EMHE PROGRAMME

FULL PhD proposals at Institut Pasteur (Paris), Institut Pasteur de Guyana Francesa y Institut Pasteur de Guadalupe

	PhD advisor	Unit and Head of Unit	Title of the project
1	ALONSO Mariana	Perception and Memory - LLEDO Pierre-Marie	Encoding of affective attributes in the olfactory system
2	AMINO Rogerio / MENARD Robert	Malaria Infection & Immunity Unit - AMINO Rogerio	Blocking the Homing of Malaria Sporozoites to the Liver
3	BELLINZONI Marco	Structural Microbiology - M. ALZARI Pedro	Hunting a metabolic supercomplex in Corynebacterium
4	BREUREC Sébastien	**Institut Pasteur de Guadeloupe** - Unité Environnement et Santé - TALARMIN Antoine	Exploration of gastric microbiota on the course of Helicobacter pylori disease.
5	BRUHNS Pierre	Unit of Antibodies in Therapy & Pathology (ATP) - BRUHNS Pierre Center for Innovation & Technological Research (CITECH) - OLIVO-MARIN Jean-Christophe	NGS analysis applied to single cell sequencing following high-throughput droplet-based microfluidic sorting: application to antibody discovery
6	DEMEURE Christian	Yersinia Research Unit - CARNIEL Elisabeth	How can Yersinia pestis become invisible to the host immune system?
7	DUSFOUR Isabelle	**Institut Pasteur de Guyane** - Unité d'entomologie médicale - GIROD Romain	Aedes aegypti populations from regions of Amazonian Basin: mapping insecticide resistance profile, selected mechanisms and population genetics.
8	SCHWIKOWSKI Benno	Systems Biology Lab - SCHWIKOWSHI Benno	Discovery of novel gene-environment interactions through monotonic models
9	ZURZOLO Chiara	Membrane Trafficking and Pathogenesis - ZURZOLO Chiara	Analysis of the functional organization of the folate receptor alpha (FRα) in ovarian cancer cells

PhD PROPOSAL 1

Title of the PhD: Encoding of affective attributes in the olfactory system
Keywords: olfaction, learning and memory, adult-neurogenesis, top-down inputs
Institut Pasteur: Institut Pasteur – Paris, France
Department: Neuroscience
Name of the lab: Perception and Memory
Head of the lab: Pierre-Marie LLEDO
PhD advisor: Mariana ALONSO
Email address: mariana.alonso@pasteur.fr
Web site address of the lab: http://www.pasteur.fr/en/research/neuroscience/units-groups/perception-and-memory
Doctoral school affiliation and University: UPMC

Presentation of the laboratory and its research topics:

The Perception and Memory laboratory focuses its research on the neural basis of sensory perception, learning, and memory. Our group applies a top-down approach to decipher mechanisms involved in adult brain plasticity using olfaction in rodents as a model system. The laboratory has acquired a strong expertise in cellular and synaptic analysis of circuit function at the early stages of the olfactory system and in revealing the link between neuronal circuit activity and behavioral responses in normal and pathological conditions.

Description of the project:

The sense of smell has to deal with numerous odors which can trigger various affective responses. We like or dislike a given odorant even before recognizing the nature of the smell. As a result of this inherent evaluation, an appropriate behavior, vital for the survival of the mammal, is triggered to either avoid or approach the scented cue. The olfactory bulb (OB) is the first central relay of the olfactory system where olfactory cues trigger complex responses encoding specified attributes such as identity, category or quantity of odorants. In addition, these odor-evoked bulbar responses are sculpted by massive top-down inputs that are thought to bring cognitive and affective dimensions according to the needs, the experience and the environment of the receiver. However, the circuit mechanism of this intricate processes in which bottom-up (*i.e.*, sensory inputs) and top-down inputs participate cooperatively to the processing of olfactory sensory information is not known.

In this project, we seek to decipher how odor affective attributes are encoded early in the olfactory system, starting from the OB, and how such enrichment of the sensory content participates to behavioral responses, including learning and memory processes. Using cutting-edge techniques such as optogenetics, imaging applied to awake animals and also psychophysics and behavior, we will examine the circuit mechanisms and the consequences of affective encoding in the olfactory system, with a particular focus on the contribution of OB adult-generated interneurons in encoding top-down information.

Understanding how the brain constructs sensory percepts is a key challenge to better clarify several basic and clinical issues, and could pave the way for new potential therapeutic approaches to treat pathological circuit dysfunction. For instance, sensory alterations are known to be key elements of important psychiatric disorders such as

attention-deficit/hyperactivity disorders, bipolar disorders and schizophrenia.

Expected profile of the candidate:

We are looking for highly motivated and talent PhD candidates who have a substantiated interest and experience in animal behavior in the field of Neuroscience. Laboratory research experience is a prerequisite. The candidate needs to exhibit a good amount of inquisitiveness, initiative and independent thinking.

Contact:

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PhD PROPOSAL 2

Title of the PhD: Blocking the Homing of Malaria Sporozoites to the Liver
Keywords: malaria, imaging, neutralizing antibodies, protective antigens, vaccine
Institut Pasteur: Institut Pasteur – Paris, France
Department: Parasites and Insect Vectors
Name of the lab: Malaria Infection & Immunity Unit
Head of the lab: Rogerio Amino
PhD Advisor: Robert Menard/ Rogerio Amino
Email address: roti@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/fr/team/group-rogerio-amino/
Doctoral school affiliation and University: Ecole Doctorale B3MI – Université Paris Diderot (Paris 7)

Presentation of the laboratory and its research topics:

Our laboratory is interested in defining the determinants involved in *Plasmodium* pre-erythrocytic stages survival in the skin and in the liver of naïve and immunized hosts using intravital imaging techniques. Our goal is to identify host and parasite determinants at cellular and molecular levels, focusing on the requirements needed to hinder the progression and development of the parasite in these tissues. Our main strategy is based on the direct and quantitative observation of the interaction of parasites and host cells *in vivo,* using transgenic - fluorescent, bioluminescent and mutant – organisms. In the last years, this approach allowed us to unravel novel steps in the life cycle of sporozoites and liver-stages[1,4,6,7] , as well as, to better understand the role of cell traversal activity in the sporozoite infection[3,5]. Now our objective is to apply this strategy to study the mechanisms of immune-protection mediated by antibodies and CD8+ T cells[2] in the malarial infection, aiming at developing better ways to elicit sterile protection in the immunized host.

Description of the project:

Malaria is a vector-borne disease caused by *Plasmodium* parasites. Half of the world's population is at risk of malaria. Despite the evolution of measures of prevention and control, malaria still affects > 200 million, and kills > 600,000 people annually. No effective licensed malaria vaccine is available so far[8]. All malaria symptoms are consequence of repetitive cycles of parasite invasion of red blood cells. However, malaria infection starts silently when a few sporozoites are inoculated into the extravascular parts of the host skin during the bite of an infected mosquito[7]. These motile stages, after actively invading cutaneous blood vessels, enter into the blood circulation and arrest specifically in the liver, where they invade and develop inside hepatocytes[1]. One sporozoite generates thousands of red blood cell-infective stages, which after gaining access to the blood circulation start the eryhtrocytic cycle of invasion[6].

Since the liver is the only visceral organ where sporozoites appreciably develop following inoculation in the skin[4], blocking the parasite arrest in the liver sinusoids seems to be of utter importance to inhibit their subsequent invasion and development inside hepatocytes. The circumsporozoite protein (CSP) is thought to be the main parasite molecule responsible for the specific arrest of sporozoites in the liver[9]. However bioluminescent midgut-derived sporozoites, which express high levels of CSP on their surface, are not specifically arrested in the liver *in vivo*.

Therefore our project proposes, using a rodent model of experimental malaria, to identify parasite molecules involved in this crucial arrest step and to target them with neutralizing antibodies to inhibit the ensuing liver infection. To accomplish these objectives the project is divided in three main parts, (i) identification *in silico* of surface protein candidates containing adhesive-motifs, (ii) screening of candidates that bind to liver cells *in vitro* and *in vivo*, and (iii) measurement of sporozoite arrest and infection inhibition in the liver, following

immunization using the selected candidates.

The first part of the project will be achieved using PlasmoDB[10], a database that compiles proteomic and transcriptomic data of *Plasmodium*, including a list of molecules expressed in the sporozoite stage. Our first set of candidates is composed of proteins containing the ThromboSpondin type-I Repeat (TSR) motif. TSR is an adhesive domain that can bind to heparin[11], a molecule known to inhibit the binding of sporozoite to hepatocytes[12]. *Plasmodium* sporozoites express at least 7 proteins containing a TSR motif. After selecting adhesive candidates, the second part of the project will test the ability of these molecules to specifically bind to liver cells. Homologous and/or heterologous systems of expression will be used to direct the candidates to the surface of fluorescent and bioluminescent cells, which will be then used to quantify by imaging, (i) the binding of these adhesin-expressing cells on hepatocytes and liver sinusoidal endothelial cells using flow chambers and (ii) the arrest of these cells in the liver using bioluminescent imaging. Finally, the third part of the project will determine if immunization using these candidates, delivered by a lentiviral platform in a prime-boost regimen, can inhibit the arrest of bioluminescent parasites in the hepatic sinusoids and liver infection.

The unraveling of molecular determinants involved in the arrest of sporozoites in the liver sinusoids, as well as, their blockage by neutralizing antibodies might represent an important step towards the discovery of new protective antigens and consequently the development of a more efficient malaria vaccine.

References:

1: Looking under the skin: the first steps in malarial infection and immunity. Nat Rev Microbiol. 2013;11:701-12.
2: In vivo imaging of CD8+ T cell-mediated elimination of malaria liver stages. Proc Natl Acad Sci U S A. 2013;110:9090-5.
3: Role of host cell traversal by the malaria sporozoite during liver infection. J Exp Med. 2013; 210:905-15.
4: Development of the malaria parasite in the skin of the mammalian host. Proc Natl Acad Sci U S A. 2010;107:18640-5.
5: Host cell traversal is important for progression of the malaria parasite through the dermis to the liver. Cell Host Microbe. 2008;3:88-96.
6: Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids. Science. 2006;313:1287-90.
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8: www.who.int/malaria/en.
9: The basolateral domain of the hepatocyte plasma membrane bears receptors for the circumsporozoite protein of Plasmodium falciparum sporozoites. Cell. 1992;70:1021-33.
10: www.plasmodb.org.
11: Heparin-binding growth-associated molecule contains two heparin-binding beta -sheet domains that are homologous to the thrombospondin type I repeat. J Biol Chem. 2000 May 5;275:13564-70.
12: The binding of the circumsporozoite protein to cell surface heparan sulfate proteoglycans is required for plasmodium sporozoite attachment to target cells. J Biol Chem. 2001;276:26784-91.

Expected profile of the candidate:

We are expecting highly motivated, dynamic and independent candidates. Previous experience in molecular biology and/or animal experimentation is desirable, but not indispensable.

Contact: roti@pasteur.fr

PhD PROPOSAL 3

Title of the PhD project: Hunting a metabolic supercomplex in *Corynebacterium*
Keywords: *Mycobacterium*; structural biology; complex; metabolism; cryo-EM
Institut Pasteur: Institut Pasteur – Paris, France
Department: Structural Biology and Chemistry
Name of the lab: Structural Microbiology
Head of the lab: Pedro M. Alzari
PhD advisor: Marco Bellinzoni
Email address: marco.bellinzoni@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/en/team/group-marco-bellinzoni/
Doctoral school affiliation and University: MTCI/Université Paris Diderot (Paris 7)

Presentation of the laboratory and its research topics:

The Structural Microbiology Unit at the Institut Pasteur has a long-lasting interest in the structural biology of mycobacteria and has been active member, for more than ten years, of three successive European Commission-funded international clusters dedicated to the identification, characterization and validation of new targets for the development of novel anti-tuberculosis molecules, *i.e.* the projects 'X-TB' (2001-2005), 'NM4TB' (2006-2011) and the ongoing project 'More Medicines for TB' ('MM4TB', 2011-2016). The activity of the lab has been focused on the elucidation of the molecular basis of signal transduction in bacteria, with several achievements on the structure and function of Ser/Thr kinases [1,2], phosphatases [3] and two-component systems [4,5]. Through the study of signal transduction pathways, and in collaboration with other groups abroad, the team has also contributed to show how mycobacteria have developed a proper mechanism of control of their central metabolism, in particular of the α-ketoglutarate 'crossroad' between the Krebs cycle and the nitrogen assimilation [6,7]. The Unit has well-established collaborations with several groups located in South America, *e.g.* the Pasteur Institute of Montevideo, Uruguay and the Institute of Molecular and Cellular Biology (IBR) in Rosario, Argentina.

Description of the project:

The tricarboxylic acid cycle, also known as the Krebs cycle, is one of the most conserved biochemical pathways in living organisms. However, despite having been known for at least eighty years, surprising new findings could still be 'round the corner', especially when we look at the spatial and temporal organization of the enzymes involved. Indeed, a few papers published in the eighties suggested that at least some of the Krebs cycle enzymes might be spatially organized in one or more clusters, likely forming multi-protein complexes [8,9], although these studies have not found much echo in more recent years. Our interest there started while looking at signal transduction pathways in mycobacteria, when we found how *Mycobacterium tuberculosis*, the etiological agent of tuberculosis, controls the activity of the α-ketoglutarate dehydrogenase complex (KDH), a tripartite complex part of the Krebs cycle and devoted to the oxidative decarboxylation of α-ketoglutarate [10,11]. While it turned out that mycobacteria control KDH through the small protein GarA [6], itself a substrate of Ser/Thr kinases [2,6] and an unknown way of regulation of central metabolism, we also accumulated evidence that, in mycobacteria and possibly in all Actinobacteria, KDH might actually be merged to PDH, *i.e.* the pyruvate dehydrogenase complex (which links glycolysis to the Krebs cycle generating acetyl-CoA), to form a unique metabolic 'supercomplex'. Since no KDH-specific E2o component (dihydrolipoamide succinyltransferase) is present, E2p from PDH has to be used as a lipoyl donor instead and is supposed to make the central core of the supercomplex. The architecture of such a complex is hard to predict, but we can expect it to be, in size, much larger than the ribosome. The student/fellow will therefore be enrolled in our project, whose goal is to isolate this complex, or reconstitute it *in vitro*, in order to

characterize it structurally by a combination of hybrid structural biology approaches, including cryo-electron microscopy, SAXS and X-ray crystallography. We have been working on *Corynebacterium glutamicum* as a non-pathogenic model, where similar evidence of the existence of a PDH/KDH mixed supercomplex has been reported [12,13], and are following two parallel approaches: 'top-down', *i.e.* isolating the complex from the source, and 'bottom-up', *i.e. in vitro* reconstitution from the recombinant isolated components. The long-term goal, however, is to go beyond a static structural picture, clarifying the dynamic processes by which the different enzymatic activities may be temporally and spatially coordinated, and to understand, in the end, by which molecular mechanisms (and in response to which stimuli) such a huge machinery could be regulated. In turn, his may open exciting perspectives for the development of new antibiotics, keeping in mind that new control mechanisms of the bacterial central metabolism, not necessarily restricted to Actinobacteria, could be unveiled.
This project is funded by a 'Young Researcher' grant from the ANR (French National Agency for Research).

References:

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[4] Albanesi, D., Martín, M., Trajtenberg, F., Mansilla, M.C., Haouz, A., Alzari, P.M., de Mendoza, D. and Buschiazzo A. (2009) *Proc. Natl. Acad. Sci. USA* 106: 16185-16190.
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[10] Wagner, T., Bellinzoni, M., Wehenkel. A., O'Hare, H.M. and Alzari, P.M. (2011) *Chem. Biol*. 18: 1011-1020.
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[12] Niebisch, A., Kabus, A., Schultz, C., Weil, B., and Bott, M. (2006). *J. Biol. Chem.* 281: 12300-12307
[13] Hoffelder, M., Raasch, K., van Ooyen, J, and Eggeling, L. (2010) *J. Bacteriol*. 192: 5203-5211.

Expected profile of the candidate:

The ideal candidate for the PhD fellowship is a brilliant and self-motivated master-level student, firmly interested in protein biochemistry and structural biology and willing to learn working with a portfolio of complementary techniques. Strong communication and presenting skills, as well as capability to work in a team are essential. Some experience in protein biochemistry, biophysics or structural biology would be a plus.

Contact: informal enquiries are welcome: marco.bellinzoni@pasteur.fr.

PhD PROPOSAL 4

Title of the PhD: Exploration of gastric microbiota on the course of *Helicobacter pylori* disease.
Keywords: *Helicobacter pylori*, gastric microbiota, histological stages, personalized medicine
Institut Pasteur: Institut Pasteur de Guadeloupe
Name of the lab: Unité Environnement et Santé
Head of the lab: Dr Antoine Talarmin
PhD advisor: Dr Sébastien Breurec
Email address: sbreurec@gmail.com
Web site address of the lab: http://www.pasteur-guadeloupe.fr/unite_env_sante/
Doctoral school affiliation and University: Université des Antilles et de la Guyane

Presentation of the laboratory and its research topics:

The Environmental- Health unit was created in 2010. It is composed of five researchers, one post-doctoral researchers, 3 PhDs and 3 technicians.
The laboratory facilities include a Biosafety level 3 laboratory, a molecular biology platform and a Maldi-Tof/Tof spectrometer.
The research topics of this unit are oriented on relation of health with the environment. Three main topics emerged :
- Medical entomology with the study of vectors responsible for viral diseases in Guadeloupe especially *Aedes aegypti* vector of Dengue and Chikungunya fevers and vectors responsible for the transmission of the West Nile valley fever.
- The environmental pathogens especially free living amoebas such as *Naegleria fowleri*
- Antibiotic resistance and in particular the role of environment in the spread of antibiotic resistance.
A new topic is being developed concerning the relation between microbiome and various environmental conditions and the proposed project is part of this program.

Description of the project:

The human stomach, an organ that was long thought to be sterile because of its acidity, is colonized by a Gram negative bacterium *Helicobacter pylori* in half of the human population. Persistent colonization of the gastric mucosa by *H. pylori* is associated with pathologies ranging from gastritis, peptic ulcer disease to gastric cancer (GC) that accounts for 800,000 deaths in the world every year. *H. pylori* infection is recognized as the main risk factor for distal gastric cancer, although just a fraction of infected patients (<3%) ever develop GC. Gastric Cancer is an inflammation-driven disease and many factors influencing the mucosal immune response may involve in the disease outcome. It is now well established that *H. pylori* virulence factors play a role in cancer pathogenesis. However, *H. pylori* is not alone in the stomach. Gastric microbiota through the inability to contain deleterious gut microbes (barrier function), through defective host immunoregulation, or both, is suspected to promote the disease and to determine the progression of the inflammation to preneoplastic stages (atrophy, metaplasia, intestinal dysplasia). Several studies in animal models have demonstrated the influence of microbiota on the severity of lesions. Only few studies have analyzed the gastric microbiota in human. Diet and lifestyle that have been shown to have profound impacts on the composition of the microbiota are clearly different between continents. The typical diet of Europe is rich in meat and saturated fats, while the African diet is reflective of those often seen in 'developing' countries: low in meat, saturated fat, and vitamins, but rich in complex carbohydrate. Thus, a ‘protective’ or a ‘deleterious’ gastric microbiota, if existing, could participate to difference of cancer risk. In Guadeloupe (French West Indies), very few data exist on the epidemiology of *H. pylori* infection. The prevalence rate was 55.2% which will be the support of a strong recruitment of patients. The main ethnic

groups were African descent/Multiracial/Creole (Primarily of European, African, Indian and Amerindian mix) (71%), Indian, mostly Tamil descent (15%) and white European (Mostly of French descent) (9%).

The aim of our study is to characterize the non-*H.pylori* flora at different histological stages of infection, from gastritis to preneoplastic lesions, to gastric cancer (GC) in human. We will develop a common questionnaire to select patients from their clinical history and to collect information on sex, age, diet, race, obesity and lifestyle. We will obtain our panel of patients from one centre in Guadeloupe. Histological examination of biopsies will be performed. One hundred gastric biopsies will be selected from patients with erosive gastritis, gastric atrophy, preneoplastic. For each selected patient, we will attempt bacteria cultivation from one biopsy. If *H. pylori* is obtained in culture, strains will be characterized by MLST, and the classic virulence factors *cagA* will be systematically searched and partially sequenced. Our experimental strategy will use high-throughput sequencing (NGS) technologies, which have increased the capability and scope of the study of complex microbiomes. They may be an ideal approach in human health to explore whole microbial communities (cultivable and not cultivable bacteria) and to suspect bacterial organisms association in particular conditions (histological stages...). We will introduce scientific visualization tools, which represent innovative methods for exploring large datasets to search which factor or combination of factors are determinant to group (by eyes) microbiota according to their relative composition of phylotypes. The conventional UniFrac distance metric and Principal Coordinates Analysis (PCoA) will be used to cluster bacterial communities consisting of similar lineages (phylogenetic distance and number of species) and to distinguish the others. The expected observation of shifts in the gastric microbiota between histological stages will have potential applications both for the understanding of the progression of the disease and the development of a prognostic test, especially because the microbiota changes can be detected easily and inexpensively. A project based on the same methodology including two centres in Africa where the infection prevalence can reach 80% (Senegal, Algeria) will be submitted in 2016 for funding.

References:

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2- Li XX1, Wong GL, To KF, Wong VW, Lai LH, Chow DK, Lau JY, Sung JJ, Ding C.Linz B, Vololonantenainab CR, Seck A, Carod JF, Dia D, Garin B, Ramanampamonjy RM, Thiberge JM, Raymond J, Breurec S. Population genetic structure and isolation by distance of *Helicobacter pylori* in Senegal and Madagascar. PLoS One. 2014 Jan 30; 9:e87355.
3- Bacterial microbiota profiling in gastritis without *Helicobacter pylori* infection or non-steroidal anti-inflammatory drug use. PLoS One. 2009;4:e7985.
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5- Weill FX, Margeridon S, Broutet N, Le Hello S, Neyret C, Mégraud F. Seroepidemiology of *Helicobacter pylori* infection in Guadeloupe. Trans R Soc Trop Med Hyg. 2002; 96:517-9.

Expected profile of the candidate:

The PhD student should have completed a Master degree or equivalent within the field of computational biology or related fields. Techniques required for this research project include NGS technologies. Familiarity with this type of data and methods for analysis as well as the relevant statistics will be an advantage.

Contact: Sébastien Breurec, Institut de Pasteur de Guadeloupe, Unité Environnement et Santé, sbreurec@gmail.com, Tél. (+590) 590 89 17 30

PhD proposal 5

Title of the PhD project: How can *Yersinia pestis* become invisible to the host immune system?
Keywords: Plague, *Yersinia pestis*, host cellular immune response, immune evasion
Institut Pasteur: Institut Pasteur – Paris, France
Department: Microbiology
Name of the lab: *Yersinia* Research Unit
Head of the lab: Prof. Elisabeth Carniel
PhD advisor: Dr. Christian Demeure
Email address: cdemeure@pasteur.fr
Web site address of the lab: https://research.pasteur.fr/fr/team/yersinia/
Doctoral school affiliation and University: Université Paris-Sud (Paris 11)

Presentation of the laboratory and its research topics:

The activities of the *Yersinia* Research Unit are primarily devoted to the analysis of:
- Mechanisms of horizontal gene transfer in *Yersinia*.
- Comparative genomics and transcriptomics between *Y. pestis* and *Y. pseudotuberculosis*.
- Molecular bases for the exceptional pathogenicity of *Y. pestis*.
- Pathophysiology of *Yersinia* infections.
- Host's mechanisms of innate and adaptive immunity.
- Genetic bases of host susceptibility to plague.
- Resistance of pathogenic *Yersinia* to antibiotics.
- Evolution of pathogenic *Yersinia*.

The Unit is also developing:
- A vaccine against plague and pseudotuberculosis.
- Typing tools for molecular epidemiology.
- Real time *in vivo* imaging technologies for pathogenic *Yersinia*.
- Tools for stable gene complementation and gene expression *in vitro* and *in vivo*.
- Techniques for molecular characterization of the various *Yersinia* species.

The Unit participates actively to the surveillance and control of enteropathogenic *Yersinia* through its activities at the National level (Reference Laboratory and French Surveillance Network), and to the fight against plague at the international level (World Health Organization Collaborating Center for *Yersinia*).

Description of the project:

Y. pestis is the agent of plague, a disease transmitted from rodents to humans by fleabites. Bubonic plague is fatal for 50-70% of patients in the absence of treatment. Pneumonic plague results from inter-human contamination through aerosols and is an acute and fulminant pneumopathy, which is systematically lethal in usually less than 3 days. *Y. pestis* is thus among the most pathogenic bacteria for humans. Despite considerable progress in plague prevention and cure, this infection has not been eradicated and natural plague foci exist in Africa, Asia and the Americas.

The key to *Y. pestis* virulence is its capacity to escape host immunity. During the first 48h of infection, the host's innate immune response is defective (pre-inflammatory phase), allowing the bacteria to multiply and invade tissues. How *Y. pestis* prevents this response and what are the bacterial virulence factors responsible for this inhibition remain open questions. The proposed project will aim at deciphering the bacteria-cell and cell-cell interactions that occur early during the infectious process to paralyze the host defense system.

The normal immune response against bacteria involves a cascade of signals starting from sentinel cells (dendritic cells (DC), macrophages), and continuing with the activation of other

cell types such as natural killer cells (NK) and T lymphocytes to produce cytokines involved in effector mechanisms. In this project, NK and DCs recruitment to infected lymph nodes will be characterized using the mouse experimental model of bubonic plague. The dynamics of recruitment and expansion or destruction of these populations in the lymph node infected with *Y. pestis* will be analyzed (flow cytometry and fluorescence microscopy). Production of cytokines and chemokines activating/attracting NK cells will be examined. Among them, IFNγ will be particularly studied because the production of this cytokine that activates essential macrophage bactericidal functions is known to be severely impaired during plague. Immunohistology will be used to define cell localization and cell-cell contacts in the lymph nodes. The presence and source of NK-inactivating factors will be determined (Immunoassays/FISH). KO or transgenic mice will be used to confirm the roles played by the identified cell populations. Once specific specific cells and factors targeted early by *Y. pestis* are identified, the interactions between these targets and the bacteria will be deciphered using in vitro cultures of single and mixed cell populations. Cell survival, mechanisms of cell death, levels of cytokine production and activation markers will be examined.
Since the capacity to prevent the triggering of an early innate immune response is specific to the plague bacillus, another part of the project will aim at identifying the bacterial factors responsible for this unique property of *Y. pestis.* Two approaches will be used. One will benefit from the very close genetic relationship between *Y. pestis* and its ancestor *Y. pseudotuberculosis* (a much less virulent enteropathogen) to identify mechanisms of immune response inhibition that are triggered by *Y. pestis* but not *Y. pseudotuberculosis*. The second approach will consist in using a set of *Y. pestis* mutants devoid of various genetic elements acquired by *Y. pestis* after its divergence from *Y. pseudotuberculosis* (already available in the laboratory) to determine which factors play a role in the observed phenotypes. If necessary, additional mutants will be constructed.
This study should provide unprecedented understanding on how a highly pathogenic bacterium circumvents innate host defense mechanisms to invade and kill its host extremely efficiently.

References:

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- Guinet, F., Ave, P., Filali, S., Huon, C., Savin, C., Huerre, M., Fiette, L., and Carniel, E. (2015) Dissociation of Tissue Destruction and Bacterial Expansion during Bubonic Plague. ***PLoS Pathogens*** 11, e1005222
- Derbise, A., Hanada, Y., Khalife, M., Carniel, E., and Demeure, C. E. (2015) Complete Protection against Pneumonic and Bubonic Plague after a Single Oral Vaccination. ***PLoS NTD*** 9, e0004162

Expected profile of the candidate:

The project is proposed for a PhD. The candidate will have interest for host-pathogen interactions, and a training in immunology. Some knowledge in bacteriology would also be appreciated. The candidate should be ready to work on highly pathogenic bacteria, in biosafety level 3 environments and on animal models.
Speaking either English or French is mandatory.

Contact: Dr C. Demeure - christian.demeure@pasteur.fr

PhD PROPOSAL 6

Title of the doctoral project: *Aedes aegypti* populations from regions of Amazonian Basin: mapping insecticide resistance profile, selected mechanisms and population genetics.
Keywords: Aedes aegypti, insecticide resistance, Amazon region, population structure
Institut Pasteur : Institut Pasteur de la Guyane – Cayenne, French Guiana
Name of the lab: Unité d'entomologie médicale
Head of the lab: Romain Girod
PhD Advisor: Isabelle Dusfour
Email address: idusfour@pasteur-cayenne.fr
Web site address of the lab: www.pasteur-cayenne.fr
Doctoral school affiliation and University: Université de Guyane

Presentation of the laboratory and its research topics:

The Medical Entomology Unit is headed by Dr Romain Girod, PhD. The team is currently composed by two researchers, one post-doctoral researcher, two PhD students, one research assistant, four technicians and regularly welcomes students. Research topics concentrate on vectors of malaria parasites, dengue, chikungunya and other arboviruses in French Guiana. Research programs are developed on bionomics, transmission, competence and insecticide resistance of those vectors. Through STRonGer European funded program (CAPACITY specific program FP7-REGPOT-2011-1) the unit has recently benefited from the construction of new research facilities including a level-3 biosafety laboratory, insectaries, insecticide testing room and molecular biology workspaces.
Team members authored 21 publications in international journals the last five years.

The most relevant publications for the project are

1: Dusfour I et al. Deltamethrin resistance mechanisms in Aedes aegypti populations from three French overseas territories worldwide. Plos Neglected Tropical Diseases, Accepted
2: Faucon F etal. Identifying genomic changes associated with insecticide resistance in the dengue mosquito *Aedes aegypti* by deep targeted sequencing. Genome Res. 2015 Sep;25(9):1347-59. doi: 10.1101/gr.189225.115.
3: Mahande AM et al. Knockdown Resistance, rdl Alleles, and the Annual Entomological Inoculation Rate of Wild Mosquito Populations from Lower Moshi, Northern Tanzania. J Glob Infect Dis. 2012 Apr;4(2):114-9. doi: 10.4103/0974-777X.96776.
4: Dusfour I et al. Multiple insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. Mem Inst Oswaldo Cruz. 2011 May;106(3):346-52.
5: Achee NL, et al. Characterization of spatial repellent, contact irritant, and toxicant chemical actions of standard vector control compounds. J Am Mosq Control Assoc. 2009 Jun;25(2):156-67.

Description of the project:

Aedes aegypti is vector of dengue, yellow fever and chikungunya viruses worldwide. This urban mosquito causes recurrent dengue outbreaks in South America (1) and is the only vector responsible of transmitting the recently introduced chikungunya virus in Amazonian region. To control this vector, mosquito control agencies have used various chemicals (organophosphates (OP), pyrethroids (PY).) or biological insecticides. Recent studies in French Guiana and Brazil conducted by our team and colleagues, have demonstrated heterogeneous distribution of resistance to PYs and OPs with high resistance levels near

country borders (2,3). These preliminary data highlighted the importance of insecticide resistance monitoring at regional level.

Several urban cities from the Amazon Basin are isolated due to dense forest barriers. Therefore it is likely that the relationship between *Ae. aegypti* populations among these localities may not be related with distance itself, but rather to circulation of people and goods. However, few is known on insecticide selective pressure in this area. Are we facing resistance local selection, migration of resistance allele or both? In a context of increasing severity and frequency of dengue outbreaks and of chikungunya and zika emergence in South America, answering these questions are essential for improving vector control strategies and insecticide resistance management. The PhD program will participate at mapping insecticide resistance profile, selected mechanisms and population genetics of *Aedes aegypti* populations in the Guiana shield in collaboration with Surinamese and Brazilian partners.

Task 1: sampling and handling mosquito populations

Mosquito collections will follow the same standardized protocols in all localities. Sampling will be made in 2-10 localities. At each locality, mosquito eggs and/or immature forms will be sampled in ca. 1km² area around the central point of each site, by ovitraps and/or larval collections using dippers and pipets. Such sampling design will allow sampling the diversity occurring in each population and limiting sampling bias in the subsequent genetic analyses.

Task 2: exploring the gene flow among *Ae. aegypti* populations

Current project in the team develop population genetics of *Aedes aegypti* populations in collaboration with the IMHT in Lisbon. The student will expand the microsatellite analyses to more population (4–6) and eventually apply new markers for his/her own purpose (7).

Task 3: evaluating insecticide resistance profiles and associated mechanisms

Mapping of resistance will be done for deltamethrin, malathion and temephos. Dose-dependent analysis will be performed using WHO bioassays (8).

Target-site changes alleles related to resistance to pyrethroids will be searched in those populations. In addition, new markers (9) related to metabolic resistance and recently developed by partners will also be characterized. Data analysis will consist of examining the occurrence of resistance markers in all populations across the study area and correlating their frequency (DNA markers) or intensity (RNA markers) with resistance data obtained for each insecticide. Cross-linking resistance data with population biology data will allow estimating the spread and flows or resistance alleles within the study area.

All this results will provide the solid scientific basis required for improving vector control strategies and insecticide resistance management in close collaboration with local authorities. This thesis work will get support from the newly created network on worldwide insecticide resistance in arboviruses vectors.

References:

1. WHO. Dengue and severe dengue. Geneva: WHO; 2012.

2. Dusfour I, Thalmensy V, Gaborit P, Issaly J, Carinci R, Girod R. Multiple insecticide resistance in Aedes aegypti (Diptera: Culicidae) populations compromises the effectiveness of dengue vector control in French Guiana. Mem Inst Oswaldo Cruz. 2011 May;106:346–52.

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4. Brown JE, McBride CS, Johnson P, Ritchie S, Paupy C, Bossin H, et al. Worldwide patterns of genetic differentiation imply multiple “domestications” of Aedes aegypti, a major vector of human diseases. Proc Biol Sci. 2011 Jan 12;278:2446–54.

5. Slotman MA, Kelly N, Harrington L, Kitthawee S, Jones JW, Scott TW, et al. Polymorphic microsatellite markers for studies of Aedes aegypti (Diptera: Culicidae), the vector of dengue and yellow fever. Mol Ecol Notes. 2007;7:168–71.

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7. Evans BR, Gloria-Soria A, Hou L, McBride C, Bonizzoni M, Zhao H, et al. A Multipurpose, High-Throughput Single-Nucleotide Polymorphism Chip for the Dengue and Yellow Fever Mosquito, Aedes aegypti. G3 Bethesda Md. 2015 May;5(5):711–8.

8. World Health Organization. Test procedures: for insecticide resistance monitoring in malaria vector mosquitoes. Geneva: World Health Organization; 2013.

9. Faucon F, Dusfour I, Gaude T, Navratil V, Boyer F, Chandre F, et al. Identifying genomic changes associated with insecticide resistance in the dengue mosquito Aedes aegypti by deep targeted sequencing. Genome Res. 2015 Sep;25(9):1347–59.

Expected profile of the candidate:

The candidate holds a Master degree in medical entomology. The applicant must have competences in molecular biology techniques. Knowledge on insecticide resistance, population genetics and/or experience in field collection is highly recommended.

Contact: Isabelle Dusfour (*idusfour@pasteur-cayenne.fr) and Romain Girod (rgirod@pasteur-cayenne.fr)*

PhD PROPOSAL 8

Title of the PhD project: Discovery of novel gene-environment interactions through monotonic models
Keywords: Statistical modeling, algorithms, biological complexity, machine learning
Institut Pasteur: Institut Pasteur – Paris, France
Department: Genomes and Genetics
Name of the lab: Systems Biology Lab
Head of the lab: Benno Schwikowski
PhD advisor: Benno Schwikowski
Email address: benno@pasteur.fr
Web site address of the lab: http://systemsbiology.org
Doctoral school affiliation and University: Frontières du Vivant (FdV)/Paris Diderot

Presentation of the laboratory and its research topics:

The Systems Biology Lab develops complex computational and statistical models of biological and biomedical complexity between genes, the environment, and higher-level phenotypes. Current projects are driven by biomedical applications in the areas of infection (dengue, malaria), and other complex diseases with a genetic component (autism, CCM). Using new computational models, algorithms, and visual analytics tools, we iterate rapidly between constructing models that include biological and biomedical intuition with large-scale data and prior knowledge, the development of statistics and algorithms that incorporate these elements, and the analysis of results with experts of the biological or medical problem, that are able to test predictions or collect new data.

Description of the project:

We aim to explore a newly accessible type of computational model to discover complex gene-environment interactions on the basis of large-scale 'omics data.
Monotonic models We will use monotonic functions for modeling complex biological relationships between environmental parameters, and molecular, physiological, and disease states. As an example, a quantitative disease state might be modeled as a function f(x,y) of two features x and y (e.g., an environmental exposure and a specific mRNA transcript level). In this case, *monotonicity* of *f* means that, under an increase of x, the phenotype f(x,y) cannot increase for some values of (x,y), and decrease for others (and similar for y).

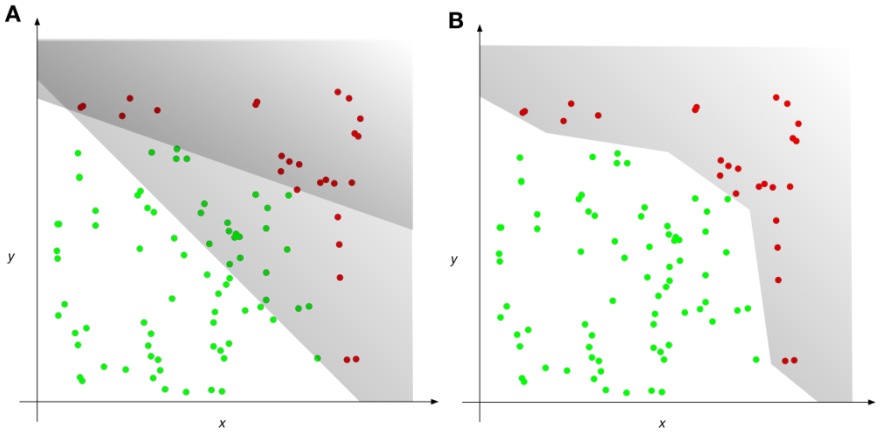

Figure 1: Simulated data representing "green" and "red" phenotype f(x,y) that depends on a combination of two features (e.g., a transcript level and an environmental parameters).
[A] Linear decision boundaries cannot properly separate green and red phenotypes.
[B] Monotonic regression is flexible enough to separate phenotypes.

Computational complexity The reason why multidimensional (d>1) monotonic (or the

closely related isotonic) models have hardly been utilized in the past on a genome scale lies in the time complexity of previous known algorithms for direct monotonic regression: At least cubic ($O(n^3)$) already for two variables[1]. Equivalent linear programming approaches were not much utilized in applications, possibly because of the additionally required feature selection[2-4].

Rationale Unlike in most commonly used models, in monotonic regression the effects of a change of mRNA concentration on f are not modeled by parameters, but learned from data. Monotonic models marry an increased ability to capture extended biological complexity (including OR and AND-like functions with discretization thresholds learned from data) with intuitive interpretability. The recent feasibility of efficient low-dimensional regression algorithms therefore offers an unprecedented opportunity to discover biomarkers and intuitively accessible hypotheses of GxE associations from combinations of environmental, omics, and phenotypic data. Furthermore, the genome-wide detection of such pairwise markers leads to yet-uncharted 'phenotype-predictive networks' whose biological significance and uses in biomedical applications wait to be explored.

Preliminary data In a first application we modeled the severity of dengue infection on the basis of clinical and omics parameters at hospital admission, and performed a screen for pairwise monotonic regression models. Figure 2 shows the best regression model, which uses HDL and another transcript to separate severe from non-severe infection. Interestingly, the region encoding the newly identified gene has been identified as a QTL region controlling HDL levels[5]. Combining both data may therefore represent an interesting candidate marker that has immediate applications in the clinic (and this can be validated rapidly through the existing close collaboration with the Pasteur Institute in Cambodia). Secondly, additional experiments triggered by our observation in cellular models may clarify the complex way in which HDL and its partner gene are related to dengue severity, thus generating deeper understanding in which molecular level, physiology, and disease states are connected in this case.

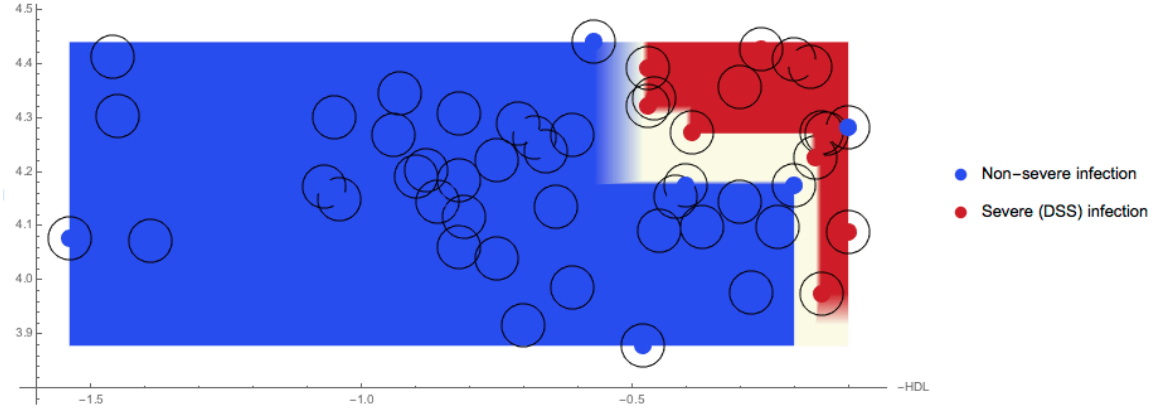

Figure 2: Joint observations of HDL and a specific mRNA transcript across 49 Cambodian dengue patients (black circles, transcriptomes courtesy A. Sakuntabhai, IP; V. Duong, IP Cambodia). Background: Interpolated model. Blue: non-severe; red: severe infection.

We will develop monotonic models further, and apply them to the modeling of gene-environment interactions. In a first step, we will [1] Assemble suitable medium- or large-scale datasets, [2] adapt statistics and corresponding fast algorithms to assess the statistical significance of the best models/markers. [3] As an extension towards more powerful, and potentially more accurate models, extend an existing algorithm towards high-quality monotonic regression models for dimensions higher than two. [4] We will aim to validate the resulting prediction from a large computational GxE screens on the datasets ([1]).
If successful, this project will lead to a powerful new type of model that can be used to describe and identify quantitative gene-environment relationships that are, on one hand, more complex than what typical current approaches can uncover, and, on the other hand,

still intuitively accessible to biologists and other experts to suggest validation and follow-up experiments.

References:

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2 Chandrasekaran, R., Ryu, Y. U., Jacob, V. & Hong, S. Isotonic separation. *INFORMS Journal on Computing* **17**, 462–474 (2005).

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4 Malar, B. & Nadarajan, R. Evolutionary isotonic separation for classification: theory and experiments. *Knowledge and Information Systems* **37**, 531-553, doi:10.1007/s10115-012-0579-5 (2012).

5 Lin, J.-P. Genome-wide scan on plasma triglyceride and high density lipoprotein cholesterol levels, accounting for the effects of correlated quantitative phenotypes. *BMC genetics* **4 Suppl 1**, S47, doi:10.1186/1471-2156-4-S1-S47 (2003).

Expected profile of the candidate:

Required: Excellent programming skills, solid knowledge in basic mathematics, statistics and basic genomic biology
Pluses: Practical experience in: handling ‘omics data, applications of machine learning, advanced algorithms, advanced statistics/biostatistics

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PhD PROPOSAL 9

Title of the PhD project: Analysis of the functional organization of the folate receptor alpha (FRα) in ovarian cancer cells
Keywords: GPI-anchored proteins, membrane microdomains, protein sorting, live imaging, protein protein interaction, signaling, cancer, epithelial cell polarity
Institut Pasteur: Institut Pasteur – Paris, France
Department: Cell Biology and Infection
Name of the lab: Membrane Trafficking and Pathogenesis
Head of the lab: Chiara Zurzolo
PhD advisor: Chiara Zurzolo/Stephanie Lebreton
Email address: chiara.zurzolo@pasteur.fr
Doctoral school affiliation and University: ParisXI

Presentation of the laboratory and its research topics:

The Unit of Membrane Trafficking and Pathogenesis headed by Chiara Zurzolo is a very young, dynamic laboratory composed of 12 people with one senior scientist besides the head, two technicians and a balance between young post docs and students (generally coming from different backgrounds and nations). Chiara Zurzolo, EMBO member, a recognized expert in the cell biology of protein and lipid trafficking, is also the head of the Department of Cell Biology and Infection at the Pasteur Institute. The laboratory combines high resolution imaging approaches in living cells with molecular biology and classical biochemistry, and excels in different assays for testing exocytic transport, endocytosis and transcytosis of proteins. They pioneered studies on apical protein sorting in polarized epithelial cells and on the intercellular spreading of prion aggregates. More recently they have proposed a role of tunneling nanotubes (TNTs) in the spreading of different prion-like aggregates, which is one of the main aims of this proposal.
The focus of the lab is on the mechanisms of protein trafficking in polarized epithelial and neuronal cells in order to understand how intracellular trafficking contributes to protein function and/or malfunction in the case of diseases, from cancer to neurodegeneration in two major research lines:

1. Mechanisms of apical sorting and membrane organization of GPI-anchored proteins (GPI-APs) in polarized epithelial cells: GPI-APs are sorted to the apical membrane in several epithelial cell lines and associate with lipid rafts during their transport to the plasma membrane. We have shown that the mechanism of GPI-AP sorting controls their function on the apical membrane of polarized epithelial cells. We use the state of the art microscopic and biochemical approaches to analyze the role of lipid domains and to identify the molecular machinery of GPI-AP sorting and organization, to understand their functional relevance in control conditions and in polarity loss like cancer.
2. Molecular mechanisms of conversion and spreading of prion and «prion-like» proteins in neurodegenerative diseases: We focus on the mechanism of prion infection, and on the role of trafficking in the pathogenesis of prion-like neurodegenerative diseases, linked to protein misfolding (e.g., Alzheimer, Parkinson, Huntington). We have shown that prions spread between cells in tunneling nanotubes (TNTs). We are studying the mechanisms of TNT formation in cell and organotypic cultures and their relevance in the intercellular transmissibility of amyloid proteins and in the pathogenesis of different neurodegenerative diseases.

Description of the project:

Analysis of the functional organization of the folate receptor alpha (FRα) in ovarian cancer cells

90% of human cancers are carcinoma that derived from epithelial cells and the epithelial ovarian cancer is the most lethal gynaecologic malignancy and the sixth most common cancer in women worldwide. The folate receptor alpha (FRα) is attached to the external leaflet of the plasma membrane via its glycosylphosphatidylinositol (GPI) anchor and is apically expressed in a restricted number of normal tissues (kidney, lung). Its function is to uptake folic acid that is critical for cell proliferation. Interestingly, FRα is over expressed in ovarian cancer patients and is thought to be a very attractive target for new anticancer agents (Kalli et al. 2008; van Dam et al. 2011; Siu et al. 2012; Walter et al. 2013). However the exact function of FRα, in ovarian cancer patients is unknown. In MA104 cells it has been shown that its capacity to uptake folic acid is depending on its plasma membrane organization.

GPI-anchored proteins are normally restricted to the apical membrane of polarized epithelial cells, where they have different functions (receptor, signalling, adhesion) (Chatterjee et al. 2001). We have recently shown that the activity of GPI-APs, at the apical surface of polarized MDCK cells, is directly regulated by their sorting in the Golgi complex (Paladino et al. 2014). Indeed in polarized MDCK cells, GPI-APs form homo-clusters in the Golgi in order to be apically sorted. This directly governs their organization in hetero-clusters and their specific activity at the apical surface. Accordingly, in non-polarized MDCK cells GPI-APs are not sorted and do not cluster in the Golgi, and as consequence remain monomeric and not fully functional at the cell surface. These data show that apical GPI-APs function is regulated by the selective sorting mechanism that occurs in the Golgi complex only when epithelial cells have established their polarity.

In this project we propose to investigate how loss of polarity in ovarian cancer cells would affect the surface organization and function of the FRα. Specifically we will investigate the Golgi and plasma membrane organization of FRα and determine how this organization affects folate uptake in ovarian cancer cells. GPI-AP organization is regulated by cholesterol and by the actin cytoskeleton, and these molecules appear to have different roles in polarized non polarized epithelial cell and fibroblasts (Goswami et al. 2008, Paladino et al. 2014; Lebreton et al. in preparation); therefore we will decipher the role of cholesterol, actin cytoskeleton as well as proteins involved in the loss of epithelial polarity (Par, Crumbs, Ecadherin, Ras, RhoA, etc.) in FRα.

To achieve this objective the applicant will use different methodologies: cell cultures, classical biochemistry, as well as state of art imaging techniques that are already set up in the laboratory. As an example, the analysis of the plasma membrane organization of FRα will be studied by performing both Number and Brightness, Fluorescence Lifetime Imaging Microscopy (FLIM) (Paladino et al. 2014) and super resolution Stochastic Optical Reconstruction Microscopy (STORM)(Lebreton et al. in preparation). These techniques will allow the reach an integrated vision of the organization and the molecular interactions of FRα in the different experimental conditions, up to single molecule level. The functionality of the receptor will be address by performing internalization assays of the receptor and its ligand (classical confocal microscopy and FACS) (Sandoval et al. 2004; Sabharanjak et al. 2004; Walter et al. 2013)

References:

1) Paladino S, Lebreton S, Zurzolo C.
Trafficking and Membrane Organization of GPI-Anchored Proteins in Health and Diseases.
Curr Top Membr. 2015;75:269-303.

2) Manuel Muniz and Chiara Zurzolo. Sorting of GPI-anchored proteins from yeast to mammals: common pathways for different sites? **Invited review JCS 2014** Jul 1;127(Pt 13):2793-801.

3) Paladino S*, Lebreton S*, Tivodar S, Formiggini F, Ossato G, Gratton E, Tramier M, Coppey-Moisan M and Zurzolo C
Golgi sorting regulates organization and activity of GPI-proteins at apical membranes. **Nat Chem Biol. 2014 Mar 30. doi: 10.1038/nchembio.1495. [Epub ahead of print]**

4) Imjeti NS*, Lebreton S*, Paladino S, de la Fuente E, Gonzalez A, Zurzolo C
N-Glycosylation instead of cholesterol mediates oligomerization and apical sorting of GPI-APs in FRT cells. **Molecular Biology of the Cell. 2011 Dec;22(23):4621-34.**

5) Paladino S, Lebreton S, Campana V, Tivodar, S, Tempre R and Zurzolo Chiara. Lipid environment and GPI-anchor are invoved in apical sorting of GPI-anchored proteins promoting protein oligomerization. **J Cell Sci. 2008 Dec 15;121(Pt 24):4001-7**

6) Lebreton S, Paladino S and Zurzolo Chiara, Selective roles for cholesterol and actin in compartmentalization of different proteins in the Golgi and plasma membrane of polarized cells. **Journal of Biological Chemistry. 2008 Oct 24;283(43):29545-53.**

***First joint author**

Expected profile of the candidate:

A background in cell biology, tissue culture, biochemistry and/or imaging is welcome, but not essential.
Most importantly the candidate should be academically outstanding, self-motivated, curiosity driven endowed with a strong work ethic, good communication skills, community spirit and ability to work in a team. He /she should show a genuine interest in cell biology and a strong motivation in applying the state of the art imaging technologies to solve biological problems.
English knowledge is required (as meetings and work is performed in English), as well as the capacity to browse the literature and critically study the scientific papers related to the subject.

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