



## GIGA Annual Report

Interdisciplinary Cluster in Applied Genoproteomics

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09

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# GIGA

## Annual Report 2009





Within our scientific landscape, GIGA, without question, occupies a very prominent place. To my knowledge, it is the only example of full integration within the scientific and technological scheme. It should be appreciated for its presence within the University of Liège as a structure that values research, building on technological transfer, company presence and the availability of an incubator. The list of subjects treated in the different research units is impressive, from Cellular and Molecular Biology to Genetics, Immunology and Neurobiology.

To lead such an enterprise, a multidisciplinary approach is necessary, but also efficient shared technical methods similar to the technology platforms open to academics and the private sector. These two necessities are the reality for GIGA.

An obvious sign of GIGA's vitality and excellence is the considerable number of research projects and scientific publications that it generates. The future of universities, research and scientific excellence evidently lies in European collaboration. Thus, the implementation of a Euroregional bioinformatics platform, in which GIGA participates, constitutes a significant advance in terms of dissemination and management of knowledge.

Finally, today there is a huge, sometimes insurmountable distance between the academic and economic worlds. Companies are already in place on the GIGA site and others are invited to join them in the future. Everything points towards excellence which is already GIGA's finest calling card.

Jean-Claude Marcourt

Minister of Higher Education

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We are in a golden age of biological research. Never before have we seen such a rapid development in technology for screening the genome of any species, or analysing their proteomes or studying complex biological processes. We have good reason to believe that major scientific breakthroughs in many areas of biology and medicine will happen in the coming ten to twenty years as the research tools we are using become sharper and sharper. But it is also a challenging time since one can make a major investment in an instrument that becomes outdated in 12 months. And how should one be able to compete with major research institutes that have many more resources and 20 next-generation sequencers rather than one or two?

Another challenge is how to create a sufficiently strong research environment that can attract the most talented young scientists who will become the future research leaders.

GIGA has been formed to tackle these challenges. By having a combination of strong research programs that can cross-fertilize with each other and well-integrated technological platforms, GIGA constitutes a research organisation that has the potential to become world leading. The grand challenge for the GIGA research leaders and their competent staff, at all levels, is to take advantage of the emerging opportunities to address unsolved biological and medical questions of importance.

*I am confident that you will make it and I therefore foresee a bright future for GIGA and its researchers.*



*Leif Andersson*

Leif Andersson  
Uppsala University, Sweden

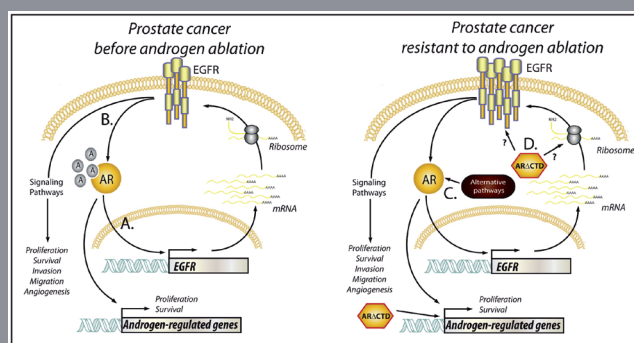
Among major causes for premature death of people living in industrialized countries, cancers represent a foremost public health problem. Because the chance of surviving an advanced cancer remains very low, there is an urgent need to develop new effective ways to detect, evaluate and treat cancers. The mission of the 175 members of GIGA-Cancer is to conduct research aiming at a better understanding of the molecular and cellular mechanisms involved in the development of malignant diseases but also allowing the cancer cells to invade and metastasize. Particular domains of expertise of GIGA-cancer are angiogenesis and lymphangiogenesis associated with the progression of cancer. The discovery and characterization of innovative biomarkers that could be used for early detection and targeted therapies is also a priority of GIGA-Cancer. Thanks to its proximity to the University hospital (CHU), GIGA-Cancer has launched several translational research programs in close collaboration with clinicians with the objective of effectively bridging basic research to patients. GIGA-Cancer is actually composed of nine groups that work synergically and share materials, expertise and ideas with the aim of becoming a center of excellence in the field of cancer research.

## HIGHLIGHT

### ANDROGEN RECEPTOR CONTROLS EGFR AND ERBB2 GENE EXPRESSION AT DIFFERENT LEVELS IN PROSTATE CANCER CELL LINES

*Cancer Res. 2009 Apr 1;69(7):2941-9*

EGFR or ERBB2 contributes to prostate cancer (PCa) progression by activating the androgen receptor (AR) in hormone-poor conditions. Here, we investigated the mechanisms by which androgens regulate EGFR and ERBB2 expression in PCa cells. In steroid-depleted medium (SDM), EGFR protein was less abundant in androgen-sensitive LNCaP than in androgen ablation-resistant 22Rv1 cells, whereas transcript levels were similar. Dihydrotestosterone (DHT) treatment increased both EGFR mRNA and protein levels and stimulated RNA polymerase II recruitment to the EGFR gene promoter, whereas it decreased ERBB2 transcript and protein levels in LNCaP cells. DHT altered neither EGFR or ERBB2 levels nor the abundance of prostate-specific antigen (PSA), TMEPA1, or TMPRSS2 mRNAs in 22Rv1 cells, which express the full-length and a shorter AR isoform deleted from the COOH-terminal domain (AR#CTD). The contribution of both AR isoforms to the expression of these genes was assessed by small interfering RNAs targeting only the full-length or both AR isoforms. Silencing of both isoforms strongly reduced PSA, TMEPA1, and TMPRSS2 transcript levels. Inhibition of both AR isoforms did not affect EGFR and ERBB2 transcript levels but decreased EGFR and increased ERBB2 protein levels. Proliferation of 22Rv1 cells in SDM was inhibited in the absence of AR and AR#CTD. A further decrease was obtained with PKI166, an EGFR/ERBB2 kinase inhibitor. Overall, we showed that AR#CTD is responsible for constitutive EGFR expression and ERBB2 repression in 22Rv1 cells and that AR#CTD and tyrosine kinase receptors are necessary for sustained 22Rv1 cell growth.



Jean-Christophe Pignon,<sup>1</sup> Benjamin Koopmansch,<sup>1</sup> Gregory Nolens,<sup>1</sup> Laurence Delacroix,<sup>3</sup> David Waltregny,<sup>2</sup> and Rosita Winkler<sup>1</sup>  
*Cancer Res. 2009 Apr 1;69(7):2941-9.*

<sup>1</sup> Laboratory of Molecular Oncology and <sup>2</sup> Department of Urology, GIGA-Cancer, CRCE, University of Liege, Liege, Belgium; and

<sup>3</sup> Institute of Genetics and Molecular and Cellular Biology, Illkirch, France

## LABORATORY OF CELLULAR AND MOLECULAR EPIGENETICS

The LCME aims at understanding the basic mechanisms of cancer and investigating novel therapeutic approaches. The expertise of the laboratory includes molecular and cellular biology techniques, animal models of cancer and the set up of clinical trials in collaboration with different hospitals. The current research topics concern leukemia (HTLV associated adult T-cell leukemia ATLL, chronic lymphocytic leukemia CLL and bovine leukemia BLV) and thoracic cancers (non-small cell lung cancer NSCLC, small cell lung cancer SCLC and mesothelioma MPM). The following projects are currently ongoing: (1) design, synthesis and evaluation of novel epigenetic inhibitors for cancer therapy, (2) gene activation therapy of cancer, (3) checkpoint therapy of ATLL, (4) epigenetic regulation of angiogenesis, (5) viral glycomics and (6) genomic markers for NSCLC response to therapy.

### MECHANISMS OF THORACIC CANCERS: EPIGENETIC THERAPY AND IDENTIFICATION OF BIOMARKERS

Lung cancer is the number one cause of cancer-related death worldwide, with more than 1 million deaths per year. Malignant pleural mesothelioma (MPM) is a neoplastic disease of the pleura strongly associated with exposure to asbestos fibers. Present chemotherapeutic regimens are marginally efficient, tumor cells being particularly resistant to radiotherapy and/or chemotherapy. We hypothesized that unresponsiveness of tumors to conventional therapeutic agents is due to inappropriate gene expression resulting from epigenetic modifications and leading to transcriptional silencing. We have demonstrated that valproate, an HDAC (histone deacetylase) inhibitor, improves efficacy of standard treatments of different thoracic cancers: MPM, non small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Ongoing projects include (1) development of targeted therapies of circulating tumor cells in NSCLC patients to reduce occurrence of metastases, (2) design of novel epigenetic inhibitors interfering concomitantly with HDAC and DNMT (DNA methyltransferase) activities and (3) identification of biomarkers for response to chemotherapy of non-small cell lung cancer.

Amélie Maincent, Chrisostome Costa, Roland Hubaux, Fabian Vandermeers, Pascale Hubert, Philippe Delvenne, Renaud Louis, Lionel Bosquée, Bernard Duysinx and Luc Willems

Collaborators : Didier Allaer, Thierry Berghmans, Jean-Paul Sculier (Belgium)

### EPIGENETIC REGULATION OF ANGIOGENESIS

Several studies have shown that histone deacetylases (HDAC) and DNA methyltransferases (DNMT) inhibitors are potent antiangiogenic compounds. Though combination of HDAC and DNMT inhibitors are now being examined in clinical trials of hematological malignancies, very little work has been done to understand the effect of this combination on normal and tumoral angiogenesis. We have designed and tested a family of twin drugs with intrinsic HDAC and DNMT inhibitory activities in relevant models of angiogenesis *in vitro* (cultures of endothelial cells and pericytes, 3D aortic ring assay) and *in vivo* (chick chorioallantoic membrane assay). We have identified a lead compound having a quantifiable antiangiogenic effect without cytotoxicity associated with increased global acetylation and decreased DNA methylation levels. This compound is presently used to develop effective approaches to treat cancer by modulating the process of angiogenesis.

Luc Willems, T.V. Shiva Shankar, Béatrice Sulka, Catherine Maillard, Silvia Blacher, Agnès Noël, Akeila Bellahcene, Vincent Castronovo  
Collaborators : Didier Lambert, Johan Wouters (Belgium)

### IMPROVEMENT OF CHRONIC LYMPHOCYTIC LEUKEMIA THERAPY

Abnormal accumulation of leukemic B-lymphocytes results from an alteration of different parameters that include cell proliferation and death, as well as migration to lymphoid tissues. Using a pulse-chase approach based on the incorporation of deuterium from deuterated-glucose into DNA, we demonstrated that B-cell turnover is reduced in CLL subjects. Activation of these quiescent leukemic cells

restores sensitivity to fludarabine in B cells from poor prognosis CLL patients who became resistant to chemotherapy.

Luc Willems, Amel Bouzar, Julien Defoiche

Collaborators : Dominique Bron, Christian Chatelain, Eric Van den Neste (Belgium)

#### MECHANISMS AND NOVEL THERAPIES OF DELTARETROVIRUS INDUCED DISEASES

It is estimated that 20 million are infected with human T-lymphotropic virus type 1 (HTLV-1) worldwide. Among them, 2-5% will develop one of the major HTLV-1 associated diseases (ATLL Adult T cell leukemia/lymphoma or HAM/TSP HTLV-associated myelopathy/Tropical Spastic Paraparesis). Bovine leukemia virus (BLV) is a closely related  $\delta$ -retrovirus infecting B lymphocytes and inducing leukemia/lymphoma in cattle. A major feature of the infection induced by both viruses is transcriptional silencing which allows proviruses to escape recognition and destruction by the host immune response. We have evaluated the effect of a novel therapeutic strategy based on transient activation of viral expression using epigenetic modulators, thereby exposing infected cells to the immune response and resulting in significant reductions in proviral loads. We have also studied another therapeutic approach includes the ability of checkpoint inhibitors in controlling HTLV-1 infection and preventing malignant disease. We have characterized the fundamental mechanisms pertaining to these therapies, which offer an option for lymphoma and relapsing acute ATLL patients.

Mathieu Boxus, Alix de Brogniez, Arnaud Florins, Carole François, Sabrina Rodriguez and Luc Willems

#### THE ROLE OF GLYCOSYLATION IN DELTARETROVIRUS INFECTION

Bovine Leukemia Virus (BLV) is an oncogenic retrovirus which is closely related to human T-cell leukemia virus types 1 and 2 (HTLV-1 and -2). In most cases, BLV infection remains clinically silent and only one-third of infected cattle develop a persistent lymphocytosis (PL). Our project aims to study the implication of the viral glycome in viral persistence and pathogenesis. In particular, we hypothesized that binding of carbohydrates to viral envelope proteins may form a «glycan shield» conferring protection against the host immune response. Ongoing experiments investigate (1) the ability of glycosylation inhibitors to interfere with viral infectivity, (2) the pathogenicity of glycosylation mutants and (3) the molecular pathways of viral entry.

Luc Willems, Amel Bouzar, Fanny Boulanger, Pierre Leprince, Catherine Sadzot-Delvaux, Jacques Piette

Collaborators : Laurent Gillet, Alain Vanderplasschen (Belgium)

#### LABORATORY OF BIOLOGY OF TUMOR AND DEVELOPMENT

We are investigating the cellular and molecular mechanisms involved in complex tissue remodeling and abnormal vascularization associated with various pathologies such as cancer growth, metastatic dissemination, pre-eclampsia, endometriosis and ocular disease (corneal graft rejection and age-related degeneration). The LBTD members mainly focus their interest on two types of mediators: angiogenic factors of the VEGF family and proteases (serine proteases and metalloproteases or MMP, ADAM and ADAMTS). Although protease function was initially viewed as the capacity to degrade extracellular matrixes and facilitate cell migration, emerging data have extended their contribution to the regulation of growth factor bioavailability, as well as the activation or inactivation of cell surface receptors or cytokine/chemokines.

#### ANGIOGENESIS IN ENDOCRINO-SENSITIVE ORGANS

The use of a progestive intra-uterine contraceptive system is commonly associated with vaginal bleeding and spotting (punctual small bleeding). LBTD has developed an *in vivo* model mimicking the effects of progestive treatments in women. This model has allowed us to study the neovascularization



in detail in order to understand the mechanisms of bleedings. LBTB has also pointed out a differential expression of MMPs in the endometrium of women treated with this contraceptive system compared to control women.

Maria-Luz Alvarez, Céline Gérard, Soraya Labied, Géraldine Brichant, Cédric Balsat, Mélanie Mestdagt, Carine Munaut, Silvia Blacher, Michelle Nisolle

### TUMORAL ANGIOGENESIS AND LYMPHANGIOGENESIS

Angiogenesis and lymphangiogenesis contribute to metastatic dissemination through blood and lymphatic vessels. Angiogenesis not only relies on the recruitment of blood endothelial cells, but also on neo-formed vessel stabilization through the vessel coverage by mural cells or pericytes. LBTB has pinpointed the unexpected contribution of several new mediators of the complex dialogue occurring between endothelial cells and mural cells: 1) MT4-MMP, a membrane type MMP produced by metastatic breast adenocarcinoma cells; (2) sVEGFR-1 and sVEGFR-2, the soluble forms of VEGF receptors overproduced in several pathologies such as pre-eclampsia and cancer; (3) hCG produced by the highly vascularized choriocarcinomas.

Nor-Eddine Sounni, Alexandra Paye, Lorin Host, Christel Pequeux, Carine Munaut, Erik Maquoi, Catherine Gèneux, Delphine As-sent, Julien Detilleux, Emily Gengoux, Sarah Berndt, Silvia Blacher, Frédéric Chantaine  
Collaborators : Sophie Perrier d'Hauterive, Vincent Geenen (Belgium)

### MIGRATION INHIBITORY FACTOR (MIF)

Glioblastomas GBMs are the most frequent primitive cerebral tumour in adults. Migration inhibitory factor (MIF) is one of the most upregulated transcripts in GBMs. The LBTB has studied the interplay between MIF and glucocorticoids which are routinely used in the treatment of these highly vascularized and aggressive tumors. It appeared that MIF and glucocorticoids exert opposite effects in GBMs through the ERK1/2 MAPK pathway. The finding that specific MIF inhibitors increase the glioma cell response to glucocorticoids is of interest. Altogether these results indicate an intricate pathway between MIF expression and glucocorticoid resistance. They suggest that MIF inhibitors could increase the response of GBMs to corticotherapy.

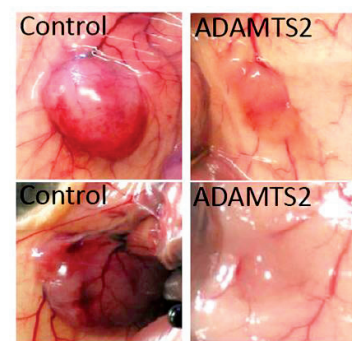
Caroline Piette, Carine Munaut  
Collaborator : Manuel Deprez (Belgium)

## LABORATORY OF CONNECTIVE TISSUES BIOLOGY

Since its creation in the sixties, the main research interest of the LCTB has been in the field of the biology of connective tissues. This includes the biochemical and molecular characterization of the main extracellular matrix (ECM) components and of the enzymes regulating their deposition and remodeling, the study of cell-ECM interactions and determination of the reciprocal regulations operated by mechanical forces developed by cells and issued from the ECM.

### REGULATION OF ANGIOGENESIS

ADAMTS-2 was the first aminoprocollagen peptidase to be cloned and characterized. The presence in ADAMTS-2 of domains known to be involved in the regulation of cell-matrix interactions prompted the LCTB to further investigate its implications during angiogenesis and pathological tissue remodeling. ADAMTS-2, used at nanomolar concentrations, reduces the attachment, spreading and proliferation of endothelial cells. It also induces their retraction and detachment from the substrate resulting in apoptosis, whereas fibroblasts and HSMC are



*ADAMTS-2 inhibits angiogenesis and tumor growth in nude mice*

not affected. Dephosphorylation of Erk1/2 and MLC largely precedes the ADAMTS-2 induced morphological alterations. In 3-D culture models, ADAMTS-2 does not alter the initial steps of formation of capillary-like structures by endothelial cells but strongly reduces their branching and long term maintenance in culture. In nude mice, growth and vascularization of tumors formed by 293-EBNA cells expressing ADAMTS-2 are drastically reduced. A similar tumor growth inhibition is also observed when using cells expressing recombinant forms of ADAMTS-2 lacking the metalloproteinase domain or deprived of some specific sequences, identifying domains that may be responsible for the observed effects. Preliminary observations point to nucleolin as a potential cell surface receptor mediating the antiangiogenic properties of ADAMTS-2.

Johanne Dubail, Christophe Deroanne, Alain Colige

## LABORATORY OF EXPERIMENTAL PATHOLOGY

Our research team has accumulated a large expertise in the diagnosis/prognosis of cancers and in the fundamental knowledge of tumor development, with a special interest for gynecopathology and hematopathology.

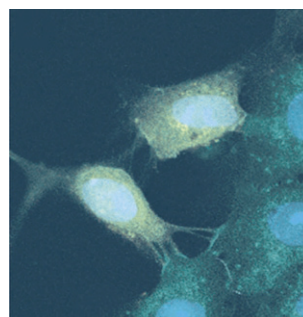
Our laboratory has an expertise to reproduce and manipulate (pre)neoplastic lesions *in vitro* (organotypic cultures) and to isolate lymphoid and other immune cells for functional studies. We also collaborate with the Human Tissue Bank of the University of Liège which includes several thousands of normal and cancer samples.

### HPV AND THE SEQUENCE “METAPLASIA-DYSPLASIA-CANCER”

Human papillomavirus (HPV) infection, particularly type 16, is causally associated with cancer of the uterine cervix. The persistence or progression of cervical lesions suggests that viral antigens are not adequately presented to the immune system. This hypothesis is reinforced by the observation that most squamous intraepithelial lesions (SILs) show quantitative and functional alterations of antigen-presenting cells, such as dendritic/Langerhans cells (DC/LC). One of the main goals of the research performed in the LEP is to determine the effects of HPV proteins on DC/LC recruitment and functions.

We showed that HPV16-induced down-regulation of CCL20 and E-cadherin observed during the cervical carcinogenesis may contribute to a diminished capacity of the immune system to control HPV infection. In addition to viral early oncoproteins, another factor potentially important for the density and function of LC is the process of epithelial metaplasia (EpM) which is observed in the transformation zone of the uterine cervix. EpM is an acquired tissue abnormality resulting from the transformation of epithelium into another tissue with a different structure and function. This adaptative process is associated with an increased frequency of (pre)cancerous lesions. We demonstrated that transforming growth factor- $\beta$ 1 is not only overexpressed in metaplastic tissues but also reduces E-cadherin expression in keratinocytes *in vitro* by inducing the promoter activity of Slug and Snail transcription factors. Finally, it was shown that *in vitro*-generated LC adhere poorly to keratinocytes transfected with either Slug or Snail DNA. We also demonstrated that these transcription factors reduce the expression of DNp63 which is important for the establishment of metaplastic epithelia, as well as for their malignant transformation.

Pascale Hubert, Anca Reschner, Michaël Herfs, Ludvine Herman, Stéphanie Demoulin, Joan Somja, Jacques Boniver, Philippe Delvenne



Confocal microscopy picture of co-culture of LC and SiHa cells transfected with siE7s and stained with E-cadherin: fluorescein isothiocyanate/LC: CM-DiI/Toto3.

### GENETIC ALTERATIONS IN COLON ADENOCARCINOMA

During colorectal tumorigenesis, several successive events, including genetic and epigenetic alterations, are involved in the transformation of the normal epithelium to a cancerous tissue with invasive and metastatic potential. The detection of these molecular alterations is important for the diagnosis and prognosis of colorectal cancer (CRC), as well as for the administration of targeted therapies aiming to enhance survival of the patients.

We used a new technique to analyze, on the one hand, EGFR gene amplification and on the other hand the presence of the variant protein EGFRvIII which present deletion of exon 2 to exon 7 of EGFR gene. This technique called MLPA (Multiplex Ligation-dependent Probe Amplification) mainly consists of fluorescent PCR amplification of probes hybridized to the gene of interest, followed by analysis of the fragments obtained on an ABI 3130XL Genetic Analyzer.

Using the MLPA technique, we observed EGFR gene amplification in 24 cases out of 95 cases of colorectal tumors. No evidence for the presence of EGFRvIII was found in any of the specimens tested. In order to validate this technique, we will perform FISH analysis on sections of paraffin-embedded tumors in parallel with the MLPA technique on DNA extracted from a frozen tumor of the same patient.

Dominique Begon, Julie Mardaga

### BIOMARKER DISCOVERY BASED ON EPIGENETIC MODIFICATIONS OF (PRE)NEOPLASTIC CELLS

Cancer is one of the most common causes of death worldwide. When detected early, current therapies often cure common cancers. Therefore the development of reliable and cost effective early detection methods is a priority in translational cancer research. Aberrant epigenetic modifications, such as DNA methylation, play a major role in the tumorigenic process, suggesting that they may also constitute valuable diagnostic tools.

Several projects have been initiated: 1) to detect new DNA methylation signatures in clinical samples obtained from endometrial and cervical cancers; 2) to identify micro RNA expression patterns and their methylation status in HPV-induced cervical cancer; 3) to identify new DNA methylation biomarkers in the sequence “metaplasia-dysplasia-cancer” and 4) to point out some biomarkers that undergo epigenetic modifications and that play a role in tumor angiogenesis.

We are currently performing high-throughput screening of DNA methylation patterns in different models. Due to intellectual property issues, lists of methylated sequences, miRNA or genes which have been identified and selected as potential biomarkers have to be kept confidential.

Pierre Dehan, Gaëlle Kustermans, Julie Horion, Samuel Guénin

### MOLECULAR PATHWAYS INVOLVED IN LYMPHOMAGENESIS

#### Peripheral T and NK cell lymphoma (PTCL)

Lymphomas derived from T and NK cells represent a heterogeneous group of aggressive malignancies with variable clinical presentation. Their molecular pathogenesis is poorly understood. The pathological criteria for delineation of the different disease entities are imprecise. This research conducted in close collaboration with Philippe Gaulard (Creteil, France) and other investigators from France, aims at characterizing the transcriptional profile of the different PTCL entities, with the purpose of identifying novel biomarkers, possible relevance to therapy, and to decipher the molecular pathways involved in these cancers. Another part of our work consists of screening for molecular alterations (gene rearrangements and mutations) targeting putative genes of interest in PTCL, not otherwise specified, and in angioimmunoblastic T-cell lymphomas. We are also implicated in the molecular and immunophenotypical characterization of CD30+ T-cell lymphomas. This project, derived from our gene expression profiling data, suggesting that CD30+ PTCLs may share some common features, relies on immunohistochemical profiling of a large series of CD30+ PTCL cases.

#### Diffuse large B-cell lymphoma (DLBCL)

DLBCL, the most common lymphoma entity, encompasses wide biological and clinical heterogeneity.

This project consists of performing the profiling of microRNA expression in DLBCL tissue samples to search for correlations with the transcriptome and the immuno-phenotype, and to identify dysregulated miRNA that might be relevant to lymphomagenesis.

#### **Establishment and characterization of novel cell lines derived from hematological malignancies**

Stéphanie Gofflot, Caroline Thielen, Bettina Bisig

Collaborators : Philippe Gaulard, Laurence Lamant, Georges Delsol (France), Teresa Marafioti (UK)

### **LABORATORY OF HISTOLOGY-CYTOLOGY**

Our research's topic is related to the analysis of the cross-talk between hematopoietic -and stromal- cells in the bone marrow in normal and pathological conditions, with a special interest in bone marrow adipocytes.

#### **NEUROPILIN-1, A NEWLY IDENTIFIED CO-RECEPTOR FOR LEPTIN FUNCTIONS DURING NORMAL AND PATHOLOGICAL HEMATOPOIESIS IN RESPONSE TO LEPTIN PRODUCED BY MEDULLARY ADIPOCYTES**

We have previously shown that bone marrow adipocytes negatively regulate granulopoiesis by inhibiting the production of granulocyte colony stimulating factor (G-CSF) by macrophages. This inhibition required Neuropilin-1 (NP-1) expression and occurred independently of the two known NP-1 ligands, VEGF and Sema3A. In addition it was possible to restore G-CSF production by neutralizing leptin, suggesting that NP-1 and leptin may be involved in a common pathway. Preliminary data confirms the hypothesis that OB-R (leptin receptor) can form a complex with NP-1. In this project, we intend to:

- Extend our study on the role of OB-R/NP-1/leptin in granulopoiesis, *in vitro* and *in vivo*.
- Analyse the signaling pathways activated by OB-R and/or NP-1
- Test the importance of leptin and OB-R/NP-1 for granulopoiesis in animals
- Investigate the possible role of OB-R/NP-1/leptin in acute myelogenous leukemia.

Marie-Paule Defresne, Chantal Humblet, Géraldine Poncin, Aurore Beaulieu, Delphine Delneuveille

### **LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGINEERING**

Angiogenesis is a pivotal process in the outgrowth and metastasis of tumors. The goal of our research is to pursue the evaluation of the inhibitors of angiogenesis previously identified and to discover new ones suitable for the treatment of angiogenesis and/or lymphangiogenesis-related diseases such as cancer. Three lines of research are ongoing.

#### **THERAPEUTIC EVALUATION OF PEPTIDES DERIVED FROM THE PROLACTIN/GROWTH HORMONE FAMILY**

We previously showed that recombinant 16-kDa N-terminal fragment of human prolactin (16K prolactin) and its derived peptides have angiostatic properties both *in vitro* and *in vivo*. Recently we showed that 16K prolactin affects the morphology of tumor blood vessel; ongoing researches are conducted in order to determine whether 16K prolactin affects vessel maturation and to identify the mechanism involved. Our work also attempts to answer the following questions: does 16K prolactin prevent lymphangiogenesis, the process of lymphatic vessel development shown to be involved in some tumorigenic processes and more importantly in metastasis? does 16K prolactin affect initial steps in tumor progression like vasculogenesis and recruitment on endothelial precursors?

Ngoc-Quynh-Nhu Nguyen, Karolien Castermans, Virginie Kinet, Michelle Lion



## UNDERSTANDING THE MECHANISM OF ACTION OF 16K FRAGMENTS OF THE PROLACTIN/ GROWTH HORMONE

The functional pleiotropy of 16K prolactin makes it important to identify one or more target molecules mediating these activities. Although a high-affinity specific binding site is present on endothelial cells, so far and despite much research performed by several laboratories, this «receptor» remains undetermined. We have thus focused on identifying the 16K prolactin «receptor».

Khalid Bajou, Salvino D'Amico, Mohammed Srahna, Stéphanie Herkenne, Jean-Yves Carabin

## IDENTIFICATION OF NEW MODULATORS OF ANGIOGENESIS

Starting from our previous research performed on inhibitors of angiogenesis, we have taken advantage of our expertise to identify new modulators of angiogenesis. In one way, new candidates are targets of angiostatic agents. Some new candidates have been selected from data obtained during our previous transcriptomic and/or proteomic analyses performed on endothelial cells or tumor samples. Among others, we also address a special interest to miRNAs. We have taken advantage of bioinformatics and zebrafish tools available in our lab and in the GIGA-Research centre to identify new miRNAs involved in angiogenesis. We have performed functional studies after having modulated their expression *in vitro* in endothelial cells and *in vivo* in zebrafish and mice. We already identified 3 miRNAs that potentially regulate angiogenesis using this approach.

Hélène Pendeville, Céline Sabatel, Ludovic Malvaux, Julie Halkein, Olivier Nivelles

## METASTASES RESEARCH LABORATORY

The Metastases Research Laboratory is engaged in three main areas of research that are interconnected and aims not only to unveil the molecular mechanisms involved in the development and progression of malignant cells but is also eager to develop new tools for the early detection, prognostic evaluation and innovative therapies that could benefit to patients suffering from cancer.

## ROLES OF SIBLINGS DURING TUMOR DEVELOPMENT, INVASION, METASTASES AND ANGIOGENESIS

Small Integrin-Binding Ligand N-linked Glycoproteins (SIBLINGS), a family of five soluble integrin-binding glycoposphoproteins comprising osteopontin (OPN), bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE), represent an emerging group of molecular tools that cancer cells use to facilitate their expansion. SIBLINGS are soluble secreted proteins that can act as modulators of cell adhesion as well as autocrine and paracrine factors by their interaction with specific cell surface receptors such as integrins. In 2009, A. Bellahcene and her team examined the role of a member of the SIBLING family, OPN, during human glioblastoma progression based on the observation that higher expression of OPN in these cancers correlated with increased aggressiveness. They demonstrated that OPN-silenced glioma cells are less able to generate tumors when tested in the chicken chorioallantoic membrane (CAM) model in correlation with a reduced proliferation rate and migration potential, indicating that OPN plays a major role in the control of the two major features of glioma progression in humans (Lamour et al., 2009, Int J Cancer). Furthermore, they studied the implications of OPN in the glioblastoma cancer stem cell phenotype acquisition and found that OPN appears to play a key role in this process. This team also has accumulated data demonstrating that DMP1 play a key negative regulatory role during angiogenesis.

Akeila Bellahcene, Virginie Lamour, Sophie Pirotte, Manuella Dewald and Vincent Castronovo

## ROLES OF HISTONE DEACETYLASES DURING TUMOR PROGRESSION AND ANGIOGENESIS

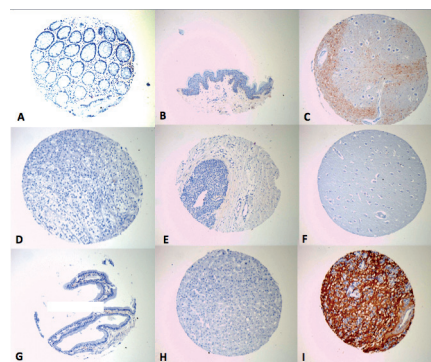
Histone deacetylases inhibitors (HDACi) have been shown to reduce tumor growth both in *in vitro* and *in vivo* preclinical studies. These molecules exert multiple and desirable anti-cancer effects by modulating the expression of a subset of genes involved in the inhibition of tumor cell proliferation and differentiation, induction of apoptosis, and inhibition of angiogenesis. For many years, MRL has been developing the tools and expertise to study the special characteristics of each HDAC and to identify which HDAC(s) may be the most relevant target(s) for intervention in oncology. Uncontrolled proliferation of cancer cells is one of the hallmarks of tumor progression. Many publications reported that inhibition of class I and II HDACs through non selective inhibitors can stop cancer cell proliferation *in vitro* through transcriptional reactivation of the p21WAF1/Cip1 gene. To identify which HDACs regulate p21WAF1/Cip1 gene expression in cancer cells, in 2009, the group of D. Mottet, used small interfering RNAs technology to target seven class I and class II HDACs (HDACs 1-7). They found that HDAC4 participates in the repression of p21WAF1/Cip1 through Sp1/Sp3-, but not p53-binding sites. HDAC4 interacts with Sp1, binds and reduces histone H3 acetylation at the Sp1/Sp3 binding site-rich p21WAF1/Cip1 proximal promoter, suggesting a key role for Sp1 in HDAC4-mediated repression of p21WAF1/Cip1. They also demonstrated that induction of p21WAF1/Cip1 is an important component of inhibition of cancer cell proliferation *in vitro* and arrest of tumor growth *in vivo* mediated by silencing of HDAC4 (Mottet D. et al., 2009, Oncogene).

Denis Mottet, Paul Peixoto, Sylvie Hastir, Nicolas Matheus and Vincent Castronovo

## IDENTIFICATION OF ACCESSIBLE BIOMARKERS FOR INNOVATIVE TARGETED THERAPIES AND DIAGNOSIS

One promising avenue towards the development of more selective, better anticancer drugs lies in the targeted delivery of bioactive compounds to the tumor environment by means of binding molecules specific for tumor-associated biomarkers. Eligibility of such markers for therapeutics ideally falls under three criteria: accessibility from the bloodstream, expression at sufficient level, and no expression in normal tissues. Most current discovery strategies provide no clue as to whether proteins of interest are accessible, in human tissues, to suitable high-affinity ligands. To address this limitation, A. Turtoi and V. Castronovo have recently developed and patented a novel, comprehensive and efficient method allowing for the identification of accessible proteins in precious pathological samples available in minute quantities. In order to isolate these proteins of interest, they have exploited the ability of chemically modified biotin to *in-vivo* label the outer membrane and extracellular proteins as well as the fact that most of these proteins are glycosylated. The approach consisted of three successive steps involving the linkage of potentially accessible proteins to biotin molecules followed by their purification. The remaining proteins were subjected to glycopeptide isolation. Finally, the analysis of the non-glycosylated peptides and the application of an *in silico* method increased the confident identification of glycoproteins. The value of the technique was demonstrated on human breast cancer tissue samples. Taken together the MS analysis delivered quantitative data on more than 800 potentially accessible proteins. In comparison to the biotinylation alone, the sequential method significantly increased the number (+ 45 %) of therapeutically and diagnostically valuable proteins. Furthermore, this team was able to characterized a new isoform of Versican (Versican 4) as a potential target for breast cancer (Kischel P. et al. 2009, Int J Cancer).

Andrei Turtoi, Yannick Greffe, Bruno Dumont, Davide Musmeci and Vincent Castronovo



*Illustration of the validation of an accessible biomarker (periostin) identified through the ex-vivo biotinylation of human breast cancer.*

*The immunohistochemistry analysis was performed on a TMA (Tissue Micro-Array) to demonstrate that elevated high periostin expression level is exclusive to breast cancer lesion (I), not detectable in various normal tissues (colon (A), skin (B), adrenal (D), mucosal epithelium (E), brain white matter (F), prostate (G), liver (H) and low in brain cortex. (original magnification: x 100).*

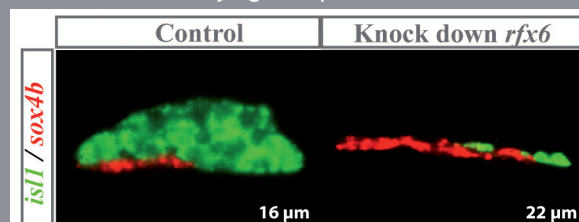
Development is the study of various cell types and organs that are generated from the zygote. A better understanding of the molecular and cellular processes involved in generating an animal or a human being from a single cell is very important, not only for basic science, but also to fruitfully imagine and/or design new therapeutic approaches to cure and stimulate regeneration in adulthood after a disease. In a few words: a better understanding of how we are built could help us to rebuild after a lesion, whatever the aetiology is. For this reason, this unit is called “Development, Stem Cells and Regenerative Medicine”.

## HIGHLIGHT

### RFX6, A NOVEL WINGED HELIX TRANSCRIPTION FACTOR REQUIRED FOR ISLET CELL DEVELOPMENT

*Development*. 2010 Jan;137(2):203-12.

Loss of the insulin-producing B-cells from the endocrine pancreas occurs in diabetes, a disease that afflicts more than 125 million people worldwide. Although diabetes is a manageable disorder, the associated complications that result in significant morbidity and mortality necessitate novel approaches of pharmacologic, cell, or gene therapy. Endocrine cell replacement is likely to be an integral step in the treatment of this disease. A major challenge lies in developing practical strategies for B-cell replacement, a task that will be facilitated by a more profound understanding of the mechanisms underlying the specification and differentiation of pancreatic endocrine cells. The discovery by our lab in collaboration with the team of Gradwohl (Strasbourg, FRANCE) of Rfx6 as a novel regulator of islet cell development might be relevant to promote and scrutinize the differentiation of stem cells to the  $\beta$  lineage.



*Rfx6 knock-down in zebrafish leads to an important decrease of endocrine cells (labelled by Isl1 in green) and an increase of the pancreatic progenitors (labelled by Sox4b in red)*

The researchers report that in both mouse and zebrafish, Rfx6, a winged helix transcription factor, is found in islet progenitor cells and maintained in developing and mature islet cells. Loss of function studies in zebrafish revealed that Rfx6 is required for the differentiation of glucagon, ghrelin and somatostatin expressing cells which, in the absence of Rfx6, are blocked at the progenitor stage. In contrast, beta cells, whose number is only slightly reduced, were no longer clustered in a compact islet. This data unveils Rfx6 as an important regulator of endocrine cell differentiation that controls the maturation of endocrine progenitor cells.

Loss of function studies in zebrafish revealed that Rfx6 is required for the differentiation of glucagon, ghrelin and somatostatin expressing cells which, in the absence of Rfx6, are blocked at the progenitor stage. In contrast, beta cells, whose number is only slightly reduced, were no longer clustered in a compact islet. This data unveils Rfx6 as an important regulator of endocrine cell differentiation that controls the maturation of endocrine progenitor cells.

Soyer, J.<sup>1</sup>, Flasse, L.<sup>2</sup>, Raffelsberger, W.<sup>1</sup>, Beucher, A.<sup>1</sup>, Orvain, C.<sup>1</sup>, Peers, B.<sup>2</sup>, Ravassard, P.<sup>3</sup>, Vermot, J.1, Voz, M. L.2, Mellitzer, G.1, and Gradwohl, G.1 (2010).

*Rfx6 is an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development.*

*Development* 137, 203-212.

<sup>1</sup>Institute of Genetics and Molecular and Cell Biology (IGBMC), Inserm U-964, CNRS UMR7104, University of Strasbourg, Illkirch, France.

<sup>2</sup>GIGA-R, University of Liege, Avenue Hospital, 1, 4000 Sart-Tilman, Belgium.

<sup>3</sup>Centre de Recherche de l'Institut du Cerveau et de la Moelle CNRS UMR7225, Inserm UMRS795, Université Pierre et Marie Curie, Paris, France.

## LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGINEERING

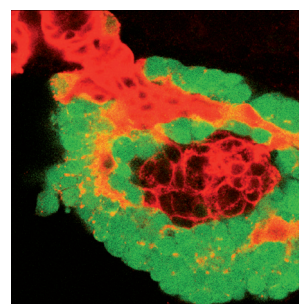
Our main research theme is understanding the molecular mechanisms controlling pancreas development. To that end, we are using mainly the zebrafish as animal model which offers several experimental advantages, including the availability of numerous translucent embryos, the possibility to observe organ morphogenesis within developing organisms and the ease of gene function studies (knock-down by morpholinos and identification of mutants). Most regulatory genes important for pancreas development are well conserved throughout evolution and have a similar function among vertebrate species. Data obtained with the fish model can generally be applied to mammals and notably humans.

### MOLECULAR DISSECTION OF PANCREAS ORGANOGENESIS

We have characterized a novel pancreatic transcription factor, Rfx6, and has shown its key role in the formation of pancreatic endocrine cells (see the selected study for detailed description). Another important finding of our laboratory was the demonstration that two homeobox genes, nkx6.1 and nkx6.2, act together in order to establish the pool of pancreatic progenitors during early embryogenesis and are required for the differentiation of these cells into endocrine  $\alpha$  and  $\beta$  cells.

Bernard Peers, Marianne Voz, Isabelle Manfroid, Lydie Flasse, Anne-Catherine Binot, Joachim Djiotso, Aurélie Ghaye, Virgine Von Berg, Nathalie Detry, François Naye, Olivier Ek

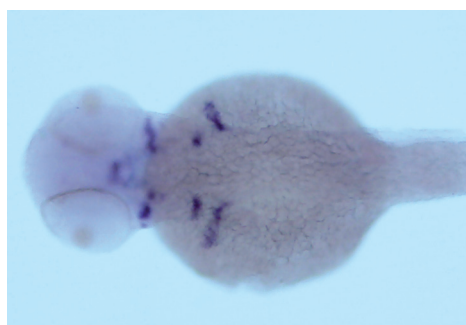
*Exocrine pancreas (green) surrounding the central endocrine islet (red) and crossed by the pancreatic ducts (red) in a late stage zebrafish embryo.*



### BONE AND PITUITARY DEVELOPMENT IN ZEBRAFISH

We use the zebrafish mainly to investigate development and maintenance of the skeletal system as well as organogenesis of the anterior pituitary. We study the function of specific genes and signaling pathways in the formation of the cartilage primordium and the subsequent bone formation, primarily in the head skeleton. We use standard genetic methods (gain and loss of function, mutants) in our studies, but we also examine the effects of specific drugs or of microgravity for a contract with the European Space Agency (ESA). Furthermore, we obtained subtractive cDNA libraries from adult zebrafish pituitary and hypothalamus and characterized the expression during development of some of the identified genes (Toro et al., 2009).

Marc Muller, Julia Dalcq, Jessica Aceto, Yobhana Quiroz, Arnaud Larbuisson



*Expression pattern of runx2b indicating the position of developing cartilage cells in the head of a 48 hpf zebrafish embryo.*

## LABORATORY OF EMBRYOLOGY

The laboratory of Embryology has two major research interests: development of novel transgenesis tools in the chicken and understanding human embryonic stem cells biology.

### CONTRIBUTION TO DECIPHERING OCT4 AND SOX2 REGULATION IN HUMAN STEM AND CARCINOMA EMBRYONIC CELLS.

OCT4 and SOX2 are transcription factors essential for the induction and maintenance of pluripotency in embryonic stem cells. Their own regulation is poorly understood. We are in the process of identifying and characterizing trans- and cis-acting elements regulating their transcription in human embryonic stem and carcinoma cells under normoxic and hypoxic culture conditions.

Luc Grobet, Delphine Connan, Virginie Marchal

### OPTIMIZING HESCS CRYOPRESERVATION CONDITIONS

In an effort to enhance the efficiency and biological safety of cryopreservation of human embryonic stem cells for regenerative medicine purposes, we are refining current slow-freezing and vitrification protocols using chemically defined media and reagents.

Luc Grobet, Fabien Ectors, Pierre Vanderzwalmen

## LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

### NEUREGULINS RECRUIT THE SWI/SNF CHROMATIN REMODELLING COMPLEXES TO MODULATE CELL FATE CHOICE DURING NEURODEVELOPMENT.

One research theme of the Laboratory of Developmental Neurobiology concerns the molecular regulation of neural stem cell proliferation and cell fate decisions during central nervous system ontogenesis. Neuregulins form a growth factor family encoded by four genes (Nrg1 to Nrg4) but encompassing more than fifty protein isoforms generated by alternative splicing and post-translational modifications. A specific isoform encoded by Nrg1 and named CRD-NRG is expressed by neural stem cells during development. We demonstrated that this transmembrane isoform could be cleaved near the internal sheet of the plasmic membrane of neural stem cells and the intracellular domain is then translocated to the nucleus where it can interact with some members of the Swi/Snf chromatin-remodelling complex. These interactions are able to modulate the cell-fate decision of the neural stem cells and their neuronal and/or oligodendroglial differentiation.

Bernard Rogister, Sabine Wislet-Gendebien, Dorothée Pirotte, Jérôme Kroonen, Emerence Laudet, Stéphanie Hody, Jessica Nassen and Linda Haugustaine

The Genetics thematic unit regroups three laboratories: Human Genetics (Prof. Vincent Bours), Hepatology and Gastroenterology (Prof. Edouard Louis), Animal Genomics (Prof. Michel Georges). The unit focuses on the forward genetic dissection of Mendelian and complex traits in human and domestic animals. It has an epigenetics program, rooted in unique animal models, with special emphasis on microRNA biology. It develops approaches for the utilization of molecular information in livestock breeding, including genomic selection and transgenics.

GIGA-Genetics totals 50 members, of which more than half are post-doctoral fellows or senior scientists. In addition to wet-lab genomicists, the unit has a strong bioinformatics/statistical genetics component. Women make up more than 50 % of the staff, and more than five nationalities are represented. Members of GIGA-Genetics cluster in seven teams: genetics of inflammatory bowel disease, human genetics, dog genetics, livestock genetics, genomic selection, epigenetics and transgenics.

### HIGHLIGHT

#### BALANCING SELECTION OF A FRAME-SHIFT MUTATION IN THE MRC2 GENE ACCOUNTS FOR THE OUTBREAK OF THE CROOKED TAIL SYNDROME IN BELGIAN BLUE CATTLE

*PLoS Genet.* 2009 Sep;5(9):e1000666

We reported the positional identification of a 2-bp deletion in the open reading frame of the MRC2/Endo180 receptor causing the recessive Crooked Tail Syndrome in cattle. The resulting frame-shift reveals a premature stop codon that causes nonsense mediated decay of the mutant messenger RNA and the virtual absence of functional Endo180 protein in affected animals. Cases exhibit skeletal anomalies thought to result from impaired extracellular matrix remodeling during ossification, and, as of yet, unexplained muscular symptoms. We demonstrated that carrier status is very significantly associated with desired characteristics in the general population, including enhanced muscular development, and that the resulting heterozygote advantage caused a selective sweep, which explains the unexpectedly high frequency (25%) of carriers in the Belgian Blue Cattle Breed. These findings highlight one of the risks associated with pushing domestic animals to their physiological limits by intense artificial selection.

Corinne Fasquelle<sup>1</sup>, Arnaud Sartelet<sup>1</sup>, Wanbo Li<sup>1</sup>, Marc Dive, Nico Tamma<sup>1</sup>, Charles Michaux<sup>2</sup>, Tom Druet<sup>1</sup>, Ivo J. Huijbers<sup>3</sup>, Clare M. Isacke<sup>3</sup>, Wouter Coppieters<sup>1</sup>, Michel Georges<sup>1</sup>, Carole Charlier<sup>1</sup>. Balancing selection of a frame-shift mutation in the MRC2 gene accounts for the outbreak of the Crooked Tail Syndrome in Belgian Blue Cattle. *PLoS Genetics*, 2009;5(9):e1000666

<sup>1</sup>Unit of Animal Genomics, GIGA-R & Department of Animal Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium.

<sup>2</sup>Unit of Bioinformatics, Department of Animal Sciences, Faculty of Veterinary Medicine, University of Liège, Belgium.

<sup>3</sup>Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, UK.



## GENETICS OF CROHN'S DISEASE

### SEARCHING FOR RARE SUSCEPTIBILITY VARIANTS FOR CROHN'S DISEASE BY MASSIVE PARALLEL RESEQUENCING

GWAS have identified more than 30 loci associated with inherited predisposition to Crohn's disease. Despite these remarkable advances, it was estimated that these loci only explain 20% of inherited predisposition, raising the question of the nature of the missing 80%. One hypothesis is that rare variants, which escape detection by GWAS, significantly contribute to genetic susceptibility to complex diseases. To test this hypothesis we developed a protocol based on massive parallel sequencing of DNA pools for the detection of rare variants in the ORF of tens of genes at once. Statistical methods were developed to reveal significant differences in cumulative frequencies of rare disruptive variants between cases and controls. We resequenced > 70 positional candidates in up to 1,000 cases and 1,000 controls. We aim to complete the analyses followed by confirmatory studies in the spring of 2010. Funding: Walloon Region (FEDER, Marshall Plan)

Myriam Mni, Yukihide Momozawa, Kayo Nakamura.

Collaborators: Leila Amininejad, Martine de Vos, Denis Franchimont, Severine Vermeire (Belgium)

### TOWARDS MULTI-TISSUE, EQTL-BASED FUNCTIONAL ANNOTATION OF LOCI ASSOCIATED WITH INFLAMMATORY BOWEL DISEASE

The susceptibility loci identified by GWAS typically span 150 Kb and encompass 3-4 genes on average. Pinpointing the causative genes and mutations remains a considerable challenge. Evaluating the effect of disease predisposing variants on the expression levels of neighboring genes via so-called eQTL analysis has proven to be a valuable source of information. As an example, we showed that SNPs in a gene desert on 5p13 associated with Crohn's disease were modulating the expression levels of PTGER4 located at 250 Kb, thereby pinpointing PTGER4 as the likely causative gene and suggesting that the predisposing variants are regulatory. In human, eQTL analyses have so far been restricted to lymphoblastoid cell lines, hence limiting their scope. To overcome this limitation we are collecting intestinal biopsies and six leucocyte populations from 400 healthy individuals. All individuals are being genotyped for SNPs spanning the genome, transcriptome analysis is being conducted on all tissue samples, genome-wide DNA methylation is being monitored for a subset of tissues, while the adherent colonic microbiome is being catalogued by high throughput sequencing for each individual. This unique data set will enable us to perform tissue-specific eQTL studies, in particular for genomic regions that have been associated with common inflammatory and intestinal disorders. It will allow us to study epigenotype-genotype and epigenotype-transcriptome, as well as microbiome-genotype and microbiome-transcriptome correlations. Funding: Walloon Region (FEDER, Marshall Plan).

Valérie Deffontaine, Alex Kvasz, Myriam Mni, Yukihide Momozawa, Catherine Reenaerts, Emilie Theatre.

Collaborators: Leila Amininejad, Martine de Vos, Denis Franchimont, Severine Vermeire (Belgium)

## HUMAN GENETICS

### IDENTIFICATION OF GENES PREDISPOSING TO MENDELIAN AND COMPLEX DISORDERS

We have conducted linkage analyses in two large multiplex pedigrees segregating for an autosomal dominant form of strabismus, and familial isolated pituitary adenoma without mutations in the AIP gene. Regions of genome-wide significant linkage were found in both cases. Resequencing of positional candidates is being conducted to identify the causative genes.

Carole Charlier, Anouk Georges, Kayo Nakamura

Collaborator: Albert Beckers (Belgium)

## GENETICS OF CYSTIC FIBROSIS

We studied African patients with cystic fibrosis-like phenotypes and identified, in addition to a novel mutation in the CF gene, several mutations in the genes coding for the sodium channel ENaC (Mutesa et al., Chest, 2009). We are currently characterizing these mutations and extending our research to a larger cohort of patients.

Léon Mutesa, Catherine Verhaeghe

## CANINE GENETICS

### FORWARD GENETIC DISSECTION OF PRIMARY CILIARY DYSKINESIA (PCD) IN DOGS IDENTIFIES A NOVEL GENE EXPLAINING AN IMPORTANT PROPORTION OF HUMAN CASES

PCD is a disorder of the motile cilia causing chronic inflammation of the respiratory tract, male sterility and situs inversus. We identified a dog pedigree with PCD. A genome-wide scan mapped the gene to a 15 Mb segment encompassing 150 genes, of which 10 were reported in the ciliome. Sequencing revealed a frame-shift loss-of-function mutation in a gene causing cardiac left-right asymmetry when knocked down in zebrafish. Sequencing the human orthologue revealed gene-disrupting mutations in a high proportion of human PCD cases with central microtubule pair defects.

Funding: European Union (LUPA project)

Géraldine Bataille, Anne-Sophie Lequarré, Anne-Christine Merveille

Collaborators: Serge Amselem (France), Cécile Clercx (Belgium), Erica Davis (USA), Frédéric Farnir (Belgium), Nico Katsanis (USA), Heymut Omran (Germany), Dominique Peeters (Belgium).

### SEARCHING FOR GENETIC DETERMINANTS OF VARIATION IN BLOOD PRESSURE, GLUCOSE AND LIPID METABOLISM IN DOG

Our aim is to collect phenotypes pertaining to cardiovascular physiology, glucose and lipid metabolism, in a cohort of 1,000 healthy dogs. QTL influencing any of the measured traits will be mapped using GWAS.

Funding: European Union (LUPA project).

Géraldine Bataille, Anne-Sophie Lequarré, Anne-Christine Merveille

Collaborators: Cécile Clercx, Dominique Peeters, Kathleen McEntee (Belgium).

## LIVESTOCK GENETICS

### GENOMIC SURVEILLANCE AND MANAGEMENT OF INHERITED DEFECTS IN LIVESTOCK

Intense selection in livestock reduces the effective population size, causing regular outbursts of recessive defects. We have established a heredosurveillance platform that collects samples from emerging defects, genetically maps the causative genes, identifies the causative mutations by resequencing positional candidates, develops and offers diagnostic tests. Our laboratory developed the first high density SNP array for livestock and demonstrated its effectiveness for positional cloning. We have pursued these efforts and have mapped 13 inherited defects and identified the causative mutation for eight (e.g. Fasquelle et al., 2009). Diagnostic tests have been developed for the 13 defects and are being offered. Large-scale utilization of the tests has allowed the eradication of the defects with major benefits to breeders.

Funding: Walloon Ministry of Agriculture.

Carole Charlier, Wouter Coppieeters, Tom Druet, Corinne Fasquelle, Sarah Geron, Arnaud Sartelet, Nico Tamma

Collaborators: Daniel Desmecht, Frédéric Rollin (Belgium)



### POSITIONAL IDENTIFICATION OF QTL AND QTN IN LIVESTOCK

We are involved in several projects aimed at identifying QTL influencing economically important traits in livestock. Examples of QTL that were identified in 2009 include parasite resistance (Coppieters et al., 2009), and female fertility in pigs. To identify QTL influencing traits of importance to the dairy industry, a large Holstein-Friesian x Jersey intercross was generated in New Zealand, characterized for hundreds of phenotypes. We are involved in the positional identification of QTN underlying several of the most relevant identified QTL. As an example, fine-mapping of a QTL influencing growth refined the map position of the QTL to a 750 Kb segment. The entire region was sequenced in six individuals with known QTL genotype. This allowed us to pinpoint a cluster of 13 SNPs out of > 8,000 with QTN-compatible segregation profiles. Further analyses are being conducted to identify the causative SNPs and elucidate its mode of action. We are also mining high density SNP genotypes of > 10,000 animals to identify genes that control inherited variation in global and local recombination rates.

Funding: Gentec, Livestock Improvement Corporation (LIC), Vialactia Biosciences (VLB).

Wouter Coppieters, Keith Durkin, Tom Druet, Latifa Karim, Li Lin, Cynthia Sandor, Haruko Takeda, Zhian Zhang  
Collaborators: Nadine Buys, Frédéric Farnir (Belgium)

### DEVELOPMENT AND APPLICATION OF MOLECULAR AND STATISTICAL TOOLS FOR GENOMIC SELECTION IN LIVESTOCK

We have been heavily involved in large scale resequencing of the bovine genome for the detection of millions of SNP that were utilized in the design of the next generation high density SNP array. We are also involved in the detection and characterization of common copy number variants (CNV) in livestock. We identified a novel type of mutation in which the duplicative transposition of a gene with only part of its regulatory elements causes a gain of function, resulting in a dominantly inherited phenotype. As part of the genomic selection program of CRV, we genotyped several thousands of animals with the 60K SNP chip that was previously designed in the laboratory. We developed a novel method based on hidden markov models for the phasing of high density SNP data that allows imputation of missing genotypes, as well as haplotype-based mapping and genomic selection (Druet & Georges, 2009). The method has been utilized within the Eurogenomics project to allow integration of SNP genotypes across countries.

Funding: Pfizer, CRV, Walloon Ministry of Agriculture.

Naima Ahariz, Nadine Cambisano, Wouter Coppieters, Tom Druet, Keith Durkin, Latifa Karim, Nico Tamma, Zhian Zhang  
Collaborators: Frédéric Farnir (Belgium)

## EPIGENETICS

### TOWARDS MOLECULAR UNDERSTANDING OF POLAR OVERDOMINANCE

The callipyge muscular hypertrophy is characterized by a unique mode of inheritance (polar overdominance), in which only heterozygotes receiving the CLPG mutation from their father express the phenotype. We showed that the CLPG mutation is a gain-of-function mutation that causes ectopic expression of the paternally expressed imprinted DLK1 gene in skeletal muscle of +Mat/CLPG-Pat animals, hence causing the phenotype. We hypothesized that the absence of phenotypic expression in CLPG/CLPG, is due to post-transcriptional silencing of DLK1 by miRNAs processed from maternally expressed non-coding RNA genes. To identify the responsible miRNA we generated miRNA catalogues of ovine skeletal muscle by high throughput sequencing, including 111 miRNAs processed from 66 precursors in the DLK1/GTL2 domain. We showed that these miRNAs are imprinted, subject to the cis-effect of the CLPG mutation, jointly account for 20% of the miRNA population in CLPG/CLPG animals, and that some of them undergo A to I editing. We are testing whether these miRNA target DLK1 using both bioinformatic and wet-lab approaches.

Funding: European Union, Politique Scientifique Fédérale, Fonds National de la Recherche Scientifique, Communauté Française de Belgique, University of Liège.

Denis Baurain, Florian Caiment, Carole Charlier, Huijun Cheng, Haruko Takeda  
Collaborators: Samuel Hiard (Belgium), Noelle Cockett (USA)

#### **PATROCLES: POLYMORPHIC MIRNA-MEDIATED GENE REGULATION**

We identified the first polymorphism that caused a mutant phenotype by perturbing miRNA-mediated gene regulation in any vertebrate: a mutation creating a potential illegitimate miRNA target site in the myostatin gene was shown to affect muscularity in sheep. We subsequently established a database ([www.patrocles.org](http://www.patrocles.org)) that compiles polymorphisms predicted to perturb miRNA mediated gene regulation (Hiard et al., 2009). Patrocles lists tens of thousands of polymorphisms that are predicted to affect miRNA targets, miRNA precursors or components of the silencing machinery. Contextual information is provided to assess the relevance of the predictions. To allow confirmation of the Patrocles predictions, we developed a biochemical procedure to identify polymorphic miRNA-target interactions *in vivo*. The method is based on the coimmunoprecipitation of miRNA targets using anti-AGO antibodies from tissues of heterozygotes for the SNPs of interest. The effect of the SNP on miRNA mediated gene regulation is assessed from the degree of allelic imbalance in the immunoprecipitate. Funding: European Union, Politique Scientifique Fédérale, Fonds National de la Recherche Scientifique, Communauté Française de Belgique, University of Liège.

Denis Baurain, Carole Charlier, Haruko Takeda, Samuel Hiard

### **TRANSGENIC ENGINEERING OF LIVESTOCK**

#### **ENGINEERING MALE-SPECIFIC DOUBLE-MUSCLING**

We have previously shown that “double-muscling” is caused by loss-of-function mutations in myostatin. We subsequently showed that it was possible to integrate dominant negative myostatin alleles onto the Y chromosome, thereby generating male-specific double-muscling in a mouse model. We are extending the same approach to livestock. To that end we characterized the bovine Y and pseudoautosomal region and developed the necessary vectors to target the bovine Y by homologous recombination followed by integration of dominant negative myostatin alleles by RMCE. Funding: Walloon Ministry of Agriculture.

Mallory Draye, Anne-Sophie Van Laere, Fabien Ectors

#### **TOWARDS SEMEN SEXING USING A TRANSGENIC APPROACH**

The ability to predetermine the sex of offspring remains an important objective. We are attempting to develop transgenic approaches towards semen sexing using the mouse as model. Our aim is to induce gene expression specifically in Y-bearing sperm. To achieve this goal, two major problems are to be solved: overcome meiotic sex chromosome inactivation (MSCI), and block diffusion of gene products between syncytial spermatides.

Funding: Walloon Ministry of Agriculture.

Prisca Feuerstein, Muralidhar Metta, Fabien Ectors





## GIGA-Infection, Immunity, Inflammation

In order to cure or improve the treatment of many human diseases, it is essential to understand the mechanisms by which the immune system maintains organism integrity against internal or external aggression.

The Infection, Immunity and Inflammation (I<sup>3</sup>) Unit of the GIGA-Research aims at stimulating synergies between research groups studying various but complementary aspects of immunity. These aspects include immune cell development, haematology, inflammation, allergy, cancer immunology and viral infections. Created in 2008, the I<sup>3</sup> Unit is composed of 11 laboratories and more than 90 researchers. Research themes span the spectrum from bench-to-bedside. Indeed, studies range from cell culture systems and animal models of disease to clinical studies, all using state-of-the-art laboratory techniques.

The I<sup>3</sup> aims at becoming an international centre of excellence in immunology -and infectious disease-related disciplines. During the years 2008-2009, members of the Unit produced or contributed to an appreciable number of significant scientific publications.

### HIGHLIGHT

#### INTERSTITIAL MACROPHAGES, GUARDIANS OF PULMONARY IMMUNE HOMEOSTASIS

*Journal of Clinical Investigation* 2009;119(12):3723-3738

Asthma is a respiratory syndrome affecting 6% of the European population. One of the most prevalent types of asthma is atopic, or allergic, asthma. Atopic asthma arises from allergic reactions of the airways against inhaled environmental allergens such as house dust mite feces or cat dander.

Allergens should in theory elicit an immune response against «non-self» in all exposed individuals. To explain the absence of allergy in non-atopic individuals, it is usually referred to as the «theory of danger». According to this theory, the immune system tolerates harmless antigens because they do not present recognizable danger signals. In these situations, dendritic cells (DCs) would induce the differentiation of regulatory T cells (Tregs) that specifically would prevent inflammatory responses against harmless antigens.

The most abundant immune cells in the lungs are macrophages. Yet, their role in the regulation of adaptive immune responses has remained relatively unaddressed. In mice, two populations of lung macrophages have been identified: alveolar (AMs) and interstitial (IMs) macrophages. IMs are located just below the lung epithelium, an ideal location to interact with DCs (Figure 1A). We therefore aimed at deciphering the function of AMs and IMs in pulmonary immune homeostasis.

We observed that IMs could be distinguished from AMs by their unique capacity to inhibit lung DC maturation and migration upon LPS stimulation, thereby preventing sensitization to concomitant aeroantigens. We furthermore demonstrated that this functional paralysis of lung DCs involves IL-10

production by IMs. Thus, in the presence of LPS, IMs, but not AMs, break the link between innate and adaptive immunity, allowing harmless inhaled antigens to escape from T cell-dependent responses. In our model, the absence of response to harmless inhaled antigens is explained by immune ignorance rather than tolerance. The corollary of this hypothesis is that IM function must be modulated during infectious episodes to allow the mounting of protective adaptive immunity. We further postulate that inhibition or dysfunction of IMs might contribute to the development of asthma in humans.

Denis Bedoret,<sup>1</sup> Hugues Wallemacq,<sup>1</sup> Thomas Marichal,<sup>1</sup> Christophe Desmet,<sup>1</sup> Florence Quesada Calvo,<sup>2</sup> Emmanuelle Henry,<sup>3</sup> Rodrigue Closset,<sup>1</sup> Benjamin Dewals,<sup>4</sup> Caroline Thielen,<sup>5</sup> Pascal Gustin,<sup>6</sup> Laurence de Leval,<sup>5</sup> Nico Van Rooijen,<sup>7</sup> Alain Le Moine,<sup>8</sup> Alain Vanderplasschen,<sup>4</sup> Didier Cataldo,<sup>2</sup> Pierre-Vincent Drion,<sup>9</sup> Muriel Moser,<sup>3</sup> Pierre Lekeux,<sup>1</sup> and Fabrice Bureau<sup>1</sup>

*Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice.*

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## LABORATORY OF CELLULAR AND MOLECULAR PHYSIOLOGY

Our research laboratory has a vast experience in the study of atopic asthma. Using the most recent mouse models of the disease, we aim at providing a better understanding of the etiology of the disease in humans, as well as proposing new pathways for therapeutic intervention. Our laboratory is working in tight cooperation with several laboratories in Belgium and abroad. We also collaborate with the ULg University Hospital (CHU) to translate our experimental results in mice to human asthma.

### INTERFERON RESPONSE FACTOR-3 IS ESSENTIAL FOR HOUSE DUST MITE-INDUCED AIRWAY ALLERGY

Pattern Recognition Receptors (PRRs) are critically involved in the pathophysiology of airway allergy. Yet, most of the signaling pathways downstream of PRRs implicated in allergic airway sensitization remain unknown. In this study, we investigated the effects of genetic depletion of Interferon Response Factor (IRF) 3 and IRF7, important transcription factors downstream of various PRRs, in a mouse model of house dust mite (HDM)-induced atopic asthma. Parameters of airway allergy due to HDM exposure were strongly attenuated in IRF3-deficient (IRF3<sup>-/-</sup>), but not in IRF7<sup>-/-</sup> mice, as compared to wild-type (WT) mice. Indeed, in HDM-exposed IRF3<sup>-/-</sup> mice, HDM-specific Th2 cell responses failed to develop, due to impaired dendritic cell maturation and migration. Thus, this study demonstrates that IRF3, a central activator of antiviral immunity, through its role in dendritic cell function, is essential for the development of Th2 type responses to airway allergens.

Thomas Marichal, Denis Bedoret, Claire Mesnil, Didier Cataldo, Pierre Lekeux, Fabrice Bureau, Christophe Desmet

Collaborators : Muriel Pichavant (France), Stanislas Goriely (Belgium), François Trottein (France), Michel Goldman (Belgium)

## LABORATORY OF HUMAN GENETICS

Our research group has initiated projects aiming at the identification of new molecular mechanisms regulating the inflammatory response, focusing on the role of receptors for extracellular ATP. We are currently studying several inflammation models, including inflammatory bowel diseases (IBD) and their evolution to cancer, sepsis as well as cystic fibrosis. We are also interested in the link existing between inflammation and thrombosis.

### ROLE OF EXTRACELLULAR ATP AND ITS RECEPTORS IN AIRWAY INFLAMMATION

ATP, released from cells as a result of multiple stress stimuli including injury and inflammation, has recently been considered as a damage-associated molecular pattern molecule. Once in the extracellular medium, ATP interacts with cell surface P2 receptors belonging to two subclasses, the G protein-coupled P2Y receptors and the P2X ion channels. In airway epithelia, extracellular ATP contributes to mucociliary clearance, a critical process required to maintain the airways clear of inhaled particles or pathogens. Targeting P2Y2 receptors is currently evaluated as a therapy for cystic fibrosis (CF) patients. Likewise, it has been suggested that  $\text{Ca}^{2+}$  entry through activated P2X ion channels would be of therapeutic benefit in this disease. In a first study, we show that ATP promotes TNF- $\alpha$ -elicited IL-8 expression through P2X ion channel-triggered  $\text{Ca}^{2+}$  entry and NF- $\kappa\text{B}$  activation (Fig. 1). P2X ion channels may therefore contribute to airway inflammation and innate immunity. We have generated transgenic mice overexpressing the ATP scavenger CD39 in the airways.

Emilie Theatre, Vincent Bours and Cécile Oury

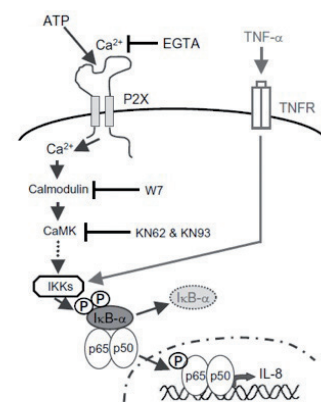


Figure 1. NF- $\kappa\text{B}$  signaling pathway downstream from P2X ion channels in human airway epithelial cells (from Theatre et al. 2009).

### ATP-GATED P2X1 ION CHAN- NELLS PROMOTE NEUTROPHIL CHEMOTAXIS THROUGH RHO KINASE ACTIVATION

Neutrophils are key cells of the innate immune system capable of quickly exiting circulation and migrating into inflamed tissues. In addition, neutrophils participate in the pathogenesis of a vast majority of inflammation-related diseases, such as atherosclerosis, coronary artery diseases, rheumatoid arthritis, inflammatory bowel diseases, cystic fibrosis and sepsis. In our study, we show that activation of P2X1 ion channels by ATP promotes neutrophil chemotaxis both *in vitro*, and, *in vivo*, in a mouse model of E.coli-induced peritonitis. These ion channels may therefore play a significant role in host defense and in inflammation. In addition to its expression on neutrophils, P2X1 ion channels are abundantly expressed on platelets and on vascular smooth muscle cells. Our goal is now to elucidate the mechanisms of P2X1 action by comparing full knock-out and cell type specific transgenic mice in relevant models of inflammatory diseases and associated thrombosis.

Christelle Lecut, Kim Frederix, Christophe Deroanne, Marc Thiry, Céline Faccinnetto, Raphaël Marée, Vincent Bours, and Cécile Oury  
Collaborators : Daniel Johnson, Paul Volders (The Netherlands), Richard Evans (UK)

## LABORATORY OF VIROLOGY AND IMMUNOLOGY

Varicella zoster virus is a human herpesvirus responsible for chickenpox and shingles. Our research is focused on the molecular mechanisms that regulate the infectious cycle and on the mechanisms developed by VZV to interfere with the cellular machinery in order to increase its replication efficacy. We also collaborate with other groups on a mouse model of asthma.

### VARICELLA-ZOSTER VIRUS IE4 PROTEIN INTERACTS WITH SR PROTEINS AND EXPORTS MRNAS THROUGH THE TAP/NXF1 PATHWAY

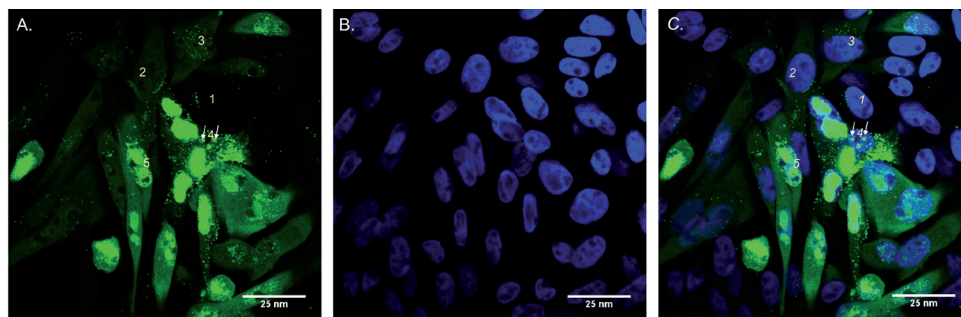
VZV encodes regulatory proteins whose role is critical in triggering and regulating the expression of the other viral genes as well as cellular genes. IE4 is one of these proteins which are expressed very early during the infectious cycle and are therefore called Immediate Early proteins. IE4 could exert its functions at a post-transcriptional level. However, the molecular mechanisms supported by this protein are not yet fully characterized. We have thus attempted to clarify this IE4-mediated gene regulation and have identified some cellular partners of IE4 by a yeast two-hybrid assay. We have shown that IE4 interacts with cellular proteins involved in various mechanisms such as cell cycle, apoptosis. Interestingly, IE4 interacts with three shuttling SR proteins, namely ASF/SF2, 9G8 and SRp20, revealing a new function of this VZV protein that we have further characterized. By Northwestern analysis, we showed that IE4 is able to bind RNA through its arginine-rich region and in immunoprecipitation experiments, the presence of RNA stabilizes complexes containing IE4 and the cellular export factors TAP/NXF1 and Aly/REF, since the interactions are RNase-sensitive. Finally, we determined that IE4 influences the export of reporter mRNAs and clearly showed, by TAP/NXF1 knockdown, that VZV infection requires the TAP/NXF1 export pathway to express some viral transcripts.

Isabelle Ote, Marielle Lebrun, Patricia Vandevenne, Sébastien Bontems, Jacques Piette, Catherine Sadzot-Delvaux  
Collaborators : Cahora Medina-Palazon, Evelyne Manet (France)

### CHARACTERIZATION OF THE FUNCTIONS OF VZV PROTEINS BY ENGINEERING OF THE VIRAL GENOME

By opposition to Herpes Simplex, for which mutants can easily be obtained, VZV is difficult to manipulate and does not recombine easily, making the construction of mutant viruses difficult. Using the Bac technology, that was adapted for our purpose, we were able to produce mutant viruses, which facilitate the characterization of some proteins in the context of the infection. In particular, we constructed simple or double-mutants in which proteins known to be components of either the capsid, the tegument or the envelope were tagged. This allows us to study the assembly of the virions and their transport in infected cells. Assembly and transportation indeed require mechanisms that are not fully characterized and involved interactions with cellular proteins.

Marielle Lebrun, Laura Riva, Jacques Piette, Catherine Sadzot-Delvaux, Franck Dequiedt, Xavier Rambout, Jean-Claude Twizere  
Collaborator : David Hill, Marc Vidal (USA)



VZV 23-GFP infected cells. Panel A: green fluorescence emitted by the 23-GFP fusion protein; Panel B: Nuclei stained with Hoechst; panel C: overlay.

## DEVELOPMENT OF INHIBITORS FOR BRONCHIAL SUBEPITHELIAL FIBROSIS TREATMENT IN CHRONIC ASTHMA

The first aim of our study was to unveil new mediators in asthma in order to better understand pathophysiology and propose or validate new potential therapeutic targets. A mouse model of asthma mimicking acute or chronic asthma disease (developed by the LBTD partner) was used to select genes undergoing a modulation in both acute and chronic conditions. Mice were exposed to ovalbumin or PBS for periods of 1, 5 and 10 weeks (short, intermediate and long term model (ST, IT, LT)) and gene expression in the lung was studied using an Affymetrix genome wide microarray and further confirmed by RT-PCR and immunohistochemistry or western-blotting for selected targets. We reported that 35 genes were positively modulated along the three time-points. These include previously cited genes in asthma pathology but also new potential new target genes such as *Agr2*, *Scin*, *C1qa* and *Cd209e* genes. We also noticed that *Arg1*, *Slc26a4*, *Ear11*, *Mmp12*, *Chi3l3* genes were highly modulated throughout the asthma pathology. These results were also exploited to develop specific microarrays (FIBROCHIP; by our URBC partner) that will be useful for future analysis.

Emmanuel Di Valentin, Jacques Piette, Céline Crahay, Didier Cataldo, Jean-Michel Foidart, Agnès Noël, Nancy Garbacki, Alain Colige  
Collaborator : Thierry Arnould (Belgium)

## LABORATORY OF EXPERIMENTAL PATHOLOGY

We study the immune response induced by innate lymphocytes such as Natural killer (NK) and  $\gamma\delta$  T cells. These lymphocytes are in the intersection between innate and adaptive immunity. Despite a recent new interest, due to a better characterization of innate lymphocyte mechanism of action, the knowledge about these cells is still limited.

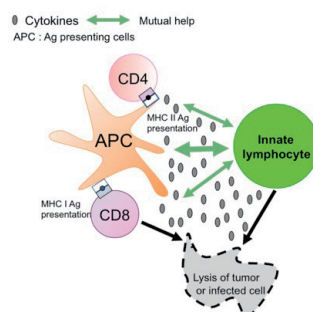
Our primary research model is cervical cancer associated with human papillomavirus infection. This model allows us to study both anti-tumoural and anti-viral immune response and our group has several years of expertise in this model.

## ROLE OF $\gamma\delta$ T CELLS IN UTERINE CERVICAL LESIONS ASSOCIATED WITH HUMAN PAPILLOMAVIRUS INFECTION

In order to study  $\gamma\delta$  T cells in the immune response against HPV infection and uterine cervical cancer development, we quantified these cells and studied the expression of ligands of  $\gamma\delta$  T cell activating receptors in cervical biopsies. This work is done in collaboration with the group of Prof. W. Kedzia of University of Poznan.

We also established a murine model by crossing HPV transgenic mice (which develop skin lesions and uterine cervical tumours after treatment with oestrogen) with mice deficient of  $\gamma\delta$  T cells (in collaboration with M. Girardi from Yale University). This model will allow us to study the impact of  $\gamma\delta$  T cells during the progression of the lesion induced by HPV.

Nathalie Jacobs, Virginie Renoux, Inge Langers, Estelle Dortu  
Collaborators : Michael Girardi (USA), Witold Kedzia (Poland)



*Schematic representation of interactions between innate lymphocytes and innate/adaptive immune cells.*

### STUDY OF ANTIGEN PRESENTING CELL (APC) AND NK CELL CROSS-TALK IN VACCINATION AGAINST HUMAN PAPILLOMAVIRUS (HPV) INFECTION

Several studies have shown that the cross talk between APC and NK cells plays a role in the induction of adaptive immune response. Recently, a vaccine against HPV infection has been developed. This vaccine, based on HPV virus-like particles (VLP), is able to activate APC such as dendritic cells. First, we studied the interactions between NK cells and HPV-VLP and observed that HPV-VLP are internalized in NK cells. We also established a co-culture model with DC and NK cells from the same donor in the presence of HPV-VLP. We observed a mutual activation between these two cell populations and our future plans are to assess the cellular and molecular mechanisms of this cell collaboration and their implication in adaptive immune responses against HPV.

### LABORATORY OF RHEUMATOLOGY

During the last few years, the laboratory of rheumatology focused its research on biomarker identification by the SELDI-TOF proteomics approach. The aim of our studies was driven along three main axes related to the identification of biomarkers specific to a studied pathology, to a common biological pathway and, finally, to a treatment response in rheumatic diseases.

Our laboratory is also studying the role of PPAR- $\gamma$  in rheumatic diseases. Besides the roles in adipocyte differentiation and energy storage, PPAR- $\gamma$ 2 and PPAR- $\gamma$ 1, are involved in cell proliferation, differentiation, and apoptosis, and their role in inflammation and cancer has been recognized.

### NEW PROTEOMICS-BASED BIOMARKERS IN KNEE OSTEOARTHRITIS

Protein expression changes were investigated by SELDI-TOF in 284 serum samples from patients with knee OA classified according to their Kellgren & Lawrence (K&L) score (0 to 4). OA serum samples were also compared to serum samples provided by healthy individuals and RA patients. Protein profiles were analysed by two statistical approaches: a univariate analysis (Mann-Whitney test) and a decision-tree multivariate analysis (a machine-learning algorithm called Extra-Trees N) that highlighted four potential biomarkers.

Dominique de Seny, Gaël Cobraiville, Michel Malaise

### ADIPOGENIC AND ANTI-INFLAMMATORY PROPERTIES OF GENISTEIN ON SYNOVIAL FIBROBLASTS

This year, we showed that a PPAR- $\gamma$  agonist, the plant hormone genistein, had anti-inflammatory properties on synovial fibroblasts (Relic et al, 2009). Indeed, in the presence of glucocorticoids, we observed high expression levels of leptin, a proinflammatory cytokine, and of leptin receptors in synovial fibroblasts despite glucocorticoids ability to downregulate TNF- $\alpha$ -induced IL-6 and IL-8. We further demonstrated that genistein alone or in combination with glucocorticoids induced synovial fibroblast adipogenesis and also highly decreased leptin expression.

Biserka Relic, Mustapha Zeddou, Aline Desoroux, Michel Malaise

### LABORATORY OF IMMUNOLOGY AND INFECTIOUS DISEASES

Our research laboratory has a vast expertise in the investigation of the cellular and molecular pathways leading to immunosuppression, in *in vitro*, *ex vivo* and *in vivo* animal models. We are particularly interested in HIV infection and immunopathogenesis. We have developed a humanized mouse model using an irradiation-free protocol and human CD34 cord blood cells. This protocol leads to the reconstitution of a human immune system in a mouse with almost all the parameters required to



stimulate immune system functions of Homo sapiens. Our laboratory is also investigating the role of several protein phosphatases in the negative regulation of T cell activation, also in cancer. We are collaborating with different laboratories (in house and international) and with the ULg University Hospital (CHU). We are mainly focusing on the investigation of the role of the LYP and VHR phosphatases in autoimmunity, cancer and angiogenesis respectively.

### HIV-HUMANIZED MICE PROJECT

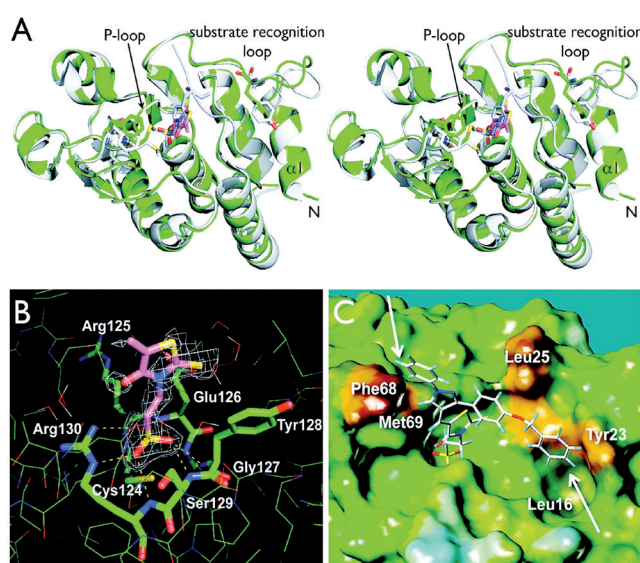
We have developed a suitable small animal model of normal human hematopoietic and immune systems in order to evaluate the anti-HIV-1 response to new immunostimulatory approaches. We are using a new strain of immunodeficient mice NOD/LtSz-Scid IL2R $\gamma$  Null NSG mice. To provide transient myelosuppressive effects and an extended window for the infusion of hematopoietic stem cells in NSG mice, we have established a reproducible radiation-free protocol of high tolerance. More than 50% human hematopoietic chimerism was achieved for over eleven months. Currently we are conducting experiments to further characterise infection and pathogenesis of HIV-1 using different subtypes. Our future plans are to conduct treatment regime and immune-based therapy trial in the HIV infected NOD/LtSz-Scid IL2R $\gamma$  Null mice model.

Maneesh Singh, Pratibha Singh, Claire Vandergeeten, Morgane Bourcy, Michel Moutschen, Souad Rahmouni, Caroline Thielen, Laurence de Leval

Collaborators : Dolorès Vaira, Yves Beguin, Etienne Baudoux (Belgium)

### VHR INHIBITORS AND CANCER

The Vaccinia H1-related (VHR) protein tyrosine phosphatase is a dual-specific Erk and Jnk phosphatase, the loss of which causes cell cycle arrest in HeLa carcinoma cells, suggesting that VHR inhibition may be a useful approach to halt the growth of cancer cells without detrimental effects on normal cells. We reported recently that VHR is upregulated in several cervix cancer cell lines as well as in squamous intraepithelial lesions and squamous cell carcinomas of the uterine cervix. In past years, we finalized and reported the development of novel multidentate small molecule inhibitors of VHR that inhibit its enzymatic activity at nanomolar concentrations *in vitro*, and are active at low micromolar concentrations on several cervical cancer cell lines.



X-ray structural analysis of the VHR-SA3 complex. (A) Stereo ribbon diagram of the overall cocrystal structure of the VHR-SA3 complex (green), aligned with X-ray coordinates of a VHR-substrate peptide complex (1J4X.pdb, VHR gray, peptide light-blue). Tyr23, Phe68, and Met69 (green and gray, respectively), as well as the oxo-thioxothiazolidinyl-ethanesulfonic acid moiety of SA3 (magenta) and pTyr from the peptide (light-blue) are highlighted in stick representation. (B) Close-up of SA3 bound to VHR's active site with visible electron density. Yellow dashed lines indicate hydrogen bond interactions of the sulfonic acid moiety with amino acid residues of the P-loop at the bottom of the catalytic pocket. (C) Modeling of SA3 into visible electron density. White arrows indicate multiple van der Waals interactions with hydrophobic regions at the surface surrounding VHR's catalytic pocket.

High throughput chemical library screening was used to identify hits that were further prioritized in profiling and kinetic experiments. In silico structure-activity relationship analysis, using a crystal structure of VHR, was applied in the search for analogs with improved potency and selectivity. These efforts resulted in the discovery of novel multidentate inhibitors, which are able to interact with both the phosphate-binding pocket and several distinct hydrophobic patches within VHR's active site.

Sofie Vossius, Souad Rahmouni, Michel Moutschen, Philippe Delvenne, Jacques Boniver  
Collaborators : Lutz Tautz, Tomas Mustelin (USA)

## LABORATORY OF BIOLOGY OF TUMOR AND DEVELOPMENT

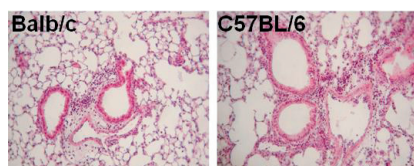
Our research laboratory focuses on mechanisms implicated in asthma pathology and lung inflammation. We use various mouse models of asthma to study mechanisms leading to asthma pathology in order to identify new potential therapeutic targets. Development of new therapeutics in humans appears to be necessary since many patients suffering from asthma are not fully controlled by currently available treatments. Moreover, some of these patients display a significant airway remodeling leading to exaggerated lung function decline and irreversible airway obstruction.

### MOUSE MODELS OF ASTHMA

We applied a mouse model of allergic asthma to two different genetic backgrounds, BALB/c and C57BL/6, in order to study the potential influence of genetic background on airway inflammation and hyperresponsiveness and the mechanisms leading to strain differences. BALB/c mice display greater levels of airway reactivity to methacholine than C57BL/6 mice and exhibit higher numbers of mast cells in lung tissue when compared to C57BL/6. On the contrary, eosinophil and neutrophil counts in bronchoalveolar lavage fluid as well as peribronchial eosinophilia were greater in C57BL/6. Production of IL (Interleukin)-4, IL-5, IL-13, CCL-5 and CCL11 in lung extracts and in BALF was different between the two strains of mice (publication 1). In collaboration with the University of Ghent, a mouse model of asthma of long exposure was applied to BALB/c and C57BL/6 and the conclusion of this study was that chronic allergen exposure induces a marked airway remodeling that parallels a decreased inflammation, which was largely comparable between the two strains.

Maud Gueders, Geneviève Paulissen, Céline Crahay, Florence Quesada-Calvo, Jonathan Hacha, Renaud Louis, Jean-Michel Foidart, Agnes Noël, Didier Cataldo

Collaborators : Chris Van Hove, Kurt Tournoy (Belgium)



*Allergen (Ovalbumin) exposure induced a significant increase in peribronchial and perivascular inflammation in lung tissue both in BALB/c and C57BL/6 mice*

### BIOMARKER DISCOVERY IN ASTHMA-RELATED INFLAMMATION AND REMODELING

The main objective of this proteomic study was to unveil lung proteins up-/down-regulated in asthma by using SELDI-TOF-MS (Surface Enhanced Laser Desorption/Ionisation-Time Of Flight-Mass Spectrometry). We point out several candidate proteins or peptides that were preferentially expressed in diseased mice. Among these candidates, we identified 5 proteins: FIZZ-1, S100A6, CC10, Ubiquitin and Histone H4.

Florence Quesada Calvo, Marianne Fillet, Dominique de Seny, Marie-Alice Meuwis, Raphaël Marée, Céline Crahay, Geneviève Paulissen, Natacha Rocks, Maud Gueders, Louis Wehenkel, Marie-Paule Merville, Renaud Louis, Jean-Michel Foidart, Agnes Noël and Didier Cataldo

### **STUDY OF THE POTENTIAL ROLE OF SPECIFIC MMPs (MMP-19 AND MMP-12) AND ADAM OR ADAMTS IN ASTHMA**

After the allergen challenge of Matrix metalloproteinase-19 (MMP-19) knockout (MMP-19<sup>-/-</sup>) mice, an exacerbated eosinophilic inflammation was detected in bronchoalveolar lavage fluid and bronchial tissue along with an increased airway responsiveness to methacholine. A shift towards increased Th2-driven inflammation in MMP-19<sup>-/-</sup> mice was demonstrated by different markers. Tenascin-C, a substrate for MMP-19, was found to accumulate in peribronchial areas of MMP-19<sup>-/-</sup> after allergen challenges and could be responsible for the exacerbated asthma phenotype.

It has been previously described that Matrix metalloproteinase-12 (MMP-12) plays a role in chronic pulmonary pathologies characterized by an intense tissue remodeling such as asthma and COPD. In a review, we summarize knowledge about MMP-12 structure, functions and mechanisms of activation and regulation, including potential MMP-12 modulation by miRNA. The relevance of silencing MMP-12 by RNA interference is highlighted.

The role of A Disintegrin And Metalloproteinases (ADAMs) and ADAMs with Thrombospondin motifs (ADAMTS) in asthma has also been investigated. These multifaceted molecules bear metalloproteinase and disintegrin domains endowing them with features of both proteinases and adhesion molecules. In 2009, we published a review which focuses on the putative roles of ADAM/ADAMTS proteinases in airway diseases such as asthma and COPD.

Geneviève Paulissen, Natacha Rocks, Maud Gueders, Celine Crahay, Florence Quesada-Calvo, Sandrine Bekaert, Jonathan Hacha, Mehdi El Hour, Jean-Michel Foidart, Agnes Noël, Didier Cataldo, Nancy Garbacki

### **LABORATORY OF HISTOLOGY-CYTOLOGY**

The laboratory of Histology-Cytology is a member of GIGA-research centre in the thematic units «Infection, Immunity and Inflammation» and «Cancer». The research topic is related to the analysis of the cross-talk between hematopoietic and stromal cells in the bone marrow in normal and pathological conditions, with a special interest in bone marrow adipocytes.

### **NEUROPILIN-1, A NEWLY IDENTIFIED CO-RECEPTOR FOR LEPTIN: FUNCTIONS DURING NORMAL AND PATHOLOGICAL HEMATOPOIESIS IN RESPONSE TO LEPTIN PRODUCED BY MEDULLARY ADIPOCYTES**

We have previously shown that bone marrow adipocytes negatively regulate granulopoiesis by inhibiting the production of granulocyte colony stimulating factor (G-CSF) by macrophages. This inhibition required Neuropilin-1 (NP-1) expression and occurred independently of the two known NP-1 ligands, VEGF and Sema3A. In addition it was possible to restore G-CSF production by neutralizing leptin, suggesting that NP-1 and leptin may be involved in a common pathway.

Marie Paule Defresne, Chantal Humblet, Géraldine Poncin, Aurore Beaulieu, Delphine Delneuve

### **LABORATORY OF HAEMATOLOGY**

#### **OPTIMIZATION OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOLLOWING NON-MYELOABLATIVE CONDITIONING REGIMEN**

Allogeneic hematopoietic cell transplantation (HCT) following myeloablative (conventional) conditioning regimen is associated with a high incidence of transplant-related morbidity and mortality, limiting its use to younger patients without medical co-morbidities.

Unfortunately, median patient age at the time of diagnosis of leukemia, multiple myeloma or non-hodgkin lymphoma ranges from 60 to 70 years. Over the past few years, it has become more and more

evident that alloreactivity of donor immunocompetent cells present in the graft against the host tumor plays a major role in eradicating malignancies after allogeneic HCT (graft-versus-tumor effects). Based on these observations, several groups of investigators have developed nonmyeloablative conditioning regimens allowing the use of allogeneic HCT in older patients, and those with co-morbidities. These approaches rely nearly exclusively on graft-versus-tumor effects for tumor eradication.

The aims of the current project are to improve our understanding, to learn how to better manipulate alloreactivity following nonmyeloablative HCT, and to study immune recovery after nonmyeloablative conditioning. The project includes several ongoing prospective clinical studies.

Yves Beguin, André Gothot, Frédéric Baron, Stéphanie Humblet-Baron, Alexandra Briquet, France Bruck, Muriel Hannon, Ludovic Belle, Sophie Servais, Sophie Dubois, Coline Daulne, Pascal Dufour



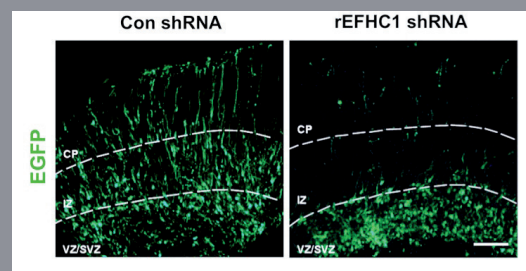
The GIGA-Neurosciences is a thematic research unit (TRU) composed of 7 laboratories. This TRU is devoted to studying a broad range of topics in Neurosciences. More than 80 senior scientists, post-doctoral researchers and graduate students collaborate to investigate the cellular and molecular aspects of the development and activity of the central nervous system in health and disease. The interdisciplinary structure of the GIGA-Neurosciences provides a framework for students to tackle a wide range of topics in Neuroscience including neuronal development and plasticity, neuronal degeneration and repair, neurophysiology and neuropharmacology.

## HIGHLIGHT

### EFHC1, A PROTEIN MUTATED IN JUVENILE MYOCLONIC EPILEPSY, INTERACTS WITH MICROTUBULES TO REGULATE CELL DIVISION AND CORTICAL DEVELOPMENT

*Nat Neurosci* 2009;12(10):1266-U1284.

Mutations in the EFHC1 gene are linked to juvenile myoclonic epilepsy (JME)<sup>1</sup>, one of the most frequent forms of idiopathic generalized epilepsies. JME symptoms appear at the onset of adolescence and include myoclonic jerks, tonic-clonic seizures and occasionally absence seizures<sup>2</sup>. JME is associated with subtle alterations of cortical and subcortical architecture<sup>3,4</sup>, called microdysgenesis, but the underlying pathological mechanism remains unknown. We have previously demonstrated that EFHC1 associates with the mitotic spindle and the centrosomes through its N-terminus<sup>5</sup>. We showed that EFHC1 is a new microtubule-associated protein (MAP) that directly interacts with microtubules (MTs) through a new type of MT binding domain located in the first 45 amino acid region. Using RNA interference (RNAi) technology and overexpression of a dominant-negative form of the protein, we showed that EFHC1 is involved in the regulation of cell division and cortical development<sup>6</sup>. *In vitro*, EFHC1 loss of function disrupted the mitotic spindle organization, impaired M phase progression, induced microtubule bundling and increased apoptosis. More importantly, EFHC1 impairment in the rat developing neocortex by *ex vivo* and *in utero* electroporation caused a dramatic disruption of radial migration (Figure 1).



**Figure 1. Essential role of EFHC1 in radial neuronal migration.** Evaluation of radial migration in cortices of embryonic rat brains electroporated *ex vivo* at E17 and analyzed 4 days later. Transfected EGFP<sup>+</sup> cells display in green. In control condition (Con shRNA), the majority of EGFP<sup>+</sup> cells have migrated into the cortical plate (CP). By contrast, when EFHC1 expression was impaired (rEFHC1 shRNA), most cells are localized in the ventricular/subventricular zone (VZ/SVZ) and intermediate zone (IZ), whereas few cells reached the CP, indicating a dramatic disruption of radial migration. Scale bar: 200  $\mu$ m.

We demonstrated that this effect resulted from failures of cell cycle exit of cortical progenitors as well as defects in the radial glia scaffold organization and in the locomotion of post-mitotic neurons. Thus, we identified EFHC1 as a new MAP that appears to be essential for normal assembly and function of mitotic spindle and for neuronal migration. This mechanism would imply a major role for EFHC1 in cerebral cortex development. We hypothesize that microdysgenesis found in patients suffering from JME3,4 partly results from radial neuronal migration defects and that it could lead to abnormal epileptogenic circuitry during cortical maturation at the onset of adolescence.

Laurence de Nijs<sup>1</sup>, Christine Léon<sup>1</sup>, Laurent Nguyen<sup>1</sup>, Joseph LoTurco<sup>2</sup>, Antonio V Delgado-Escueta<sup>3</sup>, Thierry Grisar<sup>1</sup> & Bernard Lakaye<sup>1</sup>

EFHC1 interacts with microtubules to regulate cell division and cortical development.  
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## LABORATORY OF DEVELOPMENTAL NEUROBIOLOGY

The overall goals of the Developmental Neurobiology laboratory are to uncover new cellular and molecular mechanisms involved in key developmental processes such as the production of neurons and glial cells, their differentiation and their migration in the central nervous system in health and disease. This research laboratory comprises 4 research groups. Brigitte Malgrange's group is studying the molecular mechanisms controlling the generation of hair cells and spiral ganglion neurons in the auditory portion of the mammalian inner ear in physiological and pathological conditions; new neurons during adult neurogenesis and specific neuron types from hES cells (in collaboration with L. Nguyen).

Laurent Nguyen's group uses a combination of genetic, molecular and cellular techniques to address: the genetic mechanisms of cerebral cortex development; the molecular regulation of neurogenesis in adult brain; the pathological mechanisms that underlie neurodegenerative disorders.

Using both *in vitro* and *in vivo* approaches, Bernard Rogister's group is involved in the study of the role of neuregulin in neuronal cell fate choice by a neural stem during development but also in adulthood; the cellular and molecular mechanisms allowing some mesenchymal stem cells of bone marrow to differentiate into functional neurons and the relationships between glioblastoma-initiating cells and neurogenic regions in adult brains.

Pierre Leprince's group applies comparative proteomic analysis techniques to study the structural and metabolic maturation during the differentiation of cells of the astroglial lineage in the cerebellum; the metabolic adaptations that underlie the tumoral transformation of astrocytes; the structural and metabolic alterations of muscle cells following strenuous exercise.

### INSIGHT INTO THE ROLES OF ELONGATOR IN CEREBRAL CORTICAL NEUROGENESIS

The generation of cortical projection neurons is a complex process that relies on the decision of progenitors to leave the cell cycle, migrate to appropriate laminar locations and differentiate into neurons that are stably positioned and are actively extending axonal and dendrite branches. Importantly, these concurrent steps imply dynamic cell shape remodelling which largely depends on the regulation of cytoskeleton components. Thus, identification of new cytoskeleton regulators is essential to shed more light on the molecular mechanisms responsible for the generation of fully differentiated cortical neurons. We have recently reported that the multi-subunit histone acetyltransferase Elongator complex, which contributes to transcript elongation and which is disrupted

Catherine Creppe, Lina Malinouskava, Marie-Laure Volvert, Magali Gillard, Pierre Close, Olivier Malaise, Sophie Laguesse, Isabelle Cornez, Souad Rahmouni, Sandra Ormenese, Brigitte Malgrange, Jean-Paul Chapelle, Gustave Moonen, Alain Chariot, Laurent Nguyen  
Collaborators : Shibeshih Belachew (Belgium), Ulrich Siebenlist (USA)

Deafness commonly results from a lesion of the hair cells and/or the neurons in the auditory part of the inner ear and there are currently no treatments to stop or reverse this regression in hearing abilities. A better understanding of the molecular signals that control the number of progenitors, their differentiation and their tissular organization in the organ of Corti is a prerequisite to get more insight into hair cell regeneration. Therefore, we studied the role of *sox10*, a HMG domain transcription factor, which is mutated in the human Waardenburg-Shah syndrome, which is characterized by Hirschprung's disease, resulting in pigmentary defects and neurosensory deafness. Morphological analysis of *Sox10* mutant mice revealed a significant shortening of the cochlear duct likely resulting from an ongoing depletion of cochlear progenitors. Whereas *Sox10* appears to be dispensable for the differentiation and patterning of the inner ear prosensory progenitors, our data supports a critical role for this transcription factor in the promotion of their survival. We provide genetic evidence that *Sox10*, in a concentration-dependant manner, might play a role in the regulation of *Jagged1*, a gene known to be important for inner ear prosensory development.

Ingrid Breuskin, Morgan Bodson, Nicolas Thelen, Marc Thiry, Laurence Borgs, Laurent Nguyen and Brigitte Malgrange  
 Collaborator : Philippe Lefebvre (Belgium)

Our research aims at investigating the functional organization of the cell nucleus and the development of the auditory organ in mammals. This research laboratory is also specialized in imaging approaches and in particular in immunocytological methods and in electron microscopy techniques.

The nucleolus is the main site of ribosome formation. In addition to the ribosome biogenesis, the nucleolus is involved in other functions such as the formation of various ribonucleoproteic molecules. The nucleolus is a highly organized structure, which consists of 2 or 3 distinct compartments. Our project is trying to better understand the relationships between the various components and functions of the nucleolus. Why does a third nucleolar compartment appear in amniotes in eukaryotes? Could the bipartite nucleoli be at the origin of the tripartite nucleoli during the evolution of species? Could the subdivision of nucleoli in 2 or 3 constituents be directly linked to the structure of ribosomal genes? Is the formation of the different ribonucleoproteic molecules linked to the ribosome biogenesis?

Marc Thiry, Françoise Lamaye, Nicolas Thelen  
Collaborators : Aurore Chatron-Colliet, Nathalie Lalun, Hélène Bobichon, Dominique Ploton (France)

### MORPHOGENESIS OF THE AUDITORY ORGAN IN MAMMALS

The organ of hearing in mammals is undoubtedly one of the most remarkable structures encountered in higher vertebrates. It is composed of alternating sensory cells and supporting cells. At maturity, this highly ordered structure is well described, but many question marks persist about its formation. We are trying to understand how and when the auditory organ sets up, and what are the growth factors that control the development.

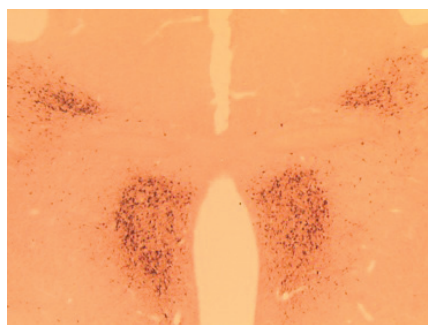
Nicolas Thelen, Ingrid Breuskin, Brigitte Malgrange, Marc Thiry

### LABORATORY OF BEHAVIOURAL NEUROENDOCRINOLOGY

Our research aims at identifying the neuroendocrine and neurochemical mechanisms that mediate the activation and sexual differentiation of reproductive behaviour in higher vertebrates. We are also interested in the metabolism of sex steroids in the brain, in particular the aromatization of testosterone into estradiol and in the interaction of steroids with neurotransmitters. Neurochemical and neuroanatomical sex differences are analyzed to determine to what extent they are able to explain sex differences in behaviour and physiology. We are also interested in the control by steroids of the neural plasticity observed during ontogeny but also in adulthood.

### MODULATION OF STEROID ACTION ON BEHAVIOR BY INTRACELLULAR METABOLISM AND STEROID RECEPTOR COACTIVATOR RECRUITMENT

Testosterone (T) activates male sexual behaviour largely through its aromatization into estrogens. However, variation in local androgen or estrogen receptor expression or in peripheral concentrations of steroid hormones often does not explain variation in the behavioural responses to hormones. We investigate how the rate of T aromatization and the recruitment of nuclear receptor coactivators modulate steroid action in the brain. Recent studies analyzed how aromatase activity is modulated by the sex of the subjects and by the endocrine conditions they were exposed to during embryonic life. We investigated in parallel how the performance of sexual behavior rapidly modifies T aromatization in specific brain nuclei. We also recently cloned and sequenced the steroid receptor coactivator 2 in quail. We are currently analyzing its function in the expression of behaviour by blocking SRC2 expression in the preoptic area via local injection of specific locked nucleic acid antisense (LNA) directed against SRC2.



*Aromatase-immunoreactive cells in the quail medial preoptic nucleus and in the bed nucleus of the stria terminalis*

Jacques Balthazart, Charlotte Cornil, Thierry Chartier, Mélanie Taziaux  
Collaborator : Gregory F. Ball (USA)

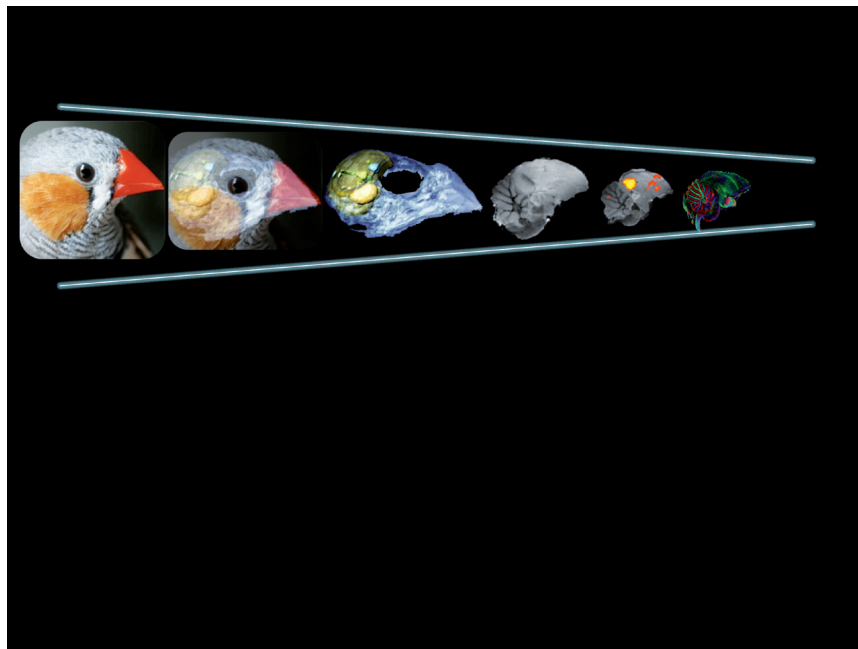
### STEROID-DEPENDENT BRAIN PLASTICITY AND SINGING BEHAVIOR IN CANARIES

The investigation of the causes and consequences of brain “plasticity” is one important topic in neurosciences. Steroid-dependent seasonal variation in the brain of songbirds such as canaries has emerged as one of the best model systems for the study of naturally occurring brain plasticity. Our laboratory investigates the cellular mechanisms underlying this plasticity at two different levels. At the cellular level, we investigate neurogenesis and proteins that influence the migration and

recruitment of new neurons such as Brain-Derived Neurotrophic Factor (BDNF), reelin and Doublecortin (DCX). In collaboration with the Bio-Imaging lab (Antwerp University), we also use magnetic resonance imaging (MRI) and functional MRI (fMRI) to analyse brain plasticity during longitudinal studies in the same subjects and identify brain areas that are activated by a variety of acoustic stimulations including the bird own song, conspecific and heterospecific songs.

Jacques Balthazart

Collaborators : Annemie Van der Linden, Vincent Van Meir, Tiny Boumans, Colline Poirier (Belgium)



*Magnetic resonance imaging allows a visualization of the brain, its various structures and connections, as well as their activity in a living animal (here a zebra finch).*

### THE ROLE OF ESTRADIOL IN FEMALE NEURAL DEVELOPMENT

A central tenet of contemporary theories of mammalian brain and behavioural sexual differentiation is that an organizational action of testosterone, secreted perinatally by the male's testes, controls male-typical facets of brain and behavioural development, whereas no active perinatal sex hormone signaling is required for female-typical differentiation. However, evidence of a possible contribution of estradiol to female-typical behavioural development involved our observation that aromatase knock out (ArKO) female mice showed significantly lower levels of female sexual behavior than wild type (WT) controls following adult ovariectomy and treatment with ovarian hormones.

Julie Bakker, Sylvie Pierman and David González-Martínez

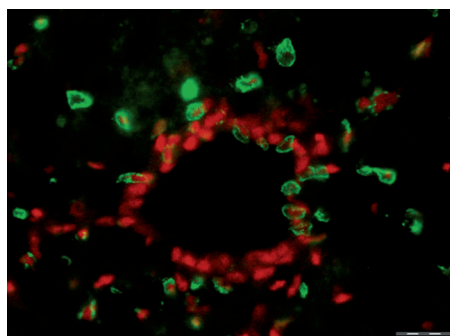
## LABORATORY OF AXONAL REGENERATION AND CEPHALIC PAIN

We are involved in 2 research projects. On the one hand, the “axonal regeneration” group steered by R. Franzen and J. Schoenen studies some of the cellular and molecular facets of post-lesional neuroplasticity using spinal cord and peripheral nerve injuries as models. These studies are based on immunocytochemical, biochemical and behavioural methods, and the use of transgenic animals. Therapeutic strategies to improve axonal regrowth and functional recovery are explored including stem cell therapy. On the other hand, the “cephalic pain” group led by S. Multon and J. Schoenen focuses on the neurobiological aspects of trigeminal nociception exploring the effects of sex hormones, tryptophan metabolism and various therapeutic strategies. The objective is to better understand the pathophysiology of headaches and migraine, and to pave the way for more effective therapies.

### SPINAL CORD INJURY AND ENDOGENOUS NEURAL STEM CELLS

Spinal cord injury remains a devastating situation due to its irreversibility. The discovery of neural stem cells (NSC) within the central nervous system raises new hopes for the development of stem cell-based reparative therapies, either by transplantation or by recruitment of endogenous NSC. Neurogenesis is regulated by various physiological and pathological conditions, like physical exercise, which, in the spinal cord, further leads to increased ependymal cell proliferation. We focused our work on the characterization of these ependymal cells, their response to spinal cord injury and the effect of treadmill training exercise on their putative NSC identity. Our results confirm that ependymal cells from the adult rat spinal cord exhibit progenitor-like characteristics. They can be recruited by spinal cord lesion, but also by treadmill training exercise, which improves locomotor recovery and maintains their stem nature.

Ariane Foret, Renaud Quertainmont, Olivier Botman, Delphine Bouhy, Philippe Amabili, Jean Schoenen and Rachelle Franzen  
Collaborator : Gary Brook (Germany)



*SOX-2 (red) / BrdU (green) ependymal cells after spinal cord injury*

### EFFECTS OF PREVENTIVE ANTI-MIGRAINE DRUGS AND SEROTONIN METABOLISM ON CORTICAL SPREADING DEPRESSION

Cortical spreading depression (CSD) is thought to underlie the migraine aura. There is no convincing evidence that CSD occurs in migraine without aura. We have studied the effect of several preventive anti-migraine drugs on CSD. Lamotrigine has the most potent suppressive effect and also a rather selective therapeutic action on the migraine aura. Valproate merely inhibits CSD propagation while riboflavin has no effect on CSD. Since both of the latter are effective in migraine without aura, this suggests that CSD is not a major causal factor in this migraine type.

Migraine is thought to be a ‘low-serotonin’ disorder. We are studying the effect of brain serotonin metabolism in the CSD and nitroglycerin models of migraine.

Sylvie Multon, Jean Schoenen, Virginie Chauvel, Vladimir Bogdanov

## LABORATORY OF ELECTROPHYSIOLOGY

Our lab is mainly dedicated to cellular and molecular electrophysiology. One of our strengths is that we work on most models used in preclinical neuropharmacology. Recording techniques used are also diverse and include patch clamp recordings, sharp microelectrode (intracellular) experiments and extracellular single-cell and multicellular (“micro-array”) recordings. We use the nucleated patch technique, which allows a very precise biophysical analysis of membrane currents of cell somata in slices. Using these techniques and preparations, we studied various aspects of ion channel physiology and pharmacology in CNS neurons.

### SK CHANNEL AND M CHANNEL PHYSIOLOGY AND PHARMACOLOGY

SK (or KCa2) channels are K<sup>+</sup> channels that are sensitive only to the intracellular concentration of Ca<sup>2+</sup> (EC<sub>50</sub> ~ 300 nM) and have a small unitary conductance (10-15 pS). In neurons, they contribute to the afterhyperpolarization that follows action potentials and underlies the “refractory period”. As other K<sup>+</sup> channels, SK channels are tetramers. Three types of subunits (SK1, SK2 and SK3) have been cloned and, interestingly, display a differential distribution within the CNS, with SK2 being present mostly in cortical areas and SK3 in subcortical areas, including in monoaminergic neurons.

In collaboration with N.V. Marrion (University of Bristol), we demonstrated a differential sensitivity of homomeric SK2 and SK3 channels to extracellular acidic pH (Goodchild et al., 2009). The half-inhibiting pH was 6.2 and 6.8, respectively, making SK3 one of the most pH-sensitive channels so far. We showed in addition that this difference could be ascribed to the presence of two histidine residues in the outer pore region in SK3 (versus one in SK2). Single-channel recordings showed that acidic pH reduces single-channel conductance without affecting open-state kinetics or open probability. We had previously shown that a blockade of SK channels increases burst-firing in dopaminergic (DA) neurons. This finding was significant because this firing pattern induces a large increase in dopamine release at the level of DA terminals. We also showed, in collaboration with a Danish group, that the excitability of DA neurons is inhibited by an opener of voltage-dependent K<sup>+</sup> channels, called M channels.

Guillaume Drion, Olivier Waroux, Jacqueline Scuvée-Moreau, Rodolphe Sepulchre, Vincent Seutin  
Collaborators : Maxime Bonjean, Jean-François Liégeois (Belgium), Terence Sejnowski (USA)

### PHYSIOLOGY OF VOLTAGE-DEPENDENT NA<sup>+</sup> CURRENTS IN DA AND GABAERGIC NEURONS OF THE SUBSTANTIA NIGRA

The focus of my current project is to characterize the functional properties of voltage-activated ion channels in neurons of the rodent substantia nigra. This brain region contains dopamine (DA) and GABA neurons and is implicated in the control of voluntary movement and in the processing of rewarding stimuli. Nigral DA and GABA neurons fulfill distinct functions and have different firing properties and action potential waveforms. To clarify the functions of these two types of neurons, the properties of ion channels underlying the action potential firing need to be described in the different compartments of these neurons. We have started to describe the time- and voltage-dependence of Na<sup>+</sup> currents in both types of neurons as the activation of Na<sup>+</sup> channels is responsible for the shaping of the rising phase of action potentials. Our results show that somatic Na<sup>+</sup> channels differ between the two types of neurons in terms of density and voltage-dependence of inactivation.

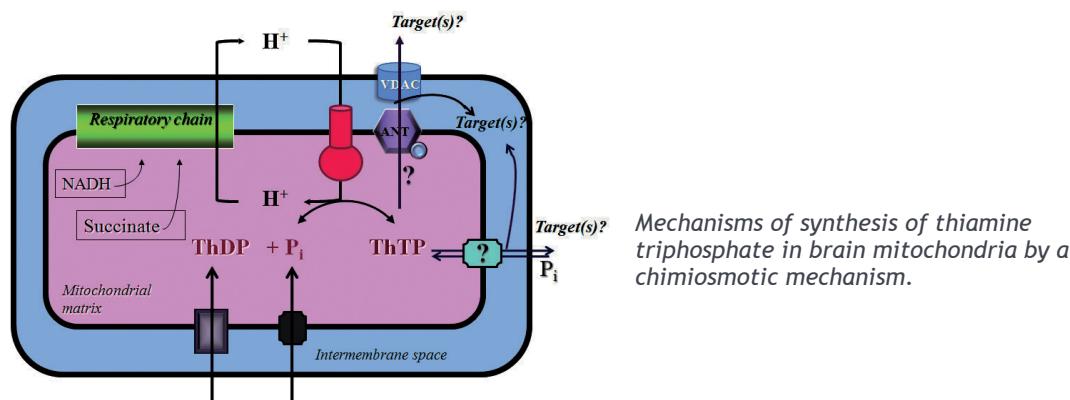
Vincent Seutin, Dominique Engel

## LABORATORY OF BIOENERGETICS, EXCITABILITY AND CEREBRAL PLASTICITY

The work of our laboratory is aimed at understanding the interplay between neuronal bioenergetics, excitability and cell survival. We want to answer the following questions: what are the roles of thiamine triphosphate, adenosine thiamine triphosphate and other phosphorylated vitamin B1 derivatives in the cell energy metabolism? Can the metabolism of phosphorylated thiamine derivatives be engineered in such a way as to counteract the harmful effects of cellular stress and neurodegeneration? We also study a peptide called melanin-concentrating hormone. Our aim is to understand how it controls behaviour and how it affects brain excitability.

### THIAMINE AND CELLULAR BIOENERGETICS

Thiamine (vitamin B1) is an essential molecule for all life forms. The main thiamine compound is thiamine diphosphate, an essential cofactor in cell energy metabolism. Other thiamine compounds are thiamine triphosphate (ThTP) and adenosine thiamine triphosphate that was discovered in our laboratory (Frédérich et al. 2009). Our work aims at understanding how ThTP is synthesized in the brain and to understand its involvement in cellular energy metabolism. Cytosolic thiamine triphosphate levels are regulated by a specific thiamine triphosphatase, an enzyme that we are in the process of characterizing. The brain is particularly sensitive to energy failure and this research may help us understand how neurons adapt to conditions of energy stress, a situation occurring in neurodegenerative diseases.



Marjorie Gangolf, Pierre Wins, Marc Thiry, Lucien Bettendorff, David Delvaux, Tiziana Gigliobianco, Gabriel Mazzucchelli, Edwin De Pauw

Collaborators : Benaïssa El Moulaj, Michel Frédéric, Georges Dive, Benjamin Elias, Luc Angenot (Belgium)

### ROLE OF MELANIN-CONCENTRATING HORMONE IN GOAL-ORIENTED BEHAVIORS

Melanin concentrating hormone (MCH) and its receptor constitute a diffuse neuromodulatory system. Gene expression profile in the brain and behavioural experiments suggest that this system participates in the control of various goal-oriented behaviour.

Our research is focused on the role of MCH in reward. We are more specifically interested in its contribution to behavioural effect of psychostimulant drugs but also on its effect on natural rewards such as food and sex. At the molecular level, our studies are focused on the regulatory effect of MCH on major neurotransmitter system such as glutamate and GABA.

Thierry Grisar, Bernard Lakaye, Sophie Harray

## LABORATORY OF DEVELOPMENTAL NEUROENDOCRINOLOGY

The laboratory of Developmental Neuroendocrinology aims at uncovering the fetal origin of some adult diseases. The team focuses on interactions between mechanism- regulating energy balance, reproduction and cerebral cortex development as well as the early effects of endocrine disrupters on those aspects.

### EARLY EFFECTS OF ENDOCRINE DISRUPTERS ON CEREBRAL CORTEX DEVELOPMENT

Endocrine disrupters are exogenous substances able to disturb endocrine functions. *In utero* exposure to Polychloro biphenyls (PCBs) has been shown to alter memory, learning and audition in rodents and human. The cellular bases of such an effect are still poorly understood. We have shown that *in utero* exposure to PCBs did not influence progenitor cell proliferation in the periventricular zone of the rat cortex but seems to affect the cell cycle exit in that region. We are currently studying the effects of such exposure on radial neuronal migration.

Jean-Pierre Bourguignon, Anne-Simone Parent, Elise Naveau

### EARLY EFFECTS OF ENDOCRINE DISRUPTERS ON SEXUAL DEVELOPMENT AND ENERGY BALANCE

We have formed a “European Society for Pediatric Endocrinology Research Unit” and got funding for developing a project on the effects of endocrine disrupters on sexual development. We have shown that the pesticide DDT was involved in precocious puberty in migrating children and confirmed this data in rodents. We are now studying the effects of endocrine disrupters on the estrus cycle. The mechanisms regulating energy balance and reproduction being strongly linked, we are studying the effects of leptin and Ghrelin on reproduction after early exposure to diethylstilbestrol.

Jean-Pierre Bourguignon, Anne-Simone Parent, Elise Naveau

## GIGA-Signal Transduction

The unit of Signal Transduction is interested in deciphering the molecular and cellular mechanisms underlying NF- $\kappa$ B activation through the classical or the alternative pathway in order to better understand how this transcription factor is deregulated in chronic inflammatory diseases as well as in cancer. We also investigate the role of phosphatases as well as of ceramides in cell apoptosis. The molecular mechanisms allowing cells to sense and to react to physico-chemical cues from the extra-cellular environment to DNA damage or to pathogens are investigated.

### HIGHLIGHT

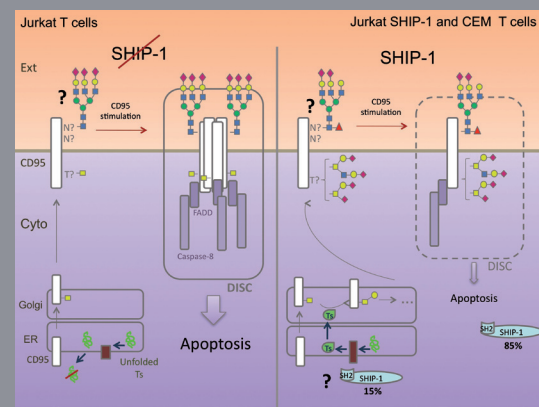
#### SHIP-1 INHIBITS CD95/APO-1/FAS-INDUCED APOPTOSIS IN PRIMARY T LYMPHOCYTES AND T LEUKEMIC CELLS BY PROMOTING CD95 GLYCOSYLATION INDEPENDENTLY OF ITS PHOSPHATASE ACTIVITY.

*Leukemia, in press.*

SHIP-1 functions as a negative regulator of immune responses by hydrolyzing phosphatidylinositol-3,4,5-triphosphate generated by PI 3-kinase activity. As a result, germ-line deletion of SHIP-1 in mice or its down regulation by miR-155, which targets SHIP-1, results in myeloproliferation and B cell lymphoma. On the other hand, SHIP-1 deficient mice have a reduced T cell population, but the underlying mechanisms are unknown. In this work, we hypothesized that SHIP-1 plays anti-apoptotic functions in T cells upon stimulation of the death receptor CD95/APO-1/Fas, the prototypical regulator of T cell fate. Using both primary T cells from SHIP-1<sup>-/-</sup> mice and several T leukemic cell lines, we report here for the first time that SHIP-1 is a potent inhibitor of CD95-induced death. We observed that a small fraction of the SHIP-1 pool is localized to the endoplasmic reticulum where it promotes CD95 glycosylation. This post-translational modification requires an intact SH2 domain of SHIP-1, but is independent of its phosphatase activity. The glycosylated CD95 fails to oligomerize upon stimulation, resulting in impaired DISC formation and downstream apoptotic cascade. These results uncover an unanticipated inhibitory function for SHIP-1 independent of its phosphatase activity at the cytoplasmic membrane and emphasize the role of glycosylation in the regulation of CD95 signaling in T cells. This work may also provide a new basis for therapeutic strategies using compounds inducing apoptosis through the CD95 pathway on SHIP-1 negative leukemic T cells.

Edith Charlier<sup>1,2</sup>, Claude Condé<sup>1,2</sup>, Jing Zhang<sup>4</sup>, Laurence Deneubourg<sup>4</sup>, Emmanuel Di Valentin<sup>1,2</sup>, Souad Rahmouni<sup>1,3</sup>, Alain Chariot<sup>1,2</sup>, Patrizia Agostinis<sup>5</sup>, Poh-Choo Pang<sup>6</sup>, Stuart M. Haslam<sup>6</sup>, Anne Dell<sup>6</sup>, Josef Penninger<sup>7</sup>, Christophe Erneux<sup>4</sup>, Jacques Piette<sup>1,2</sup> and Geoffrey Gloire<sup>1,2\*</sup>. *Leukemia*, in press.

<sup>1</sup>GIGA-Research, <sup>2</sup>Signal Transduction and <sup>3</sup>Infection, Immunity and Inflammation units, University of Liège, Belgium. <sup>4</sup>IRIBHM, Free University of Brussels, Brussels, Belgium. <sup>5</sup>Department of Molecular and Cell Biology, Faculty of Medicine, Catholic University of Leuven, Belgium. <sup>6</sup>Division of Molecular Biosciences, Imperial College London SW7 2AZ. <sup>7</sup>IMBA Institute of Molecular Biotechnology of the Austrian Academy of Sciences, A-1030 Vienna, Austria.



*Novel functions for SHIP-1 in T cells.*

Blue square: N-Acetylglucosamine; Yellow square: N-Acetylgalactosamine; Green round: Mannose; Yellow round: Galactose; Red triangle: Fucose; Violet rhombus: Sialic acid.

## LABORATORY OF VIROLOGY & IMMUNOLOGY

The LVI has been involved for many years in the understanding of the so-called canonical and alternative transduction pathways leading to NF- $\kappa$ B activation. We are currently characterizing the pathways controlled by the NOD-like receptors, ATM kinase and lymphotoxin b receptor. In addition, one project is studying the role of a phosphatase in T lymphocyte homeostasis.

### IDENTIFICATION OF NEW PARTNERS OF THE PROTEIN NOD2 AND EVALUATION OF THEIR POTENTIAL ROLE IN THE NOD2-DEPENDENT PATHWAYS

The mammalian innate immune system has evolved to detect pathogen-associated molecular patterns (PAMPS). Recognition of PAMPS involves membrane-spanning proteins like the Toll-like receptors (TLR) and cytosolic proteins such as the recently identified NOD-like receptors (NLR). This family is comprised of 23 members with important functions in immune sensing in humans, as highlighted by severe inflammatory disorders linked to polymorphisms in some NLR members. Best studied are mutations in Nod2 that are linked to Crohn's disease (CD) and Blau syndrome. Nod2 was shown to sense the bacterial peptidoglycan subunit muramyl-dipeptide (MDP) and to subsequently mediate inflammatory responses in cells by activating the NF- $\kappa$ B and MAPKs signaling pathways.

This project consists of the identification of new Nod2 partners and in the evaluation of their role in the Nod2 signaling pathways. We chose two strategies to select new Nod2 interactants. First, we performed a yeast two hybrid assay using the human ORFeome 15.1 and the protein Nod2 WT or mutated (deletion or CD mutants) as bait. The second proteomic strategy consisted in the purification of Nod2-containing complexes in stably Nod2-expressing cells after MDP treatment or infection by *Listeria monocytogenes* followed by Nod2 partner's identification by mass spectrometry.

Through the proteomic approach, we obtained several potentially interesting partners: cytoskeleton proteins, scaffold proteins, proteins shuttling between the cytosol and nucleus and interfering with transcription and membrane receptors as well as bacterial proteins from *L. monocytogenes*. By these two complementary approaches, we hope to bring new insights concerning the molecular mechanisms underlying the loss of function of CD mutants and the MDP recognition.

Aurore Lecat, Emmanuel Di Valentin, Marianne Fillet, Xavier Rambout, Jean-Claude Twizere, Franck Dequiedt, Jacques Piette, Sylvie Legrand  
Collaborators : David Hill, Marc Vidal (USA)

### DNA DAMAGE RESPONSES SIGNALING CASCADE

Genome integrity is continuously threatened by exogenous and endogenous agents that react with DNA and alter its structure; the biggest challenge comes from DNA double-strand breaks (DSB). These breaks induce a vast array of cellular responses: repair, cell cycle arrest, alternate splicing, activation of transcription factors, modulation of the balance between pro- and anti-apoptotic signals... Anticancer radio- and chemotherapies often rely on DSB-related genotoxic stress to induce the death of tumor cells. Ataxia Telangiectasia Mutated protein (ATM), a nuclear kinase, is the master regulator of the signaling pathways induced by DSB.

In an effort to identify new ATM substrates and thus new signaling cascades that could be used as pharmaceutical targets, we performed a yeast two-hybrid experiment. Sixteen previously unknown ATM interacting proteins were identified, and two of them selected for further characterization. The residues targeted by ATM were identified and confirmed by *in vitro* kinase assays with WT or mutated substrates both in the presence and absence of a specific ATM inhibitor. The identified serine targets were mutated into alanine to prevent their phosphorylation, and the functionality of the mutants assessed by different readouts. We observed that after DSB induced by  $\gamma$ -irradiation or Etoposide, an inhibitor of topoisomerase II, cell survival was modified by some, but not all of the mutants tested.

Emile Ulens, Hélène Sabatel, Emmanuel Di Valentin, Jacques Piette, Yvette Habraken

### TNFL-INDUCED P100 PROCESSING (TIPP) RELIES ON THE INTERNALIZATION OF THE COGNATE TNFR

Several members of the TNFR family can activate distinct NF- $\kappa$ B complexes through the classical and the alternative pathway. However, how a single receptor engages both pathways is still poorly understood. We have further characterized the mechanism by which TNFR induces the activation of the alternative NF- $\kappa$ B pathway. We set out to identify the cytosolic region of LTBR that mediates the activation of p100 processing. We identified a region, which does not display any known structural features, but cell imaging and biochemical studies revealed that the latter played a dual role by controlling LTBR trafficking and TRAF recruitment. Using RNA and pharmacological approaches, we demonstrated that LTBR-induced p100 processing relied on an internalization route that is dynamin- and microtubule- dependent but clathrin independent. We also identified a new atypical TRAF binding site involved in the recruitment of TRAF-2 and -3. This region allowed the alleviation of the inhibitory function of the complex TRAF2/3-c-IAP1/2 associated to NIK. *In vivo*, we found that the maturation of mesenteric stromal VCAM-1 low ICAM-1low MadCAM-1- cells into VCAM-1 high ICAM-1 high MadCAM-1+ organizer cells correlated to an activation of RelB and a down-regulation of cell surface LTBR. The dynamin-dependent internalization of TNFR is a conserved mechanism required for the induction of the alternative pathway since dynasore prevented Lymphotoxin-, CD40L-, Tweak- and BAFF-induced p100 processing while the classical NF- $\kappa$ B was unaffected.

Corinne Ganeff, Géraldine Galopin, Caroline Remouchamps, Cécile Benezech, Layla Boutaffala, Sandra Ormenese, Alain Chariot, Jacques Piette, Emmanuel Dejardin  
Collaborators : Fabrice Bouillenne (Belgium), Paula Norris (USA), Carl Ware (USA), Pascal Schneider (Switzerland), Jorge Caamano (UK).

### VARICELLA-ZOSTER VIRUS INTERFERENCE WITH THE NF-KB AND IRF SIGNALING PATHWAYS

The varicella zoster virus (VZV) is a human alpha-Herpesvirus responsible for two diseases. It causes varicella (chicken pox), establishes latency in sensory ganglia and may reactivate to cause herpes zoster (shingles) in the host. Although Herpesvirus immune evasion has been known for many years, the precise mechanisms by which VZV could interfere with two main signaling pathways, i.e. NF- $\kappa$ B and IRF have not yet been studied. VZV infection causes a severe inhibition of NF- $\kappa$ B induction as measured by NF- $\kappa$ B dimers present in the nucleus or by NF- $\kappa$ B dependent gene activation. Unexpectedly, VZV infection causes IKK activation as measured by kinase assay *in vitro*, but its classical substrate, i.e. the 32 and 36 serines of I $\kappa$ B $\alpha$  are not phosphorylated by the IKK complex.

VZV was also shown capable of interfering with IRF3 activation. In VZV-infected cells, the hyper-phosphorylated forms of IRF3 detected in the nucleus do not form active dimers, are not transcriptionally active and thereby do not lead to the release of interferon (IFN)- $\beta$  in the supernatant. The hyper-phosphorylation of IRF3 does not occur in its C-terminal part and does not involve the classical TBK-1/IKK $\epsilon$  complex. By using viral kinase-deficient mutants of VZV, an active dimeric form of IRF3 can be recovered in the nucleus of infected cells demonstrating that VZV-encoded viral kinases could participate in the viral interference with the IRF pathways.

Patricia Vandevenne, Nadia El Mjiyad, Sébastien Bontems, Marielle Lebrun, Jacques Piette, Catherine Sadzot-Delvaux.

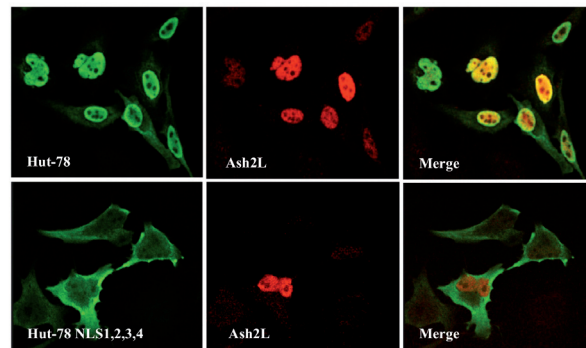


## LABORATORY OF MEDICAL CHEMISTRY

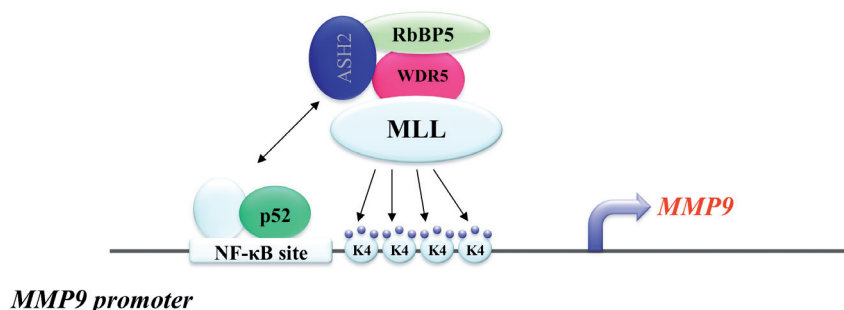
Our laboratory is interested in Signal Transduction in health and diseases. We are currently deciphering the molecular and cellular mechanisms underlying the activation of the transcription factor NF- $\kappa$ B. We elucidate the reasons why this family of proteins is constitutively activated in solid and haematological malignancies. Our work also focusses on the molecular mechanisms underlying the development and progression of human genetic diseases such as Familial Dysautonomia and various other neurodegenerative disorders.

### MATRIX METALLOPROTEINASE-9 GENE INDUCTION BY A TRUNCATED ONCOGENIC NF-KB2 PROTEIN INVOLVES THE RECRUITMENT OF MLL1 AND MLL2 H3K4 HISTONE METHYLTRANSFERASE COMPLEXES

Constitutive nuclear factor (NF)- $\kappa$ B activation in haematological malignancies is caused in several cases by loss of function mutations within the coding sequence of NF- $\kappa$ B inhibitory molecules such as I $\kappa$ B $\alpha$  or p100. Hut-78, a truncated form of p100, constitutively generates p52 and contributes to the development of T-cell lymphomas, but the molecular mechanism underlying this oncogenic potential remains unclear. We show here that MMP9 gene expression is induced through the alternative NF- $\kappa$ B activating pathway in fibroblasts and also on Hut-78 or p52 overexpression in fibroblasts as well as in lymphoma cells. p52 is critical for Hut-78-mediated MMP9 gene induction as a Hut-78 mutant as well as other truncated NF-kappaB2 proteins that are not processed into p52 failed to induce the expression of this metalloproteinase.



*The oncogenic protein Hut-78 is nuclear, colocalizes with Ash2L and recruits WDR5 and RbBP5-containing H3K4 MLL methyltransferase complexes in order to methylate Histone H3 on the MMP9 promoter and to drive gene transcription of this target gene.*



Conversely, MMP9 gene expression is impaired in p52-depleted HUT-78 cells. Interestingly, MLL1 and MLL2 H3K4 methyltransferase complexes are tethered by p52 on the MMP9 but not on the I $\kappa$ B $\alpha$  promoter, and the H3K4 trimethyltransferase activity recruited on the MMP9 promoter is impaired in p52-depleted HUT-78 cells. Moreover, MLL1 and MLL2 are associated with Hut-78 in a native chromatin-enriched extract.

Isabelle Robert, Marie Aussems, Aurore Keutgens, Xin Zhang, Benoit Nennuy, Patrick Viatour, Gaetan Vanstraelen, Marie-Paule Merville, Jean-Paul Chapelle, Laurence de Leval, Frédéric Lambert, Emmanuel Dejardin, André Gothot, Alain Chariot

### THE REPRESSING FUNCTION OF THE ONCOPROTEIN BCL-3 REQUIRES CtBP WHILE ITS DEGRADATION INVOLVES THE E3 LIGASE TBLR1

The nuclear and oncogenic BCL-3 activates or represses gene transcription when bound with NF- $\kappa$ B proteins p50 and p52, yet the molecules that specifically interact and drive BCL-3-mediated effects on gene expression remain largely uncharacterized. Moreover, constitutive GSK3-mediated phosphorylation of BCL-3 triggers its degradation through the proteasome but the proteins involved in this degradative pathway are poorly characterized. A biochemical purification of interacting partners of BCL-3 led to the identification of CtBP as a molecule required for the ability of BCL-3 to repress gene transcription. This purification also defined the E3 ligase TBLR1 as a key protein for BCL-3 degradation through a GSK3-independent pathway. Importantly, all interactions require unique motifs within the N-terminal part of BCL-3. Thus, our data defined a mechanism by which the LSD1/CtBP complex is required for the repressing ability of an I $\kappa$ B oncogenic protein and established a functional link between the E3 ligase TBLR1 and NF- $\kappa$ B.

Aurore Keutgens, Pierre Close, Xin Zhang, Benoît Hennuy, Marie Aussems, Jean-Paul Chapelle, Patrick Viatour, André Gothot, Marianne Fillet, Alain Chariot

### THE PRO-APOPTOTIC C16-CERAMIDE-DEPENDENT PATHWAY REQUIRES THE DEATH-PROMOTING FACTOR BTF IN COLON ADENOCARCINOMA CELLS

Ceramides are central molecules in sphingolipid metabolism. They are involved in the regulation of cancer-cell growth, differentiation, senescence and apoptosis. To better understand how these secondary messengers induce their biological effects, adenocarcinoma cells (HCT116) were treated with exogenous long chain ceramides (C16-ceramide) in order to mimic endogenous sphingolipids. This treatment induced a decrease of cell viability partly due to apoptosis as shown by PARP cleavage and the decrease of pro-caspase 3. Two-dimensional differential in-gel electrophoresis (2D-DIGE) revealed the differential expression of fifty-one proteins in response to C16-ceramide. These proteins are notably involved in cell proliferation, apoptosis, protein transport and transcriptional regulation. Among them, the cell death-promoting factor Btf was found to be implicated in the apoptotic signal triggered by ceramide.

Anne-Françoise Rénert, Pierre Leprince, Vincent Bours, Jean-Paul Chapelle, Jacques Piette, Marie-Paule Merville and Marianne Fillet  
Collaborators : Marc Dieu (Belgium), Jenny Renaut (Luxembourg), Martine Raes (Belgium)

## LABORATORY OF CONNECTIVE TISSUES BIOLOGY

The main research interest of the LCTB is in the field of the biology of connective tissues. This includes: the biochemical and the molecular characterization of the main extracellular matrix (ECM) components and of the enzymes regulating their deposition and remodeling, the study of cell-ECM interactions and determination of the reciprocal regulations operated by mechanical forces developed by cells and issued from the ECM.

Currently specific research projects in signal transduction mainly focus on intracellular signal mediating cross-talk between cells and their environment.

### UNRAVELLING NOVEL RhoGTPases SIGNALING PATHWAYS. INVOLVEMENT IN TUMOR PROGRESSION

The Rho-family proteins form a major branch of the Ras superfamily of small GTPases. Although this family include 23 members, most of the studies focused on the three founding members: RhoA, Rac1 and Cdc42. However, other members of the RhoGTPases family that have been less investigated may have critical functions in various physiological and/or pathological processes as suggested by recent works including ours. A precise knowledge of the cellular functions and molecular network that they



control as well as of the mechanisms of regulation of their own expression and activation represent the goals of our research programme. By using RNA interference, we have highlighted the opponent role of RhoA and RhoC in prostate cancer cells tumorigenesis *in vitro*, RhoA acting as an inhibitor of anchorage-independent growth and RhoC as a promoter. A transcriptomic analysis revealed the differential regulation of several tumor suppressor genes including NAG-1, p21 and p8. These results were confirmed by performing overexpression experiments with tetracycline-inducible clones of prostate cancer cells. Interestingly, the transfection of RhoC-silenced cells with a siRNA targeting NAG-1 reversed the observed gene regulations and restored the *in vitro* tumorigenic properties of prostate cancer cells. Tumor growth of prostate cancer cells in nude mice was significantly delayed by an intratumoral injection of siRNA targeting RhoC, an inhibitory effect relieved by co-injecting siRNA targeting NAG-1. Using siRNA and pharmacological approaches, we observed that the up-regulation of NAG-1 following RhoC silencing required p38MAPK and GSK3 $\beta$  but not ERK1,2. These results suggest that endogenous RhoC contributes to the tumorigenic phenotype of prostate cancer cells by repressing the anti-proliferative factor NAG-1.

Thi Thanh Giang Ho, Johanne Dubail, Audrey Stultiens, Betty Nusegens, Alain Colige, Christophe Deroanne

## GIGA-Systems Biology & Chemical Biology

The “Systems Biology and Chemical Biology” thematic research unit aims at fostering and synergizing the research carried out at GIGA-R in these fields. There are currently three main themes that are investigated, namely; 1) the development and application of statistical, machine learning, and systems theory methods to genoproteomics, image analysis, network inference, and modeling and control of dynamical systems, 2) the development and application of new biophysical concepts for the design of measurement and analysis methods in chemical biology, and 3) the development and application of techniques for the study of protein crystallization and the design of artificial proteins.

There are currently five groups participating in these research themes, namely; the laboratory of Systems and Modeling, the laboratory of Animal Genomics, the laboratory of Mass Spectrometry, the laboratory of Protein Engineering, and the laboratory of Cytology and Histology. The thematic research unit of Systems Biology and Chemical Biology currently totals about 60 members, among which about 30% are post-doctoral fellows or senior scientists. Women make up about 40% of the unit.

### HIGHLIGHT

#### THE ALMA-IN-SILICO INTERREG PROJECT



In 2009 the unit launched and is coordinator of the Alma-in-Silico Interreg IV project, in collaboration with the Universities of Aachen, Maastricht and Hasselt.

This 7.5M € project aims at creating the platform for bioinformatics and systems biology in the Euregio Meuse-Rhine region ([www.alma-in-silico.com](http://www.alma-in-silico.com)).

The project is organized around three main actions, namely; 1) the establishment of a bioinformatics infrastructure based

on open software using web-services, grid computing and mass data storage systems in connection with a bottom layer connected to technological platforms for sequencing, transcriptomics, imaging and proteomics, and a top layer allowing the management of workflows and services requested by end-users, 2) the integration and expansion of the technological platforms of the four centers into a single system biology oriented experiment center, and 3) the establishment of scientific and technical collaborations among academia and industry in the Euregio Meuse-Rhine region for running projects, especially in the fields of neurological, mitochondrial, cardiovascular and inflammatory disease, as well as for the development of high-throughput pipelines for toxicology and pharmacology based on Zebrafish models.

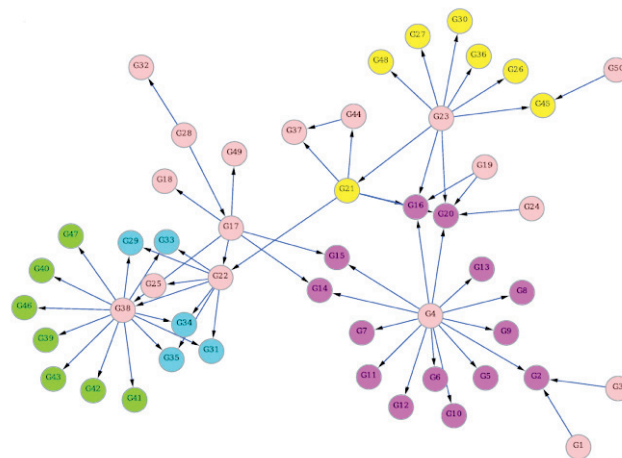
## LABORATORY OF SYSTEMS AND MODELING

The research laboratory of Systems and Modeling (Department of Electrical Engineering and Computer Science) is composed of about 50 researchers whose goal is to contribute in the general fields of modeling, control and optimization of complex systems and networks, by developing novel methods and algorithms of machine learning, statistics and optimization. Within the thematic research laboratory of Systems Biology and Chemical Biology, about 20 members of the lab focus on the development of such methods in order to exploit more efficiently the wealth of available data generated by biological experiments and their application to real dataset so as to extract reliable and quantitative models of biological relevance.

### BIOLOGICAL NETWORK INFERENCE USING STATISTICAL AND MACHINE LEARNING TECHNIQUES

In biology, physical or functional interactions between biological entities such as genes, proteins, transcription factors, micro-RNAs, tissues or diseases are conveniently represented by graphs (or networks). The objective of this project is to develop novel statistical or machine learning techniques for the inference of these networks by integrating various kinds of experimental data and to exploit these techniques in order to identify novel interactions of biological relevance. In 2009, our team won one of the tracks of the DREAM 4 reverse-engineering international competition, challenging the inference of regulatory networks from microarray expression data, and we begun to collaborate with the laboratory of Molecular Biology, MRC Cambridge, UK over the inference of epigenetic interactions in the Yeast *S.Cerevisiae*. Several other collaborations with biologists from GIGA-R are ongoing with the goal of inferring relevant interactions at work in some biological condition from the joint analysis of miRNA and mRNA expression data.

Pierre Geurts, Vân Anh Huynh-Thu, Alexandre Irrthum, Marie Schrynemackers, Louis Wehenkel  
Collaborators: Florence d'Alché-Buc (France), Madan Babu (UK)



### INFERENCE OF DYNAMIC TREATMENT REGIMES FROM CLINICAL DATA

The treatment of chronic-like diseases such as HIV infection, cancer or chronic depression implies long-lasting treatments that may have suboptimal outcome, painful side effects and expensive costs. To enhance these treatments, clinicians often adopt Dynamic Treatment Regimes (DTRs): these are made of sequential decision rules defining what actions should be taken at a specific moment to treat a patient based on his clinical outcome up to that moment. For a few years, a growing research commu-

nity has been working on the development of formal methods (mainly issued from mathematics, statistics and control theory) to infer high-quality DTRs from clinical data. Our own research focuses on developing efficient methods inspired by the reinforcement learning field for inferring optimal DTRs.

Damien Ernst, Raphaël Fonteneau, Louis Wehenkel

Collaborators: Susan Murphy (USA), Marie-José Mhawej, Claude Moog, Cécile Brunet-François, Virginie Ferré, François Raffi, Guy-Bart Stan (UK)

### MACHINE LEARNING METHODS FOR THE EXPLOITATION OF BIOMEDICAL IMAGES

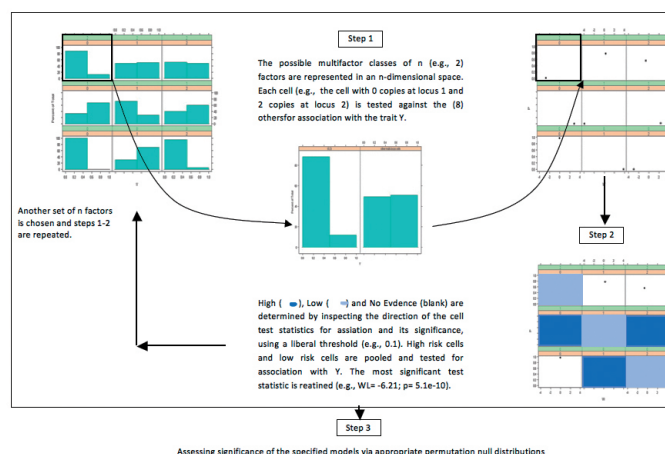
With the advent of novel biosensors and digital image acquisition technology (such as high-content screening, whole-slide virtual microscopy, etc.), scientists are now able to routinely generate terabytes of imaging data. Human interpretation of such large-scale datasets is often time-consuming and error prone. We design machine learning methods for the automatic analysis of images, including content based image retrieval from large and distributed databases, and supervised image classification and segmentation to extract quantitative information. Ongoing studies using these methods include automatic cell sorting and counting in chemotaxis assays, zebrafish physiology quantification for the assessment of developmental toxicity of compounds, whole-slide tissue quantification for the evaluation of new molecules against cancer in experimental models, high-throughput detection of protein crystals in crystallization droplets for structural genomics and structure-based drug discovery.

Pierre Geurts, Raphaël Marée, Olivier Stern, Louis Wehenkel, Didier Cataldo, Marc Muller

Collaborators: Martina Fenske and Kurt Hoffman (Germany)

### STATISTICAL APPROACHES FOR THE STUDY OF GENE-GENE AND GENE-ENVIRONMENT INTERACTIONS

There is a growing belief that disease risk can often best be modeled by interactions between biological components or, at least, that synergy between biological components can no longer be ignored. The semi-parametric Multifactor Dimensionality Reduction (MDR) strategy harbors many possibilities for future enhancements to deal with (statistical) hurdles, including the curse of dimensionality, a flexible use of different study designs (family-based and/or population-based), a flexible use of phenotypes (measured, categorical, survival, multivariate), missing genotypes and/or phenotypes, a flexible use of marker types (SNPs and/or CNVs), and allows for optimal correction for the winner's curse.



Working out the most crucial (from a data-driven point of view) enhancements in detail and extensively testing the methodologies on simulated and real-life data is currently the core business of this project, but we also work on the extension of Random Forest methodologies to better adjust for important confounding factors or to better accommodate specific characteristics of the biological problem under study, such as genetic heterogeneity. A second line of our work focuses on the integration of expression data and genomic data. For instance, we have started to investigate the complex nature

of genetic variant effects on gene expression, by extending classical eQTL approaches. In particular, we develop multi-dimensional models to link clusters of genetic loci to (clusters of) expression traits and represent them as interaction networks that allow the researcher to explore the whole spectrum of pair-wise relationships among the genes and account for them in association models linking genetic markers to expression traits.

Tom Cattaert, Jestinah Mahachie, Kristel Van Steen

Collaborators: Malu Calle (Spain), L Franke (The Netherlands) and Nuria Malats (Spain)

### FEEDBACK ASPECTS OF SPATIAL LEARNING AND MEMORY IN RATS

The Morris water maze is a standard test for studying spatial learning and memory, where a rat has to escape a circular pool by finding a platform hidden under the water surface using navigational cues. Although the test is widely used in neuroscience and psychology to decipher neuronal and conceptual structures of spatial memory, the mnemonic-navigational demands of the task are not well understood. By developing a dynamic mathematical model of rat, we found an inherent difference in the navigational control strategy for experiments where either one cue or three cues were available, and that simple cue dependent search strategies have limited effect on the escape latency as even fuzzy information on the platform location is sufficient to explain the escape latencies observed in trained rats.

Dirk Fey, Eric Bullinger

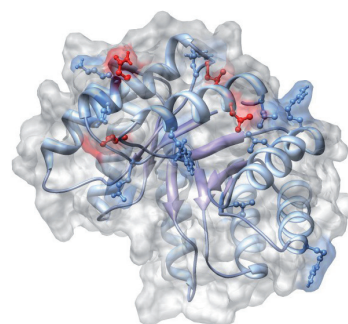
Collaborator: Sean Commins (Ireland)

## LABORATORY OF MOLECULAR BIOLOGY AND GENETIC ENGINEERING (LBMGG)

The lab of Protein Engineering is part of the Laboratory of Molecular Biology and Genetic Engineering of GIGA-R. The lab is composed of about 10 researchers focusing on the development and application of methods for protein crystallization, for the de novo design of artificial proteins, and on applications of biomimetics, in particular to steel coating problems.

### DE NOVO DESIGN OF ARTIFICIAL $\alpha/\beta$ BARREL PROTEINS: COMPUTATIONAL DESIGN AND DIRECTED EVOLUTION

One way to gain insight into the sequence-structure-function relationship in proteins is to de novo design artificial proteins. The applications of such a study are multiple. For example, in the context of the post genomic era, it would enable a more rapid interpretation of the wealth of genomic information; in medicine and industry, the ability to precisely engineer proteins to perform a specific function under a wider range of conditions. Despite impressive success in de novo protein design, designing a folded protein of more than 100 amino acids remains a challenge. In our lab, four generations of Octarellins, de novo polypeptides of more than two hundred amino acids modelled on the  $\alpha/\beta$  barrel fold, have been built and structurally characterized using biophysical and spectroscopic methods. The last generation of Octarellins was designed following a hierarchical method combining the specificity of rational design, the power of computational design, and the efficiency of directed evolution. The resulting artificial proteins have a secondary structure content typical of a TIM barrel and display a stable tertiary structure. Moreover, they are soluble, thermostable and resistant to proteolytic digestion. Crystallisation tests and NMR studies are in progress in order to determine the real structure adopted by these proteins.



*Model of Octarellin V*

Cécile Van de Weerd, Christine Evrard

Collaborators: Christian Damblon, André Matagne, Mireille Dumoulin (Belgium)

## STUDY OF CRYSTALLIZATION PROCESSES ON EARTH AND IN MICROGRAVITY

The crystallisation of proteins is essential for the analysis of protein structure-function relationships and the decoding of the genome. The main processes underlying the crystallisation of proteins, the nucleation and the growth of crystals, are not very well understood. Fundamental and applied studies of these processes are necessary for the development of more efficient approaches for the crystallisation of new proteins.

In collaboration with Belgian and Spanish groups, in work supported by the European Space Agency (ESA), we study the relationships between the growth conditions and the crystal quality, as well as the effect of microgravity on the latter. This study requires working in a multidisciplinary manner, with proteins owning different properties as model systems, using a wide range of techniques which include the production, purification and crystallisation of proteins, the analysis of protein crystals by x-ray crystallography, the visualization of the growth processes using different diagnostic tools, simulation and physico-chemical measurements. Since the 90s, our laboratory has been actively participating in crystallisation studies under microgravity conditions, notably in the context of the Octarellins project. For the last five years, our group has been involved several times in PromISS space experiments (PromISS standing for “Protein Microscope for the International Space Station”), the Granada Crystallisation Facility (GCF), and, more recently, the Protein Crystallisation Diagnostics Facility (PCDF), an instrument developed for the study of the nucleation and crystallisation processes of proteins from solutions with advanced diagnostics tools like video, high resolution microscope, dynamic light scattering device and Mach-Zehnder Interferometer. This instrument may be used to conduct detailed measurements of physical phenomena in individual reactors, and to control these phenomena through changes in temperature and concentration of the crystallising solution.

Christine Evrard, Cécile Van De Weerd

Collaborators: Dominique Maes, Klaas Decanniere (Belgium), Fermin Otalora, Jose, Antonio Gavira, Luis David Patiño Juan-Manuel García-Ruiz (Spain)

## BIOCOAT: COATING OF STEEL SURFACES BY BIOMIMETICS

The creation of new functional coatings on inorganic surfaces is a challenge in the development of new materials. The goal of the present work is to develop multifunctional coatings on large steel surfaces by biomimetics using green technologies. Biomimetics refers to human-made processes, substances, devices, or systems that imitate nature. Although biomimetics is in its infancy, with only a few applications commercialised so far, this science enables new insights into material science. In collaboration with ArcelorMittal Liege Research and two other academic laboratories of the ULg, through a multidisciplinary approach, we developed multi-layer films on steel surfaces following a bottom-up approach. Films are built layer-by-layer with designed inorganic molecules (polyelectrolytes) and natural or newly designed proteins. All these molecules are designed to be soluble in aqueous solutions and the films are built in mild conditions by dipping the metallic surfaces in baths containing the molecules of interest. By carefully choosing these molecules we confer multiple properties to the films. So far, several anti-bacterial coatings were built on stainless steel surfaces. Moreover, in GIGA-R, we genetically engineer peptides for their ability to specifically bind to inorganic materials. We fuse these peptides to fluorescent molecules to visualize their interaction with the surface (PCT/EP2009/004876 ‘Inorganic-binding peptides as tools for surface quality control detection’), magnetic beads to sort inorganic nano-powders (PCT/EP2009/004876), antibacterial peptides in order to build antibacterial coatings on steel surfaces.

Cécile Van de Weerd

Collaborators: Catherine Archambeau, Michel Beguin, Christophe Detrembleur, Anne-Sophie Duwez (Belgium)



## LABORATORY OF MASS SPECTROMETRY

The objective of the Mass Spectrometry Laboratory is to contribute to the development of new fundamental biophysical concepts and to enlighten their applications in the fields of advanced analysis methods in the field of chemical biology (proteomics, metabolomics, structural analysis).

### MASS SPECTROMETRY OF NUCLEIC ACID STRUCTURES AND COMPLEXES

Electrospray mass spectrometry has now proven to be a valid method for the study of DNA architectures and their complexes with small molecules (determination of the stoichiometry and of binding constants). The DNA structures include Watson-Crick duplexes, but also triplexes and quadruplexes, which are relevant targets in oncology. We investigate whether these DNA structures and their complexes with small ligands are conserved in the gas phase, using ion mobility spectrometry, H/D exchange and special fragmentation techniques. We continue the development of action spectrometry both in IR and UV and photophysics of ions from biomolecular edifices. Experiments are conducted with the synchrotron and the free electron beams in Paris.

Valérie Gabelica, Frédéric Rosu, Anastasia Burmistrova, Edwin de Pauw

### PROTEOMICS IN HOST-GUEST INTERACTIONS

Proteomics and metabolomic profiling is used to characterize the digestion of wood products by termites in a project called TERMITOFUEL, in collaboration with Gembloux Agrobiotech. Proteomic analysis allows characterization of the protein content of the termites' intestine. Glycans profiling allows comparison of the efficiency of the enzymatic digestion. In other projects, proteomic methods are used to determine the proteome patterns related to differential adaptations and metabolic changes of aphids to cope with plant defense mechanisms, with a focus on the role of the bacterial endo-symbionts. The proteome comparison of insects reared on different host plants and the use of artificial diets, including diverse molecules, are studied to identify the respective origin (symbionts or insect itself) and function of the identified proteins.

Edwin de Pauw

### INNOVATIVE SEQUENCING METHODS, APPLICATION TO PEPTIDOMICS, PROTEOMICS AND GLYCOMICS, INCLUDING IMAGING MS

In-source decay (ISD) in MALDI leads to a useful method for sequencing purified peptides and proteins that could, in the near future, replace Edman sequencing. A model on H radical donating capacities of the matrix was developed and new matrices elaborated. On that basis, a matrix-enhanced ISD approach was successfully applied to sequence peptides from venoms and proteins, as well as oligonucleotides and glycans. ISD is a promising tool to include in a top-down proteomic strategy. In parallel, we transfer the method to other target proteins, mostly bearing post translation modifications. Finally, the first MALDI images allowing identification of the proteins without the use of in situ enzymatic digestion were obtained.

Loïc Quinton, Gabriel Mazzucchelli, Marie-Alice Meuwis, Rowan Dobson, Nicolas Smargiasso, Delphine Debois, Edwin de Pauw

## LABORATORY OF HISTOLOGY AND CYTOLOGY

The laboratory of Histology and Cytology has a long experience in 2D and 3D cell culture, and in classical techniques to characterize cells or tissues. The cellular interactions with biomaterials (artificial lens, polymers, metallic pieces, textiles or gold nanoparticles), the cellular response to environmental contaminants and cancer cell surface markers are its fields of research. The laboratory is involved in different projects in relation to proteomics, in particular the differential proteomic profiling of cells

exposed to chemical compounds, the search of membrane targets specific to normal or cancer cells, and tissue imaging by mass spectrometry.

#### IDENTIFICATION AND CHARACTERISATION OF ENVIRONMENTAL ESTROGEN EXPOSURE BIOMARKERS USING DIFFERENTIAL PROTEOMIC TECHNIQUES ON MCF-7/BOS CELL LINE

We developed a screening test allowing the detection of estrogens and estrogen-like compounds by monitoring protein biomarkers of estrogenic activity. The use of differential proteomic techniques such as 2D-DIGE (Two Dimension Difference Gel Electrophoresis) or ICPL (Isotope Coded Protein Label) conjugated with mass spectrometry constitutes a powerful tool for the study of the protein abundance changes.

Mike Collodoro, Marie-Claire de Pauw-Gillet

#### IDENTIFICATION OF PROSTATE CANCER CELL BIOMARKERS AND TARGETING WITH A SUITABLE BIOSENSOR

This project intends to prove the concept of using optoacoustic imaging in combination with biologically functionalized nanoparticles as an integrated biosensor based system for the production of specific and sensitive data for accurate diagnosis of prostate cancer. The objective is to develop a biosensor composed of a detection element (gold nanoparticles with specific spectral properties) and a recognition element (prostate cancer specific antibody). In parallel, we seek to identify new targets for the biosensor through proteomic analysis using prostate cancer cell lines (LNCaP, PC3, DU145, C4-2B) and biopsies from healthy and cancer tissues.

Maximilien Fléron, Daureen Schol, Marie-Claire de Pauw-Gillet

### LABORATORY OF ANIMAL GENOMICS

The laboratory of Animal Genomics focuses on the forward genetic dissection of Mendelian and complex traits in human and domestic animals. It has an epigenetics program, rooted in unique animal models, with special emphasis on microRNA biology. It develops approaches for the utilization of molecular information in livestock breeding, including genomic selection and transgenics. Members of the lab collaborate in the Bioinformatics program of the thematic research laboratory in Systems Biology and Chemical biology by developing genome annotations and novel methods for gene mapping.

#### POLYMORPHIC miRNA MEDIATED GENE REGULATION

In 2006, the laboratory of Animal Genomics reported the first mutation that caused a phenotype by perturbing miRNA mediated gene regulation: the myostatin Texel mutation. In order to assist in the identification of polymorphisms that might act along the same lines in other species, we mined the public databases for SNPs destroying or creating putative miRNA target sites in 3' UTR, or affecting sequence and expression of miRNAs or components of the silencing machinery, in vertebrates. A database has been created ([www.patrocles.org](http://www.patrocles.org)) that compiles these polymorphisms and provides contextual information for prioritization.

Denis Baurain, Samuel Hiard, Michel Georges

#### DEVELOPMENT OF METHODS TO MAP GENES UNDERLYING COMPLEX TRAITS

We have developed a method based on Hidden Markov Models exploiting population and familial information for phasing and imputation of high-density SNP genotypes on large data sets. We show that the Hidden States can directly be used in a mixed model framework to map QTL influencing quantitative traits. The proposed mixed model includes a random polygenic effect with covariance structure deduced from pedigree or genome-wide SNP data. The method has been extended to binary phenotypes.

Tom Druet, Michel Georges

Collaborator: Frédéric Farnir (Belgium)





## Translational Research : GIGA/CHU

The research conducted at GIGA-R concerns life sciences and aims at a better understanding of the structure and functioning of the human being. Like many researchers, GIGA-R is from faculties where health sciences are studied (Faculty of Medicine and Faculty of Veterinary Medicine) and much of the work is dedicated to human dysfunction, i.e. disease.

This research improves the level of knowledge, but we also hope that it will contribute to improved understanding of disease and hence, better detection, diagnosis and treatment. This translation of fundamental research results into medical practice has often been by chance; this is how much progress in medicine has been made. Today's challenges in medicine are more and more demanding because we realise that it's no longer by chance or experimentation like, by way of example, the continued progression in the treatment for Leukemia: we must strive to improve our understanding of these diseases and, based on new information gathered, devise new treatments; this translation of discoveries of advanced fundamental research for the benefit of patients is now called "translational research".

Evidently, with the proximity of GIGA-R to the CHU, we expect this transitional research to be very active in Liege. This was proved by an investigation carried out both with the help of CHU doctors (by Professor M. Malaise, president of the CHU Medical Council) and GIGA-R researchers. We have thus identified close to 200 research projects that fulfil the criteria of translational research. You can find a non-exhaustive list of themes of this research below.

We must point out that several recent initiatives aim to emphasise the efforts made on transitional research. We should mention the federal government's Cancer Plan which supports 5 projects carried out with the collaboration of CHU clinicians and GIGA-R researchers in Liege. The themes of these projects are glioblastoma, cervical cancers, non-Hodgkin's lymphoma, tumor angiogenesis and mesenchymal cells.

### IMMUNOLOGY AND INFLAMMATION

- Bowel inflammatory diseases (Crohn: genetics, mechanisms, biomarkers, new treatments)
- Animal model for the study of immunomodulation in HIV+ patients
- Interstitial macrophages
- Rheumatic diseases (biomarkers, new treatments)
- Chronic rhinosinusitis (biomarkers)

### CARDIOVASCULAR SYSTEM

- Cardiac remodeling (biomarkers)
- Cardiac valves (coronary artery diseases, myxoid dégénération)
- Artherosclerosis ( mechanisms, biomarkers, aneurysm)
- Myocardial infarction (biomarkers)
- Arterial thrombosis (new treatments)

## CANCERS

- Biomarkers in cancerology : hepatic metastases, prostate cancer, DNA methylation, colon cancers
- Angiogenesis and extracellular matrix (mechanisms, *in vivo* imaging, new treatments)
- Breast cancer (interactions with skin adipocytes, mTOR inhibitors)
- Cancer of the uterine cervix (rôle of the local immunity)
- Epithelial metaplasia
- Leukemia : new approaches (stem cells, cord blood, mini-transplants, immunotherapy, mesenchymal stem cells, iron metabolism, reaction of the graft against the host, ZAP70 in myeloid leukemia)
- Non-Hodgkin lymphoma (microRNA, T cells lymphoma)
- Myeloma (proteases, new treatments)
- Glioblastoma (cancer stem cells, intelligent drugs)

## NEUROSCIENCES

- Parkinson's disease (biomarkers)
- Alzheimer's disease (new treatments)
- Brain and sexual development

## ENDOCRINOLOGY AND UROGENITAL SYSTEM

- Type 1 diabetes (Lymphoid tyrosine phosphatase)
- Endometrium, Endometriosis
- Ovarian cryopreservation
- Pituitary adenoma

## MUSCULOSKELETAL SYSTEM

- Cartilage biomarkers in childhood
- Stem cells in musculoskeletal diseases
- Tendon healing process

## GENETIC

- Genetic diseases (mucoviscidosis, pituitary adenoma)

## EXTRACELLULAR MATRIX AND ANGIOGENESIS (EXCEPT CANCERS)

- ophthalmology (age-related macular degeneration)
- dermatology (psoriasis, skin ulcers)
- ENT (vocal cords)



## 2009 Key numbers

|                                   |                       |
|-----------------------------------|-----------------------|
| <b>Members</b>                    | <b>542</b>            |
| PhD                               | 213                   |
| PhD Students                      | 201                   |
| Foreign researchers               | 85 (28 nationalities) |
| Technicians                       | 95                    |
| Administration                    | 23                    |
| Logisticians                      | 10                    |
| <b>PhD Thesis</b>                 | <b>25</b>             |
| <b>Publications</b>               | <b>208</b>            |
| FI < 3                            | 97                    |
| FI between 3 and 5                | 65                    |
| FI between 5 and 10               | 38                    |
| FI > 10                           | 8                     |
| <b>Seminars</b>                   | <b>56</b>             |
| ULg speakers                      | 6                     |
| Belgian speakers (non-ULg)        | 16                    |
| International speakers (Europe)   | 24                    |
| International speakers (non-Eur.) | 10                    |
| <b>Patents</b>                    |                       |
| Patent applications filed         | 10                    |
| Patent applications published     | 12                    |

|                                                                       |           |
|-----------------------------------------------------------------------|-----------|
| <b>FNRS (Belgian National Fund for Scientific Research) positions</b> | <b>86</b> |
| - Research Fellow                                                     | 26        |
| - Postdoctoral Researcher                                             | 15        |
| - Research Associate                                                  | 30        |
| - Senior Research Associate                                           | 6         |
| - Research Director                                                   | 6         |
| - Scientific Research Worker                                          | 3         |
| FRIA Fellowships                                                      | 44        |
| Televie Fellowships                                                   | 46        |
| FNRS Logisticians                                                     | 3         |

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### GIGA-Signal Transduction

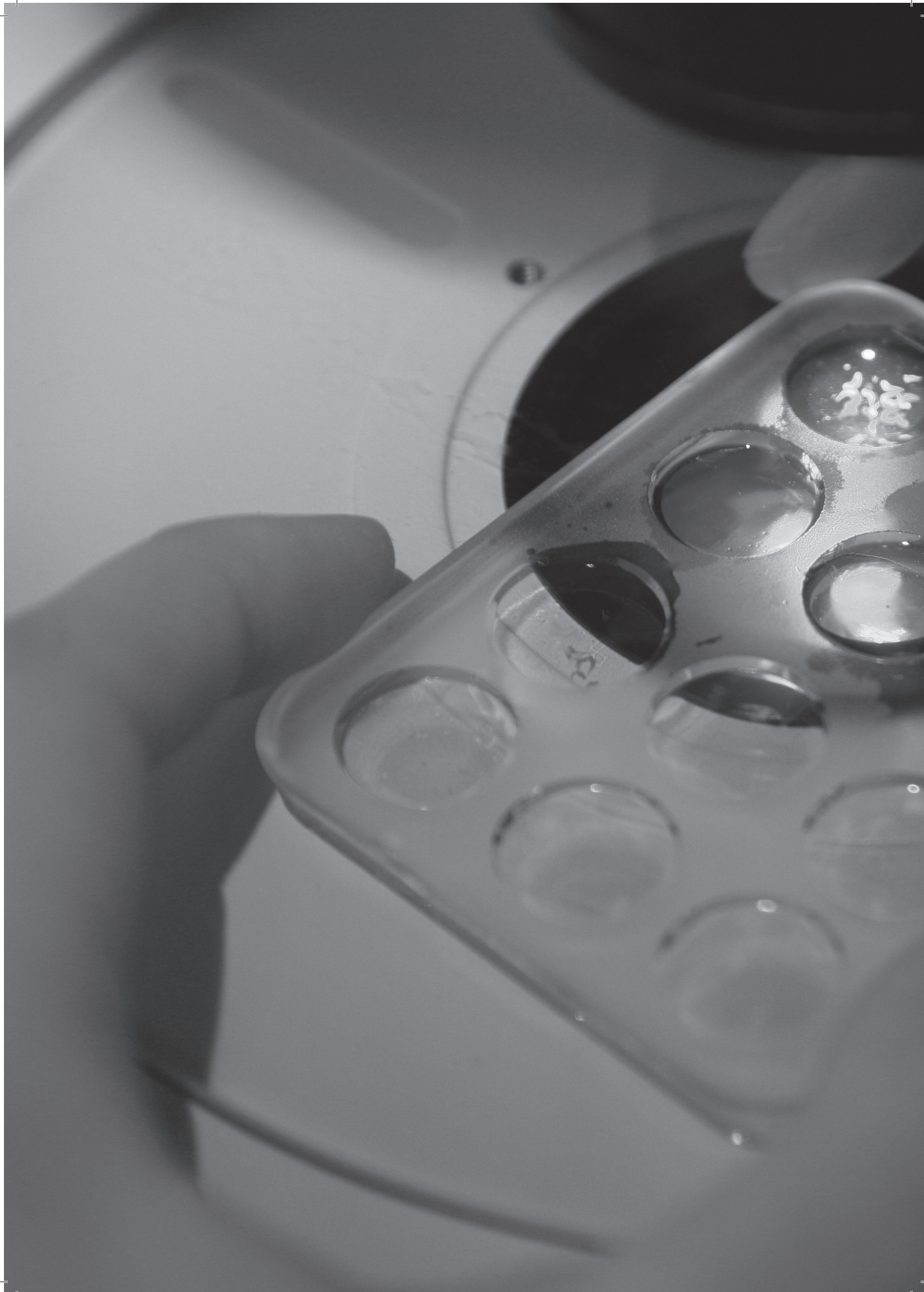
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### GIGA-Systems Biology and Chemical Biology

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Like most academic research labs, companies in the Biotech sector like Eurogentec often require access to specific technology and expertise that lie outside their core competences.

Most scientists have, at least once in their career, invested a significant amount of money and time in purchasing a novel instrument, eventually leaving it mainly unused under its protection sheet. This is particularly true for cutting edge technology which requires highly specialised technical and scientific expertise, as well as continuous operation, in order to ensure optimal performance.

The GIGA technological platforms provide the scientists in the Biotech sector with flexible and cost-effective access to cutting edge technology together with the necessary scientific competences.

Eurogentec regularly outsources Quality Control tests relating to Biopharmaceutical process developments. Along with the strict service outsourcing, the GIGA platforms offer a unique opportunity for collaborations between academic and industrial researchers for the development and commercialization of new tools and services around these platforms.



*Philippe Cronet*

Chief Scientific Officer  
Eurogentec

An efficient research requires an access to a broad range of technologies, some of them requiring specific equipment or expertise.

GIGA-technology platforms are essential to support the researcher's needs.

Each GIGA-technology platform

- is managed by an expert who is fully dedicated to the platform and can give advice and/or perform the experiment
- is equipped with state-of-the art equipment under the responsibility of the platform's manager
- offers a broad range of services, from routine to sophisticated services
- is open to academic researchers as well as to the private sector



## GIGA-GenoTranscriptomics

### HIGHLIGHT

- High throughput sequencing on Roche FLX454 or Illumina Genome analyser

In 2009, the GIGA-GenoTranscriptomics platform acquired two new Generation sequencers: the ROCHE GS FLX454 and the ILLUMINA Genome analyser. High throughput sequencing supports a broad range of applications such as deep sequencing, re-sequencing, transcriptomics, metagenomics.

A "High Throughput sequencing day" was organised on May 26th to launch this new service and gathered more than 130 researchers from both the academic world and the private sector.

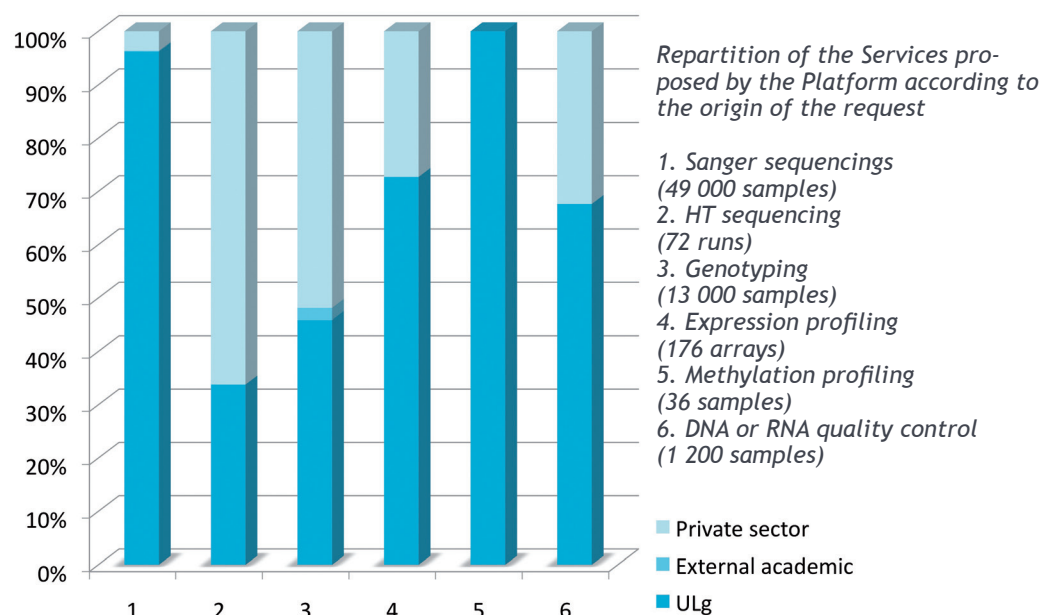
The HT sequencing technologies evolved rapidly and are continuously being improved. Benoit Hennuy and Véronique Dhennin, the two logisticians in charge of this service, interact very frequently with the technical experts from each of these companies to be continuously informed of the new developments. So far, 72 runs have been processed using one of these new technologies, about half of them for academic research programs, the others for private sector.

In addition to this new service, the platform offers conventional sequencing, genotyping and transcriptomics analyses.

In 2009, the platform performed:

- 49 800 Sanger sequencing reactions and runs
- 176 transcriptomics analyses on either the Affymetrix or the Illumina platform
- more than 13 000 genotyping analyses, half of them using an Illumina bovine SNP60K chip developed by the Animal Genomics Unit
- 36 methylation profiles on ILLUMINA platform
- more than 1 100 DNA or RNA QC

The genotyping performed directly from the mouse tail was optimized in order to allow a faster and more efficient genotyping during the transgenesis process, providing a great help for the mice facility.



## EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED IN PART THANKS TO THE GIGA GENOTRANSCRIPTOMICS PLATFORM

- Robert I, Aussems M, Keutgens A, Zhang X, Hennuy B, Viatour P, Vanstraelen G, Merville MP, Chapelle JP, de Leval L, Lambert F, Dejardin E, Gothot A, Chariot A.  
*Matrix Metalloproteinase-9 gene induction by a truncated oncogenic NF- $\kappa$ B2 protein involves the recruitment of MLL1 and MLL2 H3K4 histone methyltransferase complexes.*  
Oncogene. 2009 Apr 2;28(13):1626-38.
- Di Valentin E, Crahay C, Garbacki N, Hennuy B, Guéders M, Noël A, Foidart JM, Grooten J, Colige A, Piette J, Cataldo D.  
*New asthma biomarkers: lessons from murine models of acute and chronic asthma.*  
Am J Physiol Lung Cell Mol Physiol. 2009 Feb;296(2):L185-97.
- Katrincsakova B, Takeda H, Urbankova, H, Michaux L, Jarosova M, Vandenberghe P, Georges M, Charlier C, Wlodarska I.  
*Epigenetics Methylation analysis of the imprinted DLK1-GTL2 domain supports the random parental origin of the IGH-involving del(14q) in B-cell malignancies.*  
Epigenetics 4:7, 1-7; October 1, 2009.
- Bardiau M, Labrozso S, Mainil J G  
*Putative Adhesins of Enteropathogenic and Enterohemorrhagic Escherichia coli of Serogroup O26 Isolated from Humans and Cattle.*  
J Clin Microbiol, 2009;47: 2090-2096.

## HIGHLIGHTS

- Post-translational modification analysis
- Label free quantitative differential proteomics with nano Acquity UPCL, on line with Synapt HDMS G1

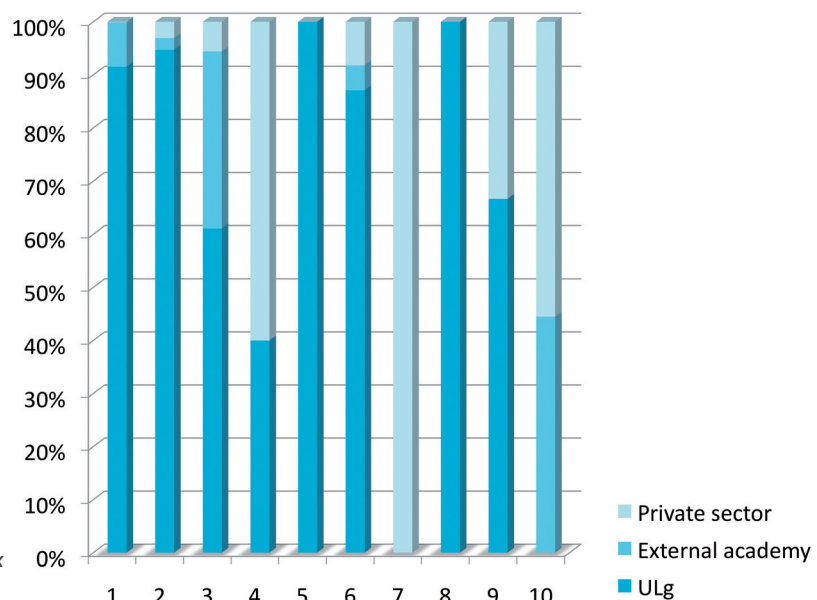
In 2009, the platform routinely performed

- 4 800 MALDI-TOF protein identifications
- 2 015 LC-MS/MS identifications of protein digest
- 193 Mass determinations by FT-MS or ESI-Q-TOF-MS
- 193 quantifications of proteins in complex mix
- 24 2D-DIGE analyses

Researchers from external academies or for the private sector frequently provide samples to be analysed by the platform, reflecting a good visibility of the team.

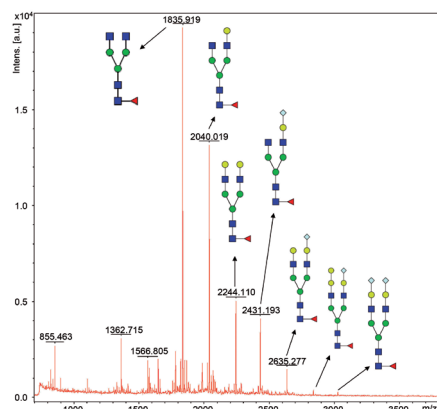
Repartition of the Services proposed by the Platform according to origin:

1. Maldi ID protein automatic (4 800 samples)
2. LC-MSMS ID protein digest (2 015 samples)
3. Maldi MS determination or manual sequencing (36 samples)
4. ISD (N- or C-Term sequencing) (5 samples)
5. Mass determination by FT-MS (22 samples)
6. Mass determination by ESI-Q-TOF-MS (171 samples)
7. Quantitation of proteins in complex mix by Heavy peptide methods (TQ-MS) - injections (193 samples)
8. 2D-DIGE (24 samples)
9. Package « Purity » or « Protein characterisation » (9 gels)
10. Pure protein multidigestion for max sequence coverages (9 samples)



One of the priorities of the platform is to achieve the characterization of post-translational modifications. To that purpose, Marie Alice Meuwis, one of the experts working on the platform completed the Glycobiology Training (2 weeks) at the Research and Infrastructure Center (GycoTRIC) at the Division of Molecular Biosciences, Faculty of Natural Sciences, Imperial College of London, UK.

The Platform is now able to perform the characterization of permethylated glycans from purified proteins and cell extracts and is working on the improvement of this technic.



*N-Glycan fragmentation spectrum for structure determination*

Finally, in 2009, the platform acquired a nano Acquity UPLC in line with Synapt TM HDMS TM G1 mass spectrometer (Waters) supporting the relative quantitative differential proteomics of protein complex protein mixtures with direct reliable identification of the proteins, as well as significant differential analysis of the relative abundance of these proteins. This technology only requires a very low quantity of samples: 2.5µg per run of total protein mix allow the identification of more than 500 different proteins, as well as their relative quantification.

A specific module of the device (Ion Mobility Spectrometry) leads to new perspectives in the field of protein folding, complex protein-ligand formation, DNA-ligand interaction analysis via a cross section of molecule or complex determination.

#### EXAMPLES OF PUBLICATIONS MADE POSSIBLE THANKS TO THE GIGA PROTEOMICS PLATFORM

- Frédérich M, Delvaux D, Gigliobianco T, Gangolf M, Dive G, Mazzucchelli G, Elias B, De Pauw E, Angenot L, Wins P, Bettendorff L.  
*Thiaminylated adenine nucleotides. Chemical synthesis, structural characterization and natural occurrence.*  
FEBS J. 2009; 276:3256-68.
- Calvo FQ, Fillet M, de Seny D, Meuwis MA, Marée R, Crahay C, Paulissen G, Rocks N, Gueders M, Wehenkel L, Merville MP, Louis R, Foidart JM, Noël A, Cataldo D.  
*Biomarker discovery in asthma-related inflammation and remodeling.*  
Proteomics. 2009; 9:2163-70.
- Smargiasso N, Gabelica V, Damblon C, Rosu F, De Pauw E, Teulade-Fichou MP, Rowe JA, Claessens A.  
*Putative DNA G-quadruplex formation within the promoters of Plasmodium falciparum var genes.*  
BMC Genomics. 2009 Aug 6;10:362.
- Le Trionnaire G, Francis F, Jaubert-Possamai S, Bonhomme J, De Pauw E, Gauthier JP, Haubruge E, Legeai F, Prunier-Leterme N, Simon JC, Tanguy S, Tagu D.  
*Transcriptomic and proteomic analyses of seasonal photoperiodism in the pea aphid.*  
BMC Genomics. 2009.

## GIGA-Imaging & Flow Cytometry

### HIGHLIGHT

- New FACSaria II to analyse and sort biohazardous samples in asptic conditions

The GIGA-Imaging and flow cytometry platform gathers 2 confocal microscopes both equipped with time-lapse devices, 4 flow cytometers and a laser microdissector.

This equipment is mostly used by the researchers themselves (more than 150 users) with the help and advice of the logistician or technician. All users are trained before their first access to ensure optimal use of the equipment.

### IMAGING

About 1 050 confocal analyses as well as 40 time-lapses have been performed for a total of more than 3 500 hours of observation, leading to publications in high impact factor journals:

- Lecut C, Frederix K, Johnson DM, Deroanne C, Thiry M, Faccinetto C, Marée R, Evans RJ, Volders PGA, Bours V, Oury C.

*P2X1 ion channels promote neutrophil chemotaxis through Rho kinase activation.*

J. Immunol., 2009 ; 183: 2801-2809.

- Creppe C, Malinouskaya L, Volvert M-L, Gillard M, Close P, Malaise O, Laguesse S, Cornez I, Rahmouni S, Ormenese S, Belachew S, Malgrange B, Chapelle J-P, Siebenlist U, Moonen G, Chariot A and Nguyen L.

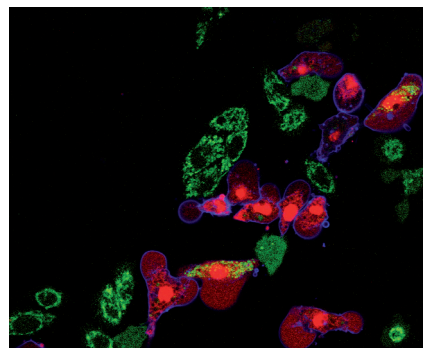
*Elongator Controls the Migration and Differentiation of Cortical Neurons through Acetylation of Alpha Tubulin.*

Cell, 2009 ; 136: 393-394.

- de Nijs L, Léon C, Nguyen L, Loturco JJ, Delgado-Escueta AV, Grisar T, Lakaye B.

*EFHC1 interacts with microtubules to regulate cell division and cortical development.*

Nat Neurosci., 2009 ; 12:1266-1274.



The platform works in close collaboration with the GIGA-Bioinformatics platform that helps with imaging data mining and computerized image classification or quantification.

These applications, as well as new techniques such as F-techniques in confocal microscopy, will be further developed in the future to reach the user's needs.

### FLOW CYTOMETRY

About 1 300 flow cytometry analyses, including phenotyping, cell cycle or cell viability analysis and CBA, as well as 40 cell sortings, were performed mostly on FACS Vantage or FACSCanto II.

In December, the platform acquired a new flow cytometer equipped with an aerosol control device (FACS Aria II) that allows the analysis and sorting of cells from biohazardous samples in aseptic conditions.

Although all this equipment is mostly used by the researchers themselves, for external users the analyses could be performed by the logistician. A company has thus outsourced a complete analysis to the platform including sample treatments, optimisation of the protocols and cell phenotyping by flow cytometry.

It is of importance to note that a High Content Analysis (HCA) platform will be installed in 2010, providing new opportunities for high throughput cell analysis.

### LASER MICRODISSECTION

Ninety four laser microdissections were performed for isolation of a single cell or a group of cells presenting some specificities (localisation, morphological characteristics,...) that could be further analysed by high sensitivity techniques.

A new microdissector will be acquired in 2010 to optimise this application, which is of high interest, especially for cancer research.



### HIGHLIGHT

- Improvement of image analyses

Since its creation, the GIGA-Bioinformatics platform works in close collaboration with the other platforms as well with researchers. Its input is indeed essential for the design of the experiments and for the analysis of the huge amount of data generated by the «omics» approaches.

In 2009, the Bioinformatics platform expanded its range of activities. Various sources of data, including transcriptomics data, microRNAs, DNA methylation, primers SNPs and images related to different pathologies or processes such as cancer, lymphoma, asthma, apoptosis or inflammation, were analyzed for GIGA members as well as for external researchers.

A close collaboration with the Proteomics platform and with clinicians has allowed the analysis of about 1050 SELDI spectra.

In collaboration with the Bioinformatics and Modeling research lab, the Bioinformatics platform has developed new computational methods to extract quantitative information from different types of biomedical images:

- software for automated cell counting in Boyden chambers
- morphometric measurements of the skeleton in zebrafish
- quantification of the myelin gains in the central nervous system (in collaboration with the University of Brandeis - USA).

This expertise in image analysis has been presented in different international meetings

- Computerized analysis of experimental pictures on zebrafish.  
Michaël Menu, Jessica Aceto, Pierre Geurts, Raphaël Marée, Louis Wehenkel and Marc Muller.  
Benelux Bioinformatics Conference, December 14-15, 2009.
- Tree-based methods for the exploitation of biomedical images.  
Raphaël Marée, Pierre Geurts, Louis Wehenkel.  
Benelux Bioinformatics Conference, December 14-15, 2009.
- Evaluation of generic classification algorithms for biomedical imaging.  
Raphaël Marée, Pierre Geurts, Louis Wehenkel.  
BioImage Informatics, Janelia Farm Research Campus, Howard Hughes Medical Institute, April 5-8, 2009.

The Bioinformatics platform also completed bioinformatics training (Introduction to Bioinformatics, 46 persons; Bioinformatics for data analysis: 22 persons) and participated to transregional trainings organized by Alma-in-Silico (Interreg).

#### EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED IN PART THANKS TO THE GIGA BIOINFORMATICS PLATFORM

- Quesada Calvo F, Fillet M, deSeny D, Meuwis MA, Marée R, Crahay C, Paulissen G, Rocks N, Gueders M, Wehenkel L, Merville MP, Louis R, Foidart JM, Noel A, Cataldo D.  
*Biomarker discovery in asthma-related inflammation and remodeling.*  
Proteomics, 2009, 9 : 2163-2170.
- Lecut C, Frederix K, Johnson DM, Deroanne C, Thiry M, Faccinetto C, Ruzicka N, Marée R, Evans RJ, Volders P, Bours V, Oury C  
*P2X1 ion channels promote neutrophil chemotaxis through Rho kinase activation.*  
Journal of Immunology, 2009 ; 15 : 183(4):2801-9.
- Schoemans R, Aigrot MS, Wu C, Marée R, Hong P, Belachew S, Josse C, Lubetzki C, Bours V.  
*Oligodendrocyte development and myelinogenesis are not impaired by high concentrations of phenylalanine or its metabolites.*  
Journal of Inherited Metabolism Disease, in press.





## GIGA-Animal facilities

Animal experimental models are of great importance for both fundamental and applied research. In fundamental research, the animal models allow to place molecular and cellular observations back into their physiological context. In applied research, these models remain a mandatory step to evaluate the efficiency and the toxicity of potential treatments, before going to clinical trials.

However, while working on animals, it is important to keep in mind the rule of the “three Rs”: Replace, Reduce, Refine. Briefly, Replacement is the most radical proposal: the use of *in vitro* or cellular techniques should always be encouraged. If not possible, non-sentient organisms (microorganisms, metazoan parasites) rather than higher animals should be used. Reduction means obtaining the best quality and most precise information with the lowest number of animals. Experiments that were well-designed and well-conducted deliver reliable results, and eliminate the need for endless repetition of the same tests. Refinement, the most subtle approach, referred to all changes in protocols that reduced the incidence or severity of distress experienced by laboratory animals. It is with this philosophy that the GIGA Animal Facility has been functioning for several years, housing mice and zebrafish. In addition, the GIGA Animal Facilities follows the guidelines of the FELASA2 regarding training of the people working on the animals (animal caretakers, technicians and researchers) and regarding the submission of all protocol to the Institutional Animal Care and Use Committee of the University of Liège.

Finally, it is important to note that, thanks to the fact that GIGA-Neurosciences joined the GIGA early in January 2009, GIGA-Animal facilities has been enriched by a zone dedicated for birds (quails and canaries).

## GIGA-MICE FACILITY AND TRANSGENESIS

### HIGHLIGHTS

- Merge of the conventional facility and the SPF mice facility
- Optimization of the embryo cryopreservation

The mice facility is divided into 2 zones:

- the **conventional** animal facility which occupies 9 floors respectively, dedicated to breeding and experimental procedures, routinely houses about 10 000 mice, 1 000 rats and 50 rabbits used for both research projects and external services. It includes biosecurity level 2 and 3 zones, as well as a zone dedicated to experimental neurosciences, large surgical rooms...
- the **Specific Pathogen-Free (SPF)** Facility currently housing around 3 000 mice, whose health is monitored every three months, according to the recommendations of the FELASA.

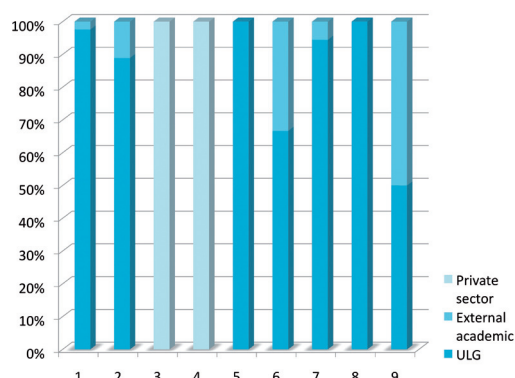
In 2009, the facility assured the management of 125 colonies and participated in 9 translational

research programs and the development of external collaborations including Biowin projects. It was also in charge of one external project of controlled pup-feeding and performed sanitisations and revitalisations. Pronucleus as well as mES cell injections were performed and the related transgenesis programs are ongoing.

The facility has also performed 1 200 MEA (Mouse Embryo Assays) for quality control of culture media used in human *in vitro* fertilization.

Highly concerned by the number of transgenic mice that have been produced, the Platform has made the effort to optimize the embryo cryopreservation by setting up the MEB (Mouse Embryo Bank). Transgenesis has indeed increased the number of mouse lines to tenfold the actual number of lines (which did not result in a tenfold increase of the number of animals) and thus, maintenance costs of mice has increased. Most of these lines have been generated for a particular purpose and may become unnecessary when the research evolves, although still useful in other works. Maintaining these is not useful and eliminating them does not make sense. The cryopreservation of these lines therefore constitutes an interesting approach. Besides the fact that cryopreservation of embryos can «resurrect» a line as needed, it can also be shared with any outside laboratory that may need it. It also allows the exchange of animals without them suffering the stress of transport, while reducing health risks between facilities. Cryopreservation of mouse lines is therefore also fully implements the rule of “3Rs” allowing a reduction of the number of animals.

So far, 18 lines have been cryopreserved and are kept in liquid nitrogen. This service is now routinely performed.



Repartition of the Services proposed by the Platform according to the origin of the request.

1. Housing (125 colonies)
2. Translational research programs (9 prog.)
3. Bottle-feeding program (1 prog.)
4. Mouse Embryo Assays (1258 assays)
5. Pronucleus injection (1)
6. mEScells injection (3)
7. Cryopreservation (18 lines)
8. Revitaliation (2 lines)
9. Sanitisation (2 lines)

## EXAMPLES OF PUBLICATIONS INCLUDING RESULTS OBTAINED, IN PART THANKS TO THE GIGA MOUSE FACILITY AND TRANSGENESIS

• Bedoret D, Wallemacq H, Marichal T, Desmet C, Quesada Calvo F, Henry E, Closset R, Dewals B, Thielen C, Gustin P, de Leval L, Van Rooijen N, Le Moine A, Vanderplasschen A, Cataldo D, Drion PV, Moser M, Lekeux P, Bureau F.

*Lung interstitial macrophages alter dendritic cell functions to prevent airway allergy in mice.*  
J Clin Invest. 2009; 119 :3723-38.

• Garigliany MM, Cloquette K, Leroy M, Decreux A, Goris N, De Clercq K, Desmecht D.  
*Modulating mouse innate immunity to RNA viruses by expressing the Bos taurus Mx system.*  
Transgenic Res 2009 ; 18: 719-732.

• El Hour M, Moncada-Pazos A, Blacher S, A, S Cal, Berndt S, Detilleux J, Host L, Obaya AJ, Maillard C, Foidart JM, Ectors F, Noel A, Lopez-Otin C.

*Higher sensitivity of Adamts12-deficient mice to tumor growth and angiogenesis.*  
Oncogene, in press.

## GIGA-ZEBRAFISH FACILITY AND TRANSGENESIS

### HIGHLIGHTS

- High throughput drug screening
- Acute toxicity testing on adult zebrafish

Zebrafish is a powerful experimental model not only for developmental research due to the rapid development and the easy observation of the transparent embryos, but also for a broad range of issues, such as toxicology, drug screening,...

Most transduction pathways are indeed conserved between all vertebrates, making the use of fish an appropriate model for a better understanding of human disease molecular mechanisms. Moreover, the use of fish in laboratory experiments is considered as a refinement compared to that of higher vertebrates as mice.

During 2009, the Zebrafish platform has:

- continuously maintained 700 strains on average
- provided 5 100 fixed embryos, 553 000 live embryos and 106 live adults for academic and non academic research
- produced 64 new transgenic or mutant strains
- performed 21 LC50 assays mainly for the private sector

Researchers from other groups interested by the zebrafish model (2 from Ulg, 1 from Maastricht University and 1 from Inserm - Bordeaux) have been trained to injection into eggs, a critical step for the production of mutant strains.

The platform has also been actively involved in courses on the use of zebrafish as an experimental model (University of Liège; FUNDP, Namur).

Mainly used by the researchers working on development (GIGA-Development, stem cells and regenerative medicine unit), the zebrafish model is more broadly used for other features and the number of requests from others than GIGA-R increases regularly.

### TWO NEW SERVICES HAVE BEEN DEVELOPED IN 2009

#### 1. HIGH-THROUGHPUT DRUG SCREENING

We have recently purchased a COPAS XL (Complex Object Parametric Analyzer and Sorter, Union Biometrica), a large particles sorter which allows analyzing and sorting objects from 0.5 to 1.5 mm based on five parameters: size, optical density, and up to three spectrums of fluorescence. Up to 15 embryos can be analysed per second, sorted and automatically dispensed into a 96 wells plate.

The larvae can then be maintained in a controlled environment, eventually in the presence of a compound to analyse and the development can be assessed after a well-defined time period, usually less than 5 days. Developmental defects can be recorded and ranked according to the severity of the observed defect (severe, moderate or none).

Based on an LC50 assay, compounds inducing severe developmental defects can be dismissed from preclinical tests. This assay could thus be time and cost saving.

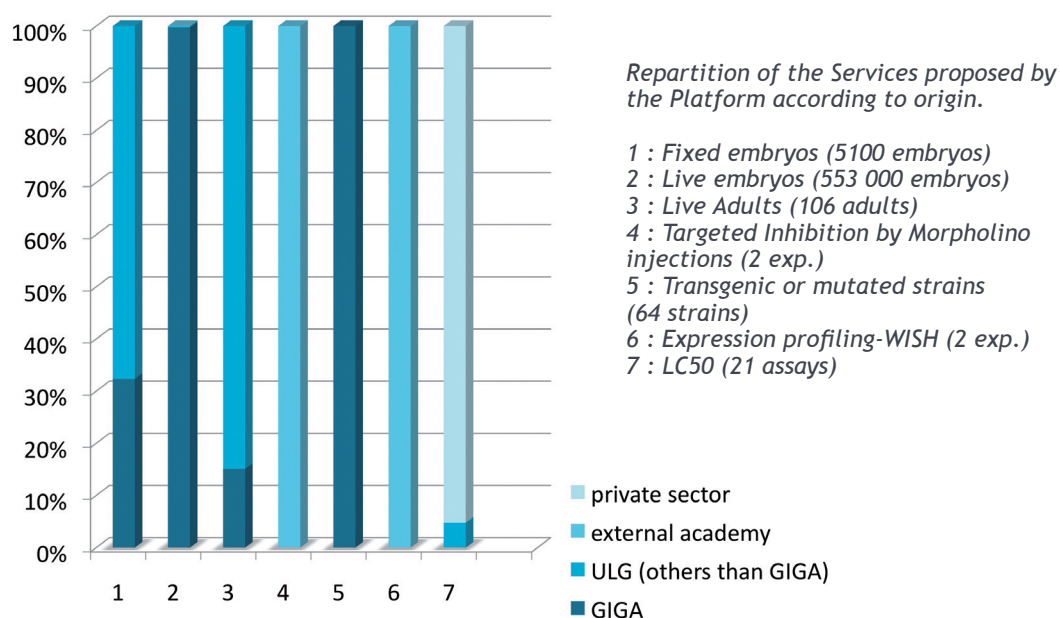
Several transgenic strains expressing fluorescent protein in a targeted organ or tissue make possible the screening of compounds for tissue-specific toxicity or activity.

## 2. ACUTE TOXICITY TESTING ON ADULT ZEBRAFISH

In the context of environmental risk or evaluation of drugs or chemicals, acute toxicity testing on adult zebrafish is one of the tools used to update the registration file of some compounds. The ULg Ethical Committee have granted us authorization to use adult fish for acute toxicity assays performed according to the OECD guideline n° 203.

Dr M. Winandy, the platform manager, has been trained at the Zebrafish International Resource Centre (University of Oregon, USA) for zebrafish sperm cryopreservation.

This new service, that will be available in 2010, will be offered to researchers throughout Europe and will help reduce live stock and facilitate the material exchange.



## IN 2009, 2 PUBLICATIONS HAVE BEEN PUBLISHED INCLUDING RESULTS OBTAINED PARTLY THANKS TO THE GIGA -ZEBRAFISH FACILITY AND TRANSGENESIS PLATFORM

• Soyer J, Flasse L, Raffelsberger W, Beucher A, Orvain C, Peers B, Ravassard P, Vermot J, Voz ML, Mellitzer G, Gradwohl G.

*Rfx6 as an Ngn3-dependent winged helix transcription factor required for pancreatic islet cell development.*

Development, 2010;137:203-12.

• Muller M, Dalcq J, Aceto J, Larbuisson A, Pasque V, Nourizadeh-Lillabadi N, Alestrom P, and Martial J  
*The function of the Egr1 transcription factor in cartilage formation and adaptation to microgravity in Danio Rerio.*

J Appl Ichthyol, 2009.

## COMMUNICATION AND NETWORKING

The technology platforms are accessible to academic researchers as well as SMEs or companies. Therefore, GIGA has always been concerned by the necessity of circulating the information broadly and of its participation in networks.

- **NEWSLETTER**

In order to keep users informed on services/equipment offered by each technology platform, we launched a newsletter, the first edition of which was sent to more than 1300 people in October. This newsletter is sent every three months and is available on the GIGA website ([www.giga.ulg.ac.be/newsletter](http://www.giga.ulg.ac.be/newsletter)) with emphasis on one specific service or equipment for each platform and gives researchers the opportunity to eventually find new solutions for the evolution of their research.

GIGA-technology platforms participate thus actively in Biowin projects and are involved in transnational programs such as :

- **FASILIS**

Facility Sharing in Life Sciences, (Interreg IV), a transnational pilot project, aims to create a framework for durable long-term cooperation between SMEs and facilities in Northwest-Europe (South East England, Øresund Region in Denmark and South Sweden, BioRegion STERN around Stuttgart, Bio-Liege in Wallonia, Health Valley and the LifetecZONE area in the Netherlands). An e-catalogue listing all the technology platforms available in the partners' regions has therefore been created and more than 80 vouchers will be distributed in 2010 to SMEs to get access from one of the partners' platform.

- **ALMA-in-SILICO (Interreg IV)**

Alma-in-Silico is a collaboration within the Euregio Meuse-Rhine area between the GIGA-Research, GIGA-Technology platforms and Biomedisch onderzoeksinstituut (BIOMED, Hasselt), Genome Center (GCM, Maastricht) and Institute für Molekular Biotechnologie (RWTH Aachen). The aim of this project is to develop an euregional bioinformatics Platform, to establish a technology platform for systems biology and to participate in a training program in bioinformatics for researchers and life science technicians. A call will be launched in 2010 allowing SMEs and research teams from one of the partners regions access to funding through vouchers.

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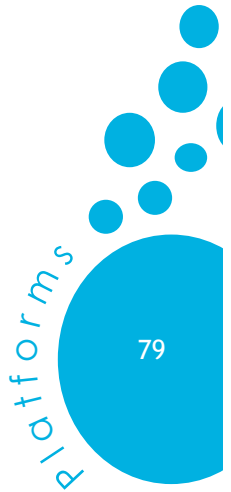
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GIGA-BUSINESS

Increasing Entrepreneurship is definitely one of the most challenging objectives of Wallonia for the years to come. GIGA contributes to this objective through its GIGA-Business division which offers top facilities to entrepreneurs of both start-ups and spin-offs. These facilities are not only space to rent, they are much more than that:

- a strongly stimulating environment for R&D departments even, and maybe more so, in the young and nascent companies
- an easy access to a vast array of exceptional equipments – with the experts to run them – without the need to invest in their immediate purchase
- open and informal forums for discussion with the experts located next door
- opportunities to join international research networks associating academics, clinicians and industrials
- the chance for the sales & marketing departments to regularly meet, in either official or casual set ups, these experts, professors, doctors to whom they will have to sell their products.

*We are very proud of the 2009 achievements of the young companies working on our premises.*

Our Business space has been full for nearly a year now and a new building is under construction (delivery date: January 2011). It is with great optimism that we look forward to the future developments of this GIGA division.



*Freddy Meurs*

Chief Executive Officer  
Meusinvest Group



## Science Park Services (SPS)

Science Park Services (SPS) is in charge of the real estate aspects for the biotech companies, which are active on the campus of the University of Liège (Belgium), or which want to locate their activities directly next to the University laboratories and scientific teams.

There are two sites: the “GIGA Espace Entreprises”, located in the GIGA Tower in the CHU (University Hospital of Liège) and building “B22”, near the Botanical Department.

GIGA-Business facilities, funded and run by Science Park Services, are located on the third floor of the GIGA building and aim at offering a development opportunity for biotech companies (spin-offs, start-ups, subsidiary companies) within the GIGA building. It offers fitted premises -usually offices and laboratories- to Belgian and foreign biotech companies.

SPS takes care of the fittings of laboratories, offices, specific rooms (cold room, dark room, meeting room, technical or storage rooms...) according to the tenants needs and requests.

The laboratories are fully equipped with benches and all fluids necessary to biotechnological activities. In addition, several services are accessible to companies located in the SPS facilities. They have privileged access to:

- the GIGA technological platforms
- more than 400 researchers from GIGA-Research and the Hospital for possible collaborations
- the scientific library of the Faculty of Medicine
- the Biosafety Management services
- the “Pi2” centre, “Patlib” center specialized in intellectual property
- the training programs in biotechnology for laboratory staff
- the recruitment of local qualified staff
- venture capital and development capital in collaboration with the MEUSINVEST Group



## Wallonia Biotech Coaching (WBC)

WBC is the bio-incubator of the Belgian Region of Wallonia. WBC aims to stimulate the creation and the maturation of biotech spin-offs, start-ups or spin-outs based on research and development activities performed in academic institutions and industrial companies.

Its mission consists in offering continuous support to entrepreneurs thanks to a panel of financial, commercial and managerial services. In 2009, WBC spent 240.000€ for its incubees.

During last year, 4 new projects joined us. WBC has now signed 8 master partnership agreements (4 spin-offs, 2 start-ups, 1 spin-out and 1 subsidiary):

- 6 companies have been incorporated and 2 more will soon be
- 3 projects are in the field of human or animal therapy, 3 offer services and 2 produce medical devices

As we looked at the figures for the first 4 incubees, we noticed a 45% increase of the number of employees (29 people) and a 17% increase of the turnover.

In addition to the support of its incubees, WBC is involved in many activities such as:

- Consulting the mission for the BioLogEurope project
- Development of a managerial software dedicated to entrepreneurs
- Economic missions with Awex in Asia and USA

For 2010, some new projects have already contacted us and we plan to welcome 4 new incubees that will benefit from our support program.



## Oncomethylome

In February and April 2009, OncoMethylome begun MGMT gene promoter methylation testing in a recently started phase II clinical trial for Cilengitide (collaboration with Merck KGaA) and a phase III clinical trial in newly diagnosed brain tumors (glioblastoma) for a US-based Radiotherapy Oncology Group (RTOG).

OncoMethylome has also got promising results from its on-going evaluation of blood-based methylation assays for screening and detection of colorectal cancer. These results were presented in September at the 15th Congress of European Organisation and 34th Congress of the European Society for Medical Oncology in Berlin.

Throughout the year, OncoMethylome has continued its collaboration with partners such as Schering-Plough, LabCorp, GSK Biologicals and ULg.

In November, the Company announced that it will be focusing its diagnostic business on 3 clinical areas : colorectal, prostate and bladder.



## Diagenode

Diagenode is a leading developer and marketer of innovative life-science tools and integrated systems for epigenetics, genomics and diagnostics. Diagenode is the only company providing a complete solution for epigenetic research, including state-of-the-art products and technology for DNA sonication, best-in-class antibodies (ChIP grade and ChIP-seq grade), and high-quality kits for chromatin immunoprecipitation (ChIP) and methylation studies, and now an automated epigenetics system, the IP-Star™.

Diagenode's customers include leading epigenetics researchers, academic institutions, high-profile genome centres and core labs, life science tools companies, molecular diagnostics players, and pharmaceutical and biotechnology companies. Our products provide researchers with the best quality and performance and allow scientists to produce consistent, cost-effective and robust results in their research. We expect that our technology will enable our customers to make cutting-edge breakthroughs in the field of epigenetics, leading to a better understanding of health, disease and development. Recently Diagenode has successfully completed its range of Chromatin ImmunoPrecipitation (ChIP) products with the launch of the HighCell # ChIP kit. This new kit improves researchers' ability to per-

form reproducible, efficient and rapid ChIP experiments from high cell numbers (1 to 10 million cells). Together with the LowCell# ChIP kit, these kits allow scientists to carry out ChIP studies from any cell numbers, substantially less manual labor, and more consistent results, making these products ideal for ChIP studies whatever the type of studied protein.

Diagenode is also proud to offer a complete range of product for methylation studies. This includes innovative antibodies such as monoclonals against 5-MethylCytidine and, most recently, against the newly discovered 5-HydroxyMethylCytidine. It also includes immunoprecipitation kits such as MethylCap™ kits and MeDIP kits. Diagenode's MethylCap kit has been optimized for the enrichment of methylated DNA using a MBD (Methylbinding domain) protein. Use of such domain allows for fractionation of methylated DNA by CpG density (directly compatible with next generation sequencing).

#### Diagenode's Molecular Diagnostics Products

Diagenode also offers DNA amplification and quantification products for the rapidly growing field of infectious disease diagnostics. The company also provides guidance to companies that develop epigenetic-based diagnostics. Our high-quality products are designed to make diagnostic assays simpler, faster, and more convenient. Our kits are CE-marked and ISO 9001 and ISO 13485 certified.



Arlenda is a spin-off from the University of Liège in Belgium, whose mission is to develop, validate and commercialise an integrated suite of statistical solutions dedicated to the life cycle of analytical methods, from optimisation to routine analysis, whilst being compliant with regulatory documents.

Arlenda provides new decision tools for the validation of analytical methods.

Its expertise is covering the following fields of activity:

- Validated Solutions using SAS for the validation of analytical methods
- Specific developments of SAS applications, and their validation
- Training in validation and statistics

Early 2009, Arlenda moved its offices to the GIGA tower and hired 3 new collaborators to reinforce the development team.

This year, Arlenda released a new version of the method validation software e.noval 3.0, which is software dedicated to the validation of both relative (LC, GC, EC, AAS,...) and absolute (titration, UV,...) physico-chemical methods.

Arlenda also has developed a strategic partnership with the Ulg (PPP) for "Design Optimisation" project research and a new commercial partnership with Morocco.



## Probiox

In 2009, Probiox conducted several clinical trials. One is investigating the company's proprietary product, the nutraceutical ProPill®, a nutraceutical composition aimed at restoring the oxidative stress conditions associated with oral contraception. Results are expected in early 2010. Other nutraceutical products are under development.

In parallel, the company has pursued the development of its service activity aimed at validating the health benefits of nutraceutical products (*in vitro* studies and clinical studies) for the nutraceutical and cosmetic industry.

The second major axis of Probiox in-house research related to the development of new diagnostic tools based on measurement of gene expression, has led to a new patent application for the prognosis of diabetes type 2 in critical subjects.



## Labage

Labage is dedicated to the development of new software and web-based tools for the management of technical expertise and R&D data and resources, using collective intelligence technologies.

Labage has launched two new products in 2009: an «electronic laboratory notebook» for biotechnology laboratories (TEEXMA-lab) and a «quality management system» for hospitals (TEEXMA-pro). They are both based on a new easy, user friendly, and flexible workflow management system, the TEEXMA knowledge management platform, and several innovative semantic tools.

Labage has also added many new supplier catalogs into its CL!P search engine.

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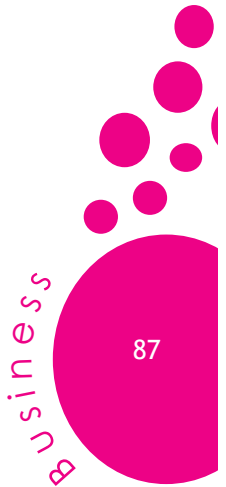
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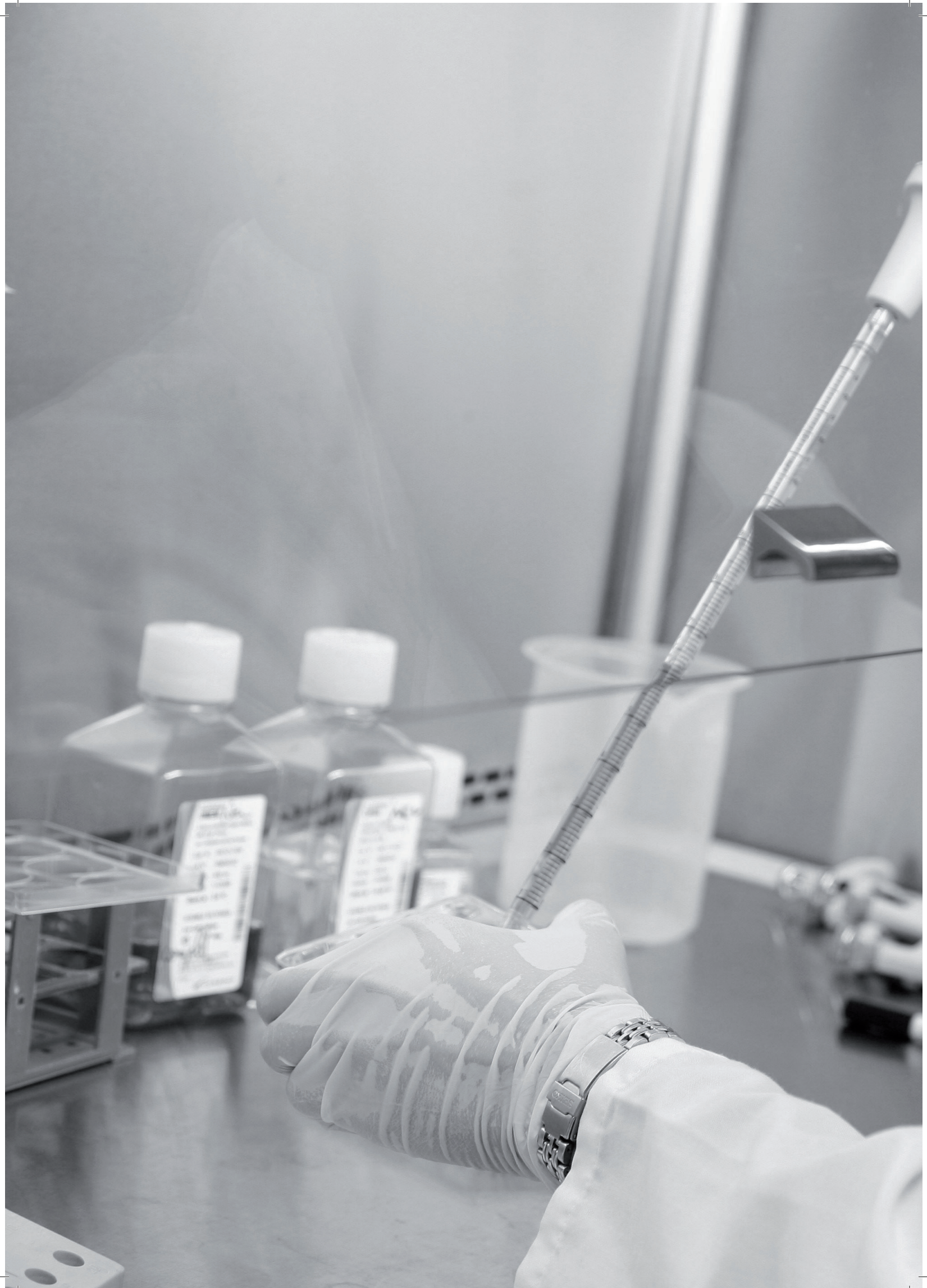


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The Biotechnology Training Centre, created in 2005, resulted from a close partnership between the GLGA Research Centre and the Forem. Since the beginning, the quality of training has been recognised by trainees from biotech companies as well as by jobseekers and students from technical colleges and universities. From our point of view, this is due to the involvement of trainers from the academic world and the facilities and knowledge of the Industrial world offered by the Forem.

As a result the amount of training hours continuously increased between 2005 and 2009 and this was followed by a stable job-insertion rate (around 75 % after one year) suggesting that, despite the higher number of trained people, most of them eventually found a job, which is the ultimate goal of the Training Centre.

Another very positive signal, from 2008, is that well-established biotech companies have started to ask the Centre for 'custom-made' sessions, highlighting quality and expertise and the fact that *this fully integrated training Centre is unique in the Walloon Region.*



*Eric Demaret*

Director  
FOREM Formation Liège-Huy

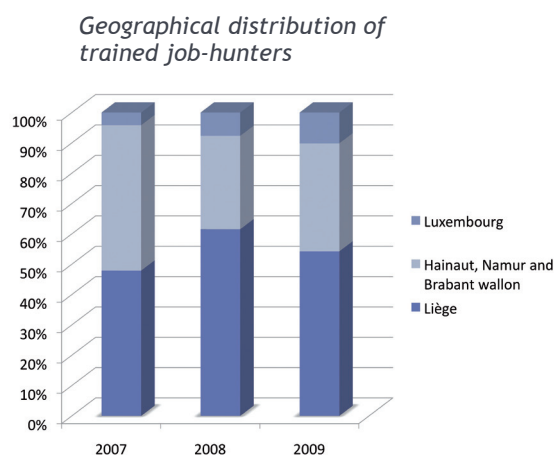
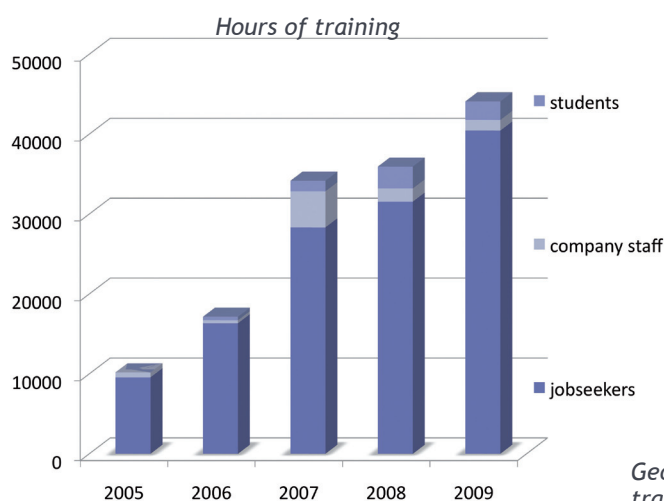
## GIGA-FOREM TRAINING CENTER

The Biotechnology Training Centre Forem-GIGA was created in 2005 by the Walloon Office for Job and Training of Liège (the FOREM) in partnership with the Interdisciplinary Cluster in Applied Genoproteomics (GIGA) of the University of Liège, supported by European Funds and the Walloon Region.

Our aims are to develop and organize biotechnology training for jobseekers and company staff, in response to market needs, and to complement the training offered by technical colleges in terms of techniques and specific expertise. Most of the company-staff training is validated by Biowin, the Health Cluster of Wallonia. To achieve these goals, the Biotechnology Training Centre works in close collaboration with both the academic and the industrial world of biotechnology.

The training centre is located inside the GIGA-Research centre itself, in the neighborhood of the Liège Science Park and in close vicinity of the increasing GIGA Business facilities. Trainees are thus in close contact with R&D laboratories and scientists. Since December 2006, the Biotechnology Training Centre has its own fully-equipped laboratory and classroom. In addition, before the end of 2011, these facilities will double, thanks to new support from the EU and the Region.

Current topics covered are: molecular biology, molecular diagnostics, immunology, protein production and purification, gas and liquid chromatography, cell culture, quality control, quality assurance, bio-safety, GxP's, bioinformatics and project management. Besides these subjects, training sessions can be tailored to customer needs.



## RESULTS FOR YEAR 2009

### 1. Jobseekers

In 2009, seven long-term training sessions were organized for 82 people from the entire Walloon Region. Besides the transverse skills (Biosafety, GxP's, QA, QC, Validation, Regulatory, scientific English, good communication and team work, informatics and foreign language training) included in each session, the technical subjects were related to:

- Project and team management in biotechnology
- PCR from A to Z
- Molecular biology techniques, in biosafety level 1 to 3 environments
- Analytical techniques: HPLC-GC-CE
- Immunology
- Protein production and characterization

To complete their training, most of the trainees performed a 2-month-long immersion program within biotech companies. This led to a high rate of job-insertion since 67% of them found a job in less than 6 months after the end of their training period, despite the economical situation encountered in 2009 (final data will be available by the end of 2010).

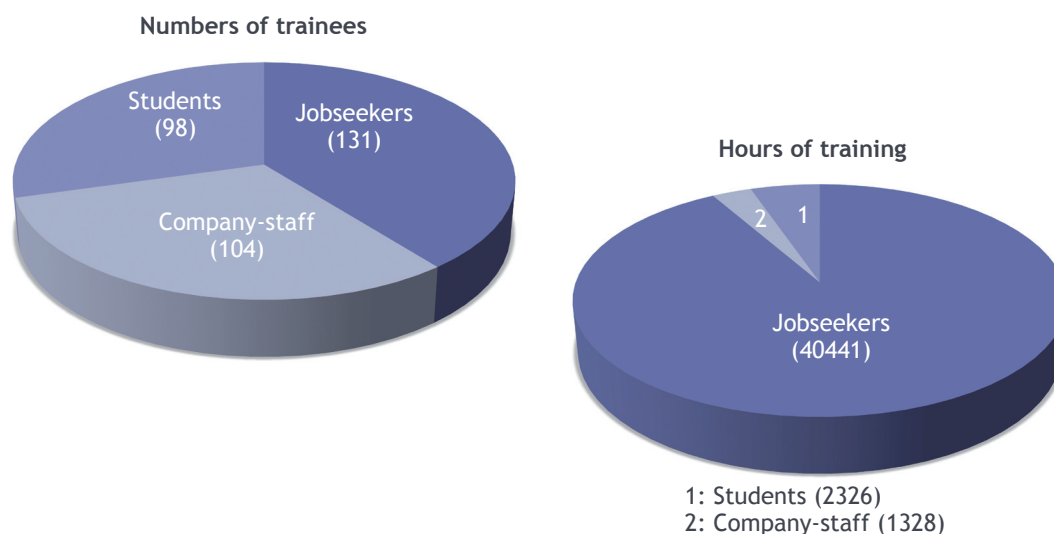
In addition, two modules focusing on biosafety and animal cell culture were offered to 22 people following a long-term training course at the Cefochim skills centre.

### 2. Company-staff

Nine training modules specifically dedicated to specific needs of the biotech companies (biosafety, bioinformatics, realtime-Q-PCR, microbiology, bacteriology, PCR) were followed by 101 people (among which 27 jobseekers, 8 students from technical colleges (Hautes Ecoles) and 3 of their teachers). Most of the sessions were given through the Biowin program.

### 3. Higher education students - Technical colleges

Four short-term modules designed for 90 students were organized in 2009, partly through Biowin, partly in collaboration with the Forem. The aim is to complete their academic courses and give them access to the state-of-the-art technology unaffordable for technical colleges, promoting interactions with experts in the field and with researchers.



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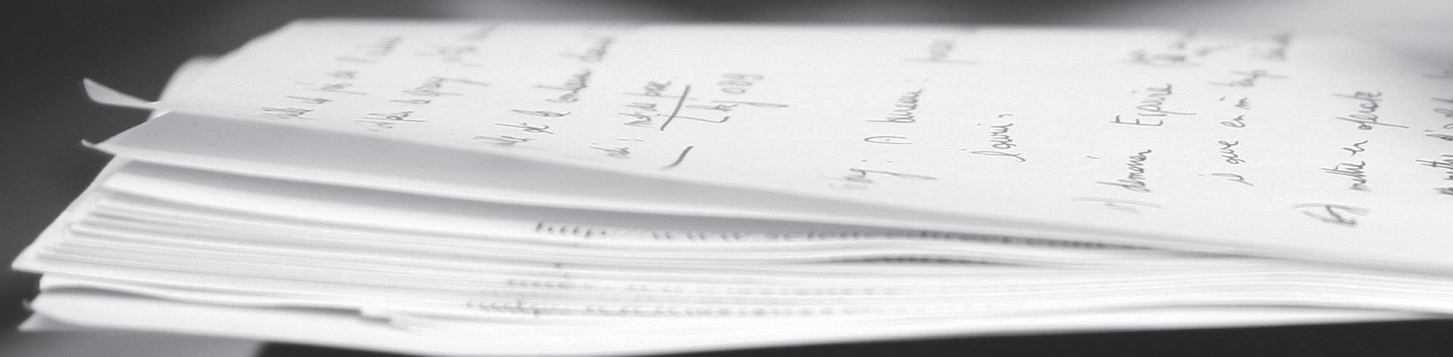
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## Patents

In 2009, GIGA-R has contributed to the filing of 10 patent applications.

In 2009, 12 patents or patent applications involving GIGA researchers have been published.

| <i>Title</i>                                                                        | <i>Inventors</i>                                                                                                          | <i>Publication Number</i> |                  | <i>TRU</i>                                      |
|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------|---------------------------|------------------|-------------------------------------------------|
| In-vitro method for screening accessible biological markers in pathologic tissues   | Castronovo Vincent<br>Waltregny David<br>Kischel Philippe                                                                 | JP                        | JP2009-522562    | GIGA-Cancer                                     |
| In-vitro method for screening accessible biological markers in pathologic tissues   | Castronovo Vincent<br>Waltregny David<br>Kischel Philippe                                                                 | US                        | US2009/0163414A1 | GIGA-Cancer                                     |
| Mutations in the myostatin gene cause double-muscling in mammals                    | Georges Michel<br>Grobet Luc<br>Poncelet Dominique                                                                        | EP                        | EP1002068B1      | GIGA-Genomics                                   |
| Mutations in the myostatin gene cause double-muscling in mammals                    | Georges Michel<br>Grobet Luc<br>Poncelet Dominique                                                                        | EP                        | EP2045322A1      | GIGA-Genomics                                   |
| Mutations in the myostatin gene cause double-muscling in mammals                    | Georges Michel<br>Grobet Luc<br>Poncelet Dominique                                                                        | JP                        | JP4368522B2      | GIGA-Genomics                                   |
| VEGF Variants                                                                       | Colige Alain<br>Mineur Pierre<br>Lambert Charles                                                                          | JP                        | JP2009-523440    | GIGA-Cancer                                     |
| Biomarker for Osteoarthritis, and/or ageing-related diseases and use thereof        | Henrotin Yves<br>Gharbi Myriam<br>Deberg Michelle<br>De Pauw Edwin                                                        | PCT                       | WO2009/146956A1  | GIGA-Systems<br>Biology and<br>Chemical Biology |
| Biomarker for Osteoarthritis, and/or ageing-related diseases and use thereof        | Henrotin Yves<br>Gharbi Myriam<br>Deberg Michelle<br>De Pauw Edwin                                                        | PCT                       | WO2009/146957A1  | GIGA-Systems<br>Biology and<br>Chemical Biology |
| Use of cyclodextrin for treatment and prevention of bronchial inflammatory diseases | Cataldo Didier<br>Evrard Brigitte<br>Noël Agnès<br>Foidart Jean-Michel                                                    | US                        | US2009/0012041A1 | GIGA-Infection,<br>Immunity and<br>Inflammation |
| Bis 1,2,3,4-tetrahydroisoquinoline derivatives and their uses as pharmaceuticals    | Graulich Amaury<br>Liègeois Jean-François<br>Seutin Vincent<br>Moreau Jacqueline                                          | JP                        | JP2009-530340    | GIGA-Neuro-<br>sciences                         |
| Bis 1,2,3,4-tetrahydroisoquinoline derivatives and their uses as pharmaceuticals    | Graulich Amaury<br>Liègeois Jean-François<br>Seutin Vincent<br>Moreau Jacqueline                                          | US                        | US2009/0093517A1 | GIGA-Neuro-<br>sciences                         |
| Multifunctional coatings                                                            | Van De Weerd Cécile<br>Detrembleur Christophe<br>Charlot Aurélia<br>Jérôme Robert<br>Vreuls Christelle<br>Zocchi Germaine | PCT                       | WO2009/147007A2  | GIGA-Systems<br>Biology and<br>Chemical Biology |

Etude du rôle des macrophages interstitiels dans l'allergie des voies respiratoires

**Bedoret Denis**, Cellular and Molecular Physiology, GIGA-Infection, Immunity and Inflammation

Implication de l'hormone chorionique gonadotrope (hCG) dans l'implantation embryonnaire normale et pathologique

**Berndt Sarah**, Biology of Tumor & Development, GIGA-Cancer

Period 2 régule la prolifération des cellules souches/progénitrices à l'origine de la neurogenèse adulte dans l'hippocampe

**Borgs Laurence**, Developmental Neurobiology, GIGA-Neurosciences

Etudes des propriétés hématosupportives *in vitro* des cellules souches mésenchymateuses

**Briquet Alexandra**, Haematology, GIGA-Infection, Immunity and Inflammation

Contribution à l'étude de la protéolyse impliquée au cours de la lymphangiogenèse

**Bruyère Françoise**, Biology of Tumor & Development

Etude de la voie de signalisation Hedgehog dans les cancers du côlon

**Chatel Guillaume**, Molecular Oncology, GIGA-Cancer

Insight into the role of Elongator in cell migration and neuronal branching

**Creppe Catherine**, Medical Chemistry, GIGA-Signal Transduction

Etude du mécanisme d'activation *in vitro* et *in vivo* du facteur de transcription NF- $\kappa$ B via la phosphorylation de I $\kappa$ B $\alpha$

**Crèvecoeur Julie**, Virology and Immunology, GIGA-Signal Transduction

Contribution à l'étude des pathologies pleurales malignes

**Duysinx Bernard**, Pneumology; GIGA-Infection, Immunity and Inflammation

Etude de l'expression, de la régulation et du rôle de la phosphatase à double spécificité VHR dans le cancer du col de l'utérus.

**Henkens Rachel**, Immunology and Infectious Diseases, GIGA-Infection, Immunity and Inflammation

Contribution à l'étude de la régulation de l'angiogenèse et de la lymphangiogenèse dans le psoriasis

**Henno Audrey**, Connective Tissues Biology, GIGA-Cancer

Evaluation du rôle des RhoGTPases dans la croissance tumorale *in vitro* et *in vivo* par les ARN interférentiels

**Ho Giang**, Connective Tissues Biology, GIGA-Cancer

Regulatory T cells in Wiskott-Aldrich syndrome and after allogeneic transplantation with nonmyeloablative conditioning

**Humblot Stéphanie**, Haematology, GIGA-Infection, Immunity and Inflammation

Geometric algorithms for component analysis with a view to gene expression data analysis

**Journée Michel**, Systems & Modeling, GIGA-Systems Biology and Chemical Biology

Contribution à l'étude du rôle des protéines SIBLINGS au cours de la progression tumorale

**Lamour Virginie**, Metastases Research

Etude des effets biologiques de facteurs physiques environnementaux

**Mineur Pierre**, Connective Tissues Biology, GIGA-Cancer

Identifications de protéines interagissant avec les facteurs de transcription AP-2 et contribuant à la surexpression du gène ERBB2 dans le cancer du sein.

**Nolens Grégory**, Molecular Oncology, GIGA-Cancer

Développement en contraception d'un modulateur sélectif du récepteur de la progestérone : le VA2914

**Pintiaux Axelle**, Biology of Tumor & Development, GIGA-Cancer

Etude du rôle des isoformes codées par les Neurégulines Nrg-1 et Nrg-2 dans la prolifération et la différenciation des cellules souches nerveuses au cours du développement

**Pirotte Dorothée**, Developmental Neurobiology, GIGA-Neurosciences

Effets des progestatifs et des modulateurs sélectifs du récepteur de la progestérone sur l'endomètre humain

**Ravet Stéphanie**, Biology of Tumor

& Development, GIGA-Cancer

Insight into the oncogenic potential of truncated NF- $\kappa$ 2 proteins through identification of their target genes

**Robert Isabelle**, Medical Chemistry, GIGA-Signal Transduction

Study of the role of extracellular ATP in airway inflammation

**Théâtre Emilie**, Human genetics, GIGA-Infection, Immunity and Inflammation

Development of the hypothalamic-pituitary axis in Zebrafish (*Danio Rerio*)

**Toro Sabrina**, Molecular Biology & Genetic Engineering, GIGA-development, Stem cells and Regenerative medicine

Etude *in vitro* et *in vivo* de la réactivation du virus de l'immunodéficience acquise de type 1 (VIH-1) par les inhibiteurs d'histones diacétylases

**Vandergeeten Claire**, Immunology & Infectious Diseases, GIGA-Infection, Immunity and Inflammation

Rôle des canaux potassiques calcium-dépendants de type SK dans la régulation du mode de décharge des neurones monoaminergiques *in vivo*

**Waroux Olivier**, Electrophysiology, GIGA-Neurosciences



## Seminars

**Abrams Jeffrey**

National Cancer Institute, USA  
*Clinical development of angiogenesis inhibitors in cancer*

**Allgower Frank**

Institute for systems theory and automatic control, University of Stuttgart, Germany  
*Assured conclusions about uncertain biological systems: How systems theoretical approaches can help*

**Andersson Leif**

Animal Genetics, Uppsala University, Sweden  
*Identification of the repressor binding to the quantitative trait nucleotide site in intron 3 of IGF2*

**Avraham Karen**

Sacker School of medicine, Tel Aviv University, Israel  
*Merging the transcriptome, proteome and microRNAs in the inner ear : implications for deafness*

**Babu Madan**

Laboratory of Molecular Biology, University of Cambridge, United Kingdom  
*Intrinsically unstructured proteins: regulation and disease*

**Bean Bruce**

Neurobiology, University of Harvard, USA  
*Electrical pacemaking of central neurons in health and disease*

**Ben-Ari Yehzekel**

Neurobiology, Institut de la Méditerranée La Ciotat, France  
*Are Neurological Disorders « Born » in Utero?*

**Bertrand Mathieu**

Molecular Biomedical research, VIB, Ghent, Belgium  
*cIAPs, E3 ubiquitin ligases regulating RIP proteins function*

**Bollen Mathieu**

Laboratory of Biosignaling and Therapeutics, KUL, Leuven, Belgium  
*Exploration of the therapeutic potential of protein phosphatases for the treatment of cancer*

**Bonizzi Giuseppina**

European Institute of Oncology, Milan, Italy  
*Extended life-span and increased frequency of symmetric self-renewing divisions in cancer stem cells due to attenuated p53 signaling*

**Braun Michel**

Institut d'Immunologie Médicale, IMI, Gosselies, Belgium  
*T cell function under persistent antigen stimulation*

**Büttner Reinhard**

University of Bonn, Germany  
*Epigenetic regulation of tumor cell growth by histone demethylases: novel molecular targets for cancer therapy*

**Ceraline Jocelyn**

Signaling pathways and prostate cancer, University of Strasbourg, France  
*Altered androgen receptor signaling pathway in prostate cancer*

**Charlier Edith**

GIGA-R, ULg, Belgium  
*Biological role of protein glycosylation in the immune system*

**Clézardin Philippe**

INSERM, Lyon, France  
*Mécanismes moléculaires de formation des micrométastases médullaires associées au cancer du sein*

**Communi David**

Institut de recherche interdisciplinaire en biologie humaine et moléculaire, ULB, Bruxelles, Belgium  
*Discovery and functional characterization of novel ligands for orphan membrane receptors involved in the immune system*

**Crèvecoeur Julie**

GIGA-R, ULg, Belgium  
*Les espèces réactives de l'oxygène et la modulation de l'ischémie cérébrale*

**David Guido**

Molecular and Developmental Genetics, VIB, University of Leuven, Belgium  
*Syndecans: platforms for receptor engagement, signaling, trafficking and down-regulation*

**Debarbieux Franck**

Département de biologie, Université de Marseille, France  
*In vivo two-photon microscopy for preclinical research: From spinal cord injury... to glioblastoma models*

**De Bolle Xavier**

Unité de biologie moléculaire, FUNDP, Namur, Belgium  
*Differentiation and intracellular adaptation of the pathogenic bacterium Brucella abortus*

**Djukanovic Ratko**

Inflammatory Cell Biology, Southampton University, United Kingdom  
*Mechanisms of T cell activation and recruitment in asthma*

**Dubuisson Jean**

Microbiologie, Institut Pasteur de Lille, France  
*Cellular and viral factors modulating hepatitis C virus entry*

**Dumoutier Laure**

Unité de Médecine Expérimentale, UCL, Louvain-la-Neuve, Belgium  
*Pro and anti-inflammatory activities of IL-22 in autoimmune diseases*

**Dürig Jan**

Department of Hematology, University of Duisburg-Essen, Germany  
*A novel NOD/SCID xenograft model for the investigation of pathophysiology and preclinical drug testing in chronic lymphocytic leukemia (CLL)*

**El Benna Jamel**

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*Regulation of NADPH oxidase (NOX2) activation : rôle of the phosphorylation of p47phox*

**Emonard Hervé**

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*LRP-1 : a new target in cancer therapy ?*

**Eyzaguirre Jaime**

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*Proteomics studies of the secretome of the lignocellulolytic fungus Penicillium purpurogenum*

**Geris Liesbet**

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*Multi-scale modeling of bone regeneration*

**Ghassibe Michella**

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*Far-associated factor-1, a protein involved in apoptosis, causes cleft palate and Pierre Robin sequence*

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*Neutralization evasion by gamma-herpesviruses*

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in control of the immune response  
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*Unique roles of ERK1 and ERK2 in  
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*Modélisation et commande de  
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la dynamique du VIH : vers une  
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*Dissection of TNF physiological  
functions using engineered mice*

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*Toponome imaging microscopy  
MELC/TIS in cell biology and trans-  
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*Embryonic stem cell research at  
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*Recent progress in the study of  
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*From data to statistical analysis:  
Explaining genetic heritability by  
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*Dendritic cells: personalized  
cancer immunotherapy from bench  
to bed*

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*Long- and short-range signals spe-  
cify, regionalize, and pattern the  
zebrafish pituitary placode*

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*Mechanisms of tumorigenesis in Rb  
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*Integration of peptide and lipid  
signaling by PDZ scaffolds, from  
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