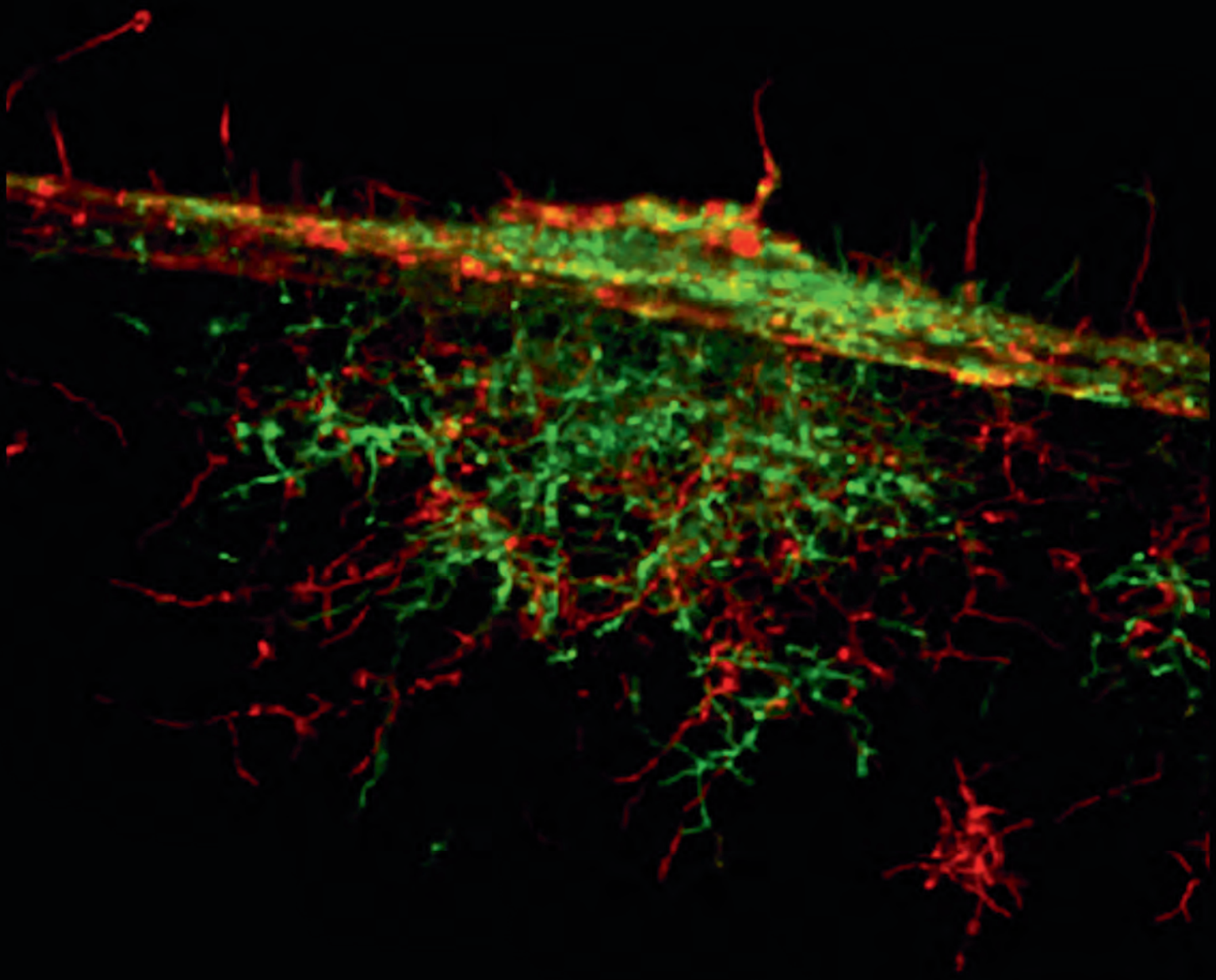
# Center for Protein Engineering

## Activity report 2011



University of Liège

CIP

'Live/Dead staining of a young *Streptomyces coelicolor* colony'
(author: Elodie Tenconi)

*This report is dedicated to Jean Dusart who died in July 2012*

Our friend Jean Dusart died on July 17, 2012. He was one of the founding members of the CIP and, for many of us, he was more a friend than just a colleague.

Jean obtained his M.Sc. from the University of Liège and, in January 1965, moved to the University of Montreal, to the group of Walter Verly, where he studied the effects of chemical mutagenesis in *Escherichia coli*. He presented his Ph.D. in September 1969 and returned to Liège in May 1970 to join the team of Jean-Marie Ghuysen. As one of the microbiologists of the group, he specialised in the study of *Streptomyces*, the pet organism of the laboratory. Due to the rather long generation time of this bacterium, it was not always easy to avoid contamination in the rather long-lasting cultures and Jean was always ready to help those of us who had less experience in microbiological techniques. In 1977, he was appointed Chercheur Qualifié (Research associate) of the FNRS, a permanent position and thus able to start to develop his own group. In 1983, in order to introduce molecular biology and genetic engineering approaches in the lab, he visited David Hopwood at The John Innes Centre in Norwich where he learned the cloning trade applied to *Streptomyces*. This rapidly yielded very successful results that enabled the cloning, sequencing and overproduction of many enzymes, including beta-lactamases and DD-peptidases for which large-scale cultures and time-consuming chemical methods had previously been necessary. In this respect, it is clear that Jean's work was instrumental in making the development of structural biology in Liège possible. He later devoted a lot of time to the study of gene regulation in *Streptomyces*, a subject which is now in the hands of his former student, Sébastien Rigali. In a gentle but rigorous way, Jean mentored many young scientists who obtained their doctorates under his direction. Most of his former PhD students greatly appreciated his permissive and at the same time paternal way of supervising. He managed to generate a competition-free working atmosphere where all members could freely develop their own project. Freedom and an ideal working environment for his PhD students were Jean's major priorities as supervisor, far before his own ambition. He was also much appreciated as an undergraduate teacher.

Jean strongly believed that cooperation was much more effective than competition. This is certainly why he was extremely active in the foundation of the CIP and its emergence as a major actor in the landscape of Biochemistry and Molecular Biology at Liège. He was a very organised and lucid member of the board of the Centre and always considered the human aspects as most important. We are all indebted to his unceasing activity to promote the Centre and his collaborators in a most sincere and disinterested manner. He tried to avoid confrontations, to solve all the problems by consensus and to put the general interest before his own.

Outside the lab, and besides his family (his wife, Suzette, and their 3 children), he had two other major centres of interest: painting and the protection of the countryside environment. The latter explains his involvement in local politics over the last years. Jean will be regretted by all his friends for his kindness, his honesty, his receptiveness and his great sense of humor.

# Contents

# INTRODUCTION

2011 has been a challenging and evolving year.
Institutional reform conducted by our University has generated changes in our Centre. Indeed, we decided to update our status and to position the Centre for Protein Engineering as a multi-disciplinary thematic research unit included in the Department of Life Sciences.
In May 2011, a new CIP website has been launched: www.cip.ulg.ac.be
During this year, we succeeded to renew our most important research funding in the frame of Interuniversity Attraction Pole program from the Belgian Science Policy Office. The new project iPros (Integrative Protein Science) will allow us to continue and develop our research within a network including the best Belgian teams in Protein Science. In a period of economical crisis, we are extremely grateful for the generous financial support that BELSPO, WBI, RW, FNRS and ULg give to the members of our group by means of the different funding programs.
Similarly, the GIGA platform for high-throughput protein mutagenesis, acquired in the frame of a FEDER program, has been installed in the CIP and its equipment was completed using funds obtained by our researchers. The acquisition of this liquid-handling robotic workstation completes and enhances the capabilities and expertise of our platform for production and purification of recombinant proteins. Dr Alain Brans, a specialist of fermentations in *E. coli* and *Bacillus* obtained a tenured position to manage this platform that the CIP proposed to turn into a cell to support research and teaching (Cellule d'Appui pour la Recherche et l'Enseignement, CARE).
In April and May 2011, the CIP was honoured by the arrival of three post-doctoral researchers coming from Australia, Argentina and France.
Our scientific production during the year 2011 resulted in forty-two publications in international peer reviewed journals and two book chapters and reviews. One patent was also registered.
Finally, I would like to thank all CIP staff members, those producing excellent science and those working in the background to keep the Centre running and improving. It is their work that makes CIP the success story that it is – and some highlights of this work are featured in this report.

While preparing this report, we have been very sad to learn the death of Jean Dusart, one of the founding members of our Centre. We will miss him.

Bernard Joris

# RESEARCH GROUPS

## APPLIED QUANTUM CHEMISTRY AND MODELLING

Group leader: **Dr Georges Dive**

Associate member: **Dr Dominique Dehareng**

## BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS

Group leader: **Prof. Bernard Joris**

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

Associate members:
**Dr Ana Amoroso**
**Dr Magda Calusinska**
**Dr Arabela Cuirolo**
**Dr Michaël Delmarcelle**
**Dr Adeline Derouaux**
**Dr Zorigto Namsaraev**
**Mr André Piette**
**Mr Olivier Verlaine**

## BIOLOGICAL MACROMOLECULES AND BIOCHEMISTRY

Group leader: **Prof. Moreno Galleni**

Permanent scientist: **Dr Georges Feller**

Associate members:
**Dr Etienne Baise**
**Dr Carine Bebrone**
**Dr Renaud Berlemont**
**Dr Marie-Eve Dumez**
**Dr Jean-Marie François**
**Dr Olivier Jacquin**
**Dr Paola Mercuri**
**Dr Noureddine Rhazi**
**Dr Frédéric Sapunaric**

# RESEARCH GROUPS

## BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY

Group leader: **Prof. Paulette Charlier**

Permanent scientist: **Dr Frédéric Kerff**

Associate member: **Dr Eric Sauvage**

## ENZYMOLOGY AND PROTEIN FOLDING

Group leader: **Prof. André Matagne**

Permanent scientist: **Dr Mireille Dumoulin**

Associate members: **Dr Alexandre Di Paolo**
**Dr Caroline Montagner**
**Dr David Thorn**
**Dr Julie Vandenameele**

## FUNCTIONAL GENOMICS AND PLANT MOLECULAR IMAGING

Group leader: **Prof. Patrick Motte**

Permanent scientist: **Dr Marc Hanikenne**

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

# EXPERTISES

## MOLECULAR BIOLOGY

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

## PROTEIN PRODUCTION

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

## PROTEIN PURIFICATION

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

## MACROMOLECULE CHARACTERISATION

### Biochemical characterisation

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

### Biophysical characterisation

- [X] Microcalorimetry
- [X] Dynamic light scattering
- [X] Analysis of peptidoglycan by HPLC
- [X] (DSC and ITC)

Protein stability, folding & aggregation:

- Spectroscopy: UV-Vis, fluorescence and circular dichroism
- Time-resolved spectroscopy

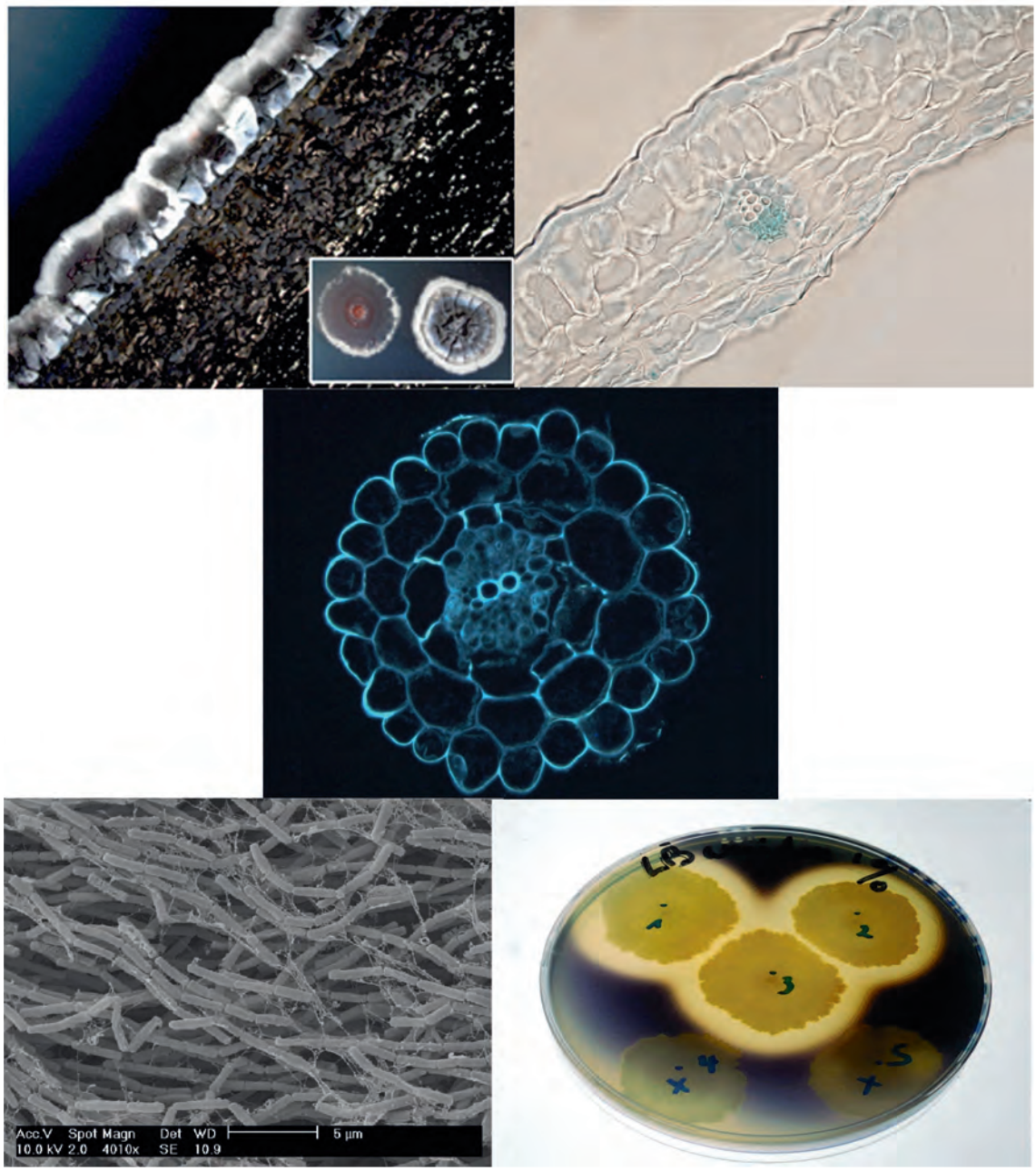
- [X] X-ray crystallography:
  - Crystallogenesis
  - de novo structure determination
  - Studies of ligand-protein complexes
  - 3D structure determination

## PLANT MOLECULAR IMAGING

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

## In Silico Studies

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



# Major Equipments

## Genetic engineering and molecular biology

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

## Green algal cultures

1 Versatile environment test chamber (Sanyo)

## Microbial cultures

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

## Plant cultures

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

## Production of proteins

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

## Purification of proteins

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

## Analytical studies

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

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

## Crystallography

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

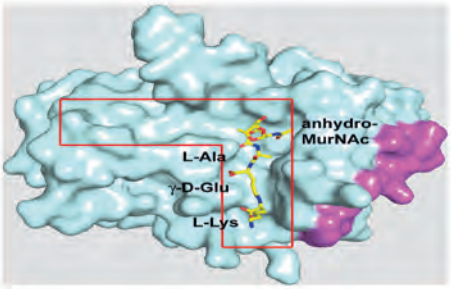
## Imaging

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

## Miscellaneous

1 Freeze-dryer (Christ)

# THEMES OF RESEARCH

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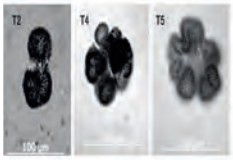
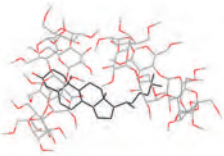
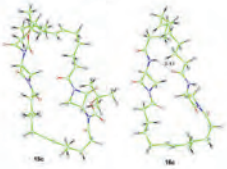
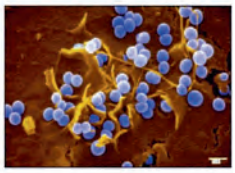
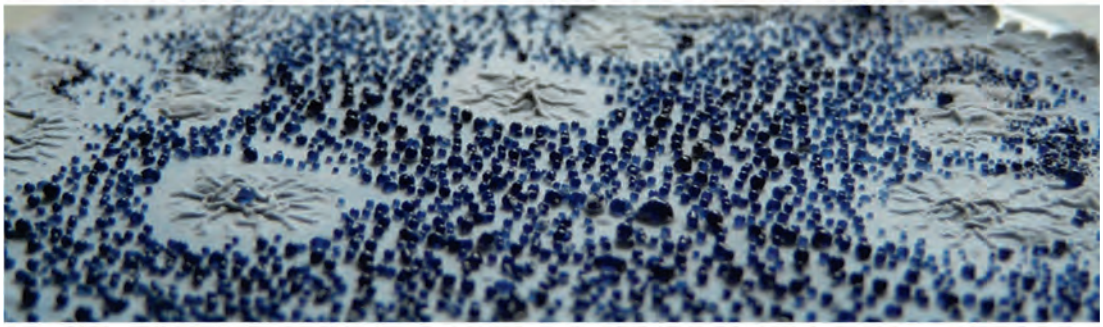
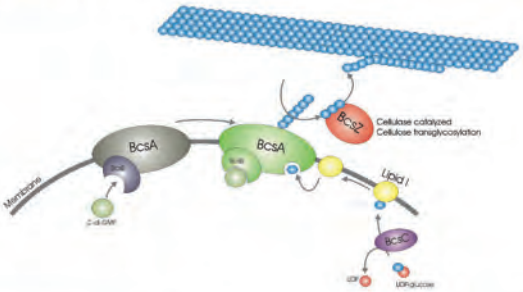


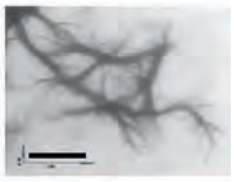



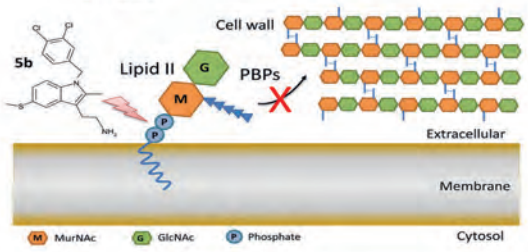


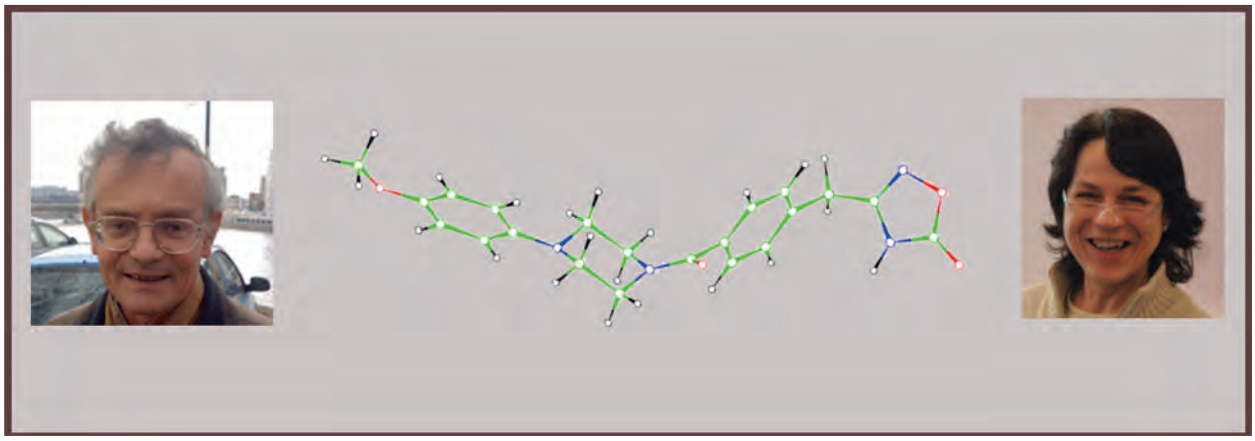










**Research group: APPLIED QUANTUM CHEMISTRY AND MODELLING**
**Group leader:** Dr Georges DIVE (FRS-FNRS)
**Associate Researcher:** Dr Dominique Dehareng
**Collaborations:** Jacqueline Marchand-Brynaert (Université Catholique de Louvain), Robert Locht (Université de Liège)

The objective is to understand, at the atomic level, the reactivity and properties of molecules, with a particular interest for biological macromolecules and their interactions either with drugs or with other macromolecules by using the theoretical tools of quantum chemistry and molecular mechanics.
At the quantum chemistry level(QM), our application studies concern interactions involving cyclodextrins and serine enzyme inhibitors.
It is now well-established that nonaqueous capillary electrophoresis (NACE) is a very powerful tool for enantioseparations and cyclodextrins (CDs) and their derivatives have been extensively used in chiral NACE. Bupivacaine and propranolol were selected as model compounds and their interactions with two single-isomer highly charged ß-cyclodextrin derivatives are studied.
Our collaboration with the laboratory of Prof. Marchand-Brynaert and her PhD student A.Sliwa about the synthesis of non-traditional 1,3-bridged ß-lactams led to unexpectedly synthesized bis-2-oxoazetidinyl macrocycles that revealed to be good inhibitors of the D,D-peptidase from *Actinomadura* R39. The 3D structures of all the dimers are studied by quantum chemistry calculations.
Molecular mechanics (MM) and molecular dynamics (DM) are used to study conformational changes in peptides and proteins as well as in protein complexes with small molecules. These techniques were used to study the conformational mobility of linkers between two protein domains.[14]
The collaboration with the Molecular Dynamics Laboratory about the spectroscopy of halogenated ethylenic compounds is continued. The geometry of the fundamental and some excited states of the neutral molecules is optimized as well as that of few cationic species, and the vibrational spectra are derived, at several QM calculation levels.

**Research group: BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS**
**Group leader:** Prof. Bernard JORIS
**Associate Researchers:** Drs Ana Amoroso, Arabela Cuirolo, Michaël Delmarcelle, Olivier Verlaine
**Ph.D. students:** Antony Argüelles-Arias, Stéphanie Berzigotti, Sébastien Dandois, Nicolas Dony, Anne Famerie, Badre Halimi, Séverine Hubert, Sarah Lebrun, Maxime Maréchal, Anne Pennartz.
**Technical assistance:** Patricia Simon
**Collaborations**: Jean-Pierre Simorre (Institut de Biologie Structurale, Grenoble, France), Andréa Dessen (Institut de Biologie Structurale, Grenoble, France), Dominique Mengin-Lecreulx et Didier Blanot (Université Paris-Sud 11, France), Marta Mollerach (Universidad de Buenos-Aires, Argentina), Stanislav Gobec (University of Ljubljana, Slovenia)

## Study of peptidoglycan metabolism - Bacterial resistance to β-lactam antibiotics – Penicillin cellular stress

Peptidoglycan is the major component of bacterial cell wall and plays an essential role in the integrity and the morphology of bacterial cell. During the bacterial cellular cycle, peptidoglycan is, at once, continuously synthesized and degraded. Peptidoglycan anabolism is a target for many antibiotics such as penicillin (β-lactam antibiotics). We are interested in the study of enzymes involved in peptidoglycan homeostasis and in the molecular events of the cellular stress generated by penicillin that unbalances this equilibrium. We also study the mechanisms of bacterial resistance to penicillin by biochemically characterizing their protein targets (Penicillin-binding proteins or PBPs) and the intrinsically highly resistant PBPs (our working models are *Enterococcus faecium* PBP5 and *Staphylococcus aureus* PBP2a). The group is also interested in the mechanism of β-lactamase induction in *Bacillus licheniformis/Staphylococcus aureus* and *Citrobacter freundii*. Beta-lactamases inactivate β-lactam antibiotics by hydrolysing their β-lactam ring and this research is linked both to bacterial antibiotic resistance and to signal transduction.
Finally the research interests in the group encompass studies of mechanisms of resistance to antibiotics, the means to circumvent them and the development of novel antibiotics.

## *Bacillus subtilis* as cell factory for the production of secondary metabolites or recombinant proteins

*Bacillus subtilis* 168 is a rod-shaped, Gram-positive soil bacterium that is a model organism for laboratory studies for cellular differentiation and secretion of numerous secondary metabolites such as enzymes and small molecules. These enzymes degrade a variety of substrates, enabling the bacterium to survive in a continuously changing environment. Some

of these enzymes are produced commercially and this production represents about 60% of the industrial-enzyme market. We develop new original molecular tools to manipulate *B. subtilis* 168 and other *Bacilllus* sp genomes to optimize production of secondary metabolites and recombinant proteins or to understand bacterial physiology.

## Waleo project: RAPARRAY: Design of a new protein microoarray support.

This project aims to build a protein microarray suitable for detection of biomolecular interactions without labeling, using a support activated by biochip molecules designed to allow attachment of proteins in an oriented manner. This new protein array will be applied to study the interaction of a peptide library from insulin against macromolecules recognizing insulin.

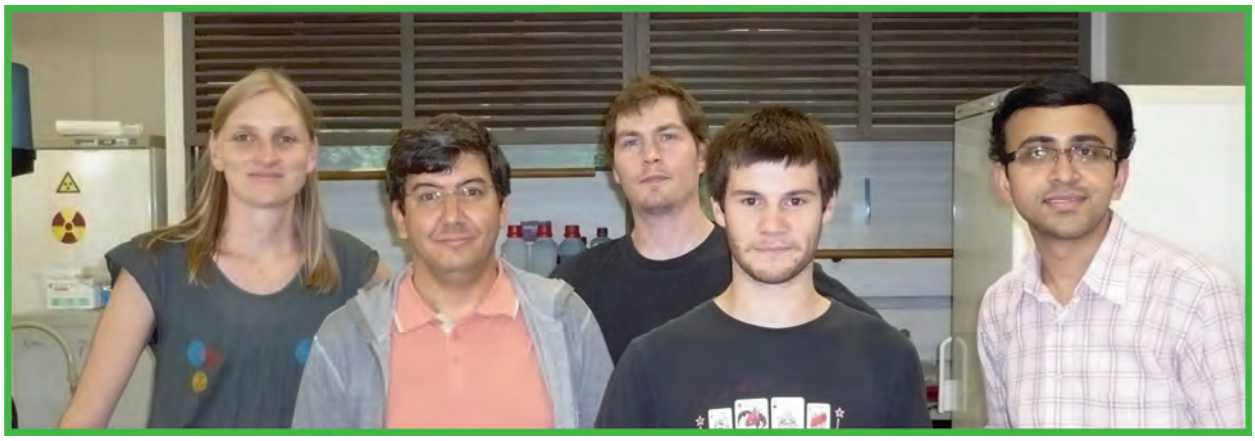
## Waleo project: RAIDGBS: Development of a test for the fast and easy detection of vaginal colonization by group B streptococci

The group B *Streptococcus* (GBS) or *Streptococcus agalactiae* is the bacterium the most frequently involved in severe infections of the newborn and, for this reason, is a public health problem. The origin of these neonatal infections is in the majority of cases, the maternal vaginal carriage at the end of pregnancy. The objective of this project is to achieve a rapid, specific and sensitive test for detecting GBS intrapartum.

**Research group: BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS**
**Group leader:** Dr Colette DUEZ (FRS-FNRS)
**Ph.D. student:** Edwige Van der Heiden

## Biofilm formation by *Bacillus subtilis* and *B. amyloliquefaciens*

The topic aims to study the roles of PBP4a and GalM in the biofilm formation by *Bacillus subtilis* and *Bacillus amyloliquefaciens*, two PGPR (Plant Growth Promoting Rhizobacteria) microorganisms.
The PBP4a (the *dacC* product) is a class C PBP possessing DD-endopeptidase and DD-carboxypeptidase activities [25]. This class of PBP is present in almost all bacteria and could be involved in processes occurring in natural niches such as the formation of biofilms. The *dacC* gene is preceded by *galM* (formerly *yoxA*), both genes belonging to a small bicistronic operon. E. Van der Heiden has deleted the whole operon in the undomesticated *B. subtilis* ATCC21332 and *B. amyloliquefaciens* FZB42, two strains that form robust biofilms. The deletion mutants were examined for their *in vitro* swarming and biofilm formation capacities but the results were not significantly different from those obtained with the parental strains. However, *in vivo* experiments on plant roots of tomatoes or *Arabidopsis thaliana* (drenching of hydroponic cultures with bacterial suspensions and detachment of bacteria from the roots after 30 day-cultures) yielded lower values of CFU/g roots with the deletion mutant compared to the parental strain or to a mutant in which the operon has been reinserted in the neutral locus *amyE*. These results are indicative of an involvement of GalM and/or PBP4a in the root colonization. This class of PBPs which seems non essential in laboratory culture conditions likely plays a role in fine remodelling of peptidoglycan preceding the biofilm formation or could be necessary for the secretion of extracellular polymeric substances present in the matrix surrounding the cells encased into biofilms. The GalM protein (a galactose mutarotase) could be involved in the synthesis or in the degradation of polysaccharides.



**Research group: BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS**
**Group leader:** Dr Mohammed TERRAK(FRS-FNRS)
**Associate Researchers:** Drs Adeline Derouaux, Badrish Soni, Mr André Piette
**Ph.D. students:** Mathieu Rocaboy, Nicolas Dony
**Collaborations:** Dr Mariana G. Pinho (Universidade Nova de Lisboa, Portugal), Prof. Eric Goormaghtigh (Université Libre de Bruxelles)

## Bacterial cell wall synthesis: Function of the monofunctional transglycosylases in *Staphylococcus aureus*

The polymerization of peptidoglycan is the result of two types of enzymatic activities: transglycosylation, the formation of linear glycan chains, and transpeptidation, the formation of peptide cross-bridges between the glycan strands. *Staphylococcus aureus* has four penicillin binding proteins (PBP1 to PBP4) with transpeptidation activity, one of which, PBP2, is a bifunctional enzyme that is also capable of catalyzing transglycosylation reactions. Additionally, two monofunctional transglycosylases have been reported in *S. aureus*: MGT, which has been shown to have *in vitro* transglycosylase activity, and a second putative transglycosylase, SgtA, identified only by sequence analysis. In collaboration with M.G. Pinho (Universidade Nova de Lisboa, Portugal), we have now shown that purified SgtA has *in vitro* transglycosylase activity and that both MGT and SgtA are not essential in *S. aureus*. However, in the absence of PBP2 transglycosylase activity, MGT but not SgtA becomes essential for cell viability. This indicates that *S. aureus* cells require one transglycosylase for survival, either PBP2 or MGT, both of which can act as the sole synthetic transglycosylase for cell wall synthesis. We have also shown that both MGT and SgtA interact with PBP2 and other enzymes involved in cell wall synthesis in a bacterial two-hybrid assay, suggesting that these enzymes may work in collaboration as part of a larger, as-yet-uncharacterized cell wall-synthetic complex [30].

## Bacterial cell division : Study of the cell division process in *Escherichia coli*

During the *E. coli* cell cycle, two morphogenetic processes alternate: cell elongation and division. Cell elongation is performed by a membrane protein complex, the elongasome that inserts new PG at a limited number of discrete sites using the peptidoglycan (PG) synthases PBP1A (class A bifunctional GT/TP) and PBP2 (class B monofunctional TP). Cell division requires a change in the mode of PG synthesis. This is accomplished by the synthesis of new PG material at mid-cell called septum that after maturation evolves to become the new cell poles of the daughter cells. Septum synthesis is directed by a complex of essential and accessory proteins called the divisome composed of cytosqueletal proteins, peptidoglycan synthases and peptidoglycan hydrolases and their corresponding regulatory proteins.

This division machinery includes at least 15 proteins: FtsZ, FtsA, ZipA, ZapA (the Z-ring stabilizing proteins ZapB and ZapC), FtsE, FtsX, FtsK, FtsQ, FtsL, FtsB, FtsW, FtsI(PBP3), FtsN, and AmiC. Division is initiated by the polymerization of the FtsZ ring and associated proteins (FtsA, ZipA, ZapA, ZapB and ZapC) at midcell underneath the plasma membrane, which subsequently functions to recruit the downstream components in hierarchical and interdependent order, either as single molecules or as preformed subgroups (e.g., FtsQ-FtsB-FtsL) and (e.g. FtsW-PBP3). PBP3 is a specific transpeptidase essential for septal peptidoglycan synthesis during cell division which function is believed to be coordination with that of the major bifunctional glycosyltransferase-transpeptidase peptidoglycan synthase PBP1b during cell division. The function of PBP1b is controlled by the inner membrane and essential divisome protein FtsN which have been shown to interact and stimulate the activity PBP1b *in vitro* and also by the outer membrane linked lipoprotein LpoB which has been also found to interact with PBP1b through the UBH2 domain and stimulate its activity. The resulting septal PG formed by the divisome is shared between two daughter cells and should be split to allow cell separation. This process is facilitated by the periplasmic peptidoglycan amidases AmiA, AmiB and AmiC which are regulated by LytM domain containing proteins such as EnvC and NlpD.

The aims of this project is to reconstitute functional complexes in lipid vesicles *in vitro* and to analyze in details the interactions and the structural changes of the main *E. coli* divisome proteins directly involved in the synthesis and their regulators. This study will provide a dynamic picture of the divisome complex and the molecular details that takes place during septal peptidoglycan synthesis.

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## Awakening cryptic antibiotic production in *Streptomyces*

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

## Study of the programmed cell death process involved in *Streptomyces* sporulation

Sporulation is the life cycle last step of bacteria who selected this process for genome preservation and dissemination. The identification of molecules regulating spore formation and the understanding of mechanisms that sense these signals and integrate them into a decision to trigger cell differentiation is a major challenge in modern microbiology. *Streptomyces* are microorganisms that, in contrast to the other model organism *Bacillus subtilis*, sporulate even without nutrient depletion or other environmental stresses. Indeed, several rounds of a programmed cell death (PCD) process directing the dismantling of *Streptomyces* colonies is connected to the build-up of the spore-forming aerial hyphae on top of the vegetative mycelium. In this field of research we raise the question whether cytoplasmic molecules liberated into the environment by the dying mycelium during PCD would carry crucial information for controlling the timing of cell differentiation. We demonstrate that phosphorylated carbohydrates, which can only be encountered in the environment due to the draining of dead cells, are driving essential messages to the PCD process and should not be regarded as just possible nutrients to satisfy survivors' cannibalism.

## Role of N-Acetylglucosamine in *Streptomyces* development

The availability of nutrients is a major determinant for the timing of morphogenesis and antibiotic production in the soil-dwelling bacterium *Streptomyces coelicolor*. N-acetylglucosamine, the monomer of chitin, is a favored carbon and nitrogen source for streptomycetes. Its intracellular catabolism requires the combined actions of the N-acetylglucosamine-6-phosphate (GlcNAc-6P) deacetylase NagA and the glucosamine-6-phosphate (GlcN-6P) deaminase/isomerase NagB. GlcNAc acts as a signaling molecule in the DasR-mediated nutrient sensing system, activating development and antibiotic production under poor growth conditions (*famine*), while blocking these processes under rich conditions (*feast*). In order to understand how a single nutrient can deliver opposite information according to the nutritional context, we carried out a mutational analysis of the *nag* metabolic genes *nagA, nagB* and *nagK*. Considering the relevance of GlcNAc for the control of antibiotic production, improved insight into GlcNc metabolism in *Streptomyces* may provide new leads towards biotechnological applications.

## Regulation of iron homeostasis in *Streptomyces*

Iron is an essential element for almost every living organism but overload is lethal. Optimized homeostasis in accordance with specific cellular needs is therefore of vital importance and iron uptake control the most appropriate mechanism. Microorganisms are no exception to this rule. Iron is one of the most abundant elements on earth but is found in poorly soluble forms not accessible to microorganisms. To subsist, they have developed iron-chelating molecules called siderophores. Siderophore are ‘secondary metabolites’ and the role exerted by these molecules in their natural environment is still intensively debated. In this research topic we heighten the versatile exploitation of siderophores in nature by challenging the authoritative dogma by which their biosynthesis is inextricably tied to iron availability and oxidative stress sensing. We unveiled, in *Streptomyces coelicolor*, the molecular basis of the first inhibitory mechanism of siderophore production that is entirely independent of intracellular iron concentration. Indeed, we demonstrated that N-acetylglucosamine (GlcNAc) represses siderophore biosynthesis via a direct expression control of the iron utilization repressor *dmdR1* by DasR, the GlcNAc utilization repressor. Excitingly, this regulatory nutrient-metal relationship seems to be conserved amongst the *Streptomyces* genera. This indicates that the link between GlcNAc utilization and iron uptake repression, however unsuspected, is not a fortuitous outcome but rather the consequence of a successful evolutionary process. The rationale for this regulatory connection depending on the environmental source of GlcNAc is still under investigations.

## The regulon of the N-Acetylglucosamine utilization regulator (NagR) in *Bacillus subtilis*

N-Acetylglucosamine (GlcNAc) is the most abundant carbon-nitrogen biocompound on earth and has been shown to be an important source of nutrients for both catabolic and anabolic purposes in *Bacillus* species. We showed that the GntR family regulator YvoA of *Bacillus subtilis* serves as a negative transcriptional regulator of GlcNAc catabolism genes expression.

YvoA represses transcription by binding a 16 bp sequence upstream of *nagP* encoding the GlcNAc-specific EIIBC component of the sugar phosphotransferase system involved in GlcNAc transport and phosphorylation, as well as another very similar 16 bp sequence upstream of the *nagAB-yvoA* locus wherein *nagA* codes for N-acetylglucosamine-6-phosphate deacetylase and *nagB* the glucosamine-6-phosphate (GlcN-6-P) deaminase. *In vitro* experiments demonstrated that GlcN-6-P acts as an inhibitor of YvoA DNA-binding activity as it occurs for its *Streptomyces* ortholog DasR. Interestingly, we observed that the expression of nag genes was still activated upon addition of GlcNAc in a *ΔyvoA* mutant background suggesting the existence of an auxiliary transcriptional control instance. Initial computational prediction of the YvoA regulon showed a distribution of YvoA binding sites limited to *nag* genes and therefore suggests renaming YvoA to NagR, for N-acetylglucosamine utilization regulator. Whole transcriptome studies showed significant repercussion of *nagR* deletion on several major *B. subtilis* regulators probably indirectly due to excess of the crucial molecules acetate, ammonia, and fructose-6-phosphate, resulting from GlcNAc complete hydrolysis. We discuss a model deduced from NagR-mediated gene expression, which highlights clear connections with pathways for GlcNAc-containing polymers biosynthesis and adaptation to growth under oxygen limitation [5].

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## Study of the cyanobacterial blooms in Belgium.

Toxic cyanobacterial proliferations ('blooms') in surface waters are an emerging problem worldwide. The BELSPO project B-BLOOMS2 aimed to study the cyanobacterial blooms in Belgium, in order to predict the putative toxicity in Belgian water bodies. This project was run in collaboration with FUNDP, VUB, UGENT and the University of Dundee (Scotland), on the basis of the monitoring of reference lakes in Flanders, Brussels and Wallonia.
A pluridisciplinary approach was used on each sample, to characterize the environmental parameters, the cyanobacterial growth and diversity, as well as the toxicity based on microcystin (*mcy*) gene detections and microcystins measurements.
The CIP team was mostly involved in the genetic determination of cyanobacterial diversity in environmental samples by cultivation and cultivation-independent methods, in the detection of the *mcy* gene cluster for the strains and the environmental samples, and in the quantification of cyanobacterial genotypes in the environment. Most samples contained putative microcystin-producers such as *Microcystis, Planktothrix* and/or *Anabaena*. Moreover, microcystins were detected in all tested samples, sometimes at values exceeding the World Health Organization guideline values for drinking water and recreational water. To study the dynamics of *Microcystis* genotypes, a Real Time PCR SYBR® Green assay on the basis of ITS sequences was designed and a multiplex Real Time PCR TaqMan® PCR assay was used. Results are currently being analyzed. Moreover, we developed a novel cultivation-independent approach, which combines an ELISA with a Whole Genome Amplification using the polymerase Phi29. We have been able to genotype isolated colonies of the enigmatic genus *Woronichinia* and to show the presence of a gene cluster for the potent toxin cyanopeptolin. The same approach showed the uniqueness of *Microcystis* colonies from Belgian water bodies, based on the analysis of several housekeeping genes.

## Cyanobacterial diversity

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 characterization 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). The DGGE (Denaturating

Gradient Gel Electrophoresis) and clone libraries are used for the molecular diversity assesment. In the BelSPO project **AMBIO**, we study the diversity and the biogeography of cyanobacteria in more 59 samples coming from the three biogeographic regions (Sub-Antarctic islands, maritime Antarctica and continental Antarctica). These samples come from water bodies with a range of ecological 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 FNRS project BIPOLES, aiming to investigate the relationships between Arctic and Antarctic cyanobacteria in similar habitats. The purpose of the BelSPO project **BELDIVA** is to realize an inventory of the cyanobacterial diversity in an area of 50 km around the new Belgian research station 'Princess Elisabeth'. Open Top Chambers were installed to study climate change impacts on the phototrophic communities. OTCs are small plastic structures that simulate climate change effects. Sensors are installed in and out of the OTCs to follow the temperature and humidity variations induced by these structures. Strains are also isolated from environmental samples and added to the **Belgian Culture Collection of (sub)polar Cyanobacteria** (BCCM/ULC). BCCM/ULC has obtained an ISO 9001 certificate in May 2011 for its services of deposition and distribution of public strains and includes 45 strains from Antarctica, Arctic and Siberia.

## Micro-H2: the potential of clostridia to produce molecular hydrogen.

During the ARC project Micro-H2, the potential of clostridia to produce molecular hydrogen; (which is regarded as a future energy carrier) was investigated. Clostridia ferment sugars to $H_2$ and $CO_2$ with acetic and butyric acid as the main electron sinks. However, depending on the strain and environmental conditions, more reduced products can be obtained e.g. ethanol, lactate, what substantially reduces the final $H_2$ yield. Additionally, the different metabolic pathways and the regulatory circuits leading to $H_2$ production in clostridia are not well resolved. Therefore, together with our project partner, the CWBI (Centre Wallon de BioIndustries), and using *Clostridium butyricum* CWBI1009 as a model species, we studied the complex $H_2$ metabolism in clostridia. Our discovery of multiple novel [FeFe] hydrogenase genes in the sequenced genomes changed our perspective on how these microbes produce $H_2$. Indeed, using different molecular tools, *e.g.* 2D-DIGE, RT-qPCR and RNA-seq, we have shown that in different environmental conditions, different hydrogenases may contribute to $H_2$ production. Additionally, under $N_2$ atmosphere during glucose fermentation in non-regulated pH, nitrogenase was proposed to contribute to the overall $H_2$ production. This was the first time that this was observed in clostridia. Despite the fact that clostridia seem to be perfectly equipped to produce hydrogen, they probably developed this capacity to quickly adapt to changing conditions, namely decreasing pH value. We concluded that, in order to maintain a constant pH inside the cell, they excrete protons (presumably in form of $H_2$) into the medium. At the same time, they get rid of the excessive reducing equivalents produced during glucose fermentation. The obtained results shed more light on the complex hydrogen metabolism in clostridia. Nevertheless, a challenge ahead is to characterize the key enzymes of hydrogen metabolism and, by means of metabolic bioengineering, to develop optimal microbial systems for biomass conversion to hydrogen.

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## Biochemical and structural studies of the type I ketohexose bisphosphate aldolases and their applications in rare sugars synthesis.

Aldolases are a specific group of lyases that form reversible carbon-carbon bonds. The reversible stereoselective addition makes aldolases interesting tools for asymmetric syntheses of rare sugars. This enzyme is an ideal alternative to traditional methods in synthetic organic chemistry.
Tagatose-1,6-bisphosphate aldolase is a class I aldolase that catalyzes the reversible condensation of dihydroxyacetone phosphate with glyceraldehyde 3-phosphate to produce four D-ketohexoses 1,6-bisphosphate: D-tagatose 1,6-bisphosphate, D-fructose 1,6-bisphosphate, D-psicose 1,6-bisphosphate and D-sorbose 1,6-bisphosphate. These four sugars are diastereoisomers and differ in stereochemistry at carbon 3 and at carbon 4 with respect of the configuration of their hydroxyl groups.
The aims of this project are the study of the tagatose 1,6-bisphosphate aldolase structure to determine amino acids that would change the specificity toward psicose/sorbose (carbon C3), tagatose/ fructose (carbon C4) and the need of a ketohexose with phosphate group.

The strategy is divided in 3 parts:
- HPLC separation and quantification ok ketohexoses
- The sequence and tridimensional structure of tagatose 1,6-bisphosphate aldolase from *Staphylococcus aureus* are known. The active site will be studied and amino acids will be selected that hopefully change the specificity. Mutations will be realized. The different mutants will be produced, purified and characterized.
- Data banks will be analysed to select aldolases, hopefully with different specificity.

Genes will be cloned and enzymes will be produced, purified and characterized. This way, activity and specificity of new aldolases will be determined. The relationship structur-activity will be studied in order to determine the essential aminoacid involved in specificity. To confirm their importance, the tagatose 1,6-bisphosphate aldolase of reference will be mutated and it specificity studied.

## Insights into the activation mechanisms and implication in mite allergy of four digestive proteases from *Dermatophagoides pteronyssinus*

Four digestive proteases which are secreted by the mite *Dermatophagoides pteronyssinus* appear to be common house dust allergens. Der p 3, Der p 6 and Der p 9 are serine proteases with trypsin, chymotrypsin and elastase activities, respectively, whereas Der p 1 is a papain-like cysteine peptidase. These enzymes are synthesized as inactive zymogens, named proDer p 1, 3, 6 and 9, which are constituted of an inhibitory propeptide before the protease domain. The activation mechanisms of proDer p 1 and proDer p 3 have been previously elucidated, but the maturation process of proDer p 6 is still unknown. ProDer p 6 was expressed in *Pichia pastoris* and purified. The inter-molecular activation kinetic was monitored depending on the presence of either HDMe, recombinant Der p 1 or rDer p 3. The activation mechanism of proDer p 6 is led by Der p 1. The specific activities of rDer p 6 and native form of Der p 6 are similar indicating that rDer p 6 can be used to further study the allergenic properties of this protease. (J. Herman)

In addition to being involved in mite allergy, the proteolytic activity of these allergens is considered pro-allergic and pro-inflammatory. In collaboration with Dr. Chevigné (CRP-Santé, Luxembourg), we have engineered “substrate phage display” libraries in order to determine the protease cleavage patterns. The screenings of the libraries by Der p 1 and Der p 3 allowed us to develop specificity models and to predict *in silico* the potential substrates of these proteases within the human proteome. (V. Campisi)

Finally, Der p 3 can be considered as a major allergen. In effect, all sera showed IgE reactivity. The IgE binding to inactive rDer p 3 (S196A) was higher than rDer p 3. These variations can be linked with the phenomenon of autolysis of Der p 3 during ELISA. (A. Bouaziz).

## Cold adapted β-galactosidase of *Pseudoalteromonas haloplanktis*: enzyme immobilization for milk lactose hydrolysis

The enzyme β-galactosidase, most commonly known as lactase, which hydrolyses lactose into its monomers that is glucose and galactose has potential applications in food processing industry. The inability to completely digest lactose by the human population is termed as lactose intolerance. In people with lactose maldigestion, a portion of lactose is not digested in the small intestine; it passes into the large intestine where it is fermented by colonic microflora. The symptoms of lactose intolerance are abdominal pain and distention, abdominal colic, diarrhoea and nausea. It is estimated that 75% of adults worldwide exhibited some decrease in lactase activity during adulthood. The frequency of decreased lactase activity ranges from 5% in northern Europe, up to 71% for Southern Europe, to more than 90% in some African and Asian countries. Technologically, lactose gets easily crystallized, which sets the limits of its applications to certain processes in the dairy industry. Cheese manufactured from hydrolyzed milk ripens more quickly than that made from normal milk. Treatment of milk and milk products with lactase to reduce their lactose content seems to be an appropriate method to increase their potential uses and to deal with the problems of lactose insolubility and lack of

sweetness. The use of enzyme immobilization technology is of significant importance from economic point of view since it makes reutilization of the enzyme, it can also help to improve the enzyme stability. Nowadays low-cost energy bio-industrial processes in biotechnology are highly desired. The isolation of pyschrophilic bacteria with cold active β-galactosidase has opened up the possibility of processing of milk and whey even at low temperatures. In our study we immobilised a cold adapted β-galactosidase from *Pseudoalteromonas haloplanctis* on a chitosan beads by glutaraldeide activation. The main scope of our project is the lactose milk hydrolysis at low temperatures.

## Unravelling the physiological function of Cel5 from *Pseudomonas stuzeri*

Cellulases are known to be involved into plant cellulose breakdown. However, the biosynthesis of this polysaccharide also requires the action of an endocellulase. In this pathway, the cellulase is encoded by the *bcsZ* gene, located on the Bacterial Cellulose Synthesis operon and the function of this enzyme is suspected to be the transglycosylation of newly produced cellooligosaccharides from the cellulose synthase complex to the existing cellulose.

The aim of this project is to investigate the molecular basis of cellulase involvement into cellulose biosynthesis. To do so, we are focused on the enzymatic and structural comparison of two cellulases supposed to be required for this polysaccharide production (RBcel1 from Antarctic metagenome and Cel5 from *Pseudomonas stutzeri*) with a well characterized hydrolytic cellulase (Cel5A from *Thermoascus aurantiacus*).

The previously determined hydrolytic parameters demonstrated that, as expected, Cel5A has the most important hydrolytic activity. Their transglycosylase ability is under investigation.

In collaboration with the Dr C. Bauvois (Protein Crystallography, IRMW, Brussels), crystals of RBcel1 were obtained for the apoenzyme and in the presence of glucose and cellobiose. The tridimensional structure of this protein is under determination. Nevertheless, a preliminary comparison of RBcel1 and Cel5A structures revealed changes in substrate binding sites along the active site cleft. These are supposed to highlight the difference of activity between the two cellulases.

We have also analyzed cellulase involvement into bacterial cellulose production *in vivo*. In collaboration with the Laboratory of Microbial Interactions (VUB, Brussels, Pr. P. Cornelis), we have deleted the *Pst_2494* locus, encoding the Cel5 cellulase in *Pseudomonas stutzeri*. The ΔPst_2494 strain was complemented with the three enzymes and then compared with the wild type strain for their ability to produce cellulose. This analysis demonstrated that all the three enzymes are able to restore cellulose production in the *P. stutzeri* mutant strain.

## Hybrid Proteins

**Development of allosteric modulator monoclonal antibodies against the extracellular domains of the G Protein-Coupled Receptors (GPCRs) :**

GPCRs are a family of integral membrane proteins located on the cell surface, whose activation induces second messenger amplification. These receptors play a pivotal role in physiological signaling pathways and are targeted by nearly 50% of currently available drugs. It is widely recognized that many GPCRs would make excellent therapeutic targets for antibodies. Raising antibodies to GPCRs has been difficult due to problems in obtaining suitable antigen because GPCRs are often expressed at low levels in cells and are unstable

when purified. We have developed a method for stabilizing extracellular structures of receptors in native-like conformations by scaffolding them into carrier proteins.
Our main objectives are:
- To develop antibodies that bind the extracellular domains of two chemokine receptors CCR5 and CXCR4 involved in HIV infection.
- To demonstrate that the antibodies can act as agonists or antagonists for these receptors.

**Development of neutralizing antibodies against *Staphylococcus aureus* and a specific assay to detect *S. aureus* in bovine mastitic milk:**

*Staphylococcus aureus* is the most common cause of contagious mastitis in cattle and induces significant economic damage worldwide. Treating such infections has been complicated by the widespread prevalence of methicillin-resistant *S. aureus* isolates. Therefore, there is an urgent need to develop: (i) potent diagnostic test to identify infections early in order to prevent spread to other animals and increase chances of a successful treatment, and (ii) novel antimicrobial agents to treat life-threatening infections caused by *S. aureus* strains. Therefore, the development of an effective vaccine or immunotherapy remains a promising alternative.
Our main objectives are:
- To develop and characterize monoclonal antibodies directed against *S. aureus* virulence factors.
- To develop a rapid diagnostic test for the detection of *S. aureus* in milk samples.

## Characterization of the human Tryptophan 2,3-Dioxygenase involved in the immune escape of liver cancer cells

Among the therapies against cancer, the use of vaccine was developed in order to stimulate the immune system of patient against cancer cells. However, the first clinical trials have shown efficacy in only 10% of patient. Cancer cells have developed a mechanism to escape the immune system by the overexpression of two heme-containing proteins, the Tryptophan 2,3-Dioxygenase (TDO) and the Indoleamine 2,3-Dioxygenase (IDO). The human TDO enzyme (hTDO), like IDO, is the first and rate-limiting enzyme of kynurenine pathway catalyzing the oxidative cleavage of L-tryptophan (L-Trp) indole ring to form the N-formylkynurenine. Their over-expression result in a local tryptophan depletion that severely affects the proliferation of T-lymphocytes and thus is immunosuppressive. A series of pyridinylvinyl-1H-indole inhibitors (such as 680C91) of rat liver TDO were described by Salter and *al.* but they exhibited a poor inhibitory activity *in vivo*.
During this project, protocols for expression, purification and kinetics of full-length human TDO without HisTag were developed. Eight to ten mg of purified protein were obtained with a purity of 80-85% and the enzyme is characterized biochemically and biophysically (spectroscopy, enzymology, circular dichroism,...). In addition, we studied the inhibitory properties of analogues of 680C91 compound (prof. Wouters and Masereel (FUNDP)). The mechanism of inhibition was determined. In parallel, crystallization assays of hTDO by the sitting-drop vapour diffusion method were realized using different kits from Hampton Research, Emerald and from our laboratory. Two compounds, the phenylimidazole used for the IDO crystallization or an inhibitor candidate, were added or not separately in protein sample to help the enzyme crystallization. Trials of crystal growth of hTDO are still underway.

## Metallo-β-lactamases inhibition by camelid single-domain antibody fragments.

The worldwide spread of metallo-β-lactamases (MβLs), especially amongst multiresistant Gram-negative strains, makes urgent a better understanding of these enzymes in order to discover new drugs. In this context, we decided in 2007 to start a project in which phage display experiments would be performed in order to select single domain antibody fragments (VHHs) able to inhibit the clinically relevant MβL VIM-4. Amongst 55 other VHHs, the only inhibiting VHH termed CA1838 has been characterized by studying its paratope, its inhibition mechanism and its epitope.

The alanine scan of CA1838's paratope showed that the binding is driven by hydrophobic amino-acids. The inhibition is in the µM range for all the β-lactams assayed and has been found to be mixed hyperbolic with a predominant uncompetitive component. Moreover, a substrate inhibition occurred only when the VHH is bound. These kinetic results are indicative of a binding site distant from the active site, which has been confirmed by the epitope mapping analysis realized by making peptide arrays. As this binding site is distant from the active-site and alters both substrate binding and catalytic properties of VIM-4, this VHH qualify to the definition of an allosteric inhibitor. Therefore, the binding CA1838 could inhibit the enzyme by interfering with a molecular motion required for efficient catalysis. Such a dynamic behaviour of MβLs has already been inferred from NMR and molecular dynamic studies (Salsbury et al.,. J Mol Model, 2009. 15(2): p. 133-45). It allows us to postulate an allosteric pathway from the VHH binding site to the active site that results in the observed inhibition through an altered mobility of the loop L7. This work is about to be published. Moreover the inhibition of BcII MβL by the VHH cABcII10 has also been characterized.

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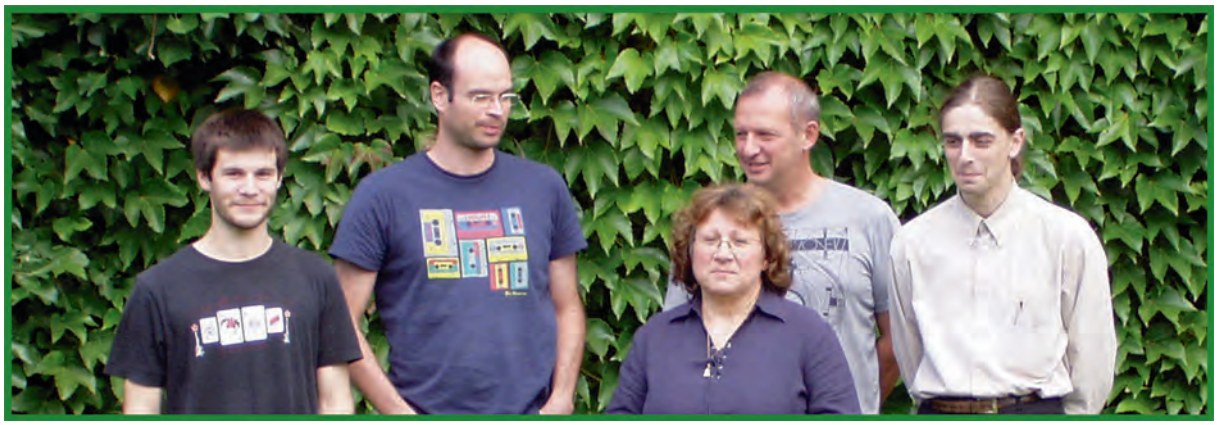
## Biochemistry of Extremophiles

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, solfatres 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 by the Laboratory are summarized below.

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

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

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



**Research group: BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY**
**Group leader:** Prof. Paulette CHARLIER
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**Technical assistance:** Raphaël Herman
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## Class A β-lactamases and their complexes with inhibitors

Bacteria exhibit a remarkable capacity to become resistant to commonly used antibacterial compounds. A very common mechanism developed by bacteria in the β-lactam resistance is the production of enzymes that efficiently hydrolyze them, the β-lactamases. These proteins belong to 4 different classes (A, B, C, D) (Bush, K. et al, 1995).
Among the strategies for circumventing the β-lactamase-mediated bacterial resistance, several compounds - such as clavulanic acid, sulbactam and tazobactam, BRL42715 and 6-β-halogenopenicillanates - able to efficiently and specifically inhibit the broad- and extended-spectrum active site serine β-lactamases were developed.
Our aim is to elucidate at the molecular level, by way of kinetic and crystallographic studies, the interactions between previously synthesized or newly designed inhibitors in order to provide clues about the pathway of the class A β-lactamase inhibition by these compounds. Our model enzymes for the class A β-lactamases are the enzyme from *B. licheniformis* BS3 and TEM variants from *Escherichia coli.*

## PBPs and their complexes with inhibitors

In our efforts to design strategies which will allow counteracting the bacterial resistance to β-lactam antibiotics, we have focused our research to protein targets that are involved in the later stages of peptidoglycan biosynthesis. The last step in PG assembly involves transpeptidation reactions which lead to peptide cross-bridges between units of pre-assembled glycan chains. Different types of peptidoglycan transpeptidases called penicillin-binding proteins (PBPs) have been identified (Tomasz, A. 1979, Sauvage, E. *et al.*, 2008). PBPs fulfil the criteria for good therapeutic targets since β-lactam antibiotics, the most used and successful drugs against bacteria, are their primary inhibitors. However, bacteria have exhibited a remarkable capacity to become resistant to β-lactams by different ways. In Gram-positive pathogens resistance towards this family of antibiotics is generated mainly by mutations or acquisition of a new low-affinity PBP (resistant PBP or rPBPs) able to fulfil the role of all other PBPs, as described in *Enterococcus sp* and methicillin-resistant

*Staphylococcus aureus*, respectively. This mechanism of resistance represents a major health threat. To date, β-lactams reported as being active against rPBPs are all based on the cephalosporin or penam/penem moiety. Our colleagues, Pr. J. Marchand-Brijnaert (UCL) and Dr G. Dive (CIP, ULg), have proposed an original approach for discovering new antibiotics. The strategy is based on the β-lactam moiety embedded in a flexible macrocycle allowing conformational adaptability of the molecules in the active site of PBPs. The first lead compounds are active against rPBPs (Sliwa A., et al. 2010, 2011a, 2011b). The efficacy of those innovative molecules invalidates all common dogma in the field of penicillins (cyclic strain, small fused cycles, and carboxylic acid function) and new compounds are under study. This work was developed in the context of IAP P6/19.

Boronic acids derivatives represent a new antibacterial class of compounds capable of inhibiting β-lactam resistant transpeptidases. For boronic acid synthesis, we collaborate with the groups of Pr. André Luxen from the University of Liege, Pr.Chris Schoffield from the University of Oxford, Pr. Fabio Prati from the University of Modena, Pr. Rex Pratt from the Wesleyan University and Pr. William Gutheil from the University of Missouri-Kansas City. This work was partly conducted within the framework of the FP6 European Integrated Project EURINTAFAR (LSHM-CT-2004-512138).

The objective of this project is to develop new leads active against PBPs insensitive to common β-lactams. Three model enzymes are considered for structural purpose: PBP2a from *Staphylococcus aureus*, PBP5fm from *Enterococcus faecium* and a sensitive PBP from *Actinomadura* R39.

## Collaborative projects

Several joint projects developed with colleagues of the University of Liège, inside and outside our centre, but also from other universities and research centres, enable us to broaden our field of investigation by interesting us in other proteins and biological phenomena.

Collaboration with **Dr Mireille Dumoulin**, ChQ FRS/FNRS, CIP, ULg
- 3D structure determination of chimeric proteins made of the beta-lactamase BlaP and polyglutamine (polyQ) stretches of different lengths.
- 3D structure determination of complexes between camelid antibody fragments (or VHHs) and human lysozyme.

Collaboration with **Prof. Moreno Galleni**, CIP, ULg
- Structural study of the Tryptophan 2,3-Dioxygenase (TDO) and the Indoleamine 2,3-Dioxygenase (IDO), two heme-containing proteins overproduced by cancer cells to escape the immune system.
- 3D structure determination of Derp 3, a digestive serine protease secreted by the mite *Dermatophagoides pteronyssinus* and identified as a common house dust allergen.

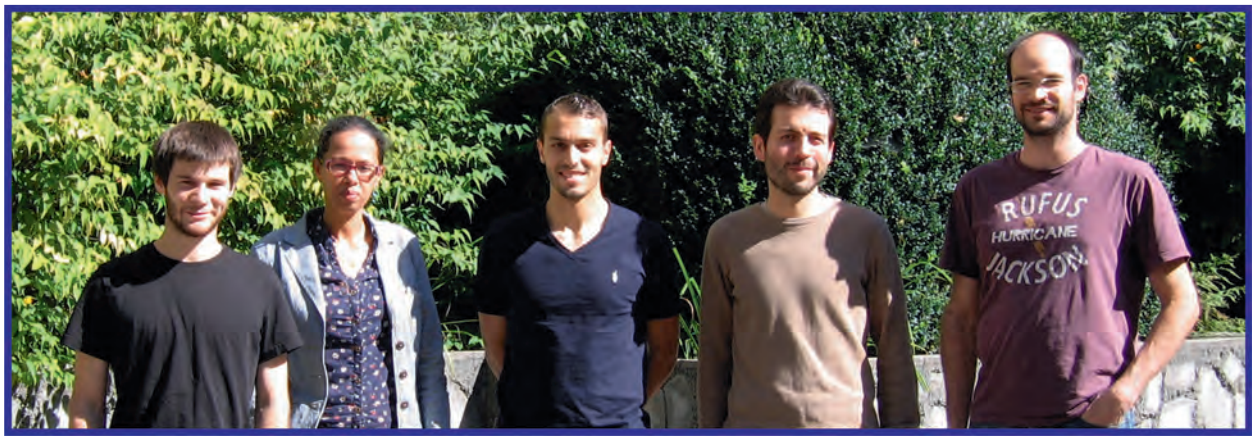
Collaboration with **Prof. Patrick Motte**, CIP, ULg
- Structural study of Ser/Arg-rich splicing factors from *Arabidopsis thaliana*.

Collaboration with **Dr Lucien Bettendorff**, ChQ FRS/FNRS, GIGA, ULg
- 3D structure determination of human Thiamine triphosphatase.

Collaboration with **Prof. Mohamed Azarkan** et **Danièle Baeyens-Volant**, Protein Chemistry Unit, Campus Erasme, ULB
- Structural study of two cysteine proteases (Bromelain and Ananaïn) from *Ananas comosus*.

Collaboration with **Prof. Abdelmounaaïm Allaoui**, Laboratoire de Bactériologie Moléculaire, Campus Erasme, ULB
- Structural study of soluble and membrane proteins of type 3 secretion system (SST3) from *Shigella flexenri*.

Collaboration with **Dr C. Hilger**, Laboratoire d'Immunogénétique et Allergologie, CRP-Santé, Luxembourg.
- 3D structure determination of mammalian lipocalins as repiratory major allergens.

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**Collaborations:** Dr Dominique Mengin-Lecreulx (University of Paris-Sud, France), Dr German Bou (Complejo Hospitalario Universitario La Coruña, Spain)

## Structural study of the divisome

The divisome is the membrane protein complex that regulates the peptidoglycan (PG) synthesis during the bacterial cell division process. In *Escherichia coli*, this machinery is composed of more than 20 different proteins that are sequentially recruited at the division site. This research project focuses on structural study of the PG hydrolases AmiC and its activator NlpD, which contribute to the separation of the daughter cells at the end of the cell division, and FtsW, the flippase that translocates PG precursors across the cytoplasmic membrane. FtsW will be studied in complex with its different partners in the divisome as well. This work will help understand a key process of the bacterial cell cycle and bring useful structural information for the search of new antibiotics.

## Undecaprenyl pyrophosphate phosphatases

The aim of this project is the structural study of the BacA and PAP2 families of undecaprenyl pyrophosphate (C55-PP) phosphatases. These membrane proteins play an essential role in the metabolism of the undecaprenyl phosphate (C55-P) which is the lipid carrier used in the synthesis of the peptidoglycan and other surface carbohydrate polymers. C55-P is synthesized *de novo* or results from a recycling. In both cases, the last step of the process is the dephosphorylation of C55-PP by C55-PP phosphatases. Multiple C55-PP phosphatases are found in the genomes of all bacteria. In *E. coli*, the LpxT C55-PP phosphatase can transfer the removed phosphate to the lipid A moiety of the lipopolysaccharide (LPS) in a phospho-transfer reaction. The multiplicity of C55-PP phosphatases observed in all bacteria could reflects the diversity of acceptor for the removed phosphate and be fundamental in the regulation of periplasmic and outer membrane activities required for the adaptation to environmental changes or other mechanisms. In *Streptococcus pneumoniae* and *S. aureus*, the disruption of the *bacA* gene results in decreased virulence and hypersensitivity to bacitracin which is an antibiotic widely used to fight skin and eyes infection that acts through the sequestration of the C55-PP lipid.
The determination of the structures of the C55-PP phosphatases from *E. coli, B. subtilis* and *H. pylori* by x-ray crystallography will bring useful information in the search for new antibiotics, as well as for a better understanding of peptidoglycan biosynthesis and other mechanisms used by bacteria to adapt to their environment.

This work will be undertaken in collaboration with the EBA-IBBMC laboratory from the University of Paris-Sud.

## Fragment-Based Screening (FBS) by x-ray

The objective of this project is the implementation of the Fragment-Based Screening (FBS) by x-ray crystallography to potentially develop new drug leads. In this method, small fragments are directly identified in the active site of the targeted protein by determining the structure of the potential complex. The library of compounds used for this screening will be prioritized using an *in silico* docking procedure centered on the area of interest of the protein. The structural information gathered about the identified compounds will then be combined with other experimental data (affinity, inhibition, ability to be chemically modified…) in order to establish the best strategy for the improvement of the compounds' stability and specificity. In this project we will apply this method to two families of proteins studied in our laboratory for several years, the class D ß-lactamases and the glycosyltransferases, which are respectively involved in the bacterial resistance to ß-lactam antibiotics and the PG synthesis.

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## Protein folding

Understanding the basic aspects of protein folding is crucial in describing many cellular processes, ranging from transcription to molecular motors and diseases associated with misfolded proteins. Rational modification and *de novo* conception of novel proteins with therapeutic or biological applications, and prediction of their three-dimensional structures from their amino acid sequence, require both a detailed description of the energetics of folding and knowledge of the driving forces and pathways that lead to the native state. Much of the progress in understanding the way in which proteins fold has been marked by intensive studies on model proteins.

Although the events in the folding of globular proteins appear to be diverse and complex, a wealth of information has been gathered from over fifty years of research. Nevertheless, some details of the folding mechanism are still unclear and, in particular, prediction of the sequence of acquisition of secondary and tertiary structural elements of multi-domain proteins (> 100 amino acids) remains a difficult task.

With the goal of contributing to a better description of the protein folding problem, we have selected five families of model proteins with essentially different folds. These include active-site serine β-lactamases, Zn(II) metallo-β-lactamases, lysozymes, camel single domain antibody fragments and *Erwinia chrysanthemi* pectine methylesterase. Folding of these proteins is analyzed by using a range of complementary spectroscopic probes. Thus, optical methods (i.e. fluorescence and circular dichroism) in combination with rapid-mixing techniques provide a first description of the folding mechanism. In the case of enzymes, this can be advantageously completed by measuring the regain of catalytic activity. Furthermore, we use pulse-labelling hydrogen/deuterium exchange experiments, in combination with 2D-NMR (collaboration with Christina Redfield, Oxford and Christian Damblon, Liège) and/or mass spectrometry (collaboration with Edwin De Pauw, Liège) measurements, to monitor the time-course of formation and stabilization of secondary structure elements.

**Research group: ENZYMOLOGY AND PROTEIN FOLDING**
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**Technical assistance:** Stéphane Preumont, Patrick Zirbes

## Protein misfolding and aggregation

The deposition of proteins in the form of amyloid fibrils, in the nervous system or in certain peripheral tissues, is associated with more than 40 degenerative diseases, referred to as amyloidoses. Our objectives are to better understand the mechanisms leading to protein misfolding and amyloid fibril formation by determining the thermodynamic and structural properties of the various species populated on the aggregation pathway. To achieve this aim, we are using a range of complementary techniques including fluorescence, circular dichroism, NMR, TEM, FTIR, QCM-D and protein engineering methods.

We are mainly working on two protein systems:
- The amyloidogenic variants of human lysozyme which are associated with a systemic non-neuropathic amyloidosis.
- Chimeric proteins comprising the beta-lactamase BlaP and polyglutamine (polyQ) stretches of various lengths. Eight distinct chimeras have been designed in order to investigate the effects of both the length and the location of the polyQ tract on the stability, dynamics and aggregation properties of BlaP. The results of these studies indicate that the aggregation properties of the BlaP chimeras recapitulate the two phenomena characteristic of proteins associated with polyQ diseases (which include Huntington's disease and several ataxias). BlaP chimeras are therefore valuable models to better understand how the protein context can influence the aggregation process. In 2011, we got a *Mandat d'Impulsion Scientifique* (FRS-FNRS) and a grant from *Crédits Spéciaux* (ULg) to systematically investigate the effects of amino-acid sequences flanking the polyQ tract on the properties of polyQ proteins.

For both systems, one particular approach involves the use of conformational camelid antibody fragments (or $V_HHs$) against the different species formed on the pathway of aggregation. In collaboration with the groups of S. Muyldermans and J. Steyaert (VUB, Brussels), we have generated more than 15 and 60 $V_HHs$, respectively specific to human lysozyme and BlaP chimeras. These $V_HHs$ are used i) as structural probes to elucidate the mechanism of fibril formation, ii) as potential inhibitor of the aggregation process and as model proteins to investigate the relationship between the amino-acid composition and aggregation propensity.

**Research group: FUNCTIONAL GENOMICS AND PLANT MOLECULAR IMAGING**
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## The SR protein family of splicing factors

In eukaryotes most nuclear genes are interrupted by introns that must be accurately excised from pre-mRNAs to give rise to functional mature protein-coding mRNAs. Splicing is catalyzed within the spliceosome, one of the most elaborate edifices in the cell whose precise assembly at each intron involves five small nuclear ribonucleoprotein particles (snRNPs).
Alternative splicing (AS) is a highly regulated mechanism that allows the synthesis of multiple mRNAs from a single gene. AS is widespread in eukaryotes and has a significant role in expanding transcriptome and proteome diversity. Recent estimates indicated that ~95% of multiexon human genes undergo AS. Global AS has been investigated in plants. More than 50% of rice AS-related genes undergo multiple AS events producing a variety of transcripts from a single gene highlighting the extremely high complexity of transcriptome regulation.
In addition to spliceosomal snRNPs, constitutive and alternative splicing requires a large number of non-snRNP-associated proteins. The serine/arginine-rich (SR) splicing factors dynamically participate in spliceosome assembly. In human, 12 SR proteins have been described. Prototypical SR proteins have a modular architecture consisting of one or two N-terminal RNA recognition motifs (RRM) and a C-terminal RS domain of low complexity enriched in Arg-Ser (or Ser-Arg) repeats. Some SR proteins contain an RNA-binding CCHC Zn-knuckle (ZnK) motif located between the RRM and RS domains.
In plants, some SR proteins are homologous to human prototypes, while others are reported to be plant-specific. The complexity of the SR protein family thus appears higher in green plants than in mammals. The functions of plant SR proteins have still to be determined in a physiological context. Moreover, much remains unknown with regards to their possible non-splicing activity. Using a multidisciplinary approach, we aim to provide fundamental insights into the functions of *Arabidopsis* SR splicing factors during growth and development.

**Research group: FUNCTIONAL GENOMICS AND PLANT MOLECULAR IMAGING**
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**Collaborations:** Prof. Ute Krämer (University of Bochum), Dr Juergen Kroymann (University Paris-Sud, Orsay), Prof. Nathalie Verbruggen (Free University of Brussels)

## Metal hyperaccumulation and hypertolerance in plants

Anthropogenic metalliferous soils are colonized by metal-tolerant plant species. Among those, a small number of species exhibit extraordinarily high levels of leaf metal accumulation. These so-called hyperaccumulators store >1% zinc or >0.01% cadmium of dry weight in above-ground biomass. Hence, zinc hyperaccumulation and zinc and cadmium hypertolerance are species-wide naturally-selected traits in the *Brassicaceae Arabidopsis halleri*, which constitutes a compelling model to study the mechanisms of speciation, adaptation to extreme environments and evolution of complex traits. In the last few years, important progress has been achieved in our understanding of the genetic mechanisms underlying metal tolerance and hyperaccumulation in *A. halleri*. Quantitative Trait Loci (QTL) analyses, comparative transcriptomics and functional analysis of candidate genes revealed the key role of a number of metal transporters in the traits. The evolution of hyperaccumulation involved the enhancement of existing functions present in the ancestor of *A. halleri* and not the creation of new functions. These enhanced functions appear to often result from gene copy number amplification and/or (cis-)regulatory changes, which are responsible for higher gene expression levels.
Combining genetic, functional genomic and biochemistry approaches, our work is aiming at identifying the molecular determinants of the differential expression of selected candidate genes in *A. halleri*, to analyse their function in metal tolerance and hyperaccumulation, to analyse metal binding by the corresponding proteins and to study how these traits evolved. The project will reveal the global functioning of metal homeostasis networks and uncover key nodes whose alterations can drastically modify metal accumulation and tolerance. This knowledge could then be applied to develop biofortification and phytoremediation technologies.

## Iron homeostasis in the unicellular green alga model *Chlamydomonas reinhardtii*

Iron deficiency represents a major nutritional issue worldwide. To better understand iron homeostasis in photosynthetic organisms which represent the main entry point of iron into the food chain, we are conducting a reverse genetic screen to identify mutants of the iron deficiency response in the unicellular alga model *Chlamydomonas reinhardtii*. The screen is

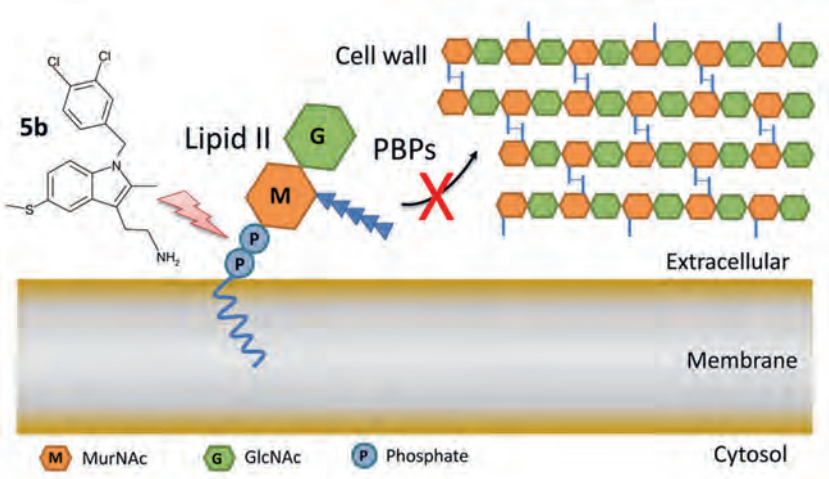
designed to isolate mutants possibly affected in the sensing and the signaling of the cellular iron status. This research will shed light on the mechanisms controlling iron homeostasis at the cellular level in photosynthetic organisms. A better understanding of iron sensing and adaptation to iron deficiency will be instrumental for the rational design of new strategies for bio-fortification of crops.

# HIGHLIGHTS OF THE YEAR 39

# Small molecule inhibitors of peptidoglycan synthesis targeting the lipid II precursor

Derouaux A, Turk S, Olrichs NK, Gobec S, Breukink E, Amoroso A, Offant J, Bostock J, Mariner K, Chopra I, Vernet T, Zervosen A, Joris B, Frère JM, Nguyen-Distèche M, Terrak M. Biochem Pharmacol. 2011 May 1;81(9):1098-105.

This work is the result of collaboration between the Centre for Protein Engineering and 5 groups involved in the European project EUR-INTAFAR: S. Gobec (University of Ljubljana, Slovenia), E. Breukink (University of Utrecht, Holland), T. Vernet (IBS Grenoble, France), I. Chopra (University of Leeds, UK) and A. Zervosen (University of Liège, Belgium). Small molecules selected using structure-based high throughput virtual screening were found to inhibit glycosyltransferase activity in the micromolar range and to exhibit antibacterial activity against several Gram-positive bacteria. Compound 5b was found to interact with the lipid II substrate via the pyrophosphate motif (see figure). These molecules are promising leads for the development of more active and specific compounds to target the essential glycosyltransferase step of the cell wall synthesis.

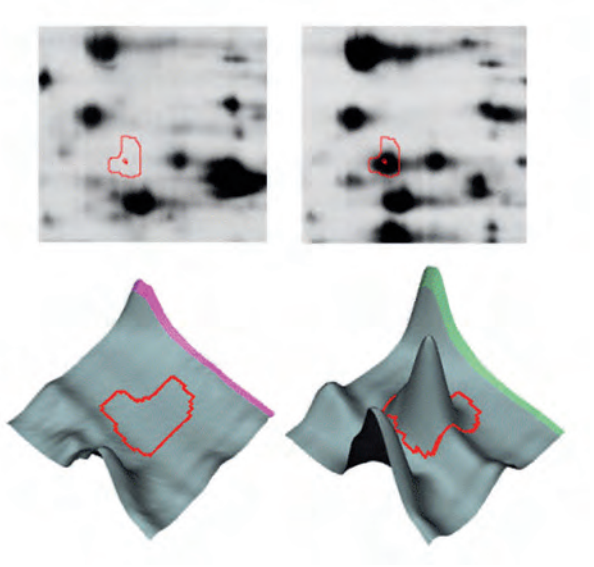



Compound 5b binds to the pyrophosphate group of lipid II and inhibits the polymerization of the peptidoglycan.

# Life in the cold: a proteomic study of cold-repressed proteins in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125

Florence Piette, Salvino D'Amico, Gabriel Mazzucchelli, Antoine Danchin, Pierre Leprince and Georges Feller (2011) Appl. And Environ. Microbiol., 77 : 3881–3883.

Our team has previously studied the cold-acclimation proteins of *Pseudoalteromonas haloplanktis*, a typical representative of γ-proteobacteria found in cold marine environments. This work has demonstrated that protein synthesis and protein folding are the main up-regulated functions at low temperature, suggesting that both cellular processes are limiting factors for bacterial development in cold environments. Here we report a proteomic survey of cold-repressed proteins at 4 °C in order to complete the metabolic pattern of the bacterium growth at low temperature. Remarkably, the major cold-repressed proteins, almost undetectable at 4°C, were heat shock proteins involved in folding assistance, showing that folding is mediated by different protein sets depending on low or high temperatures.

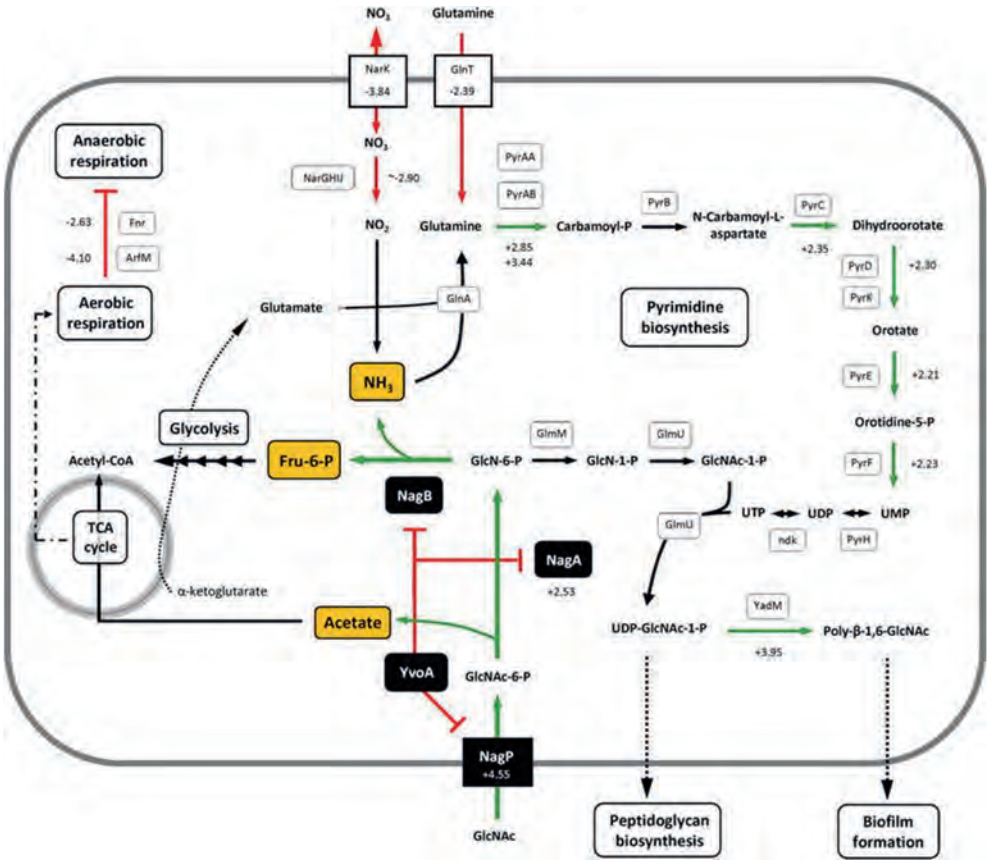


Comparative analysis of spots containing the chaperone DnaK from *Pseudoalteromonas haloplanktis* grown at 4°C (left panels) and 18°C (right panels). Spot view on 2D-gel seen in fluorescence (upper panels) and three-dimensional images (lower panels) obtained with DeCyder software.

# Regulon of the N-acetylglucosamine utilization regulator NagR (YvoA) in *Bacillus subtilis*

*Bertram R., *RIGALI S., Wood N., Lulko A.T., Kuipers O.P., Titgemeyer F. J. Bacteriol. 193:3525-3536. *Joint first authors.

The Centre for Protein Engineering has mainly built its international standing thanks to its expertise in understanding the molecular mechanism of protein functions at the amino acid or the atomic level. On the other hand, we are also developing several research themes that aim to globally comprehend the function of a protein at the 'organism' level. Together with Dr Ralph Bertram (University of Tübingen, Germany) and in a close collaboration with Prof. Fritz Titgemeyer (University of Münster, Germany), Dr Sébastien Rigali investigated the global transcriptomic response of *B. subtilis* following deletion of nagR, the N-acetylglucosamine utilization regulator.




**Summary of pathways influenced by YvoA (NagR) in *Bacillus subtilis***. N-acetylglucosamine (GlcNAc) is the most abundant carbon-nitrogen compound on earth and has been shown to be an important source of nutrients for both catabolic and anabolic purposes in *Bacillus* species. Whole-transcriptome studies showed significant repercussions of *nagR* (*yvoA*) deletion for several major *B. subtilis* pathways, probably indirectly due to an excess of the crucial molecules acetate, ammonia, and fructose-6-phosphate, resulting from complete hydrolysis of GlcNAc. Arrows in green or red indicate genes, the expression of which is up- or downregulated, respectively, in the *ΔyvoA* mutant compared to expression in the parental strain *B. subtilis*.

# SCIENTIFIC SERVICES

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# Towards BCCM/ULC: A Culture Collection of (Sub)Polar Cyanobacteria

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

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

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





**Exploration of the biodiversity**

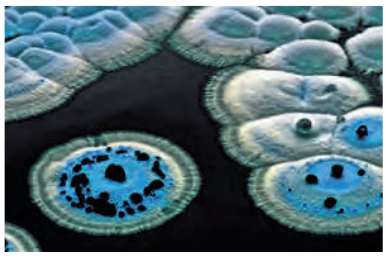
Picture and drawing of the strain ANT.LPR3, type species of the new genus *Plectolyngbya*.

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

# Protein Production and Purification Facilities

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

The platform can provide many services including:



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

For these purposes the platform is equipped with:

• Several shaking incubators for flasks from 1 ml to 2 L
• Wide range of computer controlled fermentors with working volumes from 1 to 60 L for batch and fed-batch cultures. Dissolved oxygen, pH, temperature, agitation and turbidity are controlled in all fermentors
• 2 continuous centrifuges
• 1 crossflow filtration unit
• Several systems for semi- or totally automated purification of proteins including: ÅKTA prime, ÅKTA explorer, ÅKTA explorer (2D system), ÅKTA purifier, Biopilot, Profinia Protein Purification System.

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

# High Throughput Mutagenesis Facilities

**Enzymes can naturally evolve to meet the needs of organisms in a defined environment. This natural evolution is a slow process. Laboratory evolution of enzymes can be accelerated relative to the natural process. To accelerate this evolution, we use low fidelity DNA polymerases which introduce mutations during the polymerisation step. This technique was used in our laboratory to randomly introduce mutations within a gene that encodes a psychrophilic β-galactosidase.**

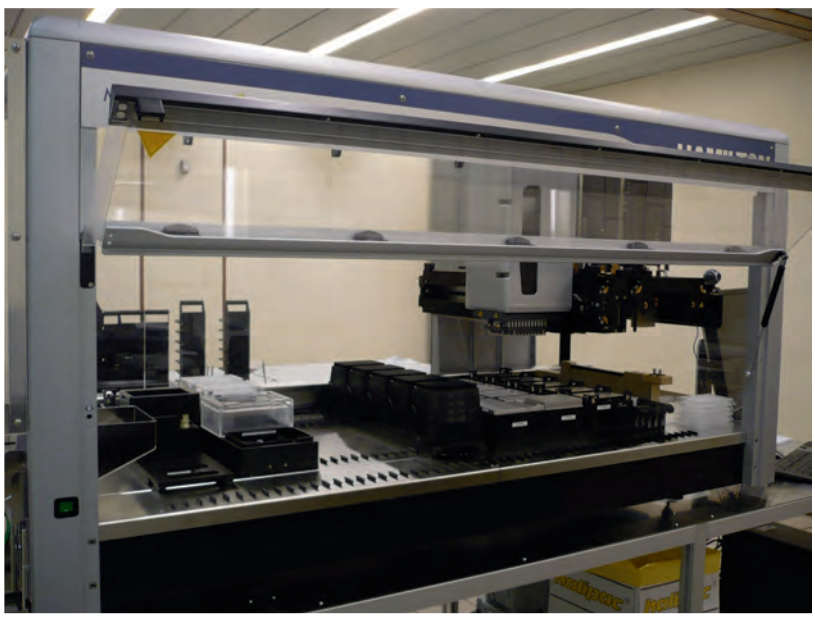
High throughput mutagenesis equipment was acquired in conjunction with the GIGA entity owing to funding from Feder. This equipment is centered on a Hamilton MICROLAB® STAR Liquid Handling Workstation. The station is supplied with an external robotic arm, a 96 channel pipetting head and 8 1000 µL independent channels. This equipment has been active since the beginning of 2011.

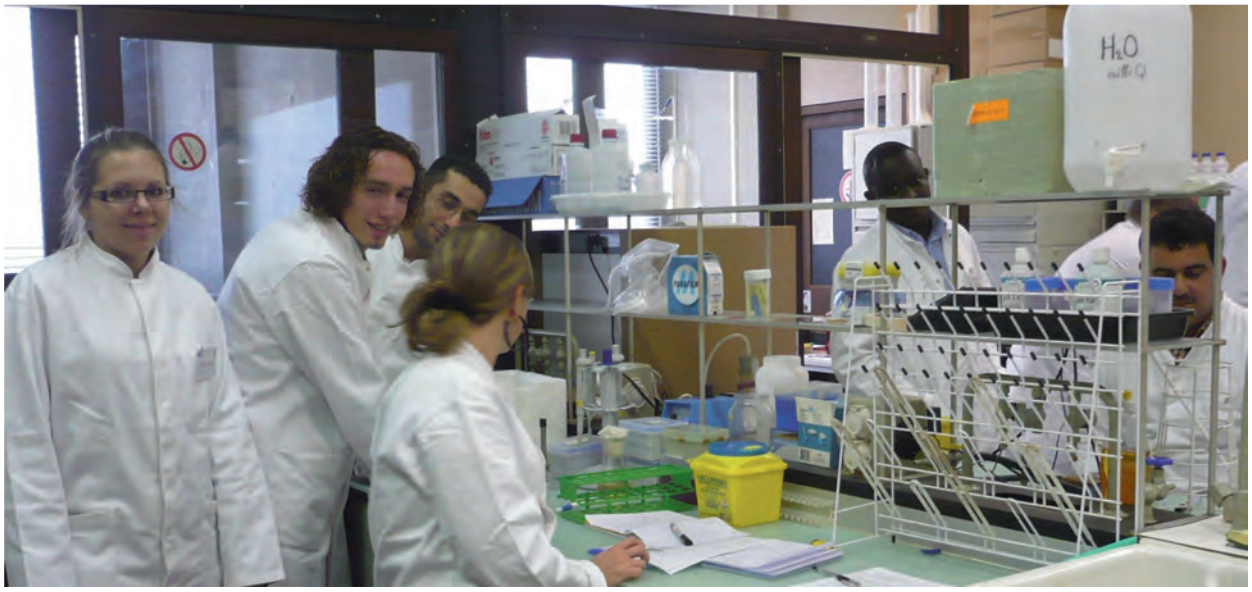
In the course of 2011, two Multimode microplate readers were acquired with funding coming from Belgian special funds for research. The first reader has 2 detection modes: one in absorbance and one in fluorescence. The second is also equipped with 2 detection modes: one in absorbance and one in luminescence. Moreover it is supplied with two injectors.

Training for programmation of the robotic handling workstation was followed by three people: Sarah Lebrun, Michaël Delmarcelle and Alain Brans. They have developed programs that allow the following applications:

- Enzymatic assays of β-lactamases
- Replicates of culture in 1 mL Deep-Well
- Enzymatic assays of human tryptophan 2.3-dioxygenase
- Enzymatic assays of xylanases
- Enzymatic assays of a psychrophilic beta-galactosidase
- Concentration determination of ketone sugars (e.g. tagatose)
- Generation of denaturation buffer for protein stability studies
- Protein purification on IMAC columns in a 96 well format.

The development of a protocol for high throughput mutagenesis of the psychrophilic beta-galactosidase from *Pseudoalteromonas haloplanktis* has been realized. It allowed us to obtain a bank of 1500 clones. This bank was characterized for its activity on ortho-nitrophenyl-ß-D-galactopyranoside (ONPG) as substrate.






## Techniques for protein production and purification

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

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

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

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

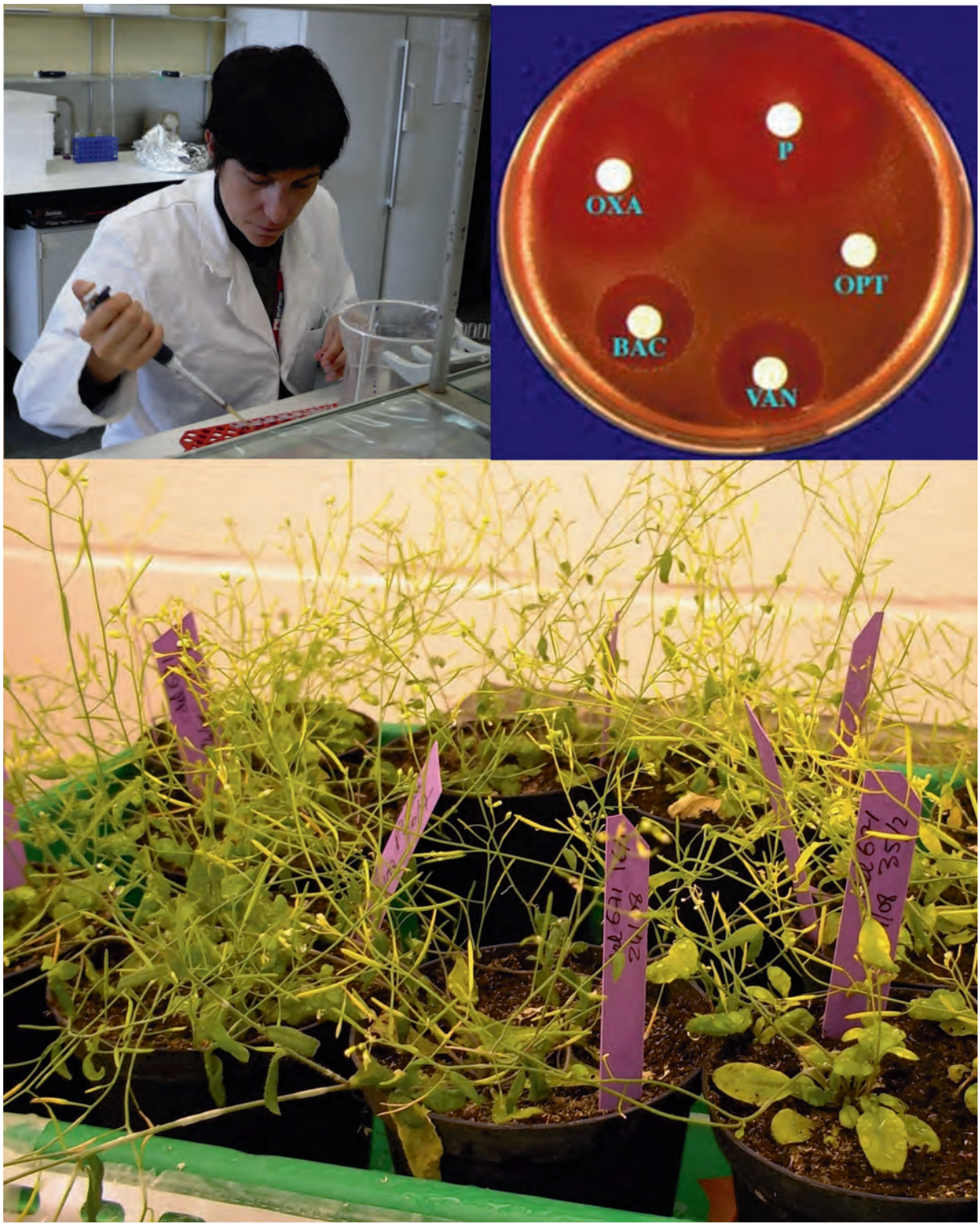
• Cell harvesting and cell disruption

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

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

LE FOREM

# SCIENTIFIC PRODUCTION



# Awards

| | |
|---|---|
| Céline Huynen | Prix Walter Verly |
| Mireille Dumoulin | Prix de l'Académie Royale des Sciences, des Lettres et des Beaux-Arts de Belgique – Concours 2011 – Groupe IV – Biologie animale |

# Invited Speakers

**Dr Alexander K. Buell**, Cambridge Nanoscience Centre, University of Cambridge, UK, "Fundamental insight into protein aggregation from accurate kinetic measurements", December 15

**Dr Jan Griesbach**, Nano Temper Technologies, Munich, Germany, "Introduction to Microscale Thermophoresis, September 21

**Dr Vincent Forge**, CEA Grenoble, France, "Insertion dans les membranes du domaine de translocation de la toxine diphtérique", November 10

**Dr Frank Sobott**, University of Antwerpen, Belgium, "Mass spec approaches to structure and assembly of biomolecular complexes", June 17

**Dr Mark van der Linden**, Institute of Medical Microbiology and National Reference Centre for Streptococci, Institute of Medical Statistics, University Hospital (RWTH), Aachen, Germany, "Current projects at the German National Reference Centre for Streptococci", October 7

# Oral Presentations

**B. Joris**, "The study of cell morphogenesis and its regulation and the design of compounds which might interfere with these processes", London, UK, January 19

**M. Dumoulin & C. Chavignon**, "Mechanism of amyloid fibril formation by human lysozyme and VHHS", IAP 6/19 meeting "Proteins: interaction involved in folding, function and supramolecular assemblages", Liege, January 25

**M. Hanikenne**, "Metal homeostasis in hyperaccumulating plants and algae", Génétique & Evolution des Populations Végétales, CNRS, Université des Sciences et Technologies de Lille, France, February 4

**J.S. Sohier**, "Caracterisation of metallo-beta-lactamases inhibition by VHHS", CRP Santé, Strassen, Luxemburg, February 10

**A. Wilmotte**, "Antarctic cyanobacterial diversity: how important are the geographical and ecological factors", BioSystematics, Berlin, Germany, February 21-26

**B. Joris**, "Engineering *Bacillus subtilis* genome", Kobe University Brussels European Centre, Brussels, Belgium, March 4

**S. Rigali**, "L'immobilité au service de l'évolution: adaptations génétiques et physiologiques des Streptomyces", Université de Liège, Belgium, March 4

**M. Hanikenne**, "Metal homeostasis in hyperaccumulating plants and algae", Plant Biology Institute Seminar Series, University of Liege, Belgium, March 14

**E. Sauvage**, "Bacterial resistance: a structural perspective", Ljubljana, Slovenia, May 6

**S. Rigali**, "Computational prediction of regulatory networks in bacteria or a rational alternative to random approaches to unveil novel cis/trans relationship (part 1)", Leiden Institute of Chemistry, Molecular Biology, University of Leiden, The Netherlands, May 13

**A. Matagne**, "Slow phases in beta-lactamase folding", Department of Molecular Biology, University of Siena, Italy, May 16

**S. Rigali**, "A la recherche des antibiotiques zombies", mini-conférence, Société Royale des Sciences de Liège, Belgium, May 19

**S. Rigali**, "Computational prediction of regulatory networks in bacteria or a rational alternative to random approaches to unveil novel cis/trans relationship (part 2)", Leiden Institute of Chemistry, Molecular Biology, University of Leiden, The Netherlands, June 2

**A. Matagne**, "Slow phases in beta-lactamase folding", 11th beta-lactamase meeting, Leonessa, Italy, June 10-14

**E. Sauvage**, "Structural basis for rearrangement of clavulanate, 6 beta-iodopenicillanate and lactivicin", 11th beta-lactamase meeting, Leonessa, Italy, June 10

**J.M. Frère**, "MICs, beta-lactamases and permeability", 11th beta-lactamase meeting, Leonessa, Italy, June 11

**M. Dumoulin,** "Engineering specific nanobodies to probe the molecular mechanism of lysozyme amyloid fibril formation", Amyloid Fibrils, Prions and Precursors: Molecules for Targeted Intervention, Halle/Saale, Germany, August 25-28

**M. Terrak**, "Specificity of *E. coli*, PBP1b for the substrate and inhibition of its GT activity" The dynamics of peptidoglycan structure and function: new insights into the "Great Wall", 2nd Symposium, Lisboa, Portugal, September 28-30

**J.M. Frère**, "Résistance et bêta-lactamases, des amies souvent inséparables", Colloque international : Emergence des bactéries multi-résistantes, Meknès, Morocco, October 8

**C. Laurent**, "Insight into the structural factors that modulate the metal binding affinity of the *Arabidopsis halleri* HMA4", SFMBBM Graduate School, 5th Annual Meeting, Brussels, October 14

**S. Rigali**, "Principles of transcriptional regulation in prokaryotes (part 2)", Université de Liège, Belgium, November 9

**S. Rigali**, "Principles of transcriptional regulation in prokaryotes (part 1)", Université de Liège, Belgium, November 16

**M. Dumoulin**, "Maladies du repliement des protéines: Cas des amyloses" mini-conférence, Société Royale des Sciences de Liège, Liège, November 17

**S. Rigali**, "Genetics and development of *Streptomyces*: adaptation génétiques d'un vieux sédentaire", FUNDP, Namur, Belgium, November 23

**V. Campisi**, "Identification of immunologically relevant human proteins as potential targets of house dust mite proteases", CRP Santé, Strassen, Luxemburg, November 25

**S. Rigali**, "Antibiotic resistance : lessons from the soil", Université de Liège, Belgium, December 5

**S. Rigali**, "Siderophore biosynthesis inhibition by the cell wall component N-acetylglucosamine counteracts *Streptomyces coelicolor* suicidal tendencies", International Symposium on the Biology of Actinomycetes (ISBA 16th), Puerto Vallarta, Mexico, December 11

# PATENTS

**Tryptamine-derived compounds as antibacterial agents**
M. Terrak, E. Breukink, S. Gobec, I. Chopra, T. Vernet
Pub. N°: WO2011/060976 A1, International Application N°: PCT/EP2010/063405
Publication Date: 26.05.2011, International Filing Date: 13.09.2010

# PhD Theses

06/05/2011 **Olivier Jacquin (Biochemistry)**
Etude des propriétés de repliement et de fixation du zinc de la métallo-ß-lactamase BcII de *Bacillus cereus* 569/H/9

24/05/2011 **Séverine Colson (Biochemistry)**
Définition du régulon DasR chez *Streptomyces coelicolor*, un réservoir pour la découverte de nouveaux gènes essentiels à l'induction de la différenciation morphologique et la production de métabolites secondaires

06/06/2011 **Marie-Eve Dumez (Biochemistry)**
Etude du mécanisme d'activation du zymogène de l'allergène Der p 3 de l'acarien *Dermatophagoides pteronyssinus*

01/07/2011 **Rafael Fernandez Carazo (Pharmacy)**
Contribution to the study of the present and past diversity of Antarctic cyanobacteria

14/10/2011 **Florence Piette (Biology)**
Etude des protéines régulées par le froid chez une bactérie antarctique : approche protéomique et biochimique de la psychrophilie

# PUBLICATIONS


1. S.A. Adediran, I. Kumar, R. Nagarajan, E. Sauvage and R.F. Pratt. Kinetics of reactions of the *Actinomadura* R39 DD-peptidase with specific substrates. Biochemistry, 50, 376-387.

2. M. Azarkan, A. Matagne, R. Wattiez, L. Bolle, J. Vandenameele and D. Baeyens-Volant. Selective and reversible thiol pegylation, an effective approach for purification and characterization of five fully active ficin (iso)forms from *Ficus carica* latex. Phytochemistry, 72, 1718-1731.

3. M. Ben Aissa, A. Herteg Fernea, H. Bouraoui, R. Ben Aissa, P. S. Mercuri and M. Manai. Molecular characterization of plasmid-encoded ACC-1a4 beta-lactamase produced by *Salmonella* strains isolated from water in a Tunisian hospital. Ann. Microbiol., 62(1), 31-36.

4. R. Berlemont, D. Pipers, M. Delsaute, F. Angiono, G. Feller, M. Galleni and P. Power. Exploring the Antarctic soil metagenome as a source of novel cold-adapted enzymes and genetic mobile elements. Revista Argentina de Microbiologia, 43, 1-10.

5. R. Bertram, Rigali S., Wood N., Lulko A.T., Kuipers O. P. and Titgemeyer F.. Regulon of the N-Acetylglucosamine utilization regulator NagR in *Bacillus subtilis*. J. Bacteriol., 193, 3525-3536.

6. A.K. Buell, A. Dhulesia, M.F. Mossuto, N. Cremades, J.R. Kumita, M. Dumoulin, M.E. Welland, T.P.J. Knowles, X. Salvatella and C.M. Dobson. Population of nonnative states of lysozyme variants drives amyloid fibril formation. J. Am. Chem. Soc., 133, 7737-7743.

7. M. Calusinska, B. Joris and A. Wilmotte. Genetic diversity and amplification of different clostridial [FeFe] hydrogenases by group-specific degenerate primers. Lett. Appl. Microbiol., 53, 473-480.

8. A. Cipolla, S. D'Amico, R. Barumandzadeh, A. Matagne and G. Feller. Stepwise adaptations to low temperature as revealed by multiple mutants of psychrophilic α-amylase from Antarctic bacterium. J. Biol. Chem., 286, 38348-38355.

9. C. Contreras-Martel, A. Amoroso, E.C.Y. Woon, A. Zervosen, S. Inglis, A. Martins, O. Verlaine, A.M. Rydzik, V. Job, A. Luxen, B. Joris, C.J. Schofield and A. Dessen. Structure-guided design of cell wall biosynthesis inhibitors that overcome β-lactam resistance in *Staphylococcus aureus* (MRSA). ACS Chemical Biology. 6(9):943-51.

10. A. Derouaux, S. Turk, N. K. Olrichs, S. Gobec, E. Breukink, A. Amoroso, J. Offant, J. Bostock, K. Mariner, I. Chopra, T. Vernet, A. Zervosen, B. Joris, J.-M. Frère, M. Nguyen-Distèche and M. Terrak. Small molecule inhibitors of peptidoglycan synthesis targeting the lipid II precursor. Biochem. Pharm., 81, 1098-1105.

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12. E. Dolušić, P. Larrieu, S. Blanc, F. Sapunaric, J. Pouyez, L. Moineaux, D. Colette, V. Stroobant, L. Pilotte, D. Colau, T. Ferain, G. Fraser, M. Galleni, J.M. Frère, B. Masereel, B. Van den Eynde, J. Wouters and R. Frédérik. Discovery and preliminary SARs of keto-indoles as novel indoleamine 2,3-dioxygenase (IDO) inhibitors. Eur. J. Med. Chem., 46, 3058-3065.

13. F. El Garch, P. Bogaerts, C. Bebrone, M. Galleni and Y. Glupczynski. OXA-198, an acquired carbapenem-hydrolyzing class D beta-lactamase from *Pseudomonas aeruginosa*. Antimicrob. Agents and Chemother., 55, 4828-4833.

14. G. Feller, D. Dehareng and J.L. Da Lage. How to remain nonfolded and pliable: the linkers in modular alpha-amylases as a case study. FEBS Journal, 278, 2333-2340.

15. R. Fernandez-Carazo, D.A. Hodgson, P. Convey and A. Wilmotte. Low cyanobacterial diversity in biotopes of the Transantarctic Mountains and Shackleton Range (80-82°S), Antarctica. FEMS Microbiol. Ecol., 77, 11-15.

16. C. Fraipont, S. Alexeeva, B. Wolf, R. van der Ploeg, M. Schloesser, T. den Blaauwen and M. Nguyen-Distèche. The integral membrane FtsW protein and peptidoglycan synthase PBP3 form a subcomplex in *Escherichia coli*. Microbiology, 157, 251-259.

17. G. Girard and S. Rigali. Role of the phenazine-inducing protein Pip in stress resistance of *Pseudomonas chlororaphis*. Microbiology, 157, 398-407.

18. M. Hanikenne and C. Nouet. Metal hyperaccumulation and hypertolerance: a model for plant evolutionary genomics. Curr. Opin. Plant Biol., 14, 252-259.

19. L. Horsfall, Y. Izougarhane, P. Lassaux, N. Selevsek, B. Liénard, L. Poirel, M.B. Kupper, K.M. Hoffmann, J.-M. Frère, M. Galleni and C. Bebrone. Broad antibiotics resistance profile of the subclass B3 metallo-beta-lactamase GOB-1, a dizinc enzyme. FEBS J., 278, 1252-1263.

20. P. Lassaux, D.A.K. Traoré, E. Loisel, A. Favier, J.-D. Docquier, JS Sohier, C. Laurent, C. Bebrone, J.-M. Frère, J.-L. Ferrer and M. Galleni. Biochemical and structural characterization of the subclass B1 metallo-beta-lactamase VIM-4. Antimicrob. Agents and Chemother., 55, 1248-1255.

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24. Z. Namsaraev, M.J. Mano, R. Fernandez and A. Wilmotte. Biogeography of terrestrial cyanobacteria from Antarctic ice-free areas. Annals of Glaciology, 51, 171-177.

25. V.V. Nemmara, L. Dzhekieva, K.S. Sarkar, S.A. Adediran, C. Duez, R.A. Nicholas and R.F. Pratt. Substrate specificity of low-molecular mass bacterial DD-peptidases. Biochemistry, 50, 10091-10101.

26. C. Nouet, P. Motte and M. Hanikenne. Chloroplastic and mitochondrial metal homeostasis. Trends in Plant Sciences, 16, 395-404.

27. FJ Pérez-Llarena, F. Kerff, O. Abian, S. Mallo, MC Fernandez, M. Galleni, J. Sancho and G. Bou. Distant and new mutations in CTX-M-1beta-lactamase affect cefotaxime hydrolysis. Antimicrob. Agents and Chemother., 55, 4361-4368.

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30. P. Reed, H. Veiga, A.M. Jorge, M. Terrak and M.G. Pinho. Monofunctional transglycosylases are not essentials for *Staphylococcus aureus* cell wall synthesis. J. Bacteriol., 193, 2549-2556.

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32. O. Savichtcheva, B. Joris, A. Wilmotte and M. Calusinska. Novel FISH and quantitative PCR protocols to monitor artificial consortia composed of different hydrogen-producing *Clostridium spp*. International Journal of Hydrogen Energy, 36, 7530-7542.

33. I. Sosič, H. Barreteau, M. Simčič, R. Šink, J. Cesar, A. Zega, S. Golič Grdadolnik, C. Contreras-Martel, A. Dessen, A. Amoroso, B. Joris, D. Blanot and S. Gobec. Second-generation sulfonamide inhibitors of D-glutamic acid-adding enzyme: activity optimization with conformationally rigid analogues of D-glutamic acid. Eur. J. Med. Chem., 46, 2880-2894.

34. PM Tiber, O. Orun, C. Nacar, UO Sezerman, F. Severcan, M. Severcan, A. Matagne and B. Kan. Structural characterization of recombinant bovine Goα by spectroscopy and homology modeling. Spectroscopy, 26, 213-229.

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36. M. Vandevenne, G. Gaspard, M. El Belgsir, Y. Cenatiempo, D. Marechal, M. Dumoulin, JM Frère, A. Matagne, M. Galleni and P. Filée. Effects of monopropanediamino-β-cyclodextrin on the denaturaion process of the hybrid protein BlaPChBD. Biochim. Biophys. Acta, 1814, 1146-1153.

37. E.C.Y. Woon, A. Zervosen, E. Sauvage, K.J. Simmons, M. Zivec, S.R. Inglis, C.W.G. Fishwick, S. Gobec, P. Charlier, A. Luxen and C.J. Schofield. Structure guided development of potent reversibly binding penicillin binding protein inhibitors. ACS Med. Chem. Lett., 2, 219-223.

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39. A. Zervosen, R. Herman, F. Kerff, A. Herman, A. Bouillez, F. Prati, R.F. Pratt, J.M. Frère, B. Joris, A. Luxen, P. Charlier and E. Sauvage. Unexpected tricovalent binding mode of boronic acids within the active site of a penicillin binding protein. J. Am. Chem. Soc., 133, 10839-10848.

40. M. Vandevenne, V. Campisi, A. Freichels, C. Gillard, G. Gaspard, JM Frère, M. Galleni, and P. Filée. Comparative functional analysis of the human macrophage chitotriosidase. Protein Science, 20, 1451–1463.

41. Taton, A., Wilmotte, A., Smarda, J., Elster, J., & Komarek, J. *Plectolyngbya hodgsonii*: a novel filamentous cyanobacterium from Antarctic lakes. Polar Biology, 34, 181-191.

42. Wilmes B., Kock H., Glagla S., Albrecht D., Voigt B., Markert S., Gardebrecht A., Bode R., Danchin A, Feller G., Hecker M. and Schweder T. Cytoplasmic and periplasmic proteomic signatures of growing cells of the psychrophilic bacterium Pseudoalteromonas haloplanktis TAC125. Applied Environ. Microbiol. 77, 1276-1283.

# BOOK CHAPTERS AND REVIEWS

F. Roulling, F. Piette, A. Cipolla, C. Struvay and G. Feller. Psychrophilic enzymes: cool responses to chilly problems. In: Extremophiles Handbook. Ed K. Horikoshi, Springer, 2011, 892-913

R. Berlemont and C. Gerday. Extremophiles In: Comprehensive Biotechnology (2nd edition). Ed. M. Moo-Young, Elsevier, 2011

# Protein Structures deposited within the Protein Data Bank

| PDB ID | STRUCTURE TITLE | AUTHOR |
|---|---|---|
| 2XLN | CRYSTAL STRUCTURE OF A COMPLEX BETWEEN ACTINOMADURA R39 DD-PEPTIDASE AND A BORONATE INHIBITOR | Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 2XK1 | CRYSTAL STRUCTURE OF A COMPLEX BETWEEN ACTINOMADURA R39 DD-PEPTIDASE AND A BORONATE INHIBITOR | Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 2Y59 | UNEXPECTED TRICOVALENT BINDING MODE OF BORONIC ACIDS WITHIN THE ACTIVE SITE OF A PENICILLIN BINDING PROTEIN | Sauvage, E., Zervosen, A., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 2Y55 | UNEXPECTED TRICOVALENT BINDING MODE OF BORONIC ACIDS WITHIN THE ACTIVE SITE OF A PENICILLIN BINDING PROTEIN | Sauvage, E., Zervosen, A., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 2Y4A | UNEXPECTED TRICOVALENT BINDING MODE OF BORONIC ACIDS WITHIN THE ACTIVE SITE OF A PENICILLIN BINDING PROTEIN | Sauvage, E., Zervosen, A., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 3ZVW | UNEXPECTED TRICOVALENT BINDING MODE OF BORONIC ACIDS WITHIN THE ACTIVE SITE OF A PENICILLIN BINDING PROTEIN | Sauvage, E., Zervosen, A., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 3ZVT | UNEXPECTED TRICOVALENT BINDING MODE OF BORONIC ACIDS WITHIN THE ACTIVE SITE OF A PENICILLIN BINDING PROTEIN | Sauvage, E., Zervosen, A., Herman, R., Kerff, F., Rocaboy, M., Charlier, P. |
| 2Y91 | CRYSTAL STRUCTURE OF CLASS A β-LACTAMASE FROM BACILLUS LICHENIFORMAIS BS3 WITH CLAVULANIC ACID | Power, P., Sauvage, E., Herman, R., Kerff, F., Charlier, P. |

# SYMPOSIA

Symposium “Microbial biogeography and diversity patterns: extending classical ecology theories or defining new paradigms”, BioSystematics, Berlin, Germany, February 25
**Co-organizer: Dr A. Wilmotte**

Workshop “Antarctic Biodiversity: Status and Trends”, University of Liege, Belgium, May 31
**Main Organizer: Dr A. Wilmotte**

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

# EDUCATION

# Academic Courses

## Bachelor and Preparation to Masters

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Masters

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

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

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

Astrobiologie, 30h Th + 15h Pr. – GEOL0263 – V. Dehant, **M. Galleni**, E. Javaux, Y. Nazé, P. Claeys and **A. Wilmotte**. Master 1 Sciences spatiales et master 1 en sciences géologiques.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## Inter University Thematic Weeks

Antibiotic resistance. 25h – BIOC0716-1 - **J.-M. Frère**, **M. Galleni** et **B. Joris**. Master 2 Biochimie et Biologie Moléculaire et Cellulaire.

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

Microorganismes extrémophiles. 25 + 25h Pr - MICR0713-1 - **M. Galleni**, **G. Feller** and **A. Wilmotte**. Master 2 BBMC.

## Complementary Masters

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

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

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

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

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

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



## Third Cycle

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

Advanced course on “Protein Purification: What to do and how” (Coordinated by **Prof. J.-M. Frère**), 15h. Prof. E. Depauw, **J.-M. Frère**, **M. Galleni**, **B. Joris** et **A. Matagne**. 17-19 May.

## Courses given abroad

Bioinformatique, 35 h - **A. Brans**. Bac 3 DUT Génie biologique. IUT de Mont de Marsan, Université de Pau et des Pays de l’Adour, France. 14-21 November 2011.

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

Enzyme kinetics, Protein folding and Protein Purification. A**. Matagne**, **J.-M. Frère** et **M. Galleni**. Masters en Biotechnologie et Microbiologie. Cycle de 3 ans, 15h/an. Università degli Studi di Siena, Siena, Italy. June 2011.

Nanobodies or Camelid Antibody Fragments: Properties and Application, 7 h – **M. Dumoulin** Department of Pharmaceutical Sciences, University of Padova, Italy, 28-29 november 2011.

Production de protéines recombinantes, 8 h - **A. Brans**. Bac 3 DUT Génie biologique. IUT de Mont de Marsan. Université de Pau et des Pays de l'Adour, France. 21-22 November 2011.

Statistique multivariée, 30 h - **G. Dive**. Masters1 et 2 en Chimie et thérapeutique. Université Paris VII Denis-Diderot, France.

## Courses given in another Belgian university

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

An introduction to microcalorimetric studies of proteins, University of Namur – FUNDP, Belgium , **G. Feller,** 2h., 28 February 2011

## Courses given in Technical High Schools

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

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

# Trainees and Students

## Master I Trainees

BAPTISTE Stacy
GILLET Erik
JOURDAN Samuel
KUBICA Annick
LAMBERT Jérémy
LEKEUX Gilles
MARTINET Loïc
MASSOZ Simon
MENZER Linda
PALM Sarah
PAQUES Cécile
RENAULD Justine
RIGAUX Maïlys
SCHILS Nicolas
SERVAIS Laurence

## Master II Students

**DELBRASSINE François** — MasterII BBMC à finalité approfondie, ULg.
Relation activité-flexibilité-stabilité au sein d'une famille d'alpha-amylases chlorure-dépendantes.

**DEWARD Adeline** — MasterII BBMC à finalité approfondie, ULg.
Taxonomie moléculaire de souches d'Oscillatoriacées Antarctiques.

**GERARD Méline** — MasterII BBMC à finalité approfondie, ULg.
Etude fonctionnelle des facteurs d'épissage SR d'*Arabidopsis thaliana* de la sous-famille RS2Z.

**HAMACHER Morgane** — MasterII BBMC à finalité approfondie, ULg.
Etude du mode d'action et de l'expression transcriptionnelle du régulateur global DasR chez *Streptomyces coelicolor.*

**HUYNEN Céline** — MasterII BBMC à finalité approfondie, ULg.
Caractérisation des propriétés amyloïdogènes du peptide Aß42(associé à la maladie d'Alzheimer) replacé dans un environnement protéique.

**JERIDI Souraya** — MasterII BBMC à finalité approfondie, ULg.
Etude des protéines GalM et PBP4a de *Bacillus amyloliquefaciens* FZB42.

**LAMBERT Stéphany** — MasterII BBMC à finalité approfondie, ULg.
Etude des bases moléculaires du contrôle de la production des sidérophores par la N-acétylglucosamine chez *Streptomyces coelicolor.*

**MURAT Elise** MasterII BBMC à finalité approfondie, ULg.
Caractérisation du Trigger Factor psychrophile de *Pseudoalteromonas haloplanktis* et des ses homologues mésophile (*Escherichia coli*) et thermophile (*Thermotoga maritima*).

**SEPULCHRE Jérémy** MasterII BBMC à finalité approfondie, ULg.
Etude du récepteur BlaR1 impliqué dans la reconnaissance de la pénicilline chez *Bacillus licheniformis* 749/I

## Erasmus Students - Master II

**BERTOLDO Davide** Master II. Faculty of Pharmacy. University of Padova, Italy.
Effects of the binding of trigger factor and VHHs on the aggregation of BlaP chimeras containing polyglutamine stretches.
February-September 2011.

**FAILLACI Francesca** Biotecnologie per l'Industria e la Ricerca Scientifica.
Università degli Studi di Palermo, Italy.
Développement d'une technologie de production de lait délactosé.

## Technical high schools – Bachelor III

**BOULEAU Cindy** Licence Professionnelle de Biotechnologie, option biologie moléculaire appliquée à la sécurité alimentaire. Université de Pau et des Pays de l'Adour, Mont De Marsan, France.
Clonage, mutagenèse et étude de l'arabinose isomérase pour la production de tagatose.

**CREPIN Sophie** Bachelier en Agronomie, finalité environnement. Haute Ecole de la Province de Liège. Catégorie Agronomique, La Reid
Etude du gène FRD3, un gène de l'homéostasie des métaux chez *Arabidopsis halleri* et *Arabidopsis thaliana*.

**DOMBIER Cécile** Bachelier-technologue de laboratoire médical, option cytologie. HEPL André Vésale. Quai du Barbou, 2, 4000 Liège.
Cartographie d'épitopes de l'a-hémolysine de *Staphylococcus aureus*.

**GRAIDE Hélène** Bachelier-technologue de laboratoire médical, option chimie clinique. Haute Ecole Charlemagne CHU-B36/Tour 4.
Clonage et production des protéines FtsW et NlpD impliquées dans la division cellulaire bactérienne.

**MFEUZA Marguerite** Bachelier en chimie, finalité biotechnologie. HEPL Rennequin Sualem, quai Gloesner,6, 4020 Liège.
Transformation de *Bacillus licheniformis* 749I par un transposon mini Tn10 dans le but d'étudier la résistance aux ß-lactames.

| | |
|---|---|
| **PAULUS Virginie** | Bachelier – Technologue de laboratoire médical. HELMo-Saint Laurent, Quai Mativa 38, 4020 Liège.<br>Etude de cellulases impliquées dans la synthèse de cellulose bactérienne |
| **ROPPE Aurélie** | Bachelier-technologue de laboratoire clinique, option biotechnologie. HELMo-Saint Laurent, Quai Mativa 38, 4020 Liège.<br>Caractérisation de la Tryptophane 2,3-dioxygénase humaine |
| **SOMJA Sarah** | Bachelier-technologue de laboratoire médical, option chimie clinique. Haute Ecole Charlemagne CHU-B36/Tour 4<br>Etude de la transglycosylase monofonctionnelle de *Staphylococcus aureus*. |
| **VANDEN BROECK Arnaud** | Bachelier-technologue de laboratoire médical, option cytologie. HEPL André Vésale. Quai du Barbou, 2, 4000 Liège.<br>Délétion dans *Bacillus subtilis* de l'opéron *pbpE-racX* et caractérisation des mutants en relation avec la formation de biofilms. |

# General Public Activities

## Activities for students of secondary schools

'Journée des Rhétos' (organized by the'Administration de l'Enseignement et des Etudiants'). 15 February 2011. Participation of **C. Bebrone, F. Bouillenne, P. Charlier, M. Delsaute, J. Herman, F. Kerff, M. Maréchal** and A. **Wilmotte**.

'Soirée Masters' (organized by the 'Administration de l'Enseignement et des Etudiants'). March 29th. Participation of **Patrick Motte**.

Les recherches scientifiques réalisées à la station antarctique Princess Elisabeth. April 1st.Conference by **A. Wilmotte** to the 5th and 6th levels at the secondary school Notre-Dame de Tournai.

'Portes ouvertes Parents-Rhétos-Passerelles '(organized by the 'Administration de l'Enseignement et des Etudiants').
May 7th. Participation of **D. Dehareng** and **P. Motte**.

Course on Antarctica by **A. Wilmotte** to the 6th grade of the primary school, Athénée de Vielsam on May 19th.

## Dream Day (www.dreamday.be)

La vie de chercheur. 24 March 2011:
Participation of **Georges Dive**, Athénée Sainte Marie à Huy.
Participation of **A. Wilmotte** and **M. Delsaute**, Institut St Roch à Theux.

## Newspaper article or press conference

Interview of **A. Wilmotte** and **Z. Namsaraev**: 'Les premiers habitants de l'antarctique' for a BELSPO publication 'Les Belges aux pôles. La quatrième année polaire internationale'. D2011/1191/28
http://www.belspo.be/belspo/organisation/Publ/pub_ostc/ANTAR/PolarBrochure_fr.pdf

## Scientific blog

BELDIVA expedition: www.antarcticabelgium.blogspot.com

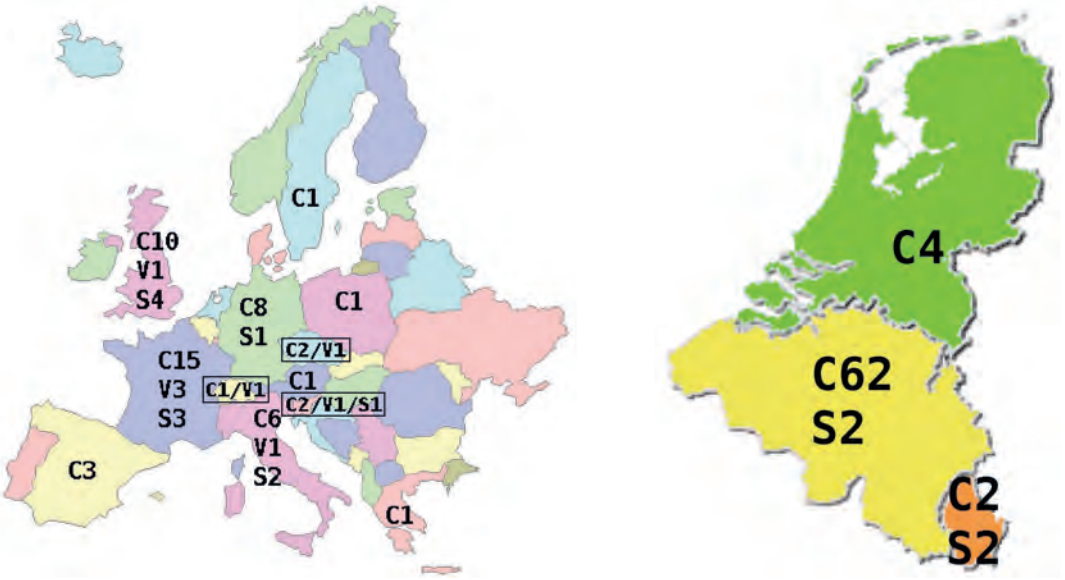
## Wide audience conferences

**Annick Wilmotte**: organisation of the session 'L'Antarctique dans tous ses états', 6 lessons from September 20th till October 18th. Collège Belgique, Royal Belgian Academy of Sciences, Namur.
Lesson: L'Antarctique est un continent microbien. **A. Wilmotte** and A. Willems, October 11th.

**Jean-Marie Frère**: La résistance bactérienne: pour la poursuite sans relâche de la recherche de nouveaux antibiotiques. Collège Belgique, Royal Belgian Academy of Sciences, Namur on November 7th.

**Jean-Marie Frère**: Étude de cas : impact de l'utilisation d'antibiotiques sur les résistances bactériennes. Cours-conférence, Collège Belgique, Namur on November 24th. http://www.academieroyale.be

# INTERNATIONAL EXCHANGES


**C** Collaborations
**V** Visitors
**S** Stays in other Institutions

# Postdocs In

**Dr Arabela CUIROLO**

**Research group: Bacterial diversity, physiology and genetics**
**Group leader: Prof. B. Joris**

My name is Arabela Cuirolo, I´m an Argentinian citizen working at the Center for Protein Engineering, University of Liege with a post-doctoral scholarship from FNRS since April 2011. What can I say about me and why I am here? Well, I was tired of living in a place where the sun shines almost every day in a city that never sleeps, and no, it was not NYC, it was Buenos Aires!

My PhD project was focused on the altered expressions of resistance in methicillin resistant *Staphylococcus aureus*. It was carried out at the Laboratory of Research on MRSA (Methicillin Resistant *Staphylococcus aureus*), Virginia Commonwealth University (VCU), USA under direction of Dr. Adriana Rosato and also at the University of Buenos Aires (UBA), Argentina, under guidance of my co-advisor Dr. Marta Mollerach.

So I thought, after fighting some years on the altered expressions of resistance in *Staphylococcus aureus* over there, why not to try a new life-work experience….so here I am! I started my postdoctoral training at the Centre for Protein Engineering (CIP) with Professor Bernard Joris. I am working right now in areas such as protein engineering, production and purification and *Bacillus* spp physiology, which I've never explored before.

**Dr Adeline DEROUAUX**

**Research group: Bacterial diversity, physiology and genetics**
**Group leader: Dr M. Terrak**

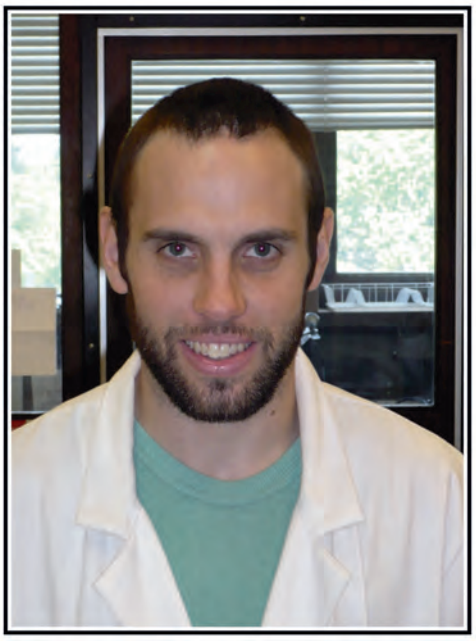
I'm a bit different PostDoc "in" than the others. I completed my master in Biochemistry at the University of Liège in 2000 with an internship realized at CIP with Sébastien Rigali in Jean Dusart's lab on XlnR, a transcriptional regulator of *Streptomyces*. At the end of that first lab work experience with those very interesting bacteria, I decided to do a PhD in the same lab on CRP, another transcriptional regulator of *Streptomyces*, financed by the FRIA. Meanwhile, I got married and had my first child. I could finish my PhD in 2005 and got the chance to stay at the CIP to work with Martine Nguyen-Distèche and Mohammed Terrak on a big European project called EUR-INTAFAR on "Inhibition of new targets for fighting antibiotic resistance". So I shifted from the *Streptomyces* world to *E. coli* cell division, peptidoglycan synthesis and their inhibition, a completely new topic for me but an old one in the CIP. I remember that it took me some time to enter in this vast topic with so many papers already published compared to my PhD topic. I started to study glycosyltransferases, their activities, their inhibition, their interactions with other proteins, etc. This project allowed me to meet and work with the best European scientists in the field. I found these collaborations very interesting and fruitful. From such collaborations, I thought it would be great to leave the CIP for a PostDoc abroad already with the idea to come back with new ideas and know-how. I decided to apply for an EMBO fellowship to go in to Waldemar Vollmer's lab in Newcastle, UK, and study the structure of the peptidoglycan of the septum. I got the fellowship and so, in 2009, I moved to Newcastle with family (I got a second child at the beginning of the European project) for one year at the beginning. I was lucky as my husband managed to have a sabbatical leave. As he was a laboratory technician, we worked together in the lab at Newcastle. So we were not very good in improving our English and the kids were learning it a lot quicker than us. After a couple of months in the UK, I realized that one year of PostDoc wouldn't be enough to obtain interesting results, particularly on the challenging topic I had chosen. So we decided to extend our stay one more year. This experience abroad has been very interesting for the whole family. I found it important to see how we work in a new environment. I had the opportunity to meet a lot of interesting scientists, I learned many techniques either specific to peptidoglycan or not. Even if the results were not always there, I enjoyed working there particularly with such a passionate boss. To facilitate my return in Belgium, in 2011, I applied for a return grant from Belspo to rejoin Mohammed Terrak's lab and work on interactions of *E. coli* divisome proteins in membranes. Because of the absence of Belgian government, approval of the projects was delayed. I returned to Belgium without any project as my husband had to come back after his 2-year sabbatical leave. I finally got the return grant for 2 years and I'm back in Mohammed's lab. Presently, I am purifying membrane proteins involved in *E. coli* cell division, reconstitute them in vesicles and analyze their structure and interactions by infrared spectroscopy, hydrogen/deuterium exchange and mass spectrometry in collaboration with Erik Goormaghtigh at ULB. And as usual, I am already worrying about the next financial source in the hope to be able to continue the research topic I want.

**Dr Caroline MONTAGNER**

**Research group: Enzymology and Protein Folding**
**Group leader: Prof. A. Matagne**

I did my PhD at the CEA in Grenoble where I was studying membrane-protein interactions using a biophysical approach. This involved the use of optical spectroscopic techniques as well as Hydrogen/Deuterium exchange combined with mass spectrometry. I was then a post-doctoral fellow at the Biozentrum in Basel (Switzerland) in the group of Prof. Guy R. Cornelis working on the type III secretion system of *Yersinia enterocolitica*. I was studying the formation of protein complex in the host cell membrane, thus applying my expertise in a more *in vivo* context. Then I decided to come back to biophysics and more precisely protein folding. In this frame, the CIP offered an unrivalled opportunity. Indeed the centre is devoted to protein studies from their structure determination to their function. This combination of high expertise and the availability of all the equipment necessary to these studies make the CIP a great place to perform cutting edge research. Furthermore, the tight interactions between all the groups is a great chance to learn more about complementary fields in protein studies and acquire new expertise.

I am now a post-doctoral fellow in the group of Prof. André Matagne.
This group has a very good expertise in protein folding, and state-of-the-art equipment needed to perform such studies. I'm currently studying the folding process of a zinc ß-lactamase from *Bacillus cereus* (known as BcII). This metallo-ß-enzyme (~25 000 Da) is among the largest proteins studied in detail so far and is therefore a good model for studying the folding mechanism of relatively large monomeric globular proteins. The equilibrium folding properties have already been characterized.
In order to obtain a complete picture of the folding process of BcII, stopped-flow optical spectroscopy is used in conjunction with quenched-flow hydrogen exchange labelling experiments analyzed by mass spectrometry (in collaboration with the group of Prof. Edwin de Pauw in Liège) and NMR (in collaboration with Prof. Christina Redfield, in Oxford). Preliminary refolding kinetics showed that the tertiary structure is acquired in five observable steps, which time constant values could be determined. In the earliest refolding phases (dead time of the stopped-flow device and phase 1) the protein was shown to adopt a molten-globule structure. The limiting step for regain of activity could also be identified. Test experiments aiming at setting the proper conditions for the quenched-flow hydrogen exchange labelling experiments are ongoing.



**Dr David THORN**

**Research group: Enzymology and Protein Folding**
**Group leader: Dr M. Dumoulin**

I joined the ranks of the CIP in May 2012, within a week of graduating with a PhD from the University of Adelaide, Australia. Whilst joining the laboratory of Protein Folding and Enzymology, my research interests lie more on the dark side of protein behaviour entailing misfolding, aggregation, and the formation of rope-like assemblies called amyloid fibrils. Originally from Sydney, I studied Biotechnology at the University of Wollongong with every hope of becoming a 'genetic engineer'. That was before I was introduced to amyloid fibrils, and I must admit it was love at first sight. Since then I have mainly studied fibril formation by the casein proteins of bovine milk with Prof. John Carver.

Now, as part of the 'polyQ group' led by Dr. Mireille Dumoulin, I investigate the aggregation behaviour of chimeric proteins consisting of the β-lactamase BlaP with polyglutamine (polyQ) insertions. PolyQ expansion above a threshold length in proteins leads to an increased propensity to form amyloid fibrils. The role that non-polyQ regions of the protein play in modulating this aggregation is the focus of the *Mandat d'Impulsion Scientifique* funded by the FNRS and through which I am supported. The broad aim of this research is to gain a better understanding, at the molecular level, of how polyQ proteins give rise to several neurological disorders including Huntington's disease.

Having worked previously with milk-derived proteins, I sought a project where I could get back to my Biotech roots so to speak and develop my skills in molecular cloning and protein expression. This, and because I love Belgium and wish to learn French (albeit rather slowly), is why I chose to join the CIP.

# COLLABORATIONS

**ARGENTINA**

University of Buenos Aires – Laboratory for Bacterial Resistance – **G. Gutkind, M. Mollerach & P. Power**

**AUSTRIA**

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

**BELGIUM**

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

CER Groupe – Marloie – **A. Collard & C. Fanchamps**

Euroscreen – Bruxelles – **S. Blanc**

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

FUNDP – Département de Pharmacie - Namur – **B. Masereel & R. Frederik**

FUNDP – Département de Chimie - Namur – **C. Michaux**

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

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

KUL – Laboratory Biomolecular Dynamics – Leuven – **Y. Engelborghs**

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

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

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

Ludwig Institute for Cancer Research – Brussels – **B. Van den Eynde**

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

Nutrilab NV – Heusden-Zolder – **C. Vastenavond & J.M. François**

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

SCK-CEN – Unit of Microbiology – Mol – **M. Mergeay, N. Leys & R. Van Houdt**

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

UCL – Earth and Life Institute – Louvain-la-Neuve – **A.C. Mailleux**

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

UCL – Biochemistry and Molecular Genetics of Bacteria – Louvain-la-Neuve – **P. Soumillion**

UGent – Laboratory for Protein Chemistry and Biomolecular Engineering – Ghent – **B. Devreese**

UGent - Department of Organic Chemistry, Organic and Bioorganic synthesis - Ghent - **J. Van der Eycken**

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

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

ULB – Unité de Recherche d'Immunobiologie - Laboratoire d'Allergologie Expérimentale – Gosselies – **E. Adam & D. Walgraffe**

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

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

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

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

ULB – Structure et Fonction des Membranes Biologiques (SFMB) - Bruxelles – **E. Goormaghtigh, V. Raussens & A. Gustot**

ULB – Unité de Toxicologie – Bruxelles – **R. Kiss**

ULB – Physiologie animale – Gosselies – **M. Moser**

ULB – Laboratoire de Génétique des Procaryotes – Bruxelles – **L. Van Malderen**

ULg – GIGA Neurosciences – Liège – **L. Bettendorf**

ULg – Centre de Biophysique Moléculaire Numérique – Gembloux – **R. Brasseur**

ULg – GIGA-R – Physiologie Cellulaire et Moléculaire – Liège – **F. Bureau**

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

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

ULg - Département de Chimie – Nano-chimie et Systèmes Moléculaires – Liège – **A.S. Duwez**

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

ULg – Département clinique des animaux de compagnie et des équidés – Liège – **T. Franck**

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

ULg – Département des Sciences Cliniques – Liège – **A. Gothot**

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

ULg – Département de Chimie – Chimie des Macromolécules et des Matériaux Organiques – Liège - **C. Jérôme**

ULg – GIGA-Neuroscience – Liège – **P. Leprince**

ULg – Département de Chimie – Laboratoire de Dynamique Moléculaire– Liège – **B. Leyh**

ULg – Département des Sciences Cliniques / Pneumologie-Allergologie – Liège – **R. Louis**

ULg – Centre de Recherches du Cyclotron – Chimie Organique de Synthèse – Liège – **A. Luxen**

ULg – Département des Sciences Biomédicales et Précliniques/Bactériologie, mycologie, parasitologie, virologie – Liège – **P. Melin**

ULg – Centre de l'Oxygène : Recherche et Développement (C.O.R.D.) – Liège – **A. Mouithys-Mickalad**

ULg – GIGA-R – Biologie et Génétique Moléculaire – Liège – **M. Muller**

ULg – Chimie et Bio-industries / Chimie Biologique Industrielle – AgroBioTech Gembloux – **M. Paquot**

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

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

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

ULg – GIGA-R – Biologie et Génétique Moléculaire – Liège – **C. Van de Weerdt**

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

VUB – Department of Biochemistry – Laboratory of Biomolecular Dynamics – Leuven – **Y. Engelborghs**

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

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

**BRAZIL**

University of Sao Paulo – **M. Fiore & C. Sant'Anna**

Federal University of Rio de Janeiro – Institute of Microbiology Paulo de Goes – **A.S. Rosado**

**CANADA**

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

**CZECH REPUBLIC**

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

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

**FRANCE**

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

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

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

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

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

Université Joseph Fourier – Institut de Biologie Structurale – Laboratoire de Cristallographie Macromoléculaire - Grenoble – **A. Dessen**

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

Université Joseph Fourier – Institut de Biologie Structurale – Laboratoire de Résonance Magnétique Nucléaire – Grenoble – **J.P. Simorre**

Université Joseph Fourier – Institut de Biologie Structurale – Laboratoire d'Ingénierie des Macromolécules – Grenoble – **T. Vernet**

Université Paris VI – Laboratoire de Recherche Moléculaire sur les Antibiotiques – **M. Arthur**

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

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

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

Université René Descartes – Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques – **Y. le Merrer**

Université de Technologie de Compiègne – Compiègne – **B. Bihan-Lavalle & S. Padiolleau**

**GERMANY**

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

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

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

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

University of Kaiserslautern – Department of Microbiology – Kaiserslautern – **R. Hakenbeck**

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

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

University of Tübingen – Department of Microbial Genetics – **R. Bertram & F. Götz**

**GREECE**

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

**ITALY**

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

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

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

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

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

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

**LUXEMBURG**

Centre de Recherche Public de la Santé – Laboratoire de Rétrovirologie – Strassen – **A. Chevigné**

Centre de Recherche Public de la Santé – Laboratoire d'Immunogénétique et d'Allergologie – Strassen – **F. Hentges**

**POLAND**

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

**SLOVENIA**

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

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

**SPAIN**

INIBIC – Microbiology – University College Hospital A Coruña – **G. Bou**

University of León – Institute of Biotechnologia – León – **J.F. Martin**

University of Oviedo – Faculty of Medicine – Department of Functional Biology – Oviedo – **A. Manteca**

**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**

**THAILAND**

Chulalongkorn University – Division of Allergy and Clinical Immunology - Bangkok – **A. Jacquet**

**THE NETHERLANDS**

Groningen Biomolecular Sciences and Biotechnology Institute – Department of Genetics – Groningen – **O. Kuipers**

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

Leiden University - Microbial Development - Leiden Institute of Chemistry - Leiden - **G. van Wezel, E. VijgenBoom & G. Girard**

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

**UNITED KINGDOM**

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

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

Sekisui Diagnostics UK – West Malling – Kent – **E. Asilonu**

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

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

University of Essex – School of Biological Sciences – Colchester – **J.A.R. Worrall**

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

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

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

University of Oxford – Oxford Centre for Molecular Sciences – Oxford – **C. Schofield**

**USA**

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

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

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

University of Missouri-Kansas City – Division of Pharmaceutical Sciences – Kansas City – **W. G. Gutheil**

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

The Pennsylvania State University – Department of Biology – University Park – **D. Cosgrove**

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

# VISITORS

**Arsovska Emilija**, University of Ljubljana, Slovenia, November 7-December 9

**Baudelet Paul-Hubert**, ENSAIA Nancy, France, January 4– 15 February 15

**Buell Alexander,** University of Cambridge, Department of Chemistry, UK, December 12-16

**Da Lage Jean-Luc**, CNRS, Gif-sur-Yvette, France, February 17-25

**Di Gregorio Sabrina Noelia**, University of Buenos Aires, Argentina, September 2-November 30

**Faillaci Francesca**, University of Palermo, Italy, May 2– December 20

**Forge Vincent**, CEA Grenoble, France, November 9-13

**Lahiani Sadja**, UMBB, Boumerdes, Algeria, June 3-November 25

**Mares Jan**, University of South Bohemia, Czec Republic, February 28-May 28

**Mollerach Marta**, University of Buenos Aires, Argentina, February 1-18

**Power Pablo**, University of Buenos Aires, Argentina, October 11-30

# Stays in other Institutions

**Bouaziz Ahlem**, Laboratoire d'allergologie expérimentale, ULB, Gosselies, Belgium, several stays between April and December

**Campisi Vincenzo**, Laboratory of Retrovirology, CRP-Santé, Luxemburg, Luxemburg, May 2-August 31

**Delsaute Maud**, Institut de Recherches Microbiologiques JM Wiame, ULB, Bruxelles, February 28-March 18

**Dumont Janice**, Institut de Biochimie et Biophysique Moléculaire et Cellulaire, Université Paris-Sud, Orsay, France, January 10-20

**Dumont Janice,** Department of Chemistry, University of Cambridge, UK, February 21-March 2

**Dumoulin Mireille**, Department of Chemistry, University of Cambridge, UK, February 1-3

**Feller Georges**, Wyatt Technology Corporation, Dernbach, Germany, November 7-9

**Huynen Céline**, CRIBI Biotechnology Centre, University of Padua, Italy, March 1-June 20

**Laurent Sophie**, P3 Workshop, University of Zurich, Switzerland, September 5-9

**Matagne André**, Department of Biochemistry, University of Oxford, June 27-29

**Montagner Caroline**, Department of Biochemistry, University of Oxford, UK, September 11-22

**Pain Coralie**, CRIBI Biotechnology Centre, University of Padua, Italy, September 1-December 31

**Rhazi Noureddine**, Division Bio-Recherche, Marnes-la-Coquette, France, November 9-10

**Sauvage Eric**, University of Buenos Aires, Argentina, February 13-20

**Sauvage Eric**, Ljubljana, Slovenia, 5-6 May

**Sohier Jean-Sébastien**, Laboratory of Retrovirology, CRP-Santé, Luxemburg, Luxemburg, February 22-25 and June 20-30

**Struvay Caroline**, Institut Laue-Langevin, Grenoble, France, November 20-December 2

# FUNDING

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## Les Pôles d'Attraction Interuniversitaires

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

## Politique Scientifique Fédérale Belge

**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
**PRODEX (ESA) / BELSPO C90360** (2010-2011) – EXANAM: Gene exchange between anthropogenic and native microbial communities at Mandats de doctorants Concordia, role in biosafety and environmental protection.
**BELSPO C4/00/03** (2011) - Consolidation of the Belgian « Biological Resource Centre (BRC)» and its integration in a «Knowledge-based Belgian Biological Resource Engine (BBRE)».
**Mandats de doctorant** : Pedro De Carvalho Maalouf (AMBIO)

## Les Actions de Recherche Concertées

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

## Fonds de la Recherche Scientifique - FNRS

**Projets de recherche**
**FNRS 1670** (2008-2011) Fibres amyloïdes : fonctions, structure et mécanisme de formation.
**FNRS 4.4509.090.F** (2009-2012) - Etude structurale des protéines impliquées dans le métabolisme et la dynamique de la paroi bactérienne.
**FNRS 1.5139.11** (2010-2012) PYROCYANO: Antarctic cyanobacterial diversity.
**IISN 4.4503.11** (2011-2014) "Use of synchrotron radiation. Development of structural biology projects applying macromolecular crystallography."
**F.4505.11- Mandat d'Impulsion Scientifique** (2011-2013) Mechanism of fibril formation by proteins containing polyglutamine expansions: role of the sequences flanking the expansions
**Cooperation bilatérale FNRS/MINCYT** (Argentina) (2010-2011) New insights on peptidoglycan structure and the enzymes involved in their biosynthesis in clinical isolates of vancomycin-resistant *Staphylococcus aureus*.
**Coopération bilatérale FNRS/CONICET** (Argentina) (2011-2013) Characterization of new beta-lactamases produced by clinical and environmental bacteria
**Cooperation bilatérale FNRS/CNPq** (Brésil) (2010-11) Diversity and functional activity of cyanobacteria from South Shetlands Islands, Antarctic Continent

**Mandats de recherche**

**Mandats de doctorants FRIA** : Anthony Argüelles, Vincent Campisi, Jean-Benoît Charlier, Chloé Chavignon, Alexandre Cipolla, Matthias Craig, Morgane Dehousse, Maud Delsaute, Nicolas Dony, Janice Dumont, Régine Freichels, Amandine Godin, Jessica Guillerm, Julie Herman, Céline Huynen, Marine Joris, Stéphany Lambert, Yannick Lara, Clémentine Laurent, Sarah Lebrun, Marie-José Mano, Maxime Maréchal, Coralie Pain, Florence Piette, Delphine Pipers, Mathieu Rocaboy, Jean-Sébastien Sohier, Nancy Stankovic, Caroline Struvay, Elodie Tenconi, Edwige Van der Heiden.

**Mandat de doctorant Télévie** : S. Laurent

**Mandats d'aspirant** : C. Struvay et C.Laurent

**Mandats de Chargé de Recherche** : C. Bebrone et V. Tillemans.

**Mandat FNRS de Chercheur Temporaire Postdoctoral** pour le Dr Arabela Cuirolo (08/04/2011 au 07/04/2013), avenant à la convention FRFC 2.4588.11

**Mandat FNRS de 'Post-doctoral Scientific Collaborator'** pour le Dr David Thorn (02/05/2011 au 30/04/2013, FRS-FNRS MIS F-4505-11)

## Fonds de la Recherche Fondamentale Collective

**FRFC 2.4506.08** (2008-2011) - Study of the glycosyltransferase enzymes catalyzing the glycan chain polymerization of the bacterial wall peptidoglycan

**FRFC 2.4535.08** (2008-2011) - Exploration du génome d'une bactérie de l'Antarctique : approches protéomiques et biophysiques de la psychrophilie et de l'adaptation aux basses températures

**FRFC 2.4.583.08** (2008-2011) Heavy metal tolerance in zinc and cadmium hyperaccumulators: analysis of overexpression mechanisms of metal transport genes and functional characterization of the corresponding proteins.

**FRFC 2.4558.09** (2009-2011) Fossilisation of cyanobacteria: phylogenetic, micropaleontological and sedimentological approaches, and implications for the evolution of the primitive biosphere.

Cooperation to the project of Prof. Emmanuelle Javaux (Geology, ULg, BE)

**FRFC 2.4.642.09** (2009-2012) Regulation of gene expression at the post-transcriptional level in photosynthetic eukaryotes: study of a family of conserved SR splicing factors and functional organization of the nucleus

**FRFC 2.4530.09** (2009-2012) - Etude des cinétiques de folding de trois protéines modèles, à l'aide des méthodes d'échange hydrogène/deutérium, couplées à la spectrométrie de masse et à la RMN

**FRFC 2.4548.10** (2009-2013) - Caractérisation des interactions entre biomolécules par résonance plasmonique de surface.

**FRFC 2.4570.09** (2009-2013) BIPOLES : Geographic and ecological distribution of Antarctic and Arctic cyanobacteria.

**FRFC 2.4588.11** (2010-2013) Induction de la β-lactamase BlaP

**FRFC 2.4523.11** (2011) – Contribution à l'analyse de la conformation des macromolécules par diffusion de lumière.

**FRFC 7057031** (2011-2012) - *Enterococcus* résistant aux β-lactames

**FRFC 2.450412.** (2011-2014) - Conception et développement d'un système opto-fluidique à fibres optiques pour l'étude des propriétés de transport des membranes biologiques.

**FRFC n°2.4631.11** (2011-2014) "Facteurs d'épissage SR de *Danio rerio*"

## Région Wallonne

**RW BIOWIN 5678** (2008-2011) – CANTOL: Discovery and validation of indoleamine 2,3-dioxygenase inhibitors to overcome tolerance in cancer immunotherapy
**SPW-DG06 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)
**SPW- DG06 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
**SPW-DG06 516265** (2010-2011) – MEDATR : Dosage en temps réel de substances pharmacologiques dans des fluides physiologiques (avenant)
**Partenariat-Public-Privé (P.P.P.) n°1117354** (2011-2013) RECOINS – Renaturation in vitro de protéines recombinantes produites dans Escheirichia coli, après solubilisation des corps d'inclusion. Stabilisation des protéines au cours de la purification et du stockage à long terme.
**SPW-DGA** Projet D31-1253 "Développement d'une technologie de production de lait délactosé"

## Bilateral Cooperation Wallonie/Bruxelles

**International-Slovenia** (2010-2012) CMP-2009/22676 : Recherche d'inhibiteurs de glycosyltransférases à potentiel antibactérien.
**International-Pologne** (2011-2012) : Diversité moléculaire et changements saisonniers des cyanobactéries dans les tourbières hautes acides de type baltique.
**International-France** (2011-2012) Tournesol : Étude des relations structure-fonction des undécaprényl pyrophosphate phosphatases bactériennes.
**Mandats de doctorant**: Ahlem Bouaziz

## Région Flamande

**IWT project 100558** (2011-2012) Comparative study of the enzymatic production of tagatose from lactose in whey permeate versus the "in vivo" biosynthesis of tagatose from glucose (Vergelijkende studie van het enzymatisch productieproces van tagatose uit lactose in weipermeaat versus de "in vivo" productie van tagatose uit glucose)

## Union Européenne

**Concerted Action CAREX** A European platform for research on life in extreme environments (http://www.carex-eu.org/)

## Université de Liège

**Crédit de démarrage**
Projet R.CFRA.1237 (2010-2012) - Dr S. Rigali
Identification de nouvelles voies d'induction des métabolites secondaires chez les *Streptomyces*.
**Crédits classiques**
Projet C-11/108 (2011-2013) - Dr M. Dumoulin
Mécanisme de formation de fibres amyloïdes par les protéines contenant des expansions polyglutamine : rôle des séquences adjacentes aux expansions
**Mandat de post-doctorat à l'ULg pour chercheur étranger**
Dr C. Nouet (2009-2011)

# Missions of Expertise

## Member of Research Councils

**Prof. Moreno Galleni**
Membre du Conseil sectoriel de la Recherche "Science et Technique" (2009-)
Membre du Conseil universitaire de la Recherche (2009-)

**Prof Jean-Marie Frère**
Membre de la Commission Sciences de la Vie et de la Santé 8, Agence Nationale de la Recherche (ANR), France

## Member of Editorial Board

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

## Member of the Editorial Advisory Panel

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

# Commitees and Societies

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

**Galleni Moreno**
BioLiège (Vice-president)

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

**Motte Patrick**
Espaces botaniques de Liège (Vice-President)

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

# Composition of the Center

## Managing Committee

**Director**
Bernard Joris

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

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

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

## Administrative Staff

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

## Technical Assistance

Anne-Marie Matton
Marie Schloesser
Iris Thamm

## Temporary Members

Researchers

Dr Ana Amoroso
Dr Etienne Baise
Dr Carine Bebrone
Dr Renaud Berlemont
M. Fabrice Bouillenne
Dr Arabela Cuirolo
Dr Dominique Dehareng
Dr Michael Delmarcelle
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