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How the kinetochore's unexplored Fibrous Corona ensures error-free chromosome segregation

Division (delete as appropriate):	Biomedical Sciences
Degree (delete as appropriate):	PhD
Mode of Study (delete as appropriate):	Full time
Project suitability (delete as appropriate):	Home / EU / Overseas
1st Supervisor:	Andrew McAinsh
Additional Supervisors:	TBC
Funding body for tuition fees (please tick as appropriate):	Chancellors International Scholarship X WMS Scholarship X WCPRS ☐ Self-Funded ☐ Other (please specify)
Funding Body for Stipend: (please tick as appropriate):	Chancellors International Scholarship X WMS Scholarship X WCPRS ☐ Self-Funded ☐ Other (please specify)
Has the funding been awarded?	Yes

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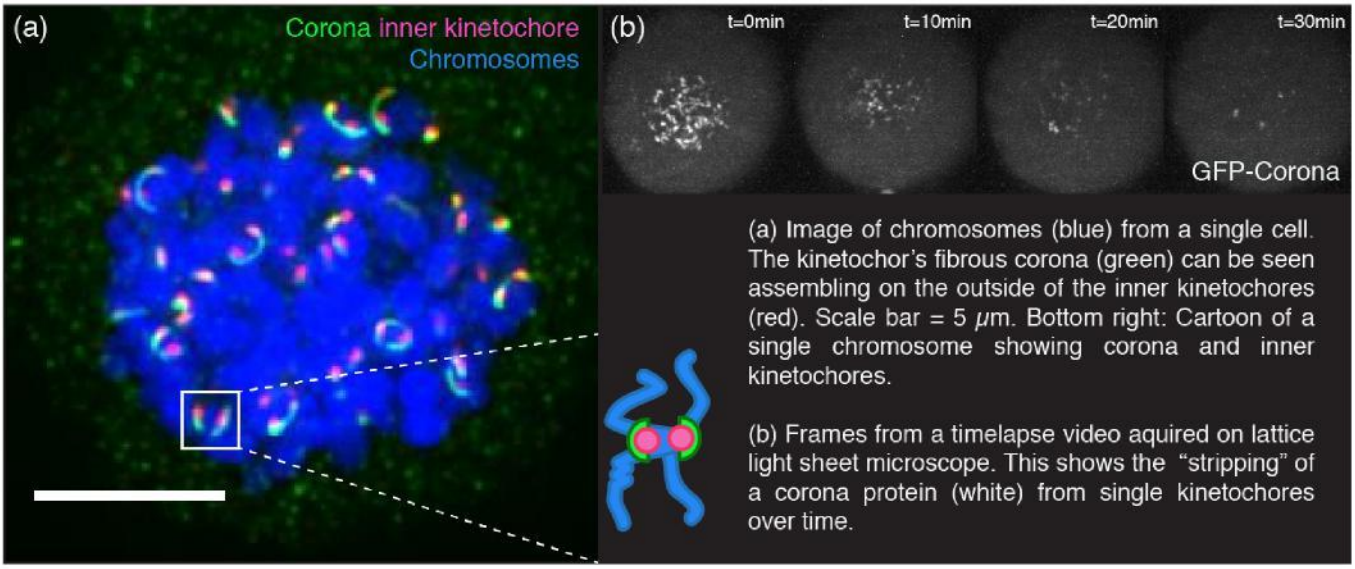
Project Summary including key research questions, aims and anticipated outcomes (max 300 words)

Successful mitotic cell division requires the accurate segregation of chromosomes into daughter cells. Errors in this process are associated with a myriad of pathologies, such as tumour evolution, metastasis and recurrent miscarriage. Chromosome movement during mitosis is governed by kinetochores, large centromere associated protein complexes that form attachment sites for spindle microtubules.

While the major complexes that build the inner kinetochore domains are heavily studied, the outermost proteins that form a region termed the "fibrous corona" are poorly understood (see **Figure 1a**). In our latest work we made a leap forward in understanding how the CENP-F protein controls the composition of the corona: CENP-F through a poorly understood mechanism is able to restrain Dynein motors in the corona. This preventing Dynein from prematurely "striping" kinetochore proteins from the corona as microtubule attachments form (see Auckland & McAinsh, BioRxiv, 627380, 2019). Our initial experiments show that this mechanisms is important for kinetochores to "correct" improper attachments thus allowing normal chromosome segregation.



(a) Image of chromosomes (blue) from a single cell. The kinetochor's fibrous corona (green) can be seen assembling on the outside of the inner kinetochores (red). Scale bar = 5 μm. Bottom right: Cartoon of a single chromosome showing corona and inner kinetochores.

(b) Frames from a timelapse video aquired on lattice light sheet microscope. This shows the "stripping" of a corona protein (white) from single kinetochores over time.

In this PhD project you will work towards answering three key questions:

1. **What are the dynamics of corona disassembly?** We will develop a next-generation live-cell light sheet based assay (building on the proof-of-principle experiment shown in **Figure 1b**) in order to quantify stripping kinetics of Dynein cargos (i.e. CENP-E, Mad1) and thus allow us to visualise the cycles of corona assembly and disassembly at single kinetochores as they form microtubule attachments.
2. **How does CENP-F restrict Dynein motor activity?** We will use CRISPR/Cas9 to mutate candidate proteins (e.g. Nde1, Ndel1 & Lis1) that we suspect form a bridge between CENP-F and Dynein. We will test how these mutants impact corona assembly/disassembly. We will also search for new factors that recruit the CENP-F-Nde1-Ndel1-Lis1 module to kinetochores and define why, and how, this module is important for error correction processes.
3. **Can we rebuild the minimal machinery?** We will aim to reconstitute these physical interactions with purified proteins, and if time allows, test how these complexes affect Dynein activity *in vitro* (in collaboration with Dr A. Straube).

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Describe the methodology and techniques to be employed (max 200 words)

You will be trained to use our state-of-the-art Lattice light sheet microscope (LLSM) to image subcellular events at unprecedented spatiotemporal resolution. For this we will also use CRISPR/Cas9 gene editing to build cell lines in which genes are tagged and/or mutated at the endogenous locus with fluorescent proteins.

As the project develops you may also use protein expression and purification along with microscope-based assays to visualise single molecules.

Throughout the project you will continually develop core molecular and cell biology skills and be part of a dynamic, supportive, international laboratory with a mix of students, postdocs and lab manager.