Structure of ART Toxins: understanding toxicity through host receptor interactions

Theme: Infection, Immunity & Repair

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The ADP-ribosyltransferase toxin (ARTT) family is a large group of bacterial exotoxins that include the diphtheria (DT), cholera and pertussis toxins. These toxins target eukaryotic cells with a receptor-binding domain, and deliver the enzymatic component into the host cytoplasm, where addition of ADP-ribose to the target substrate results in the inhibition of normal protein synthesis, and leads to cell death.

The DT-like group includes P.aeruginosa exotoxin A (ExoA), and V.cholerae cholix toxin. These toxins bind to specific receptors on the surface of epithelial cells, subsequently undergoing endocytosis which coincide with a rapid drop in pH. The vesicular acidic pH causes the toxin to change conformation and be proteolytically activated into binary components. The enzymatic subunit then follows a retrograde pathway to the endoplasmic reticulum, where it is ultimately translocated into the cytoplasm allowing it to reach its substrate. Although the cell surface receptors for ExoA and cholix have been partly identified, no molecular details are available on these toxin-receptor interactions. Part of this project will be to characterize the interactions of ExoA with the GM1 ganglioside, and the LRP1 protein receptor. LRP1 (low-density lipoprotein receptor-related protein 1) is a membrane-anchored protein with a large cytoplasmic domain which is involved in a number of important physiological processes, including bone repair, and has anti-inflammatory roles in disease.

The first goal of this project will be the production of the material needed: bacterial expression of recombinant non-toxic derivatives of ExoA and isolation of LRP1 from mammalian cell cultures. The complex formed by ExoA and LRP1 will be studied by single particle electron microscopy, while the ExoA interaction with the GM1 carbohydrate will be analysed by X-ray crystallography. This work will involve access and training at state-of-the-art facilities such as synchrotron radiation sources (Diamond, ESRF) and high-end microscopes (Talos Arctica, Titan Krios). The structural analysis will be followed up by mutational studies and characterisation of the interactions using toxin-receptor binding assays. Effects of these mutations on the toxin's cell trafficking properties will also be assessed. The discovery of modifications that improves cell binding or alter cell trafficking may lead to the development of novel tools for protein drug delivery or new anti-toxin approaches.

The project will also be extended to homologues of ExoA that have not yet been characterised. Through this research project the student will gain experience in a wide range of techniques across different disciplines, including protein production and characterisation, biophysical methods such as X-ray crystallography and single particle cryoEM, as well as cell culture and imaging. Results should lead to impactful research publications, and will be presented at international conferences.

IMPORTANT: In order to apply for this project, you should apply using the DTP's online application form: https://cardiff.onlinesurveys.ac.uk/gw4-biomed-mrc-doctoral-training-partnership-student-appl

You do NOT need to apply to the University of Bath at this stage – only those applicants who are successful in obtaining an offer of funding form the DTP will be required to submit an application to study at Bath.

More information on the application process may be found here:
https://www.gw4biomed.ac.uk/doctoral-students/

APPLICATIONS CLOSE AT 17:00 ON 25 NOVEMBER 2019.