MARIE SKLODOWSKA-CURIE ACTIONS

Co-funding of regional, national and international programmes (COFUND)

Imaging cytoskeletal filament organization at the molecular scale

Financing : EU funding COFUND – Aix Marseille University

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Laboratories involved:

http://www.fresnel.fr/spip/spip.php?article1102

http://crcm.marseille.inserm.fr/en/researchteams/ali-badache/

1. SUMMARY

This PhD project aims at developing new optical and biological tools to decipher the organization of septins, which are essential proteins of the cell cytoskeleton, together with their interaction with other cytoskeletal filaments actin and microtubules during cell division. The topic addressed is highly interdisciplinary in co-supervision between **Institut Fresnel** (Ecole Doctorale 352 Physique et Sciences de la Matière) and **Centre de Recherche en Cancérologie de Marseille (CRCM)** (Ecole Doctorale 062 Sciences de la Vie et de la Santé). Addressing the nanoscale organization of such proteins will ultimately contribute to better understand mechanisms involved in cell division, which is essential for multiple fundamental biology aspects such as cell proliferation and differentiation, tissue growth and repair. To do so, a new optical scheme capable of imaging filaments with super-resolution (lower than the optical diffraction limit) together with their nano-scale orientation and organization will be implemented, based on polarized fluorescence microscopy. This project is based on the knowledge developed since many years by the co-supervisor in complementary fields covering optical instrumentation, molecular biology and cell biology. It will therefore be highly multidisciplinary and offer opportunities to collaborate with an industrial partner as well as collaborators in biophysics at the international level.

2. DESCRIPTION

Cell division is a fundamental biological process that is essential for multiple aspects of animal physiology, including cell proliferation and differentiation, tissue growth and repair. Its dysfunction leads to several human pathologies, including tumour development and tissue degeneration. Cell division involves strong cell shape changes and thus heavily relies on the redistribution and cooperation of force-generating cytoskeletal filamentous proteins, notably actin filaments and microtubules (Fig.1). While the requirement of these proteins is not questioned, little is known about the functional contribution of septins, a family of proteins which was

recently recognized as a novel component of the animal cytoskeleton. Septins form protomers which can polymerize into filaments (Fig.1), and they also associate with actin and with microtubules. A complete understanding of how cytoskeletal filaments contribute to biological functions in cell division requires a deeper knowledge of how septins, actin filaments and microtubules organize with respect to each other, which is still lacking. Addressing this question ultimately boils downs to the challenge of measuring inter-filament organization (orientation, spacing, alignment); however, quantitative imaging tools that combine molecular specificity, high spatial resolution and real-time measurements of filament organization are currently limiting.

This project aims at developing optical and biological tools to decipher the organization of septins and their interaction with actin and microtubules during cell division. The topic addressed is highly interdisciplinary in co-supervision between I. Fresnel (ED 352 Physique et Sciences de la Matière) and Centre de Recherche en Cancérologie de Marseille (CRCM) (ED 062 Sciences de la Vie et de la Santé).

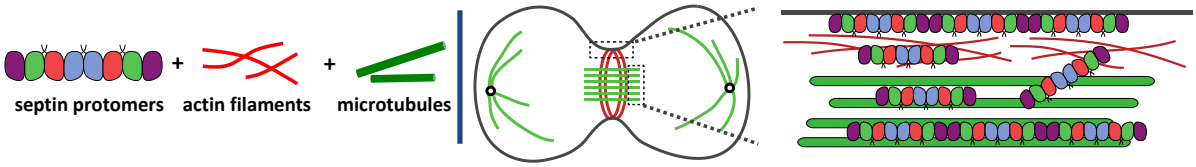

Fig.1. Left : schematic representation of cytoskeletal filaments. Right: assembly at the cytokinetic ring during cell division.

Optics. In this project, dedicated optical imaging methods will be implemented with the project co-supervisor at I. Fresnel, in collaboration with the company HORIBA, with the goal to measure filament organization at high spatial resolution and potentially in real time. The method will be based on polarized fluorescence imaging, in which I. Fresnel has developed a recognized expertise [1,2]. It will consist in implementing new optical schemes that will considerably improve the existing methods, for instance with the use of several project polarizations on a single camera chip.

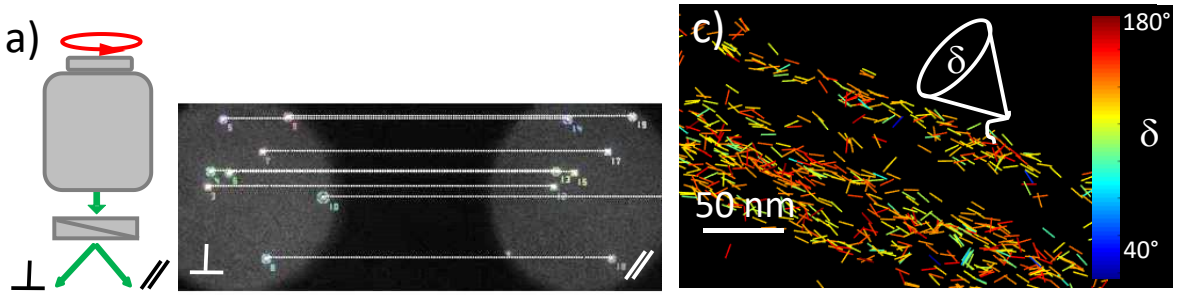

Fig.2. a) Single molecule orientation detection: the excitation is circularly polarized and the emission is split into sub-images of localized single molecules, projected on different polarization states. c) Each single molecule is represented by a stick (its mean orientation) and a color (its angular fluctuation extent). Adapted from [2].

Biology. This method will be applied to two systems: in vitro reconstituted cytoskeletal filaments from purified components, in collaboration with the international partner (AMOLF, The Netherlands), and dividing mammalian cells, with the co-supervisor at CRCM. In vitro studies will decipher the behaviour and local structure of single filaments and of filament assemblies, providing reference signatures for studies in cells. Functional studies in cells will then be performed at different stages of cell division. The molecular mechanisms at the origin of inter-filament organization will be investigated using septin mutants impaired for polymerization or with modified selectivity for actin or microtubules.

2. EXPECTED PROFILE OF THE CANDIDATE

This project being highly interdisciplinary, the candidate is expected to be either of physics or biophysics background, with either knowledge in optics (with interest in biology) or biology (with interest in experimental optics). The work will involve both optical instrumentation developments and biological sample preparation, the student will thus be involved in different facets of the work where in vitro / cell sample developments, experimental imaging and data analysis will be equally important. A previous experience with interdisciplinarity during the master thesis is thus very favorable, in particular involving optical microscopy. He/She should be motivated by the exploration of novel questions in biology. He/She should also be interested in the development of an instrument of interest for industry, since the detection device envisioned in this project can possibly lead to industrial development. The candidate will work for part of his/her time with an international collaborator and participate in several national and international conferences: he/she should therefore be capable of integration in international environments.

3. SUPERVISORS

Supervisor 1: Institut Fresnel, Marseille, France. The MOSAIC team of I. Fresnel is dedicated to the development of optical microscopy and endoscopy tools for bio-imaging, from the single molecule scale to tissues. It is in particular known for its important advances in nonlinear vibrational microscopy and its leading position in polarized microscopy, in particular for polarized confocal and super-resolution microscopies which exists only in a few laboratories around the world. **Sophie Brasselet** (DR2 CNRS) is an optical physicist, with more than 15 years of experience in the field of optical microscopy and polarized imaging. She will be in charge of the supervision for the development of the polarized and super-resolution imaging modality, together with data analysis and processing tools. **Manos Mavrakis** (CR1 CNRS), a biochemist working on septin and actin biology who joined I. Fresnel in 2015, will supervise the molecular biology and biochemistry aspects of the project.

Supervisor 2: Centre de Recherche en Cancérologie de Marseille (CRCM). The team of Ali Badache works on the molecular mechanisms of tumor cell motility dependent on microtubules. It deciphered an original signalling pathway from the ErbB2 oncogenic tyrosine kinase receptor to proteins interacting with the +end of microtubules. **Pascal Verdier-Pinard** (CR1 Inserm) has extensive experience in the pharmacology, biochemistry and cell biology of microtubules and human septins over the last 20 years. He will supervise the cell biology aspects of the project.

International partner: FOM Institute AMOLF, The Netherlands. Gijsje Koenderink (Professor and group leader) is an experimental biophysicist focusing on the physical principles that underlie the self-organization of cells. She will contribute expertise on the reconstitution of septins with hybrid actin/microtubule networks. During his/her project, the candidate will have the opportunity to go regularly to AMOLF in order to (1) learn about in vitro reconstitution, (2) prepare his/her own samples to be characterized at AMOLF and later at I. Fresnel, (3) transfer the necessary knowledge for the completion of the project at I. Fresnel and CRCM.

Inter-sectorial partner : HORIBA will bring a unique expertise in the dimensioning of optical devices dedicated to polarized detection. It will in particular give access to its know-how on the existing system to evolve towards an imaging-compatible polarimeter. The doctoral candidate will visit frequently the involved colleagues at HORIBA, with several goals: (1) learn how to properly dimension a polarimetric detection system, (2) apply it to super-resolution imaging, (3) expand his/her knowledge on the industrial environment.

4. RECENT PUBLICATIONS

1. Kress A., Wang X., Ranchon H., Savatier J., Rigneault H., Ferrand P. and **S. Brasselet**. Mapping the local organization of cell membranes using generalized polarization resolved confocal fluorescence microscopy. Biophysical Journal 105, 127-136 (2013)
2. Valades Cruz, C.A, H. A. Shaban, A. Kress, N. Bertaux, S. Monneret, **M. Mavrakis,** J. Savatier**, S. Brasselet**, Quantitative nanoscale imaging of orientational order in biological filaments by polarized superresolution microscopy. Proceedings of the National Academy of Sciences of the United States of America 113, E820-828 (2016)
3. **Mavrakis, M.** Y. Azou-Gros, F-C. Tsai, J. Alvarado, A. Bertin, F. Iv, A. Kress, **S. Brasselet, G.H. Koenderink** and T. Lecuit. Septins promote F-actin ring formation by crosslinking actin filaments into curved bundles. Nature cell biology 16, 322-334 (2014)
4. **Mavrakis, M.,** Tsai, F.C. & **Koenderink, G.H.** Purification of recombinant human and Drosophila septin hexamers for TIRF assays of actin-septin filament assembly. Methods in cell biology 136, 199-220 (2016)
5. **Mavrakis, M.** Visualizing septins in early Drosophila embryos. Methods in cell biology 136, 183-198 (2016)
6. Chanez B., Gonçalves A., **Badache A.** and P. **Verdier-Pinard.** Eribulin targets a ch-TOG-dependent directed migration of cancer cells. Oncotarget. 6, 41667-41678 (2015)
7. Connolly D., Yang Z., Castaldi M., Simmons N., Oktay M.H., Coniglio S., Fazzari M.J., **Verdier-Pinard P.** and C. Montagna. Septin 9 isoform expression, localization and epigenetic changes during human and mouse breast cancer progression. Breast Cancer Res. 13 :R76 (2011)
8. Connolly, D., Abdesselam, I., **Verdier-Pinard, P.** & Montagna, C. Septin roles in tumorigenesis. Biological chemistry 392, 725-738 (2011)