

Program and Abstract Book

- 2nd Conference -
ImaBio Young Scientist Network



September 28th-30th 2020
Institut Pasteur
(Duclaux Amphitheatre)
Paris, France



Summary

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WELCOME FROM THE ORGANIZERS

Dear colleagues,

We are happy to welcome you to the second meeting of the ImaBio Young Scientists Network (IYSN) at the Institut Pasteur in Paris on the 28th-30th of September 2020.

During MiFoBio, the summer school for functional microscopy in biology, in October 2018, we realized that we were many young researchers working on closely linked subjects. IYSN is the result of our desire to form a network of young researchers within the ImaBio GDR. This first and second meeting were organized by people coming from a large range of research domains (physics, biology, super resolution, image processing, chemistry...) to bring together young researchers. It is an occasion for you to network (virtually this time) with other scientists in the bio-imaging community, meet more experienced researchers and open your perspectives for future work. We sincerely hope you will enjoy these opportunities!

IYSN is bringing together three keynote speakers working on genome structure and functions, viral assembly and phagocytosis by immune cells, but also career development workshops as well as student oral or poster presentations.

This conference is organized with the financial support of CNRS, Institut Pasteur and StaPa, we thank them for that.

We would also like to thank Ignacio Izeddin and the ImaBio team for their support, constant motivation and for their fruitful help and advices for the workshop organization.

ORGANIZING TEAM

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	<p>The event is co-organized by Stapa – young researchers of the Institut Pasteur http://stapa.ovh</p>

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Program

Monday, September 28th 2020

13.45-14.00

Welcome from the ImaBio YSN and GDR ImaBio.

14.00-15.00

Keynote lecture

Interdisciplinary approaches to understand the 4D genome

Antoine Coulon, *Institut Curie*

15.00-16.00

Short talks

A VHH directed against tau as a novel therapeutic approach in tauopathies

Orgeta Zejneli, *University of Lille*

Multi-Template Matching: a simple and generic object-detection solution for microscopy

Laurent Thomas, *Acquifer/University of Heidelberg*

16.00-17.00

Poster Session

Tuesday, September 29th 2020

11.00-12.00

Workshop

Exploring the job market and developing your career by networking

Kristina Berkut, *ABG*

12.00-13.00

Short talks

Deciphering viral DNA fates in the nuclear landscape restructured by HIV-1

Viviana Scoca, *Institut Pasteur*

Through the microscope: novel approaches in host-pathogen interaction direct observation and inferred modeling in Zebrafish

Valerio Laghi, *Institut Pasteur*

13.00-14.00

Break

14.00-14.30

Software presentation

DIVA and its recent projects on microscopy and medical applications

Oumaima Sliti, Charlotte Godard, *Institut Pasteur*

14.30-15.30

Short talks

Exploring the active state of the NADPH oxidase by live-cell FRET-FLIM

Hana Valenta, *University Paris-Saclay*

Local principles of gene co-regulation within the 3D genome

Maciej Kerlin, *Institut Curie*

15.30-16.30

Keynote lecture

Quantitative monitoring of HIV-1 assembly in living T cells using dynamic nanoscopies

Cyril Favard, *Institut de Recherche en Infectiologie de Montpellier*

Wednesday, September 30th 2020

11.00-12.00

Workshop

The skills as a tool to develop/plan my career
MAASC

12.00-13.00

Keynote lecture

TBD
Florence Niedergang, *Institut Cochin*

13.00

Closing remarks

Prizes for the best short talk and poster

KEYNOTE SPEAKERS



Antoine Coulon

Institute: Institut Curie

Speech: 28th September at 14.00

Topic: Interdisciplinary approaches to understand the 4D genome

Antoine's team aims to understand how the physical organization of the genome in the eukaryotic nucleus relates to the regulation of its expression. They explore this topic at multiple spatial and temporal scales, with an approach combining real-time single-molecule imaging technologies and physical/mathematical analysis and modeling techniques.



Cyril Favard

Institute: Institut de Recherche en Infectiologie de Montpellier

Speech: 29th September at 15.30

Topic: Quantitative monitoring of HIV-1 assembly in living T cells using dynamic nanoscopies

Cyril's group aim at characterizing the respective roles of viral proteins and host-cell lipids or sub-plasma membrane constituents during the assembly of enveloped viruses affecting human beings (such as HIV-1 and influenza viruses). They are biologists and biophysicists using multi-disciplinary approaches.



Florence Niedergang

Institute: Institut Cochin

Speech: 30th September at 12.00

Topic: TBD

Florence's lab studies the mechanisms of capture and degradation by phagocytic cells, their impact on immune responses and their alterations by viral infections, in the context of the development of bacterial co-infections or, more recently, in the cross-talk between microglia and neurons.

LIST OF PARTICIPANTS

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Keynote Speakers: Abstracts

Quantitative monitoring of HIV-1 assembly in living T cells using dynamic nanoscopies.

C. Floderer¹, J. Chojnacki², JB Sibarita³, JB Masson⁴, C. Eggeling⁵, D. Muriaux¹, **C. Favard¹**.

¹Membrane Domains and Viral Assembly, IRIM, CNRS UMR 9004, Montpellier, France

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⁴Decision et processus Bayesiens, Institut Pasteur, Paris, France

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Until recently, images of viruses were mainly obtained using electron microscopies, providing important structural information. The development of nanoscopies (SMLM and STED) these last 20 years allowed direct imaging of fluorescently (multi)labelled viruses in their host cells. Although these photonic nanoscopies won't reach ultimate structural details provided by electron microscopy, they allow direct visualization of viruses and virus components in living cells.

HIV-1 is a retrovirus, an enveloped RNA virus. Production of new HIV-1 particles are mainly driven by the self-assembly of HIV-1 Gag proteins, on the associated genomic RNA, at the inner leaflet of the host T cell plasma membrane, Gag recognizing mainly the phospholipid PI(4,5)P₂.

Combining dynamics at the single (few) molecules levels to fluorescent nanoscopy we studied the time course of HIV-1 Gag assembly and its consequence on the plasma membrane of living host CD4 T-cells. Using multiple particle tracking PALM, we deciphered the respective role of Gag-Gag and Gag-RNA interactions. Interestingly, we observed directed instead of Brownian motions of single Gag protein towards the assembly site. Using Bayesian inference analyses of the trajectories, we were able to quantify the respective attractive energies due to Gag/Gag and Gag/RNA interactions (1).

Using scanning STED-FCS, we also showed that during assembly, HIV-1 reorganizes the plasma membrane of living CD4 T cells. HIV-1 specifically sorts PI(4,5)P₂ and cholesterol at the assembly site to generate its own envelop instead of targeting pre-existing lipid domains (2). Moreover, we showed that HIV-1 Gag only is sufficient to generate these nanodomains as it was previously described *in vitro* on model membranes (3).

Overall, dynamic nanoscopies helped us to quantitatively decipher the roles of the different components of HIV-1 during the production of new viruses in the host cells. Interestingly, our results are nicely mimicked by recent coarse grained molecular dynamics simulations opening new perspectives in understanding and quantifying complex mechanisms at the single molecule level.

¹ Floderer C., Masson JB., Boiley E., Georgeault S., Merida P., El Beheiry M., Dahan M., Roingeard P., Sibarita JB., Favard C., Muriaux D. *Single molecule localisation microscopy reveals how HIV-1 Gag proteins sense membrane virus assembly sites in living host CD4 T cells.* **Sci Rep.** 8:16283, (2018).

² C. Favard, J Chojnacki, P. Merida, N. Yandrapalli, J. Mak, C. Eggeling, D. Muriaux, *HIV-1 Gag specifically restricts PI(4,5)P₂ and cholesterol mobility in living cells creating a nanodomain platform for virus assembly.* **Science Adv**, 5(10), eaaw8651 (2019).

³ Yandrapalli N, Lubart Q, Tanwar HS, Picart C, Mak J, Muriaux D*, Favard C*. *Self-assembly of HIV-1 Gag protein on lipid membranes generates PI(4,5)P₂/Cholesterol nanoclusters.* **Sci Rep.**;6:39332 (2016).

Interdisciplinary approaches to understand the 4D genome

Antoine Coulon

Sorbonne Université, Institut Curie, PSL Research University, CNRS, Nuclear Dynamics unit, Physical Chemistry unit, F-75005 Paris, France

To be presented on site

To be presented on site

Florence Niedergang

Institut Cochin, Paris, France

To be presented on site

Short talks: Abstracts

A VHH directed against tau as a novel therapeutic approach in tauopathies.

Orgeta Zejneli^{1,2}, Clément Danis^{1,2}, Elian Dupré¹, Alexis Arrial³, Jean-Christophe Rain³,
Morvane Colin¹, Luc Buée², Isabelle Landrieu²

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**Equal contribution*

Tauopathies, including Alzheimer's disease (AD), are neurodegenerative diseases characterized by the accumulation of aggregated tau into the brain cells of the patients. Tau is a soluble, unfolded microtubule-associated protein that regulates cytoskeletal dynamics of neurons in the central nervous system. Under pathological conditions, tau becomes abnormally phosphorylated and aggregates into filamentous brain inclusions. Although the mechanisms leading to the pathological tau species are not clearly understood, different molecular features have been identified as involved in the aggregation process, including the identification of the peptide motif PHF6 (306-VQIVYK-311) that compose the nuclei of tau aggregation. One of the new highly promising therapeutic approaches towards tauopathies is the immunotherapy directed against tau. This strategy has shown good outcomes in tauopathies and AD mouse models. Further, clinical trials targeting different tauopathies such as progressive supranuclear palsy (PSP) and AD are ongoing. Here, we use VHHs (or nanobodies, Variable Heavy- chain of the Heavy-chain-only-antibody), for targeting Tau and preventing its aggregation. In partnership with Hybribody Company, a synthetic phagedisplay library of VHHs was screened against recombinant full length Tau protein. The epitopes recognized by the selected VHHs, were defined using Nuclear Magnetic Resonance (NMR) spectroscopy. A VHH targeting an epitope in the microtubule binding domain of tau, which corresponds to the nuclei of taufilaments, was selected. Further, yeast two-hybrid was performed to increase its biochemical properties. The encouraging results of inhibitory effect towards tau aggregation both in vitro and in a cellular model of seeding with this VHH raised the hopes for a potential novel therapy in tauopathies.

Multi-Template Matching: a simple and generic object-detection solution for microscopy.

Laurent Thomas^{1,2}, Jochen Gehrig¹

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Object-detection algorithms allow to automatically detect regions of interests in microscopy images, and can be used for automated feedback microscopy or as a generic processing step in image-analysis workflows. While many algorithms for object-detection are available from the field of computer vision, they usually require expert knowledge, annotated ground-truth data or powerful hardware resources. To simplify object-detection tasks for life-science researchers, we developed Multi-Template Matching, a generic object-detection algorithm that allows to robustly localize object of interests provided one or a few template images. The tool is available as a Fiji plugin, a KNIME workflow and python package and does not require powerful hardware. Moreover, the toolset is supplemented by a detailed documentation, video tutorials and examples. We illustrate Multi-Template Matching for the detection of organs like head and yolk in zebrafish screening data and the detection of medaka embryos in 96 well plate. Importantly, the tool can be used for other samples without much effort for installation or usage. Using different templates, the tool can also be used for the classification of the detected regions. In this presentation, we will introduce multi-template matching as well as other open-source contributions to ImageJ/Fiji for object-detection and annotation.

Deciphering viral DNA fates in the nuclear landscape restructured by HIV-1.

Viviana Scoca^{1,2}, Marion Louveaux^{3, 4}, Renaud Morin⁵, Dmitry Ershov^{4,6}, Jean-Yves Tinevez⁴, Francesca Di Nunzio¹

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Upon infection, Human Immunodeficiency Virus 1 (HIV-1) retrotranscribes its RNA genome into double-stranded DNA and permanently integrates it into the host DNA. HIV-1 integration has evolved to optimize the release of high levels of viral progeny in the first weeks of infection and to discretely carry on a lifelong coexistence with the host. Studies in fixed cells allow only a partial understanding of the fate of HIV-1 DNA, once imported into the nucleus. Thus, we adapted a DNA-tagging bacterial system, called "HIV-1 ANCHOR", to label HIV-1 retrotranscribed DNA. This approach provides the first live-imaging tool to study the spatio-temporal regulation of nuclear HIV-1 forms. This system is highly specific and quantitative for the tracking of both episomal and integrated forms. We unraveled the spatio-temporal dynamics of proviral DNA co-detecting the viral foci of transcription that occupy the same nuclear space. Live tracking of the episomal forms showed the viral DNA aggregating into flower shapes. Real-time imaging of actively transcribing viral foci showed that they can converge at the same site, establishing a "proviral kiss", probably to hijack the same transcriptional factory. Interestingly, we observe that HIV-1 infection reshapes the nuclear environment in macrophages, constituting membraneless organelles, formed by CPSF6 and SC35 factors, from which HIV-1 proviruses are excluded. On the other hand, HIV-1 DNA localize in speckle domains neighboring CPSF6/SC35 clusters, known to be chromatin regions of transcriptional boost. Besides HIV-1 biology, the lentiviral vector-based nature of this new imaging method makes it highly customizable for studying the cellular entry of other relevant viruses, such as SARS-CoV2.

Through the microscope: Novel approaches in host-pathogen interaction direct observation and inferred modelling in Zebrafish.

Valerio Laghi¹, Lena Kleij¹, Rene Doursat, Jérémie Guedj², Emma Colucci¹, Jean-Pierre Levraud¹

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How do viruses propagate from organ to organ – particularly to the brain, and how does the innate host response, or drugs, counter this?

Our project starts from biology and develops towards modelling and bioinformatics. We will exploit an established model of viral infection of zebrafish larvae, in which the distribution of infected cells can be imaged in the whole body over time. We aim to create a new method of analysis and reconstruction of viral spreading from organ to organ, and later from cell to cell, and to understand the relative impact of the host interferon (IFN) response and antiviral drugs on the mechanism of viral dissemination, with a particular focus on brain invasion.

We use SINV strains encoding for fluorescent proteins co-expressed with late viral proteins and we are generating new tools to monitor early events of infection. Target cells, and the innate immune response, are monitored using transgenic reporter zebrafish. We analyze in the infection course in control animals, in larvae made unable to respond to type I IFNs, and in larvae treated with antiviral drugs.

Firstly, we use high throughput methods to screen large number of samples infected with SINV to determine the general route followed by the virus to spread in the host and lay down a first mathematical model based on two compartments (CNS and Outside CNS).

Based on assessments of viral burdens and modelling of dynamics using ordinary differential equations, we will derive the parameters of viral growth (burst size, half-life of infected cells, estimation of target cells). Another key parameter that we want to infer from our model is the frequency of virus crossing from periphery to CNS.

After having determined the temporal frames of infection, the involved tissues and the base model, we move towards high resolution confocal imaging to be able to acquire long time lapses of the infection spreading in vivo and identify the key cells types involved. The 3D reconstruction will be used to create a mathematical model of SINV movement in the body, with a focus on discovering the molecular mechanism used to invade the central nervous system, the cellular population involved, the kinetic rules associated to the viral biophysics in each compartment of the specified model.

Exploring the active state of the NADPH oxidase by live-cell FRET-FLIM.

Hana Valenta

Institut de Chimie Physique, Université Paris-Saclay

In living cells, dynamic interactions between proteins play a key role in regulating many signaling pathways and biochemical events. It is also the case of **the phagocyte NADPH oxidase (NOX)**, a key enzyme of the innate immune system. It generates superoxide anions ($O_2^{\cdot-}$), precursors of reactive oxygen species (ROS) that are critical for host responses to microbial infections. The NOX is a protein complex composed of five subunits (membrane-bound: gp91^{phox}, p22^{phox} and cytosolic: p67^{phox}, p47^{phox} and p40^{phox}) and a small GTPase Rac. The activation mechanism of the NOX relies on the assembly of all cytosolic proteins with the membrane-bound components, whereby **protein-protein interactions** play an important role. Lack of the NADPH oxidase activity leads to chronic granulomatous disease (CGD) characterized by severe and recurrent infections. On the other hand, enhanced levels of ROS contribute to cardiovascular and neurodegenerative diseases. The NOX activity needs to be tightly regulated in order to maintain physiological levels of ROS.

In this project we investigated the active state of the NOX in living cells using state of the art of fluorescence microscopy strategies. To obtain constitutive, robust NOX activity in cells, we used a chimeric protein called "**Trimer**", which is composed of domains of the cytosolic proteins p47^{phox}, p67^{phox} and Rac1 and works as a single activating protein of the NOX. We observed that FP-labeled Trimer is localized in the plasma membrane of COSNOX cells (COS-7 cell stably expressing gp91^{phox} and p22^{phox}). To elucidate **spatial organization** of the FP-labeled Trimer in the plasma membrane we opted for Förster Resonance Energy Transfer (FRET) measured by fluorescence lifetime imaging microscopy (FLIM). As the FRET phenomenon occurs only between fluorophores in close proximity (< 10 nm), it is well-suited to detect protein-protein interactions and also provide information about topology & geometry of the Trimer proteins in the active complex. **Live-cell FRET-FLIM** experiments revealed that the FP-Trimer forms clusters in the plasma membrane.

Local principles of gene co-regulation within the 3D genome.

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The eukaryotic genome is highly organized in both space and sequence. From entire chromosomes to individual genes the 3D organization of the genome is linked to transcription and many regulatory mechanisms likely coexist at different scales. At the sub-megabase scale, the eukaryotic genome is physically organized into self-interacting domains ('contact domains' or TADs) that are thought to constrain the range of action of gene regulatory elements called 'enhancers'. Perturbations of TAD structures affecting enhancer-gene contacts are involved in many cancers.

Current data suggest that TADs serve as 'regulatory units' to co-regulate multiple genes by exposing them to the same landscape of enhancers. Genes from the same TAD indeed often display correlated expression across cells. Interestingly, correlated expression is seen between functionally related genes. However, how 3D organization at an individual locus plays a mechanistic role in co-regulating functionally related genes is unknown.

Using single-molecule imaging techniques, we observe the spatial positions and transcriptional activities of pairs of genes co-regulated by the same – or by different – enhancers in single cells. We use estrogen stimulation in breast cancer cell line MCF7 as a model system to study hormone-responsive genes and enhancers. We selected three enhancer-dependent functionally-related AP-1 family genes. Using single-molecule FISH techniques, we measure the coupling between genes as the correlation *in cis* of their transcriptional activity. To investigate the role of the TAD structures in co-regulation, we perturbed TAD boundaries at the locus of interest. Together, this will shed light on the mechanisms by which multiple enhancers and multiple genes communicate and coordinate their activity within the 3D genome.

Posters: Abstracts

Tracking exogenous dsRNA in mosquitoes and flies bodies

Sabrina Fletcher, Yasutsugu Suzuki, Cassandra Koh, Maria-Carla Saleh

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Since the discovery of RNA interference (RNAi), extensive research has been done to fully understand how this biological process works. RNAi has been shown to play a major role in cell cycle, oncogenesis, immunity and more. Moreover, it has provided us with a new tool to manipulate gene expression in different organisms. Nowadays, gene knockdown in cell cultures and in organisms is a widely used technique that relies on the incorporation of dsRNA into cells and downstream processing. This approach is often used in mosquito research to knockdown specific genes *in vivo*. However, there are still some uncertainties about the mechanism of action. For instance, after dsRNA injection into a mosquito, where does it go? Does it enter all cells of the body or is there specificity for certain cell type/tissue/organ? To start answering these questions, we injected dsRNA labelled with a fluorescent molecule into *Aedes aegypti* mosquitoes. We also tested dissemination of the dsRNA in the widely used model organism *Drosophila melanogaster* or fruit fly. At different timepoints, we dissected the midguts and ovaries, and we performed wholemount histofluorescence and confocal microscopy imaging. Surprisingly, in mosquitoes we were able to detect dsRNA inside cells in the midgut and ovaries after only 1 hour-post-infection. Moreover, there seemed to be some specificity within each tissue, since not all cells incorporated the dsRNA. On the contrary, in the fruit fly, we didn't find internalization of dsRNA in the analyzed tissues. Our results show that dsRNA internalization is not a general cellular event, even in mosquitoes, and therefore identifying the target cells/tissues will help us perfect and expand this widely used experimental technique.

Poly(ADP-ribose)-dependent chromatin unfolding facilitates the association of DNA-binding proteins with DNA at sites of damage

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The poly (ADP-ribose) polymerase, PARP1, is a key enzyme for DNA damage detection and repair mediation. The addition of poly (ADP-ribose) (PAR) chains along the chromatin fiber due to PARP1 activity regulates the recruitment of multiple factors to sites of DNA damage. In this work we investigated how, aside from direct binding to PAR, early chromatin unfolding events controlled by PAR signaling contribute to protein recruitment to DNA lesions. Using time-lapse microscope, we could assess PARP1 recruitment and release kinetics and we could observe that different DNA-binding domains accumulate at damaged chromatin in a PAR-dependent manner, and that this recruitment correlates with their affinity for DNA. We also show that this recruitment is promoted by early PAR-dependent chromatin remodeling rather than direct interaction with PAR. Fluorescence Correlation Spectroscopy (FCS) allowed us to show that the recruitment originates from facilitated binding to more exposed DNA. Finally, the relevance of this new mode of PAR-dependent recruitment to DNA lesions is demonstrated by the observation that reducing the affinity for DNA of both CHD4 and SMARCA5, two proteins shown to be involved in the DNA-damage response, strongly impairs their recruitment to DNA lesions.

Extracellular vesicles in *Cryptococcus neoformans*: from structural insights to vaccine strategy

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Extracellular vesicle (EV) research has become commonplace in different biomedical fields. In medical mycology, EV research is still in its infancy. Using cutting-edge methodological approaches including cryogenic electron microscopy and cryogenic electron tomography imaging techniques, proteomics, and nanoscale flow cytometry, we provide a robust set of data regarding the structural and compositional aspects of EVs from the encapsulated pathogenic yeast *Cryptococcus neoformans*. Our analysis suggested a new EV structural model, in which the vesicular lipid bilayer is covered by a 16nm thick mannoprotein-based fibrillar decoration, bearing the capsule polysaccharide as its outer layer. About 10% of the EV population is devoid of fibrillar decoration, adding another aspect to EV diversity. By analyzing EV protein cargo from three cryptococcal species, we characterized the typical *Cryptococcus* EV proteome. It contains several membrane-bound protein families, including some tetraspanin (Tsh proteins) bearing a SUR7/Pall motif. The presence of protective antigens on the surface of *Cryptococcus* EVs, resembling the morphology of encapsulated virus structures, suggested their potential as a vaccine. Indeed, mice immunized with EVs obtained from an acapsular *C. neoformans* mutant rendered a strong antibody response and significantly prolonged survival of mice upon *C. neoformans* infection.

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