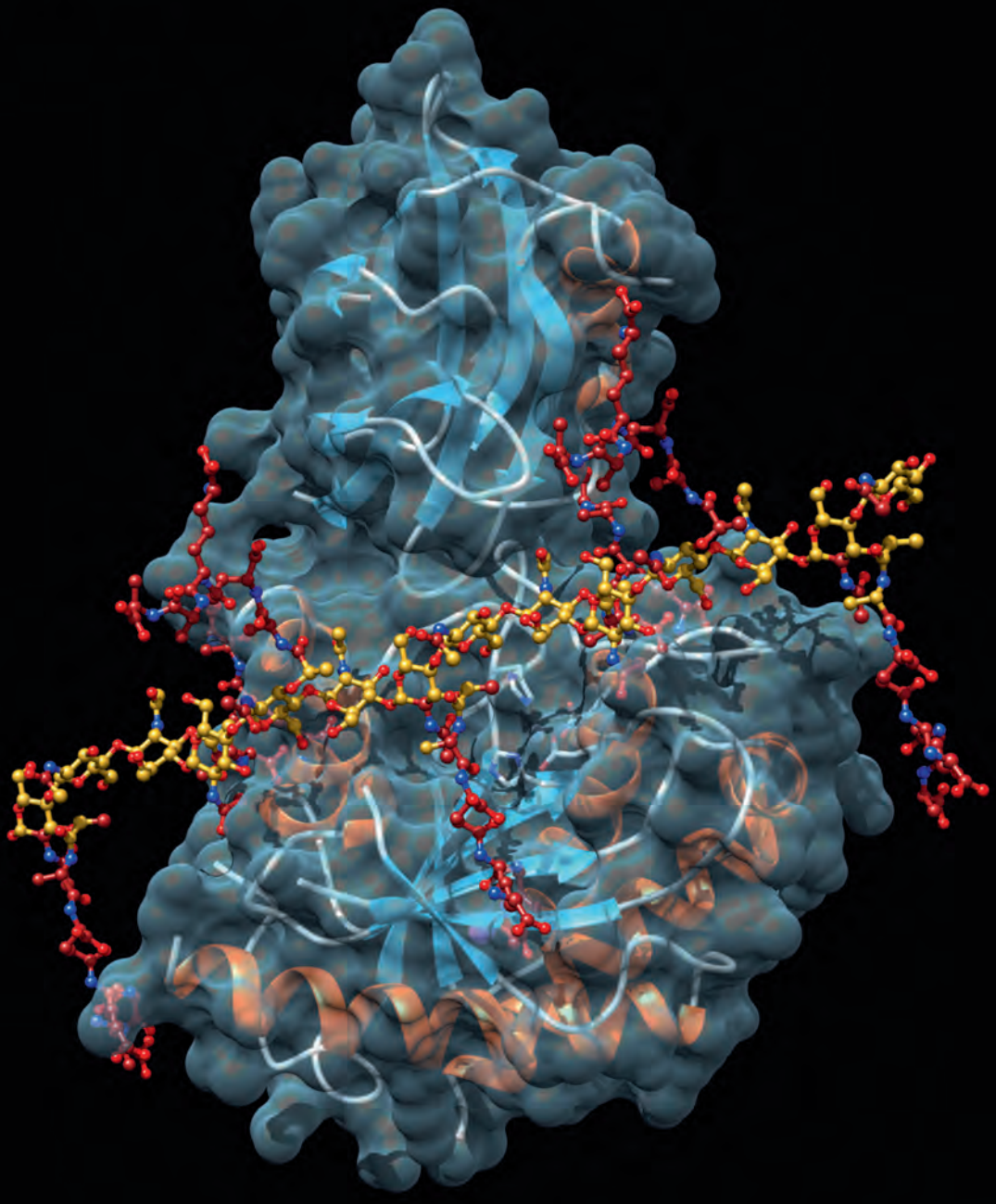
Center for Protein Engineering

Activity report 2012



University of Liège

CIP

"Crystal structure of the E.coli cell division amidase AmiC with a modeled peptidoglycan strand"
(authors: M. Rocaboy, E. Sauvage)

Contents

INTRODUCTION

The Centre for Protein Engineering (Centre d'Ingénierie des Protéines-CIP) is a multidisciplinary research centre merging several laboratories of the faculty of science of ULg (University of Liège).The CIP was founded in 1990 by Professor Jean-Marie Ghuysen to develop basic biomedical research with potential medical applications. The principles on which the center was founded were important for Ghuysen's own scientific career and were reinforced by our two former directors, Professors Jean-Marie Frère and Bernard Joris.

Excellence, freedom of the researchers to choose their own line of investigation, collaborative work and interdisciplinary research remain the major objectives driving the development of the centre. This policy has a corollary which is, when possible, to translate our discoveries into biotechnological or medical progresses. Some of our senior scientists hold a Faculty position and have teaching appointments. The influx of graduate, master and doctoral students, but also postdoctoral fellows supported by various grants (from the University, Belspo, the F.R.S.-FNRS, the Walloon region and the European commission FP programs) are key elements for the success of the CIP. We are also committed to the development of longlife learning programs since we know that, in a rapidly changing world, they are essential for employment, economic success and allowing people to fully participate in society.

The Centre for Protein Engineering is managed by a board which comprises all the permanent senior scientists of the centre. The board nominates a directorate presently composed of Professors Paulette Charlier (Vice-Director), André Matagne (Secretary), Moreno Galleni (Director) and Dr Marc Hanikenne. The board has the major ambition to maintain technical and scientific environments allowing successful long-term projects to be pursued but also the emergence of new and original research of high quality. Research at the CIP is supported by the public, regional, federal and international bodies as well as by private companies. Most of our funds are awarded on a competitive basis.

The activity report of 2012 is a good testimonial of our ambitions. During this year, our team was reinforced by hosting three new postdoctoral fellows from France, India and the United States of America. Our collaboration network was reinforced via the funding of the "Interuniversity Attraction Poles" (IAP) Program, which aims to provide support for teams of excellence in basic research from Belgian universities belonging to both linguistic communities (Lab. for Protein Biochemistry and Biomolecular Engineering - UGent, Biochimie et Génétique Moléculaire Bactérienne-UCL, Biophysique Moléculaire Numérique-ULg-GxABT, Lab. of Medicinal Chemistry – KULeuven, Organic and Bioorganic Synthesis –UGent, Research Group of Functional Genomics and Proteomics-KULeuven) and also to three international teams from France, The Netherlands and the United Kingdom. This program, named Ipros, is coordinated by the CIP (the official coordinator is Professor B. Joris). Beside this, all researchers of the CIP contribute to strengthen our international network of collaborations, which has expanded to teams from Australia, Bulgaria, France, Italy, Spain, Switzerland, Germany, the United Kingdom and USA.

Finally, in the frame of the European Strategy Forum on Research Infrastructures (ESFRI), a collaborative consortium between the Structural Biology Brussels and VIB department for Structural Biology (Vrije Universiteit Brussel / VIB), the Structural Biology and Bioinformatics Center (Université Libre de Bruxelles) and the Centre for Protein

Engineering, hosts the Belgian Instruct Affiliate Centre (BIACe). It provides an integrated structural biology platform, to the Belgian and European research communities ranging from protein production to crystallography and NMR.

We address our thanks to the former director of the Board, Prof B. Joris for his management of the CIP during the last three years and we are grateful to all of those who support the CIP.

Moreno Galleni

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 Dail Laughinghouse IV
Dr Serge Leimanis
Mr Olivier Verlaine

BIOLOGICAL MACROMOLECULES AND BIOCHEMISTRY

Group leader: **Prof. Moreno Galleni**

Permanent scientist: **Dr Georges Feller**

Associate members:
Dr Etienne Baise
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**
Dr Meriem El Gachi

ENZYMOLOGY AND PROTEIN FOLDING

Group leader: **Prof. André Matagne**

Permanent scientist: **Dr Mireille Dumoulin**

Associate members: **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**

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

PLANT MOLECULAR IMAGING

- [X] Plant physiology
- [X] Plant genetic transformation
- [X] Molecular imaging
- [X] Plant genetics and genomics

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 : VP-DSC and ITC200 (GE Healthcare)
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)

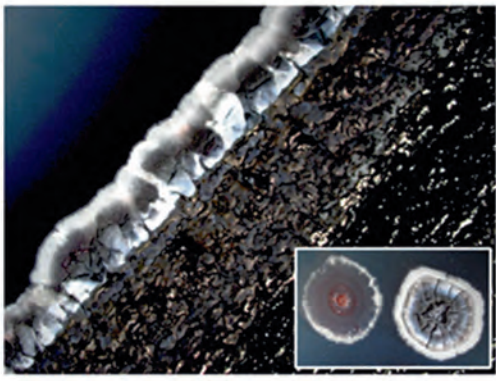
Imaging

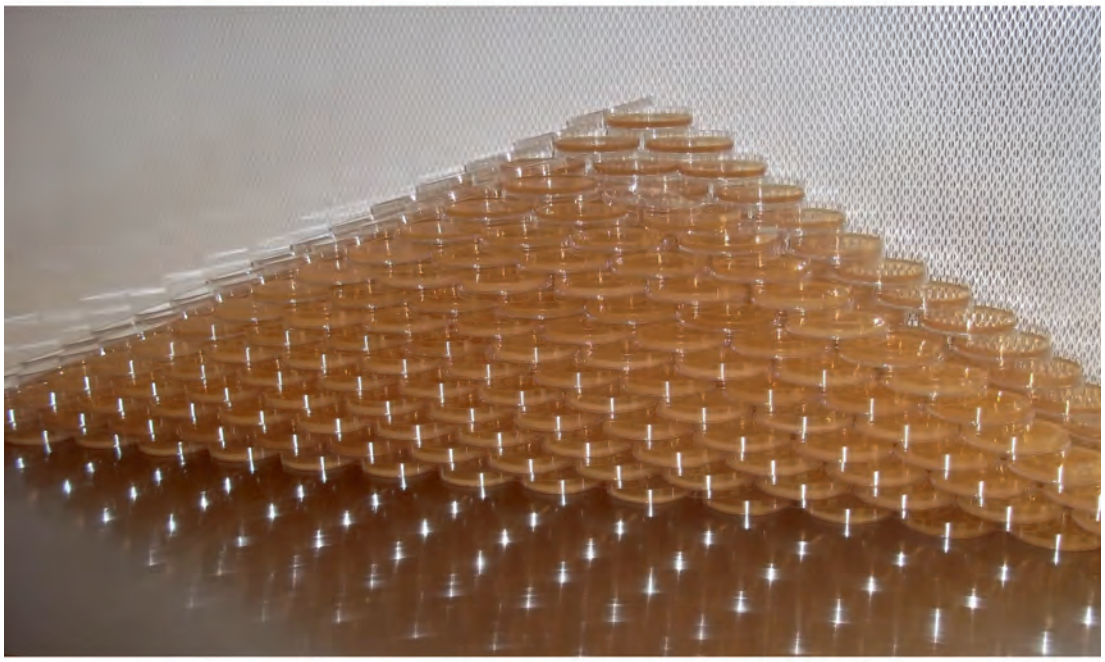
1 Axio Imager Z1 fluorescent microscope (Zeiss)
1 Camera for digitalisation of images and analytical analyses (Deltapix Scandinavia)
1 CKX 31 inversed microscope (Olympus)
1 DMLB2 microscope (Leica)
1 Molecular Imager FX system (Biorad)
1 Phase contrast microscope (Reichert)
1 Binocular microscope (model 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)

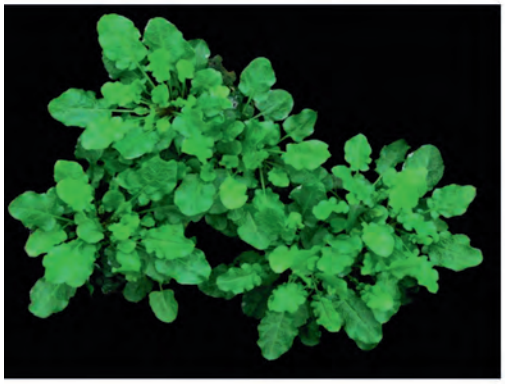
Miscellaneous

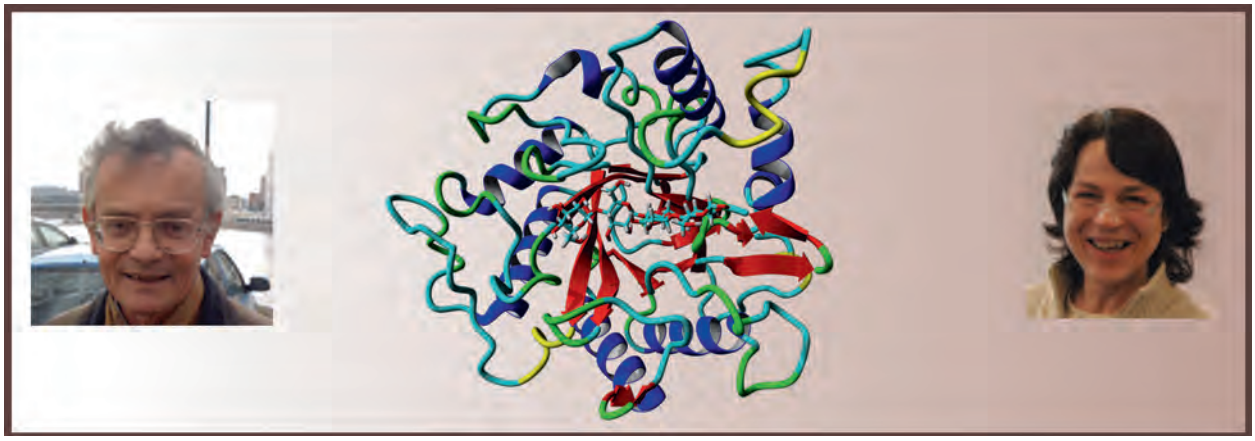
1 Freeze-dryer (Christ)

THEMES OF RESEARCH









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, serine enzyme inhibitors and energy surfaces. A review about quantum chemistry applied to the study of ß-lactams has been published [42].
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 [29]. The mechanism of inclusion of Artemisinin and several cyclodextrins has been studied. The spectroscopic and theoretical results confirm that the most favourable orientation of the alkaloid is not an inclusive one [39].
The interactions between two methyl-cyclopentadienylcarboxylate lead to dimers in two geometric arrangements depending on the reaction coordinate followed by the Cope rearrangement. The energy surface has been calculated along two coordinates and the stationary points have been located and their ratio is in accordance with the NMR experimental data [8,13].
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 several models of the active site and the ligand have been studied by quantum chemistry calculations [30,31].
Several ways could be involved in the synthesis of non-symmetrically substituted phenoxazines. They have been analyzed at an experimental and theoretical levels [5].

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 are being used to study different complexes between glucosides and amylases. The collaboration with the Molecular Dynamics Laboratory about the spectroscopy of halogenated ethylenic compounds is still going on. 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 [18].

Research group: BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS
Group leader: Prof. Bernard JORIS
Associate Researchers: Drs Ana Amoroso, Arabela Cuirolo, Michaël Delmarcelle, Serge Leimanis, Olivier Verlaine
Ph.D. students: Antony Argüelles-Arias, Stéphanie Berzigotti, Sébastien Dandois, Nicolas Dony, Anne Famerie, Séverine Hubert, Sarah Lebrun, Maxime Maréchal, Anne Pennartz, Marjorie Dauvin, Patric Stefanic
Technical assistance: Patricia Simon
Collaborations: Dr Jean-Pierre Simorre, Dr Thierry Vernet, Dr André Zapun (Institut de Biologie Structurale, Grenoble, France), Prof. Dominique Mengin-Lecreulx, Prof. Didier Blanot (Université Paris-Sud 11, France), Prof. Gabriel Gutkind, Prof. Marta Mollerach (Universidad de Buenos-Aires, Argentina), Prof. Edwin De Pauw, Prof. André Luxen, Prof. Pierrette Melin (University of Liège, Belgium)

Study of peptidoglycan metabolism - Bacterial resistance to β-lactam antibiotics

Peptidoglycan is the major component of bacterial cell wall and plays an essential role in the integrity and morphology of bacterial cell. During the bacterial cellular cycle, peptidoglycans are, 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 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 *Entrococcus faecium* PBP5 and *Staphylococcus aureus* PBP2a). 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.

β-lactamase induction as model for signal transduction and gene regulation

To survive and continuously adapt their cellular machinery to the external environment, living cells have acquired membrane proteins which allow communication between the outside and the inside of the cell. These proteins perform a diversity of functions as different as metabolite uptake and cell signalling. In several bacteria, the synthesis of one or several β-lactamase(s) is the main factor of β-lactam antibiotic resistance and is induced by the presence of the antibiotic. As this type of antibacterial agents does not significantly cross the cytoplasmic membrane, bacteria in which the β-lactamase is inducible have a mechanism which signals the presence of the antibiotic outside the cell. The group studied three different mechanisms of β–lactamase induction : one in Gram-negative bacteria (*Citrobacter freundii* model) and two in two Gram-positive bacteria (*Bacillus licheniformis/Staphylococcus aureus*

and *Streptomyces cacaoi* models). The aim of this research is to understand the mechanism of bacterial resistance and the coupling between signal transduction and gene regulation.

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.

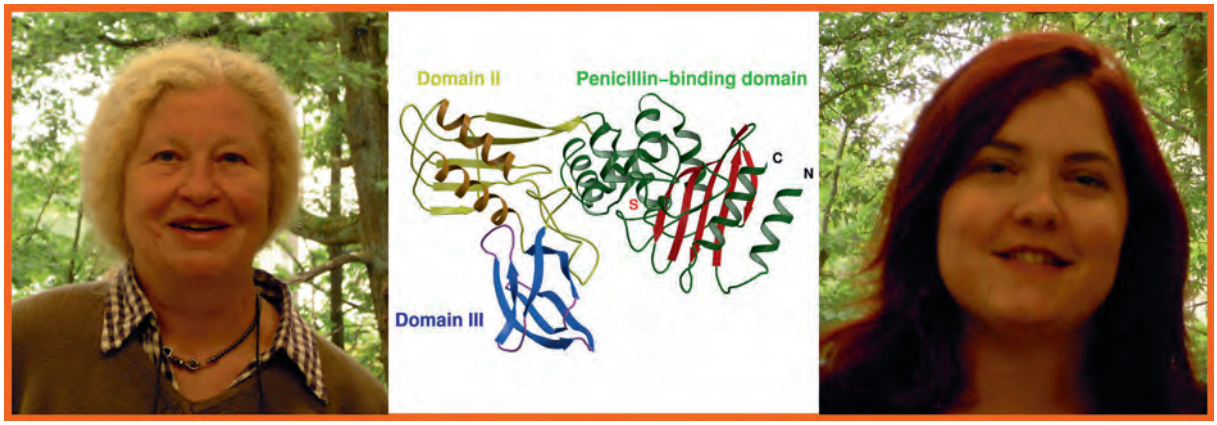
This project aims to build a protein microarray suitable for detection of biomolecular interactions without labeling, using a support activated by biochip molecules designed to allow attachment of proteins in an oriented manner. This new protein array will be applied to study the interaction of a peptide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

MED-ATR : Development of a bedside technique for rapid determination of antibiotics in the bloodstream by using attenuated total reflectance (ATR) infrared spectroscopy

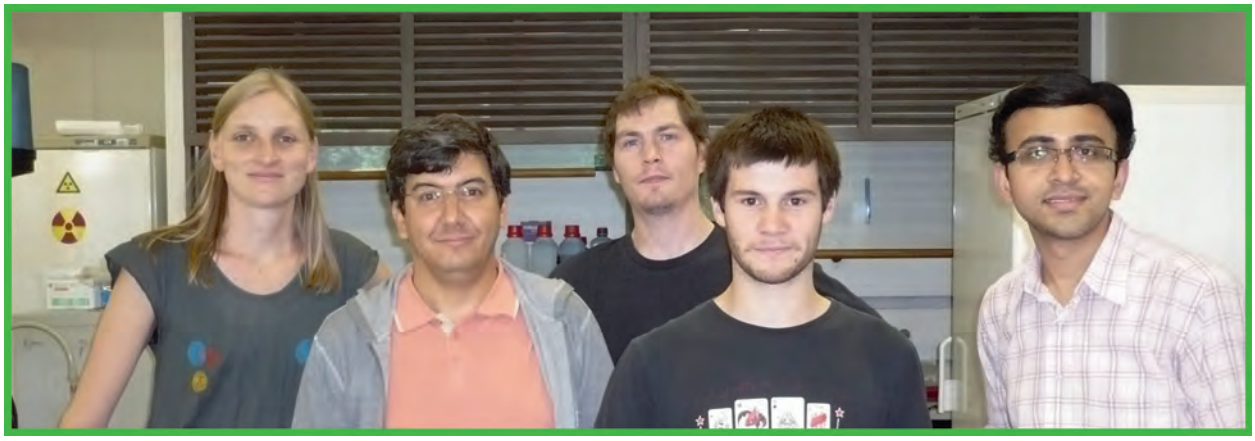
The current public health issues related to opportunistic infections in patients at risk and the dramatic rise of bacterial resistance require, in the hospitals, the application of an optimized antibiotic administration such as continuous infusion. This technique of administration remains limited due to the difficulty of quickly measuring serum free antibiotics. The aim of MED-ATR project is to find a method to overcome this difficulty.



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

Study of the *yoxA-dacC* products from *Bacillus subtilis* (Bs) and *Bacillus amyloliquefaciens* (Ba).

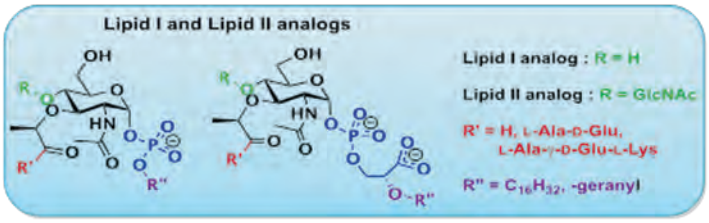
In *Bacillus subtilis* and *B. amyloliquefaciens*, the *yoxA* and *dacC* genes form an operon. The first gene codes for a 37 kDa protein homologous to aldose-1- epimerases or galactose mutarotases. Its product was renamed GalM. We are currently determining the 3D structures of GalM complexes with carbohydrates. The kinetic characteristics are also under study. The GalM activity precedes that of three others enzymes belonging to the Leloir pathway and it was recently shown that the galactose metabolism plays a crucial role in the biofilm formation by *B. subtilis*. The second gene initially referred to as *pbp* because of a clear homology of its product with penicillin-binding proteins (PBPs) encodes a DD-carboxypeptidase and was renamed *dacC*. According to its position on a SDS-polyacrylamide gel, the *dacC* product is referred to as PBP4a. Like the *E. coli* PBP4, the prototype of this class (C1) of low-molecular mass PBPs, the BsPBP4a is a DD-endopeptidase able to cleave the D-alanyl-γ-meso-2,6-diaminopimelyl (D-Ala-γ–m-A2pm) in the peptidoglycan cross-bridges formed by the DD-transpeptidases. The PBP4a is composed of three domains: the penicillin-binding domain (domain 1) and two additional domains whose we try to establish the functions. Under laboratory growth conditions, the *B. subtilis* PBP4a is dispensable. However, this class of PBPs is present in almost all bacteria and seems to be part of the minimal arsenal of PBPs as suggested by the case of *Neisseria gonorrhoeae* which has only four PBPs, one class A (PBP 1), one class B (PBP2), one class C3 (PBP 4) and PBP3 that belongs to class C1. Such conservation may indicate an essential role in natural niches. A similar study is performed with the homologous proteins from *B. amyloliquefaciens*. Their cellular localizations remain to be elucidated.



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: Prof. Piet Herdewijn (Katholiek Universiteit Leuven, Belgium), Prof.Waldemar Vollmer (University of Newcastle, United Kingdom)

Synthesis of modified peptidoglycan precursor analogues for the inhibition of glycosyltransferase

The peptidoglycan glycosyltransferases (GTs) are essential enzymes that catalyze the polymerization of glycan chains of the bacterial cell wall from lipid II and thus constitute a validated antibacterial target. Their enzymatic cavity is composed of a donor site for the growing glycan chain (where the inhibitor moenomycin binds) and an acceptor site for lipid II substrate. In order to find lead inhibitors able to fill this large active site, a series of substrate analogues of lipid I and lipid II have been synthesized (in collaboration with P. Herdewij, KUL) with variations in the lipid, the pyrophosphate, and the peptide moieties and evaluated their biological effect on the GT activity of *E. coli* PBP1b and their antibacterial potential. We found several compounds able to inhibit the GT activity *in vitro* and cause growth defect in *Bacillus subtilis*. The more active was C16-phosphoglycerate-MurNAc-(L-Ala-D-Glu)-GlcNAc, which also showed antibacterial activity. These molecules are promising leads for the design of new antibacterial GT inhibitors [14].



Cooperativity of peptidoglycan synthases active in bacterial cell elongation

During the *Escherichia col*i cell cycle, two morphogenetic complexes called elongasome and divisome govern cell elongation and division respectively. They are composed of peptidoglycan synthases, hydrolases and cell morphogenesis proteins allowing the coordination of these activities. We have contributed (collaboration with W. Vollmer, University of Newcastle) to the study showing that the *E. coli* peptidoglycan glycosyltrasferase-transpeptidase PBP1A interacts with the cell elongation-specific transpeptidase PBP2 *in vitro* and in the cell. PBP2 stimulates the glycosyltransferase activity of PBP1A, and PBP1A and PBP2 cooperate to attach newly synthesized peptidoglycan to sacculi. PBP2 has peptidoglycan transpeptidase activity in the presence of active PBP1A. Cells lacking PBP1A are thinner and initiate cell division later in the cell cycle. PBP1A localizes mainly to the cylindrical wall of the cell, supporting its role in cell elongation [2].

Research group: BACTERIAL DIVERSITY, PHYSIOLOGY AND GENETICS
Group leader: Dr Sébastien RIGALI (FRS-FNRS)
PhD students: Matthias Craig, Elodie Tenconi, Stéphany Lambert, Marta Maciejewska
Collaborations: Gilles van Wezel and Magdalena Swiatek (University of Leiden, NL); Fritz Titgemeyer (University of Munsteer, D), R. Cavicchioli, K.S. Siddiqui (University of New South Wales, Australia), E. Marcellin, C. Licona-Cassani (University of Queensland, Brisbane, Australia)

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 are 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.

Regulation of N-Acetylglucosamine utilization in *Streptomyces*

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.

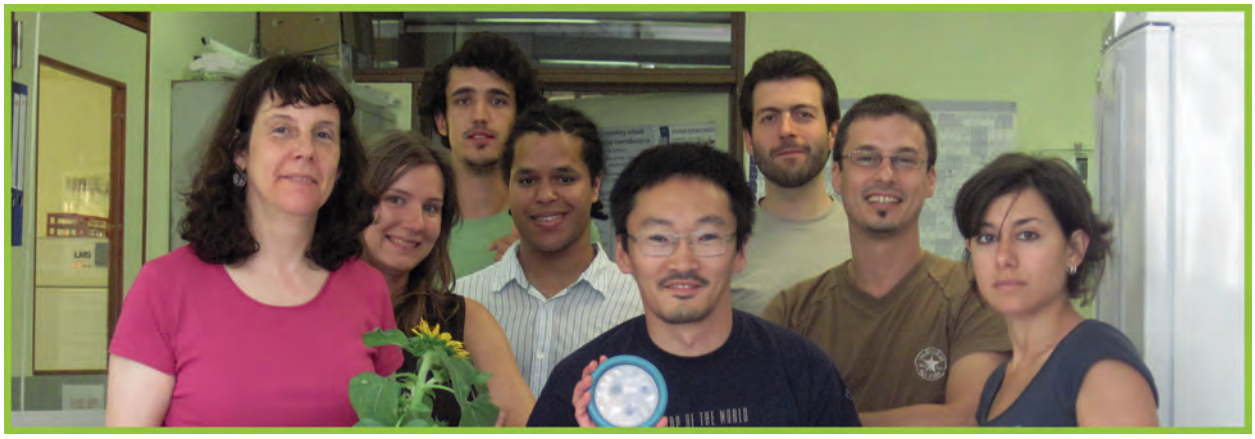
Role of iron and siderophore in *Streptomyces* development

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. Siderophores 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.

Polysaccharide-degrading enzymes and antibiotic production from karstic actinomycetes

Actinomycetes are soil-dwelling Gram-positive bacteria that have a key role in the mineralization of the plant residual biomass thanks to their diversified enzymatic arsenal which includes cellulases, xylanases, pectinases, alpha-amylases, mannanase, and many enzymes that belong to other polysaccharide hydrolytic systems. Unexpectedly, in carbonate (karstic) caves, which are primarily inorganic, and therefore carbon sources-limited habitats, the dominance of actinomycetes have been reported. The abundance of these major dead plant material bio-

decomposers in inorganic habitats is highly intriguing. Elucidation of the adaptations that could evolve in a response to highly oligotrophic environment, can lead to discovery of novel bioactive molecules (antibiotics…) and novel enzymes which may be key weapons for actinomycetes to outcompete the nutritional rivals.



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The BelSPO project **CCAMBIO**: study of the diversity and the biogeography of cyanobacteria in relation to Climate Change

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 assessment. In the BelSPO project **AMBIO**, we study the diversity and the biogeography of cyanobacteria in more than59 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.

ARC project 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, which 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.

The FRFC project **BIPOLES**: study of the bipolar geographic distribution of cyanobacteria

The FRFC BIPOLES project aims to study the geographical and ecological distribution of cyanobacteria from Antarctica and the Arctic. The central question is whether dispersal barriers influence the cyanobacterial distribution, or their presence is only driven by the ecological parameters of the biotopes where they are established. Presently, we aim to refine our data for the Antarctic cyanobacteria and complement this dataset with more Arctic sequence data, from more diverse biotopes, taking advantage of the novel ‘deep sequencing’ techniques.

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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 structure-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 its 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 haloplanktis* 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 stutzeri*

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.

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 results 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 was 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 was 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 (V_HHs) able to inhibit the clinically relevant MβL VIM-4. Amongst 55 other V_HHs, the only inhibiting V_HH 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 V_HH 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 V_HH qualifies 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 V_HH 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 V_HH cABcII10 has also been characterized.

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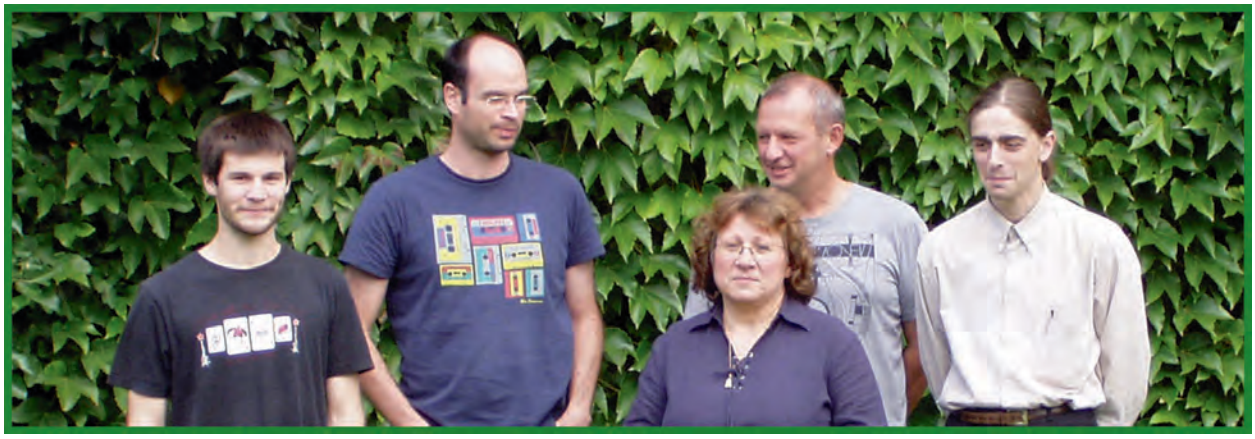
Biochemistry of Extremophiles

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, solfatares or hot springs. Life at these extreme biological temperatures obviously requires a vast array of adaptations.
The Laboratory of Biochemistry focuses its research activities on the molecular adaptations displayed by extremophilic biomolecules, with special reference to proteins and enzymes. The scientific staff has produced pioneering contributions in the field of cold-adapted proteins. The three main research topics currently developed 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.



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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 on 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.
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*.
β-lactam based new drugs are still in development. We are studying structural complexes with ceftaroline and ceftobiprole as examples for fifth generation cephalosporins developed by pharamaceutical companies. Monobactams of new generations, such as siderophore monobactam, are developed and investigated as well.
Boronic acids derivatives represent a new antibacterial class of compounds capable of inhibiting beta-lactam resistant transpeptidases. For boronic acid synthesis, we collaborate with the groups of Prof. André Luxen from the University of Liege, Prof. Chris Schoffield from the University of Oxford, Prof. Fabio Prati from the University of Modena, Prof. Rex Pratt from the Wesleyan University and Prof. William Gutheil from the University of Missouri-Kansas City.

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
a. 3D structure determination of chimeric proteins made of the beta-lactamase BlaP and polyglutamine (polyQ) stretches of different lengths.
b. 3D structure determination of complexes between camelid antibody fragments (or V_HHs) and human lysozyme.

Collaboration with **Prof. Moreno Galleni**, CIP, ULg
a. 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.
b. 3D structure determination of Derp 3, a digestive serine protease secreted by the mite *Dermatophagoides pteronyssinus* and identified as a common house dust allergen.

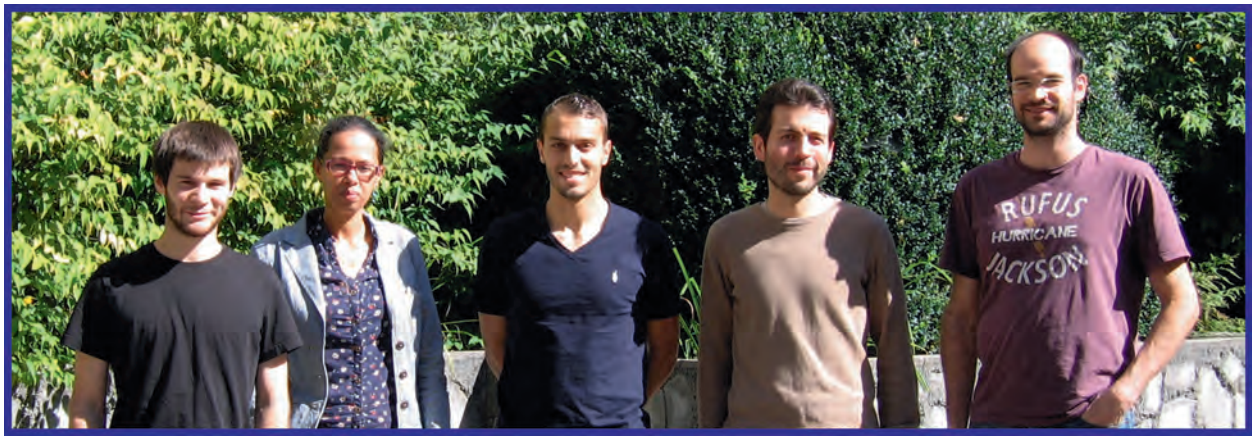
Collaboration with **Prof. Patrick Motte**, CIP, ULg
Structural study of Ser/Arg-rich splicing factors from *Arabidopsis Thaliana*.

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.



Research group: BIOLOGICAL MACROMOLECULE CRYSTALLOGRAPHY
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Associate Researchers: Dr Meriem El Ghachi
PhD students: Mathieu Rocaboy, François Delbrassine
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Collaborations: Prof. Denis Baurain (ULg), Dr Lucien Bettendorff (GIGA, ULg), Prof. Johan Van der Eycken (University of Gent), Dr Mohamed Azarkan, Dr German Bou, Dr Dominique Mengin-Lecreulx, Dr Thierry Touze (University of Paris-Sud, Paris, France), Dr Ivo Gompers-Boneca (Institut Pasteur, Paris, France) , Dr Maryline Foglino (Marseille Université, Marseille, France).

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.

Structural study of 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), 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 is undertaken in collaboration with Drs Mengin-Lecreulx and Touze from the EBA-IBBMC laboratory, University of Paris-Sud, Paris (France), the Dr Gompers-Boneca from Biology and Genetics of Bacterial Cell Wall laboratory, Institut Pasteur, Paris (France) and the Dr Foglino from the laboratory of bacterial chemistry UMR7283, Aix – Marseille Université, Marseille (France).

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. This project is done in collaboration with the Prof. Van der Eycken from the Department of Organic Chemistry, Organic and Bioorganic synthesis, UGent, Ghent (Belgium).

Research group: ENZYMOLOGY AND PROTEIN FOLDING
Group leader: Prof. André MATAGNE
Associate Researchers: Drs Caroline Montagner, Julie Vandenameele
PhD students: Roya Barumandzadeh, Jessica Guillerm, Natacha Scarafone
Technical assistance: Juliana Kozarova
Collaborations: Dr Catherine Michaux and Guillaume Roussel (FUNDP), Dr Mohamed Azarkan and Danielle Baeyens-Volant (ULB), Prof. Christina Refield (University of Oxford, U.K.), Dr Lucien Bettendorff, (ULg), Dr Jean-Francois Collet (Université Catholique de Louvain), Prof. Erwin De Genst (University of Cambridge, U.K.), Dr Jean-Denis Docquier (Università di Siena ; Italy), Dr Filip Meersman (University of Antwerp, Rousselot, Belgium, U.C.London, U.K.), Dr Michaël Nigen (Montpellier SupAgro, INRA, France), Séverine Padiolleau (UTC Compiègne, France), Lorna J. Smith (University of Oxford, U.K.), Profs Edwin de Pauw et Christian Damblon (ULg)

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.

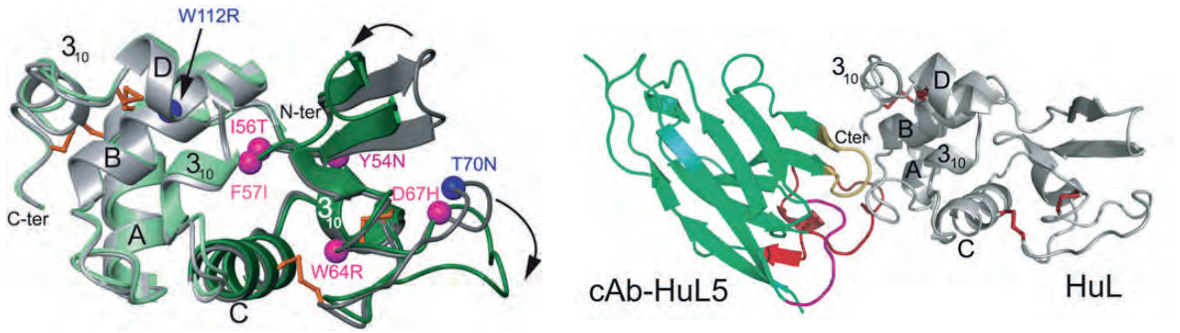
Research group: ENZYMOLOGY AND PROTEIN FOLDING
Group leader: Dr Mireille DUMOULIN (FRS-FNRS)
Associate Researchers: Dr David Thorn
PhD students: Natacha Scarafone, Chloé Chavignon, Janice Dumont, Céline Huynen, Linda Menzer, Coralie Pain
Technical assistance: Stéphane Preumont

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. This family of diseases includes Alzheimer's, Parkinson's and Huntington's diseases as well as type II diabetes. 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, DLS, NMR, QCM, TEM, AFM and protein engineering methods.

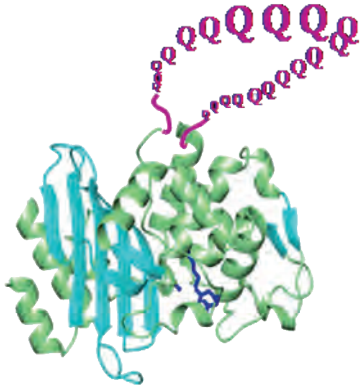
Use of camelid heavy-chain antibody fragments to investigate the mechanism of amyloid fibril formation by the amyloidogenic variants of human lysozyme

Five single-point mutants (Y54N, I56T, F57I, W64R and D67H) and two double mutations (F57I/T70N and W112R/T70N) in the gene of human lysozyme are associated with a hereditary non-neuropathic systemic amyloidosis, in which fibrillar deposits accumulate in a wide range of tissues including the liver, spleen and kidneys. In collaboration with the groups of **S. Muyldermans** and **J. Steyaert** (VUB, Brussels), and **C.M. Dobson** (Cambridge, UK) we have generated a series of ~20 conformational camelid heavy-chain antibody fragments (V_HHs or Nanobodies) specific of human lysozyme. These V_HHs are used i) as structural probes to elucidate the mechanism at the molecular level of fibril formation, and ii) as potential inhibitors of the aggregation process.



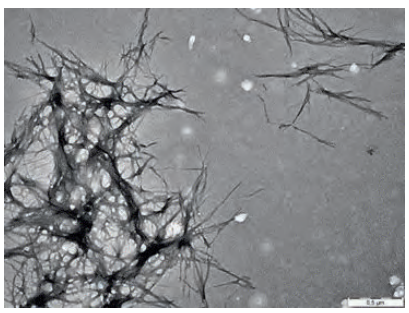
Design and characterisation of model polyglutamine proteins made of the beta-lactamase BlaP and polyglutamine (polyQ) stretches

The expansion of polyglutamine (polyQ) repeats is associated with an increased propensity of the protein to aggregate into amyloid fibrils. There are ten human proteins presently known within which polyQ expansion above a threshold length, e.g. 35-50 residues, leads to ten distinct neurodegenerative disorders, the most well-known being Huntington's disease. While repeat length, aggregation, and disease are well correlated, recent studies suggest the non-polyQ regions of these proteins can also play a significant role, both preventative and facilitative, in the aggregation process. With the aim of exploring this role in more detail, we have engineered chimeric proteins via the insertion of polyQ repeats of various length (23, 30, 55, 79 Q) into two sites of BlaP, a β-lactamase from *Bacillus licheniformis 749/C*. We are investigating the effects of both the length and the location of the polyQ tract on the structure, 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: (i) the existence of a polyQ length threshold above which BlaP chimeras readily form amyloid fibrils, (ii) above this threshold, the longer the polyQ, the faster the aggregation. BlaP chimeras are therefore valuable models to better understand how the non-polyQ regions influence the aggregation process. Moreover, like for human lysozyme, we have generated a series of V_HHs specific of BlaP and we used them i) as structural probes to elucidate the mechanism, at the molecular level, of fibril formation, and ii) as potential inhibitors of the aggregation process. This work is carried out in collaboration with the groups of J. Steyaert (VUB, Brussels), M. Galleni (C.I.P, ULg), F. Kerff and P. Charlier (C.I.P, ULg), C. Damblon (Chimie Biologique Structurale, ULg), N. Willet and A-S Duwez (Nano-Chimie et Systèmes Moléculaires, ULg), C. Jérôme (CERM, ULg) and G. Esposito (Univ. of Udine, Italy). This work is funded by a Mandat d'Impulsion Scientifique (FRS-FNRS) and a grant from Crédits Spéciaux (ULg).



Mechanism of amyloid fibril formation by a series of camelid antibody fragments

We are investigating the aggregating properties of a series of V_HH in order to better understand the relationship between the amino-acid sequence and aggregation propensity.



Functionalization of gadolinium nanoparticules for amyloidosis diagnosis

Amyloidoses remain a considerable clinical challenge due to their variable existing forms and involvement in different organs and tissues. They are, therefore, often misdiagnosed or diagnosed very late. Moreover, there is a body of evidence showing that protein deposition and irreparable damages occurred many years before the significant symptoms appear. Thus, the treatment of patients should ideally start before the apparition of symptoms and this requires novel diagnostic methods, preferably non-invasive, allowing an early detection of these diseases. The aim of this project, carried out within the frame of an EraNet project (Dia-AMYL), is a proof of concept, consisting in developing and validating innovative nanoparticles with multifunctional properties for the early diagnosis of amyloidosis and for related innovative therapies.

It is carried out in collaboration with the groups of V. Forge (CEA, Grenoble, France), O. Tillemant (Université Claude Bernard Lyon 1, France), C. Louis (NanoH, Lyon, France) and E. Alleamn (Université de Genève, Switzerland).

Research group: FUNCTIONAL GENOMICS AND PLANT MOLECULAR IMAGING
Group leader: Prof. Patrick MOTTE
Associate Researchers: Dr M. Hanikenne, Dr M. Muller, Prof. P. Charlier
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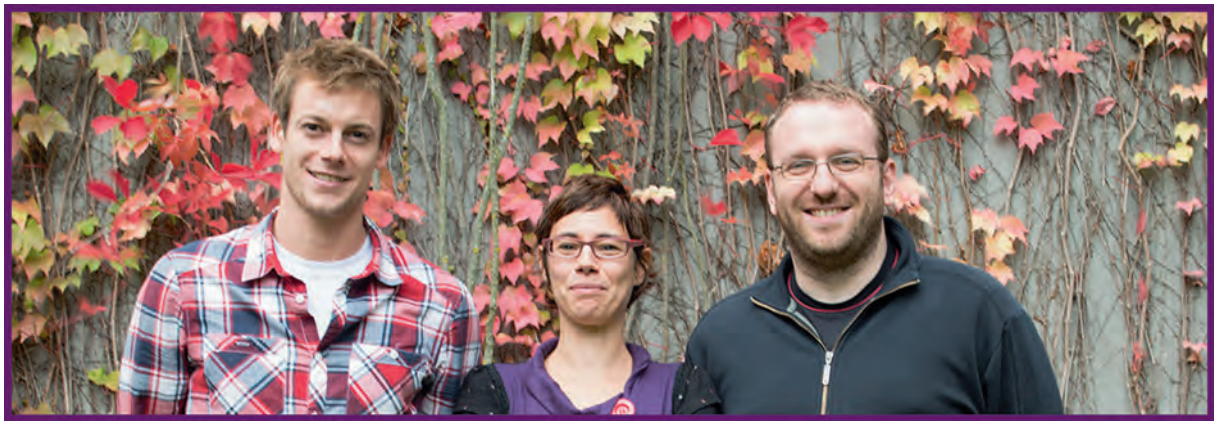
Technical assistance: Marie Schloesser

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 humans, 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
Group leader: Dr Marc HANIKENNE
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PhD students: Jean-Benoît Charlier, Clémentine Laurent
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

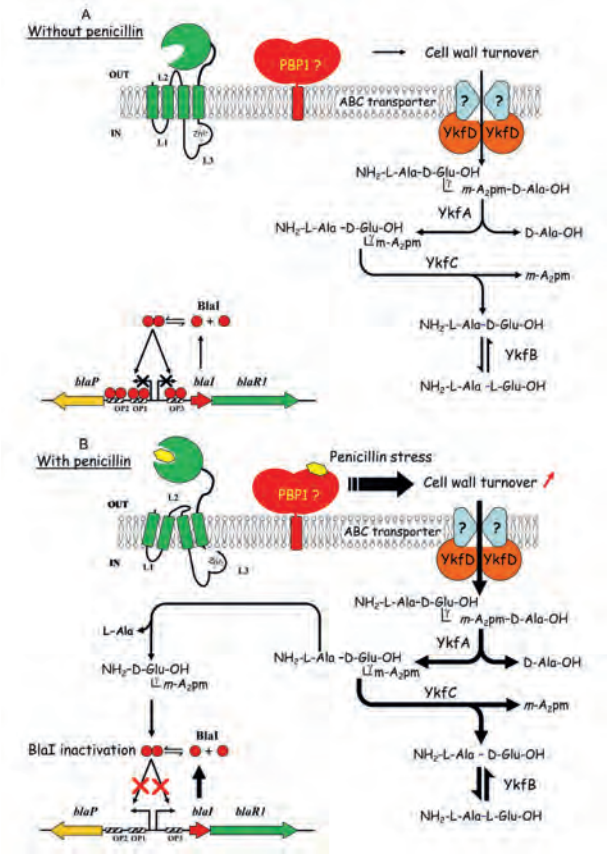
designed to isolate mutants possibly affected in the sensing and the signaling of the cellular iron status. This research will shed light on the mechanisms controlling iron homeostasis at the cellular level in photosynthetic organisms. A better understanding of iron sensing and adaptation to iron deficiency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

HIGHLIGHTS OF THE YEAR 39

A Peptidoglycan Fragment Triggers β-lactam Resistance in *Bacillus licheniformis*

Ana Amoroso, Julien Boudet, Stéphanie Berzigotti, Valérie Duval, Nathalie Teller, Dominique Mengin-Lecreulx, André Luxen, Jean-Pierre Simorre and Bernard Joris.
PLoS Pathog. 2012;8(3):e1002571. doi: 10.1371

To resist to β-lactam antibiotics Eubacteria either constitutively synthesize a β-lactamase or a low affinity penicillin-binding protein target, or induce its synthesis in response to the presence of antibiotic outside the cell. In *Bacillus licheniformis* and *Staphylococcus aureus*, a membrane-bound penicillin receptor (BlaR/MecR) detects the presence of β-lactam and launches a cytoplasmic signal leading to the inactivation of BlaI/MecI repressor, and the synthesis of a β-lactamase or a low affinity target. We identified a dipeptide, resulting from the peptidoglycan turnover and present in bacterial cytoplasm, which is able to directly bind to the BlaI/MecI repressor and to destabilize the BlaI/MecI-DNA complex. We propose a general model, in which the acylation of BlaR/MecR receptor and the cellular stress induced by the antibiotic, are both necessary to generate a cell wall-derived coactivator responsible for the expression of an inducible β-lactam-resistance factor. The new model proposed confirms and emphasizes the role of peptidoglycan degradation fragments in bacterial cell regulation.



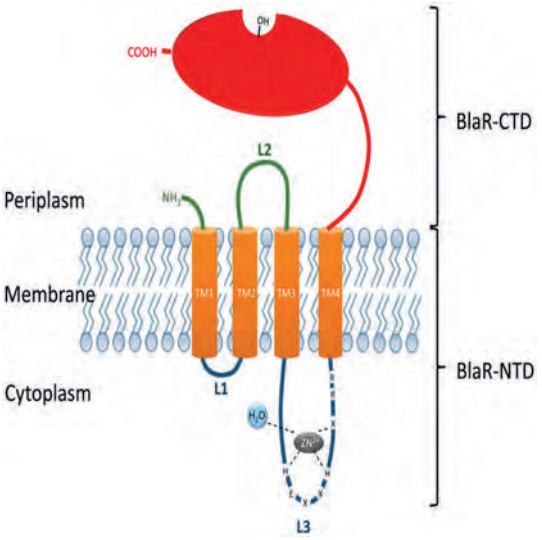
(A) Without penicillin outside the cell, the BlaR penicillin receptor is not activated, BlaI dimer interacts with its nucleic operators (OP1, OP2 and OP3) and BlaP β-lactamase expression is maintained at a low level. PBP1 involved in the latest step of peptidoglycan biosynthesis is fully active and indirectly involved in cell wall turnover. The cytoplasmic YkfA, B, C and D proteins are probably part of a catabolic network involved in the use of peptidoglycan fragments generated by cell wall turnover.

(B) In presence of a concentration of penicillin such that the BlaR receptor and PBP1 are fully and partially acylated, respectively, two signals are generated into the bacterial cell. Partial PBP1 inactivation would generate a penicillin stress that would increase the cell wall turnover and the accumulation of peptidoglycan fragments in the cytoplasm. The penicillin-activated BlaR receptor would hydrolyze the L-Ala-γ-D-Glu-m-A2pm tripeptide, resulting from the activity of YkfA, to generate the γ-D-Glu-m-A2pm dipeptide. The binding of the coactivator to BlaI leads to the inactivation and subsequent release of the repressor in the cytoplasm where it can be hydrolyzed by cytoplasmic proteases.

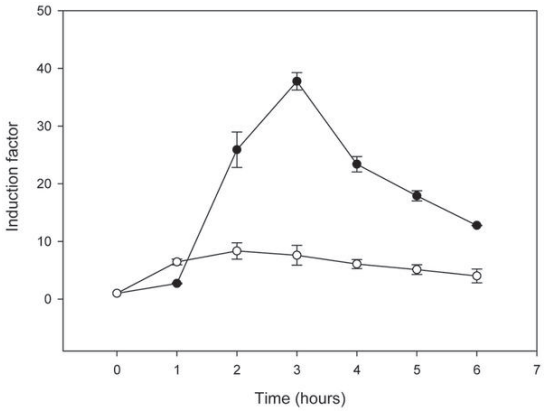
Bacillus licheniformis BlaR1 L3 Loop Is a Zinc Metalloprotease Activated by Self-Proteolysis

Stéphanie Berzigotti , Kamal Benlafya , Jérémy Sépulchre, Ana Amoroso and Bernard Joris.
PLoS One. 2012;7(5):e36400. doi: 10.1371

In Bacillus licheniformis 749/I, BlaP β-lactamase is induced by the presence of a β-lactam antibiotic outside the cell. The first step in the induction mechanism is the detection of the antibiotic by the membrane-bound penicillin receptor BlaR1 that is composed of two functional domains: a carboxy-terminal domain (BlaR-CTD) exposed outside the cell, which acts as a penicillin sensor, and an amino-terminal domain anchored to the cytoplasmic membrane, which works as a transducer-transmitter (BlaR-NTD).

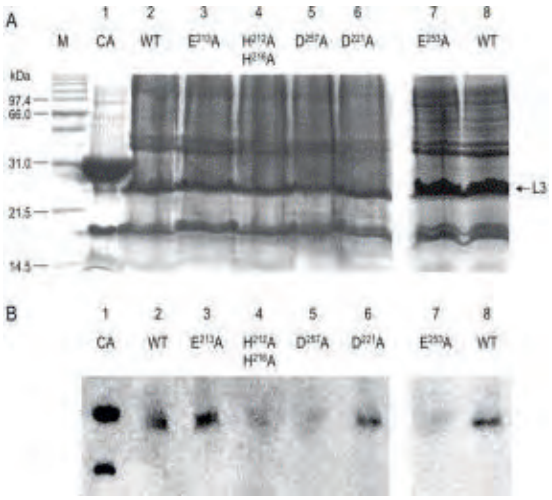


The acylation of BlaR1 sensor domain by the antibiotic generates an intramolecular signal that leads to the activation of the L3 cytoplasmic loop of the transmitter by a single-point cleavage. The exact mechanism of L3 activation and the nature of the secondary cytoplasmic signal launched by the activated transmitter remain unknown. However, these two events seem to be linked to the presence of a HEXXH zinc binding motif of neutral zinc metallopeptidases. By different experimental approaches, we demonstrated that the L3 loop binds zinc ion, belongs to Gluzincin metallopeptidase superfamily and is activated by self-proteolysis.



Induction factor of β-lactamase synthesis for the B. subtilis strains transformed with pDML995 (wild-type divergeon; •) or pDML3045 (mutation R304A/R305A;○), showing that the β-lactamase synthesis is not induced when BlaR1 L3 loop is not activated by self-proteolysis .

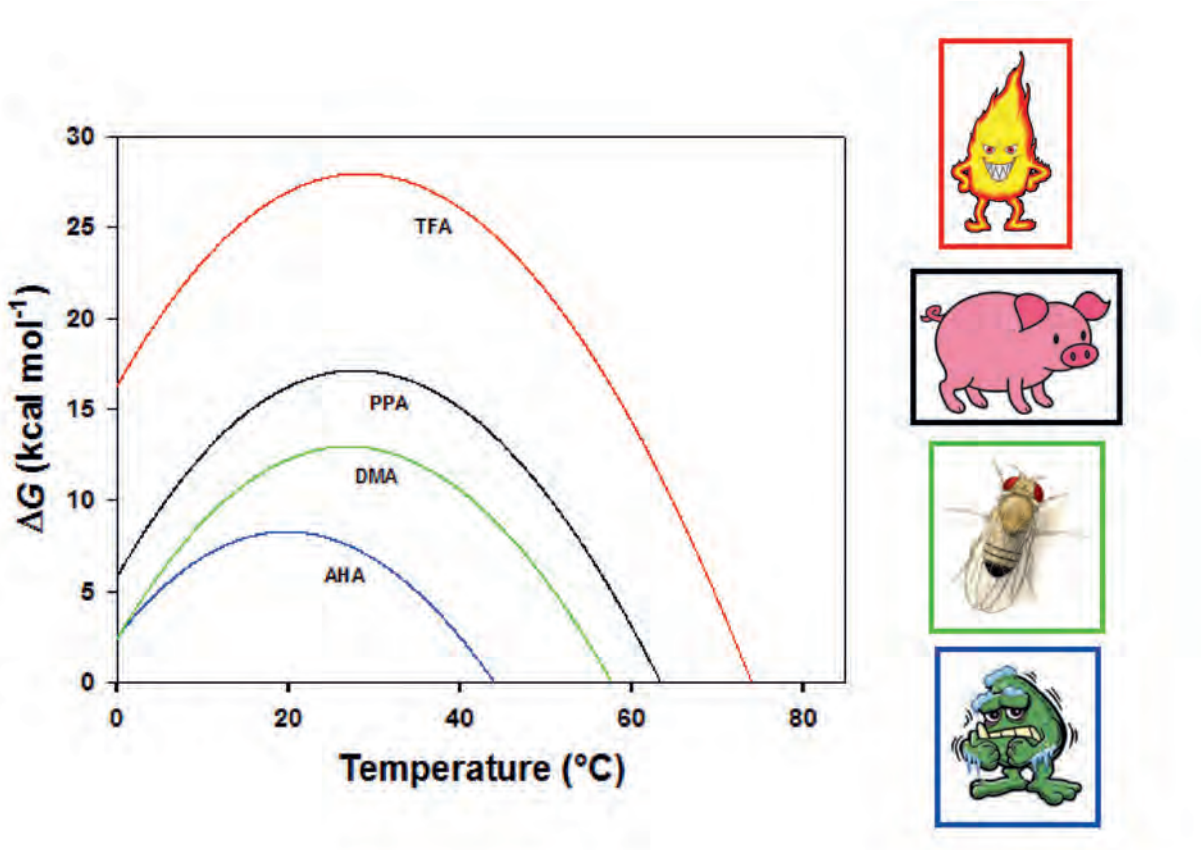
(A) Coomassie Blue-stained SDS-PAGE of partially purified inclusion bodies of wild-type and L3 loop mutants (B) Zinc blot analysis of (A) to show that BlaR1 L3 loop is a Zinc-metalloprotease



Temperature adaptations in psychrophilic, mesophilic and thermophilic chloride-dependent alpha-amylases

Alexandre Cipolla , François Delbrassine , Jean-Luc Da Lage and Georges Feller
Biochimie, 2012, 94 : 1943-1950.

The functional and structural adaptations to temperature have been addressed in homologous chloride-dependent α-amylases from a psychrophilic Antarctic bacterium (AHA), the ectothermic fruit fly (DMA), the homeothermic pig (PPA) and from a thermophilic actinomycete (TFA). This series covers nearly all temperatures encountered by living organisms. We report a striking continuum in the functional properties of these enzymes coupled to their structural stability and related to the thermal regime of the source organism. In particular, thermal stability recorded by intrinsic fluorescence, circular dichroism and differential scanning calorimetry (DSC) appears to be a compromise between the requirement for a stable native state and the proper structural dynamics to sustain the function at the environmental/physiological temperatures. The thermodependence of activity, the kinetic parameters, the activation parameters and fluorescence quenching support these activity-stability relationships in the investigated α-amylases.

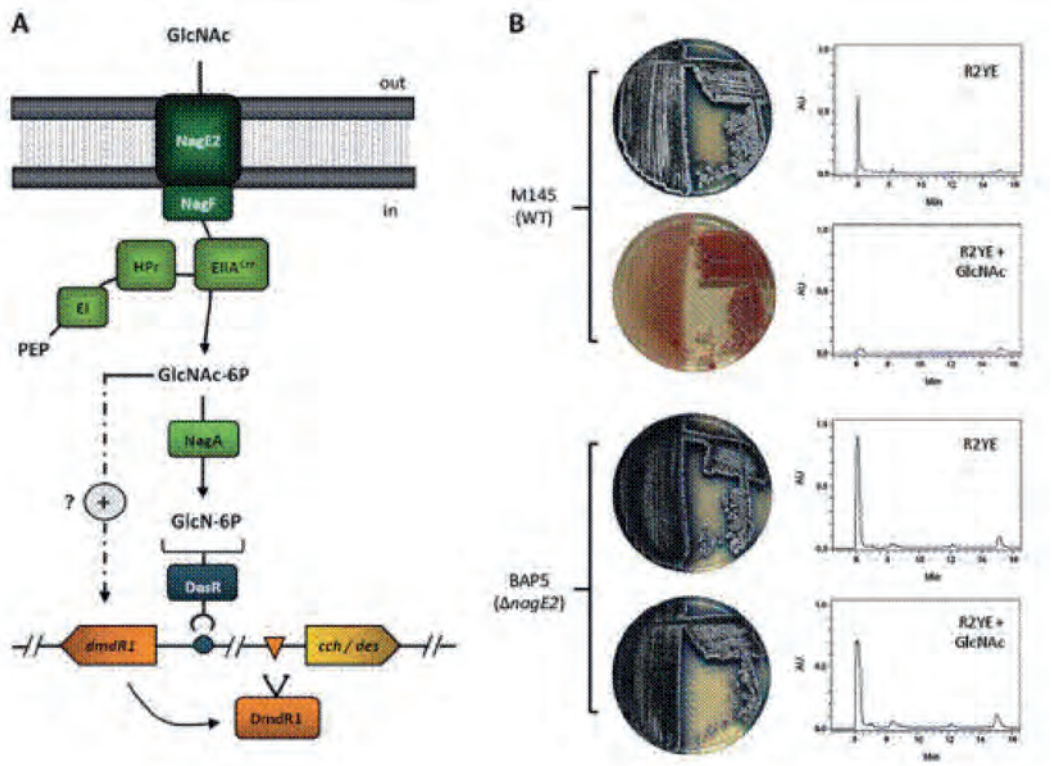


Stability curves calculated from DSC data. The Gibbs free energy of unfolding corresponds to the work required to unfold the native state as a function of temperature. The increased stability of the α-amylases and of Tm (at ΔG=0) is essentially gained via uplifting the curves towards higher free energy values.

Unsuspected Control of Siderophore Production by N-acetylglucosamine in Streptomycetes

Matthias Craig , Stéphany Lambert, Samuel Jourdan, Elodie Tenconi, Séverine Colson, Marta Maciejewska, Marc Ongena, Juan-Francisco Martin, Gilles van Wezel and Sébastien Rigali.
Environmental Microbiology Reports. Volume 4, Issue 5, Pages 512-521.

Microorganisms have developed iron-chelating molecules called siderophores that capture this element in the environment and the resulting complexes are internalized by specific uptake systems. While biosynthesis of siderophores in many bacteria is regulated by iron availability and oxidative stress, we describe here a new type of regulation of siderophore production. We show that in *Streptomyces coelicolor*, their production is also controlled by N-acetylglucosamine (GlcNAc) via the direct transcriptional repression of the iron utilization repressor *dmdR1* by DasR, the GlcNAc utilization regulator. This regulatory nutrient-metal relationship is conserved among streptomycetes, which indicates that the link between GlcNAc utilization and iron uptake repression, however unsuspected, is the consequence of a successful evolutionary process. We describe here the molecular basis of a novel inhibitory mechanism of siderophore production that is independent of iron availability. We speculate that the regulatory connection between GlcNAc and siderophores might be associated with the competition for iron between streptomycetes and their fungal soil competitors, whose cell walls are built from the GlcNAc-containing polymer chitin. Alternatively, GlcNAc could emanate from streptomycetes' own peptidoglycan that goes through intense remodelling throughout their life cycle, thereby modulating the iron supply according to specific needs at different stages of their developmental programme.



(A) Signaling cascade from N-acetylglucosamine transport by the PTS transport system (EI, HPr, EIIA, NagF, NagE2) to siderophore synthesis (*cch, coelichelin/des, desferrioxamines*) inhibition by DmdR1 in *S. coelicolo*r. (B) Deletion of *nagE2* encoding the GlcNAc permease, prevents the blocking effect of GlcNAc on *S. coelicolor* development and the inhibitory effect of GlcNAc on siderophore biosynthesis (peaks on the HPLC profiles).

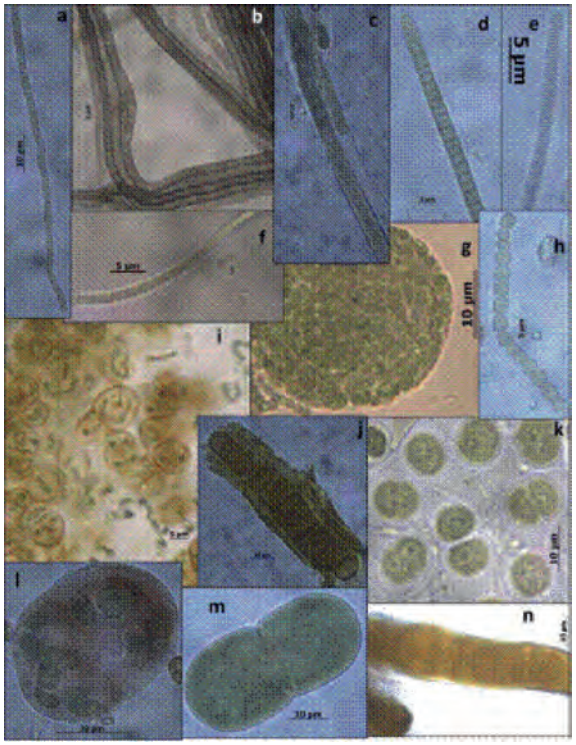
Cyanobacterial diversity for an anthropogenic impact assessment in the Sør Rondane Mountains area, Antarctica

Rafael Fernandez-Carazo, Zorigto Namsaraev, Marie-José Mano, Damien Ertz and Annick Wilmotte.
Antarctic Science, 24, 229-242

The Antarctic 'Princess Elisabeth' research Station has been built in 2007-09 in the Sør Rondane Mountains (Dronning Maud Land). The construction site is situated close to the Utsteinen Nunatak (71857'S, 23820'E), on a granite ridge. Prior to the survey and construction activities, the site had been subject to limited or no direct human impacts. Therefore, a biological inventory has been started for future evaluations of human impacts in the area.

This study presents the cyanobacterial diversity of 10 samples from the Utsteinen ridge and the Utsteinen Nunatak in the vicinity of the station's site and the more distant Ketelersbreen Dry Valley. The terrestrial crusts, including mosses, lichens and cyanobacteria, and thin biofilms on gravels were collected. All samples were taken before the station was built and were studied by light microscopy and Denaturing Gradient Gel Electrophoresis. Two strains were also isolated. Two different phenol-based extraction methods, with and without detergent-based steps, were tested to improve the quality of molecular detection from these environmental samples.

We observed a rather high cyanobacterial diversity (10 morphotypes and 13 operational taxonomic units) in comparison to other Antarctic terrestrial locations with a milder climate, and a widespread distribution pattern within the Sør Rondane Mountains area. The latter might reflect the habitat similarities and/or the easy local dispersal capabilities of microorganisms within the region. The high diversity and proportion of potential endemic (46%) operational taxonomic units, of which 23% were newly described, suggest that this area could have acted as a biological refuge during past glaciations.

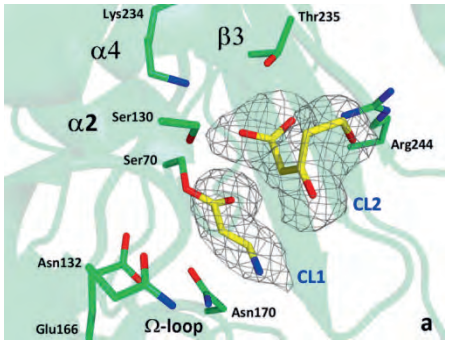


Diversity of cyanobacterial morphotypes identified in the environmental samples and cultures. a. *Phormidium priestley*i, b. strain *Phormidium priestley*i ANT-UTS195, c. & d. *Phormidium autumnale*, e. *Phormidium sp.*, f. *Leptolyngbya Antarctica*, g. *Nostoc sp.*, h. & i. strain *Nostoc sp.* ANT-UTS183, j. *Coleodesmium sp.*, k. *Chroococcus sp.*, l. *Asterocapsa sp.*, m. *Cyanothece aeruginosa*, and n. *Stigonema sp.*

Novel fragments of clavulanic acid observed in the structure of the class A β-lactamase from *Bacillus licheniformis* BS3

Pablo Power, Paola Mercuri, Raphaël Herman, Frédéric Kerff, Gabriel Gutkind, Georges Dive, Moreno Galleni, Paulette Charlier and Eric Sauvage
J Antimicrob Chemother. 2012 Oct;67(10):2379-87.

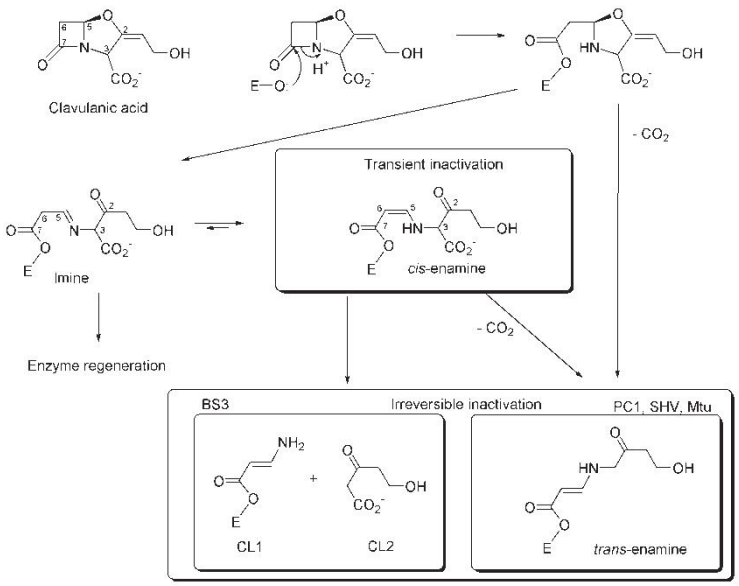
Clavulanic acid, a naturally occurring β-lactam that displays potent β-lactamase inhibitory properties represents a major weapon for circumventing the β-lactamase-mediated bacterial resistance. Different pathways have been proposed to explain the inhibition mechanism of class A β-lactamases by clavulanic acid. In this work, we report the 2 Å resolution crystal structure of the adduct of the class A β-lactamase from *B. licheniformis* BS3 with clavulanic acid, and provide clues about the pathway of class A β-lactamase inhibition by clavulanate.



Detailed view of the structure of the active site of *B. licheniformis* BS3 β-lactamase in complex with clavulanic acid.

Unlike structural results obtained from crystals of the SHV-1 β-lactamase or the β-lactamases from *S. aureus* and *M. tuberculosis*, the structure of clavulanic acid observed after its reaction with the β-lactamase from *B. licheniformis* BS3 is made of two moieties. Hydrolysis of clavulanate in two fragments results from the interactions of the carboxylate with active site residues.

The analysis of the X-ray structure of the complex yielded by the reaction between clavulanic acid and BS3 indicates that the transient inactivated form, namely the cis-trans enamine complex, is hydrolyzed in an ethane-imine ester covalently linked to the active site serine and a pentan-3-one-5-ol acid. It is the first time that this mechanism is observed in inactivated β-lactamase. Furthermore, the ionic interactions made by the carboxylic group of pentan-3-one-5-ol may allow the understanding of the decarboxylation process of the trans-enamine of the non-productive complex observed for the interaction between clavulanate and the SHV-1 and *Mycobacterium tuberculosis* β-lactamase (Mtu).



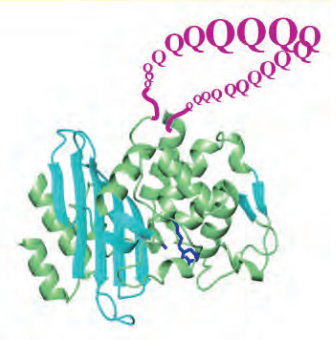
Updated mechanisms for the inactivation of class A β-lactamase by clavulanic acid based on X-ray structures.

This work was supported by the Fonds de la Recherche Scientifique (IISN 4.4509.11) and by a bilateral scientific agreement (V4/325C) between the Belgian Funds for Scientific Research (FRS-FNRS) and the Argentinean National Scientific and Technical Research Council (CONICET).

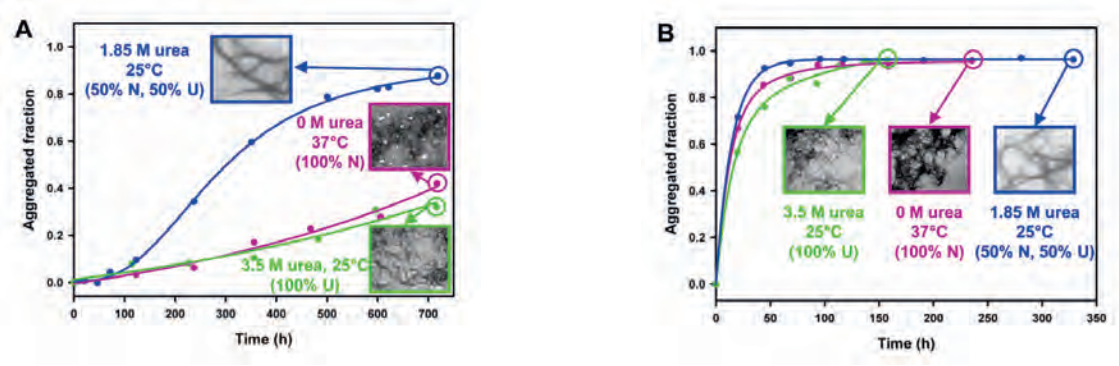
Amyloid-like fibril formation by polyQ proteins: a critical balance between the polyQ length and the constraints imposed by the host protein

N. Scarafone, C. Pain, A. Fratamico, G. Gaspard, N. Yilmaz, P. Filée, M. Galleni, A. Matagne, and M. Dumoulin.
PLoS One 7(3):e31253.

Nine neurodegenerative disorders, called polyglutamine (polyQ) diseases, are characterized by the formation of intranuclear amyloid-like aggregates by nine proteins containing a polyQ tract above a threshold length. These insoluble aggregates and/or some of their soluble precursors are thought to play a role in the pathogenesis. The mechanism by which polyQ expansions trigger the aggregation of the relevant proteins remains, however, unclear. In this work, polyQ tracts of different lengths were inserted into a solvent-exposed loop of the β-lactamase BlaP and the effects of these insertions on the properties of BlaP were investigated by a range of biophysical techniques. The insertion of up to 79 glutamines does not modify the structure of BlaP; it does, however, significantly destabilize the enzyme. The extent of destabilization is largely independent of the polyQ length, allowing us to study independently the effects intrinsic to the polyQ length and those related to the structural integrity of BlaP on the aggregating properties of the chimeras. Only chimeras with 55Q and 79Q readily form amyloid-like fibrils; therefore, similarly to the proteins associated with diseases, there is a threshold number of glutamines above which the chimeras aggregate into amyloid-like fibrils. Most importantly, the chimera containing 79Q forms amyloid-like fibrils at the same rate whether BlaP is folded or not, whereas the 55Q chimera aggregates into amyloid-like fibrils only if BlaP is unfolded. The threshold value for amyloid-like fibril formation depends, therefore, on the structural integrity of the β-lactamase moiety and thus on the steric and/or conformational constraints applied to the polyQ tract. These constraints have, however, no significant effect on the propensity of the 79Q tract to trigger fibril formation. These results suggest that the influence of the protein context on the aggregating properties of polyQ disease-associated proteins could be negligible when the latter contain particularly long polyQ tracts.



X-ray structure of the host protein BlaP showing the insertion of the polyQ tract

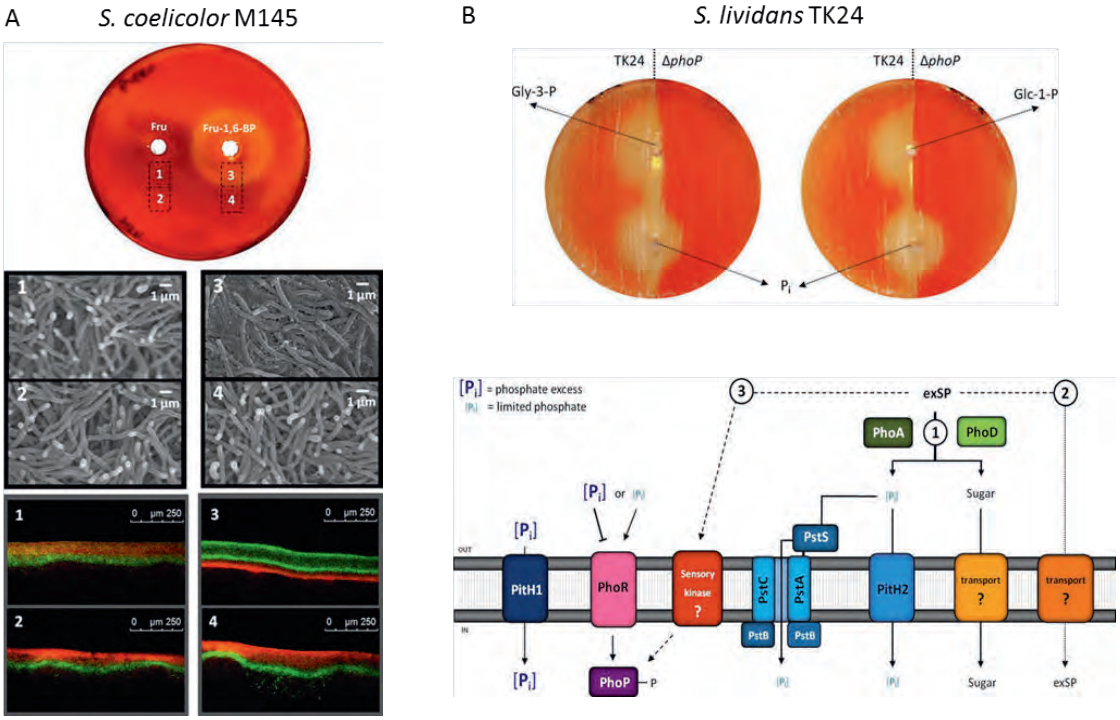


Comparison between the aggregation kinetics and the morphology of aggregates obtained with BlaP(Gln)55 (A) and BlaP(Gln)79 (B) under the following conditions of incubation: (i) PBS, pH 7.5 and 0 M urea at 37°C (pink),(ii) PBS, pH 7.5 and 1.85 M urea at 25°C (blue) and (iii) PBS, pH 7.5 and 3.5 M urea at 25°C (green). N is the native state and U is the unfolded state.

Extracellular sugar phosphates are assimilated by Streptomyces in a PhoP-dependent manner

Elodie Tenconi, Samuel Jourdan, Patrick Motte, Marie-Joëlle Virolle and Sébastien Rigali
Antonie van Leeuwenhoek, 2012 102(3):425-33.

Filamentous microorganisms of the bacterial genus Streptomyces have a complex life cycle that includes physiological and morphological differentiations. It is now fairly well accepted that lysis of Streptomyces vegetative mycelium induced by programmed cell death (PCD) provides the required nutritive sources for the bacterium to erect spore-forming aerial hyphae. However, little is known regarding cellular compounds released during PCD and the contribution of these molecules to the feeding of surviving cells in order to allow them to reach the late stages of the developmental program. In this work we demonstrated that the supply of phosphorylated sugars (exSPs) delays the occurrence of the second round of PCD, blocks streptomycetes life cycle at the vegetative state and inhibits antibiotic production. The mechanism by which sugar phosphates affect development was shown to involve genes of the Pho regulon that are under the positive control of the two component system PhoR/PhoP.



(A) *S. coelicolor* M145 culture plate exposed to fructose (Fru) or fructose-1,6-biphosphate (Fru-1,6-BP). Note the reduced production of prodiginines around the well containing the exSP. Zones 1, 2, 3, and 4 were analyzed by cryo-scanning electron microscopy (middle part) or by confocal laser-scanning microscope after the LIVE/DEAD Bac-Light Bacterial Viability Kit (Invitrogen) staining. (B) In *S. lividans* TK24 the inactivation of *phoP*, a transcriptional regulator that governs the adaptation of the bacteria to Pi limitation prevents the 'exSPs effect' but not that of Pi on development. The lower part shows the favored model (1, continuous lines) of PhoP-dependent sugar phosphate assimilation via alkaline phosphatase (PhoA, PhoD) enzymatic activity, alternative model of putative PhoP-dependant direct sugar phosphate uptake (3, dotted lines) and model of putative sugar phosphate sensing (2, dashed lines).

SCIENTIFIC SERVICES

CONTACTS

- <u>BCCM/ULC: Culture collection for cyanobacteria</u>:
http://bccm.belspo.be/about/ulc.php


Manager: Dr Annick Wilmotte
awilmotte@ulg.ac.be
Tel: +32 (0)4 366 33 87/38 56

Technical assistance: Marine Renard

- <u>Protein Factory platform</u>:
http://www.proteinfactory.ulg.ac.be/

Manager: Dr Alain Brans
abrans@ulg.ac.be
Tel : +32 (0)4 366 34 50

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

- <u>High throughput mutagenesis facilities</u>

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

Biotechnology Training Centre:
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forem-biotech@skynet.be
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Fabrice Bouillenne
F.bouillenne@ulg.ac.be
Tel: +32 (0)4 366 33 15

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

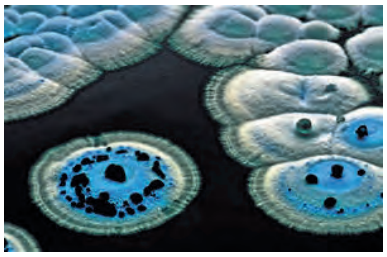
Picture and drawing of the strain ANT.LPR3, type species of the new genus *Plectolyngbya*.

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 FACTORY PLATFORM

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.

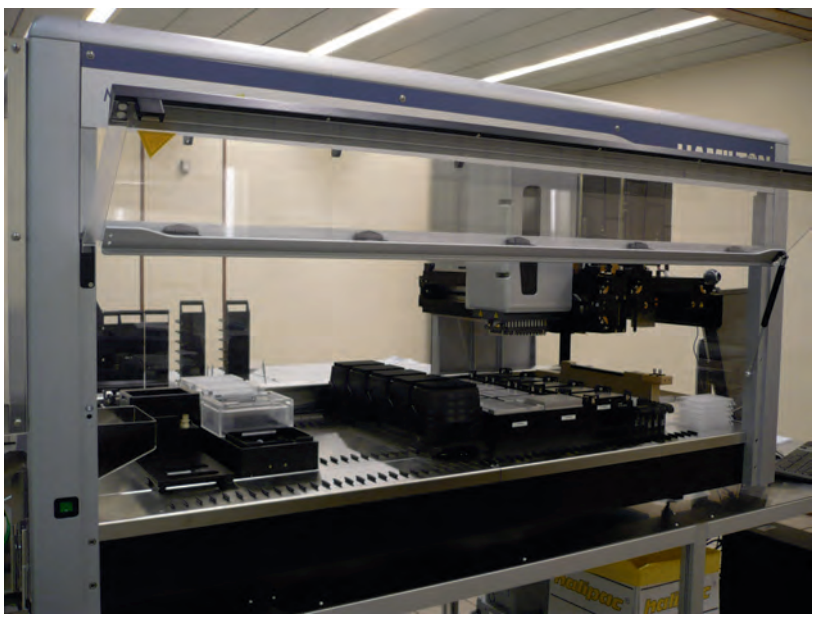
High throughput mutagenesis equipment was acquired in conjunction with the GIGA entity owing to funding from Feder. This equipment is centered on a Hamilton MICROLAB® STAR Liquid Handling Workstation. The station is supplied with an external robotic arm, a 96 channel pipetting head and 8 1000 µL independent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

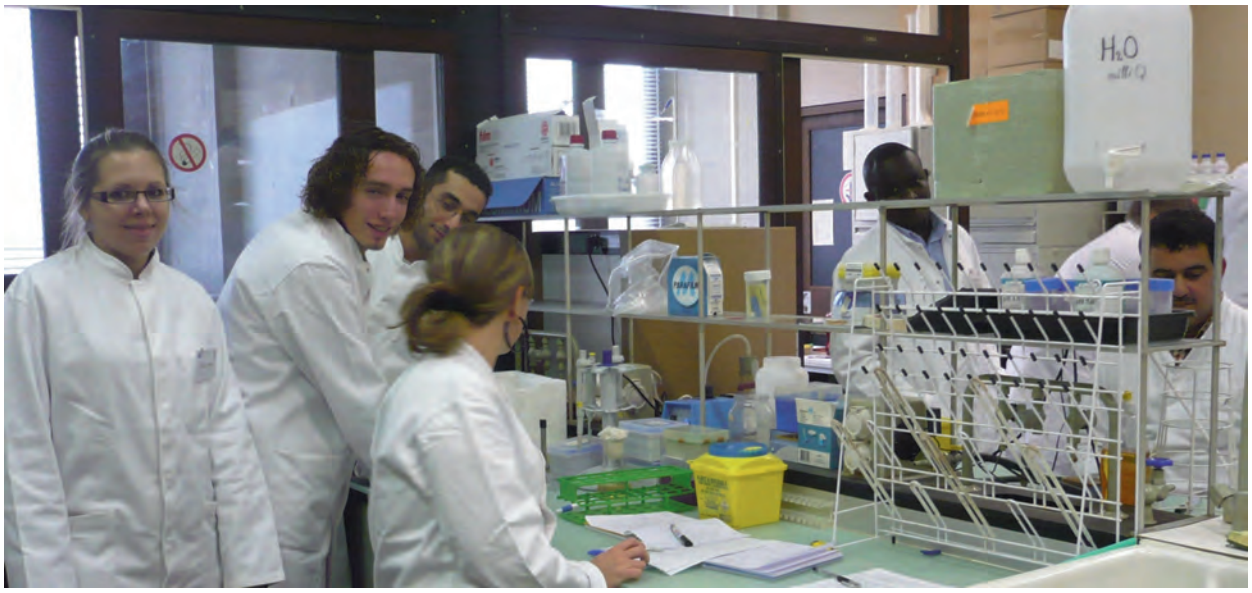
In the course of 2011, two Multimode microplate readers were acquired with funding coming from Belgian special funds for research. The first reader has 2 detection modes: one in absorbance and one in fluorescence. The second is also equipped with 2 detection modes: one in absorbance and one in luminescence.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

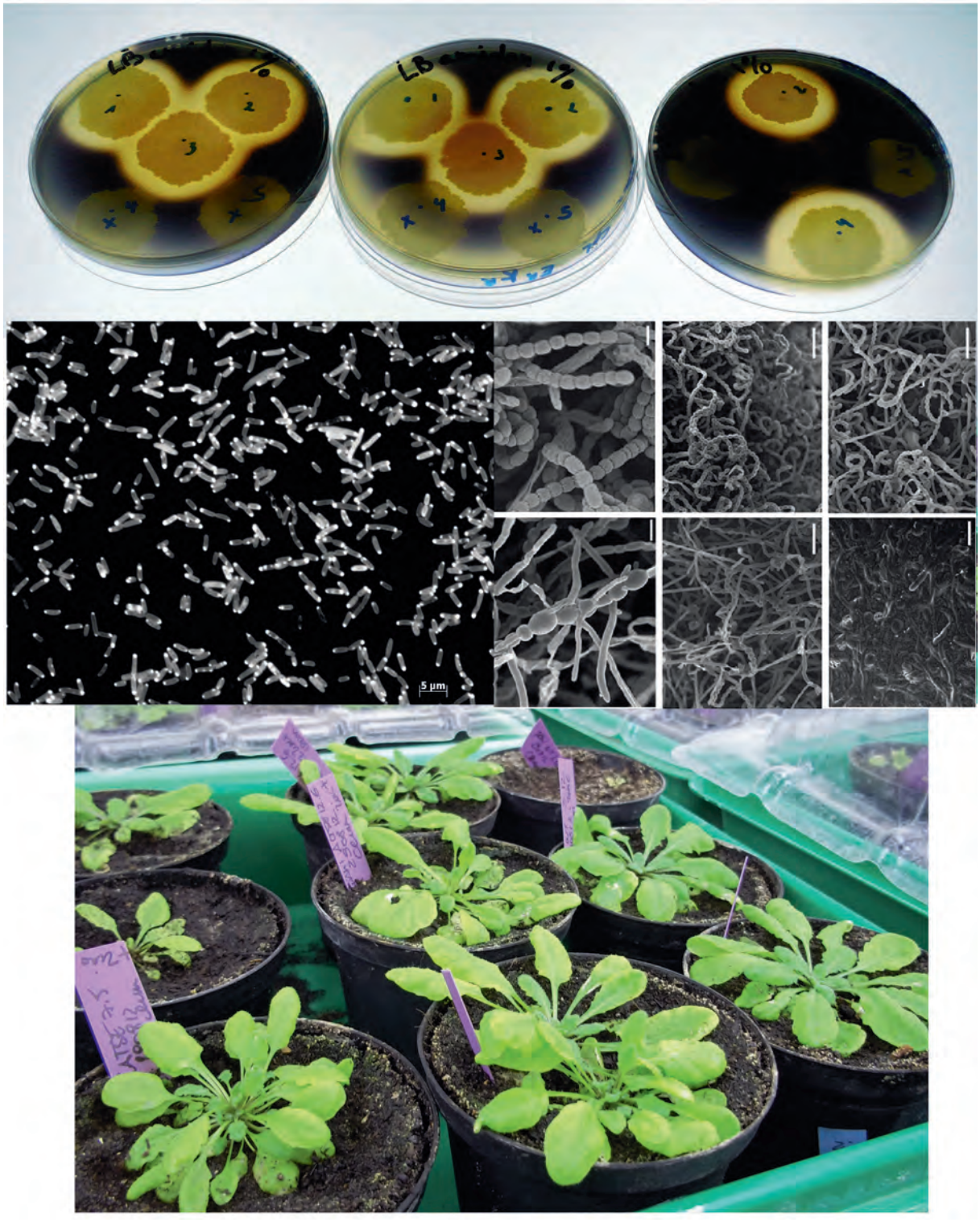
• Cell harvesting and cell disruption

• 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

Mathias Craig	Igor Stojiljkovic award for "outstanding post-doctoral presentation" at the biometals symposium, VUB, Brussels, Belgium, July 15-19
Maud Delsaute	"Cellulase involvement in the bacterial cellulose biosynthesis": best poster presentation at "Biomedica 2012: Where Science & Business meet", Section "Biomaterials", Liège, Belgium, April 18-19

Invited Speakers

Dr Mohamed Azarkan, ULB, Brussels, Belgium, "La PEGylation des groupements thiols comme alternative à la chromatographie d'affinité: application aux protéases à cystéine de tiges d'*Ananas comosus*", April 20

Dr Marc Beauregard, Université du Québec, Trois-Rivières, Canada, "A novel carboxylesterase : EstGtA2 – Controlling stability at alkaline pH", October 1

Dr Renaud Berlemont, University of California, Irvine, USA, "La dégradation de la cellulose comment ça marche… approches phylogénomique et métagénomique", June 11

Dr Andy Chevigné, CRP-Santé, Luxembourg, "Chemokine recognition site 1: diversity, function and therapeutic potential", November 23

Dr Patrice Courvalin, Institut Pasteur, Paris, France, "L'échec des staphylocoques résistants à la vancomycine", November 30

Dr Erwin de Genst, University of Cambridge, UK, "Nanobodies as structural probes for protein mis-folding and fibril formation", October 12

Dr Meriem El Ghachi, Institut Pasteur, Paris, France, "Rôle bi-fonctionnel d'une D,D-endopeptidase (HdpB) dans la morphogenèse de Helicobacter pylori ", February 3

Dr Gennaro Esposito, University of Udine, Italy, "Application of H-D exchange to the determination of the energy landscape of proteins and their complexes", February 6

Dr Gennaro Esposito, University of Udine, Italy, "Metabonomics by NMR", February 8

Dr Paul R. Gooley, University of Melbourne, Australia, "Role of dynamics in the optimization of carbohydrate binding by the beta-subunit of AMP-activated protein kinase", August 27

Dr Piet Herdewijn, KUL, Leuven, Belgium, "Introduction to a Xenome project", February 10

Prof. Jeffrey R. Johansen, John Carroll University, Ohio, USA "What are cyanobacterial species?", August 29

Dr Pierre Lebrun, VUB-VIB, Brussels, Belgium, "Etudes structurales de HBHA, une adhésine majeure de Mycobacterium tuberculosis », April 3

Dr Séverine Padiolleau, Université de Technologie de Compiègne, France, "Avantages et limites des systèmes d'expression prokaryote périplasmique et cytoplasmique", February 14

Dr Patrizia Polverino de Laureto, University of Padova, Italy, "Protein complex analysis using mass spectrometry", February 7

Dr Patrizia Polverino de Laureto, University of Padova, Italy, “Docosahexaenoic acid induces the formation of alpha-synuclein toxic aggregates. Structural characterization and biological activity”, February 7

Dr Patrizia Polverino de Laureto, University of Padova, Italy, “Applications of mass spectrometry in functional proteomics”, February 9

Dr Loïc Quinton, ULg, Liège, Belgium, “Les venins animaux: du mélange toxique à la fabrication des nouveaux médicaments”, January 20

Dr Vincent Raussens, ULB, Brussels, Belgium, “Amyloid and FTIR: from hate to love?”, September 21

Dr Savvas Savvides, University of Ghent, Belgium, “The ultimate molecular “Wuxi Finger Hold” ”, December 7

Dr Badrish Soni, INRA-CNRS Toulouse, France, “Characterization of cyanobacterial phycobiliproteins and pchR-like transcriptional regulators”, February 16

Dr Nicolas Willet, ULg, Liège, Belgium, “Investigation of mechanochemical processes by single-molecule AFM”, March 23

Oral Presentations

M. Hanikenne, "Evolution of metal hyperaccumulation in Arabidopsis halleri", Molecular Ecology, Vienna, Austria, February 4-7

G. Feller, "Adaptation des protéines aux basses temperatures chez les psychrophiles", Laboratoire Evolution, Génomes et Spéciation, CNRS Gif sur Yvette, France, February 10

S. Rigali, 'L'immobilité au service de l'évolution: adaptation génétiques et physiologiques des Streptomyces', Université Libre de Bruxelles, Belgium, March 5.

S. Rigali, Computational Prediction of Regulatory Networks in Bacteria or a rational alternative to random approaches to unveil novel cis/trans relationships, University of Leiden, The Netherlands, April 13 and 20.

M. Delsaute, "Cellulase involvement in the bacterial cellulose biosynthesis", Biomedica 2012: Where Science & Business meet, Liège, Belgium, April 19

M. Dumoulin, "Creation of chimeric proteins to investigate the mechanism of aggregation of polyglutamine proteins which are associated with neurodegenerative diseases", CFP-CeProMa lecture, University of Antwerp, Belgium, May 10

A. Wilmotte, "Antarctic cyanobacterial diversity: how important are the geographical and ecological factors?", 8e Journées scientifiques du Comité National Français de Recherches Antarctiques, Brest, France, May 10

M. Dumoulin, "Properties and applications of Nanobodies: prospects in systemic and neurodegenerative protein aggregation diseases." Nano-hybrides, Moltrasio, Italy, May 21

A. Argüelles Arias, "Amylolysin, a novel lantibiotic from Bacillus amyloliquefaciens GA1 with potent antilisterial activity", International Symposium on antimicrobial Peptides, Villeneuve d'Ascq, France, June 13-15

M. Hanikenne, "Plants and algae metal homeostasi in Liège", GDRI LOCOMET kick-off meeting, Lille, France, July 4-6

A. Wilmotte, "Out of sight, out of mind? Diversity of microscopic organisms as an overlooked criterion for conservation purposes", XXXII Open Science Conference of the Scientific Committee on Antarctic Research Meeting, Portland, USA, July 14-20

M. Craig, "Unsuspected control of siderophore biosynthesis by the cell wall component N-acetylglucosamine in Streptomyces, VUB, Brussels, Belgium, July 18

A. Argüelles Arias, "The Bacillus amyloliquefaciens lantibiotic amylosysin : a potent weapon against multi-drug resistant pathogens », International Meeting on Antimicrobial Peptides, University of Leipzig, Germany, August 30-31

A. Matagne, "A young person's guide to protein folding", FEBS Sofia School of Protein Science: Structure and Dynamics of Biological Macromolecules, Sofia, Bulgaria, September 9-14

C. Huynen, “Influence of the protein context on the propensity of polyglutamine tracts to trigger protein aggregation into amyloid fibrils” (poster selected for a short oral presentation). Sofia School of Protein Science: Structure and dynamics of biological macromolecules, Sofia, Bulgaria, September 14

M. Hanikenne, “Zinc hyperaccumulation: a model to examine metal homeostasis in plants”, 5th biannual International Meeting of the Institute of Metals in Biology, Grenoble, France, September 18-21

M. Hanikenne, “Zinc hyperaccumulation: a model to examine metal homeostasis in plants”, Université de Lorraine, Nancy, France, September 28

M. Dumoulin, “Investigation of the mechanism of aggregation of polyglutamine proteins which are associated with neurodegenerative diseases”, Conférence Universitaire de Suisse Occidentale, University of Geneva, Switzerland, October 12

A. Matagne, “Le repliement des protéines, une surprenante gymnastique moléculaire qui prête vie aux protéines”, Collège Belgique, Palais des Académies, Brussels, Belgium, October 25

E. Tenconi, Role of DasR and N-acetylglucosamine in the onset of *Streptomyces coelicolor* development, Journées Streptomyces, Université de Lorraine, Nancy, France, October 25

A. Wilmotte, “Antarctic cyanobacterial diversity: how important are the geographical and ecological factors?”, XXI Congresso Latinoamericano de Microbiologia – ALAM 2012, Santos, Brazil, October 31

C. Montagner, “Equilibrium unfolding of Bacillus cereus beta-lactamase gives insight into the role loops in the enzyme activity”, 3rd annual East-NMR user meeting, Lasko, Slovenia, November 13-16

C. Huynen, “Influence of the protein context on the propensity of polyglutamine tracts to trigger protein aggregation into amyloid fibrils”, Annual meeting of the SFMBBM doctoral school of Structure and Function of the Biological Macromolecules, Biochemistry and Modeling, Louvain-la-Neuve, Belgium, November 16

M. Dumoulin, “Investigation of the mechanism of aggregation of polyglutamine proteins which are associated with neurodegenerative diseases”, University of Padova, Italy, November 23

S. Rigali, Antibiotic resistance: lessons from the soil. University of Liège, Belgium, December 3.

M. Hanikenne, “Zinc hyperaccumulation: a model to examine metal homeostasis in plants”, INRA-Suprago, Montpellier, France, December 4

F. Kerff, “Cristallographie des protéines: principe de base et résultats”, Mini-conférence de la Société Royale des Sciences de Liège, Liège, Belgium, December 20

Patents

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

P. Filée, N. Rhazi, M. Galleni, B. Taminiau, O. Jolois, A. Collard, A. Jacquet

Pub. N°: US2012/0014983 A1, Application N°: 13/143, 795,

Publication date: 19.01.12, Filing date: 15.01.2010

PhD Theses

24/02/2012 **Magdalena Calusinska (Biotechnology)**
Application of molecular tools to study the hydrogen production by the bacteria of the genus Clostridium

06/06/2012 **Mathias Craig (Zoology)**
Regulation of siderophore biosynthesis and role in Streptomyces development

19/06/2012 **Natacha Scarafone (Biochemistry)**
Creation of model proteins to investigate the mechanism of aggregation of expanded-polyglutamine proteins - Insertion of polyglutamine tracts into the β-lactamase BlaP

08/10/2012 **Séphanie Berzigotti (Biochimie)**
Etude de la voie de signalisation menant à l'induction de la β-lactamase BlaP chez *Bacillus licheniformis* 749l

24/10/2012 **Alexandre Cipolla (Zoology)**
Adaptations structurales et fonctionnelles aux températures extrêmes au sein de la famille des alpha-amylases chlorure-dépendantes

PUBLICATIONS

1. A. Amoroso, J. Boudet, S. Berzigotti, V. Duval, N. Teller, D. Mengin-Lecreulx, A. Luxen, J.P. Simorre et B. Joris
A peptidoglycan fragment triggers beta-lactam resistance in Bacillus licheniformis
PLOS Pathogens, 8, 1-15

2. M. Banzhaf, B. van den Berg van Saparoea, M. Terrak, C. Fraipont, A. Egan, J. Philippe, A. Zapun, E. Breukink, M. Nguyen-Distèche, T. den Blaauwen and W. Vollmer
Cooperativity of peptidoglycan synthases active in bacterial cell elongation
Mol. Microbiol., 85, 179-194

3. A. Barabasz, A. Wilkowska, A. Ruszczynska, E. Bulska, M. Hanikenne, M. Czarny, U. Krämer and D.M. Antosiewicz
Metal response of transgenic tomato plants expressing P1B-ATPase
Physiol. Plant., 145, 315-331

4. S. Berzigotti, K. Benlafya, J. Sépulchre, A. Amoroso and B. Joris
Bacillus licheniformis BlaR1 L3 loop is a zinc metalloprotease activated by self-proteolysis
PlosOne, 7, e364000

5. F. Bruyneel, G. Dive, J. Marchand-Brynaert
Non-symmetrically substituted phenoxazinones from laccase-mediated oxidative cross-coupling of aminophenols: an experimental and theoretical insight
J. Org. Biomol. Chem., 10, 1834-1846

6. S. Califice, D. Baurain, M. Hanikenne an P. Motte
A single ancient origin for prototypical serine/arginine-rich splicing factors
Plant Physiology, 158, 546-560

7. S. Chakraborty, B. Asgeirsson, R. Minda, L. Salaye, JM Frère and BJ Rao
Inhibition of a cold-active alkaline phosphatase by imipenem revealed by in silico modeling of metallo-beta-lactamase active sites
FEBS Letters, 586, 3710-3715

8. A. Chenel, G. Dive, C. Meier, M. Desouter-Lecomte
Control in a Dissipative Environment: The Example of a Cope Rearrangement
J. Phys. Chem. A, 116, 11273-11282

9. A. Cipolla, F. Delbrassine, JL Da Lage and G. Feller
Temperature adaptations in psychrophilic, mesophilic and thermophilic chloride-dependent alpha-amylases
Biochimie, 94, 1943-1950

10. M. Craig, S. Lambert, S. Jourdan, E. Tenconi, S. Colson, M. Maciejewska, M. Ongena, J.F. Martin, G. van Wezel and S. Rigali
Unsuspected control of siderophore production by N-acetylglucosamine in streptomycetes
Environ. Microbiol. Rep., 4(5), 512-521

12. S. Dawarakanath, A.K. Chaplin, M.A. Hough, S. Rigali, E. Vijgenboom and J.A.R. Worrall
Response to copper stress in Streptomyces lividans extends beyond genes under direct control of a copper-sensitive operon repressor protein (CsoR)
J. Biol. Chem., 287, 17833-17847

13. G. Dive, R. Robiette, A. Chenel, M. Ndong, C. Meier, M. Desouter-Lecomte
Laser control in open quantum systems: preliminary analysis toward the Cope rearrangement control in methyl-cyclopentadienylcarboxylate dimer
Theor. Chem. Acc., 131, 1236

14. S. Dumbre, A. Derouaux, E. Lescrinier, A. Piette, B. Joris, M. Terrak and P. Herdewijn
Synthesis of modified peptidoglycan precursor analogues for the inhibition of glycosyltransferase
JACS, 134, 9343-9351

15. J.R. Kumita, L. Helmfors, J. Williams, L.M. Luheshi, L. Menzer, M. Dumoulin, D.A. Thomas, D.C. Crowther, C.M. Dobson and A.-C. Brorsson
Disease-related amyloidogenic variants of human lysozyme trigger the unfolded protein response and disturb eye development in Drosophila melanogaster
FASEB Journal, 26, 192-202

16. M. Kupper, C. Bauvois, JM Frère, K. Hoffmann, M. Galleni and C. Bebrone
The CphAII protein from Aquifex aeolicus exhibits a metal-dependent phosphodiesterase activity
Extremophiles, 16, 45-55

17. V. Larosa, N. Coosemans, P. Motte, N. Bonnefoy and C. Remacle
Reconstruction of a human mitochondrial complex I mutation in the unicellular green alga Chlamydomonas
The Plant Journal, 70, 759-768

18. R. Locht, D. Dehareng et B. Leyh
The spectroscopy of the geminal ethylene difluoride (1,1-C2H2F2): the HeI, threshold and constant ion state photoelectron spectroscopies
J. Phys. B At. Mol. Opt. Phys., 45, 115101

19. J. Masset, M. Calusinska, C. Hamilton, S. Hilligsmann, B. Joris, A. Wilmotte and P. Thonart
Fermentative hydrogen production from glucose and starch using pure strains and artificial co-cultures of Clostridium spp
Biotechnol. Biofuels, 5, 1-15

20. L. Moineaux, S. Laurent, J. Reniers, E. Dolusic, M. Galleni, J.M. Frère, B. Masereel, R. Frédérick and J. Wouters
Synthesis, crystal structures and electronic properties of isomers of chloro-pyridinylvinyl-1H-indoles
Eur. J. Med. Chem., 54, 95-102

21. K. Pauwels, M.M. Sanchez del Pino, G. Feller and P. Van Gelder
Decoding the folding in Burkholderia glumae lipase: folding intermediates en route to kinetic stability
PlosOne, 7, e36999

22. FJ Pérez-Llarena, A. Fernandez, L. Zamorano, F. Kerff, A. Beceiro, B. Aracil, E. Cercenado, E. miro, A. Oliver, J. Oteo, F. Navarro and G. Bou
Characterization of anovel IMP-28 metallo-beta-lactamase from a Spanish Klebsiella oxytoca clinical isolate
Antimicrob. Agents Chemother., 56, 4540-4543

23. F. Piette, P. Leprince et G. Feller
Is there a cold shock response in the Antarctic psychrophile Pseudoalteromonas haloplanktis?
Extremophiles, 16, 681-683

24. P. Power, P. Mercuri, R. Herman, F. Kerff, G. Gutkind, G. Dive, M. Galleni, P. Charlier and E. Sauvage
Novel fragments of clavulanate observed in the structure of the class A beta-lactamase from Bacillus licheniformis BS3
J. Antimicrob. Chemother., 67, 2379-2387

25. Y. Quiroz, M. Lopez, A. Mavropoulos, P. Motte, J.A. Martial, M. Hammerschmidt and M. Muller
The HMG-Box transcription factor Sox4b is required for pituitary expression of gata2a and specification of thyrotrope and gonadotrope cells in zebrafish
Mol. Endocrinol., 26, 1014-1027

26. G. Roussel, A. Matagne, X. De Bolle, E.A. Perpète and C. Michaux
Purification, refolding and characterization of the trimeric Omp2a outer membrane porin from Brucella melitensis
Protein Expr. Purif., 83, 198-204

27. T. Salinas, F. duby, V. Larosa, N. Coosemans, N. Bonnefoy, P. Motte, L. Maréchal-Drouard and C. Remacle
Co-evolution of mitochondrial tRNA import and codon usage determines translational efficiency in the green alga Chlamydomonas
PLOS Genetics, 8, e1002946

28. N. Scarafone, C. Pain, A. Fratamico, G. Gaspard, N. Yilmaz, P. Filée, M. Galleni, A. Matagne et M. Dumoulin
Amyloid-like fibril formation by polyQ proteins: a critical balance between the polyQ length and the constraints imposed by the host protein
PlosOne, 7, e31253

29. A.C. Servais, A. Rousseau, G. Dive, M. Frederich, J. Crommen, M. Fillet
Combination of capillary electrophoresis, molecular modelling and nuclear magnetic resonance to study the interaction mechanisms between single-isomer anionic cyclodextrin derivatives and basic drug enantiomers in a methanolic background electrolyte.
J. Chromatogr. A., 1232, 59-64

30. A. Sliwa, G. Dive, J. Marchand-Brynaert
12- to 22-Membered Bridged ß-Lactams as Potential Penicillin-Binding Protein Inhibitors
Chemistry - An asian Journal 7, 425–434

31. A. Sliwa, G. Dive, A. Zervosen, O. Verlaine, E. Sauvage, J. Marchand-Brynaert
Unprecedented inhibition of resistant penicillin binding proteins by bis-2-oxoazetidinyl macrocycles
Med. Chem. Commun., 3, 344-351

32. M.A. Swiatek, E. Tenconi, S. Rigali and G. van Wezel
FuncTIonal analysis of the N-Acetylglucosamine metabolic genes of Streptomyces coelicolor and role in control of development and antibiotic production
J. Bacteriol., 194, 1136-1144

33. M.A. Swiatek, M. Urem, E. Tenconi, S. Rigali and G. van Wezel
Engineering of N-acetylglucosamine metabolism for improved antibiotic production in Streptomyces coelicolor A3(2) and an unsuspected role of NagA in glucosamine metabolism
Bioengineered, 3, 280-285

34. L. Tavel, L. Jaquillard, A. I. Karsisiotis, F. Saab, L. Jouvensal, A. Brans, A.F. Delmas, F. Schoentgen, M. Cadene and C. Damblon
Ligand binding study of human PEBP1/RKIP: interaction with nucleotides and raf-1 peptides evidenced by NMR and mass spectrometry
PlosOne, 7, e36187

35. E. Tenconi, S. Jourdan, P. Motte, M.-J. Virolle and S. Rigali
Extracellular sugar phosphates are assimilated by Streptomyces in a PhoP-dependent manner
Antonie Van Leeuwenhoek, 102, 425-433

36. L. Van Oudenhove, K. De Vriendt, J. Van Beeumen, P. Mercuri and B. Devreese
Differential proteomic analysis of the response of Stenotrophomonas maltophilia to imipenem
Appl. Microbiol. Biotechnol., 95, 717-733

37. A. Zervosen, A. Bouillez, A. Herman, A. Amoroso, B. Joris, E. Sauvage, P. Charlier and A. Luxen
Synthesis and evaluation of boronic acids as inhibitors of Penicillin Binding Proteins of classes A, B and C
Bioorg. Med. Chem., 20, 3915-3924

38. A. Zervosen, E. Sauvage, JM Frère, P. Charlier and A. Luxen
Development of new drugs for an old target – The penicillin binding protein
Molecules, 17, 12478-12505

39. H. Zime-Diawara,, G. Dive, G. Piel, M. Moudachirou, M. Frédérich, J. Quetin-Leclercq, B. Evrard
Understanding the interactions between artemisinin and cyclodextrins: spectroscopic studies and molecular modeling
J. Incl. Phenom. Macrocycl. Chem., 74, 305–315

BOOK CHAPTERS AND REVIEWS

40. S. Baurin, X. Henry, N. Hoyez and J.M. Frère
Are there unknown classes of beta-lactamases? Sensitivity of beta-lactams to nucleophilic agents and other enzymes
Beta-lactamases, chapter 21, pp 507-514
Ed. J.M. Frère
Nova Science Publishers
New-York

41. C. Bebrone, G. Garau, I. Garcia-Saez, L. Chantalat, A. Carfi and O. Dideberg
X-ray structures and mechanisms of metallo-β-lactamases
Beta-lactamases, chapter 3, pp 41-77
Ed. J.M. Frère
Nova Science Publishers
New-York

42. D. Dehareng et G. Dive
Quantum chemistry applied to the study of beta-lactamases
Beta-lactamases, chapter 12, pp 315-340
Ed. J.M. Frère
Nova Science Publishers
New-York

43. J.D. Docquier, GM Rossolini, H. Nikaido and J.M. Frère
Interplay between beta-lactamase activity, outer membrane permeability and active efflux system
Beta-lactamases, chapter 16, pp 401-418
Ed. J.M. Frère
Nova Science Publishers
New-York

44. G. Feller and R. Margesin
Polar microorganisms and biotechnology
In: Polar Microbilogy: Life in a Deep Freeze
Eds R.V. Miller and L.G. Whyte, ASM Press, Washington DC, 2012, 166-180

45. J.M. Frère
Kinetics of beta-lactamases in theory and practice
Beta-lactamases, chapter 6, pp 139-160
Ed. J.M. Frère
Nova Science Publishers
New-York

46. B. Joris and J. Dusart
The induction of beta-lactamases in eubacteria
Beta-lactamases, chapter 17, pp 421-435
Ed. J.M. Frère
Nova Science Publishers
New-York

47. F. Kerff, E. Sauvage, L. Vercheval and P. Charlier
Structures of class D beta-lactamases
Beta-lactamases, chapter 5, pp 103-135
Ed. J.M. Frère
Nova Science Publishers
New-York

48. A. Matagne and R.H. Pain
Folding and stability of class A beta-lactamases
Beta-lactamases, chapter 19, pp 459-477
Ed. J.M. Frère
Nova Science Publishers
New-York

49. F. Piette, C. Struvay, A. Godin, A. Cipolla and G. Feller
Life in the cold: proteomics of the Antarctic bacterium Pseudomonas haloplanktis
In: Proteomic applications in biology
Eds Heazlewood J.L. and Petzold C.J., InTech, Rijeka, 2012, 93-114

50. C. Struvay and G. Feller
Optimization to low temperatures activity in psychrophilic enzymes
Int. J. Mol. Sci., 13, 11643-11665

51. M. Vandevenne, M. Galleni and P. Filée
How to make good use of “bad” enzyme: utilization of efficient beta-lactamases for the benefits of biochemical research
Beta-lactamases, chapter 20, pp 479-503
Ed. J.M. Frère
Nova Science Publishers
New-York

Protein Structures deposited within the Protein Data Bank

PDB ID	STRUCTURE TITLE	AUTHOR
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 B-LACTAMASE FROM BACILLUS LICHENIFORMIS BS3 WITH CLAVULANIC ACID.	Power, P., Sauvage, E., Herman, R., Kerff, F., Charlier, P.
4A5R	CRYSTAL STRUCTURE OF CLASS A BETA-LACTAMASE FROM BACILLUS LICHENIFORMIS BS3 WITH TAZOBACTAM	Power, P., Sauvage, E., Herman, R., Kerff, F., Charlier, P.
3TVL	COMPLEX BETWEEN THE HUMAN THIAMINE TRIPHOSPHATASE AND TRIPHOSPHATE	Delvaux, D., Herman, R., Sauvage, E., Wins, P., Bettendorff, L., Charlier, P., Kerff, F.
4BEN	R39-IMIPENEM ACYL-ENZYME CRYSTAL STRUCTURE	Van Elder, D., Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P.
3ZCZ	CRYSTAL STRUCTURE OF A COMPLEX BETWEEN ACTINOMADURA R39 DD-PEPTIDASE AND A TRIFLUOROKETONE INHIBITOR	Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P.
4B4X	CRYSTAL STRUCTURE OF A COMPLEX BETWEEN ACTINOMADURA R39 DD-PEPTIDASE AND A SULFONAMIDE BORONATE INHIBITOR	Canella, S.E., Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P.
4B4Z	CRYSTAL STRUCTURE OF A COMPLEX BETWEEN ACTINOMADURA R39 DD-PEPTIDASE AND A SULFONAMIDE BORONATE INHIBITOR	Canella, S.E., Sauvage, E., Herman, R., Kerff, F., Rocaboy, M., Charlier, P.

SYMPOSIA

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

Workshop “Next-generation Sequencing at the Poles”, University of Liège, Belgium, November 21
Main Organizers: Dr Annick Wilmotte & Dr Dail H Laughinghouse

Bioinformatic training on the analysis of NGS data, University of Liège, Belgium, November 22, 23
Main Organizers: Dr Annick Wilmotte & Dr Dail H Laughinghouse

EDUCATION

Academic Courses

Bachelor and Preparation to Masters

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

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

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

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

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

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

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

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

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.

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.

Bioinformatique et modélisation, 10h +10h TD - CHIM0625-1 - **D.Dehareng**. Master 2, finalité approfondie.

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, 20h + 20h Pr – GENE0003-1 - **M. Hanikenne**. Master 1 BBMC

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.

Microorganismes extremophiles, 25h Th - MICR1713-1 - **G. Feller**, **M. Galleni** and **A. Wilmotte**. Master 2 BBMC.

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**. May 22-24.

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

Courses given abroad

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

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.

Mécanisme d’action de la pénicilline - 3h, **J.-M. Frère**, IBS Grenoble, France, November 26

Nanobodies or Camelid Antibody Fragments: Properties and Application, 7 h – **M. Dumoulin** Department of Pharmaceutical Sciences, University of Padova, Italy, November 21-22.

Nanobodies or camelid Antibody Fragments: Properties and Application, 7 h – **M. Dumoulin** Department of Pharmaceutical Sciences, University of Udine, Italy, November 25-28.

Production de protéines recombinantes, 8 h - M.Delmarcelle. Bac 3 DUT Génie biologique. IUT de Mont de Marsan. Université de Pau et des Pays de l'Adour, France.

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., February 28.

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

Bac 3 Trainee

RITACCO Caroline

Master I Trainees

ABDELMOUMENE Chahinez
ANTOLIN URIARTE Juan Carlos
BARTHOLOME Odile
COURTOIS Justine
DI FRANCO Arnaud
DJIAHA Sylviane
ERDOGAN Tiffany
FANARA Steven
HEUKEMES Delphine
HOEBRECK Charline
KAY Jennifer
LAVERGNE Arnaud
LECLERCQ Sophie
LEGRAND David
LE PAIGE Ulric
MISTIAEN Kevin
NOIRFALISE Pauline
POLLENUS Thomas
RICH Bryan
SCHEEPERS Maxime
STAUTEMAS Jennifer
TRUSSART Charlotte
VANDEN BROECK Arnaud

Master II Students

CRASSON Oscar
Master en Biochimie et Biologie Moléculaire et Cellulaire, à finalité approfondie ULg.
Cartographie des épitopes majeurs de l'α-hémolysine de *Staphylococcus aureus.*

DAUVIN Marjorie
Master BBMC à finalité approfondie, ULg.
Etude de l'induction et du retour à l'état basal de la β-lactamase BlaP chez *Bacillus licheniformis*.

DETILLOUX Gaëtan
Master en Science Chimiques.
Elaboration de supports nanoporeux à base de chitosane pour la catalyse enzymatique et organométallique. Collaboration avec le CERM, ULg

HENRARD Nicolas	Master en Sciences de l'ingénieur industriel finalité biochimie HEPL ISIL. Quai Gloesener, 6 4020 Liège. Développement et informatisation d'un système de contrôle d'un fermenteur associé à un dosage en continu d'une enzyme extracellulaire.
JEROUNDI Najia	Master en Physiologie - Biologie Cellulaire et Moléculaire. Faculté des Sciences Ain Chock. Université Hassan II, Casablanca, Maroc. Folding de la β-lactamase BS3 de *Bacillus licheniformis*.
JOURDAN Samuel	Master BBMC à finalité approfondie, ULg. Contribution à l'étude du rôle des sucres phosphorylés dans le développement chez Streptomyces.
KUBICA Annick	Master BBMC à finalité approfondie, ULg. Recherche et caractérisation de mutants de la carence en fer chez *Chlamydomonas reinhardtii*.
LEKEUX Gilles	Master BBMC à finalité approfondie, ULg. Expression de protéines recombinantes en cellules BY2.
MARTINET Loïc	Master BBMC à finalité approfondie, ULg. Etude du rôle du fer dans l'induction du développement chez *Streptomyces coelicolor.*
MASSONET Philippe	Master en Sciences Chimiques ULg Protéolyse et allergie aux acariens: étude des spécificités des protéases allergéniques Derp 1 et Derp 9 produites par l'acarien *Dermatophagoides pteronyssinus*.
MENZER Linda	Master BBMC à finalité approfondie, ULg. Etude du mécanisme de formation des fibres amyloïdes chez le zebrafish (*Danio rerio*).
MONTIGAUD Pauline	Master en Ingénieur Agro-alimentaire. Ecole Oniris, rue de la Géraudière BP 82225 44322 Nantes cedex 3 France. Clonage, expression et caractérisation de l'arabinose isomérase avec une séquence polyhistidine en position N-terminale.
OULD AMER Yasmine	Master BBMC à finalité approfondie, ULg. Etude du potentiel de synthèse de composés secondaires bioactifs par des cyanobactéries.
RIGAUX Maylis	Master BBMC à finalité approfondie, ULg. Etude d'YkfD et d'YkfA impliqués dans le catabolisme du peptidoglycane chez *Bacillus subtilis*.

Erasmus Students - Master II

CANNELLA Sara Master II. Faculty of Pharmacy, University of Padova, Italy.
The effects of insertion of expanded polyglutamine tracts on the structure, stability and aggregation of the β-lactamase BlaP from *Bacillus licheniformis* 739/C.

COSOLO Andrea Master II. Faculty of Medecine and Chirurgy, University of Udine, Italy.
Effects of the binding of a series on VHHs on the properties of the chimeric proteins made from the β-lactamase BlaP and polyglutamine tracts.

DE FRANCO Simona Master II. Università degli studi di Napoli Federico II, Napoli, Italie. Evaluation of five monoclonal antibodies against *Staphylococcus aureus* Clumping Factor A: neutralizing effects and epitopes interactions.

FAILLACI Francesca Master en Biotecnologie per l'industria e la ricerca scientifica. Université de Palerme, Italie.
Cold adapted β-galactosidase of *Pseudoalteromonas haloplanktis*: enzyme immobilization for milk lactose hydrolysis.

GUARINO Carla Master in Molecular and Industrial Biotechnology, faculty of sciences, biotechnology. Università degli studi di Napoli Federico II, Napoli, Italie.
Cloning, expression, purification and characterization of five class I aldolases for the production of ketohexoses-1,6-bisphosphate.

Technical high schools – Bachelor III

GAUTHIER Nicolas Bachelier – Technologue de laboratoire médical (option chimie clinique). Haute école Charlemagne, CHU – B36 /Tour 4. Avenue de l'Hôpital, 1. 4000 Liège (Sart Tilman).
La transglycosylase monofonctionnelle de *Staphylococcus aureus*. Mutations, productions, purifications.

GUISSARD Valérie Bachelier – Technologue de laboratoire médical.
HELMo – Saint Laurent. Quai Mativa 38, 4020 Liège.
Etude génétique et fonctionnelle du facteur d'épissage SRSF2b au cours du développement embryonnaire de *Danio rerio*.

HANON Kevin Bachelier – Technologue de laboratoire médical (option cytologie). HEPL André Vésale. Quai du Barbou 2, 4020 Liège.
Etude des domaines II et III des PBP4a de *Bacillus subtilis* et *Bacillus amyloliquefaciens*.

HEYNEN Stéphanie	Bachelier – Technologue de laboratoire médical. Haute Ecole Charlemagne. CHU – B36 /Tour 4. Mise au point de la transformation d'*Enterococcus hirae* et étude des protéines découlant de l'opéron *ftsw-psr-pbps*.
LE DOUJET Typhaine	Licence Professionnelle en Biotechnologie, option Biologie Moléculaire appliquée à la Sécurité Alimentaire. IUT Université de Pau et des Pays de l'Adour. Mont-de-Marsan, France. Mise en place d'une méthode de mutagénèse aléatoire d'une β-galactosidase psychrophile.
LEGRAND François	Bachelier Technologue de laboratoire médical, HELMo-Saint-Laurent. Quai Mativa, 38 4020 Liège. Etude de l'utilisation d'une β-galactosidase psychrophile dans le traitement du lait.
NAWLE Anand	Génie Biologique, option industries alimentaires et biologiques. Université de Pau et des Pays de l'Adour. Mont-de-Marsan, France. Etude de la voie métabolique du tagatose de *Bacillus liheniformis*.
NOLLET Laetitia	Bachelier en Chimie, finalité Biochimie. HEPL Rennequin Sualem. Quai Gloesener, 6 4020 Liège. Clonage et production de FtsW impliquée dans la division cellulaire bactérienne.
PEREIRA Ophélie	Licence Professionnelle en Biotechnologie, option Biologie Moléculaire appliquée à la Sécurité Alimentaire. IUT Université de Pau et des Pays de l'Adour. Mont-de-Marsan, France. Étude du gène HMA4 dans l'hyperaccumulation de zinc chez *Arabidopsis halleri*.
SCHIMANSKI Andréas	Bachelier en Chimie, finalité Biochimie-Biotechnologie. HEPL Rennequin Sualem. Quai Gloesener, 6 4020 Liège Impact des changements climatiques dans les communautés de cyanobactéries en antarctique/ Caractérisation des souches d'Antarctique.
TILMAN Elodie	Bachelier Technologue de laboratoire médical, HELMo-Saint-Laurent, Liège. Mutagenèse dirigée de la L-arabinose isomérase de *Geobacillus stearothermophilus* et introduction d'un gène reporteur (GFP) dans un plasmide de production.

WELSCHEN Laure

Bachelier Technologue de laboratoire médical, option cytologie. HEPL André Vésale. Quai du Barbou 2, 4020 Liège.
Génération et analyse de mutants de la L-arabinose isomérase de *Geobacillus stearothermophilus* T6 visant à améliorer son activité vis-à-vis du galactose.

General Public Activities

On ULg.TV

Comprendre la protéine pour mieux saisir le vivant : a general presentation of the main themes of research in CIP. http://www.ulg.ac.be/cms/c_1922518/fr/comprendre-la-proteine-pour-mieux-saisir-le-vivant since February 2012

Les bactéries vaincront elles les antibiotiques ? By Prof. B. Joris
http://www.ulg.ac.be/cms/c_2535015/fr/les-bacteries-vaincront-elles-les-antibiotiques since November 2012

Activities for students of secondary schools

Journée Enseignement Secondaire « La biologie moléculaire et la biotechnologie en région liégeoise » (an organization of the 'Service de Promotion et Information sur les Etudes' de l'AEE), February 7. Participations of **Prof. P. Motte Dr A. Wilmotte, Prof. J.-M. Frère, Dr A. Brans** and **Dr F. Kerff**.

Dream Day (http://www.dream-it.be/fr/index.html), March 15.
Rencontre du **Dr A. Wilmotte** avec une classe de l'école Sainte-Marie de Huy.

Soirée Masters, March 26
Participation of **Prof. P. Motte**.

ULg Journée Portes Ouvertes, May 5
Participation of **Prof. P. Motte**.

Matinée « Portes Ouvertes » Rhétos, October 20
Participation of **Prof. P. Motte**.

Articles and interviews

Prof. André Matagne. Un partenariat public-privé pour un laboratoire universitaire.
Université Liège - http://le15ejour.ulg.ac.be - Janvier 2012 - N°210

Dr Georges Feller. Des psychrophiles à toutes les sauces.

Réflexions, le site de vulgarisation de l'Université de Liège
© Université de Liège - http://reflexions.ulg.ac.be/ April 11

Dr G. Feller. Misons sur les basses températures. FNRS news n°89, juin 2012, pp 26-27

Prof. J.-M. Frère. Les bêta-lactamases, armes bactériennes contre les antibiotiques.
Réflexions, le site de vulgarisation de l'Université de Liège
Université de Liège - http://reflexions.ulg.ac.be/ November 14

Prof. J.-M. Frère. Interview sur la résistance aux antibiotiques dans le programme « O Positif » RTBF, December 12

On RTBF3: La résistance bactérienne aux antibiotiques by **Profs. B. Joris** and **M. Galleni**.
Les Niouzz, February 6

Participation of **Dr M. Hanikenne** and **Dr A. Wilmotte** (workshop on cyanobacteria: microscopy and extraction of pigments) to the:
Plant Fascination Day,
Observatoire du Monde des Plantes ULg, May 18

Scientific blog

Dr A. Wilmotte, editor of the scientific blog BELDIVA: www.antarcticabelgium.blogspot.com

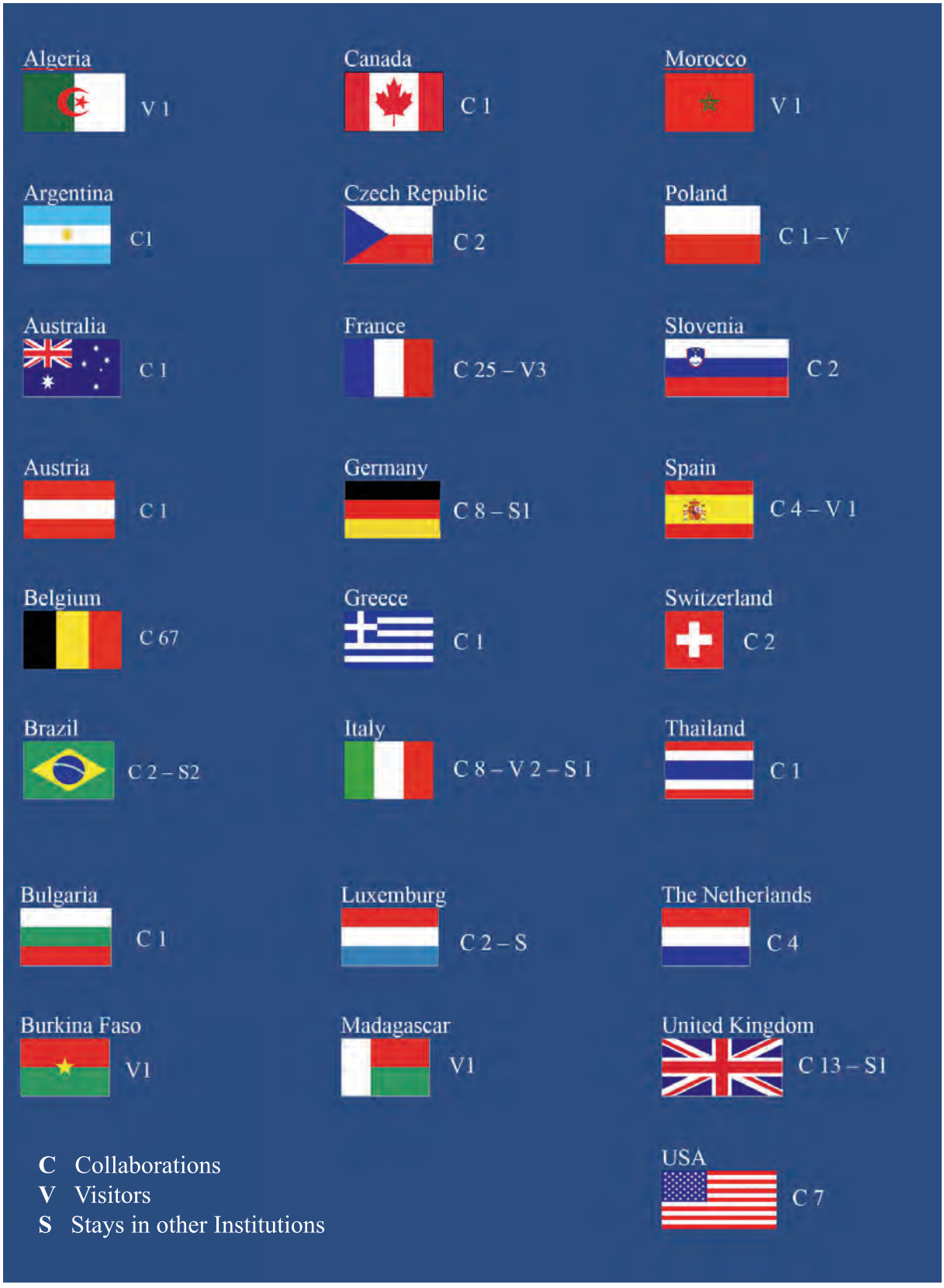
Wide audience conferences

Prof. J.-M. Frère : Résistance aux antibiotiques.
Collège Belgique, Royal Belgian Academy of Sciences, Namur, November 21 and Bruxelles, December 12.

Prof. J. Coyette: Mode d'action des antibiotiques. Structure et synthèse de la paroi bactérienne.
Collège Belgique, Royal Belgian Academy of Sciences, Bruxelles, November 21.

Dr C. Duez: Biofilms et résistance.
Collège Belgique, Royal Belgian Academy of Sciences, Bruxelles, December 12.

INTERNATIONAL EXCHANGES



Postdocs In

Dr Meriem El ghachi

Research group: Biological Macromolecule Crystallography
Group leader: Dr F. Kerff

My name is Meriem El ghachi and I am post-doc. During my PhD in the laboratory of «Enveloppes bactériennes et Antibiotiques» (in the University Paris sud France) I worked on the metabolism of undecaprenyl-phosphate a key lipid intermediate involved in the synthesis of various bacterial cell wall polymers such as the peptidoglycan, lipopolysaccharides, and teichoic acids. The metabolism of this lipid was not known at the time. I identified the proteins required for the dephosphorylation of undecaprenyl-pyrophosphate into undecaprenyl phosphate. In *E. coli* this dephosphorylation involves four integral membrane proteins, BacA, and three members of the type 2 phosphatidic acid phosphatase family (PAP2): PGPB, YbjG and YeiU. Characterizing these proteins required the combination of different methods, fully taking advantage of the different experts in chemistry, genetic and biochemistry working in the lab. Then I did a short post-doc in the lab to find the target of the Colicin M, a toxin secreted by some *E. coli* strain.

In 2006, I moved to Dr Ivo Boneca's lab at the Pasteur institute in Paris but stayed in peptidoglycan biosynthesis field. The goal of my research was to investigate the determinants of cell shape of the helical bacterium, *Helicobacter pylori*, which is known to provoke stomach cancer and ulcers. I focused on the further characterization of the interaction between the proteins MreC and PBP2 involved in the elongation of bacteria using different *in vivo* and *in vitro* approaches (notably surface plasmon resonance and ultratcentifugation). We collaborate also with Dr Andrea Dessen for SAXS experiments. Moreover the minimal interacting domain of MreC required for interaction was defined and its specific expression in trans provoked the loss viability of *H. pylori*. Hence the surface of the complex could constitute a new target for the development of new kind of inhibitors. During this postdoc, I worked on a zinc metalloprotease, involved in both the helical form of *H. pylori* and cell division. Pasteur institute was a great place to open my mind on other fields such as neurology or immunology.

In 2012, I joined Dr. Frederic Kerff's lab to work again on my favorite proteins: the undecaprenyl pyrophospahate phosphatases. This time, the aim is to determine the structure of members of these two families of proteins from *E. coli*, *B. subtilis* and *H. pylori* in order to help identify their catalytic mechanism and their substrate specificity. This project is challenging but we have already purified several of these membrane proteins with a good yield and screened numerous detergents to solubilize them and get the smallest and most homogenous micelles. This work will be undertaken in close collaboration with the laboratory of "Enveloppes Bactériennes et Antibiotiques."

So I am here to learn crystallography! And the CIP is one of the best places to pursue my exploration of the peptidoglycan synthesis mechanism.

Dr Dail Laughinghouse IV

Research group: Bacterial diversity, physiology and genetics
Group leader: Dr Annick Wilmotte

My name is Dail Laughinghouse, and I am a 'mix' from Brazil and the USA working in the Lab of Cyanobacteria at the Center for Protein Engineering, University of Liege with a post-doctoral fellowship from FNRS since August 2012 working on the biogeography and molecular diversity of cyanobacteria of the Arctic and Antarctic.

A little about my background…I studied Biological Sciences in Brazil and dabbled around with paleontology, polymorphisms of the DRD2 receptor of alcoholics, and vascular plants before focusing more on cyanobacteria and algae. However I still work on medicinal plants in my spare time with collaborators in Brazil. Thus, in Brazil, my main work focused on the systematics of bloom-forming cyanobacteria, as they are known for noxious blooms and there is a well-known case where almost a 100 people died in northeastern Brazil. However, I had worked on algae from rocks, trees, tadpole and fish intestines, plankton, walls, soils, etc.

I left Brazil for graduate studies in the USA (Ohio) to continue on the molecular biological aspects/genetics of cyanobacteria focusing on 16s rRNA and ITS at the time, including secondary structure. Here the cyanobacteria came from deserts and soil crusts, thus more like something I would return to in my post-doc. These studies were used to differentiate taxa at the specific-level, since many species are morphologically poor.

I changed areas a little for my PhD. I carried out my PhD at the University of Maryland while being contracted for the Dept of Botany, NMNH, Smithsonian Institution in Washington, DC to work on cyanobacteria and algae from freshwater to marine systems. Here I worked on systematics of tropical cyanobacteria and algae, as well as the use of algal turf scrubbers for water remediation, then conversion of the algal biomass into useful by-products…biofuels, fertilizers, nutraceutical products, etc.

Currently, I still collaborate with the Smithsonian working on the Historic collections they maintain on cyanobacteria from expeditions from the late 1800's to early 1900's…trying to bring them back to life and extract DNA, and how we should safeguard current expeditions so that people in the future can use our material with novel technologies in 100-200+ years.

So why change from the tropics for the poles? That is a good question. I still don't know the answer and why I did this. I miss working in the tropics, when I would be snorkeling the coral reefs in a speedo and sleeping among the mangroves on a small Caye in Belize. Now I am under kilos of clothing in the poles. However, after one spends time working in the poles, we understand the great importance that these studies have on a global scale.

Dr Badrish Soni

Research group: Bacterial diversity, physiology and genetics
Group leader: Dr Mohammed Terrak

I did PhD in Microbiology (February 2003 – February 2008) on "Cyanobacterial phycobiliproteins: production, purification, crystallization, structure determination and application" with Prof. Datta Madamwar and Dr. Ujjval Trivedi from Sardar Patel University, India.

I did my first post-doc research (June 2008 – May 2010) to work on "Role of *pchR* transcriptional regulators in tolerance to metal and oxidative stresses in *Synechocystis* sp. PCC 6803" and "Expression of yeast ethanol genes in *Synechocystis* sp. PCC 6803 for the production of solar ethanol" with Dr Franck Chauvat, Integrative Biology Laboratory, Centre of Atomic Energy (CEA), Saclay, France. Then after I did my next post-doc (August 2010 – May 2012) on "Cyclic AMP signalling and the control of infection in the Medicago symbiont *Sinorhizobium meliloti*" with Dr Jacques Batut, Laboratory Plant-Microorganisms Interactions, INRA, Toulouse, France.

In June 2012, I joined the group of Dr Mohammed Terrak to work on "Expression and purification of bacterial membrane proteins". My main focus was to clone, express and purify FtsW and RodA proteins from several organisms. The Spr protein was expressed, purified and crystal screens were made.
The CIP has been a very memorable place for me not just because of extremely helpful colleagues and positive working environment, but also for personal developments - as my son "Om" was born on July 1, 2012. I enjoyed working with the joyful working culture in the CIP and shall miss all the Friday evening "Trappist Club" and belgian beers!

COLLABORATIONS

ARGENTINA

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

AUSTRALIA

University of New South Wales – School of Biotechnology and Biomolecular Sciences – **R. Cavicchioli & K.S. Siddiqui**

University of Queensland, Brisbane – Australian Institute for Bioengineering and Nanotechnology AIBN – **E. Marcellin & C. Licona-Cassani**.

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 & G. Roussel**

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**

Rousselot – Ghent – **F. Meersman**

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

UCL – de Duve Institute – Bruxelles – **J.F. Collet**

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 - Laboratoire de Bactériologie Moléculaire – Bruxelles – **A. Allaoui**

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

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

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 Biologie, Ecologie et Evolution – Morphologie ultrastructurale – Liège – **P. Compère**

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 – **N. Willet & 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 – Laboratoire de pharmacognosie – Liège – **M. Frédérich**

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 Neurosciences – Biologie cellulaire et tissulaire – Liège – **M. Thiry**

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 – **S. Muyldermans, E. Pardon, L. Wyns & J. Steyaert**

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

BRAZIL

University of Sao Paulo – **M. Fiore**

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

BULGARIA

Stefan Angeloff Institute of Microbiology – Sofia – **M. Angelova**

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

Aix Marseille Université – Laboratoire de Chimie bactérienne – Marseille – **M. Foglino**

CEA – Institut de Recherche Technologique et des Sciences du Vivant, Laboratoire de Chimie et Biologie des Métaux – **V. Forge & C. Marquette**

CEA Saclay - Laboratoire Léon Brillouin – Gif-sur-Yvette – **S. Longeville**

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 – Unité de Biologie et Génétique de la Paroi bactérienne – Paris – **I. Gomperts-Boneca**

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

Montpellier SupAgro – Montpellier – **M. Nigen**

Nano-H S.A.S. – Lyon – **C. Louis**

Université de Bretagne Occidentale – Brest – **M. Le Romancer**

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

Université Claude Bernard Lyon 1 – Laboratoire de Physico-Chimie des Matériaux Luminescents – Lyon – **O. Tillement & F. Lux**

Université Denis Diderot, Paris VII – Laboratoire ITODYS – Paris - **F. Maurel**

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 de Chimie Physique – Orsay – **M. Desouter**

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 – **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

International School for Advanced Studies – Trieste – **P. Calligari**

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**

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**

University of Udine – Department of Biomedical Science and Technology – Udine – **G. Esposito**

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, C. Hilger & A. Kuehn**

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 Granada – Química Física – **F. Conejero-Lara**

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

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

SWITZERLAND

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

Université de Genève – Faculté des Sciences Pharmaceutiques – Genève – **E. Alleman**

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 College of London – Department of Chemistry – **F. Meersman**

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

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

University of Oxford – Department of Chemistry – Oxford – **L.J. Smith**

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

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

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 Florida – Center for Heterocyclic Chemistry – Gainesville – **A. Katritzky**

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

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

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

VISITORS

Da Lage Jean Luc, CRNS Gif sur Yvette, France, October 23-25

Esposito Gennaro, University of Udine, Italy, February 5-8

Joncour-Génicot Sabine, CNRS Roscoff, France, February 10-17

Padiolleau Séverine, Université de Technologie de Compiègne, France, February 13-14

Perez Llarena Francisco José, CHU A Coruña, Spain, October 17 – January 20

Polverino de Laureto Patrizia, University of Padova, Italy, February 4-9

Lahiani Sadja, UMBB, Boumerdes, Algeria, June 1 – September 28

Randrianarivo Hanitra Ranjàna, University of Antananarivo, Madagascar, November 25 – December 25

Rhazi Filali Fouzia, University of Meknès, Morocco, September 22-29

Waleron Krzysztof and **Malgorzata**, University of Gdansk, Poland, January 2-8, August 15-25

Zeba Boukare, June 1 – July 15

Stays in other Institutions

Calusinska Magdalena, Sao Paulo University, Brazil, July 9-22

Campisi Vincent, Centre de Recherche Public de la Santé, Luxemburg, Luxemburg, July 9-August 10

Amandine Godin, University of Bayreuth, Germany, January 15-21

Matagne André, Department of Biochemistry, University of Oxford, UK, May 17-20

Pain Coralie, CRIBI, University of Padova, Italy, January 1- February 29

Wilmotte Annick, Sao Paulo University, Brazil, October 20 - November 2

FUNDING

FUNDING

Les Pôles d'Attraction Interuniversitaires

PAI P7/44 (2012-2017) - Integrative Protein Science: from small molecules to complex biological systems (the CIP is the Coordinator)

Politique Scientifique Fédérale Belge

BELSPO SD/BA/01A (2012-2016) - CCAMBIO: Antarctic Microbial Diversity and Climate Change
Mandats de doctorant : Igor Stelmach Pessi (CCAMBIO)
BCCM/ULC Culture Collection of (sub) polar cyanobacteria

Les Actions de Recherche Concertées

ARC 07/12-04 (2007-2012) – MICRO-H2: Microbiological production of hydrogen: study of microalgal and bacterial processes
ARC-SF 12/16-04 (2012-2015)– NetRBI : Modelling of the Network Regulating *Bacillus licheniformis* BlaP β-Lactamase Induction

Fonds de la Recherche Scientifique - FNRS

Projets de recherche
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
F.4518.12- Mandat d'Impulsion Scientifique (2012-2013) Structural study of the undecaprenyl pyrophosphate phosphatases involved in the metabolism of the lipid carrier required for the biosynthesis of the bacterial cell-wall carbohydrate polymers
Coopération bilatérale FNRS/CONICET (Argentina) (2011-2013) Characterization of new beta-lactamases produced by clinical and environmental bacteria
Coopération bilatérale FNRS/CNPq – Brésil (2011-2012) Diversity and functional activity of cyanobacteria in the South Shetlands Archipelago, Antarctica

Mandats de recherche
Mandats de doctorants FRIA : Anthony Argüelles, Vincent Campisi, Jean-Benoît Charlier, Chloé Chavignon, Matthias Craig, Oscar Crasson , Maud Delsaute, Janice Dumont, Régine Freichels, Amandine Godin, Julie Herman, Céline Huynen, Marine Joris, Stéphany Lambert, Clémentine Laurent, Sarah Lebrun, Marta Maciejewska, Marie-José Mano, Maxime Maréchal, Coralie Pain, Florence Piette, Delphine Pipers, Mathieu Rocaboy, Nancy Stankovic, Caroline Struvay, Elodie Tenconi, Edwige Van der Heiden.
Mandat de doctorant Télévie : S. Laurent
Mandats d'aspirant FRS/FNRS : S. Jourdan, C. Laurent et C. Struvay

Mandats FNRS de Chercheur Temporaire Postdoctoral:
- Dr Arabela Cuirolo (08/04/2011 au 07/04/2013), avenant à la convention FRFC 2.4588.11
- Dr David Thorn (02/05/2011 au 30/04/2013), convention MIS F.4505.11
- Dr Meriem El Ghachi (11/06/2012 au 04/04/2014, convention MIS F.4518.12
- Dr Badrish Soni (04/06/2012 au 30/04/2013), convention FRFC 2.4543.12
- Dr Dail Laughinghouse IV (01/08/2012 au 30/06/2014), convention FRFC 2.4570.09

Fonds de la Recherche Fondamentale Collective

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 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*"
FRFC 2.4581.12 (2012-2013) - Ultracentrifuge.
FRFC 2.4543.12 (2012-2015) - Etude de la polymérisation du peptidoglycane de la paroi bactérienne.

Région Wallonne

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 (2010-2014) – MEDATR : Dosage en temps réel de substances pharmacologiques dans des fluides physiologiques (avenant)
SPW-DGA Projet D31-1253 (2011-2013) "Développement d'une technologie de production de lait délactosé"

SPW-DGA XXX (2012-2014) : Détection des mammites bovines à *S. aureus* en vue de minimiser son impact socio-économique chez l'éleveur, la chaîne de transformation du lait et le consommateur.
Partenariat-Public-Privé (P.P.P.) n°1117354 (2011-2013) RECOINS – Renaturation in vitro de protéines recombinantes produites dans *Escherichia coli*, après solubilisation des corps d'inclusion. Stabilisation des protéines au cours de la purification et du stockage à long terme.

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/)
COST Action ES1105 : Cyanobacteria booms and toxins in water resources : Occurrence, impacts and management (CYANOCOST)
CEE-Bio-NMR n° BIO-NMR-00081 (2012). Cellular regulation: beta-lactamase induction and bacterial penicillin stress.
Dia-AMYL (EuroNanoMed) Amyloid peptide grafted to GD-nanoparticles for amyloidosis diagnosis (2012-2015)

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*.

Projet SFRD-12/03 (2012-2014) – Dr M. Hanikenne

Rôle de la méthylation de l'ADN dans l'adaptation locale chez la plante hyperaccumulatrice de métaux *Arabidopsis halleri*.

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

Projet FSRC-12/38 (2012-2014) – Prof. P. Charlier

Cristallisation et analyse des cristaux de protéines à haut débit.

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-)

Member of the Evaluation Committee

Prof. André Matagne, Member of the evaluation Committee for the Picardie Region, "Santé, vivant" (2012-)

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 (Board member)
National committee of Biophysics (President)
F.R.S.-FNRS Contact group on Structural Biology (President)
Graduate school (F.R.S.-FNRS) on Structure and Function of Biological Macromolecules, Bioinformatics and Modelling (SFMBBM) (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
Moreno Galleni

Current Executive Committee:
Paulette Charlier, Moreno Galleni, Marc Hanikenne, André Matagne

Current Managing Committee:
Alain Brans, Paulette Charlier, Dominique Dehareng, Georges Dive, Colette Duez, Mireille Dumoulin, Georges Feller, Moreno Galleni, Colette Goffin, Marc Hanikenne, Bernard Joris, Frédéric Kerff, André Matagne, Patrick Motte, Sébastien Rigali, Mohammed Terrak, Annick Wilmotte

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

Administrative Staff

Paola Catanzaro (Executive secretary)
Stéphanie Hanson (Administrative 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
M. Fabrice Bouillenne
Dr Magdalena Calusinska
Dr Arabela Cuirolo
Dr Dominique Dehareng
Dr Michael Delmarcelle
Dr Adeline Derouaux
Dr Meriem El Gachi
Dr Dail Laughinghouse IV
Dr Serge Leimanis
Dr Paola Mercuri
Dr Caroline Montagner
Dr Cécile Nouet
Dr Noureddine Rhazi
Dr Eric Sauvage
Dr Badrish Soni
M. Patrick Stefanic
Dr David Thorn
Dr Julie Vandenameele
M. Olivier Verlaine

PhD students

Anthony Argüelles Arias
Roya Barumandzadeh
Stéphane Baurin
Stéphanie Berzigotti
Ahlem Bouaziz
Vincent Campisi
Jean-Benoît Charlier
Chloé Chavignon
Alexandre Cipolla
Matthias Craig
Oscar Crasson
Ismahene Dahmane
Sébastien Dandois
Marjorie Dauvin
Pedro De Carvalho Maalouf
Simona De Franco
Morgane Dehousse
François Delbrassine
Maud Delsaute
Nicolas Dony
Janice Dumont
Anne Famerie
Adriana Fernea
Régine Freichels
Amandine Godin
Jessica Guillerm
Julie Herman
Céline Huynen
Adrien Jehaes
Najia Jeroundi
Marine Joris
Samuel Jourdan
Stéphany Lambert
Yannick Lara
Clémentine Laurent
Sophie Laurent
Sarah Lebrun
Marta Maciejwska
Marie-José Mano
Maxime Maréchal
Linda Menzer
Coralie Pain
Anne Pennartz
Mathieu Rocaboy
Frédéric Roulling
Natacha Scarafone
Jean-Sébastien Sohier
Nancy Stankovic
Igor Stelmach Pessi
Caroline Struvay
Elodie Tenconi
Edwige Van der Heiden

Technical assistance

Caroline Bortuzzo
Astrid Freichels
Gilles Gaspard
Nicole Gérardin-Otthiers
Nicolas Henrard
Raphaël Herman
Juliana Kozarova
Alexandre Lambion
Stéphane Preumont
Marine Renard
Patricia Simon
Patrick Zirbes