation. We have fully characterised the effects of binding three of these 19 V_HHs [**11, 107**, unpublished results] and have shown that they inhibit fibril formation by two different mechanisms.

CHIMERIC PROTEINS AS MODELS TO BETTER UNDERSTAND DISEASES ASSOCIATED WITH POLYQ EXPANSIONS (Work done in collaboration with P. Filée and M. Galleni)

Nine progressive neurodegenerative disorders, including Huntington's disease, are associated with anomalous expansion of a polyglutamine (polyQ) tract above a threshold size into nine different proteins. These proteins with expanded polyglutamine repeats have been found to form intranuclear amyloid-like aggregates and the formation of these aggregates could play an important role in the pathogenesis.

In order to get further insight into the mechanism of aggregation of polyQ proteins, we have inserted polyQ sequences into a permissive loop of a well studied protein, the β-lactamase BlaP from *B. licheniformis* 749/C [**92**]. The structural, thermodynamic and aggregating properties of chimeric proteins containing 23, 30, 55 and 79 glutamines have been investigated. The results obtained suggest that like for the nine proteins associated with the diseases, there is a threshold size of repeats above which the chimeric β-lactamase aggregates into amyloid-like fibrils. They also show a complex interplay between the effects of the length of the polyQ tract and the structural context conferred by the host protein on the aggregating properties of polyQ proteins.

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STREPTOMYCES GENETICS AND DEVELOPMENT

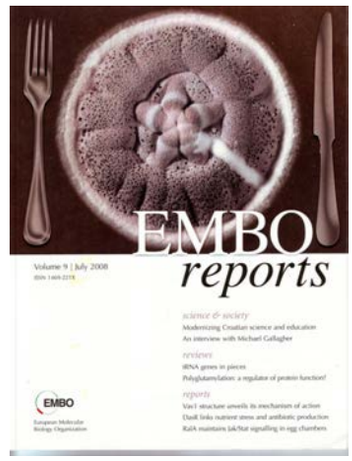
Streptomycetes are filamentous, sporulating, Gram-positive soil bacteria. As producers of some two-thirds of known antibiotics, anti-cancer agents, enzymatic inhibitors, or herbicides, streptomycetes are also considered as a paradigm of secondary metabolite-producing microorganisms. Analysis of *Streptomyces* 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. They are likely to be regulated in subtle ways that adapt the organisms to life in the soil, with its diverse physical, chemical and biological stresses. Therefore streptomycetes will continue to be major sources of important natural compounds, and, in such a promising context, huge efforts are devoted to understand the mechanisms that govern the production of secondary metabolites.

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. 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 aim of our project is to answer the following question:

"What are the mechanisms that regulate Streptomyces life cycle or monitor the environment nutrient status and what are the signalling cascades that lead to a decision to enter physiological differentiation?"

Our work has highlighted the first complete signalling cascade from nutrient sensors to antibiotic biosynthesis pathways in *S. coelicolor*. Each step of this cascade is controlled by the transcriptional repressor DasR and the N-acetylglucosamine which results from cell wall autolysis is now regarded as a "signal" molecule triggering secondary metabolism [**54, 123**]. Since DasR controls the expression of membrane sensors as well as antibiotic pathway-specific activators, it has to be regarded as an obligatory check point for the onset of secondary metabolism.

Rigali et al., (2006) Mol. Microbiol. 61(5):1237-51



Rigali et al., (2008) EMBO rep. 9(7): 670-5

Related web links for additional information:

www.nature.com/nrmicro/journal/v6/n8/full/nrmicro1963.html
www.ulg.ac.be/cms/c_188297/les-contes-de-la-crypte
www.ulg.ac.be/cms/c_151910/vers-la-decouverte-de-nouvelles-molecules-dinteret-therapeutique-ou-industriel

This work is carried out in close collaboration with the group of Prof. Gilles van Wezel (Leiden, NL).

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APPLIED PROJECTS

α-BUSTEC

ADJUVAC

ALLERVAC

APTARRAY

AUREA

CANTOL

COLD LACTASE TECHNOLOGY TRANSFER

GOCELL

GPCR-like

LACOMAT

LPSens

MED-ATR

Micro-H_2

OPARRAY

OPTIMISATION DE *B. cereus* EN TANT QU'USINE CELLULAIRE

PURIFICATION DES PROTEINES ANTIGENIQUES

RAIDGBS

RAPARRAY

SCALAB

SENSOTEM

TOLEDIAB

VACCINS

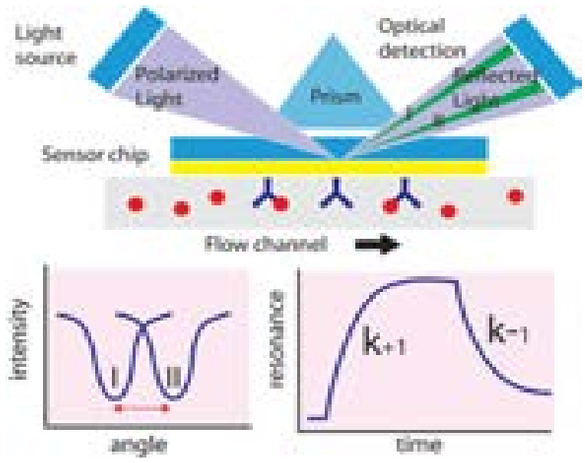
The projects mentioned in coloured boxes are described in the following pages.

House dust mite allergens have been shown to be causative factors of allergic manifestations such as atopic dermatitis, perennial rhinitis or bronchial asthma. More than 80% of patients suffering from allergic asthma are positive for mite extracts and have large amounts of IgE specific for mite allergens. In Europe, the most prevalent species of house dust mites are *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, depending on relative humidity and temperature.

ALLERVAC aims at developing an experimental vaccine against dust mite allergies with the required characteristics to test it in animals and then to adapt it to human. For this purpose, we want to select the best « immunosome» resulting from the association between an antigen and an adjuvant. Our objective is to determine the optimal adjuvant/antigen combination. The adjuvant is a cationic lipid supposed to orientate and enhance the immune response. The antigen is Derp1, a cysteine proteinase allergen contained in dust mite faeces.

The interactions between the lipid and the protein are measured by Surface Plasmon Resonance (SPR) phenomenon.

The selection of adjuvants is performed with the help of a «Biacore» apparatus detecting the SPR signal. Briefly, an analyte, here **ProDerp1**, an inactive form of **Derp1** is captured by a ligand (cationic liposomes) that has been previously immobilized on the sensor surface. The event of analyte capture by the ligand gives rise to a measurable signal by the device and allows the determination of the complexation **kinetic constants** (k_{on} and k_{off}). Those parameters will help to select the most appropriate liposomes for vaccination experiments in mice.



Partners :
Dr Vandenbranden M. (coordinator) - Structure et Fonction des Membranes Biologiques (SFMB) - ULB
Dr Jacquet A. - Laboratoire d'Allergologie Expérimentale - ULB
Dr Lensink M. - Bioinformatique des génomes et des réseaux (BiGRe) – ULB

Industrial collaborator:
GSK Biologicals

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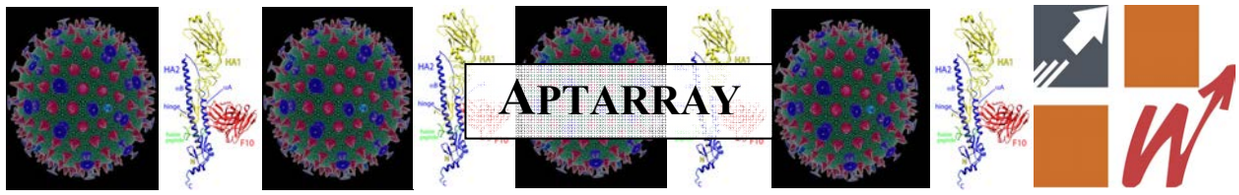
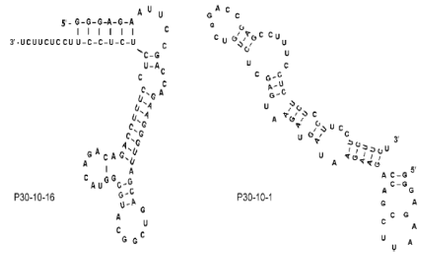
R. Barumandzadeh

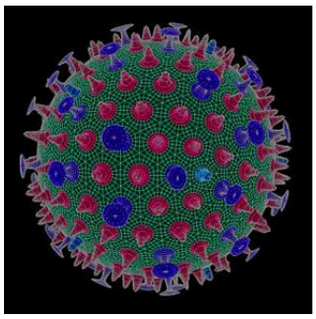


Development of RNA-aptamer biochips for identification and typing of animal viruses

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

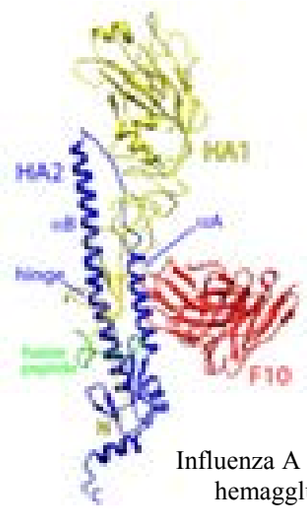




Influenza A Virus

This project consists in the identification of the RNA aptamers directed against the hemagglutinin of influenza A virus. These RNA aptamers will be attached on a microchip allowing the identification and typing of several viral strains in one single step.

The influenza A avian virus is considered as potentially able to generate highly pathogenic viruses. Such events have taken place at least five times over 15 years in: Hong-Kong in 1997 (H5N1), China in 1999 (H9N2), the Netherlands in 2003 (H7N7), Southeast Asia in 2004 (H5N1) and Mexico in 2010 (H1N1). At the present stage, the scientific community estimates that avian viruses H5 and H7 are most likely to yield very pathogenic human viruses. As far as the public health is concerned, the critical point for the management of a crisis is the precocity of the diagnosis of an infection by the influenza virus. However, the present procedure allowing reliable identification of the influenza virus requires several weeks. This project aims at designing an assay to detect and rapidly identify the virus.



Influenza A virus hemagglutinin

Scientific and technological goals:

- Bioinformatics identification of the very high conserved aminoacid sequences of H1, H2, H3, H5, H6, H7, and H9 hemagglutinin sub-types of the influenza A viruses.
- Production and purification of the conserved fragment of H1, H2, H3, H5, H6, H7, and H9 hemagglutinins.
- Selection of RNA aptamers with high affinity for the conserved fragments of H5, H7 and H9 hemagglutinins.
- Production of polyclonal antisera by rabbits and mice injected with the conserved fragments of H1, H3, H5, H6, H7 and H9 hemagglutinins.
- Characterisation of polyclonal antisera fixed on ELISA supports or spotted on microarrays.
- Starting a process of identification of monoclonal antibodies raised against conserved fragments of H3, H5, H6, H7 and H9.

To date and to our knowledge, our laboratories have the broadest collection of conserved fragments of influenza A virus hemagglutinins ever produced.

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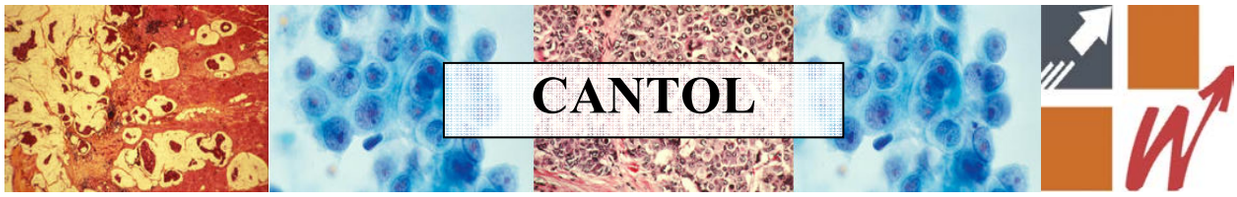
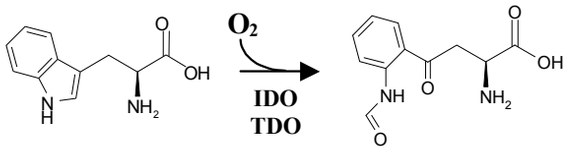
Collaborator: Dr D. Drescher



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) in tumors.

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 2,3 double bond in the indole moiety of L-tryptophan.

Recently, IDO has been found to play an important role in the process of immune evasion by tumors. IDO dependent T-cell suppression and tolerance induction by dendritic cells suggest that L-Trp catabolism has profound effects on T-cell proliferation and differentiation, which implicate the immunotherapeutic manipulations designed for patients with cancer and chronic infectious diseases.

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.

Inhibitors:

In collaboration with the partners, several series of innovating 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

- The Centre for Protein Engineering is responsible for producing and purifying the enzyme in adequate quantity and quality for the high throughput screening conducted by Euroscreen.
- Secondly, we are in charge of crystallization trials of the enzyme with and without inhibitors,
- Thirdly, the mechanism of inhibition is studied in details by kinetic measurements using different detection methods,
- Finally, the tryptophan 2,3-dioxygenase (TDO) an enzyme with identical biochemical activity to that of IDO is studied for its potential to interfere with tumor therapy by IDO blocking agents.

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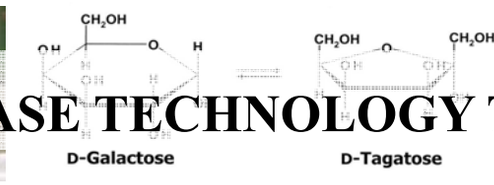
PhD student: S. Laurent



COLD LACTASE TECHNOLOGY TRANSFER

Sugar is one of the major factors causing various adult diseases, such as obesity, although nowadays these problems, due to bad nutrition habits and little physical activity start to occur more readily at ages below 14 years. 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 and healthy life. D-tagatose is a sweet-tasting, natural monosaccharide (keto-hexose) 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 maximum value of 1.5 kcal/g compared to 4 kcal/g for sucrose. D-tagatose has a very low glycemic index (GI ≤ 3). D-tagatose does not induce an increase in blood glucose or insulin levels which makes D-tagatose a desirable sugar substitute for type 2 diabetics[1]. Oral intake of D-tagatose in prebiotic food induces production of butyrate and stimulates the growth of beneficial bacteria in the human colon. Therefore it is believed to be useful in food or functional additive food to prevent colon cancer[2]. D-tagatose is only slowly converted to organic acids by tooth plaque bacteria and so, 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[3] and Nutrilab NV[4] are focused on the development, production and commercialisation of functional food ingredients and consumer products. 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.

The next challenge is the selection and development of the economically most appropriate industrial production process of D-tagatose. The production will start from lactose which is present in whey permeate. The enzymatic hydrolytic conversion of lactose will generate large quantities of galactose followed by enzymatic isomerization of galactose to tagatose. The optimal β-galactosidase (or lactase) for treatment of whey permeate is preferably active at a pH of 6-7 and a temperature of 4-25°C. Therefore, the use of cold active lactases from psychrophilic organisms are preferred for hydrolysis of lactose.

The data collected by the Laboratory of Biochemistry show that the β-galactosidase from *Pseudoalteromonas haloplanktis* (cold lactase) is quite efficient in hydrolyzing lactose at low temperatures[5]. In view of the potential interest in the food processing industry, the cold lactase and its applications were protected by a patent[6].

Nutrilab identified the cold lactase as a potential candidate for the development of an enzymatic D-tagatose production process. After a first meeting in May 2009, a cold lactase was provided to Nutrilab, produced by Lonza (Czech Republic) and tested by KaHoSL R&D (Ghent). Based on the results, Nutrilab concluded a "Know-How and Biological Material License Agreement" with the University of Liège (Interface). The following technology transfer resulted in the development of an industrial production process of the cold lactase and in its validation in an enzymatic production of D-tagatose. The collaboration with Nutrilab also involved data exchange on the regulatory aspects of the use of cold lactase in food.

In view of the successful technology transfer additional collaborations are being initiated between the CIP and Nutrilab.

Collaborators: G. Aerts (KaHoSL R&D), N. Antheunis (Interface, ULg), G. Schoofs (Damhert), I. Van de Voorde (KaHoSL R&D), C. Vastenavond (Nutrilab)

References:

[1] D-tagatose is known as an anti-hyperglycaemic agent that can be used to inhibit formation of advanced glycosylation in products in mammals, as described in U.S. Pat. Nos. 5,356,879 and 5,447,917 to Zehner, Lee R. (Brookeville, MD) Levin, Gilbert V. (Annapolis, MD), Saunders, James P. (Rockville, MD) ,Beadle, James R. (Elkridge, MD.) 1994

[2] Bornet *et al.* 1994. Rev Prat 44(8):1051–1055 ; [3] http://www.damhert.be/index.cfm; [4] http://www.nutrilab.be/index.php

[5] Hoyouxer al. 2001. Appl. Environ. Microbiol., **67**, 1529-153.

[6] *'Cold-active beta galactosidase, the process for its preparation and the use thereof'*. Patent number: WO01004276 Publication date: 2001/01/18. Inventor(s): François Jean-Marie, Baise Etienne, Hoyoux Anne, Dubois Philippe, Genicot Sabine, Gerday Charles, Jennes Isabelle.

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Associate researchers: Dr E. Baise, Dr A. Brans, Dr M. Galleni

The Hybrid Protein technology is a highly convenient and functional tool to display polypeptide(s) or protein domain(s) within the structure of a carrier protein allowing the exogenous polypeptide to be refolded as a biologically active molecule. As a result, Hybrid Protein technology should be considered as a very useful tool for the study of protein domains. The core of the Hybrid Protein technology is based on the use of:

A. A class A β-lactamase with a unique insertion site as a protein scaffold

The insertion site is a highly permissive loop situated far away from the active site. This ensures solvent accessibility for the exogenous polypeptide and limits steric hindrance of the β-lactamase catalytic site. Since this technology involves an actual insertion and not an end to end fusion, the folding of both the exogenous polypeptide or protein and the enzyme scaffold are strongly interdependent. Consequently, the β-lactamase activity is conditioned by the correct folding of the exogenous polypeptide of interest.

The use of a β-lactamase as carrier has been validated by multiple research projects related to final industrial products. The research projects financed by the Walloon Region are **VACCINS**, **SENSOTEM** and **GOCELL**.

This technology has been used for the development of immunoassays and vaccines [**27**], for epitope mapping [**72**] and production of protein domains [**92**].

Moreover, hybrid proteins can be expressed at the surface of the phage fdTet in fusion with the minor coat protein PIII and screened for their affinities towards specific targets [**72**]. Since the hydrolysis of β-lactams generates protons, the β-lactamase is also useful for the development of potentiometric biosensors detecting signal transduction.

Bifunctional hybrid β-lactamases can be used either in drug screening, drug targeting, vaccine development, or production and selection of hybridoma.

B. An Inactivated Carrier Hemolysin Alpha as a protein scaffold with multiple insertion sites

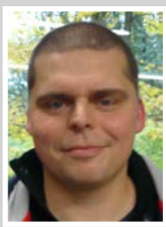
A modified form of the staphylococcal alpha-hemolysin (HA) termed ICHA for Inactivated Carrier Hemolysin Alpha was obtained by introducing numerous restriction sites in the coding sequence. The aims were to suppress the haemolytic activity of this protein and to create a permissive insertion site of large heterologous polypeptides without interfering with the other biological properties of the carrier.

This recombinant protein is still able to interact with bilipidic layers and to form oligomers. The restriction sites introduced in the ICHA encoding gene allow to display at least one heterologous polypeptide or protein fragment either in N- or C-terminal fusion with the carrier protein or into ICHA at the surface of the recombinant protein.

The use of ICHA as a carrier protein has been validated by the development of multivalent recombinant vaccines including several antigens in a same vaccine formulation. The research projects supported by the Walloon Region are **AUREA** and **GPCR-Like**.

For example, with this technology we have developed a multivalent vaccine against several *S. aureus* virulence factors. Presently, the administration of ICHA polytopes to mice results in a significant protection against *S. aureus*.

In conclusion, hybrid protein technology is a new tool with multiple applications.



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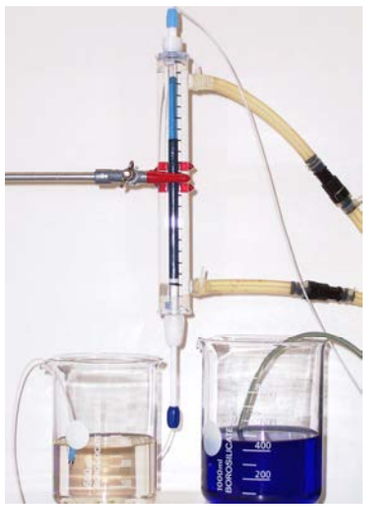
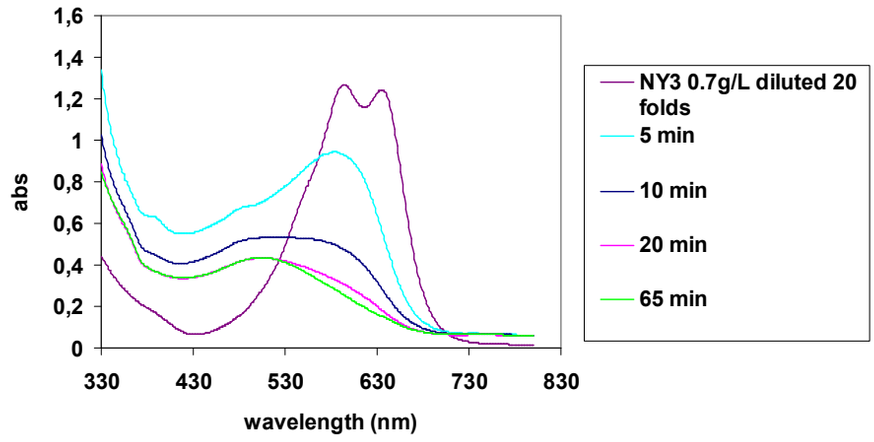
The Centre for Protein Engineering has participated to the Walloon Region LACOMAT project led by the Catholic University of Louvain (Microbiology Unit, Dr. S. Vanhulle).
This project aims at developing a new biocatalytic material for the treatment of industrial effluents containing chemically resistant and toxic dyes. This research focuses on: i) the selection of microorganisms (White-Rot Fungi) competent in environmental clean-up bioprocesses, ii) the production and characterisation of their enzymes and iii) the design of a granular material with immobilized enzymes as well as a process to perform wastewater treatments.

White-rot basidiomycetes are characterized by their ability to degrade complex and recalcitrant plant biopolymers such as lignin, hemicelluloses and cellulose. Beyond the ecological role of these enzymes in recycling the residual biomass, they are also widely studied because of their potential use in several applied areas such as textile, paper, and food industries. Notably, ligninolytic enzymes of white-rot fungi reveal unique biodegradation capabilities in the textile industry where they are used to degrade the excess of unfixed dyes that render industrial effluents highly coloured and toxic.

Strains belonging to the basidiomycetes genus *Pycnoporus* were known to produce laccases as the only enzyme responsible for lignin degradation since no other components of the ligninolytic system have ever been identified previously such as lignin peroxidases, manganese peroxidases, versatile peroxidases or glyoxal oxidases. *Pycnoporus sanguineus* MUCL 41582 was selected for its remarkable efficiency in decolourizing dyes. In order to understand its particular capability, we attempted to identify and characterize the responsible enzymes involved in this process. The laccase enzyme has been purified and characterized. Moreover in addition to the expected laccase activity, enzymatic assays on culture supernatants and on purified enzymes have shown the presence of manganese peroxidase and glyoxal oxidase activities, therefore highlighting for the first time a complete ligninolytic arsenal in *P. sanguineus*.





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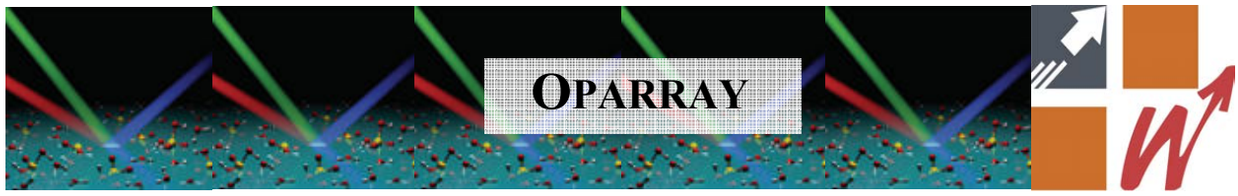
Technical assistance: A.-M. Matton



Protein microarrays are based on the technology of coupling molecules (e.g. proteins including antibodies and antigens) onto a support in an array format for multiplexed analysis. Detection of captured molecules is usually accomplished by proteins labelled with a fluorescent probe. Imaging in these cases is accomplished with a CCD camera or a laser scanner.

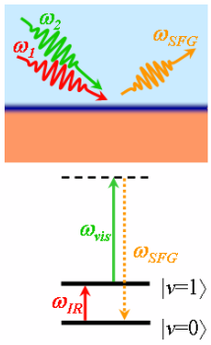
During the OPARRAY project, three laboratories have joined their expertises:

- the CIP,

- the Laboratory of Medecinal and Organic Chemistry (UCL, Louvain-la-Neuve, Belgium),

- the Laboratory "Lasers et Spectroscopies" (FUNDP, Namur, Belgium).

These groups have collaborated in the development of a new and original strategy to capture proteins in an oriented fashion and of a new method of imaging them based on Sum Frequency Generation (SFG) spectroscopy. SFG is a nonlinear optical technique used to study the details of molecular structure and dynamics at surfaces and interfaces. The extremely high surface selectivity of the SFG process allows researchers to focus on the thin layer of a few atoms or molecules near the interface with no interferences from the bulk media on either side.



In a second-order vibrational sum frequency experiment an infrared photon and a visible photon interact with the molecules on the interface, resulting in a background free sum frequency signal in the visible region.

During this program, we have: i) demonstrated the chemisorption of 6-[(R,S)-5-(1,2-dithiolan-3-yl) pentanoyl-amino]-penicillanic acid (DTPA), a β-lactam antibiotic derivative, as self-assembled monolayers on gold surface without any alteration of the penicillin pharmacophore [75] and ii) documented its reactivity towards a penicillin-binding protein monolayer. The integrity of the penicillin pharmacophore upon adsorption and its sensitivity to the BlaR-CTD enzyme were highlighted by PM-IRRAS measurements. This work is a first step towards the development of novel biosensors and well-ordered protein arrays, both based on the high affinity of penicillin for particular proteins.

SFG spectroscopy equipment
from the LLS laboratory

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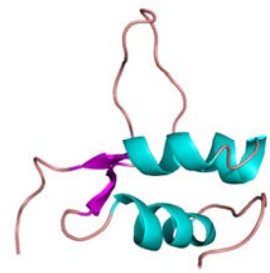
Technical assistance: S. Grubisic & E. Goukens

TOLEDIAB

TOLEDIAB is the acronym of a research project entitled 'Développement d'un vaccin tolérogène/régulateur contre le diabète auto-immun de Type 1' aiming at the development of a tolerogenic vaccine against Type 1 diabetes. It is a WALEO 3 project funded by « la Direction générale des Technologies, de la Recherche et de l'Énergie » attributed to Prof. V. Geenen (Centre d'Immunologie de l'ULg, CIL). In addition to Prof. M. Galleni (Macromolécules Biologiques, CIP), the other partners are Prof. E. De Pauw (Centre d'Analyse des Résidus en Traces de l'ULg ; CART), Dr. A. Jacquet (Service de Génétique Appliquée, SGA ; ULB) and Prof. M. Goldman, Director of the « Institut d'Immunologie Médicale (IMI) » of ULB. The project is supported by GlaxoSmithKline (GSK) Biologicals of Rixensart. This 4-year project was officially launched in January 2006. At the end of 2009, in view of the promising results, it has been renewed for an additional year.

For a proper reaction against non-self infectious agents, the immune system must be able to distinguish, through an educational process, between the host molecular structure (self) and material that originates from outside (nonself). This critical process of recognition and the immunological tolerance to self antigens take place in the thymus where stromal cells allow for the selection of a functional and self-tolerant T-cell repertoire. It is one of the primary functions of the thymus to prevent autoimmunity through the induction of immunological tolerance to self antigens.

In type 1 diabetes (T1D), a defect of thymus in self-tolerance programming of pancreatic insulin-secreting islet β cells has been identified as the origin of the development of an autoimmune diabetogenic response. This results in a highly selective autoimmune response that ends with the death of insulin-secreting islet β cells in the pancreas. T1D autoantibodies target three major autoantigens identified as (pro)insulin, the 65-kDa isoform of glutamic acid decarboxylase (GAD65), and the tyrosine phosphatase IA-2.



Insulin-like growth factor 2 (IGF-2) is the dominant member of the insulin family expressed during fetal life by the thymic epithelium. The very low level of insulin gene transcription in normal thymus can be correlated to the poor tolerogenicity of the corresponding protein and to the failure of all clinical trials that have attempted to restore immune tolerance to islet β cells via various methods of insulin administration. Based on the high structural homology and crosstolerance between insulin and IGF-2, the development of a novel type of vaccination, so-called 'negative/tolerogenic self-vaccination', for the prevention and cure of T1D is investigated in this project. If this approach proves to be effective in re-programming T1D immunological tolerance, it could pave the way for the design of new self-vaccines against other autoimmune diseases. Using the same rationale, GAD67 and tyrosine phosphatase IA-2 variant respectively members of the GAD65 and the tyrosine phosphatase IA-2 families that are, similarly to IGF-2, expressed in the thymus, appear as pertinent additional candidates for the development of a 'negative/tolerogenic' vaccine against T1D.

The main CIP contribution to the project is the design, cloning, expression, production and purification of the selected protein candidates in a formulation compatible with animal vaccination. Several protein variants based on the human IGF-2 have been designed and cloned for expression in *E. coli*, *Pichia pastoris* and *Arabidopsis thaliana*. Production and purification protocols have been developed as well as several LPS removal methods. GAD67 has been cloned in *E. coli* and a production protocol is being finalised. These proteins have been supplied to the TOLEDIAB partners throughout the project for T1D animal model and *in vitro* testing.

This work was done in collaboration with Dr Pierre Tocquin (ULg).

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Associate researcher: Dr E. Baise

PhD student: J.S. Sohier

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 specific disadvantages in terms of protein yield. Difficulties in handling, the time required from gene modification and transformation to protein purification, the cost of production and scaling-up, the risk of incorrect folding or instability of the recombinant protein, the inherent bacterial inability to introduce post-translational modifications… rank among the drawbacks impeding the development in 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 very 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 most traditional system 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 therapeutic 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. Besides the purification process, the following steps include 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 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 goal is the discovery of very efficient untranslated sequences as well as promoters and regulatory regions to flank the coding sequences we are interested in.

Micro-algae such as these *Dunaliella species* are considered as promising eukaryotes for growth in bioreactors and mass-production of therapeutic proteins.

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- **Protein production et purification facilities:**

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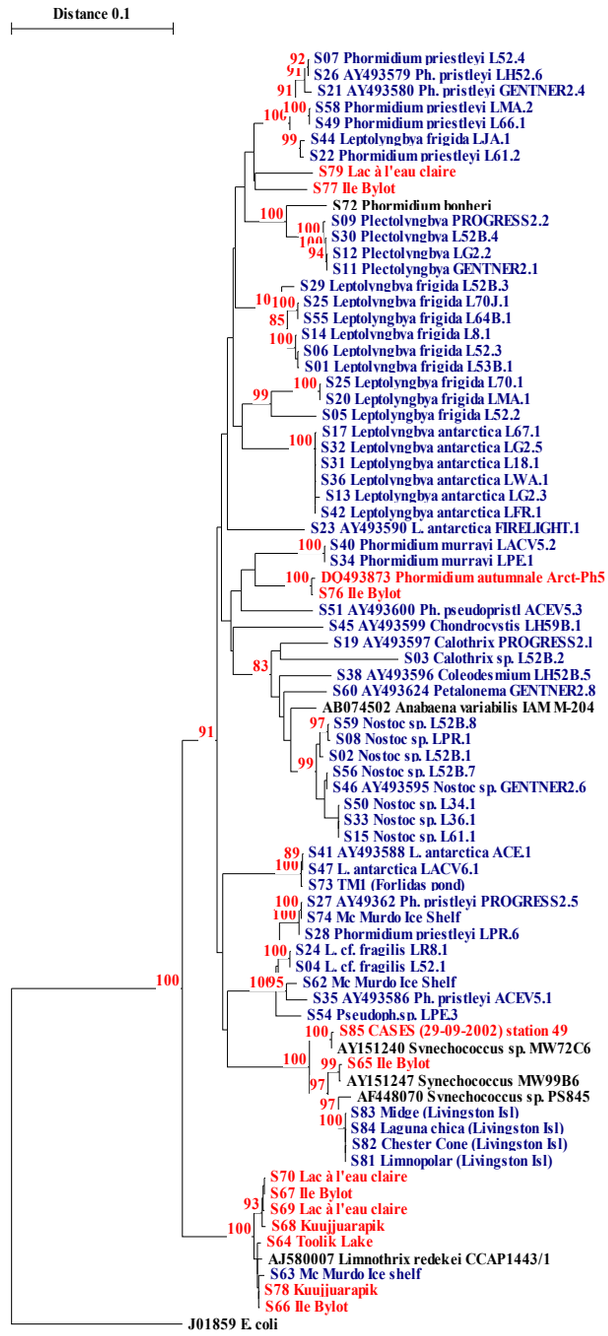
CULTURE COLLECTION OF CYANOBACTERIA

Since 2005, the BCCM (Belgian Co-ordinated Collections of Microorganisms) has suppported the elaboration of a collection of polar cyanobacteria.

125 unicyanobacterial strains coming from different Antarctic (South Victoria Land, East Antarctica, Transantarctic Mountains, James Ross Island), Arctic regions (North Canada, Arctic Ocean, Alaska) as well as different biotopes (microbial mats, lakes, ice shelves, dry valleys, hypolithic and endolithic habitats). Twelve strains originate from Siberian lakes in the sub-Arctic aera. The three main orders of cyanobacteria (Chroococcales, Oscillatoriales, Nostocales) are represented.

Tests for viability, purity, and authenticity include the sequencing of the 16S rRNA gene and the spacer between the 16S and 23S (ITS) rRNA genes, as well as genomic fingerprints obtained with variants of the HIP repeats, RFLP of rpoC1 and recA gene fragments. The HIP repeats (Highly Iterated Palindrome) (5'- GCGATCGC - 3') are overrepresented in cyanobacterial genomes and were used to differentiate related strains (Robinson *et al.*, Nucl. Acids. Res. 1995; Smith *et al.*, Microbiol. 1998). We tested several HIP variants with two additional bases at the 3' end, including a degeneracy, to increase the number of bands to be compared. The PCR conditions were also optimized. However, the visualisation of the band patterns remains problematic.

To ensure strain maintenance, usually carried out by regular transfers to fresh culture medium, cryopreservation and lyophilisation tests were also performed (maximum 1 year). The latter did not succeed. Cryopreservation is now carried out at –70 °C and in liquid nitrogen, with and without cryprotectant (DMSO). Methanol was tested but the success rates were lower than with the two other protocols.

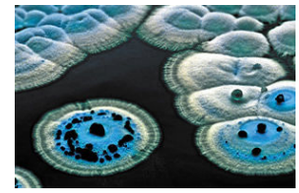


Neighbor-joining tree based on 1061 16S rRNA positions, with a Jukes-Cantor correction for multiple mutations. Indels were not taken into account. Bootstrap values higher than 80 % are indicated besides the nodes. Blue sequences are from Antarctic strains and the Arctic ones are in red.

PROTEIN PRODUCTION AND PURIFICATION FACILITIES

An efficient research requires access to a broad range of technologies, some of which require expertise and specific equipments. The "Protein Production and Purification Platform" is opened 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 fungi strains, followed by their purification.

The platform can provide many services including:



- 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*

- Analytical scale or pilot-scale recombinant protein production from these organisms

- Cell harvesting or supernatant cleaning using continuous centrifugation or hollow fiber filtration

- Cell disintegration to recover proteins produced in the intracellular compartment

- Protein purification at the analytical and pilot scale

To perform these tasks 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 on 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.

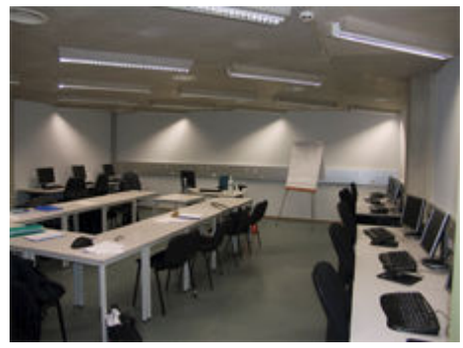
The Protein Production and Purification Platform has provided proteins for : kinetic studies, protein structure determination by NMR or crystallography, protein-protein or protein-ligand interaction studies, secondary metabolite productions, enzyme-inhibitor studies, amyloid fibril formation and protein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

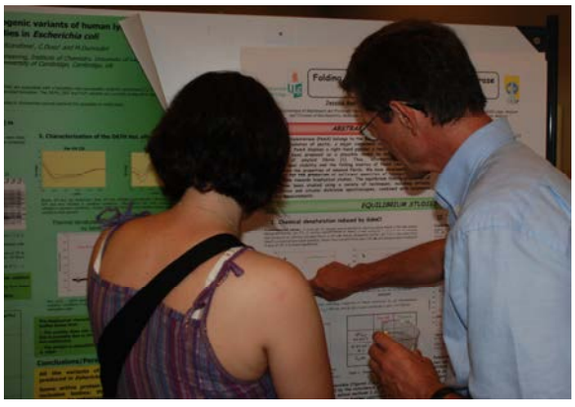
Techniques for protein production and purification

Since 2006, the CIP has been working 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 in *Escherichia coli* and *Bacillus subtilis*.
- Protein production in flasks and in 20 liters fermentors (batch and fed-batch cultures) with *E. coli, B. subtilis* and *Pichia pastoris*.
- Cell harvesting and cell disruption.
- Protein purification by various technologies including: ion exchange, molecular sieve, hydrophobic and affinity chromatography.
- Protein identification by SDS-PAGE, enzymatic testing and Western blotting.





AWARDS

2005

Marylène Vandevenne, Prix Walter Verly

Jean-Denis Docquier, ICAAC Young Investigator Award

2006

Nadia Ruth, Région wallonne poster award, 10th Bioforum, Liège, Belgium

Nadia Ruth, Originality prize at the poster session of the 7th workshop on Biosensors and Bioanalytical µ-Techniques in Environmental and Clinical analysis, Kusadasi, Turkey

Jean-Denis Docquier, Agathon de Potter award from the Belgium Royal Academy of Sciences of Belgium

Mireille Dumoulin, Agathon de Potter award from the Belgium Royal Academy of Sciences of Belgium

Rafael Fernandez Carazo, Original Research poster award from Eurogentec, 10th Bioforum, Liège, Belgium

2007

Andy Chevigné, Puratos-Beldem award for protein engineering and bioprocess development, 11th Bioforum, Liège, Belgium

Nicolas Morin, Student prize, 12th Annual Meeting of the French Association for Cytometry - Clermont-Ferrand, France

Séverine Hubert, ASM Student Grant, 47th ICCAC, Chicago, USA

Xavier Henry, ASM Student Grant, 47th ICCAC, Chicago, USA

2008

Renaud Berlemont, ASM Student Grant Award, 108th ASM General Meeting, Boston, USA

Renaud Berlemont, BSM Award for Excellent Poster, Annual Scientific Meeting "Stress response in the microbial world", Brussels, Belgium

2009

Julie Gielis, ASM Student Grant, 49th ICCAC, San Fransisco, USA

INVITED SPEAKERS

2005

Dr Kerensa Broersen, Wageningen Centre for Food Sciences, The Netherlands, "Implications of protein glycosylation on kinetic partitioning", February 3

Dr Carolien Dirix, Department of Chemistry, KUL, Belgium, "High pressure as a tool to study/induce unfolding and aggregation phenomena", February 11

Dr Rolf Daniel, Institut für Mikrobiologie und Genetik, University of Göttingen, Germany, "Metagenomics: a way to explore and to exploit the unknown", March 2

Dr Sami Yunus, Physico-Chimie et Physique des Matériaux, UCL, Belgique, "La synthèse de transduction et d'acquisition multiplexé du signal pour la mise au point d'un biosenseur conductimétrique ou potentiométrique basé sur la polyaniline", June 17

Dr Filip Meersman, Department of Chemistry, Katholieke Universiteit Leuven, Belgium, "Is the amyloid fibril the global minimum in free energy?", November 25

Dr Stéphane Stenuite, Unité de Recherche en Biologie des Organismes, FUNDP Namur, Belgique, "Etude du picoplancton d'un grand lac africain", December 2

Dr Marc Vanhove, Dyax, Belgium, "Generation of fully human antibodies derived from F_{ab}-on-phage display libraries", December 7

Dr Allel Chedad, Interdisciplinary Research Center, Katholieke Universiteit Leuven Campus Kortrijk, Belgium, "Influence of Trp mutation on native, intermediate and transition states of goat α-lactalbumin: an equilibrium and kinetic study", December 8

2006

Dr Jean-François Collet, de Duve Institute Université Catholique de Louvain, Belgium, "La thiorédoxine 2 d'*Escherichia coli*, un exemple unique de thiorédoxine à zinc", February 17

Dr Catherine Michaux, Department of Chemistry, FUNDP, Belgium, 'Une nouvelle méthode de renaturation de protéines", May 5

Dr John Barlow, Department of Molecular and Cellular Interactions, VUB, "Nucleoside Hydrolase: fast kinetics at cold temperatures", May 19

Dr René Wintjens, Institut de Pharmacie, Chimie Générale, ULB, Belgium, "Etudes structurales de protéines végétales extrêmement stables", June 16

Prof. Franz Schmid, Department of Biochemistry**,** University of Bayreuth, Germany, "The gene-3-protein of filamentous phage. A host for evolutionary protein stabilization and a model to study prolyl *cis/trans* isomerization in protein folding and function", October 13

Dr Danny Jans, Laboratory of Physiology, Faculty of Medicine, University of Hasselt, Belgium, "How Bacteria Use Epithelia", October 27

2007

Dr Gilles van Wezel, Microbial Development, Leiden University, The Netherlands, "Snap shots from a family album: SsgA-like proteins control *Streptomyces morphogenesis*", January 26

Dr Ludovic Bannwarth, CNRS-Paris 6, France, "Une stratégie alternative pour inhiber la protéase du VIH-1 : le ciblage de l'interface entre sous-unités", October 29

Dr Ghislain Schyns, Biotechnology R&D, DSM Nutritional Products Ltd., Kaiseraugst, Switzerland, "(White) biotech at DSM: overview and selected examples", November 7

Dr Patrice Courvalin, Institut Pasteur, Paris, France, "Regulation of vancomycin resistance expression in Gram-positive cocci", November 9

Dr Isabelle Georges, Département de chimie et des bioindustries, UCL, Belgium "Exploring the new subdivision Acidobacteria", November 27

2008

Sylvie Bun, Université de Technologie de Compiègne, France, "Evolution dirigée d'un fragment d'anticorps anti-idiotypique à activité de type β-lactamase", February 29

Dr Anne-Catherine Mailleux, ULB, Belgium, "Social organization in dust mites", March 7

Prof. Beki Kan, Marmara University, Turkey, "Purification and characterisation of recombinant heterotrimeric G of alpha protein", June 20

Dr Marc Vanhove, Dyax, Liège, Belgium, "Surface Plasmon Resonance: a user's perspective", September 5

Dr Cécile Van de Weerdt, GIGA Research, University of Liège, Belgium, "Les Octarellines : histoire de leurs évolutions", November 21

Dr Michaël Nigen, INRA Rennes, France, "Interactions et assemblages entre biomolécules", December 12

2009

Jochen Govaert and Dr Dirk Saerens, VUB, Brussels, Belgium, "Nanobodies and beyond: from fundamental research to medical applications", March 3

Hayat El Hajjaji, ICP-UCL, Louvain la Neuve, Belgium, "Caractérisation de deux protéines d'*Escherichia coli* présentant un centre zinc", March 18

Dr Jaime Eyzaguirre, Universidad Andrés Bello, Chile, "Proteomics studies of the secretome of the lignocellulolytic fungus *Penicillium purprogenum*", June 7

Lorena Varela, University of Granada, Spain, "Amyloid fibril formation by the N47A mutant of the alpha-spectrin SH3 domain", June 29

Jean-Michel Brunel, Université Paul Cézanne, Marseille, France, "New insights on the mechanism of action of squalamine against Gram+ and Gram- bacteria", October 16

ORAL PRESENTATIONS

2005

S. Rigali, "A simple approach to decipher bacterial regulatory codes and reveal new cis/trans relationships", Leiden Institute of Chemistry, The Netherlands, January 5

S. Rigali "A simple approach to decipher new bacterial regulatory codes", Freie Universität Berlin, Germany, January 15

A. Wilmotte, "Some like it cold: cyanobacteria in Byers Peninsula, Livingston Island, Maritime Antarctica", Centre d' Etudes nucléaires SCK/CEN, Mol, Belgium, March 25

C. Bebrone "CphA, un exemple pratique de beta-lactamase à zinc", Institut de Recherches Microbiologiques Wiame, Brussels, Belgium, May 24

A. Taton, "Cyanobacterial diversity in Antarctica based on taxonomic molecular markers", 3rd International Workshop on Space Microbiology, Mol, Belgium, May 25

G. Dive, "Application de la chimie quantique à l'étude de mécanismes réactionnels en chimie et en catalyse enzymatique **"**, 19èmes journées franco-belges de pharmacochimie, Liège, Belgium, May 26-27

P. Charlier, "Host-Ligand Relationships: The experimental approaches", University of Louvain-la-Neuve, Belgium, June 1

J.M. Frère, "Beta-lactamases! Not again!" Symposium on bacterial physiology, Stockholm, Sweden, June 3-4

M. Galleni, "Hybrid beta-lactamases", 9th beta-lactamase workshop, Leonessa, Italy, June 6-8

A. Taton, "Cyanobacterial ecology in Antarctica", Joint European and National Astronomy Meeting, Liège, Belgium, June 7

J.M. Frère, "Beta-lactamases as the major resistance factor", 9th beta-lactamase workshop, Leonessa, Italy, June 11-15

A. Taton, "Cyanobacterial diversity in Antarctica based on 16S rRNA sequences", ASLO Summer Meeting, Santiago de Compostela, Spain, June 20

G. Dive, "Méthodes spectroscopiques et quanto-chimiques appliquées à l'étude des molécules d'intérêt biologique", Aussois, France, August 28 - September 2

A.Wilmotte, "Molecular taxonomy of cyanobacteria based on the 16S rRNA gene", 6th European Workshop on the Molecular Biology of Cyanobacteria, Gdansk, Poland, September 26

S. Rigali, "Prédiction des relations cis-trans chez les prokaryotes", Unité de Recherche en Biologie Moléculaire, University of Namur, Belgium, October 7

A. Wilmotte, "Investigating Life in Extreme Environment", ESF Workshop Sant Feliu de Guixols, Spain, November 5-8

E. Sauvage, "Crystal structure of a penicillin-binding protein, the *Actinomadura* R39 DD-peptidase", Groupe de contact FNRS « Rayonnement synchrotron », Namur, Belgium, November 18

J.M. Frère, "The expanding world of carbapenemases", 45th ICAAC, Washington DC, USA, December 17

2006

A. Wilmotte, "Introduction à B-BLOOMS, résultats principaux" et "Recommandations pratiques BBLOOMS ", Meeting des end-users des projets MANSCAPE/B-BLOOMS, Réunion Fondation Universitaire, Brussels, Belgium, March 9

A. Wilmotte, "Monitoring of toxigenocity of blooms and toxin analyses", Colloque MANSCAPE/B-BLOOMS, Royal Belgian Institute of Natural Sciences, Brussels, Belgium, March 10

A. Wilmotte & R. Fernandez-Carazo, "Diversity and distribution of cyanobacteria in

Antarctic microbial mats", Workshop BE-POLES sur la recherche polaire belge, Royal

Belgian Institute of Natural Sciences, Brussels, Belgium, March 23

M. Dumoulin, "Investigation of the mechanism of amyloid fibril formation by human lysozyme", Osaka University, Institute for Protein Research, Osaka, Japan, April 10

S. Rigali, "From nutrient sensors to routes of antibiotic production in *Streptomyces coelicolor*", Congrès:"Journées *Streptomyces*", Ecole Centrale de Lyon, Ecully, France, June 15

A. Wilmotte, "Advances in molecular diversity and taxonomy of cyanobacteria in the last decennia", 12th International Symposium on Phototrophic Prokaryotes, Pau, France, August 29

J.M. Frère, "Metallo-beta-lactamases", Antibiotic workshop, Novartis Institute, London, UK, September 21-22

S. Rigali, "Sensing worsening conditions in streptomycetes", Friedrich-Alexander-University Erlangen-Nuremberg, Germany, October 23

M. Dumoulin, "Investigation of the mechanism of amyloid fibril formation by human lysozyme", Umeå University, Department of Chemistry, Umeå, Sweden, October 31

R. Fernandez-Carazo, "A Belgian collection of polar Cyanobacteria: novel diversity and pharmaceutical screening of isolates", Symposium de la Société Belge de Microbiologie, Brussels, Belgium, November 24

M. Dumoulin, "Investigation of the mechanism of amyloid fibril formation by human lysozyme using camelid antibody fragments", 17th Faltertag, Halle, Germany, December 31

2007

A. Wilmotte, "La puce à ADN MIDI-CHIP pour l'identification et le suivi des cyanobactéries potentiellement toxiques ", Journée du Groupe d'Intérêt Scientifique Cyanobactéries, Institut Pasteur, Paris, France, January 31

M. Terrak, "Bacterial cell wall biosynthesis", 1st International fungal/plant cell meeting: cell wall polysaccharides of fungi and plants, Biarritz, France, March 10-14

J.M. Frère, "Combatting bacterial resistance", Biovision, Lyon, France, March 13-14

P. Lassaux, " Mercapto-phosphonate compounds as broad spectrum inhibitors of the metallo-β-lactamases", 17th European Congress of Clinical Microbiology and Infectious Diseases, Munich, Germany, March 31-April 3

D. Dehareng, "Modèles et modélisation moléculaire", Université de Laval, Québec, Canada, April 25

R. Fernandez-Carazo, "Diversity and distribution of cyanobacteria in Antarctica", 17th Symposium of the International Association for Cyanophyte Research, Merida City, Mexico, June 25-29

R. Fernandez-Carazo, "The last lakes on earth: Aquatic life in Dufek Massif, Antarctica", British Ecological Society Annual Meeting, Glasgow, UK, September 10-12

G. Dive, "Applied quantum chemistry to design antibiotics", International Conference of Computational Methods in Sciences and Engineering, Corfou, Greece, September 25-30

A. Wilmotte, "A Belgian collection of polar cyanobacteria: search for novel diversity and pharmaceutical screening of isolates", 11th International Conference on Culture Collections 'Connections between Collections", Goslar, Germany, October 8

N. Morin, "Response of *Arthrospira* sp. PCC8005 to oxidative stress using flow cytometry", 12th Annual Meeting of the French Association for Cytometry, Clermont-Ferrand, France, October 10

M. Dumoulin, "Probing the mechanism of human lysozyme amyloid fibril formation using camelid antibody fragments", Catholic

University of Leuven, de Duve Institute, Belgium, November 29

M. Dumoulin, "Investigation of the mechanism of amyloid fibril formation by human lysozyme", University College Dublin, Conway Institute, Dublin, Ireland, December 13

2008

C. Bebrone, "Emerging antimicrobial resistance: mechanisms, management and control", 1st International Workshop – Alpha Project Bacterialnet, Florence, Italy, January 17-19

M. Dumoulin, "Probing the mechanism of human lysozyme amyloid fibril formation using camelid antibody fragments", Université Libre de Bruxelles, Belgium, March 13

N. Morin, "The genome of *Arthrospira* sp. PCC8005: An insight into the role of repeated sequences in cyanobacterial genomes", Annual Meeting of the Society for General Microbiology, Edinburgh, Scotland, April 2

A. Matagne, "Single-Domain Antibody Fragments that Inhibits Fibril Formation by Stabilizing Human Lysozyme Variants", Department of Molecular Biology, University of Siena, Italy, May 16

M. Terrak, "Structure and function of peptidoglycan transglycosylases", Symposium "Peptidoglycan and Bacterial Shape", University of Liège, Belgium, May 21

P. Charlier, "Structure and function of DD-peptidases", Symposium "Peptidoglycan and Bacterial Shape", University of Liège, Belgium, May 21

M. Vandevenne, "The hybrid protein BlaPChBD as a versatile tool to study protein/ligand interactions", Brussels, May 26

A. Chevigné, "Relationship between propeptide pH unfolding and inhibitory ability during proDer p 1 activation mechanism", Brussels, May 26

J.M. Frère, "Beta-lactamases as the major resistance factor", 10th Meeting β-Lactamases, Eretria, Greece, May 31- June 4

P. Lassaux, "Structural characterisation of the sub-class B1 metallo-β-lactamase", 10th Meeting β-Lactamases, Eretria, Greece, May 31- June 4

L. Vercheval, "Crystallographic and kinetic studies of carboxylated lysin in class D beta-lactamase OXA-10", 10th Meeting β-Lactamases, Eretria, Greece, May 31- June 4

S. Colson, "Le processus de différenciation morphologique chez *S. coelicolor* en réponse à la disponibilité nutritionnelle", Journées Actinomycètes, Castanet-Tolosan, Toulouse, France, June 12-13

S. Dandois, "Etude de l'induction des beta-lactamases chez *Streptomyces cacaoi* : le rôle de la protéine BlaB", Journées Actinomycètes, Castanet-Tolosan, Toulouse, France, June 12-13

N. Scarafone, "Chimeric proteins as models to study the mechanism of aggregation associated with polyglutamine expansions", 22nd Annual Symposium of the Protein Society, San Diego (CA), USA, July 19-23

N. Scarafone, "Chimeric proteins as models to study the mechanism of aggregation associated with polyglutamine expansions", 20th Faltertage: Folding *in vitro* and folding *in vivo*, Wittenberg, Germany, September 26-28

J.M. Frère, "Getting the zinc out: metallo-β-lactamases", 48th Annual ICAAC Meeting, Washington DC, USA, October 25-28

G. Dive, "Mécanismes moléculaires en biochimie", Ecole thématique CNRS, Biarritz, France, December 8-12

M. Terrak, "Penicillin-binding proteins: function in the bacterial cell wall assembly and their targeting with antibacterial agents", Università degli Studi dell'Insubria, Varese, Italy, December 15

2009

P. de Carvalho Maalouf, "Diversité moléculaire des cyanobactéries Antarctiques', Gembloux, Belgium, April 10

S. Colson, "Identification of novel transporters involved in cell differenciation or antibiotic production in *Streptomyces coelicolor*, Université de Louvain-la-Neuve, Belgium, May 8

E. Sauvage, "Crystal structure of *Escherichia coli* penicillin-binding protein 3", Université de Louvain-la-Neuve, Belgium, May 8

A. Matagne, Conference on Protein Folding, Royal Society of Sciences, University of Liège, Belgium, May 28

M. Dumoulin, "Investigation of human lysozyme fibril using camelid antibody fragments as structural probe", University of Udine, Department of Biomedical Science and Technology, Italy, June 9

M. Dumoulin, "Combining biophysical techniques to investigate amyloid fibril formation", University of Padova, CRIBI Biotechnology Centre, Italy, June 10

S. Rigali, "Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces*", Symposium "Penicillin-recognizing enzymes: from enzyme kinetics to protein folding, University of Liège, Belgium, July 3

M. Dumoulin, "Understanding the mechanism of amyloid fibril formation by human lysozyme", Symposium "Penicillin-recognizing enzymes: from enzyme kinetics to protein folding, University of Liège, Belgium, July 4

J.M. Frère, "Ah non ! pas encore des bêta-lactamases ", Société Française de Biochimie et Biologie Moléculaire Annual Congress, Nancy, France, August 21-29

Z. Namsaraev, « Molecular diverstiy of cyanobacteria in glacial environments", University of Bristol, UK, October 8

M. Dumoulin, "Creation of chimeric proteins to investigate the mechanisms involved in polyQ diseases: effects of the polyQ insertions on the properties of the β-lactamase BlaP", University of Cambridge, Department of Chemistry, Cambridge, UK, November 21

E. Sauvage, "Resistencia bacteriana", Buenos Aires, Argentina, December 2

S. Rigali, "L'immobilité au service de l'évolution: adaptations génétiques et physiologiques des *Streptomyces*", University of Namur, Belgium, December 7

G. Feller, "Stability-activity relationships in natively unstable proteins", Workshop on Thermodynamically unstable proteins: chance or necessity? Trieste, Italy, December 14

J.M. Frère, "Organic and bioorganic reaction mechanisms", University of Huddersfield, UK, December 16

PATENTS

Hybrid proteins of active-site serine beta-lactamase
Giannota Fabrizio (BE), Filée Patrice (BE), Galleni Moreno (BE), Frère Jean-Marie (BE), Joris Bernard (BE), Brans Alain (BE), and Ruth Nadia (BE).
Pub. N°: WO/2005/078075, International Application N°: PCT/EP2005/050174
Publication Date: 25.08.2005, International Filing Date: 17.01.2005

Methods and means for metabolic engineering and improved product formation by micro-organisms
Rigali Sébastien (BE), Titgemeyer Mannfred Friedrich Bruno (DE), Van Wezel Gilles Philippus (NL).
Pub. N°: WO/2007/094667, International Application N°: PCT/NL2007/050061
Publication Date: 23.08.2007, International Filing Date: 14.02.2007

PHD THESES

2005

Bauvois Cédric, (Biology) β-lactamase à sérine active : caractérisation cinétique et structurale de 4 β-lactamases de classe C plasmidiennes. Etude cristallographique du mutant K70C de la protéine OXA-10, implication au niveau du mécanisme réactionnel des β-lactamases de classe D

Bebrone Carine, (Biochemistry) Caractérisation des résidus impliqués dans le mécanisme d'action et la spécificité de la beta-lactamase de classe B CphA

Chahboune Aïcha, (Biochemistry) Caractérisation biochimique des produits des gènes de l'opéron yajG-ampG d'*Escherichia coli*

de Lemos Esteves Frédéric, (Biology) Amélioration des performances d'une xylanase en condition de pH extrêmes

Derouaux Adeline, (Biochemistry) Etude du régulateur CrpSco : Mise en évidence des processus biologiques contrôlés, recherche du ligand et des gènes cibles chez *Streptomyces coelicolor*

Duval Valérie, (Biochemistry) Etude du récepteur BlaR et du signal cytoplasmique transmis lors de l'induction de la β-lactamase BlaP chez *Bacillus licheniformis* 749/I

Géron Christine, (Chemistry), Relations entre instabilité Hartree-Fock triplet et structure moléculaire.

Pastoret Soumya, (Biochemistry) Etude fonctionnelle de la protéine FtsW dans la formation du septum chez *Escherichia coli*

Ruth Nadia, (Biochemistry) La β-lactamase TEM-1 comme protéine porteuse dans le cadre de la vaccination contre l'entérotoxine STaI d'*Escherichia coli*

2006

Boutte Christophe, (Biology) Diversité génotypique des cyanobactéries planctoniques de lacs d'eau douce européens

Delmarcelle Michaël, (Biochemistry) La D-aminopeptidase *d'Ochrobactrum anthropi* et la DD-carboxypeptidase-transpeptidase de *Streptomyces* R61 comme modèle pour l'étude de la spécificité des protéines à boucle 3 étendue reconnaissant la pénicilline

Taton Arnaud, (Botany) Diversité des cyanobactéries dans les tapis microbiens des lacs antarctiques

2007

Horsfall Louise, (Chemistry) Study of the Subclass B3 and Inhibitors of the Metallo-β-Lactamases

Joseph Gangoué Piéboji, (Biology) Caractérisation de bêta-lactamases et leur inhibition par les extraits de plantes médicinales

2008

Chevigné Andy, (Biochemistry) Etude du mécanisme d'activation du zymogène de l'allergène Der p 1 de l'acarien *Dermatophagoïdes pteronyssinus*

Wolf Benoît, (Biochemistry) Caractérisation de la protéine de division FtsW et étude de ses interactions avec certaines protéines des machineries de synthèse du peptidoglycane chez *Escherichia coli*.

2009

Baise Etienne, (Biochemistry) Contribution à l'étude de l'activité antivirale et du mécanisme moléculaire de la MX1 bovine

Berlemont Renaud, (Zoology) Etude de la diversité enzymatique des microorganismes du sol par l'approche métagénomique

Morin Nicolas, (Biosciences) Studies to the response to spaceflight related conditions in the cyanobacterium *Arthrospira sp.*PCC8005 using a genomic approach

Vandenameele Julie, (Chemistry) Caractérisation des propriétés de folding de la β-lactamase de classe A *Bacillus licheniformis* BS3

Vandevenne Marylène, (Biochemistry) Characterisation of the human macrophage chitotriosidase

PUBLICATIONS

2005

[1] M.E.G. Aarsman, A. Piette, C. Fraipont, T.M.F. Vinkenvleuger, M. Nguyen-Distèche and T. Den Blaauwen. Maturation of the *E. coli* divisome occurs in two steps. *Mol. Microbiol*, **55**, 1631-1645.

[2] S.A. Adediran, M. Nukaga, S. Baurin, J.M. Frère and R.F. Pratt. Inhibition of class D β-lactamases by acyl phosphates and phosphonates. *Antimicrob. Agents Chemother.*, **49**, 4410-4412.

[3] C. Bauvois, A.S. Ibuka, A. Celso, J. Alba, Y. Ishii, J.M. Frère and M. Galleni. Kinetic properties of four plasmid-mediated AmpC β-lactamases. *Antimicrob. Agents Chemother.*, **49**, 4240-4246.

[4] C. Bebrone, C. Anne, K. De Vriendt, B. Devreese, G.M. Rossolini, J. Van Beeumen , J.M. Frère and M. Galleni. Dramatic broadening of the substrate profile of the *Aeromonas hydrophila* CphA metallo-β -lactamase by site-directed mutagenesis. *J. Biol. Chem.*, 280, 28195-28202.

[5] P. Bertholet, M. Gueders, G. Dive, A. Adelin, V. Barillaro, B. Perly, D. Cataldo, G. Piel, L. Delattre and B. Evrard. The effect of cyclodextrins on the aqueous solubility of a new Mmp inhibitor: phase solubility, 1 H-NMR spectroscopy and molecular modeling studies, preparation and stability study of nebulizable solutions. *J. Pharm. Pharmaceut. Sci.*, **8**, 164-175.

[6] C. Boutte, J. Komarkova, S. Grubisic, P. Znachor, A. Bataille, E. Zapomelova, A. Gullukaya, J. Jezberova and A. Wilmotte. A polyphasic approach to assess the cyanobacterial diversity of summer samples from Czech reservoirs. *Algological Studies*, **117**, 177-195.

[7] A. Chahboune**,** M. Decaffmeyer, R. Brasseur and B. Joris. Membrane topology of the *Escherichia coli* AmpG permease required for recycling of cell wall anhydro-muropeptides and AmpC β-lactamase induction. *Antimicrob. Agents Chemother.*, **49**, 1145-1149.

[8] F. de Lemos Esteves, T. Gouders, J. Lamotte-Brasseur, S. Rigali and J.M. Frère. Improving the alkalophilic performances of the Xyl1 xylanase from *Streptomyces* sp. S38: structural comparison and mutational analysis. *Protein Sci*., **14**, 292-302.

[9] M. Delmarcelle, M.C. Boursoit, P. Filée, S. Baurin, J.M. Frère and B. Joris. Specificity inversion of *Ochrobactrum anthropi* D-aminopeptidase to a D,D-carboxypeptidase with new penicillin binding activity by directed mutagenesis. *Protein Sci*., **14**, 2996-2303.

[10] P. Douette, R. Navet, P. Gerkens, M. Galleni, D. Levy and F. Sluse. *Escherichia coli* fusion carrier proteins act as solubilizing agents for recombinant uncoupling protein 1 through interactions with GroEl. *Biochem. Biophys. Res. Commun.*, **333**, 686-693.

[11] M. Dumoulin, D. Canet, A.M. Last, E. Pardon, D.B. Archer, S. Muyldermans, L. Wyns, A. Matagne, C.V. Robinson, C. Redfield and C.M. Dobson. Reduced global cooperativity is a common feature underlying the amyloidogenicity of pathogenic lysozyme mutations. *J. Mol. Biol.*, **346**, 773-788.

[12] J.M. Frère, M. Galleni, K. Bush and O. Dideberg. Is it necessary to change the classification of β-lactamases ? *J. Antimicrob. Chemother.*, **55**, 1051-1053.

[13] J.M. Frère and P. Marchot. Inactivators in competition. How to deal with them... and not ! *Biochem. Pharmacol.*, **70**, 1417-1423.

[14] G. Garau, C. Bebrone, C. Anne, M. Galleni, J.M. Frère and O. Dideberg. A metallo-β-lactamase enzyme in action: crystal structure of the monozinc carbapenemase CphA and its complex with biapenem. *J. Mol. Biol.*, **345**, 785-795.

[15] E. Gridelet, D. Dehareng, R. Locht, A.J. Lorquet, J.C. Lorquet and B. Leyh. Ground and excited state dissociation dynamics of ionized1,1-difluoroethene. *J. Phys. Chem. A*, **109**, 8225-8235.

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[20] R. Locht, B. Leyh, D. Dehareng, H.W. Jochims, H. Baumgärtel. The vacuum UV photoabsorption spectrum of methyl bromide (CH_3Br) and its perdeuterated idotopomer CD_3Br. A vibrational analysis. *Chem.Phys*., **317**, 87-102.

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[22] P.R. Moreira, C. Duez, D. Dehareng, A. Antunes, E. Almeida-Vara, J.M. Frère, F. Xavier Malcata and J.C. Duarte. Molecular characterization of a versatile peroxidase from a *Bjerkandera* strain. *J. Biotechnol.*, **118**, 339-352.

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[24] A. Piette, A. Derouaux, P. Gerkens, E. Noens, G. Mazzuchelli, S. Vion, H. Koerten, F. Titgemeyer, E. De Pauw, P. Leprince, G.P. van Wezel, M. Galleni and S. Rigali. From dormant to germinating spores of *Streptomyces coelicolor* A3(2) : new perspectives from the crp null mutant. *J. Proteome Res*., **4**, 1699-1708.

[25] P. Power, M.Galleni, J. Di Conza, J. Ayala and G. Gutkind. Description of In116, the first blaCTX-M-2-containing complex class 1 integron found in *Morganella morganii* isolates from Bueno Aires, Argentina. *J. Antimicrob. Chemother.*, **55**, 461-465.

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[28] D. Saerens, M. Pellis, R. Loris, E. Pardon, M. Dumoulin, A. Matagne, L. Wyns, S. Muyldermans and K. Conrath. Identification of a universal VHH framework to graft non-canonical antigen-binding loops of camel single-domain antibodies. *J. Mol. Biol*., **352**, 597-607.

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[30] E. Sauvage, R. Herman, S. Petrella, C. Duez, F. Bouillenne, J.M. Frère and P. Charlier. Crystal structure of the *Actinomadura* R39 DD-peptidase reveals new domains in penicillin-binding proteins. *J. Biol. Chem.*, **280**, 31249-31256.

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[52] A. Mussagy, A. Wilmotte et G. Cronberg. The cyanophyte *Arthrospira fusiformis* from Mozambique, Africa: morphological and molecular characterization. *Algological studies*, **121**, 59-73.

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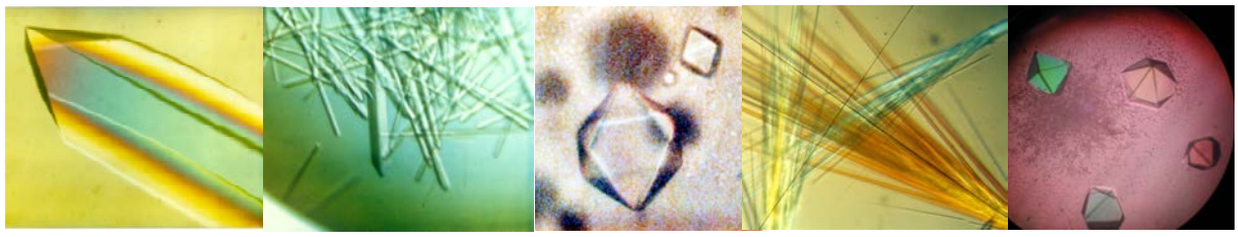
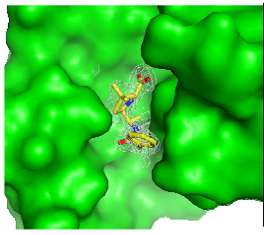
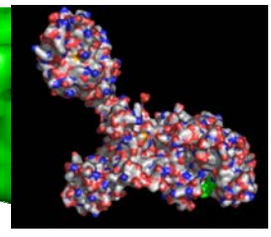
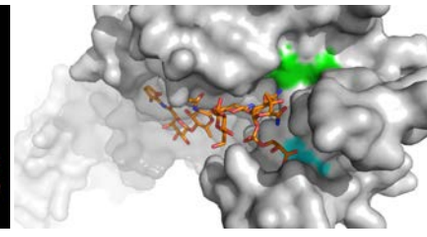
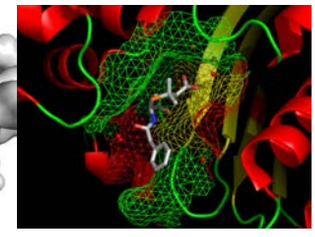
Protein structures deposited within the Protein Data Bank

Over the 5 years, 30 protein structures have been deposited within the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).



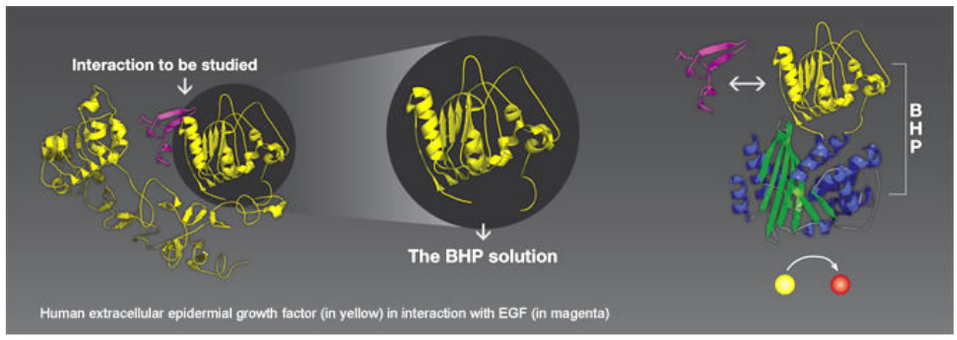
PDB ID	STRUCTURE TITLE	AUTHOR
2BH0	CRYSTAL STRUCTURE OF A SEMET DERIVATIVE OF EXPA FROM *BACILLUS SUBTILIS* AT 2.5 Å	Petrella S, Herman R, Sauvage E, Filée P, Joris B, Charlier P.
2BH7	CRYSTAL STRUCTURE OF A SEMET DERIVATIVE OF AMID AT 2.2 Å	Petrella S, Herman R, Sauvage E, Joris B Généreux C, Pennartz A, Charlier P.
2CC1	CRYSTAL STRUCTURE OF THE CLASS A BETA-LACTAMASE FROM *MYCOBACTERIUM FORTUITUM*	Sauvage E., Fonzé E., Charlier P.
2HP5	CRYSTAL STRUCTURE OF THE OXA-10 W154G MUTANT AT PH 7.0	Kerff F, Falzone C, Herman R, Sauvage E, Charlier P.
2HP6	CRYSTAL STRUCTURE OF THE OXA-10 W154A MUTANT AT PH 7.5	Kerff F, Falzone C, Herman R, Sauvage E, Charlier P.
2HP9	CRYSTAL STRUCTURE OF THE OXA-10 W154A MUTANT AT PH 6.0	Kerff F, Falzone C, Herman R, Sauvage E, Charlier P.
2HPB	CRYSTAL STRUCTURE OF THE OXA-10 W154A MUTANT AT PH 9.0	Kerff F, Falzone C, Herman R, Sauvage E, Charlier P.
2J9P	CRYSTAL STRUCTURE OF THE *BACILLUS SUBTILIS* PBP4A, AND ITS COMPLEX WITH A PEPTIDOGLYCAN MIMETIC PEPTIDE	Sauvage E, Herman R, Kerff F, Duez, C, Charlier P.
2QZ6	FIRST CRYSTAL STRUCTURE OF A PSYCHROPHILE CLASS C B-LACTAMASE	Michaux C, Massant J, Kerff F, Charlier P, Wouters J.
2RL3	CRYSTAL STRUCTURE OF THE OXA-10 W154H MUTANT AT PH 7.0	Vercheval L, Kerff F, Herman R, Guiet R, Sauvage E, Charlier P, Frère J-M, Galleni M.
3B3X	CRYSTAL STRUCTURE OF CLASS A B-LACTAMASE OF *BACILLUS LICHENIFORMIS* BS3 WITH AMINOCITRATE	Sauvage E, Herman R, Kerff F, Charlier P
2VGK	CRYSTAL STRUCTURE OF *ACTINOMADURA* R39 DD-PEPTIDASE COMPLEXED WITH A PEPTIDOGLYCAN-MIMETIC CEPHALOSPORIN	Sauvage E, Herman R, Kerff F, Charlier P
3DEY	COMPLEX OF THE N-ACETYLMURAMYL-L-ALANINE AMIDASE AMID FROM *E. COLI* WITH THE SUBSTRATE ANHYDRO-N-ACETYLMURAMIC ACID-L-ALA-D-GAMMA-GLU-L-LYS	Kerff F, Petrella S, Herman R, Sauvage E, Mercier F, Luxen A, Frère J-M, Joris B, Charlier P.
3DEZ	COMPLEX OF THE N-ACETYLMURAMYL-L-ALANINE AMIDASE AMID FROM *E.COLI* WITH THE PRODUCT L-ALA-D-GAMMA-GLU-L-LYS	Kerff F, Petrella S, Herman R, Sauvage E, Mercier F, Luxen A, Frère J-M, Joris B, Charlier P.

3D30	STRUCTURE OF AN EXPANSIN LIKE PROTEIN FROM *BACILLUS SUBTILIS* AT 1.9 Å RESOLUTION	Kerff F, Petrella S, Herman R, Sauvage E, Joris B, Charlier P.
3F9O	CRYSTAL STRUCTURE OF THE DI-ZINC CARBAPENEMASE CPHA FROM *AEROMONAS HYDROPHILA*	Delbruck H, Bebrone C, Hoffmann KMV.
3FAI	THE DI-ZINC CARBAPENEMASE CPHA N220G MUTANT	Delbruck H, Bebrone C, Hoffmann KMV.
2WGI	CRYSTAL STRUCTURE OF THE ACYL-ENZYME OXA-10 W154A-BENZYLPENICILLIN AT PH 6	Vercheval L, Falzone C, Sauvage E, Herman R, Charlier P, Galleni M, Kerff F.
2WGV	CRYSTAL STRUCTURE OF THE OXA-10 V117T MUTANT AT PH 6.5 INHIBITED BY A CHLORIDE ION	Vercheval L, Kerff F, Bauvois C, Guiet R Sauvage E, Charlier P, Galleni M.
2WGW	CRYSTAL STRUCTURE OF THE OXA-10 V117T MUTANT AT PH 8.0	Vercheval L, Kerff F, Bauvois C, Guiet R Sauvage E, Charlier P, Galleni M.
2WHG	CRYSTAL STRUCTURE OF THE DI-ZINC METALLO-BETA-LACTAMASE VIM-4 FROM *PSEUDOMONAS AERUGINOSA*	Lassaux P, Traore D.A.K., Galleni M, Ferrer J.L.
2WIX	CRYSTAL STRUCTURE OF *ACTINOMADURA* R39 DD-CARBOXYPEPTIDASE COMPLEXED WITH THE SQ26445 MONOBACTAM	Rocaboy M., Sauvage E., Herman R., Duez C., Joris B., Charlier P.
2WK0	CRYSTAL STRUCTURE OF THE CLASS A B-LACTAMASE BS3 INHIBITED BY 6-B-IODOPENICILLANATE.	Sauvage E, Zervosen A, Dive G, Herman R, Kerff F, Amoroso A., Fonzé E, Pratt R.F, Luxen A, Charlier P.
2WKE	CRYSTAL STRUCTURE OF THE *ACTINOMADURA* R39 DD-PEPTIDASE INHIBITED BY 6-B-IODOPENICILLANATE	Sauvage E, Herman R, Kerff F, Charlier P.
2WKH	CRYSTAL STRUCTURE OF THE ACYL-ENZYME OXA-10 K70C-AMPICILLIN AT PH 7.0	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	Vercheval L, Bauvois C, Kerff F, Sauvage E, Guiet R, Charlier P, Galleni M.
2WKX	CRYSTAL STRUCTURE OF THE NATIVE *E. COLI* ZINC AMIDASE AMID	Petrella S, Kerff F, Herman R, Généreux C, Pennartz A, Sauvage E, Joris B, Charlier P.
2WUQ	CRYSTAL STRUCTURE OF BLAB PROTEIN FROM *STREPTOMYCES CACAOI*	Dandois S, Herman R, Sauvage E, Charlier P, Joris B, Kerff F.
2X01	CRYSTAL STRUCTURE OF THE OXA-10 S67A MUTANT AT PH 7	Vercheval L, Kerff F, Bauvois C, Sauvage E, Guiet R, Galleni M, Charlier P.
2X02	CRYSTAL STRUCTURE OF THE CLASS D B-LACTAMASE OXA-10 AT 1.35 Å RESOLUTION	Vercheval L, Kerff F, Sauvage E, Herman R, Galleni M, Charlier P.

SPIN OFF : PROGENOSIS

The company was set-up in 2006 as a spin-off of the University of Liège, based on technologies developed in the Centre for Protein Engineering. ProGenosis (www.progenosis.com) is devoted to the analysis of protein interactions. The proprietary technology platform is dedicated to developing efficient, innovative and convenient Bifunctional Hybrid Proteins (BHPs), which can be used for protein domain studies. The core of the BHP technology is based on the use of an active-site serine **β-lactamase enzyme as the protein scaffold**. This enzyme catalyzes the hydrolysis of the β-lactam ring of penicillin-related antibiotics. The insertion site is a highly permissive loop diametrically opposed to the site of enzyme activity. This ensures solvent accessibility for the exogenous polypeptide and limits steric hindrances into the active site of the β-lactamase.



ProGenosis provides R&D customized services to Life Science companies in three main areas:

- Protein engineering
- Antigen & antibody development
- Epitope mapping

ProGenosis technology has been validated by multiple research projects related to industrial final products such as therapeutics antibodies, IVD reagents or vaccine components.

ProGenosis possesses exclusive worldwide rights on bifunctional hybrid protein technology (CA2555393; EP1713907; US2007161040).

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SYMPOSIA

2005

Fourth Meeting of the Belgian Biophysical Society on “Protein Folding and Stability”, University of Liège, Belgium, September 2
Main Organizer: Prof. A. Matagne

2006

Fifth Meeting of the Belgian Biophysical Society on “Protein Folding and Stability”, University of Liège, Belgium, September 1
Main Organizer: Prof. A. Matagne

2007

Mini symposium to celebrate the career of Professor Jacques Coyette, University of Liège, Belgium, January 25
Main Organizer: Prof. J.M. Frère

Sixth Meeting of the Belgian Biophysical Society on “Protein Folding and Stability”, University of Liège, Belgium, August 31
Main Organizer: Prof. A. Matagne

Joint meeting of the Belgian Biophysical Society, the National Committee for Biophysics, the Belgian Society for Biochemistry and Molecular Biology and the F.W.O. Contact groups on Protein Structure, Folding and Dynamics, and on Crystallography, on “Proteins and membranes: a joint venture”, Palais des Académies, Brussels, Belgium, December 7
Co-organizer and Chairman: Prof. A. Matagne

Annual Meeting of the “Groupe de Contact Rayonnement Synchrotron” F.R.S.-FNRS, University of Louvain, Louvain-La-Neuve, Belgium, December 19
Co-Organizer: Prof. P. Charlier

2008

One-day symposium in the frame of "The SFMBBM thematic doctoral school", "The EUR- INTAFAR European project" and "IUAP programme (P6/19)" on Peptidoglycan and Bacterial Shape, University of Liège, Belgium, May 21
Main organizer: Prof. J. M. Frère

Seventh Meeting of the Belgian Biophysical Society on “Protein Folding and Stability”, University of Liège, Belgium, August 29
Main Organizer: Prof. A. Matagne

Fifth Belgian Crystallography Symposium BCS-5, Academy House, Brussels, Belgium, October 15
Co-Organizer: Prof. P. Charlier

Annual Meeting of the « Groupe de Contact Rayonnement Synchrotron” F.R.S.-FNRS, University of Brussels, Brussels, Belgium, December 12
Co-Organizer: Prof. P. Charlier

2009

Symposium in honour of Professor Jean-Marie Frère on "Penicillin-recognizing enzymes: from enzyme kinetics to protein folding", Centre for Protein Engineering, University of Liège, Belgium, July 1-3
Organizers: Prof. P. Charlier, Prof. M. Galleni, Prof. B. Joris and Prof. A. Matagne

Annual Meeting of the « Groupe de Contact Rayonnement Synchrotron" F.R.S.-FNRS, Facultés Universitaires Notre Dame de la Paix, Namur, Belgium, December 27
Co-Organizer: Prof. P. Charlier

EDUCATION

ACADEMIC COURSES

Bachelor and Preparation to Masters

Bactériologie, 20h + 15 h TP - **J. Coyette**. Première licence Biochimie, 1e Licence Biologie végétale, 1e Licence Biologie animale et DES en Biotechnologie.

Biochimie, 30h + 30 TP - **J.-M. Frère**. 2ème Candidature/Bac 2 Sciences de l'ingénieur.

Biochimie des micro-organismes, 30h - **J. Coyette**. Deuxième licence en Biochimie.

Biochimie, 30 h + 30 h TP - BIOC0002-1 - **P. Charlier**. Bac 2 Sciences de l'ingénieur, orientation ingénieur civil, option Génie Biomédical.

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

Biochimie générale, 30h - **J.-M. Frère**. 1ère Licence/Bac 3 Chimie.

Biochimie, 30 h - CHIM0678-1 - **A. Matagne**. Bac3 Sciences chimiques et année préparatoire aux Sciences Chimiques.

Biologie I, 20h - **J. Coyette**. Première candidature en Chimie.

Biologie II, 30h + 30h TP - **J. Coyette**. Deuxième candidature en Chimie.

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

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

Chimie des macromolécules biologiques, 60h + 40h TP + 4h visite d'usine - BIOC0209-3/4/6 - **M. Galleni** et **A. Matagne**. Bac 3 Sciences biologiques. Année préparatoire en Sciences biologiques et en Biochimie et Biologie Moléculaire et Cellulaire.

Chimie des macromolécules biologiques et thermodynamique des systèmes biologiques, 70h + 40h TP + 4h 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 en biochimie et biologie moléculaire et cellulaire.

Génétique, biologie moléculaire et chimie des macromolécules, 30h + 30h TP - BIOC0001-1 - J. Dommes et **M. Galleni**. Master générique en Sciences biologiques. Année préparatoire au master en biologie des organismes et écologie et au master en Sciences biologiques.

Microbiologie - MICR0711-1 Partim 2 : Bactériologie : 20h + 10h TP - **B. Joris**. Bac 3 Biochimie et Biologie Moléculaire et Cellulaire. Année préparatoire aux masters en Biochimie et Biologie Moléculaire et Cellulaire, en Biologie des organismes et Ecologie et 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 TP - GBIO0007-1 - **B. Joris**. Master 2 en Bioinformatique et modélisation, finalité approfondie et master 2 en Ingénieur civil biomédical, finalité approfondie.

Antibiotic resistance, 25h + 25h TP - BIOC0716-1 - J.-D. Docquier (University of Siena, Italy), **J.-M. Frère**, **M. Galleni** et **B. Joris**. Master 2 Biochimie et Biologie Moléculaire et Cellulaire.

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

Aspects génétiques et biochimiques de l'évolution, 25h + 20h TP - GENE0432-3 - V. Demoulin, **M. Galleni** et C. Remacle, suppl. : D.

Baurain. Masters 1 en Biochimie et Biologie moléculaire et Cellulaire et en Sciences biologiques.

Astrobiologie, 30h + 15h TP - GEOL0263-1 - P. Claeys, V. Dehant, **M. Galleni**, E. Javaux, Y. Nazé, **A. Wilmotte**. Masters 1 en Sciences spatiales et en Sciences géologiques.

Astrobiologie, 30h - GEOL0263-2 - P. Claeys, V. Dehant, **M. Galleni**, E. Javaux, Y. Nazé, **A. Wilmotte**. Master 2 Biologie des organismes et Ecologie, finalité approfondie.

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

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

Biochimie et physiologie des microorganismes, 20h + 20h TP - BIOC0003-2 - **B. Joris**. Masters 1 en Biochimie et Biologie Moléculaire et Cellulaire et en Sciences biologiques.

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

Bioinformatique appliquée, 36h.- BIOC0717-1 - M. Georges et **B. Joris**. Master 2 en Biochimie et Biologie Moléculaire et Cellulaire, finalité Biochimie Industrielle.

Chimie générale biologique, partim enzymologie, 15h - **J.-M. Frère**. 2ème Licence/M 1 Chimie.

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

Compléments d'enzymologie, 15h - **J.-M. Frère**. 2ème Licence Biochimie.

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

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

Génétique, biologie moléculaire et chimie des macromolécules, 30h + 30h TP - BIOC0001-1- J. Dommes et **M. Galleni**. Master générique en sciences biologiques.

Génétique, biologie moléculaire et chimie des macromolécules, 30h - BIOC0001-2 - Sciences biologiques. J. Dommes et **M. Galleni**.

Génomique et bioinformatique, 25h - GENE0208-2 - M. Georges, **B. Joris**. Master 2 en Biochimie et Biologie Moléculaire et Cellulaire.

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

Microorganismes extrémophiles, 25h + 25h TP - MICR0713-1 - **G. Feller**, **M. Galleni** et **A. Wilmotte**. Master 2 Biochimie et Biologie Moléculaire et Cellulaire.

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

Principes généraux de la biologie et de la biochimie, 15h - **J.-M. Frère**. Master 2 Ingénieur civil chimiste.

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, 20h - **J.-M. Frère**. 1ère Licence Biochimie/ Master 1 Biochimie et Biologie Moléculaire et Cellulaire.

Propriétés fonctionnelles des macromolécules biologiques, 30h+15h TD+ 20h TP - BIOC0210-4/2 E. De Pauw et **A. Matagne**. Master 1 Biochimie et Biologie Moléculaire et Cellulaire.

Relations structure-fonction dans les biomolécules, 15h - **J.-M. Frère**. Master 2 Ingénieur civil biomédical.

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

Structure et dynamique des macromolécules biologiques, 20h + 20h TD + 20h TP - BIOC9233-1 - **P. Charlier** et **A. Matagne**. Master 1 Biochimie et Biologie Moléculaire et Cellulaire, finalités didactique, approfondie et industrielle.

Structure et fonction des protéines, 25h + 25h TP - BIOC0715-1 - **P. Charlier** et **M**

. Dumoulin. Master 2 Biochimie et Biologie Moléculaire et cellulaire, finalités approfondie, didactique et industrielle.

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

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

Complementary Masters

Biochimie, 30h + 30h TP - 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 (y compris plasmides et éléments transposables), 15h – **J. Coyette**. DES en Biotechnologie.

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

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

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

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

Third Cycle

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

Advanced course on "Protein Purification: What to do and how" (Coordinated by Prof. **J.-M. Frère**), 15h **-** Profs E. Depauw**, J.-M. Frère, M. Galleni, B. Joris** et **A. Matagne.** 9-12 May 2005, 10-12 May 2006, 8-10 May 2007, 28-30 April 2008 and 5-7 May 2009, Liège.

Physico-chimie des molécules d'intérêt biologique. Aspects théoriques et expérimentaux **- D. Dehareng.** Deux cours de 3 heures en Chimie physique moléculaire. Louvain-la-Neuve, 30 mai- 3 Juin 2005.

Physico-chimie des systèmes inorganiques et organométalliques : Aspects théoriques et expérimentaux **-G. Dive**, coorganisateur des cours de 3ème cycle interuniversitaire F.R.S. en Chimie physique moléculaire – Session 2007.

Physico-chimie des polymères : Aspects théoriques et expérimentaux **- G. Dive**, coorganisateur des cours de 3ème cycle interuniversitaire F.R.S en Chimie physique moléculaire. Louvain-la-Neuve, 25-29 mai 2009. Functional density theory, 2h **- D. Dehareng**.

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

Courses given abroad

Antibiotic resistance, 3h **- J.-M. Frère**. Master en Microbiologie. Cours organisé dans le cadre du contrat Bacterialnet (programme UE Alfa), 5-6 Mars 2009, Université de Buenos Aires, Argentine.

Bioinformatique, 35h **- A. Brans**. Bac 3 Licence Professionnelle en Génie Biologique, 2005-2009, IUT de Mont de Marsan, Université de Pau et des Pays de l'Adour, France

Camelid Antibody Fragments: Properties and Application, 8h - **M. Dumoulin**. May 2007, University of Pavia, Italy.

Chimie des Protéines, 8h **- M. Galleni**. Licence en sciences Alimentaires, IUT Chimie Biologique, Mont de Marsan- Université de Pau et des Pays de l'Adour, France.

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

Production de protéines recombinantes, 8 h **- A. Brans**. Bac 3 Licence Professionnelle en Génie Biologique. IUT de Mont de Marsan, 2009, Université de Pau et des Pays de l'Adour, France.

Statistique multivariée, 30h **- G. Dive**. Masters 1 et 2 en Chimie et thérapeutique. 2006-2009, Université Paris VII Denis-Diderot, France.

Protein chemistry **-** 30h **- M. Galleni**. Master in Biochemistry. 2009, Université de Hanoi, Vietnam.

Bioinformatics **-** 30h **- B. Joris**. Master in Biotechnology. 19-25 April 2009, Université de Hanoi, Vietnam.

Courses given in Technical High Schools

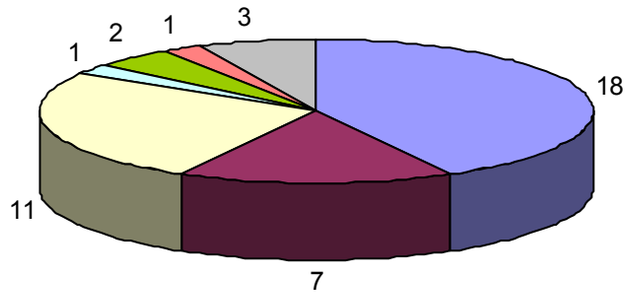
Bioinformatique appliquée, 30h. – **G. Moutzourelis** en 2006, **S. Rigali** en 2007 et 2008 et **S. Colson** en 2009. Spécialisation en Biotechnologies Médicales et Pharmaceutiques. Haute Ecole André Vésale, Liège.

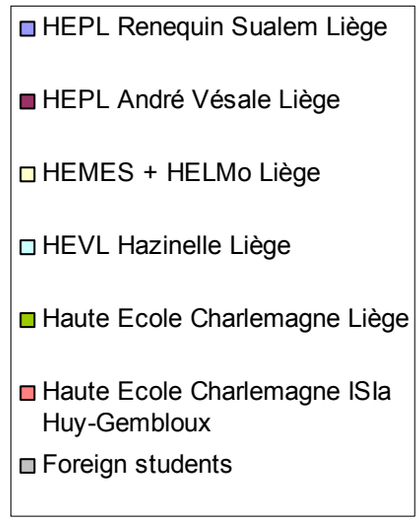
Génie génétique et enzymatique, 30h – Partim 1 **- A. Brans.** Master 1 en Sciences de l'ingénieur industriel, finalité biochimie. Haute Ecole de la Province de Liège, Liège.

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, Liège.

'GRADUAT' AND TECHNICAL BACHELOR STUDENTS

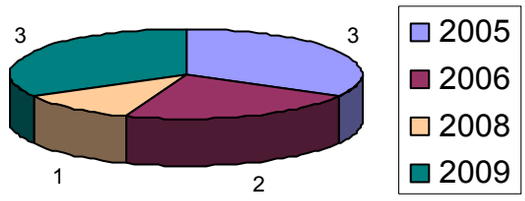
Total number of students: 46



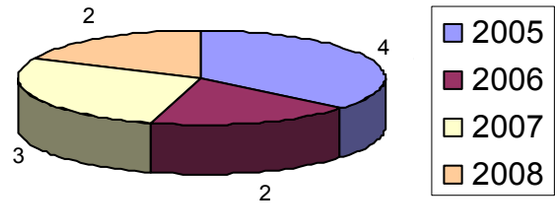


FIRST LICENCE AND BACHELOR III TRAINEES

ULg
(Chemical Sciences)



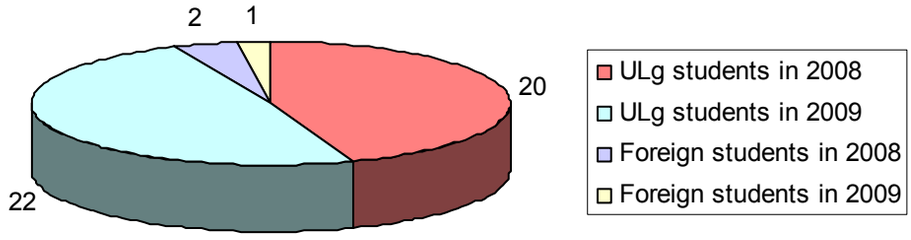
Foreign students
(IUT and CUST)



Total number of students: 20

IUT: Institut Universitaire de Technologie; CUST: Centre Universitaire Scientifique et Technique de Clermont-Ferrand

MASTER I TRAINEES



Total number of students: 45 (including 3 foreign students)

DEA, DES, LICENCE, MASTER II AND COMPLEMENTARY MASTER STUDENTS

2005

Antionotti Ingrid, Construction et caractérisation de bêta-lactamases TEM-1 hybrides (Licence en Sciences biologiques)

Bastien François, Etude du gène *ponB* codant pour une PBP de classe A et de son produit chez *Enterococcus hirae* (Licence en Sciences biologiques)

Berkane Chaïbia, Analyse de la transcription de l'opéron *blaI-blaR1* au cours de l'induction de la bêta-lactamase BlaP chez *Bacillus licheniformis* et *Bacillus subtilis*. Mise en évidence d'un atténuateur potentiel (Licence en Sciences biochimiques)

Berlemont Renaud, Etude de la diversité enzymatique des microorganismes du sol par l'approche métagénomique (DEA en Sciences)

Calusinska Magdalena, Diversity of mat-forming cyanobacteria from meltwaters on Livingston Island, Antartic Peninsula: a molecular and morphological approach (Licence in Biotechnology, Erasmus student, University of Gdansk, Poland)

Colson Séverine, DasR, le premier régulateur global chez *S. coelicolor* (Licence en Sciences biochimiques)

Dandois Sébastien, Etude du récepteur BlaR de *Bacillus licheniformis* impliqué dans l'induction de la bêta-lactamase BlaP (Licence en Sciences biochimiques)

Famerie Anne, Etude de l'interaction entre la boucle L2 et le domaine C-terminal de BlaR1 chez *Bacillus licheniformis* (DEA en Sciences)

Falzone Claudia, Etude structurale du mutant W154A de la bêta-lactamase de classe D OXA-10 de *Pseudomonas aeruginosa* (Licence en Sciences biochimiques)

Fanchamps Christophe, Etude de la diversité enzymatique des microorganismes du sol par approche métagénomique (Licence en Sciences biochimiques)

Ferber Florence, Etude des PBP2 et PBPZ chez *Enterococcus faecium* et *Enterococcus hirae* (Licence en Biochimie)

Gobardhan Laurence, Etude de la stabilité et du repliement de la Thiorédoxine 2 (Master en Chimie, Université Pierre et Marie Curie, Paris VI, France)

Halimi Baddre, Modifications génétiques de souches de *Bacillus subtilis* pour l'optimisation de la production de lipopeptides (DES Biotechnologie)

Izougarhane Youssef, Etude de la métallo-bêta-lactamase GOB-1 chez *Chryseobacterium meningosepticum* (Licence en Sciences biochimiques)

Jeanjean Sylvie, Etude de la protéine 1b liant la pénicilline (PBP1b) chez *E. coli* (Licence en Sciences biochimiques)

Kayath Aimé Christian, Etude de la PBP4, une protéine membranaire de la classe B d'*Enterococcus hirae* (DES en Biotechnologie)

Knapen Sarah, Diversité génotypique des cyanobactéries d'eau douce au sein du lac du Ri Jaune (Complexe de l'Eau d'Heure) (Licence en Sciences botaniques)

Libert Coralie, Sélection d'inhibiteurs peptidiques de bêta-lactamases à sérine active et de métallo-bêta-lactamases (Licence en Sciences chimiques)

Pennartz Anne, Les protéines reconnaissant le peptidoglycane: les PGRPs (DEA en Sciences)

Pipers Delphine, Construction d'une protéine hybride bifonctionnelle adaptée au dosage des immunoglobulines (Licence en Sciences biochimiques)

Ska Géraldine, Interaction entre la protéine PBP3 et les protéines FtsW tronquées chez *Escherichia coli* (Licence en Sciences biochimiques)

Van der Heiden Edwige, Etude du rôle physiologique de la protéine YajG d'*Escherichia coli* (Licence en Sciences biochimiques)

Vercheval Lionel, Production, purification et caractérisation des mutants H203A et R104A de la bêta-lactamase OXA-10 de *Pseudomonas aeruginosa* (Licence en Sciences biochimiques)

Wathelet Jennifer, Développement d'une bêta-lactamase hybride de haute affinité pour les immunoglobulines de type E en vue de la

construction d'un biosenseur (Licence en Sciences chimiques)

Wolf Benoît, Interaction entre les protéines de la division FtsW et PBP3 chez *E. coli* (DEA en Sciences)

2006

Berzigotti Stéphanie, Etude du divergeon *BC1074-1075-1076* de *Bacillus cereus* apparenté au divergeon *blaP-blaI-blaR* de *Bacillus licheniformis* (Licence en Sciences biochimiques)

Chevigné Andy, Caractérisation de l'interaction entre la protéase Derp1 et son propeptide (DEA en Sciences)

Dandois Sébastien, Etude de la protéine BlaB impliquée dans l'induction des bêta-lactamases BlaL et BlaU chez *Streptomyces cacaoi* (DEA en Sciences)

Di Paolo Alexandre, NMR studies of the Lambda lysozyme (DEA en Sciences)

Dony Nicolas, Etude de la formation de fibres amyloïdes par le lysozyme humain (Licence en Sciences biochimiques)

Gielis Julie, Etude de la fonction de la protéine Psr chez *Enterococcus hirae* (Licence en Sciences biologiques)

Jehaes Adrien, Développement d'un bio-polymère bioactivé (Licence en Sciences biochimiques)

Pipers Delphine, Etude de la protéine bi-fonctionnelle BlaPPL en vue de la mise au point d'un biosenseur électrobiochimique (DEA en Sciences)

Scarafone Natacha, Insertion de séquences amyloïdogéniques dans la bêta-lactamase de *Bacillus licheniformis* 749/C (Licence en Sciences biochimiques)

Vandenameele Julie, Introduction à l'étude du folding d'une bêta-lactamase à serine active (DEA en Sciences)**.**

2007

Argüelles-Arias Anthony, Contribution à l'étude d'un métabolite secondaire de *Bacillus subtilis* possédant une activité antimicrobienne (Licence en Sciences biochimiques)

Banzubazé Emmanuel, Construction de souches de *Bacillus subtilis* 168 dépourvues des PBP3 (*pbpC*), PBP4a (*dacC*) et PBP4* (*pbpE*). Caractérisation des mutants, étude de la formation de biofilms et de la localisation du PBP4a (DEA en Sciences)

Colson Séverine, Etude de la différenciation morphologique chez *Streptomyces coelicolor* en réponse à la disponibilité nutritionnelle (DEA en Sciences)

Craig Matthias, La protéine DasA est impliquée dans la perception des signaux nutritionnels contrôlant la différenciation morphologique de *Streptomyces coelicolor* (Licence en Sciences biologiques, orientation zoologie)

Dumez Marie-Eve, Etude du mécanisme d'activation du zymogène ProDer P 3 de l'acarien *Dermatophagoides pteronyssinus* (DEA en Sciences)

Fendri Linda, Etude d'expression différentielle de gènes en fonction de stress lumineux chez *Arthrospira* sp PCC8005 (Licence en Sciences biochimiques)

Jehaes Adrien, Développement d'un polymère à réticulation réversible (DEA en Sciences)

Kyembwa Michèle-Catherine, Caractérisation du signal transmis par BlaR chez *Bacillus licheniformis* (Licence en Sciences biochimiques)

Pisart Fabrice, Etude de la biodiversité enzymatique des microorganismes du sol par la métagénomique (Licence en Sciences biochimiques)

Sohier Jean-Sébastien, Anticorps à chaînes lourdes de camélidés comme source

d'inhibiteurs peptidiques dirigés contre les bêta-lactamases de classe A et B (DEA en Sciences)

Vandevenne Marylène, Caractérisation de la chitotriosidase de macrophage humain (DEA en Sciences)

2008

Berzigotti Stéphanie, Etude du signal transmis par le récepteur BlaR1 impliqué dans l'induction de la bêta-lactamase BlaP de *Bacillus licheniformis* (DEA en Sciences)

Defosse Chloé, Purification et caractérisation d'un peptide antibiotique produit par *Bacillus subtilis* (DES en Biotechnologie)

Genin Alexis, Production, purification et caractérisation de l'alpha-amylase de *Drosophila melanogaster* AmyD (Master complémentaire en Biotechnologie)

Gielis Julie, Etude de l'opéron *ftsW-psr-pbp5* chez *Enterococcus hirae* (DEA en Sciences)

Mathéus Nicolas, Production, purification et caractérisation des protéines hybrides (ES) BlaP-domaine D, D1, D2 et D3 de la Fibronectin binding Protein de *Staphylococcus aureus* (Licence en Sciences biochimiques)

Verchevalˆ Lionel, Production, purification et caractérisation de différents mutants de la beta-lactamase OXA-10 de *Pseudomonas aeruginosa* (DEA en Sciences)

Reriouedj Fares, Caractérisation des gènes de biosynthèse d'un lantibiotique chez *Bacillus subtilis* et mise au point d'une méthode de délétion génique *cre*/*lox* (DES en Biotechnologie)

Scarafone Natacha, Chimeric proteins as models to study the mechanism of aggregation associated with polyglutamine expansions (DEA en Sciences)

Tukumbane Kabasele, Production, purification et caractérisation du trigger factor de *Pseudoalteromonas haloplanktis* TAC125 (Master complémentaire en Biotechnologie)

2009

Balsamo Giuliana, Epitope mapping des anticorps anti-alpha hémolysine par phage display (Erasmus student, Università degli studi di Siena, Italia)

Cloes Marie, Caractérisation des propriétés de repliement de la bêta-lactamase à zinc de *Bacillus cereus* 569/H/9 (Master II BBMC)

Dehousse Morgane, Etude du processus de formation de fibres amyloïdes par le lysozyme humain (Master II BBMC)

Delsaute Maud, Recherche de nouvelles cytidylate kinases par criblage de séquences d'une banque métagénomique (Master II BBMC)

Godin Amandine, Mutagenèse et caractérisation d'oxydases bleues extrémophiles impliquées dans la résistance au cuivre (Master II BBMC)

Herman Julie, Etude du rôle des prolines du propeptide dans le repliement, la stabilité et l'activation de l'allergène ProDer P 3 de l'acarien *Dermatophagoides pteronyssinus* (Master II BBMC)

La Sala Marcello, Identificazione e caratterizzazione di un' α/β idrolasi psicrofila mediante approcio metagenomico (corso di laurea specialistica in Biotecnologie Industriali, Università di Napoli, Italia)

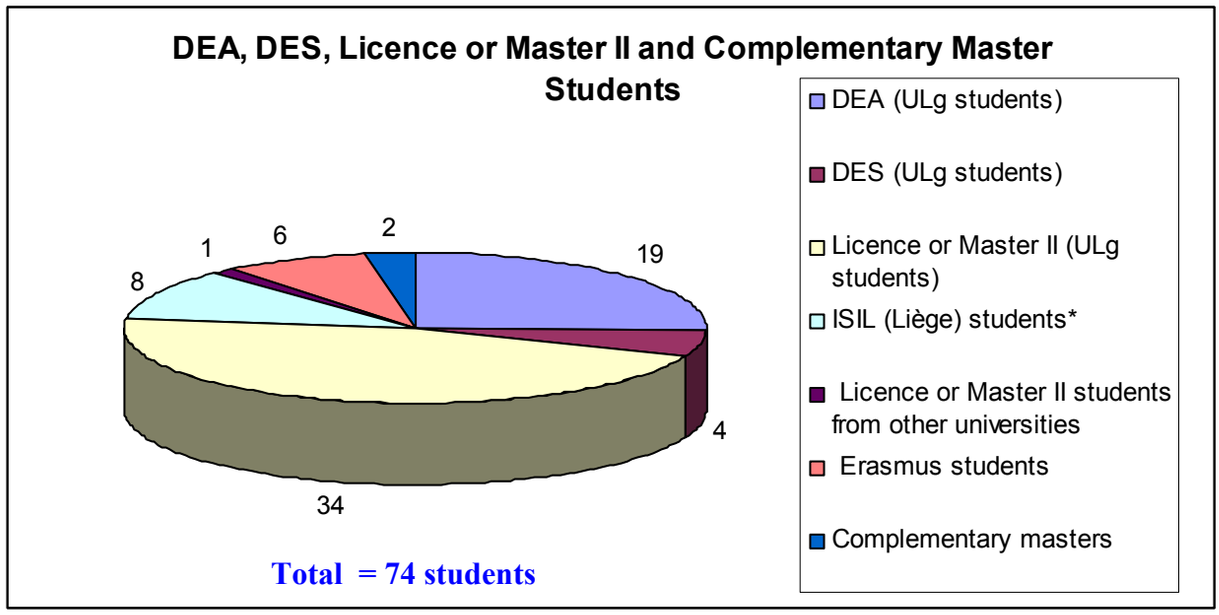
Negro Sonia, Production, purification et caractérisation d'un trigger factor psychrophile et de ses homologues mésophile et thermophile (Master II BBMC)

Pain Coralie, Production et caractérisation de chimères de la bêta-lactamase de *Bacillus licheniformis* 749/C contenant des inserts polyglutamine (Master II BBMC)

Rocaboy Mathieu, Etude structurale et enzymatique de complexes entre la DD-peptidase R39 d'*Actinomadura* sp R39 et les antibiotiques à noyau β-lactame de types pénicilline, céphalosporine et monobactame (Master II en Biochimie Structurale, Protéomique et Métabolomique, Université Paul Sabatier Toulouse, France)

Tedesco Pietro, Produzione ricombinante e purificazione di un mutante della β-lattamasi bacterica pasmidica CMY-2 (Erasmus student, Scienze biotecnologiche Università degli studi di Napoli Federico II, Italia)

Teveroni Emmanuela, Sviluppo della tecnologia delle proteine ibride bifunzionali applicate all'immunodosaggio (Erasmus student, Università degli studi di Siena, Italia)



Legend: **DEA** : Diplôme d'Etudes Approfondies, **DES** : Diplôme d'Etudes Spécialisées
BBMC : Biochimie et Biologie Moléculaire et Cellulaire
ISIL: Institut Supérieur Industriel Liégeois, Liège. *: Engineer in Biotechnology

GENERAL PUBLIC ACTIVITIES

Participation to Futuris, the European Reserach Programm on Euronews

http://www.euronews/en/sci-tech/futuris

In **June 2006**, Dr Bernard Joris introduced the Eur-Intafar project in the broadcast entitled 'Countering bacterial resistance to antibiotics'.

Activities for secondary school

November 11 2007: Dr Bernard Joris participated to a meeting between ULg researchers and secondary school students of the 'Institut Saint Roch' in Theux, Belgium. Various themes and the related ethical problems were approached.

Printemps des Sciences

Institute of Zoology, Quai van Beneden, Liège, Belgium

An organization of Réjoui**sciences** www.ulg.ac.be/sciences

- **March 19-23 2007:** The cyanobacteria group represented by Cédric Lassenge and Dr Annick Wilmotte organized a workshop and presented the International Polar Year and some related projects: MERGE and AMBIO. The role of Antarctic cyanobacteria has been emphasized. Hydrosoluble and liposoluble photosynthetic pigments from polar cyanobacterial strains were extracted and separation of pigments by Thin Layer Chromatography was performed.

- **March 10-16 2008**: during a hands-on workshop, the AMBIO project was presented by the Cyanobacteria group (Dr F. Zakhia, P. Simon and Dr Annick Wilmotte). The Photosynthetic pigments from Antarctic cyanobacterial strains were extracted and analyzed by Thin Layer Chromatography. In addition, a general introduction on the Cyanobacteria and a description of the International Polar Year 07/08 was given by Dr A. Wilmotte.

- **March 23-27 2009**: 'Cyanobactéries et évolution de la Terre et de la Vie', a workshop animated by Dr A.Wilmotte and Pr E. Javeaux (ULg Geology Department). Fossil and modern cyanobacteria were observed under the microscope and a power point presentation was followed by a discussion.

For more information, see: http://www.ambio.ulg.ac.be/activities.html and the blog created in 2009: http://www.antarcticabelgium.blogspot.com

Semence de curieux

Two RTBF radio broadcasts presented an interview in French with Dr Annick Wilmotte on the Cyanobacteria in polar zones. **March 25** and **April 1 2007**.

Wide audience conferences

- **April 23 2008**: 'La guerre des étoiles ou la résistance bactérienne aux antibiotiques' presented by Prof. Bernard Joris at the University of Liège, amphithéatres de l'Europe, Sart Tilman. An organization of the 'asbl Sciences et Culture': http://www.sci-cult.ulg.ac.be/Activites.html

- **September 9 2007**: **'**L'Antarctique, un continent microbien**'** presented by Dr Annick Wilmotte at 'Tour et Taxis' in Brussels for the inauguration of the Belgian Princess Elisabeth Antarctic base**.** The development of microbial communities, especially the cyanobacteria in Antarctica was emphasized.

Nuit des chercheurs

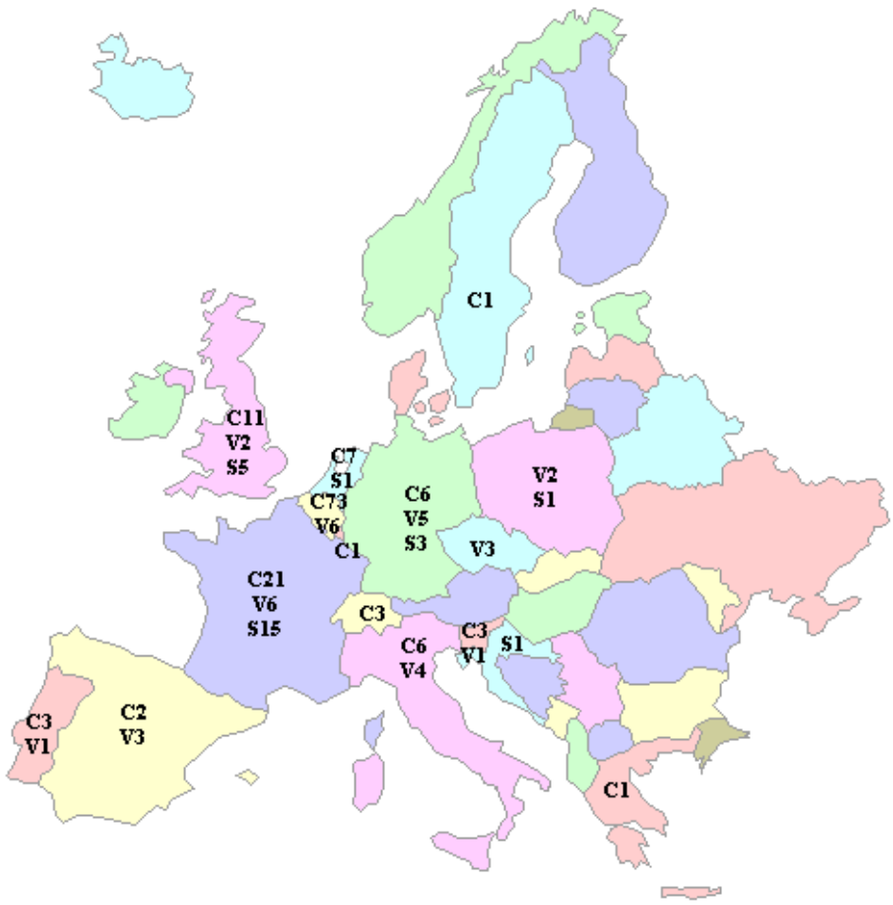
www.ulg.ac.be/nuitdeschercheurs

September 28 2007: several wide audience activities took place at the Institute of Zoology, Quai Van Beneden, Liège, Belgium. Dr Rafael Fernandez, Yannick Lara, Dr Annick Wilmotte and Dr Frédéric Zakhia showed polar cyanobacteria under the microscope and explained the cyanobacterial blooms at the visitors. Dr Colette Duez illustrated the negative or positive Gram colorations with slides of *Escherichia coli* or *Bacillus subtilis*. Petri dishes with bacteria from different genera were exhibited and the white/blue screening of recombinant bacteria was presented.

Participation to annual events organized by the SIEP

In 2007, 2008 and 2009, Adrien Jehaes PhD student at the Centre for Protein Engineering was a representative of the ULg Science Faculty at the exhibition organized in Bruxelles, Namur and Liège by the 'Service d'Information sur les Etudes et les Professions'.

INTERNATIONAL EXCHANGES



C Collaborations
V Visitors
S Stays abroad

COLLABORATIONS

Argentina

University of Buenos Aires - Laboratory of Microbiology - Faculty of Pharmacy and Biochemistry - Buenos Aires - **G. Gutkind**

Australia

University of New South Wales - School of Biotechnology and Biomolecular Sciences - Sydney - **R. Cavicchioli**

Belgium

Beldem-Puratos Group - Andenne - **J. Georis**

Belovo S.A. - Bastogne- **J. Lignan**

Biocode Hycel S.A. - Liège - **P. Stephanic**

C.E.R.I.A. - Institut des Industries de Fermentation - Bruxelles - **J. P. Simon**

Centre de Formation en Biotechnologies - GIGA-Forem - Liège - **L. Corbesier**

Centre d'Economie Rurale - Département Biotechnologie - Marloie - **B. Quinting & A. Collard**

Damhert NV/SA - Heusden- Zolder - **G. Schoofs**

D-Tek S.A. - Mons - **A. Vigneron**

Eumedica SA - Manage - **S. Carryn**

Eurogentec - Seraing - **D. Maréchal**

Euroscan Instruments SA – Vedrin- **B. Thibaut**

Euroscreen SA - Brussels - **S. Blanc**

FUNDP - Département de Pharmacie - Namur - **B. Masereel**

FUNDP - Laboratoire de Chimie Biologique Structurale - Namur - **J. Wouters**

FUNDP - Laboratoire Lasers et Spectroscopies - Département de physique - Namur - **P. Thiry**

FUSAGx - Centre de Biophysique Moléculaire Numérique - Gembloux - **R. Brasseur**

Glaxo Smith Kline Biologicals - Rixensart - **C. Gérard**

Institut de Recherches Microbiologiques Jean-Marie Wiame (IRMW) - Bruxelles - **C. Bauvois**

Isogen Life Science Belgium - Sint-Pieters-Leeuw - **J. Fevery**

IT4IP S.A. - Seneffe - **E. Ferain**

Kitozyme S.A. - Herstal - **M.-F. Versali**

KaHo Sint-Lieven - Laboratory of Enzyme and Brewing Technology - Faculty Industrial Engineering Biochemistry Department - Ghent - **G. Aerts**

KaHo Sint-Lieven – Departement of Chemistry and Biochemistry - Ghent - **I. Van de Voorde**

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**

KUL - Laboratory of Biomolecular Dynamics - Department of Biochemistry - Leuven - **Y. Engelborghs**

KUL - Laboratory of Medicinal Chemistry - Leuven - **P. Herdewijn**

Ludwig Institute for Cancer Research - Woluwe-Saint-Lambert - **B. Van den Eynde**

Nutrilab - Heusden-Zolder - **C. M. J. Vastenavond**

Prayon - Engis - **K. Tantaoui Elaraqi**

Progenosis - Liège - **F. Giannotta**

RUG - Department of Organic Chemistry, Organic and Bioorganic Synthesis - Ghent - **J. Van der Eycken**

RUG - NMR and Structure Analysis Unit – Department of Organic Chemistry - Ghent - **J. C. Martins**

RUG - Laboratory for Protein Biochemistry and Biomolecular Engineering - Ghent - **B. Devreese**

UCB - Braine l'Alleud - **J.-B. Hubert**

UCL - De Duve Institute - Louvain-la-Neuve - **J.-F. Collet**

UCL - Laboratoire de Biochimie Physique et des Biopolymères - Département de Chimie - Louvain-la-Neuve - **J. Fastrez**

UCL - Molécules, Solides et Réactivité – Institut de la Matière Condensée et des Nanociences - Louvain-la-Neuve - **D. Peeters**

UCL - Pharmacologie Cellulaire et Moléculaire - Département des Sciences Pharmaceutiques - Louvain-la-Neuve - **P. Tulkens**

UCL – Unité de Chimie Structurelle (CSTR) – Département de Chimie - Louvain-la-Neuve - **J.-P. Declercq**

UCL - Unité de Chimie et de Physique des Hauts Polymères - Institut de la Matière Condensée et des Nanosciences - Louvain-la-Neuve - **S. Demoustier**

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

ULB - Hôpital Erasme - Bruxelles - **J.-M. Boeynaems**

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

ULB - Service de Chimie Quantique et Photophysique - Bruxelles - **J. Liévin**

ULB - Service de Génétique Appliquée - Gosselies - **E. Godefroid**

ULB - Service de Physiologie Animale - Gosselies - **M. Moser**

ULB - Structure et Fonction des Membranes Biologiques - Faculté des Sciences - Bruxelles - **E. Goormaghtigh**

ULB - Unité de Toxicologie - Institut de Pharmacie - Bruxelles - **R. Kiss**

ULg - Biochimie et Microbiologie Industrielles - Département des Sciences de la Vie - Liège - **P. Thonart**

ULg - Biochimie et Physiologie Humaine et Pathologique - Département des Sciences Biomédicales et Précliniques - Liège - **L. Bettendorff**

ULg - Centre de Recherches sur les Protéines Prion - Département des Sciences Biomédicales et Précliniques - Liège - **W. Zorzi & B. Elmoualij**

ULg - Centre de Recherches du Cyclotron, Chimie Organique de Synthèse - Liège - **A. Zervosen** & **A. Luxen**

WOW Company - Naninne - **J. Demarteau**

Zentech S.A. - Angleur - **A. Bosseloir**

ULg - Centre d'Immunologie - Département des Sciences Biomédicales et Précliniques - Liège - **V. Geenen**

ULg - Chimie Analytique - Département de Pharmacie - Liège - **P. Hubert**

ULg - Chimie Biologique Structurale - Département de Chimie - Liège - **C. Damblon**

ULg – Département d'Electricité, Electronique et Informatique - GIGA-R - Liège - **L. Wehenkel**

ULg - Génomique Fonctionnelle et Imagerie Moléculaire Végétale - Département des Sciences de la Vie - Liège - **P. Motte**

ULg - GIGA-R : Biologie et Génétique Moléculaire - Département des Sciences de la Vie - Liège - **A. Lejeune, C. Evrard & C. Van de Weerdt**

ULg - GIGA-R - Rhumatologie Liège - **D. De Seny**

ULg - Laboratoire de Biologie des Tumeurs et du Développement - Département des Sciences Cliniques - Liège - **C. Généreux**

ULg - Laboratoire de Dynamique Moléculaire - Liège - **B. Leyh**

ULg - Laboratoire de Spectrométrie de Masse - Département de Chimie - Liège - **E. De Pauw**

ULg – Laboratoire d'Analyses - Département de Sciences des Denrées Alimentaires - Liègre - **G. Maghuin-Rogister**

ULg - Pharmacognosie – Faculté de Médecine - **L. Angenot**

ULg - Physiologie de la Reproduction - Département de Sciences Fonctionnelles - Liège - **J.-F. Beckers**

ULg - Physiologie végétale - Département des Sciences de la Vie - Liège - **F. De Lemos Esteves**

ULg - Technologie Pharmaceutique – Faculté de Médecine - **G. Piel**

ULg - Virologie - Immunologie - GIGA-R - Liège - **E. Dejardin**

UMH - Laboratoire de Physique des Surfaces et Interfaces - Mons - **J. De Coninck**

VUB - Institute for Molecular Biology and Biotechnology - Brussels – **D. Saerens & S. Muyldermans**

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

France

CEA – CNRS - Université Joseph Fourier - Laboratoire de Cristallographie et Cristallogenèse des Protéines - Institut de Biologie Structurale J.-P. Ebel - Grenoble - **J.-L. Ferrer**

CEA Grenoble - Laboratoire Chimie et Biologie des Métaux - Grenoble - **V. Forge**

CEA-CNRS UMR 5075-UJF - Laboratoire de Cristallographie Macromoléculaire - Institut de Biologie Structurale Jean-Pierre Ebel - Grenoble - **G. Garau**

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

CNRS IBS - Laboratoire d'Ingénierie des Macromolécules - Institut de Biologie Structurale Jean-Pierre Ebel (CEA-CNRS UMR 5075 UJF) - Grenoble - **T. Vernet**

CNRS IBS - Laboratoire de Cristallographie Macromoléculaire - Institut de Biologie Structurale Jean-Pierre Ebel (CEA-CNRS UMR 5075 UJF) - Grenoble - **A. Dessen**

CNRS IBS - Laboratoire de Résonance Magnétique Nucléaire - Institut de Biologie Structurale Jean-Pierre Ebel (CEA-CNRS UMR 5075 UJF) - Grenoble - **J.-P. Simorre**

CNRS Orléans - Centre de Biophysique Moléculaire - Orléans - **C. Damblon**

Inserm – Trafic, Signalisation et Ciblage Intracellulaires - Paris - **L. Johannes**

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

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

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

Laboratoire des Amino acides, Peptides et Protéines - Faculté de Pharmacie, Montpellier - Montpellier - **J.-F. Hernandez**

Novexel – Romainville - **T. Stachyra & C. Miossec**

UMR 6022 CNRS Compiègne - Génie Enzymatique et Cellulaire - Compiègne - **B. Bihan-Avalle**

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

Université de Nantes - ProtNeteomix - Nantes – **V. Sakanyan**

Université des Sciences et Technologies de Lille - Laboratoire de Procédés Biologiques, Génie Enzymatique et Microbien - Polytech'Lille - Villeneuve d'Ascq - **P. Jacques**

Université Paris 7 - Laboratoire de Pharmacochimie Moléculaire - Unité de Recherche Pharmacochimie Moléculaire et Systèmes Membranaires - Paris - **F. Heymans**

Université René Descartes - Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques - UMR 8601 CNRS - Paris - **Y. Le Merrer**

Université Paris VI, LRMA-INSERM E004 - Laboratoire de Recherche Moléculaire sur les Antibiotiques (LRMA) - Paris - **M. Arthur**

Université Paris-Sud, - UMR 8619 CNRS, Enveloppes Bactériennes et Antibiotiques - Institut de Biochimie - Orsay - **D. Mengin-Lecreulx**

Germany

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

RWTH-Aachen - Bioanalytics - Institute for Molecular Biotechnology - Aachen - **K. Hoffmann**

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

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

University of Kaiserslautern – School of Biology - Department of Microbiology - Kaiserslautern - **R. Hakenbeck**

University of Tübingen – Microbial Gentics - Faculty of Biology - Tübingen - **F. Götz**

Greece

University of Crete - Division of Applied Biology and Biotechnology - Department of Biology - Heraklion - **V. Bouriotis**

Italy

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

University of Siena – Department of Microbiology - Siena **– G. Rossolini**

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

University of Bologna – Department of Chemistry "G. Ciamician" - Bologna - **D. Giacomini**

University of Modena and Reggio Emilia - Department of Chemistry - Modena - **F. Prati**

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

University of Pavia - Department of Genetics and Microbiology - Pavia - **A. Galizzi**

Luxembourg

Centre de Recherche Public-Santé - Laboratoire d'Immunogénétique et d'Allergologie -Val Fleuri - Luxembourg – **C. Hilger**

Japan

Chiba University - Graduate School of Advanced Integration Science - Chiba - **A. Saito**

Portugal

Catholic University of Portugal - Superior School of Biotechnology - Porto - **A. Bernardino de Almeida**

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

National Institute of Engineering, Technology and Innovation - Department of Biotechnology - Lisbon - **J. Duarte**

Russia

Winogradsky Institute of Microbiology RAS, - Moscow – **Z. Namsaraev**

Slovenia

Lek Pharmaceuticals d.d.-Drug Discovery - Ljubljana – **U. Urleb**

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

University of Ljubljana - Department of Pharmaceutical Chemistry - Faculty of Pharmacy - Ljubljana - **S. Gobec**

Spain

CHU Juan Canalejo - Laboratory of Microbiology-INIBIC - La Coruña - **G. Bou**

University of Granada - Molecular Biophysics and Biotechnology Group - Department of Physical Chemistry and Institute of Biotechnology - Granada - **F. Conejero-Lara**

Sweden

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

Switzerland

Basilea Pharmaceutica International Ltd - Basel - **M. G. P. Page**

DSM Nutritional Products Ltd. - Basel – **G. Schyns**

Lonza Ltd - Basel - **D. Elbers**

The Netherlands

Leiden University - Center for Electron Microscopy - Leiden - **A. M. Mommaas**

Leiden University - Clusius Laboratory - Institute of Biology - Leiden - **G. Bloemberg**

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

Maastricht University Medical Centre - NUTRIM School for Nutrition, Toxicology and Metabolism - Maastricht - **C. Vreuls**

Promega Benelux BV - Leiden - **A. Loriaux**

University of Amsterdam - Molecular Dynamics of the Cell Cycle - Swammerdam Institute for Life Sciences - Amsterdam - **T. den Blaauwen**

Utrecht University - Institute of Biomembranes - Department Biochemistry of Membranes, Bijvoet Center, - Utrecht - **E. Breukink**

United Kingdom

AbD Serotec - Kidlington - **A. Lane**

John Innes Centre - Molecular Microbiology - Department of Molecular Microbiology - Norwich - **D. Hopwood**

University of Cambridge - Cambridge - **C. V. Robinson**

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

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

University of Leicester - Biological NMR Centre - Department of Biochemistry - Leicester - **C. Damblon & G. Roberts**

University of Newcastle upon Tyne - Institute of Cell and Molecular Biosciences - Newcastle upon Tyne - **W. Vollmer**

University of Nottingham - School of Biology - Nottingham - **M. Alcocer**

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

University of Oxford - Oxford Center for Molecular Sciences and Dyson Perrins Laboratory - Oxford - **C. J. Schofield**

University of Strathclyde - Microbiology - Strathclyde Institute of Pharmacy and Biological Science - Glasgow **- P. Hoskisson**

USA

Bio-Rad - Hercules - **W. Liu**

Cerexa - Cookland -, CA - **D. Biek**

Neogen - Food Safety Division - Lansing - **F. Klein & J. Rice**

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

The Pennsylvania State University - Mueller Lab - Department of Biolgy - Pennsylvania - **D. J. Cosgrove**

University of Missouri-Kansas City - School of Pharmacy, Division of Pharmaceutical Sciences - Kansas City - **W. G. Gutheil**

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

VISITORS

Al Ahmadi Jami, University of Tehran, Iran, 11 November 2006 - 4 June 2007

Bannwarth Ludovic, Université de Paris 6, France, 18-19 November 2007

Banzhaf Manuel, University of Newcastle, UK, 5-9 October 2009

Ben Achour Nahed, University of Tunis, Tunisia, 8 May 2007 – 7 November 2007

Ben Aissa Melek, University of Tunis, Tunisia, 1 October–15 December 2006, 17 March - 16 June 2007

Benmedhi Houcine, Université de Paris VII, France, 2-13 July 2007

Bili Daniela, University of Rome, Italy, 14-17 October 2009

Borgianni Luisa, University of Siena, Italy, 1-30 March 2007 and 12-20 May 2009

Boudet Julien, IBS, Grenoble, France, October 2005 (1 week)

Bourry David, University of Ghent, Belgium, 4-17 November 2007, 9-14 December 2007

Bun Sylvie, UTC Compiègne, France, 1-28 February 2008

Coejero-Lara Francisco, University of Granada, Spain, 26-28 March 2009

Druyer François, Université de Pau et Pays de l'Adour, Mont-de-Marsan, France, April 7- July 7, 2008

El Hajjaji Hayat, Université Catholique de Louvain, Belgique, 9-21 January 2006, 13-26 March 2006, 21 May -6 June 2007, 1-19 November 2007, 26 November - 16 December 2007, 17-27 January 2008

Elster Josef, University of South Bohemia, Czech Republic, 3-12 December 2007

Ervenich David, RWTH-Aachen, Germany, 23-24 April 2008

Foma Kibwega Roland, CWBI Gembloux, Belgique, 1-31 December 2009

Forge Vincent, CEA, Grenoble, France, 3-8 December 2006

Gallo Gianluca, University of Naples, Italy, 08 May- 30 June 2007

Gangoué Piéboji Joseph, University of Yaounde, Cameroon, 9 January - 9 March 2007, 8 October - 9 December 2007

Giustini Cécile, IBS, Grenoble, France, 1 week in February 2008

Hosking Edan, Neogen, USA, 7-19 September 2009

Kan Beki, Marmara University, Turkey, 18-23 May 2008

Koch Michel, EMBL, Hamburg, Germany, 19-21 September 2007

Komarek Jiri, University of South Bohemia, Czech Republic, 3-12 December 2007

Maurer Patrick, University of Kaiserslautern, Germany, 2-6 October 2005

Meersman Filip, Catholic University of Leuven, Belgium, 19-21 September 2007

Mendes da Silva Ferreira Rui Manuel, University of Aveiro, Portugal, 01 October 2005-31 March 2006

Pain Roger, Jozef Stefan Institute, Llubljana, Slovenia, 31 August-6 September 2005, 31 August-6 September 2006, 30 August -2 September 2007, 28 August-2 September 2008, 30 June-5 July 2009, 2 October-4 October 2009

Pellegrini Cristina, University of L'Aquilla, Italy, 02 May-31 July 2006

Perez Llarena Francisco José, University A Coruña, Spain, 4-10 May 2008, 22 July- 22 August 2008

Raji Kaoubab Abdelmoughit, Université Libre de Bruxelles, Belgique, 9-11 November 2009, 7-11 December 2009.

Redfield Christina, University of Oxford, UK, 30 November – 1 December 2006

Réjiba Samia, University of Tunis, Tunisia, 15 February – 15 September 2008

Schmid Franz, University of Bayreuth, Germany, 12-13 October 2006

Strunecky Otakar, Institute of Botany, Czech Academy of Science, Czech Republic, 16-27 March 2009

Tantaoui Elaraqi, Khadija, Prayon SA, Belgium, 1-31 December 2009; November 2009 - May 2010

Tischer Alexander, University Halle-Wittenberg, Germany, 21 July-7 October 2005

Varela Lorena, University of Granada, Spain, 12 May- 10 July 2009

Waleron Krzystof, University of Gdansk, Poland, 18-31 January 2009

Waleron Malgorzata, University of Gdansk, Poland, 4-10 March 2007, 14-27 October 2007, 18-31 January 2009

Yilmaz Meral, Anadolu University, Turkey, 9 October 2006- 9 March 2007

Zawadzka-Skomial Joanna, University of Warsaw, Poland, 4-29 April 2005

Zeba Boukaré, University of Ouagadougou, Burkina Faso, 1 October - 24 November 2008

STAYS ABROAD

Amoroso Ana, IBS, Grenoble, France. February 23-27, 2009

Barumandzadeh Roya, Modélisation, Interactions et Repliement Laboratoire Chimie et Biologie des Métaux (LCBM), CEA, Grenoble, France, June 22-26, 2009

Bebrone Carine, Different stays at the Institute for Molecular Biotechnology, RWTH-Aachen, Fraunhofer IME, Aachen, Germany, March 15-30, 2007 and one or two days every two weeks till December 2009

Berlemont Renaud, Genomic and Applied Microbiology, University of Göttingen, Germany, September-December, 2005

Magdalena Calusinska, Department of Biotechnology of the Intercollegiate Faculty of Biotechnology, University of Gdansk, Poland, December 8-24, 2008 and July 26-31, 2009

Charlier Paulette, ten stays at ESRF, Grenoble, France, for x-ray diffraction data collections at FIP/BM30a beamline.

Colson Séverine, Chair for Microbiology, University Friedrich Alexander, Erlangen, Nuremberg, Germany, November 20-28, 2006

Dandois Sébastien, ESRF, Grenoble, France, July 26-28, 2008

Di Paolo Alexandre, Department of Biochemistry, University of Oxford, UK, March- September, 2006

Dumez Marie-Eve, Institut de Biologie Structurale Jean-Pierre Ebel, Laboratoire de Cristallographie et Cristallogenèse des Protéines (LCCP), December 9-14, 2007

Dumoulin Mireille, Department of Chemistry, University of Cambridge, UK, April 13-17, 2009

Joris Bernard, IBS, Grenoble, France. February 23-27, 2009

Herman Raphaël, several stays at ESRF, Grenoble, France, for X-ray diffraction data collections at FIP/BM30a beamline

Kerff Frédéric, several stays at ESRF, Grenoble, France, for x-ray diffraction data collections at FIP/BM30a beamline.

Lassaux Patricia, Institut de Biologie Structurale Jean-Pierre Ebel, Laboratoire de Cristallographie et Cristallogenèse des Protéines (LCCP), January 1-June 30, 2008

Matagne André, University of Oxford, UK, 25-29 June 2006 and 23-26 January 2008

Matagne André, University of Cambridge, UK, 24-28 July 2006 and 13-14 November 2007

Matagne André, CEA Grenoble, France, October 22-28, 2006 and November 9-11, 2009

Namsaraev Zorigto, Field trip to Svalbard field station of the Institute of Botany AS CR, Norway, July 18-29, 2009

Rigali Sébastien, Summer school in Applied Molecular Microbiology, Mediterranean Institute for Life Science, Split, Croatia, June 23-July 1, 2007

Sauvage Eric, several stays at ESRF, Grenoble, France, for x-ray diffraction data collections at FIP/BM30a beamline

Scarafone Natacha, Department of Chemistry, University of Cambridge, UK, April 13-17, 2009

Vandenameele Julie, Biological NMR Centre, University of Leicester, U.K., April 23 –May 20, 2006 and July 19-26, 2006

Vandenameele Julie, CEA, Laboratoire de Biophysique Moléculaire et Cellulaire, Grenoble, France, May 22- June 4, 2005 ; November 5-18, 2006 and December 10-16, 2006

Vandenameele Julie, CNRS, Centre de Biophysique Moléculaire, Orléans, France, June 8-13, 2008

Vandevenne Marylène, Institut de Biologie Structurale Jean-Pierre Ebel, Laboratoire de Cristallographie et Cristallogenèse des Protéines (LCCP), March 30 - April 10, 2008

Vercheval Lionel, two stays at IBS and ESRF, Grenoble, France, for x-ray diffraction data collections at FIP/BM30a beamline. December 8-14, 2007 and March 30-April 10, 2008

Wilmotte Annick, Antarctic Station Princess Elisabeth, Utsteinen, Dronning Maud Land, Antarctic, January 20-February 20, 2009

Wolf Benoit, Institute for Life Sciences, University of Amsterdam, The Netherlands, September 2-16, 2006

FUNDING


FNRS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FUNDING

Les Pôles d'Attraction Interuniversitaires

PAI P5/33 (2002-2006) - Protein structure and function in the post-genomic, proteomic era (the CIP was the Coordinator)

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

Politique Scientifique Fédérale Belge

SSTC EV/12/34A (2003-2006) - Algal bloom: emerging problem for health and sustainable use of surface

BELSPO C3/00/14 (2005-2010) - Elaborating public culture collections of diatoms, polar cyanobacteria and mycobacteria in Belgium

BELSPO SD/CA/01A (2005-2009) - HOLANT: variabilité du climat et changements écologiques dans la zone côtière de l'Antarctique est et maritime pendant l'holocène

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 EA/00/05A (2009-2011) - BELDIVA : Exploration belge de la diversité microbienne dans la région de la Station Princesse Elisabeth, Antarctique

Bourse POST-DOC pour chercheurs hors EU : Z. Namsaraev (03/03/2007 au 10/07/2007 et 15/10/2007 au 7/05/2008)

Return Grant from the Belgian Science Policy: M. Terrak **(**2004-2006)

Return Grant from the Belgian Science Policy: F. Sapunaric (2005-2007)

Les Actions de Recherche Concertées

ARC 03/08-297 (2003-2008) - L'usine cellulaire d'assemblage de la paroi bactérienne (Promotor)

ARC 07/12-04 (2007-2012) – MICRO-H_2: 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 (2003-2006) - Utilisation du rayonnement synchrotron : développement de la recherche en biologie structurale par l'étude des macromolécules via la cristallographie

FNRS 2.4550.05 (2007-2008) - Etude des mécanismes moléculaires associés à la conversion d'une protéine soluble en fibres amyloïdes

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 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 pour le Dr Ana Amoroso (1/05/2004 au 30/04/2005), **Convention n° 2.4530.03 F/15-MCF/SD-15051**

Mandat FNRS de Chercheur Temporaire Postdoctoral pour le Dr Mireille Dumoulin (1/01/2005 au 31/12/2005)), **Convention n° 2.4550.05**

Mandat FNRS de Chercheur Temporaire Postdoctoral pour le Dr Mireille Dumoulin (1/01/2006 au 31/12/2007), **Convention n° 2.4550.05**

Mandat FNRS de Chercheur Temporaire Postdoctoral pour le Dr Mireille Dumoulin (1/01/2007 au 30/9/2008), Référence F 6/15 – MCF/OL – P 12, **Convention n° 2.4550.05**

Mandat FNRS de Chercheur Temporaire Postdoctoral pour le Dr Joseph Gangoué Piéboji (1/10/2008 au 1/10/2009), **Convention n° 2.4511.06**

Mandat FNRS de Chercheur Temporaire Postdoctoral pour le Dr Michaël Nigen (1/01/2009 au 31/12/2010), avenant à la **Convention F.R.F.C. n° 2.4530.09**

Mandats de doctorants FRIA : 35

Mandats d'aspirant : Chevigné A., Vandevenne M. et Struvay C.

Mandats de Chargé de Recherche : Fickers P., Kerff F. et Bebrone C.

Fonds de la Recherche Fondamentale Collective

FRFC 2.4530.03 et 9.4527.05 (2003-2006) - Induction des bêta-lactamases et des xylanases chez les bactéries à gram positif. Etude du signal transmis et de la réponse cellulaire

FRFC 2.4515.00 (2004-2007) - Les stratégies d'adaptation au froid d'enzymes de l'Arctique et de l'Antarctique

FRFC 2.4580.05 (2005-2007) - Diversité des picocyanobactéries du lac Tanganyika

FRFC 2.4550.05 (2005-2008) - Etude des cinétiques de folding de quatre types de protéines modèles à l'aide des techniques de mélange rapide combinées à diverses méthodes spectroscopiques et aux méthodes d'échange hydrogène/deutérium

FRFC 2.4511.06 (2006-2009) – Structure et mécanismes catalytiques des enzymes impliquées dans la résistance aux antibiotiques

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 2.4530.09 (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

Fonds de la Recherche Scientifique Médicale

FRSM 3.4586.05 (2005-2008) - Solubilisation de protéines membranaires par modélisation in silico et mutagenèse dirigée. Caractérisation biochimique et structurale des mutants solubles

Région Wallonne

RW 4768 (2003-2006) - Purification des protéines auto-antigéniques

RW 4778 (2003-2006) - Optimisation de *B. subtilis* en tant qu'usine cellulaire

RW 215056 (2002-2005) - LPSens : détection et purification des endoxines bactériennes

RW 215120 (2003-2006) - OPARRAY : Développement d'un protein-array orienté et d'une détection quantitative intrinsèque

RW 215123 (2003-2006) - VACCINS : Méthodologie pour le développement des vaccins recombinants

RW 215365 (2003-2006) – α-BUSTEC : Conception d'un "α-helix bundle" soluble comme système support de boucles hydrophiles de protéines membranaires

RW 415701 (2004-2008) - SENSOTEM : Mise au point d'un biosenseur pour le diagnostic des maladies virales

RW 415896 (2005-2009) - APTARRAY : Elaboration de biopuces aptamériques destinées à l'identification et au typage des virus animaux (Réseau II)

RW 415897 (2005-2009) - AUREA : Prévention vaccinale des mammites bovines à *Staphylococcus aureus* (Réseau II)

RW 415951 (2005-2009) - LACOMAT : Mise au point d'un matériau biocatalytique granulaire recyclable pour l'épuration des eaux

RW 516208 (2005-2009) - ADJUVAC : Identification, caractérisation et validation de molécules adjuvantes pour la vaccination néonatale

RW 516025 (2006-2009) - GOCELL : Pansement acellulaire bioactivé pour le traitement des ulcères cutanés

RW 516073 (2006-2009) - TOLEDIAB : Développement d'un vaccin tolérogène/régulateur contre le diabète de type 1

RW 516265 (2006-2010) - MED-ATR : Dosage en temps réel de substances pharmacologiques dans des fluides physiologiques

RW 616293 (2006-2009) - ALLERVAC : Mise au point d'une méthode vaccinatoire anti-allergique basée sur l'utilisation d'immunosomes

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 (2008-2010) SCALAB : systèmes de production d'anticorps à grande échelle

Union Européenne

HPRN-CT-2002-00264 (2002-2006) - MEBEL: Metallo-β-lactamases as model Zn enzymes (Coordinator)

LSHM-CT-2003-503335 (2004-2006) - COBRA: Combating resistance to antibiotics by broadening the knowledge on molecular mechanisms behind resistance to inhibitor of cell wall synthesis (Partner)

LSHM-CT-2004-512138 (2005-2009) - EUR-INTAFAR: Inhibition of new targets for fighting antibiotic resistance (Coordinator)

LSHM-CT-2006 – 037525 (2009) - EURAMY: Systemic Amyloidosis in Europe (Partner)

Université de Liège

Crédits classiques

Projet C-06/19 (2005) - Etude structurale des protéines impliquées dans le stress cellulaire induit par la pénicilline

Projet C-05/68 (2005) - Etude des cinétiques de folding des protéines, à l'aide des techniques de mélange rapide combinées à diverses méthodes spectroscopiques

Projet C-09/75 (2009) - 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 β-lactamases à sérine et à zinc

Mandat de post-doctorat à l'ULg pour chercheur étranger : K. Waleron (1/12/2007 au 30/11/2008).

MISSIONS OF EXPERTISE

Member of Research Councils

Prof. Jean-Marie Frère
Conseil de la Recherche (ULG, Belgium): 2005-2006

Prof. Moreno Galleni
Membre du conseil facultaire de la Recherche (2005-2009)
Commissaire à la recherche du Département des Sciences de la Vie (2005-2009)
Membre du Conseil sectoriel de la Recherche "Science et Technique" (2009-)
Membre du Conseil Universitaire de la Recherche (2009-)

Member of editorial board

Prof. Jean-Marie Frère, Antimicrobial Agents and Chemotherapy (2005-2009), Biochemical Journal (2005-2008), Biochemical Pharmacology, 2005-2008

Dr Georges Feller, Extremophiles

Prof. Moreno Galleni, Antimicrobial Agents and Chemotherapy

Dr Annick Wilmotte,
FEMS Microbiology Reviews (2002-2005)
Guest Editor of a special issue of Polar Science: Kanda Merge (Polar Science, **3**, November 2009)

Member of the editorial advisory panel

Prof. André Matagne, The Biochemical Journal

Dr Georges Feller, Biologia (Bratislava)

Reviewing activity for the following journals

Acta Crystallographica
Algological Studies
Analytical Chemistry
Applied Microbiology and Biotechnology
Archiv der Pharmazie
Archives of Microbiololgy
Biochemical and Biophysical Research Communication
Biochemical Journal
Biochemical Pharmacology
Biochemistry
Biochimie
Bioorganic and Medicinal Chemistry Letters
Bioorganic and Medicinal Chemistry
Biophysica and Biochemica Acta
Biophysical Journal
Biotechnology Advances
Biotechnology and Bioengineering
Canadian Journal of Microbiology
Chemical and Medicinal Chemistry
Current Opinion in Chemical Biology
Drugs
Environmental Technology
Enzyme and Microbial Technology
European Journal of Biochemistry
European Journal of Medicinal Chemistry
Extremophiles
FEBS
FEBS Journal
FEBS Letters
FEMS Microbiology Ecology
FEMS Microbiology Letters
International Journal of Biological Macromolecules
Journal of the American Chemical Society

Journal of Bacteriology
Journal of Biotechnology
The Journal of Biological Chemistry
Journal of Chemical Theory and Computation
Journal of Molecular Biology
Journal of Molecular Catalysis
Journal of Phycology
Journal of Photochemistry and Photobiology
Journal of Solid State Chemistry
Marine Genomics
Medical Science Monitor
Microbiology
Microbiological Research
Molecular Microbiology
Nature Reviews Microbiology
Photochemical and Photobiological Sciences
PloS Biology
Polar Science
Proceedings of the National Academy of Sciences
Protein Engineering Design and Selection
Process Biochemistry
Proteins
Protein Science
Proteomics
Sedimentary Geology
Tetrahedron
Thermochimica Acta
Trends in Microbiology

Reviewing Activity of Research Projects/ Expertises for Scientific Commissions

Agence Nationale de la Recherche ANR (France)
Agence Nationale de la Recherche ANR programme jeunes chercheurs (France)
Austrian Science Fund
BBSRC (UK)
BQR Université Henri Poincaré Nancy
CNRS
Dutch Technology Foundation
EMBO
EUROPOLAR (ESF)
F.R.S-F.N.R.S.
Fondation de la Recherche Médicale FRM (France)
FP6 Mobility 8-9-10 mars-avril 2006
F.R.I.A.
FWO
INSERM (France)
MURST (Italy)
National Science Foundation (USA).
Programme Blanc de l'ANR (France)
Région Bruxelloise: Impulse Program in Life Sciences (Belgium)
Région Bretagne
Sultan Qaboos University Postgraduate Studies and Research et en Belgique
Slovak Research and Development Agency
The Eli and Edythe L. Broad Foundation
The Netherlands Organisation for Scientific Research (NWO), Pays-Bas
The United States-Israel Binational Agricultural Research and Development Fund
Wellcome Trust (UK)

Expertise of research unit activities

Comité d'Evaluation de l'Unité de Glycobiologie Structurale et Fonctionnelle UMR CNRS/USTL n° 8576, 5 et 6 janvier 2009

COMMITTEES AND SOCIETIES

Charlier Paulette

Comité National Belge de Cristallographie (représentant ULg), Vice-president since 2009

Groupe de contact F.R.S.-FNRS « Rayonnement Synchrotron », Secretary since June 2007

Galleni Moreno

Vice-president of BioLiège (2009-)

Matagne André

Belgian Biophysical Society, Vice-president 2002- 2006; President 2007 -

National Committee of Biophysics (Belgium, since 2003; President 2009- …)

Wilmotte Annick

Vice-chair of the 'ad hoc' Committee for the preparation of the IPY (Academy of Belgium) (2006-2009)

Scientific expert of the Belgian Delegation to the Committee of Environmental Protection of the Antarctic Treaty (since 2008)

Belgian National Committee for Antartic Research (2008-)

Subcommittee for the Taxonomy of Pathotrophic bacteria of the International Committee on Systematic Bacteriology (ICSB)

COMPOSITION OF THE CENTRE

Managing Committee

Director

Jean-Marie Frère (-2008)

Bernard Joris (2008-)

Current Executive Committee:

Paulette Charlier, Mireille Dumoulin, Moreno Galleni, André Matagne, 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, André Matagne, Paola Mercuri, 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)

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 Claudine Fraipont
Dr Jean-Marie François
Melle Astrid Freichels
Dr Patricia Lassaux
Melle Sarah Lebrun
Dr Paola Mercuri
Dr Zorigto Namsaraev
Dr Noureddine Rhazi
Dr Frédéric Sapunaric
Dr Eric Sauvage
Dr Brahim Semane
Dr Alexandre Di Paolo
Dr Julie Vandenameele
M. Olivier Verlaine

PhD students

Anthony Argüelles Arias
Roya Barumandzadeh
Stéphane Baurin
Stéphanie Berzigotti
Ahlem Bouaziz
Magdalena Calusinska
Chloé Chavignon
Alexandre Cipolla
Séverine Colson
Matthias Craig
Sébastien Dandois
Pedro De Carvalho Maalouf
Morgane Dehousse
Maud Delsaute
Nicolas Dony
Marie-Eve Dumez
Janice Dumont
Anne Famerie
Rafael Fernandez Carazo
Adriana Fernea
Amandine Godin
Jessica Guillerm
Badre-eddine Halimi
Julie Herman
Séverine Hubert
Olivier Jacquin
Adrien Jehaes
Yannick Lara
Sophie Laurent
Marie-José Mano
Georges Moutzourelis
Coralie Pain
Anne Pennartz
André Piette
Florence Piette
Delphine Pipers
Mathieu Rocaboy
Frédéric Roulling
Natacha Scarafone
Jean-Sébastien Sohier
Caroline Struvay
Elodie Tenconi
Edwige Van der Heiden

Technical assistance

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