Project Details	
Project Code	MRCIIAR25Ca Parmeggiani
Title	Sweet disposition: combatting bacterial infections and antimicrobial resistance with designer proteins that target biofilm matrix carbohydrates
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Microorganisms grow as biofilms on surfaces, from teeth to surgical implants to the lining of lungs. Biofilms are dense cell aggregates embedded in a complex matrix of molecules that surrounds and protects the cells and provides resistance to antimicrobials. This project will hijack this protection to design specific matrix-binding proteins that deliver agents to trigger biofilm degradation and improve the delivery of antimicrobials. The student will develop and apply computational methods to design novel proteins able to recognise specific biofilm matrix polysaccharides, and then exploit microbiology and microscopy techniques to test their efficacy in the lab using bacterial biofilm models.
Description	Microorganisms grow as biofilms on surfaces, from teeth to surgical implants or even the lining of the lungs. Biofilms are dense cell aggregates embedded in a complex matrix of molecules that surrounds and protect the cells. Critically, this matrix confers resistance to antimicrobial treatments with both chemical (e.g. antibiotics) and physical (e.g. acidic/basic conditions, detergents) mechanisms of action, with the matrix eventually sacrificing only the external layers, leaving the underlying microbes unharmed. Complex carbohydrates have long been recognised as key components of the biofilm matrix and therefore viable targets for therapeutic strategies to combat biofilm formation. However, such approaches have been hindered by the fact that these carbohydrates are traditionally difficult to recognise and bind, meaning that they effectively provide a shielding effect similar to the glycan coverage of viruses. In this project, we will address this issue by leveraging our expertise in the design of proteins that can bind specific carbohydrates to effectively hijack the protective biofilm matrix and use it to facilitate delivery of agents that will trigger biofilm matrix degradation and improve access by antimicrobial treatments. To deliver this proof-of-concept project, three major bacterial pathogens have been selected for which biofilm formation is a key virulence factor: Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans. P. aeruginosa and S. aureus are also high priority pathogens on the World Health Organization 2024 list because of their global threat due to antimicrobial resistance (AMR). P. aeruginosa is notorious for causing chronic lung infections in immunocompromised individuals, patients suffering from burn wounds or cystic fibrosis patients. It produces three distinct extracellular polysaccharides (EPS): Psl, Pel, alginate. Psl is a neutral repeating pentameric saccharide built up from D-mannose, L-rhamnose and D-glucose. Pel is a cationic linear homopolymer of partially de-N-acetylated α-1,4-GalNAc built up from predominantly dimeric repeats of α-1,4-linked galactosamine and N-acetylgalactosamine. Alginate is an anionic polymer composed of β-1,4-linked D-mannuronic and α-L-guluronic acids on which the C-2 and C-3 hydroxy groups of the mannuronic acid residues can be acetylated to a varying degree. S. aureus is responsible for a range of conditions,

	including bacteraemia, infective endocarditis, osteomyelitis, and skin and soft tissue infections. Its predominant EPS is partially deacetylated poly-β-1,6-N-acetylglucosamine (dPNAG). S. mutans is a leading cause of dental caries, a global disease that affects 3.1 billion people worldwide, with major impacts on quality of life, and can also cause heart disease (infective endocarditis). Its key biofilm constituent is a polymer of α-1,3-linked glucose, with an increase in 3-linked branch points (e.g. 2,3-, 3,4-, 3,6- and 3,4,6-linked glucose) when formed on a surface. The project will proceed through the following objectives: 1) Analysis of designability for targets. The student will explore available literature and investigate the structural characteristics of candidate EPS to identify the requirements for protein design and to prioritise the targets. 2) Computationally design novel protein carbohydrate-binding domains. The student will learn and further develop physics-based and machine-learning tools in molecular docking, structure prediction and protein design (e.g. Autodock Vina, Rosetta, RFdiffusion). 3) Selection of binders. The student will subject the pool of designed proteins to selection processes (e.g. yeast display) to identify binding candidates. 4) Expression and characterisation of selected clones. The student will express the selected clones in Escherichia coli, purify and biophysically characterise the proteins, and assess binding to carbohydrates in vitro via fluorescence polarisation and isothermal titration calorimetry. 5) Binding to biofilms. The student will use established biofilms models for each bacterium and assess binding of the designer proteins labeled with fluorophores. 6) Biofilm degradation. Proteins that display the most effective binding will be fused with exo- and endo-glucanases and these fusion proteins then tested for their capacity to degrade carbohydrate and disrupt bacterial biofilms. 7) Improved antimicrobial activity. Fusion proteins will be applied to bacterial biofilms in combination with existing antimicrobial treatments (e.g. antibiotics) to assess if the fusion proteins can enhance antimicrobial penetration into the biofilms and thus improve biofilm sensitivity. The student and supervisory team will have regular meetings at which next steps for project development will be discussed. It is expected that the balance of these sessions will shift from supervisor to student during the project life cycle as the student builds skills and confidence as an independent researcher.

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