

## MASTER INTERNSHIP PROPOSAL

**Department:** Physics of Cells and Cancer (PCC), Institut Curie

**CNRS identification code:** UMR168

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**Location:** Institut Curie, 11 rue Pierre et Marie Curie 75005 Paris - FRANCE

**PhD after internship:** YES

**PhD funding:** pending (ANR)

**TITLE:** Active self-organization of actin-filament bundles driven by myosin motors: spontaneous wave-like beating, chiral rotations, active droplets.

**BACKGROUND:** Biological cells can change shape, move and respond to their environment by coordinating the mechanical activity of molecular motors on filament networks of the cytoskeleton. Although the interaction between single motors and filaments has been extensively characterized, the emergent self-organized behaviors in large assemblies of motors and filaments is far less understood. In our group, we develop minimal in-vitro molecular systems based on myosin motors and actin filaments. We have recently discovered that polymerizing actin filaments can self-assemble into polar bundles of hundreds of filaments that show spontaneous wave-like beating, with waveforms that resemble those observed in the beating of eukaryotic flagella [1]. Fluorescent labeling of both actin and myosin allows us to study the dynamic interplay between motor dynamics (attachment, detachment, processivity) and bending of the actin-filament bundle. Polymerization from a surface micropattern of an actin-nucleation-promoting factor on the flat surface of a glass slide or from the curved surface of micron-sized beads result in 2D left-right symmetric beating or 3D helical beating with chiral rotations, respectively. Using DNA-based, liquid-liquid phase separation to direct actin nucleation inside liquid droplets, we have also very recently produced emulsions of active droplets containing an actin-

filament network whose size, internal architecture and dynamics evolves in time in a manner that depend on actin polymerization and active force production by myosins.

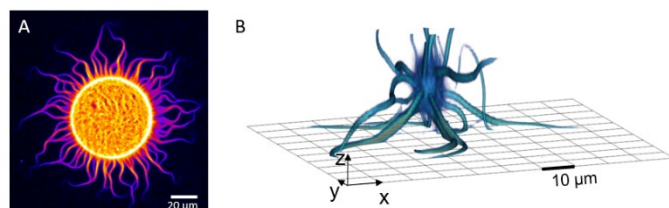


*Fig. 1: (A) Actin-filament bundles undulating in 2D near the surface of a glass slide; they have grown radially from the border of a 60- $\mu$ m disk of actin nucleation. (B) Octopus-like object, with actin-filament bundles ("tentacles") projecting in all directions from a microbead surface; the bundles rotate counter-clockwise.*

**WORKING HYPOTHESES:** [H1] Preliminary observations suggest that myosin motion toward the filament-bundle's tip results in funnelling, an increase in motor concentration and the resulting formation of myosin clusters. [H2] 3D chiral rotations of filament bundles, but not 2D left-right symmetric beating, lead to accumulation of twist, limiting the number of beating periods that can be observed before the bundle collapses. [H3] Bundling of actin filaments by myosin and the emergence of a 3D chiral component to motion depends on myosin properties (size, flexibility, stepping on actin). [H4] Droplets encapsulating actin and myosin mature and interact via active processes that violate expectations from passive thermodynamics.

**OBJECTIVES:** [O1] Study the formation of myosin clusters and their dynamics within a beating bundle using Fluorescence Recovery After Photobleaching (FRAP). [O2] Characterize the internal structure of a beating actin-filament bundle using (i) sparse labelling of actin filaments, (ii) FLIM-FRET microscopy and (iii) electron microscopy. [O3] Compare self-organized beating driven by motors of different biophysical properties, specifically myosin X (large head-to-head distance, flexible) and myosin V. [O4-for active emulsions] Study droplet maturation, internal structure and interactions with neighbors depending on actin polymerization and myosin activity.

**METHODS:** protein characterization, protein micro-patterning, microscopy (spinning disk, TIRF, FLIM-FRET microscopy, SEM), image analysis (ImageJ, Python, MATLAB), theoretical modeling.

**COLLABORATIONS:** Antoine Jégou and Guillaume Romet Lemonne (IJM, Paris; protein purification), Raju Regmi (PCC; FLIM-FRET microscopy), Koyomi Nakazawa (PCC; active emulsions), Aurélie Bertin (I2BC, Gif; scanning-electron microscopy), Jean-François Joanny (PCC/Collège de France; theory) and Frank Jülicher (MPIPKS, Germany; theory).

### REFERENCE:

[1] Pochitaloff M, Miranda M et al (2022) Flagella-like beating of actin bundles driven by self-organized myosin waves. Nat Phys 18:1240–1247.