Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

Supervisor(s):
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Host laboratory:
Laboratoire de Physiologie Cellulaire & Végétale
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Title of the M2 research internship:
Determination of the biophysical and biomechanical properties of liquid-liquid phase separating proteins from Arabidopsis thaliana

Project summary:
Plant growth and reproduction are finely tuned to changes in abiotic stimuli such as light, temperature, nutrient concentrations and water availability. These factors act as sensory inputs, altering plant developmental processes to optimize reproductive success under changing environmental conditions. Recent studies by our team and collaborators suggest that one important mechanism allowing fast response to small changes in the environment is the formation of a dynamic liquid-liquid phase separated state (liquid-liquid phase separation-LLPS) (Jung, 2020). In vivo, LLPS forms membraneless organelles that consist of an immiscible protein and/or nucleotide rich phase and a protein/nucleotide depleted phase. LLPS formation and dynamics are sensitive to environmental variables including salinity, pH and temperature. As such, it is an ideal mechanism for tuning plant response to abiotic inputs. However, relatively little is known concerning the underlying mechanisms that trigger LLPS. The proposed project will investigate the biophysical mechanisms and biomechanical properties of the LLPS-forming protein, EARLY FLOWERING 3 (ELF3), a key thermosensor in Arabidopsis. The project will use biochemical and biophysical techniques to characterize naturally occurring ELF3 variants under different salt, pH and temperature conditions. The student will recombinantly express and purify ELF3 domains and natural variants. The proteins will be expressed with fluorescent proteins tags for downstream characterisation using confocal microscopy and fluorescence recovery after photobleaching experiments to probe the dynamics of the phase separated state. The potential long range ordering of molecules in the droplet phase will be determined using a combination of small angle X-ray and neutron scattering experiments. The biomechanical properties of the droplets will be determined using atomic force microscopy. These experiments will allow the detailed molecular description of the phase separated sate and allow for the prediction of ELF3 LLPS under a physiologically important range of conditions.

Keywords:
liquid-liquid phase separation, temperature sensing, Arabidopsis thaliana

Relevant publications of the team:

Silva, C. S.; Nayak, A.; Lai, X.; Hutin, S.; Hugouvieux, V.; Jung, J.-H.; López-Vidriero, I.; Franco-Zorrilla, J. M.; Panigrahi, K. C. S.; Nanao, M. H.; Wigge, P. A.; Zubieta, C. Molecular Mechanisms of Evening Complex Activity in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 2020, 117 (12), 6901–6909. https://doi.org/10.1073/pnas.1920972117.

Jung, J.-H.; Barbosa, A. D.; Hutin, S.; Kumita, J. R.; Gao, M.; Derwort, D.; Silva, C. S.; Lai, X.; Pierre, E.; Geng, F.; Kim, S.-B.; Baek, S.; Zubieta, C.; Jaeger, K. E.; Wigge, P. A. A Prion-like Domain in ELF3 Functions as a Thermosensor in Arabidopsis. Nature 2020, 585 (7824), 256–260. https://doi.org/10.1038/s41586-020-2644-7.