PhD PROJECT PROPOSAL

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Muscle building at the nanoscale: how molecular order guides sarcomere morphogenesis

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

Sarcomeres are highly ordered molecular machines enabling muscle contraction. Despite their importance, how sarcomeric components assemble during muscle development remains unknown. The main goal of the project is to decipher how the molecular order of key sarcomeric proteins emerges during muscle development. Combining advanced polarized super-resolution imaging with novel labelling technologies in *Drosophila* will reveal the protein's localization and its orientation at the nanoscale. The PhD student will first focus on the localization and orientation of actin using established tools, then precisely localize sarcomeric proteins α-actinin, myosin and titin, using nanobodies, which will be subsequently modified to determine their molecular orientation. The originality of this project relies on the integration of novel quantitative approaches that bridge from the molecular nano- to the macroscopic scale, to understand sarcomere morphogenesis during *Drosophila* muscle development.

Keywords*
Muscle; sarcomere; *Drosophila*; super-resolution microscopy; polarization resolved microscopy; nanobodies; self-organization

Scientific question and Objectives (10 lines)*

The objective is to reveal how key sarcomeric proteins in *Drosophila* flight muscle build up into well-defined organizations at the molecular level, to form a macroscopic organized sarcomere assembly.

Aim 1: Apply existing actin probes to quantify the actin order build-up during sarcomere assembly in *Drosophila* at different developmental stages, from the single-molecule scale to the tissue scale.

Aim 2: Decipher the nanoscopic localization of sarcomeric proteins in developing *Drosophila* muscles by combining nanobody labelling, DNA-PAINT and dedicated optical sectioning methods to achieve deep-tissue super-resolution imaging of entire muscle tissue.

Aim 3: Develop strategies to adapt existing nanobodies against sarcomeric proteins to orientation imaging, from the ensemble to the single-molecule level. DNA-PAINT tools and photoactivable nanobody probes will be modified to rigidify their link to fluorochromes.

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

Biology: We quantified the build-up of actin orientational order in *Drosophila* muscles using fixed probes (Loison et al., 2018). We will use novel actin probes developed for fixed tissues or in live imaging

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established in the Brasselet lab (Rimoli et al. 2022, Silva Martins et al. 2024) for single molecule actin order imaging. Our strategy introduces rigid linkers to reduce the fluorescent label's mobility. We will analyze protein conformational and organizational modifications before, during, and after sarcomere assembly in wild-type and possibly mutants affecting efficient muscle force production. Recently, we have developed nanobodies against key sarcomeric components that can be expressed in living muscles to follow the dynamics of the target protein *in vivo* (Loreau et al. 2023). We will use these nanobodies to precisely localize the sarcomeric proteins at the single-molecule level deep in the muscle tissue and observe how these proteins come together during muscle development. This technique was recently developed for mature muscles (Schueder et al. 2023). Then, we will engineer the nanobodies to enable orientational order imaging of the different sarcomeric proteins to define their local order.

Physics: We will use the recently developed super-resolution technique called Single Molecule Orientation and Localization Microscopy (SMOLM) (Brasselet et al. 2023, Rimoli et al. 2022) to determine the orientation and wobbling of individual fluorescent probes, which report the orientation and conformation of proteins at the single-molecule level. The assessment of wobbling will allow the selection of appropriate probes for nanoscale orientation imaging. A dedicated instrument will be developed for SMOLM images in tissues, which will require specific sectioning illumination strategies.

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)

The success of this project depends on a tight collaboration between the group of Sophie Brasselet, a physicist at the Institute Fresnel, and Pierre Mangeol, a biophysicist in the Schnorrer group at the IBDM. Both groups provide complementary expertise in genetics, molecular biology, biophysics and advanced quantitative imaging. The collaboration stands on the long experience in interdisciplinary research between the Brasselet and Schnorrer groups (Loison et al., 2018, Silva Martins et al., 2024). The proposed project is only feasible by continuous interactions between both collaborating groups to link the biology and genetics of sarcomere assembly with a molecular understanding of how proteins self-organize into regular supramolecular complexes across dimensions – from micrometer-sized sarcomeres to centimeter-large muscle fibers. This will require developing dedicated tissue labelling approaches at IBDM, and dedicated polarized super resolution microscopy tools at I. Fresnel. Establishing this link in *Drosophila* muscles will enable future modelling approaches to myofibrillogenesis.

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

The PhD student will collaborate with group members of Sophie Brasselet's lab (I. Fresnel) on the instrumentation development aspects, in particular adapting tissue-imaging modalities of polarized super-resolution microscopy; with group members of Pierre Mangeol's and Frank Schnorrer's lab (IBDM) on the development of dedicated nanobody approaches and the biology of muscle development; Manos Mavrakis (I. Fresnel) for advice on the development of dedicated rigid probes; Ralf Jungmann (Ludwig Maximilian University, Munich) for advice on the development of dedicated

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PAINT probes and with Dirk Görlich (Max Planck Institute Göttingen on the modifications of the nanobodies for order imaging (both collaborations are already established with the Schnorrer group).

PhD student's expected profile*

This is an interdisciplinary PhD project. The selected candidate should enjoy working in two interactive teams, one with a physics focus and a strong interest in imaging and building high-end microscopes (Brasselet), and one with a biological focus and a strong interest in mechanical forces (Mangeol). Importantly, the microscope will be developed to be entirely dedicated to the biology question addressed. Hence, the student will be preferably an experimental physicist with a very strong interest in biological systems, with a strong advantage if the Master experience has involved working at the interface with biology. Experimental experience with *Drosophila* or genetics is not essential, while basic principles of cell biology are of advantage.

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 project is related to a project previously coordinated a while ago by F. Schnorrer (with P.F. Lenne, S. Brasselet), called "Muscle building: bridging molecular order to macroscopic morphogenesis", in which we investigated molecular actin order in the developing sarcomere of *Drosophila* flight muscles. While this was dedicated to actin ensemble measurements in fixed images only, here we will investigate nanoscale order and we will extend the analysis to various key sarcomeric proteins, at the single protein level, using radically different labelling, analysis and microscopy tools.

Two to five references related to the project*

O. Loison, M. Weitkunat, A. Kaya-Çopur, C. Nascimento Alves, T. Matzat, M. L. Spletter, S. Luschnig, S. Brasselet, P.-F. Lenne and F. Schnorrer, Polarization resolved microscopy reveals a muscle myosin motor independent mechanism of molecular actin ordering during sarcomere maturation. PLoS Biol 16(4): e2004718 (2018) DOI: 10.1371/journal.pbio.2004718

S. Brasselet, M.A. Alonso, Polarization microscopy: from ensemble structural imaging to single molecule 3D orientation and localization microscopy. Minireview. Optica 10 (11), 1486-1510 (2023) doi.org/10.1364/OPTICA.502119

C. Silva Martins, …., S. Brasselet, M. Mavrakis, Genetically encoded reporters of actin filament organization in living cells and tissues (2024) ⟨hal-04563732⟩, Biorxiv DOI: 10.1101/2024.04.26.591305

Pleiner, T., Bates, M., Trakhanov, S., Lee, C. T., Schliep, J. E., Chug, H., Böhning, M., Stark, H., Urlaub, H., & Görlich, D. (2015). Nanobodies: Site-specific labeling for super-resolution imaging, rapid epitope- mapping and native protein complex isolation. eLife, 4 (2015). https://doi.org/10.7554/ELIFE.11349

Schueder, F., Mangeol, P., Chan, E. H., Rees, R., Schünemann, J., Jungmann, R., ... & Schnorrer, F. (2023). Nanobodies combined with DNA-PAINT super-resolution reveal a staggered titin nanoarchitecture in flight muscles. *Elife*, *12*, e79344.

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

Sophie Brasselet:

C. Rimoli, C. Valades Cruz, V. Curcio, M. Mavrakis, S. Brasselet. 4polar-STORM polarized super-resolution imaging of actin filament organization in cells. Nat. Communications 13, 301 (2022) DOI: 10.1038/s41467-022-27966-w

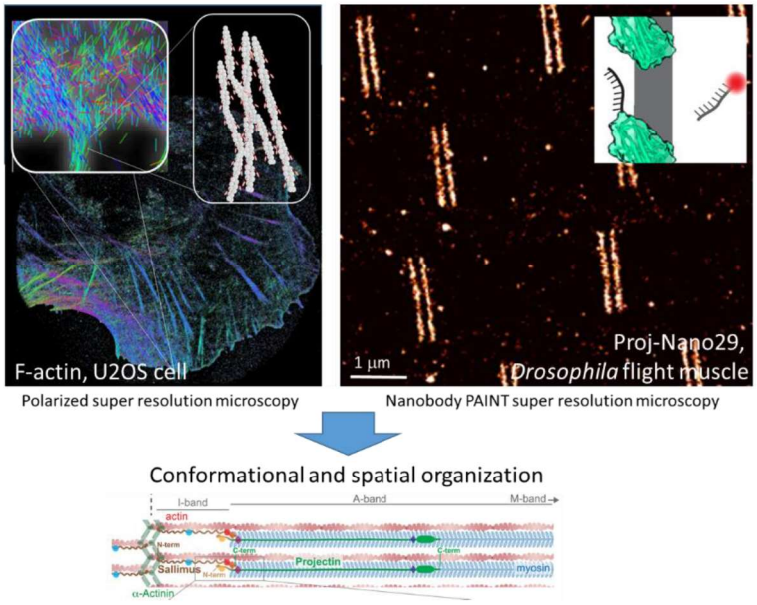
V. Curcio, L. A. Aleman-Castaneda, T. G. Brown, S. Brasselet, M. A. Alonso, Birefringent Fourier filtering for single molecule Coordinate and Height super-resolution Imaging with Dithering and Orientation (CHIDO). Nat. Communications 11 (1) (2020) DOI: 10.1038/s41467-020-19064-6

Pierre Mangeol:

P. Mangeol, D. Massey-Harroche, M. Sebbagh, F. Richard, A. Le Bivic, P.F. Lenne. (2024). The zonula adherens matura redefines the apical junction of intestinal epithelia. *Proceedings of the National Academy of Sciences*, *121*(9), e2316722121.

F. Schueder*, P. Mangeol*, E. HoYee Chan, R. Rees, J. Schünemann, R. Jungmann, D. Görlich, F. Schnorrer. Nanobodies combined with DNA-PAINT super-resolution reveal a staggered titin nanoarchitecture in flight muscles - Elife, e79344 (2023) DOI: 10.7554/eLife.79344

Project's illustrating image



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