The molecular basis of regulating branched microtubule nucleation

Supervisory team:
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Project description:
Mitosis, the accurate alignment and segregation of duplicated chromosomes is an essential cellular process that, when perturbed, contributes to diseases including cancer. Branched microtubule (MT) nucleation plays a vital role in mitosis, generating the bulk of the mitotic spindle. We have been working on two key protein complexes involved in this process, Augmin and the gamma-tubulin ring complex (gamma-TuRC). We hypothesize that when Augmin binds to the gamma-TuRC, it induces a structural alteration that enhances the probability of lateral interactions occurring between adjacent tubulin dimers. This, in turn, promotes the initiation of MT nucleation through the gamma-TuRC.
In this project, you will test this hypothesis by elucidating the structures of the gamma-TuRC and Augmin, isolated from Drosophila (fruit fly) embryos, using cryo-electron microscopy (cryo-EM). The protein complexes will be isolated using a straight-forward, one-step biochemical purification procedure called cleavable-affinity purification (cl-AP), pioneered in our lab1,2. The samples will then be screened by conventional room-temperature transmission electron microscopy (TEM). This will reveal if the gamma-TuRC/Augmin purification is of sufficient concentration, purity and homogeneity for data acquisition or if the purification protocol needs to be improved. Once optimal gamma-TuRC/Augmin samples have been obtained, they will be prepared for cryo electron microscopy (cryoEM). Thousands of high magnification images of gamma-TuRC/Augmin will be recorded, and high-resolution 3D maps will be generated. The atomic model of g-TuRC/Augmin will be built into the cryoEM map, guided by other research in the lab, including cross-linking and HDX-mass spec data and AI-guided protein structure modelling software. This work will provide testable hypotheses as to the structural consequences of Augmin upon the gamma-TuRC. It will allow the design of modified Augmin or gamma-TuRC subunits that will be reintroduced into the Drosophila embryo, with the aim of activating or suppression branched MT nucleation.

This PhD project is an opportunity for an ambitious and interdisciplinary scientist to join our team of researchers. The student will be supported and assisted by other members of the Wakefield and Daum groups, including cell biologists, protein engineers and cryo-EM experts. The student will learn structural techniques, Drosophila cultivation, biochemistry, plus familiarization with several programming languages, mathematical modelling and statistical skills. The Living Systems Institute is an interdisciplinary home for agile researchers across traditional disciplines. It brings together mathematicians, physicists, cell and molecular biologists, biomedical scientists and engineers. The student will thus be equipped with a rare and highly transferrable set of skills in both structural mass spectrometry of proteins and in vivo cell biology.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.