Title of Project:	Structure-driven dissection and inhibition of mitotic regulators in the fungal pathogen *Cryptococcus neoformans*
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Summary of project
Cryptococcus neoformans is an understudied fungal pathogen with an unusual life cycle, forming polyploid Titan cells during infection in a bid to evade the immune system. It kills hundreds of thousands of immune-compromised AIDS patients each year, mainly in Africa. Bub1 is a central player in the mitotic spindle checkpoint and the Bub1 gene of *Cryptococcus* (CnBub1) is a strong, candidate drug target as the null mutation is almost lethal. You will express, purify, crystallise and solve the structures of the highly conserved kinase domain of CnBub1 and of its conserved N-terminal TPR (tetratricopeptide repeat) domain that is likely to drive dimerisation. These structures will be used to screen for and identify small molecule inhibitors of CnBub1 kinase. Our working model is that CnBub1 dimerisation activates its kinase activity, leading to significant auto-phosphorylation and spindle checkpoint signalling. To test this model, you will carry out *in vivo* structure-function studies on CnBub1. CRISPR-mediated genome engineering will be employed to determine the consequences of specific bub1 mutations in the TPR and kinase domains. How do they impact viability, polyploidy, Titan cell formation and infectivity? Small molecule inhibitors will be screened for that produce similar loss of function phenotypes. Longer term these Bub1 inhibitors should prove useful in combination therapy treatments, alongside anti-microtubule drugs.

What quantitative skills will the student acquire or develop during their PhD project?
Recombinant protein purification, crystallisation and structure determination Quantitative live-cell imaging (spinning-disc confocal microscopy) and microfluidics Quantitative *in vitro* titan cell formation assays and *in vivo* infectivity assays CRISPR-mediated genome engineering and Molecular Genetics in *Cryptococcus neoformans* *In vitro* kinase assays and the use of bulky ATP analogues, plus specific (BAY-320 and BAY-524) Bub1 kinase inhibitors, to specifically and quantitatively inhibit CnBub1 kinase.