Queen's Doctoral Training Programme - Multi-dimensional approaches to understanding microbe/host interactions in the context of disease, therapeutics and community resilience

Title of studentship	**Elucidating the role of the Legionella glycosyltransferase toxin LtpM for microbe/host interactions – a chemical biology approach**
Project summary (max 250 words – this will be used to advertise the project if selected).	The glycosylation of host protein substrates by pathogenic bacteria is a common feature of microbe/host interactions. In this project, we will develop novel and generally applicable chemical tools to study the role of host protein glycosylation for infection. We will focus on the bacterial pathogen *Legionella pneumophila*, the causative agent of Legionnaires' disease, a severe form of pneumonia. We have recently shown that *L. pneumophila* uses a novel type of glycosyltransferase effector called LtpM to subvert host defence mechanisms. We will develop chemical inhibitors and activity-based probes of LtpM – currently lacking – and use these chemical tools to establish the catalytic mechanism of LtpM, to identify host targets of LtpM and their role for *Legionella* virulence *in vivo*, and to identify new LtpM-like glycosyltransferase toxins. Outcomes of the project will also establish if LtpM and related glycosyltransferase toxins may be suitable targets for a novel anti-virulence therapeutic strategy. The project will provide training in a wide range of techniques at the interface of chemistry, chemical biology and microbiology, including chemical probe design and synthesis, protein biochemistry, assay development, and the application of infection models. It will also offer exciting opportunities for collaboration both nationally and internationally, including with research groups at Public Health England and the Complex Carbohydrate Centre (Georgia, USA).
Supervisor(s)	1. Prof Gerd Wagner (Pharmacy) 2. Dr Gunnar Schroeder (SMDBS, WWIEM)
What types of new collaborative relationships would this studentship support (e.g. development of national and international collaborations or industrial involvement/financial support) (100 words max)	The studentship will support the development of new and existing national and international collaborations with academic and non-academic partners e.g. at Public Health England (Dr Mark Sutton) and the Complex Carbohydrate Centre (Georgia/USA, e.g., Christine Szymanski, Bob Haltiwanger). The studentship also aligns with ongoing research in a Franco-British network on "Glycans in AMR", which the PI has recently established with pump-prime funding from the French embassy in London. These collaborations will enable us to widely apply the chemical tools developed in this project with a range of bacterial pathogens, beyond Legionella (e.g., Public Health England: Klebsiella pneumoniae; Szymanski: Campylobacter).
Research centre / School	School of Pharmacy

Subject area	Chemical Biology, Immunobiology and Microbes, Cellular Microbiology
Candidate requirements / Key skills required for the post. Please note for the QUB-DTP awards applicants must have a 1st or 2.1 Honours degree (or equivalent) in a relevant subject.	Applicants must have an excellent background in chemical biology, medicinal/organic chemistry, biochemistry, pharmaceutical sciences, or a closely related subject.
Relevant links for project advertisement/ more information	https://www.qub.ac.uk/schools/SchoolofPharmacy/Research/find-a-phd-supervisor/dr-gerd-wagner.html https://pure.qub.ac.uk/en/persons/gunnar-neels-schroeder
Keywords for search filters	chemical biology, medicinal chemistry, anti-virulence, antimicrobial resistance, host-pathogen interaction
Training provided through the research project	organic synthesis, chemical probe design, protein biochemistry, assay development, infection models, experimental design, data analysis, scientific writing, presentation skills
Expected impact activities	Academic impact • High quality publications in internationally recognised peer-reviewed journals (e.g., Nature Chemical Biology) • Data acquisition for collaborative follow-on grant applications • Communication of results at national and international conferences,, promoting the recognition of life science research at QUB. • Development of inhibitors and diagnostic probes for infection for translational research applications and commercialisation Non-academic impact • Regular outreach activities on topics such as bacterial infections, hygiene, AMR, and medicines development to non-specialist audiences, e.g. for Science Week NI. These activities will build on a workshop on these topics which the PI delivered this year for primary school children.

Project details including expertise in area and workplan (max 1200 words)

BACKGROUND. The glycosylation of host protein substrates by pathogenic bacteria is a common feature of microbe/host interactions (Cell Microbiol 2015). Typically, the bacterial pathogen secretes a toxin with glycosyltransferase activity, which manipulates cellular functions of eukaryotic target host cells by glycosylation of host proteins. Important examples include the *Clostridium difficile* toxins A and B, which modify guanine nucleotide-binding proteins of the Rho family. Similar glycosyltransferase toxins have also been identified in other bacterial pathogens including *Escherichia coli*, *Yersinia*, *Photorhabdus* and *Legionella*. In response, human cells have evolved mechanisms, which sense the inactivation of, for example Rho GTPases, and trigger innate cellular defence mechanisms (Nature 2014).

Legionella pneumophila is the causative agent of Legionnaires' disease, a severe form of pneumonia (Cell Microbiol 2015). Each *L. pneumophila* strain injects hundreds of effector proteins into host cells, including homologues of classical glycosyltransferase toxins, which have important roles in pathogenesis and immune modulation (Curr Top Microbiol Immunol 2013). Most effectors are uncharacterised and of unknown function. One of the applicants (Schroeder) has recently demonstrated that the *Legionella* effector protein LtpM is a new type of phosphoinositide-activated glucosyltransferase toxin that acts through glycosylation of host protein substrates (J Biol Chem 2019). Like all glycosyltransferases, LtpM catalyses the transfer of a sugar from a glycosyl donor to acceptor molecules, despite sharing no obvious sequence homology. The preferred donor substrate of LtpM is the sugar-nucleotide UDP-glucose (UDP-Glc). Uniquely amongst glycosyltransferase toxins, LtpM does not require a divalent metal cofactor (e.g., Mg^{2+}, Mn^{2+}) for catalytic activity, and the exact chemistry of this new active site remains unknown. It also remains to be established if *Legionella* species encode more LtpM-like cryptic glycosyltransferase toxins.

AIMS. In this project, we will use a chemical biology approach

(i) to establish the catalytic mechanism of LtpM,
(ii) to identify host targets of LtpM and their role for *Legionella* virulence *in vivo*,
(iii) to identify new LtpM-like glycosyltransferase toxins.

We will develop bespoke chemical inhibitors and activity-based probes for LtpM – currently lacking – to achieve these aims. Outcomes of the project will also establish if LtpM may be a suitable target for the development of a novel class of anti-virulence agents.

AREAS OF EXPERTISE. The supervisory team is ideally placed to carry out the proposed, highly interdisciplinary work programme:

Wagner joined the QUB School of Pharmacy in 2019 as Chair in Chemical Biology & Medicinal Chemistry. He has a long-standing track record in the development of inhibitors and chemical probes for glycosyltransferases, including for bacterial enzymes. Notable previous work in this area includes the discovery of an entirely novel mode of allosteric inhibition for glycosyltransferases (Nat Chem Biol 2010; J Med Chem 2012, J Biol Chem 2013, J Biol Chem 2015), the development of a novel class of drug-like covalent inhibitors (Bioorg Med Chem 2017, Bioorg Med Chem 2018), and the development of several operationally simple and generally applicable glycosyltransferase assays (ChemBioChem 2010, MedChemComm 2014, MedChemComm 2017). He has established an externally funded research collaboration with Public Health England, as well as a Franco-British research network on "Glycans in AMR" (15 PIs), supported by pump-prime funding from the French embassy in London.

Schroeder is Lecturer in Microbial Pathogenesis at the WWIEM and has more than 17 years of experience in cellular microbiology research with focus on the manipulation of host cells, e.g. macrophages, by bacterial pathogens. His main model organism is *Legionella pneumophila.* These projects resulted in several publications on *Legionella* genomics (Gomez-Valero, 2019; Schroeder, 2010), a new *Galleria mellonella in vivo* model (Harding, 2012; Harding, 2013a, Harding, 2013b), a new method to determine effector-interactomes under physiological infection conditions (Mousnier & Schroeder, 2014; So, 2016; So, 2019) and the characterisation of novel T4SS effectors (Harding, 2013a; Harding, 2013b; Schroeder, 2015) including the glycosyltransferase LtpM (Levanova, 2019). His group is currently supported by MRC NIRG and an EU MCSA funding and has the biochemical and cellular microbiology tools and assays in place to provide training and advance this PhD project swiftly.

WORK PROGRAMME.

Activity 1 *Development of a non-radioactive biochemical assay for LtpM*. Previous biochemical work with LtpM relied on a cumbersome radioassay. In order to facilitate the development of inhibitors and chemical probes, we will establish an operationally simple, non-radioactive biochemical assay for LtpM. The assay will be based on a generalisable assay format for glycosyltransferases previously developed by Wagner (MedChemComm 2014, MedChemComm 2017). It will be adapted for a microplate format and can be used for the identification of inhibitors and probes as well as mapping the active site of LtpM by site-directed mutagenesis. All relevant materials and protocols for the expression and purification of recombinant LtpM are available in the Schroeder lab.

Activity 2 *Development of LtpM inhibitors and activity-based probes.* We will use the new assay developed in Activity 1 for the development of two classes of tool compounds:

(i) Inhibitors. We will use different strategies previously applied successfully with other bacterial glycosyltransferases to identify small molecular inhibitors of LtpM, including the development of allosteric inhibitors (Nat Chem Biol 2010) and covalent inhibitors (Bioorg Med Chem 2018). We will evaluate their activity and target selectivity for LtpM and other known classical Legionella glycosyltransferase effectors.

(ii) Activity-based probes. We will exploit the long-standing experience of Wagner with sugar-nucleotide chemistry to develop analogues of the LtpM donor UDP-Glc that can be used for monitoring LtpM activity and the identification of LtpM host protein substrates. Specifically, we will introduce a chemical tag (e.g., an azide group) at the D-glucose portion of UDP-Glc. Upon transfer of the azidosugar to acceptor substrates, we will use this tag for the specific labelling of the glycosylated substrates using established Click chemistry. This target-agnostic approach will allow us to enrich and identify novel host protein substrates of LtpM e.g., from wild-type samples.

Activity 3 *Application of the chemical tools in cell assays/host-pathogen interaction models*.

(i) We will use the activity-based probes with *Legionella* lysates or extracts of infected host cells and chemical proteomics to identify additional LtpM-like glycosyltransferase toxins in *L. pneumophila* prototype strains and other *Legionella* species that have remained underinvestigated. New effector candidates will be validated using *Legionella* translocation assays, routinely run in the Schroeder Team, and their glycosyltransferase activity/specificity analysed using our non-radioactive assay.

(ii) We will use a microplate, fluorescence based assay established in the Schroeder Team to measure the effect of our inhibitors on the replication of *L. pneumophila* expressing fluorescent proteins. We will use the same assay to analyse the role of any of the targets of LtpM, which we identify, by gene silencing or deletion. Immunofluorescence microscopy will be used to dissect, which stage of the infection process effective inhibitors disrupt.

(iii) For newly identified host targets, we will validate the modification *in vitro* and depending on the nature of the protein, determine the effect of the modification on e.g. enzymatic activity or change to the interactomes of the proteins. Tools for this are established in the Schroeder Team.

OUTLOOK. The chemical tools and approaches developed in this project will be generally applicable to study microbe/host interactions with many different bacterial pathogens, including species from clinical samples. We will realise these opportunities through the collaborations outlined above (e.g., Public Health England; Complex Carbohydrate Centre/Georgia).