Title of Project:	Deciphering the SPOTs complex; understanding the cellular regulation of sphingolipid metabolism
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Summary of project
Sphingolipids (SLs) are essential components of eukaryotic cell membranes that also play important roles as potent signal molecules. They have been identified as molecular markers in a number of diseases such as Alzheimer's, diabetes and cancer. Recently it has been discovered that bacteria found in the human microbiome can also produce complex SLs. The precise metabolic pathways that control how SLs are made and broken down are unknown. The SL pathways in yeast and humans are similar with key components involved in regulation displaying some homology. We will begin by using S. cerevisiae as a model to explore the molecular details of SL regulation. The key first essential enzyme in all SL-producing organisms is serine palmitoyltransferase (SPT). SPT is pyridoxal 5'-phosphate (PLP)-dependent that catalyses the condensation of L-serine with palmitoyl-CoA to give sphingosine. In bacteria, SPT is a cytoplasmic homodimeric enzyme the x-ray structure of which we determined. In contrast, eukaryotic SPTs are multimeric, membrane bound complexes consisting of at least 4 subunits; SPT1 and SPT2, which form the PLP-dependent, catalytic core. This core is regulated by 2 subunits; ssSPT, a small protein activator which increases enzyme activity 100x fold and an ORM protein which has been shown to inhibit activity of the complex in a SL-dependent manner. Also associated with this is a Sac1 phosphatase whose exact role is unclear. Taken together, this multi-subunit "SPOTs" complex controls SL metabolic flux and somehow responds to cellular SL concentrations to increase or decrease SL biosynthesis on demand. The aims of the project are to use combined synthetic biology, informatics, metabolic labelling and structural biology approaches to reveal the mechanism of how the SPOTs complex controls cellular SL levels.

What quantitative skills will the student acquire or develop during their PhD project?
Cell biology ● We will use a synthetic biology approach to assemble expression constructs of all the required parts (SPT1, SPT2, ssSPT, ORM, Sac1) that encode the SPOTs constructs ● Screen S. cerevisiae strains (wild type and labelled-fusions) for expression of SPOTs enzymes. Use microscopy to measure co-localisation of subunits. ● Generate mutant gene libraries of the regulatory subunits (ssSPT and ORM) and determine their phenotypic impact yeast growth. Quantitative skills ● Use mass spectrometry (MS) analysis to measure the SL production from the various yeast strains. Here we will use L-serine isotopologue labelling (13C, 15N, 2H). ● We will control the expression of the SL genes in the synthetic circuits by optimised promoter/ribosome binding site combinations. ● Measure SPT enzyme activity of the SPOTs complex constructs. ● Isolate SPOTs complexes for cryo-EM analysis (at Edinburgh and Diamond, Oxford).