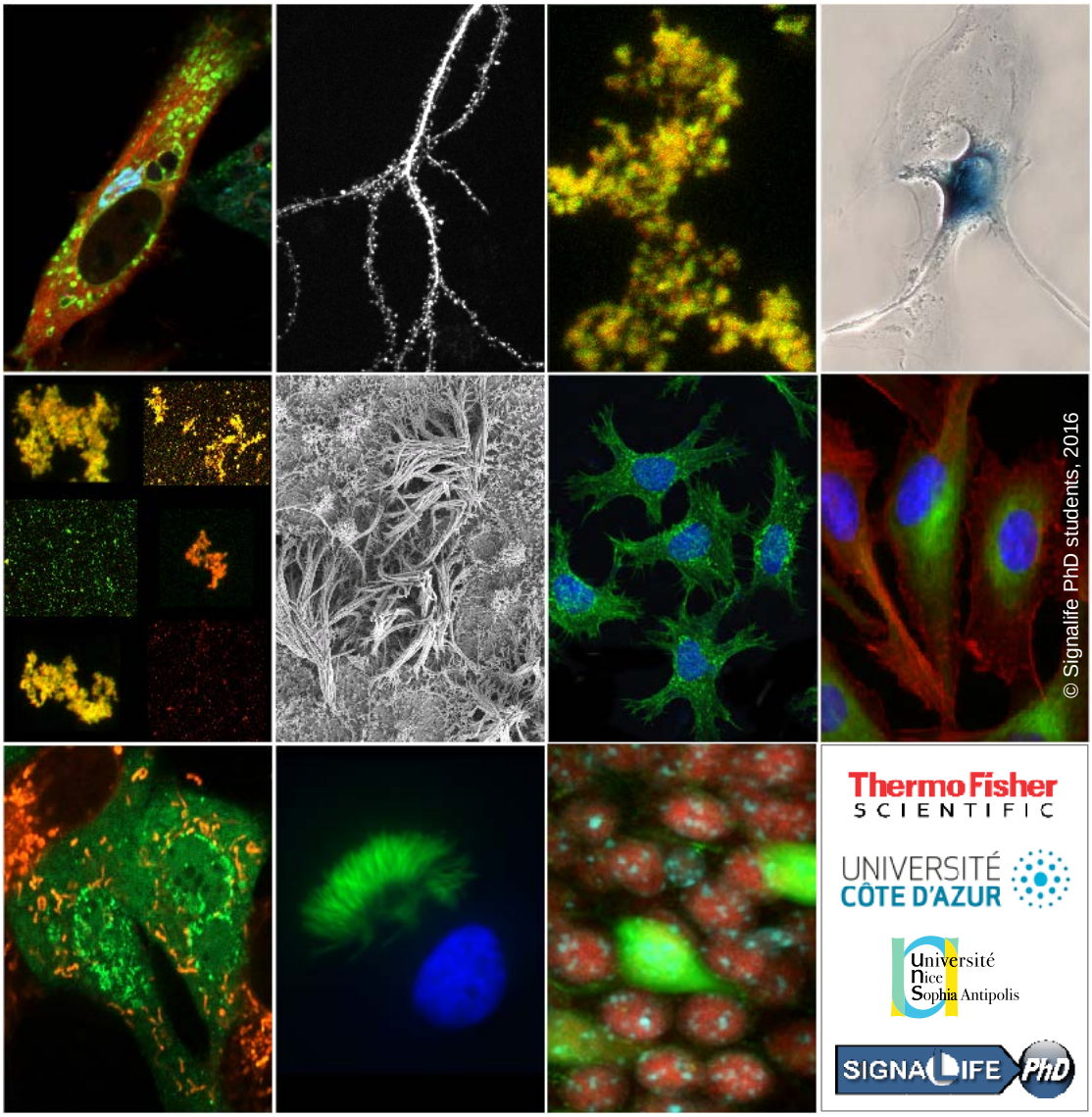
Labex SIGNALIFE
Student Conference/Retreat

14th - 15th November 2016



ThermoFisher SCIENTIFIC

UNIVERSITÉ CÔTE D'AZUR

Université Nice Sophia Antipolis

SIGNALIFE PhD

Château Valrose, Nice, France

Program schedule

Monday, November 14th

9.00	Welcome coffee, registration
9.30	Opening introduction

SESSION 1: 'Nervous & immune system'

9.40	Guest Speaker: **Dr Valérie Verhasselt,** Tolérance Immunitaire Team, Université de Nice Sophia-Antipolis, Hopital de l'Archet 1, Nice *Mother-child interaction through breast milk: a critical step for immune system maturation*
10.20	Student talk 1: **Derya Deveci** *Allatostatin- A as a coordinator of growth and maturation*
10.40	Student talk 2: **Aidan Falvey** *The potential role of the carotid body in homeostatic regulation of inflammation*
11.00	**Coffee break**
11.30	Student talk 3: **Scherazad Kootar** *Blocking glucocorticoid receptors prevents acute effects of amyloid-beta oligomers at hippocampal synapses*
11.50	Student talk 4: **Lazaro Emilio Aira Diaz** *Implication of Lyn tyrosine kinase in Psoriasis*
12.10	Student talk 5: **Jozef Bossowski** *Low-protein diet suppresses cancer progression through immune system modulation*
12.30	**Lunch buffet**

SESSION 2: 'Organelles & lipids'

13.30	**KEYNOTE speaker 1: Dr Aurelien Roux,** University of Geneva, Switzerland *Buckling of the cell membrane by ESCRT-III*
14.20	'Signalife' Principal investigator: **Dr Bruno Antonny,** IPMC, Sophia-Antipolis *Fishing transport vesicles with a string and a hook, the GMAP-210 example*
15.00	Student talk 6: **Denisa Jamecna** *Investigation into the function of the N-terminal region of OSBP*
15.20	Student talk 7: **Cynthia Lebeaupin** *Lack of Bl-1 predisposes to ER stress-induced Non-Alcoholic Fatty Liver Disease*
15.40	**Coffee break**
16.00	**Bowling/snooker at Acropolis**
19.00	**Dinner at Villa d'Este**
après dîner	**Drinks in the Old Town**

Tuesday, November 15th

9.00 Welcome coffee

9.25 Introduction of the second day

SESSION 3: 'Mass spec. & protein/DNA/RNA interactions'

9.30 **KEYNOTE speaker 2: Dr Jeroen Krijgsveld**, German Cancer Research Center, Heidelberg, Germany
Proteome meets genome: dynamics of chromatin composition in embryonic stem cells

10.20 Guest Speaker: **Dr Sabrina Pisano**, Institute for Research on Cancer and Ageing,IRCAN, Nice
Atomic force microscopy as tool to achieve insights into nucleic acids

11.00 **Coffee break**

11.20 Student talk 8: **Nadia Formicola**
CamKII: a novel regulatory component of Imp neuronal RNA granules?

11.40 Student talk 9: **Agustina Razetti**
Drosophila gamma neuron branching process during development: a mechanistic approach

12.00 Student talk 10: **Sandra Ruiz-Garcia**
Airway epithelium defferentiation: digging inside each single cell

12.20 Student talk 11: **Raphaël Mategot**
Investigating the function of nuclear miRNAs

12.40 **Lunch buffet - Poster preparation**

13.30 POSTER SESSION

SESSION 4: 'Modeling & Population studies'

15.00 Student talk 12: **Sofia Almeida**
Analysis and modeling of biorhythms

15.20 Student talk 13: **Johan Hallin**
Powerful decomposition of complex traits in a diploid model

15.40 Student talk 14: **Nikita Lukianets**
Statistical study of the morphological properties of pyramidal sensorimotor neurons in mice

16.00 **Awards**

16.15 **Closing remarks & drinks**

18.00 **End**

Guest Speaker: **Dr Valérie Verhasselt**

Mother-child interaction through breast milk: a critical step for immune system maturation

V. Verhasselt, MD, PhD, Director of Immune Tolerance team, Nice , France

The talk will illustrate how breastfeeding can complement early life immune system for optimal immune function during physiological breastfeeding period but also contribute actively and directly to neonate immune system education and maturation.

We have been studying in mouse model how maternal milk can influence immune tolerance establishment, a process which is critical to prevent inappropriate immune responses to self antigens and innocuous environmental antigens. We have demonstrated that both dietary but also respiratory antigens can be transferred orally to the neonate through breast milk and impact offspring immune response in the long term. We have identified that immune outcome in the offspring exposed to tiny amounts of antigens through breast milk will be critically dependent on maternal milk co-factors. These are impacting neonate immune system maturation and differentiation. Data obtained in human birth cohorts are validating our results obtained in experimental mouse models.

The identification of factors required in early life for immune education should help to improve strategies of prevention of both infectious and immune-mediated disease.

Student talk 1: **Derya Deveci**

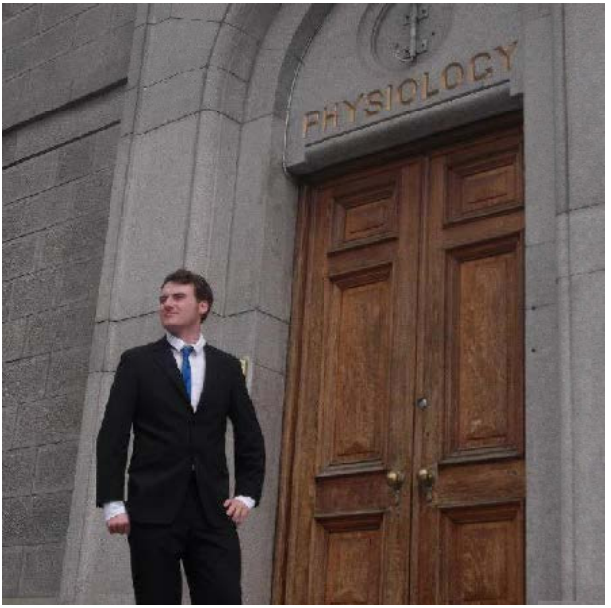
Allatostatin- A as a coordinator of growth and maturation

How does an organism know when to undergo puberty? This is a fundamental question in biology that remains unanswered. Puberty marks the metamorphosis of a child into an adult, which is referred to as the juvenile maturation transition (JMT). In addition, in organisms that undergo determinate growth this transition is associated with cessation of growth, fixing its final body size. In vertebrates and insects, both events occur due to a peak of steroid hormones that induce maturation at the same time as growth inhibition. In Drosophila melanogaster, a peak in the prothoracicotropic hormone (PTTH) by the PTTH producing neurons induces the production of the insect steroid hormone ecdysone. Modulating PTTH levels affects the timing of JMT and subsequently final body size. In order to understand the mechanism controlling the onset of puberty we focused on understanding the regulation of PTTH since it is the first known signal to activate the cascade of events leading to JMT . To do this, we conducted a biased RNAi screen where we identified Allatostatin A receptor 1 (AstA-R1), that delays JMT once downregulated in the PTTH neurons. AstA-R1 is a GPCR that is known to bind its ligand allatostatin-A (AstA) which is produced by AstA neurons that are in close proximity to PTTH neurons. Interestingly, AstA-R1 is the homologue of mammalian kisspeptin receptor, important in puberty onset. In order to understand how an organism knows when to undergo puberty we will now focus on understanding the regulation of AstA.

Student talk 2: **Aidan Falvey**

The potential role of the carotid body in homeostatic regulation of inflammation

Homeostasis; the ability of the body to control the fluctuation of its environment, is aimed at maintaining multiple physiological variables - temperature, Ph and glycemia within a narrow range of values. Recent evidence shows inflammation, induced by activated immune cells in tissues, is homeostatically monitored by peripheral nerves via the inflammatory reflex. The current inflammatory reflex is composed of an afferent vagal paraganglia that detect inflammation, a signal to the brain producing an efferent vago-spleen response attenuating inflammation. The efferent arm is well studied. However, the afferent arm is less convincing - data is limited and inconsistent. Peculiarly, there is one paraganglia that remains unconsidered as part of this reflex despite strong evidence to the contrary. The carotid body (CB) is a polymodal sensor and the largest paraganglia in the body. The CB is innervated by the carotid sinus nerve (CSN), which connects to the glosopharyngeal nerve - entirely separate to the vagus nerve. Perhaps this is the reason it remains unconsidered. However, the CB detects and responds to several inflammatory markers (IL-6, IL-1ß, zymosan and LPS), which are capable of inducing CSN discharge (IL-1ß, zymosan and LPS). Furthermore, loss of the bilateral CSN produces an exaggerated inflammatory response and exacerbates the survival to sepsis. Considering this evidence, the CB and its CSN are worthwhile targets of further investigation - potentially providing an alternative or even true afferent pathway to the inflammatory reflex. Full elucidation of this pathway will be required to determine the carotid bodies true role.



Student talk 3: **Scherazad Kootar**

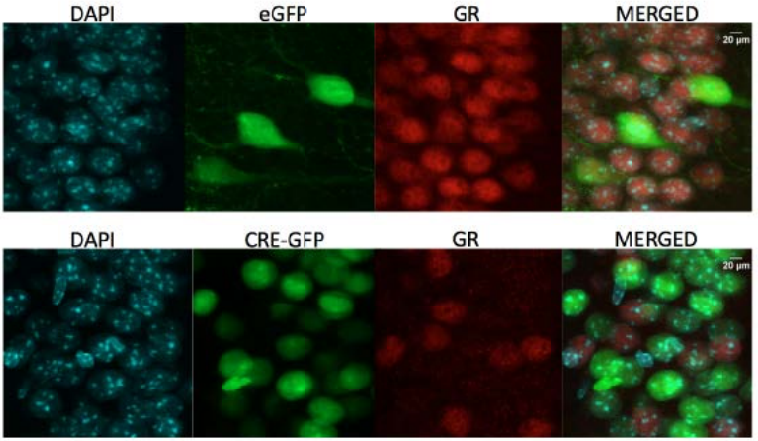
Blocking glucocorticoid receptors prevents acute effects of amyloid-beta oligomers at hippocampal synapses

Scherazad Kootar, Marie-Lise Frandemiche, François Tronche, Jacques Barik, Hélène Marie

Alzheimer's disease (AD) is associated to dysregulation of the Hypothalamus- Pituitary- Adrenal (HPA) axis in human patients and in mouse models. HPA dysregulation in AD produces an increase in glucocorticoids, which bind to the glucocorticoid receptors (GRs). We recently showed that inhibiting these receptors in an AD transgenic mouse model prevented early episodic memory and synaptic plasticity deficits (Lanté et al, Neuropsychopharmacology 2015). Also, both corticosterone and amyloid-beta oligomers (oAß) modulate hippocampal synaptic plasticity, but the functional relationship between oAß and GRs at synapses is still mostly unknown.

To explore this functional relationship, we studied how oAß modulates post synaptic density (PSD) protein content of hippocampal neurons, including GR levels, in absence of GR activity. We also asked how oAß modified long term potentiation (LTP) in adult mouse hippocampal sections in absence of GR activity. We showed that when synthetic oAß (100nM) exogenously added to cultured hippocampal neurons there was an increase in GR levels within the PSD. This GR increase was prevented when a selective GR antagonist (CORCEPT Therapeutics) was added, suggesting an intricate relationship between oAß and GR signaling at synapses. Also, this GR antagonist prevented the acute effects normally induced by oAß on PSD content. Furthermore, we showed that blocking GR signaling, pharmacologically and genetically, prevented the LTP deficit caused by oAß. These results indicate that GR activity is necessary for oAß to perturb LTP.

Together, these data identify an important role of GRs in acute Aß-induced synapto-toxicity.



In-vivo eGFP and CRE-GFP virus infected CA1 hippocampal neurons in mice and glucocorticoid receptor (GR) staining by immunofluorescence.

Student talk 4: **Lazaro Emilio Aira Diaz**

Implication of Lyn tyrosine kinase in Psoriasis

Aira Lazaro Emilio Aira[1,2]; Diogo Gonçalvès[1,2]; Pascal Colosetti[1,2]; Jean-Paul Ortonne[3]; Jean-Philippe Lacour[3]; Patrick Auberger[1,2]; Sandrine Marchetti[1,2]

[1] INSERM U1065, Centre Méditerranéen de Médecine Moléculaire (C3M), Cell death, Differentiation, Inflammation and Cancer, Nice, France

[2] Université de Nice Sophia-Antipolis, Faculté de Médecine, Nice, France

[3] Centre Hospitalier Universitaire de Nice, Service de Dermatologie, Hôpital Archet II, Nice, France

The Src family kinase, composed of 9 members, has a key role in the control of many biological processes. Lyn, one of these proteins, has a well-established function in hematopoietic cells; but recent studies have shown that Lyn can also control other tissues. Recently, it has been demonstrated that caspases can cleave Lyn in its N-terminal domain and that the cleaved-form of Lyn can be relocalized from membrane to cytosol. The ubiquitous overexpression of this protein in mice leads to a skin inflammatory syndrome, which has several features common with human psoriasis. Based on this result, we wanted to know whether the Src tyrosine kinase Lyn has a role in this autoimmune disease. To this end, we induced a psoriasis-like disease in WT mice through ear injection of recombinant protein IL-23 or by the application of TLR 7/8-agonist imiquimod on mice backs. In both models, an increase of Lyn expression was observed at both mRNA and protein levels, which was not seen for Src and Fyn, two other members of Src family kinase. When lesional human psoriasis skin biopsies were analyzed, we also detected an increase in Lyn expression with respect to non-lesional biopsies and to healthy donors. Therefore, we investigated what kind of cells was involved in this modulation.

In this context, we observed that the expression of Lyn was increased both in the dermis and the epidermis in human and mice, indicating that the recruitment of immune cells within the injured skin but also the modulation of Lyn in keratinocytes were both implicated. Interestingly, an increase in Lyn expression in human keratinocytes stimulated in vitro with TNF-α and IL-17, the two principal cytokines implicated in psoriasis pathogenesis, was observed. These preliminary results show that Lyn could be an important regulator of psoriasis.

Student talk 5: **Jozef Piotr Bossowski**

Low-protein diet suppresses cancer progression through immune system modulation

Diet is well known to be the major factor in cancer onset and progression. It has been shown that reducing the amount of food intake, called Calorie Restriction (CR) suppresses effectively cancer development in various animal models and at the same time improves general health and expends lifespan.
However, introducing CR along with standard treatment raises many clinical challenges. CR has been proposed to act through a variety of mechanisms and metabolic alterations. That's why there is an urgent need to decipher the molecular actors of CR, which would enable to design targeted therapies, resulting in obtaining benefits of CR without the risk and drawbacks of performing actual CR.
For that reason, we designed and tested the impact of diets reduced in particular macronutrients (-25% carbohydrates; -25% proteins) on the Eμ-Myc transgenic mouse model of B-cell lymphoma and the CT26/BALB/c colorectal cancer. We demonstrate that macronutrient modulation in spite of general calorie intake results in reduction in cancer progression. Surprisingly, that effect was partially immune system dependent, as we lose this protection in a model of immunodeficient mice. Under Low protein diet, we observed an enhanced immune response towards tumour cells that resulted in inflammation, T-cells activation and cytotoxic effects. We are now trying to identify the metabolic pathway that could explain this effect.
We have shown that macronutrient modulation impacts cancer progression, which is the consequence of immune system modification. Identifying which molecular pathway is implicated in this effect is our main goal right now.

KEYNOTE speaker 1: **Dr Aurelien Roux**

Buckling of the cell membrane by ESCRT-III

Department of Biochemistry, University of Geneva, CH-1211 Switzerland

Cells and organelles are delimited by lipid bilayers. Since these membranes are impermeable to most solutes, in order to exchange material with their environment, organelles and cells have developed a large protein family involved in budding membranes to form membrane carriers. These carriers transport material between organelles. Proteins involved in intracellular membrane traffic can remodel the membrane by several ways. Clathrin, for example, polymerizes into a spherical cage onto the membrane, forcing it to curve. Here we describe a recently discovered protein complex called ESCRT-III, which has the property of forming spirals at the surface of the lipid bilayer. This unique structural feature did not suggest any known mechanism by which it could deform the membrane. It was theoretically proposed that, while growing into a spiral, it accumulates stress energy which can be released by buckling of the central part of the spiral[1]. By using high-speed AFM and biophysical tools to measure membrane elasticity we show how the elastic and polymerization properties of the ESCRT-III filament are compatible with such model[2]. We further investigated the dynamics of the complex when the Vps4 - an ATPase that causes the ESCRT complexe to disassemble - is present. We found that Vps4 promotes a dynamic instability within the ESCRT polymers in a similar way than for actin or microtubules. We propose that this instability is necessary for assembly in presence of growth inibiting subunits Vps2 and Vps24, and to allow constriction by relaxation of elastic stress within large ESCRT assemblies.

[1] MLenz, D. Crow, and J.-F. Joanny, Physical Review Letters 103 (2009).

[2] N. Chiaruttini, L. Redondo-Morata, A. Colom, F. Humbert, MLenz, S. Scheuring, and A. Roux, Cell 163, 866 (2015).

'Signalife' Principal investigator: **Dr Bruno Antonny**

Fishing transport vesicles with a string and a hook, the GMAP-210 example

The membrane of organelles of the early secretory pathway (ER, cis Golgi) differ from that of other organelles in bulk features, including low sterol, low electrostatics and high level of unsaturation. Here, we present evidence that the unsaturation level of phospholipids in combination with membrane curvature plays an important role for the control and selectivity of vesicle traffic at the ER - cis Golgi interface. First, the Golgi partitioning of ArfGAP1, which regulates Arf1 effectors such as COPI, is very sensitive to the ratio between unsaturated and saturated phospholipids. The recruitment of ArfGAP1 to the Golgi is modulated by fatty acid diet and by targeting specific phospholipid acyltransferases for saturated and unsaturated acyl chains. Second, we show that the capture of vesicles by the golgi matrix also takes advantage of the high unsaturation and high curvature of transport vesicles. For this, we use a protocol developed by Kobayaski and Pagano in 1988, which consists in incubating pure liposomes with perforated cells. This approach indicates that the Golgi has an exceptional avidity for curved membranes without stereospecific interactions. The liposome tethering properties of the Golgi resembles that of the golgin GMAP-210: both prefer small (radius < 40 nm) liposomes made of monounsaturated but not saturated lipids. Reducing GMAP-210 levels decreases liposome capture by the Golgi. Extensive mutagenesis analysis suggests that GMAP-210 tethers authentic transport vesicles via a mechanism whereby its N-terminal ALPS motif senses lipid-packing defects at the vesicle surface through its regularly spaced hydrophobic residues. Molecular dynamics simulations of lipid membranes with the same curvature and lipid composition as that of COPI vesicles suggest that such membranes display the highest level of defects in lipid packing and thereby can be selectively recognized by ALPS motifs.

Student talk 6: **Denisa Jamecna**

Investigation into the function of the N-terminal region of OSBP

Denisa Jamecna, Joëlle Bigay, Bruno Mesmin, Bruno Antonny
Institut de Pharmacologie Moléculaire et Cellulaire (IPMC) - CNRS UMR 7275

Oxysterol binding protein (OSBP) is a membrane tethering and lipid transfer protein that participates in cholesterol homeostasis. OSBP consists of a pleckstrin homology (PH) domain, two central coiled-coils, a "two phenylalanines in acidic tract" (FFAT) motif and a C-terminal lipid binding OSBP-Related Domain (ORD). The PH domain recognizes PI(4)P and a small G protein Arf1-GTP at trans-Golgi membranes, whereas the FFAT motif interacts with ER-resident protein Vap-A. By binding all these determinants simultaneously, OSBP tethers ER and Golgi membranes and transports cholesterol from the ER to the Golgi and PI(4)P from the Golgi to the ER.

In addition to aforementioned domains, OSBP also contains an 87-amino acid long N-terminus. Most (64%) of the N-terminal amino acids are glycine, proline or alanine and this region is predicted to be unstructured with zero net charge. Its function in membrane tethering and lipid transfer regulation is unknown.

To elucidate this issue, we compared liposome tethering activity of two truncated OSBP constructs. The constructs called "N-PH-FFAT" and "PH-FFAT" contained the PH domain, the coiled-coils and the FFAT motif but differed in presence/absence of N-terminus. In experiments with PI(4)P-containing liposomes of Golgi-like composition, we observed that N-PH-FFAT (N-terminus present) was not capable of liposome tethering, whereas PH-FFAT promoted aggregation of liposomes.

This finding was confirmed using confocal microscopy with fluorescent Golgi-like liposomes, when large aggregates formed only in presence of PH-FFAT. Dimerization of the protein was crucial for tethering because a PH-FFAT mutant lacking the coiled-coils did not promote liposome aggregation.

Our observations suggest that the N-terminus creates a steric hindrance around PH domains that prevents symmetric tethering of PI(4)P-containing liposomes. In cells, N-terminus could play a role in proper spacing of OSBP on Golgi membrane and preventing abnormal membrane stacking.

Student talk 7: **Cynthia Lebeaupin**

Lack of BI-1 predisposes to ER stress-induced Non-Alcoholic Fatty Liver Disease

Endoplasmic reticulum (ER) stress responses are linked to metabolic dysfunctions and the activation of cell death and inflammatory mechanisms associated with the development of chronic liver disease. We therefore hypothesized that the genetic ablation of Bax Inhibitor (BI)-1, an evolutionarily conserved and cytoprotective ER-membrane protein, would render the liver vulnerable to unresolved ER stress. To induce an acute but reversible ER stress response, we injected tunicamycin into BI-$1^{+/+}$ and BI-$1^{-/-}$ male mice and observed the hepatic consequences after 48h. Challenging BI-1-deficient mice with tunicamycin overwhelmed ER stress response signaling leading to dysregulated hepatic glucose and lipid homeostasis, exacerbated inflammation and pyroptosis, fibrosis and ultimately, liver failure. In order to more closely mimic the pathological changes that occur in obese humans, we also used a high-fat diet (HFD)-induced obesity model to provoke chronic ER stress in BI-$1^{+/+}$ and BI-$1^{-/-}$ mice. Our findings show that BI-1-deficient mice fed a HFD are insulin resistant with livers presenting the pathophysiological features of Non-Alcoholic Steatohepatitis (NASH). We therefore confirm an important role for BI-1 that may present a new therapeutic perspective for chronic liver disease.

Keynote speaker 2: **Dr Jeroen Krijgsveld**

Proteome meets genome: dynamics of chromatin composition in embryonic stem cells

German Cancer Research Center (DKFZ), Proteomics of Stem Cells and Cancer, & Heidelberg University, Medical Faculty, Heidelberg, Germany

One of the key questions in genome biology is to understand how transcription of every single gene in the genome is regulated, each in a context-dependent manner. Such regulation is largely determined by transcription factors, along with a great variety of accessory proteins that dynamically interact with chromatin. In this presentation I will show recent work from our lab introducing methodologies to study protein interactions in chromatin, with a focus on stem cells and pluripotency. Specifically, we introduced a novel approach for the selective identification of chromatin-associated proteins (SICAP) by combining chromatin immuno-precipitation (ChIP) and mass spectrometry to identify the proteins that co-localize with a DNA-binding protein of interest. We have used this to explore chromatin-bound protein networks around the pluripotency facors Oct4, Sox2 and Nanog. Moreover, by investigating how the composition of these networks depends on the pluripotent state, this has allowed us to identify novel regulators of pluripotency. Since these methods are highly generic, they will also prove powerful tools to characterize chromatin in many other biological contexts.

Guest Speaker: **Dr Sabrina Pisano**

Atomic force microscopy as tool to achieve insights into nucleic acids

Molecular and Cellular Imaging Facility Core (PICMI), Institute for Research on Cancer and Ageing, Nice (IRCAN)

For 30 years atomic force microscope (AFM) has been a valuable tool in life science to study biological samples.
AFM is a non-optical microscopy that, among other possibilities, enables the achievement of a 3D topographical image of the sample at the nanoscale level.
In addition to high resolution, this technique offers also a remarkable versatility, allowing the imaging of native biological samples both in air and in the liquid, with no need of labeling or staining.
In particular, AFM has been widely used for structural studies of nucleic acids and nucleic acid complexes.
The morphometric analysis performed on the images can unveil specific structural and functional aspects of the sample, such as the multimerization state of a binding protein on nucleic acids or a DNA conformation change due to a binding protein.
Herein, some examples of both accomplished and current projects in the field of nucleic acids will be presented.

Student talk 8: **Nadia Formicola**

CamKII: a novel regulatory component of Imp neuronal RNA granules?

Ribonucleoprotein (RNP) granules are supramolecular structures composed of RNA and proteins, found in many cellular contexts, and known to be tightly regulated in space and time. Neuronal granules, in particular, appear to contain components of the translational machinery, and to switch from packed to translationally active conformations in response to stimulation. To date, the various factors controlling RNP dynamics remain to be elucidated.

In the lab, we focus on cytoplasmic ribonucleoprotein particles characterized by the presence of the Imp protein during Drosophila CNS development. Imp is a conserved RNA binding protein and its function is required for axonal regrowth during development. Furthermore, Imp accumulates together with target mRNAs in RNA granules that are dynamically transported to axons.

In order to find components of axonally-localized Imp RNP granules, we isolated Imp particles from brain lysates and identified 69 Imp interacting proteins using Mass Spectrometry approaches. To test their involvement in Imp RNP dynamics, we systematically knocked-down these candidates in Mushroom Body neurons, using inducible RNAi lines.

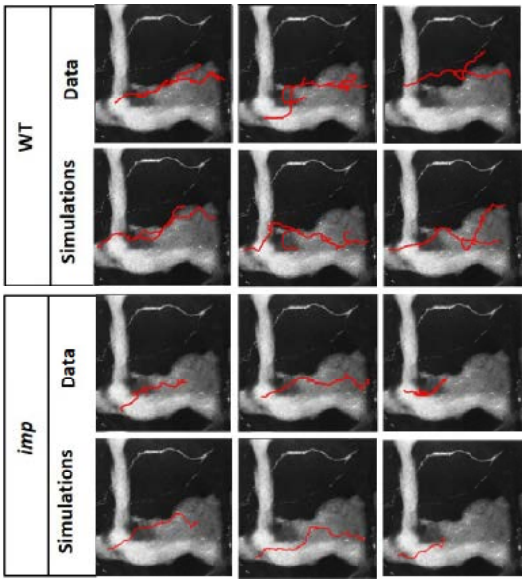
One potential interactor seems to be particularly intriguing: the Calcium (Ca^{2+}) and Calmodulin (CaM)-dependent serine/threonine kinase II (CaMKII). This protein has been involved in synaptic plasticity, learning and memory in Drosophila and is known to respond to Ca oscillations.

We are currently studying the impact of CamkII activation/ inactivation on Imp expression and Imp RNPs dynamics, using a combination of transgenic lines. As CamkII is regulated by neuronal activity, we are also testing if Imp granules are sensitive to changes in synaptic activity, using different techniques: genetic gain and loss-of-function approaches, biochemical purifications, live imaging and Drosophila S2 cell culture assays.

Student talk 9: **Agustina Razetti**

Drosophila Gamma Neuron Branching Process During Development: a mechanistic approach

Important advances have already been achieved in identifying the main factors involved in neuron development. The next step that has to be done is concerning how we approach the question. In this work we intend to close the gap between classic in vitro experimental assumptions and real in vivo situations, where the final neuronal morphology is acquired through a dynamic and environmental-dependent process. In particular, the branch formation process - how or why branches are created - has been belittled or over-simplified by neuron development models. In our opinion, this represents a constraint in the general understanding of neuron development, hierarchy of the neuronal tree and adult functionality.
Our goal is to bring light to the mechanisms of branch formation during development in realistic conditions. We study the particular case of Drosophila Gamma neuron remodeling and analyze, for the first time to our knowledge, the mechanical situation of a whole population of Gamma neurons (650 individuals) growing together in a constraint space (i.e. medial lobe of the Mushroom Body). We hypothesize that one kind of branches are born when the growing tip encounters a mechanical obstacle (i.e. other neurons or the lobe limits), enhancing the probability that at least one neurite reaches the end of the lobe. We design a simple but still as-realistic-as-possible stochastic model to test this hypothesis. We show that the proposed mechanistic branch generation process is plausible, and explore unsolved problems concerning the understanding of two particular Gamma neuron mutation phenotypes.



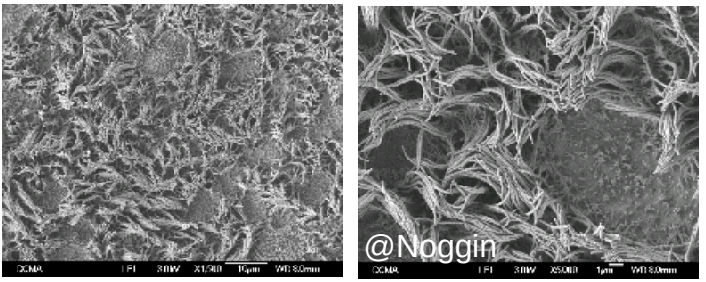
Student talk 10: **Sandra Ruiz Garcia**

Airway epithelium differentiation: digging inside each single cell

Ruiz Garcia Sandra, Arguel Marie-Jeanne, Paquet Agnès, Lebrigand Kevin, Cavard Amélie, Marcet Brice, Barbry Pascal, Zaragosi Laure-Emmanuelle.
CNRS, Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), UMR-7275, 660 route des Lucioles, 06560 Sophia-Antipolis, France
University of Nice-Sophia-Antipolis (UNS), Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, Valbonne, 06560 Sophia-Antipolis, France. (Corresponding author: ruiz-garcia@ipmc.cnrs.fr)

Key words: airway epithelium, multiciliogenesis, differentiation, progenitor/stem cells, gene expression.

The airway epithelium is mainly composed of 3 cell types: multiciliated cells (MCCs) which exhibit at their surface hundreds of motile cilia, goblet (or secretory) cells, and basal cells. Mucociliary clearance, an essential mechanism that protects the respiratory system from external aggressions, is orchestrated by coordinated beating of motile cilia. In chronic airway diseases, the injured epithelium frequently displays defective repair characterized by a loss of MCCs associated with goblet cell hyperplasia.
After respiratory injuries, the tissue must repair to reproduce a functional mucociliary epithelium through processes involving proliferation and differentiation of a subpopulation of progenitor cells. During regeneration, the accurate molecular events leading to terminal differentiation have not yet been fully characterized. We aimed to establish a molecular signature of the different progenitor cells involved in this process. We have applied a microfluidics-based approach that allows a comprehensive and unbiased measurement of the transcriptome from isolated cells derived from our 3D model of human airway epithelium. Using this method, the 3 main cell types of the fully differentiated airway epithelium were correctly identified. Distinct populations of MCCs, goblet and basal cells can be clearly characterized by the expression of several specific markers such as FOXJ1, MUC5AC and KRT5.
This single cell approach can now be used to analyze the airway epithelium at different stages of regeneration. Our results at the single-cell resolution should decipher the exact stages of basal cell differentiation and identify novel molecular actors which could be critical to drive full regeneration of an injured epithelium.



Student talk 11: **Raphaël Mategot**

Investigating the function of nuclear miRNAs

The discovery of RNA interference (RNAi) has unravelled a new principle for regulation of gene expression, as well as producing new tools for medicine. MicroRNAs (miRNAs) are 19-22 nt small RNAs that post-transcriptionnally suppress gene expression by a sequence-specific mechanism known as RNAi. Mature miRNAs are loaded into an Argonaute-2 protein (Ago2) in the cytoplasm to target messenger RNAs 3'UTR through Watson-Crick base pairing, leading to translational repression and/or degradation of the target mRNA.

In mammals, miRNAs roles have been dogmatically confined to the cytoplasm because of initial observations that excluded RNAi components from the nucleus, leading to widespread skepticism on the existence of a nuclear RNAi pathway. However, emerging data sheds light on the occurence of a mammalian nuclear RNAi pathway.

Herein we show that endogenous RNAi components such as Ago2 and miRNAs also localize to the nucleoplasm of mammalian cells, and potentially contact chromatin. In the nucleoplasm, miRNAs act to destabilize mRNAs through a post-transcriptional mechanism potentiated by paraspeckles proteins such as SFPQ, an RNA-binding protein.

Moreover, miRNAs nuclear localization is not restricted to cell lines but also occurs in primary cells. In fact, LPS-stimulated bone-marrow derived macrophages show nuclear localization of miR-155, a miRNA whose expression and function is related to activated immune cells, raising the question of the nuclear targetome of miRNAs in-vivo and in physiopathological context.

Taken as a whole, our work extend the scope of RNA interference to the nucleus, and anticipates novel mechanisms by which cells control nuclear gene expression.

Student talk 12: **Sofia Almeida**

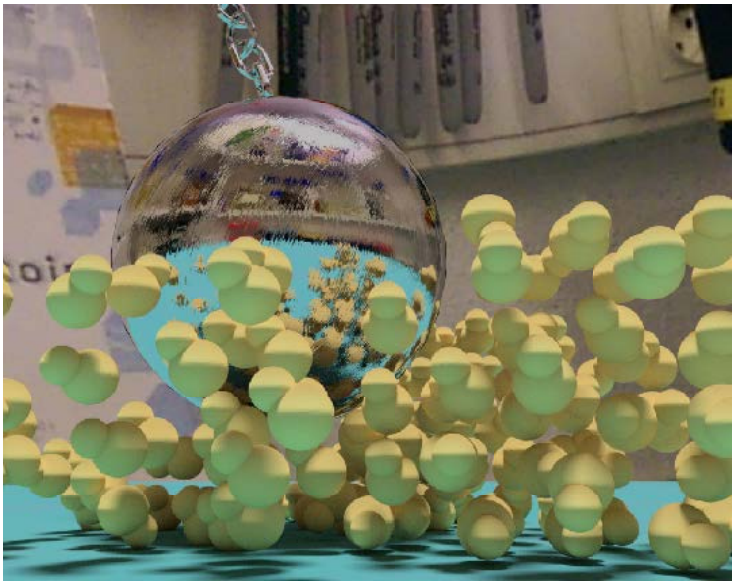
Analysis and Modeling of Biorhythms

The cell division cycle and the circadian clock are two essential life rhythms that ensure the health of cells. These can be interpreted as oscillators and have been widely studied through dynamical mathematical modeling. Recently, two studies combining single live cell imaging with computational methods have demonstrated that the cell cycle influences the clock in mammals, causing synchronization and phase-lock between the two. The mechanism by which this coupling happens is still unknown. As uncontrolled cell proliferation is one of the key features leading to cancer, understanding the coupling mechanism could allow to synchronize or slow down tumoral cell growth through interference with the clock. In this work, we propose mathematical models for the mammalian cell cycle and the circadian clock, analyze and reduce them. The cell cycle model is based on post-translation modifications of cyclin B-cdk1, also called mitosis promoting factor (MPF), the known essential protein of the mammalian cell cycle. The circadian clock model is based on two essential feedback loops of the circadian clock involving the proteins BMAL1, Rev-ErbAα and the PER:CRY complex. Finally, we propose a coupling mechanism in which MPF phosphorylates Rev-ErbAα leading to its degradation. We observe the synchronization between the oscillators as well as different phase-lock configurations consistent with observations.

Student talk 13: **Johan Hallin**

Powerful decomposition of complex traits in a diploid model

Understanding where trait variation within a population originates from is a long term goal of life sciences. To this end, we devise a powerful framework for decomposition of trait variation into its genetic causes in diploid model organisms. We construct 6642 Saccharomyces cerevisiae Phased Outbred Lines (POLS) with fully assembled diploid genomes by crossing sequenced advanced intercrossed lines, and demonstrate the capacity of this approach by decomposing 18 fitness traits. We achieve near complete trait heritability and precisely estimate additive (73%), dominance (10%), second (7%) and third (1.7%) order epistasis components. Mapping quantitative trait loci (QTLs) we find nonadditive to outnumber additive loci and extensive pleiotropy. We find best parent heterosis to the same extent as worst parent heterosis and dominant contributions to heterosis to outnumber overdominant. Our POL framework offers the most complete decomposition of diploid traits to date and can be adapted to most model organisms.



Wrecking ball plowing through some yeast in the lab

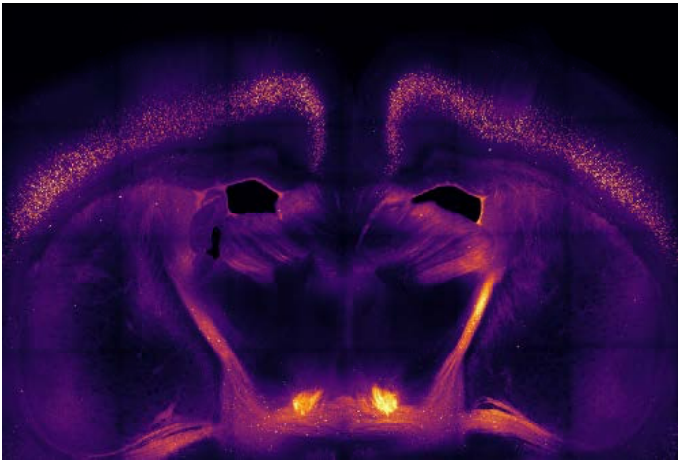
Student talk 14: **Nikita Lukianets**

Statistical study of the morphological properties of pyramidal sensorimotor neurons in mice

Nikita Lukianets (1-2), Franck Grammont (2), Michèle Studer (1)
Institut de Biologie Valrose, Nice (1) ; Laboratoire J.A. Dieudonné (2) ; SIGNALIFE PhD Program

The neocortex is subdivided functionally into areas, each playing a particular role, and anatomically, into six distinct layers with specialized neuronal cell types. Distinct sets of gene expression gradients are necessary for such organization. It has been shown, that the transcriptional regulator Nr2f1 (COUP-TFI) is necessary and sufficient for the development of neocortical somatosensory areal identity and that the lack of Nr2f1 function causes a drastic expansion of cortical motor area at the expense of the somatosensory one. Nr2f1 conditional mice exhibit a significant increase of Ctip2-labeled corticofugal neurons and emergence of a novel cell population, co-labelled by Ctip2 and Satb2. We have shown that, Ctip2/Satb2 postnatal co-localization defines two distinct neuronal subclasses projecting either to the contralateral cortex or to the brainstem.

The heterogeneity of Ctip2/Satb2 neurons with respect to their projection targets raises another question of whether this heterogeneity is reflected in neuron morphology. The logic is that the neurons, projecting to different regions of the brain, have a particular morphological identity, reflecting different signal integration capabilities and thus distinct computational properties. In the first part of my work, I address the question of morphological cell identity of the pyramidal neurons of the primary somatosensory cortex and its relation to the neurons co-expressing Ctip2/Satb2, using unsupervised machine learning techniques. In the second part, I address the question of voluntary motor control defects, observed in the Nr2f1 mutant mice. By developing and applying novel methods of quantitative image analysis, I test the hypothesis that Nr2f1 function is necessary for the correct establishment of voluntary motor control circuits, in particular, of the cortico-ponto-cerebellar pathway, the descending efferent copy pathway involved in prediction of optimal body states.



A scream of the soul

Notes

Poster 1: **Rania Ben Jouira**

Extracellular matrix produced by BRAF inhibitor-resistant melanoma cells promotes therapeutic resistance to drug-sensitive cells

Rania Ben Jouira, Christophe Girad, Ilona Berestjuk, Aude Mallavialle, Damien Alcor, Sophie Tartare-Deckert and Marcel Deckert
INSERM, U1065, Centre Méditerranéen de Médecine Moléculaire, Team : Microenvironment, Signaling and Cancer, Université de Nice - Sophia Antipolis, Faculté de Médecine, Nice, France.

Cutaneous melanoma remains one of the most challenging and difficult cancers to treat because of its high plasticity, metastatic potential and resistance to treatment. New therapies targeting oncogenic BRAFV600E mutation have shown remarkable clinical efficacy. However, drug resistance invariably develops. Thus, the need for improving existing therapies remains critical. Recent studies have indicated that tumor resistance arises from (epi)genetic cancer cell alterations and from the tumor microenvironment in which the extracellular matrix (ECM) is a determinant factor. Both stromal and tumor cells contribute to ECM deposition and remodeling during disease progression. Here, we found that invasive BRAF inhibitor (BRAFi)-resistant melanoma cells, but not BRAFi-sensitive cells, abundantly produced matrix proteins and remodeled a 3D ECM displaying fibronectin (FN) and collagen fibers. Interestingly, this resistant melanoma-derived ECM is able to induce a phenotype switching of the therapy-sensitive cells and more importantly protect them from the anti-proliferative effect of the BRAFi Vemurafenib. Our results suggest that resistance to targeted therapy is associated with the production by melanoma cells of a pathological fibrotic matrisome that may affect cell behavior and therapeutic response.

Poster 2: **Tomas de Garay**

CD98hc (SLC3A2) presents a novel longer variant with an alternative promoter

Tomas de Garay, Chloe Feral

CD98hc, encoded by SLC3A2 gene, is a type II transmembrane protein implicated in cell proliferation and migration as well as extracellular matrix assembly. It is overexpressed in tumor cells. It regulates the expression at the membrane of the catalytic chain of a heteromeric amino acid transporter and simultaneously modulates integrin outside-in signaling, by direct interaction via its extra and intracellular domains respectively. The rise of high throughput sequencing studies allowed the identification of a longer CD98hc mRNA variant, including an extra exon at the 5' end, in the mouse genome. This unexpected variant is not characterized yet. Our work aims at exploring (1) the existence of CD98hc long variant at a protein level, (2) its possible differential functionality and (3) its transcriptional regulation by an alternative promoter upstream of that controlling the short variant expression. We were able to detect this long variant at both mRNA and protein level in mouse dermal fibroblasts. Current work is focusing on identifying possible new protein interactions for this longer version. In parallel, we test the functionality of each variant separately using reconstituted knock out dermal fibroblasts, a model well stablished in the lab. We evaluate the mRNA expression levels of both variants in multiple cell types and conditions; this information will be used to choose candidate transcription factors from a list obtained in an in silico analysis of both variant promoters to assess their differential transcriptional regulation. Altogether, this work will characterize CD98hc function and expression regulation in view of those two variants.

Poster 3: **Gaia Fabris**

Amino acid availability controls miRNA biogenesis in rat hepatocytes

Fabris G, Dumortier O, Lebrun P, Van Obberghen E
Aging and Diabetes group (Pr. Van Obberghen Emmanuel),Institute of Research on Cancer and Aging in Nice(IRCAN), CNRS UMR7284-INSERM U1081-UNS, Faculté de Médecine
28, Avenue de Valombrose (7th foor), 06107 Nice Cedex 2 - France

MicroRNAs (miRNA) are small molecules containing 20-22 nucleotides which act as post-transcriptional repressors by interfering with mRNA translation. Their biogenesis is governed by a complex protein network which includes Dicer, Drosha and Ago2. Although their mode of action is well described, the regulation of their expression is still elusive. Recently we showed in rat progeny that maternal amino acid (aa) restriction during pregnancy increases miRNA expression in the fetal pancreas, decreases β-cells proliferation and favors at adulthood the development of type 2 diabetes. We observed a similar miRNA upregulation in the fetal liver of these descendants together with a reduction in hepatocytes proliferation. Interestingly, we found a comparable miRNA expression in the fetal liver of other maternal malnutrition models. We suggest that cells, when exposed to nutritional restriction, increase miRNA expression in order to reduce their proliferation. We found that aa deprivation directly upregulates miRNA expression in rat primary hepatocytes. In parallel, we observed an increase in Drosha and a decrease in mTOR phosphorylation. Remarkably, the restoration of the essential aa in the depleted culture medium normalizes mTOR phosphorylation, Drosha protein level and miRNA expression. We are currently investigating the relationship between miRNA biogenesis and hepatocyte proliferation. Taken together, our results strongly support the idea that aa could regulate miRNA biogenesis by controlling the Drosha protein level via the mTOR pathway.

Poster 4: **Ramona Galantonu**

A link between steroid signaling pathways and L1 retrotransposition

Galantonu Ramona, Pizarro Javier, Monot Clément, Lagha Nadira, Montandon Margo, Kazakyan Serdar, Valfort Aurore-Cécile, Vidalain Pierre-Antoine, Cristofari Gaël
Institute for Research on Cancer and Aging of Nice (IRCAN), France

Mobile genetic elements play important roles in the evolution and function of the human genome. Among them, the Long Interspersed Nuclear Element-1 (LINE-1 or L1) retrotransposon contributes to the genetic diversity of the human population, and occasionally leads to inherited genetic diseases. L1 elements are also reactivated in many tumors. L1 jumps through a 'copy and paste' mechanism. This process involves two L1-encoded proteins, ORF1p and ORF2p, which associate with the L1 mRNA to form a ribonucleoprotein particle, the core of the retrotransposition machinery. However, little is known about the cellular factors involved in L1 replication. Our laboratory has discovered by yeast 2-hybrid screens that ORF2p, an L1 protein with endonuclease and reverse transcriptase activities, interacts with the estrogen-related receptor α (ERRα), a member of the nuclear receptor family. This observation suggests a model by which ERRα could regulate retrotransposition, possibly by tethering the L1 machinery to chromatin or to specific genomic locations. The existence of several ERRα paralogs prompted us to test whether ORF2p could also interact with other members of this superfamily. To achieve this goal, we used a fluorescent two-hybrid assay (F2H) in mammalian cells. Our results indicate that ORF2p interacts with several other members of the steroid receptors group.

To further explore the potential role of this interaction in targeting L1 to chromatin, we artificially tethered ERRα to a unique LacO array and we measure de novo L1 insertions by a cellular retrotransposition assay.

Collectively, these data identify steroid signaling pathways as a potential regulatory mechanism for genome instability in human cells.

Poster 5: **Anida Hasanovic**

Patched as a new therapeutic target for adrenocortical cancer

Anida Hasanovic[1], Carmen Ruggiero[1], Nelly Durand[1], Marco Volante[2], Mabrouka Doghman[1], Enzo Lalli[1] and Isabelle Mus-Veteau[1]

[1] Institut de Pharmacologie Moléculaire et Cellulaire, CNRS et Université de Nice-Sophia Antipolis (UMR 7275), Sophia Antipolis , France

[2] Department of Oncology, University of Turin and San Luigi Hospital, Turin, Italy

Adrenocortical cancer is a rare heterogenous malignacy which affects about one person in a million and whose 5-year survival rate is only 35% on average. The best treatment available at the present time is composed of a mixture of chemotherapeutic agents, including doxorubicin, combined with adrenolytic substance mitotane. However, the response to this treatment remains modest, which means that more effective therapy is urgently required. Hedgehog receptor Patched is overexpressed in aggressive cancers, including adrenocortical cancer, which we have shown in vitro in adrencortical cell line H295R and in TMA from adrenocortical cancer patients. Recently we have demonstrated that Patched is also involved in the efflux of drugs such as doxorubicin, a chemotherapeutic agent used for clinical management of recurrent cancers. This suggests that Patched could contribute to chemotherapy resistance of cancer cells (Bidet et al. 2012). In order to identify small molecules that are able to inhibit multidrug resistance activity of Patched and to increase chemotherapeutic treatment efficiency, we developed a screening based on the ability of small molecules to inhibit growth of yeast overexpressing human Patched in medium containing doxorubicin). One of the identified compounds had the ability to increase cytotoxic, antiproliferative and apoptotic effect of doxorubicin in a dose- and time - dependent manner in adrenocortical cancer cell line H295R. We have shown that these effects of the compound are due to the inhibition of doxorubicin efflux activity of Patched and its combination with doxorubicin could represent a new approach in the treatment of adrenocortical cancer.

Poster 6: **Sokchea Khou**

Tumor-associated neutrophils in skin carcinoma

The tumor microenvironment shapes cancer progression across all stages of the disease and immune suppressive pathways that restrain anti-tumor immunity have been identified. Known to carry a suppressive effect, the myeloid-derived suppressor cells (MDSCs) comprise two main populations: monocytic MDSCs (M-MDSCs) and polymorphonuclear MDSCs (PMN-MDSCs) also named tumor-associated neutrophils (TAN). Interestingly, a recent meta-analysis of human tumors linked the presence of TAN with the worst prognostics in cancer patients. They are believed to be polarized into N2 pro-tumoral TAN under the influence of TGF-β. Yet, their role in the tumor microenvironment remains controversial as N1 anti-tumoral TAN have also been described. A better discrimination of N1 and N2 TAN is needed to help understand their role in cancer progression. To address these questions, we performed comparative transcriptomic analyses of four highly purified populations of TAN infiltrating precancerous and established cutaneous carcinomas or surrounding skin. Our data reveal a specific gene signature of TAN present within lesions compared to their respective skin controls. The analysis of the differentially expressed genes points toward a pro-tumoral role of TAN during skin carcinogenesis progression. Based on these data, we have identified markers of N1 and N2 that are currently being investigated both in vitro and in vivo using mouse skin carcinoma models. These studies will provide new insights on the role of TAN in cancer and should open new opportunities in human cancer therapy.

Poster 7: **Tiziana Napolitano**

Deciphering the role of FT1 during pancreas morphogenesis and throughout adulthood

Tiziana Napolitano, Fabio Avolio Andhira, Vieira, Noemie Druelle, Biljana Hadzic and Sergi Navarro.
Univ. Nice Sophia Antipolis, Inserm, CNRS, iBV, 06100 Nice, France.

Aiming to identify genes of importance for pancreatic beta-cell (neo-)genesis, we initiated several screens by combining in vitro and in vivo approaches. Here, we report the characterization of FT1, a transcription factor, whose role was investigated during pancreas morphogenesis and throughout adulthood. Gene expression analyses demonstrated that FT1 expression is initiated at the early stages of embryonic pancreas development and is maintained throughout the entire adult life. In order to gain further insight into the role and function of FT1, we generated a transgenic mouse line allowing its specific inactivation in the pancreas from the first phases of organ development. The resulting animals were found to be viable, fertile, and did not exhibit any premature death. Combining functional studies, immunohistochemistry, lineage tracing, and electron microscopy approaches, we accumulated evidences suggesting a functional role of FT1 in pancreatic cells fate specification and phenotype maintenance. Importantly, using models of type I and II diabetes, we demonstrate that the sole loss of FT1 induces a protection against chemically-induced type I diabetes, but also against high fat diet-induced type II diabetes. Together, these results suggest that FT1 could represent a very important target in the context of type I and II diabetes research.

Poster 8: **Katharina Stobbe**

Effect of Lipid Nature on the Establishment of Diet-Induced Obesity in Mice. Role of Chemokine RANTES/CCL5

Katharina Stobbe[1], Ophélia Le Thuc[1,2], Cécilia Colson[1], Nicolas Blondeau[1], Jean-Louis Nahon[1], Carole Rovère[1]
[1] Université Côte d'Azur, CNRS, IPMC, France. [2] Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany.

Obesity is defined by the excessive accumulation of body fat and accompanied by chronic low-grade inflammation of the peripheral metabolic tissues, especially of adipose tissue. Adipocytes secrete inflammatory mediators such as cytokines and chemokines, which can act at the cerebral level and modulate neuronal activity. The hypothalamus is an important region of the brain, which contains neural networks involved in the control of energy metabolism and feeding behaviour. Emerging evidence indicates that inflammation occurs also at the level of the hypothalamus.
We were interested in the inflammatory response of the hypothalamus and adipose tissues to high-fat feeding and the development of diet-induced obesity as well as the role of the chemokine RANTES and different lipid nature within this context. We addressed this question by comparing the effect of various high-fat diets (HFD) with different lipid sources and quality of fatty acids. Wild type and knockout CCL5 mice were fed either a standard diet or HFD. After 8 and 16 weeks of feeding, animals were sacrificed and peripheral and cerebral tissues collected. Metabolic parameters, locomotor activity, expression levels of proinflammatory cytokines/chemokines and peptides involved in feeding behavior were measured. Our results suggest that lipid nature has a distinctive effect on the development of obesity and alteration of metabolic parameters. Variations in lipid nature and composition induce different profiles of inflammatory and neuropeptide mRNA expression in the hypothalamus. Furthermore the absence of RANTES seems to have a protective, age dependent effect on the development of obesity and associated metabolic impairment.

Poster 9: **Serena Testi**

The Phytophthora parasitica effector Avh195 interferes with autophagy in distant eukaryotes

Serena Testi1, Marie-Line Kuhn1*, Nathalie Zuccini-Pascal3, Pascaline Auroy2, Fantao Kong2, Gilles Peltier2, Harald Keller1, et Franck Panabières1*

[1]INRA, Université Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France; [2]Institut de Biologie Environnementale et Biotechnologie, UMR 7265 CEA-CNRS-Aix Marseille Université, CEA Cadarache, 13108 Saint-Paul-lez-Durance, France; [3]INRA, Laboratoire de Toxicologie Cellulaire, Moléculaire et Génomique des Pesticides, UMR 1331 TOXALIM, 06903 Sophia-Antipolis Cedex, France.* contributed equally

Oomycetes from the genus *Phytophthora* are plant pathogens, which have devastating impacts on agriculture and natural ecosystems. During infection, they produce an arsenal of secreted proteins called "effectors" which manipulate host plants either by inducing cell death or by repressing defence reactions. *Phytophthora parasitica* is a root pathogen with a hemibiotrophic lifecycle: during the initial stages of infection (biotrophy) the oomycete establishes an intimate contact with the living cells of the host, before inducing plant cell death to complete its life cycle (necrotrophy). cDNA libraries that were obtained from *P. parasitica*-infected tomato plants and onion epidermis cells display several sequences that encode putative effectors, such as Avh195. The gene encoding this effector belongs to a multigene family comprising 9 genes. The predicted protein possesses two potential binding sites for ATG8, a key protein in the process of autophagy. Heterologous expression of Avh195 in tobacco plants slows down cell death responses such as those induced by proapoptotic BAX and the HR inducers, cryptogein and AvrPtoB. On this basis, we investigate the antagonism between death-inducing agents and Avh195 aiming at identifying the manipulated host signaling pathways, with a particular focus on the link between Avh195 and the autophagy machinery. To identify the molecular targets of Avh195, we initiated a trans-phylum analysis on plants (tobacco and A. thaliana), human cells (HeLa), and green microalgae (*Chlamydomonas reinhardtii*). Genetic expression of Avh195 dramatically alters the cellular phenotype in all these organisms, indicating that the effector targets an evolutionary-conserved mechanism.

Poster 10: **Nathalie Yazbeck**

Modulation of PD-L1 expression on lung cancer cells according to KRAS mutation subtypes and correlation with immune cell infiltration

Yazbeck N, Falk A, Thon L, Mograbi B, Ilie M, Brest P, Hofman P
Research Team " Carcinogenesis-related chronic active inflammation ", Pr Paul Hofman, PU-PH, Team Leader

Lung cancers are the second most diagnosed cancers and the leading cause of cancer-related death worldwide with a low 5-years survival rate. Despite the progresses in EGFR and ALK targeting therapies, patients harboring KRAS mutations are still refractory to treatments. However, the discovery of immunechekpoints, such as the PD-1/PD-L1 pathway, shed the light on the immunotherapy as a potential approach to treat patients, in particular those with KRAS mutations.
Preliminary data in our lab showed that KRAS mutations are differentially associated with PD-L1 expression and immune infiltrate in the lungs. So the aim of our study was to elucidate the mechanism behind this differential expression of PD-L1 and neutrophils recruitment depending on the KRAS mutation. However, the inducible expression model of KRAS we used has shown to be inconvenient due to the protein degradation by the proteasome.
Another factor affecting PD-L1 expression is the differentiation status of the cells. Cancer stem cells (CSCs) have been shown to overexpress PD-L1 and to be more responsive to signals modulating its expression. Therefore our aim is to test the effect of chemotherapeutic agents and anti-PD-1/PD-L1 treatments on PD-L1 expression by CSCs and their potential contribution to the immune evasion. We hope this study would allow a better understanding of PD1/PDL1 pathway implication in tumor resistance and relapse, which will improve personalized immune based-therapies to cure lung adenocarcinoma.

Poster 11: **Caterina Novelli**

Genetic and cell biological dissection of Hedgehog morphogen secretion mechanisms in Drosophila melanogaster

Caterina Novelli, Tamas Matusek, Pascal Therond

In our laboratory we are studying the Hedgehog (Hh) morphogen, in particular, we are interested in how this dually lipid modified molecule is secreted and travels in the extracellular environment. Currently there are three main systems accounting for Hh transport: formation of Hh multimers, integration of Hh into lipoprotein particles (Lpp), and finally exovesicle mediated Hh secretion which require the function of the ESCRT machinery. As an additional level of regulation, recently cytonemes, which are dynamic thin long cellular extensions rich in actin, were shown to be involved in the transport of Hh and its co-receptor Ihog within exovesicles (Gradilla et al. 2014). We investigate the role of Ihog, a transmembrane protein, in the stabilization of filopodia-like structures using two different models: the epithelial sheet in the pupal abdomen (called histoblast nests) and the wing imaginal disc. Our goal is to explain the relationship between Hh release, Ihog protein and cytonemes formation.

Poster 12: **Lenka Schorova**

Investigating deSUMOylation at the neuronal synapse

Lenka Schorova, Marie Pronot, Gwenola Poupon, Carole Gwizdek and Stéphane Martin
Laboratory of 'Sumoylation in neuronal function and dysfunction', Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), Sophia Antipolis, Valbonne, France

The subcellular molecular structure is extremely complex. And despite this complexity a healthy cell is able to correctly orchestrate in time and space all signaling pathways so it can respond to its physiological demands. The fine control of signaling processes is mediated by posttranslational modifications. In neurons, this regulation must be particularly efficient as synaptic transmission occurs within milliseconds. The reversible covalent attachment of SUMO (Small Ubiquitin-like Modifier) proteins, called sumoylation, has emerged as a vitally important posttranslational modification that regulates target proteins' functions. Sumoylation is a three step enzymatic cascade, analogous to ubiquitination, which is carried out by the E1 activating enzyme, E2 conjugating enzyme (Ubc9) and potentiated by the activity of E3 ligases. Sumoylation is reversed by the action of SUMO deconjugating proteases. We have previously reported that the Ubc9 enzyme is regulated by activation of the metabotropic glutamate receptor 5 (mGluR5) leading to PKC-dependent transient trapping of Ubc9 at synapses increasing synaptic sumoylation levels. However, little is known about the regulatory mechanisms of SUMO deconjugation.
Here we use live-cell imaging approaches to study the synaptic regulation of the SUMO deconjugating enzyme SENP1 (SENtrin-specific Protease 1). We show that an increase in synaptic activity alters SENP1 dynamics. Furthermore, SENP1 synaptic regulation is conducted on a much slower timescale compared to Ubc9. This is in agreement with the hypothesis that sumoylation and desumoylation are sequential processes regulating synaptic function. In the next steps, pharmacology approaches will be used to uncover the signaling pathways involved in SENP1 synaptic regulation.

Poster 13: **Liudmyla Lototska**

Investigation of human Rap1 role in cellular senescence and metabolism

Liudmyla Lototska, Aaron Mendez-Bermudez, Eric Gilson
Institute for Research on Cancer and Aging (IRCAN), Nice; Liudmyla.LOTOTSKA@unice.fr

Shelterin is a multiprotein complex essential for telomere protection against DNA damage response (DDR) activation and prevention of chromosome fusions. Rap1 is one of the six proteins that form mammalian shelterin complex. It has been demonstrated that Rap1 is important for prevention from homologous recombination at telomeres. Moreover, we have recently shown that presence of RAP1 at telomeres is a backup mechanism to prevent non-homologous end-joining repair, when topology-mediated telomere protection is impaired (Benarroch-Popivker D. et al., Mol Cell. 2016; 61(2)). In addition, it can be recruited to extra-telomeric DNA sites, where it behaves as a transcriptional regulator. A direct evidence for extra-telomeric functions of Rap1 is the change in cell metabolism of Rap1 null mice via modulation of expression of its target genes. However, whether this transcriptional function of Rap1 is involved in telomere-mediated replicative senescence is unknown.
Here we demonstrate that knockdown of human Rap1 in young fibroblasts (MRC5) triggers premature senescence. Interestingly, this senescent phenotype appears to be telomere-independent. These findings suggest that upon Rap1 knockdown young primary human fibroblasts activate pathways that are different from those provoking replicative senescence. We also show that these Rap1-compromised cells exhibit an altered expression of some genes that were previously shown to be bound by Rap1. Interestingly, one of these genes is PGC1a (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), which is well-known to control metabolism and mitochondria biogenesis.
Altogether, these results suggest a transcriptional role of Rap1 in the metabolism switch from respiration to glycolysis, which occurs in senescent cells.

Poster 14: **Pierre Bourdely**

Tenascin-C promotes oral squamous cell carcinoma by modulating the distribution and activation of dendritic cells

*Pierre Bourdely[1], Caroline Spenlé[2], Christiane Arnold[2], Annick Klein[2], Olivier Lefebvre[2], Anne Sudaka[3], Fabienne Anjuère[*1] Ellen van Obberghen-Schilling[*4] and Gertraud Orend[*2], * co-corresponding authors.*
[1]Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne, [2]INSERM U1109, MN3T, Strasbourg, [3]Centre Antoine Lacassagne, Nice, [4]Institut de Biologie de Valrose, Nice

Tenascin-C (TNC), an extracellular matrix protein (ECM) with known immunomodulatory properties, is up-regulated during inflammation and carcinogenesis. This is one major component of the neoplastic stroma of human Head and Neck Squamous Cell Carcinomas (HNSCC) associated with bad prognosis. Based on these observations, we hypothesized that tumoral TNC participates in the immunoediting of tumors. As dendritic cells are key actors of the antitumoral immunity, but are regulated by components of the tumor environment, we focused our study on the regulation and function of dendritic cells by TNC during oral carcinogenesis using complementary experimental approaches.
We developed a 4NQO-induced OSCC model in genetically modified mice expressing or not the TNC matrix protein. In this chemical model of carcinogenesis that mimics the human pathology, TNC KO mice had reduced tumor frequency and never developed invasive carcinomas. TNC assembled into matrix tracks in WT mice. Noticeably, TNC served as niches for CD45+ leukocytes including dendritic cells (DC). In TNC deficient mice, DC and other immune cells strongly invaded the tumorigenic epithelium, without affecting their overall abundance. Complementary ex vivo and in vitro experiments allowed us to show how TNC modulates the migratory and functional properties of tumor-associated dendritic cells.
Altogether our results suggest a role of TNC in promoting tumor onset and progression of OSCC by trapping immune cells within the TNC tracks thus preventing them from reaching and targeting the tumor epithelium.

Poster 15: **Rohan Wakade**

Ypt6, a small GTPases is required for filamentous growth and virulence in Candida albicans

R. Wakade, S. G. Filler, R.A. Arkowitz and M. Bassilana
University Côte d'Azur, CNRS, Inserm, iBV, Nice France
Los Angeles Biomedical Research Institute at Harbor-UCLA Medical center, Torrance, CA, USA, California, United States

Candida albicans is a harmless commensal that can become pathogenic. The dimorphic switch, a key feature critical for its virulence, requires cytoskeleton reorganization and sustained membrane trafficking. Rab G Proteins (Ras related protein in Brain) have important functions in membrane traffic, yet little is known regarding their roles in external signal mediated polarized growth and pathogenicity. *C. albicans* has 8 Rab G-proteins, which is the minimal set of Rab proteins among fungal species, making it an ideal model to study the role of membrane traffic. Here, we focused on 2 Rab proteins most likely to function in Golgi to plasma membrane traffic: Ypt6, the Rab6 homolog, and Ypt31, the Rab11 homolog, and examined their role in filamentous growth, antifungal sensitivity and virulence. Our results indicate that Ypt31, but not Ypt6, is essential for viability. Using the pTet repressible promoter, we generated strains in which the level of expression of *YPT31* could be regulated and determined that Ypt31 is required for filamentous growth, in addition to being critical for antifungal sensitivity. In contrast, Ypt6 is not required for antifungal sensitivity, yet is necessary for filamentous growth; the *ypt6* mutant exhibits shorter hyphae, compared to the wild type strain. We also show that Ypt6 is critical for virulence, using murine models for hematogenously disseminated and oropharyngeal candidiasis.

Poster 16: **Anil Chougule**

Identification and characterization of new LR determination factors in Drosophila

Anil Chougule, Charles Geminard and Stephane Noselli

Keywords: Left-Right asymmetry, *Drosophila*, Myosin, Actin cytoskeleton

In *Drosophila*, chirality is manifested in the looping of the gut, genitalia and testis. Our laboratory identified the conserved type ID myosin (*myoID*) gene as a major Dextral determinant of LR asymmetry in flies. Indeed, *myoID* null mutant show an asymmetric but totally inverted Sinistral development. Interestingly, this phenotype indicates that an underlying, *recessive* Sinistral pathway exists controlling LR asymmetry in the absence of *myoID*.
To try identify new factors controlling LR development in *Drosophila*, and in particular those involved in the yet uncharacterized Sinistral pathway, we have designed a specific genetic screen based on a double RNAi approach. A tester line expressing *myoID* RNAi (making flies Sinistral) is crossed to a collection of RNAi lines targeting collections of genes of interest. Candidates Sinistral factors are expected to turn Sinistral flies into symmetric ones. Because, genetic evidence suggests an important role for the actin cytoskeleton for proper *Drosophila* LR determination, we have started by screening genes involved in cytoskeleton function. We will present our current results on the analysis of some actin regulators including the small G-actin binding protein Profilin (*chickadee*), whose loss-of-function affects both Sinistral and Dextral asymmetry involving *myoID* function. Our current results support a model in which subsets of actin filaments are assembled specifically and required for chirality determination.

Poster 17: **Maria Isabel Acosta Lopez**

A study on the phospho-regulation of the tumor suppressor HACE1 and its role in epithelial integrity

M.I. Acosta Lopez1, J. Boudeau2, S. Urbach2, P. Munro1, A. Mettouchi1, A. Debant2, O. Visvikis1, and E. Lemichez1
1-INSERM U1065, C3MUniversité de Nice Sophia-Antipolis
2-CNRS, UMR 5237, Centre de Recherche de Biochimie Macromoléculaire

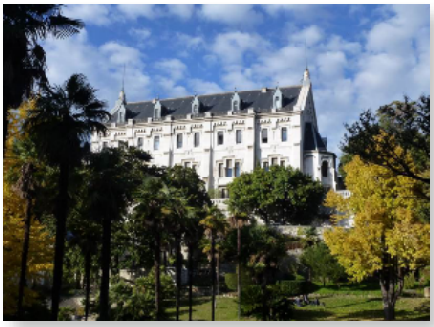
Rac1 is a small GTPase instrumental in the regulation of actin-based processes. Rac1 is frequently the target of bacterial toxins such as CNF1, a virulence factor produced by Uropathogenic E. coli that constitutively activates Rac1 by locking it in a GTP-bound form. Studying CNF1, our group identified the selective ubiquitin-mediated proteasomal degradation of Rac1 GTP-bound form, a novel way of blunting Rac1 signaling. As a major follow-up, we and other teams recently identified Rac1-GTP as the first target of HACE1, an HECT E3 ligase known as a tumor suppressor whose gene expression is down-regulated in cancers of numerous origins. In contrast, little is known about the regulation of HACE1 activity at the protein level. Therefore, we undertook the analysis of the post translational modifications of HACE1 and identified a phospho-site that is modulated in response to cell intoxication by CNF1. In addition, we have observed that HACE1 downregulation affects E-cadherin protein levels and modifies cell monolayer permeability. Our ongoing work is aimed at determining how phosphorylation modulates the activity of HACE1 and how this controls the adhesion between cells.

PRACTICAL INFORMATION

Château Valrose, Nice

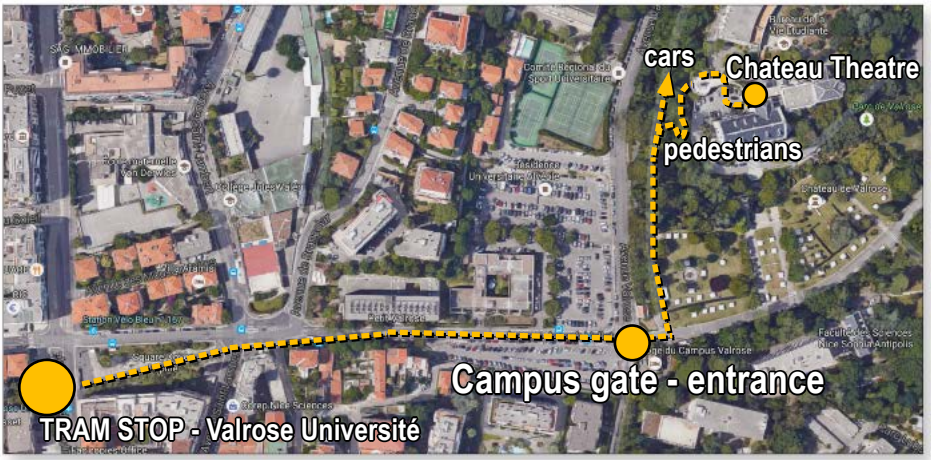
Valrose is a University 'Life Sciences' campus that is remarkable for its natural and historical surroundings. Notably, the Château which was built between 1860-1870 by order of Baron Paul von Derwies (counselor of Tsar Alexander II) and jointly designed by three significant architects: David Grimm, Antonio Crocci and Constantin Scala.

This Signalife Conference/Retreat will be held in the Château's Theatre and our sponsor ThermoFischer Scientific as well as the refreshments will be located in the Salle à Manger on the first floor of the Château.



IMPORTANT!

For security reasons all participants will be checked at the campus entrance gate by their ID/student cards.



PRACTICAL INFORMATION

Acropolis

Since this is not only a conference but also a student retreat we wil socialize in other than scientific environment - for this purpose bowling and snooker games have been arranged at Acropolis - be there on the first day of the conference at 4pm.

Address: Nice Bowling Acropolis, 5 esplanade Kennedy, 06300 Nice

Restaurant Villa d'Este

After a hard science- and sports-filled day it is time to lean back and enjoy delicious food at Villa d'Este.

Be there on the first day from 7pm.

Address: 6 Rue Masséna, 06000 Nice

Acknowledgements

The Signalife conference/retreat organizing committee would like to express our sincere gratitude to **Dr Konstanze Beck**, Labex Signalife PhD officer, who was helping us from the initial idea to organize the Signalife retreat, until the very last moment of the realization of this event.

We also thank **Dr Stéphane Noselli**, Labex Signalife Scientific coordinator and president of Labex Signalife council, for his support.

We would like to gratefully acknowledge **Dr Hélène Marie** and **Dr Marcel Deckert**, president and vice president of the Labex Signalife Educational Committee, for their advice and help.

Further, we would like to thank **Franck Aguila** for his graphic artwork on the program booklet.

Finally, our thanks and appreciation go to all invited speakers, students who presented their work in talks and posters, as well as students who contributed to the organization of the event. Without **YOU** sharing your outstanding research projects and participating in the Signalife retreat, this event could never happen, and therefore we extend our sincerest gratitude to all Signalife PhD students.

The Signalife retreat/conference organizing committee would also like to thank our supporters and sponsors:

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Notes