Project Details	
Project Code	MRC23IIARBr Spencer
Title	Exploiting glycan interactions for bacterial detection
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	Bacterial infections acquired in the community (e.g. tuberculosis (TB)) or healthcare settings (e.g. Staphylococcus aureus/MRSA) are a global public health burden exacerbated by growing antimicrobial resistance and slow antimicrobial drug development. Bacteria exploit specific carbohydrates (glycans) during the infection process. This project explores how bacteria interact with synthetic glycans, and how these might be developed as tools for detecting infections.
Description	Bacterial infections are a global public health challenge exacerbated by growing antimicrobial resistance (AMR) and numbers of vulnerable (immunocompromised) patients, and the weak antibiotic development pipeline. Combatting AMR requires informed prescribing to make best use of current drugs, rapid pathogen identification in patient samples will slow resistance development by avoiding inappropriate antibiotic use. This multidisciplinary proposal explores bacterial interactions with synthetic carbohydrates (glycans), aiming to identify specific interactions on which to base pathogen identification tools and characterise glycan interactions with target receptors. We focus on interactions of trehalose-based glycans as these are known targets of Mycobacterium tuberculosis (M. tb, the cause of tuberculosis (TB)), but also bind Staphylococci, including S. aureus responsible for opportunistic infections. Specific objectives are: i) to test a panel of glycan (trehalose) analogues for binding to a panel of target bacteria; ii) to identify the bacterial receptor(s) responsible for glycan binding and iii) to characterise glycan:receptor interactions by biophysical and, potentially, structural (X-ray crystallographic) methods. Co-I Galan (MCG) has already synthesised a panel of trehalose analogues which will be available to the student. These will be conjugated (using click chemistry and EDC-coupling) to latex beads for detection of bacterial binding under Objective i using agglutination, and to fluorescent labels for detection by microscopy and in 96-well plates. Bacterial targets include BSL2-compatible M. tb homologues (M. smegmatis, M. bovis BCG), S. aureus (methicillin-susceptible and resistant strains), S. epidermidis and S. saphrophyticus (due to importance as a urinary pathogen) as well as comparator Gram-negative bacteria (e.g. Escherichia coli, Klebsiella pneumoniae) not expected to bind trehalose. Under Objective ii the student will identify bacterial glycan receptors. The primary approach will be to employ analogues of glycan ligands identified as above, modified with diazirine photo-affinity labels to facilitate covalent proximity labelling of receptors by UV-induced cross-link formation. Labelled bacteria will be fractionated and labelled fractions retrieved for receptor identification using mass spectrometry at the University of Bristol proteomics facility. Involvement of candidate receptors in glycan binding will be verified using knockouts where these are available (e.g. for S. aureus) along with complementation experiments that are expected to abolish and restore glycan binding, respectively. Glycan:receptor interactions will be characterised biophysically, and investigations of glycan:receptor complex structures initiated, under

	Objective iii. Identified receptors will be produced in recombinant E. coli. Glycan interactions will be characterised biophysically by fluorescence spectroscopy, surface plasmon resonance, isothermal titration calorimetry and thermal stability measurements using circular dichroism spectroscopy. Suitable high affinity complexes will be used in crystallisation experiments with the aim of determining structures of glycan:receptor complexes. These data will establish the utility of trehalose analogues as probes for specific bacteria, motivating future exploration of their suitability for diagnostic use. Characterisation of bacterial receptors will aid development of more specific and higher affinity probes and identify glycan-responsive pathways in our target species. The student will have full involvement in decisions about project direction, including the extent of focus on individual Objectives; e.g. balancing screening glycan "hits" against a more extensive panel of bacteria under physiological conditions (Objective i) with characterising interactions with specific receptors (Objectives ii, iii); as well within Objectives (selecting receptor interactions to purse under Objective iii).
Supervisory Team	
Lead Supervisor	
Name	Professor James Spencer
Affiliation	Bristol
College/Faculty	Life Sciences
Department/School	Cellular and Molecular Medicine
Email Address	Jim.Spencer@bristol.ac.uk
Co-Supervisor 1	
Name	Professor Carmen Galan
Affiliation	Bristol
College/Faculty	Science
Department/School	Chemistry
Co-Supervisor 2	
Name	Dr Maisem Laabei
Affiliation	Bath
College/Faculty	
Department/School	Biology and Biochemistry
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	