M2 internship proposal

Actomyosin-driven active liquid droplets

Liquid-liquid phase separation (LLPS) is a key process through which cells can spatially compartmentalize macromolecules. Some proteins associated with the nucleation of cytoskeletal filaments, such as actin, can form phase-separated droplets in vitro, and actin growth leads to deformation of these droplets [1]. Recently, we have developed a platform through which an actin nucleation promoter can be targeted to artificial phase-separated RNA-polypeptide droplets; growing actin filaments self-assemble into criss-crossing rings within the droplets (Figure 1(A)), and bundles outside of it (Figure 1(B)).

On the other hand, the host team has also developed a minimal molecular system where polymerizing actin filaments and various myosin motors (myosin II, V, and X) self-assemble into filament bundles that undergo a wave-like beating similar to the beating of sperm flagella (Figure 1(C)) [2]. So far, these beating bundles can be anchored to the surface of a glass coverslip or of a polystyrene microbead.

In this internship, we propose to explore how myosin motor activity may lead to the remodeling of liquid droplets from which actin filaments are growing. A possible goal is to observe the beating of droplet-anchored bundles, which could be compared qualitatively and quantitatively to microbead-anchored bundles. Another possibility is the observation of actin ring remodeling and contraction in a manner reminiscent of the closure of the cytokinetic ring during cell division.

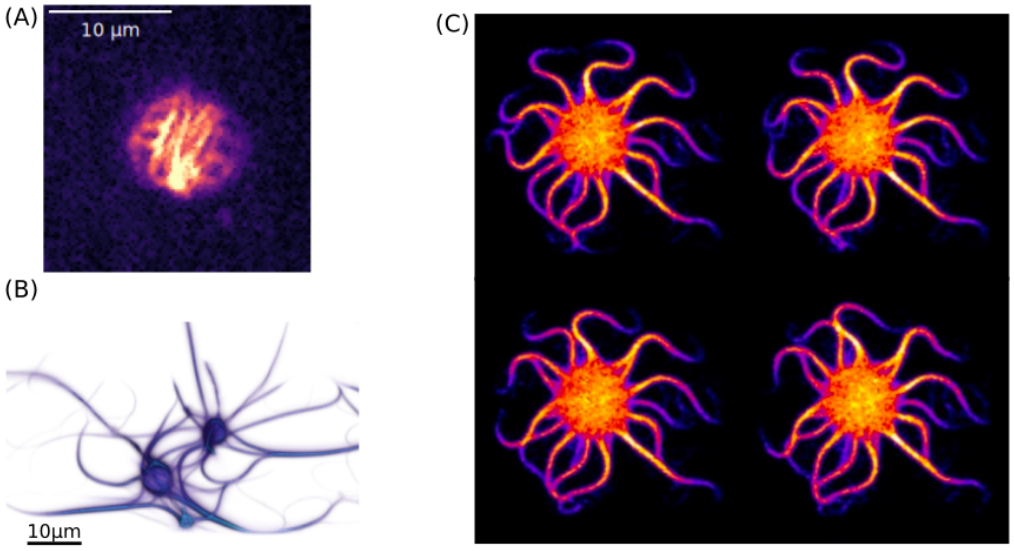

Figure 1: (A) Criss-crossing actin rings confined in a phase-separated RNA-polypeptide droplets. (B) Actin-filament bundles grown from droplets. (C) Autonomous 2D undulations of actin-filament bundles driven by myosin II motors [2].

References

[1] K. Graham, A. Chandrasekaran, L. Wang, N. Yang, E.M. Lafer, P. Rangamani, J.C. Stachowiak. Liquid-like condensates mediate competition between actin branching and bundling. *PNAS* **121**:e2309152121 (2024).

[2] M. Pochitaloff, M. Miranda, M. Richard, A. Chaiyasitdhi, Y. Takagi, W. Cao, E.M. De La Cruz, J.R. Sellers, J.-F. Joanny, F. Jülicher, L. Blanchoin L, P. Martin. Flagella-like beating of actin bundles driven by self-organized myosin waves. *Nat. Phys.* **18**:1240–1247 (2022).

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Keywords:
Self-organization, molecular motors, cytoskeleton, liquid-liquid phase separation, reconstituted systems, bottom-up approaches.

Skills:
Protein biochemistry, fluorescence microscopy.

This internship can be followed by a thesis.