PhD PROJECT PROPOSAL

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ACTIN-SHIFT: Molecular mechanisms by which actin isoforms control sarcomere assembly

Supervisor 1 (with name, email, affiliated laboratory and doctoral school affiliation)
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Supervisor 2 (with name, email and affiliated laboratory and doctoral school affiliation)
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Abstract (10 lines)*

Muscle cells initially contain very dynamic actin filaments and then assemble stable contractile sarcomeres at a defined stage during development. Each sarcomere contains highly ordered actin filaments and is mechanically connected to neighbouring sarcomeres forming long periodic chains (left image). The peptide sequence of muscle actin isoforms present in sarcomeres is highly similar to nonmuscle actin isoforms, which are present in the early dynamic filaments. Yet, these two actin isoforms have different biochemical properties. **Here, we would like to study the mechanism by which molecular differences in actin isoforms lead to a dramatic shift in actin organization and dynamics in muscle cells.** We aim to combine *Drosophila* genetics and *in vivo* imaging with actin biochemistry and *in vitro* actin assembly imaging to understand how the molecular choreography of the components drives sarcomere assembly in muscles.

Keywords*
actin; sarcomere; *Drosophila*; muscle; TIRF microscopy; live imaging; CRISPR; biochemistry; evolution;

Scientific question and Objectives (10 lines)*

1. Characterise the functional differences between the *Drosophila* muscle and nonmuscle actin isoforms by performing CRISPR-based knock-in swaps of their coding regions in *Drosophila* and analyse the resulting muscle phenotypes by confocal microscopy.
2. Manipulate the temporal dynamics of selected actin regulators to test their actin isoform specificity during sarcomere assembly *in vivo* by live imaging.
3. Characterise the biochemical properties of the different actin isoforms and their interactions with selected actin binding proteins *in vitro* using single filament polymerisation and TIRF microscopy (right image).

Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

This project uses *Drosophila* genetics to investigate the generally important question, why different actin proteins exist in muscle cells. This is exciting, because all animals that contain organized sarcomeres also contain muscle actin and non-muscle actin isoforms. However, despite the fact that actin protein is extremely well studied and essential for every eukaryotic cell, the functional reasons

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for the molecular differences between muscle and non-muscle actin are not known. CRISPR-based knock-ins in *Drosophila*, followed by phenotypic analysis will for the first time functionally investigate the relevance of the molecular differences (Aim 1). We have established an efficient knock-in strategy in the lab and house a suite of antibodies and nanobodies for confocal imaging to make this aim straight forward.

Actin works with accessory proteins that modulate its polymerization dynamics. We have identified the temporal expression dynamics of key regulators (including formins and cofilins) during *Drosophila* flight muscle development. This will allow us to manipulate the expression of these regulators at defined times in the context of the swapped actin isoforms to test *in vivo* which regulator is specific to a certain actin isoform (Aim 2).

Using our established single-molecule actin polymerization assay in combination with TIRF microcopy, we will dissect the biochemical specificity between actin isoforms and these regulators. We will test how different purified polymerization regulators interact with muscle and non-muscle actin isoforms and modulate polymerization rates and filament stability (Aim 3). Together, this synergistic project will identify the molecular mechanisms how actin isoforms control sarcomere assembly.

Interdisciplinarity and Implication of the two labs (15 lines)*
(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

This interdisciplinary project is an international collaboration between two groups based in Marseille and in Singapore.

The homebase for you will be the **group of Frank Schnorrer at the Developmental Biology Institute of Marseille (IBDM)**. The Schnorrer group is expert in muscle biology and *in vivo* imaging. Aims 1 and 2 will be performed in Marseille. The group is part of the Turing Centre of Living Systems, Centuri, which brings together biologists, physicists, and computational scientists. Thus, you will benefit from an interdisciplinary environment, including collaborations, courses, seminars and meetings in Marseille.

Your second base will be **the group of Alphée Michelot at the Mechanobiology Institute in Singapore (MBI)** to achieve Aim 3. The Michelot group is expert in actin biochemistry and *in vitro* imaging. The MBI is a leading multidisciplinary institute devoted to developing new paradigms for understanding biological functions in health and diseases from the perspective of cell, tissue and organ mechanics/dynamics and mechano-signal transduction. The institute operates on a fully integrated open-lab philosophy, with an extensive infrastructure supported by core facilities dedicated to technology such as state-of-the-art light microscopy, nano- and micro- fabrication, and computing.

Specify with whom the person recruited will collaborate and on what aspects *

The PhD student will spend the first 1.5 years in Frank Schnorrer's lab at the IBDM in Marseille to perform aims 1 and 2. The student will interact with all the lab members and will be advised on CRISPR based knock-ins, muscle biology and confocal microscopy. Aim 3 will be performed in Alphee Michelot's lab at the MBI in Singapore, including support for housing. The student will learn how to perform the

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actin single filament polymerization assays and TIRF imaging.

PhD student's expected profile*

This is an interdisciplinary PhD project. You are a biologist, biochemist, physicist, chemist or engineer with a core interest in quantitative biology. You are ambitious, curious, enjoy to learn novel techniques and like to find answers to problems. You want to take advantage of the opportunity to carry out your doctorate in two different countries and learn from different cultures and expertise.

Is this project the continuation of an existing project or an entirely new one?
In the case of an existing project, please explain the links between the two projects (5 lines)*

This is a new project that is funded by a CNRS grant to the Michelot and Schnorrer labs.

Two to five references related to the project*

1- Spletter et al. A transcriptomics resource reveals a transcriptional transition during ordered sarcomere morphogenesis in flight muscle. ***eLife*** 2018. doi: 10.7554/eLife.34058
2- Gressin et al. Architecture dependence of actin filament network disassembly. ***Curr Biol.*** 2015. doi: 10.1016/j.cub.2015.04.011.
3- Boiero-Sanders et al. Specialization of actin isoforms derived from the loss of key interactions with regulatory factors. ***EMBO J.*** 2022. doi: 10.15252/embj.2021107982

Two main publications from each PI over the last 5 years*

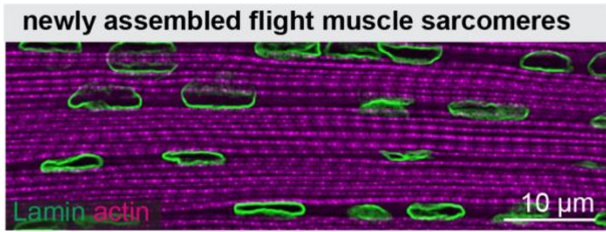
Frank Schnorrer:
1- Zhang et al. Mechanoresponsive regulation of myogenesis by the force-sensing transcriptional regulator Tono. ***Curr Biol*** 2024. doi: 10.1016/j.cub.2024.07.079

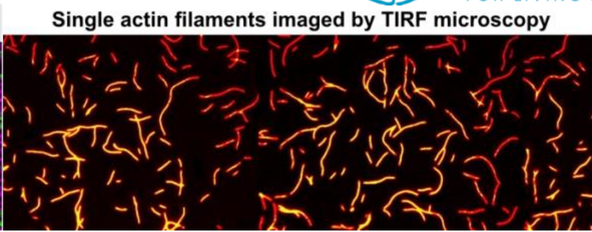
2- Schueder et al. Nanobodies combined with DNA-PAINT super-resolution reveal a staggered titin nanoarchitecture in flight muscles. ***eLife*** 2023. doi: 10.7554/eLife.79344

Alphee Michelot:
1- Boiero-Sanders et al. Specialization of actin isoforms derived from the loss of key interactions with regulatory factors. ***EMBO J.*** 2022. doi: 10.15252/embj.2021107982

2- Colombo, Antkowiak et al. A functional family of fluorescent nucleotide analogues to investigate actin dynamics and energetics. ***Nature Comm.*** 2021. doi: 10.1038/s41467-020-20827-4

Project's illustrating image





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