OXFORD INTERDISCIPLINARY BIOSCIENCE
Doctoral Training Partnership

**Industrial CASE Studentship Advertisement – 2023-24**

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| **Project Title:** | An Integrative structural biology approach towards understanding the molecular basis of N-glycosylation |

**Brief description of project:**

The integration of structural techniques on a molecular and cellular scale such as X-ray crystallography and single-particle cryo-electron microscopy alongside X-ray fluorescence of the cell is a powerful approach towards understanding the molecular basis of cellular mechanisms. The enzyme complex composed of DPAGT1, ALG13 and ALG14, which catalyses the first committed steps of N-glycosylation presents an ideal model for taking such an integrative approach. N-glycosylation is one of the most common and fundamental forms of post-translational modification in eukaryotes. N-glycosylation is required for the correct folding, trafficking and function of many proteins with dysregulation of clinical significance. Many enzymes involved in N-glycosylation, including DPAGT1, OST and GntI are dependent on $Mg^{2+}$ and/or $Mn^{2+}$. The level of N-glycosylation and concentration of these ions is tightly regulated in the endoplasmic reticulum (ER) and the Golgi. Dysregulation of N-glycosylation and $Mn^{2+}$ is associated with diseases such as congenital myasthenic syndromes, congenital diseases of glycosylation, cancer and neurodegenerative disorders.

We have previously solved the X-ray crystal structure of DPAGT1 in apo, substrate bound and antibiotic inhibitor bound states partly explaining the catalytic mechanism behind glycosylation. The Neurosciences Group in the Weatherall Institute of Molecular Medicine (WIMM) has established methods to express, purify and assess the function of DPAGT1 and the wider DPAGT1/ALG13/ALG14 complex. The project aims to

1. Probe the structural basis for the phosphotransferse mechanism of DPAGT1 using X-ray crystallography supported by functional analysis of functional and disease mutants.
2. Characterise the wider DPAGT1/ALG13/ALG14 complex using a combination of functional assays and single-particle cryo-EM

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3. Determine the relationship between N-glycosylation levels, ER and Golgi $Mn^{2+}$ concentrations and the unfolded protein response.

This project brings together experts in the biochemistry of N-linked glycosylation and membrane protein structural biology with the ultimate aim of presenting an integrative approach to characterize the mechanism and phenotypic effects of N-glycosylation on a molecular, biochemical and cellular scale through use of a wide range of approaches available through the Macromolecular crystallography, Biological cryo-imaging and Imaging and microscopy groups at Diamond. Specifically, the project will showcase a conjoined approach using the beamlines VMXi and VMXm to collect data from small membrane protein crystals, the electron bioimaging centre to characterise the DPAGT1/ALG13/ALG14 complex, I14 to understand metal ion distribution in the cell, the Membrane Protein Laboratory (https://www.diamond.ac.uk/Instruments/Mx/MPL.html) to support production of high-quality samples and the WIMM to provide the biochemical basis and molecular biological tools such as gene editing.

**Attributes of suitable applicants:**

The suitable applicant needs to have at least a bachelor degree or equivalent in biochemistry, preferably with experience in structural biology. They need to have a demonstrable passion for understanding the fundamental mechanisms of biology and a track record of academic excellence.

This project requires working with several different teams of scientists at Diamond Light Source and the University of Oxford; therefore, the suitable applicant needs to be able to work in a team and enjoy working in a multidisciplined scientific environment.

**Funding notes:**

This project is funded for four years by the Biotechnology and Biological Sciences Research Council UKRI-BBSRC. UKRI-BBSRC eligibility criteria apply (https://www.ukri.org/files/funding/ukri-training-grant-terms-and-conditions-guidance-pdf/). Successful students will receive a stipend of no less than the standard UKRI stipend rate, currently set at £18,622 for the academic year 2023 to 2024 (the rates for subsequent academic years have not been published), plus a £2,000 per annum stipend top-up.

*This project is supported through the Oxford Interdisciplinary Bioscience Doctoral Training Partnership (DTP) studentship programme. The student recruited to this project will join a cohort of students enrolled in the DTP's interdisciplinary training programme, and will participate in the training and networking opportunities available through the DTP. For further details, please visit www.biodtp.ox.ac.uk. The DTP and its associated partner organisations aim to create a community that is innovative, inclusive and collaborative, in which everyone feels valued, respected, and supported, and we encourage applications from a diverse range of qualified applicants.*

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