

Protease-sensitive polydiacetylene (PDA) vesicles

PhD student: Claire Parkinson. Oct 2005 to October 2010.

Glasgow University, Dr Graeme Cook. Mologic supervisor Prof Paul Davis.

PhD title: Functionalised PDA liposomes as biosensors for proteins (2010), theses.gla.ac.uk/2499/

A synthetic membrane is an organized supramolecular membrane that encompasses molecular recognition with signal transduction analogous to a natural biosensing system in a cell membrane. These synthetic based models allow the study and application of receptor-ligand binding to biosensor design. In order to enable a recognition event and a response the liposome incorporates a known ligand with a suitable receptor interaction that upon complementary binding can elicit a measurable response. Polydiacetylene based sensors have been previously considered and utilised for the detection of biologically important species due to the stimuli-responsive colour changing properties. These colorimetric biosensors are self-assemblies of diacetylene lipids mixed with natural or synthetic biological receptor molecules. Polydiacetylene liposomes functionalised with molecular recognition groups can bind and thus detect colorimetrically if the binding is complementary. Biodetection of an analyte in aqueous media requires that the structure of the diacetylenic compound is able to form a stable dispersion in water, polymerizes efficiently yielding a coloured material, incorporating a suitable receptor that binds with an analyte and transduction of the binding interaction by means of a colour change. The structural features to be considered are chain length, solubility, amphiphilicity, functional group for modification. Publication: A dual-responsive ferrocene-functionalised PDA liposome, C E Maclean et al, *Tetrahedron Letters* (2012), 53(24), 2948-2950.

Bacterial quorum sensing in acute wounds

PhD student: Robert Goldstone. Oct 2007 to Sep 2010

University of Nottingham, Prof Paul Williams & Dr Miguel Camara.

Mologic supervisors: Prof Paul Davis & Dr Malcolm Stratford

PhD title: Investigating the relationship between Quorum Sensing,

Motility, and the Type 3 Secretion System of *Yersinia pseudotuberculosis* (2011),
http://etheses.nottingham.ac.uk/2471/1/RJG_thesis_final_version.pdf

Over the course of the last two decades, research into the role of quorum sensing (QS) in regulating diverse bacterial behaviours has exploded, and around twelve years ago, a QS network was identified in the enteropathogenic bacterium *Yersinia pseudotuberculosis*, which was shown to control motility and cellular clumping. This thesis seeks to expand this regulatory relationship and explore the causes and consequences of the link between QS and motility, which affects pleiotropic processes including the type 3 secretion system (T3SS) and biofilm formation. Indeed, the clumping phenotype first explored by Atkinson *et al.* (1999), is linked to QS-dependent regulation of the T3SS, since the deletion of several QS genes results in liquid culture biofilm (LCB) formation. This is concomitant with T3S protein secretion into culture supernatant, which occurs under normally non-inducing conditions, while deleting the T3SS structural component *ycsJ* prevents secretion and LCB formation. De-repression of the T3SS and the development of LCBs also occurs following mutation of the flagella regulators *flhDC* and *fliA*, revealing that QS and the flagella system co-regulate LCBs. However, interestingly it was found that LCB formation and secretion also occurs following mutation of the flagella structural gene *flhA*. The $\Delta flhA$ mutant represents a flagella-minus strain, in which the underlying regulatory circuit mediated by FlhDC and FliA is intact, suggesting that an element of the flagella structure that depends on FlhA activity acts as a check-point governing expression of the T3SS. Both QS and the flagella system positively regulate biofilm formation by *Y. pseudotuberculosis* on the surface of the nematode worm, *Caenorhabditis elegans*. Surprisingly, the up-regulated T3SS was found to be responsible for mediating down-regulation of biofilm formation by *Y. pseudotuberculosis* QS mutants, since subsequent deletion of *ycsJ* could restore biofilms to wild-type levels. This suggested that a component of the injectisome was capable of influencing cellular processes in addition to its role in secretion. In light of the link regulatory link between flagella and T3S, this raised the possibility

that the injectisome could play a role in the reciprocal regulation of motility. Since the genetic regulatory network underpinning expression of the T3SS is intact in the $\Delta yscJ$ mutant, like the $\Delta flhA$ mutant for flagella, the $\Delta yscJ$ mutant can reveal the role of the injectisome structure in modulating gene expression. By phenotypic observation, it was determined that the $\Delta yscJ$ mutant displayed aberrant flagella mediated motility, swimming vigorously under conditions in which the wild-type did not, and, similar to the over-production of Yop proteins in the $\Delta flhA$ mutant, the $\Delta yscJ$ mutant over-produces flagellin. This suggests that a component of the T3SS injectisome acts as a checkpoint to regulate motility, which appears to be at the level of transcription, since the $\Delta yscJ$ mutant displays up-regulation of the flagella regulators *flhDC* and *fliA*. Indeed, the relationship between T3S and motility appears to require a direct influence on QS, since subsequent mutation of *ypsI* and *ytbI* abolishes $\Delta yscJ$ -dependent hyper-motility, the $\Delta yscJ$ mutant displays altered expression of the QS system genes. Furthermore, for the emerging transcriptional relationship between these systems, the flagella and QS mutants which are up-regulated for the production of Yop proteins also over-express the virulence regulator *virF*, completing the transcriptional regulatory circuit which appears to be crucial for the regulation of lifestyle choices by *Y. pseudotuberculosis*.

Publications:

Atkinson S, Goldstone R J et al. (2011). Biofilm Development on *Caenorhabditis elegans* by Yersinia Is Facilitated by Quorum Sensing-Dependent Repression of Type III Secretion. PLoS Pathogens 7: e1001250.

Goldstone R J, Popat R, Fletcher M P, Crusz S A, Diggle S P, (2012) Quorum sensing and social interactions in microbial biofilms. In Microbial Biofilms: current research, methods and applications (Ed. Lear & Lewis). Caister Academic Press.

Modified peptidoglycan precursors for rapid detection of MRSA

PhD student: Sandeep Sandhu. Dec 2007 to Dec 2010

University of Warwick, Prof Tim Bugg. Mologic supervisors: Prof

Paul Davis & Dr James Schouten

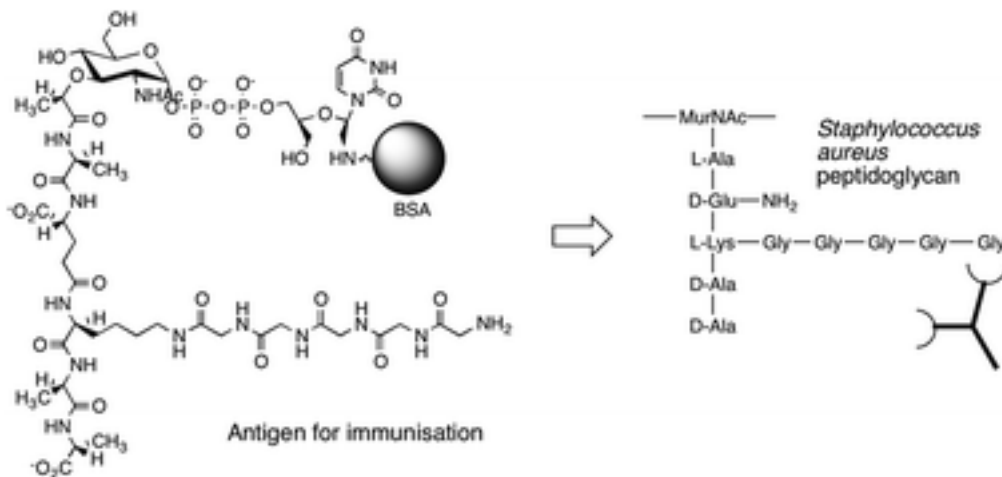
PhD title: Development of an antibody-based assay for methicillin resistant *Staphylococcus aureus* using synthetic peptidoglycan precursors (2011), wrap.warwick.ac.uk/36857/

Methicillin-resistant *Staphylococcus aureus* (MRSA) are isolates of the bacterium *Staphylococcus aureus* that have acquired genes encoding antibiotic resistance to all penicillins, including methicillin and other narrow-spectrum β -lactamase-resistant penicillin antibiotics. Outbreaks of MRSA occur quite frequently as there is no quick screening test for the presence of MRSA. The aim of this project is to try and develop an antibody based detection test for rapid detection of MRSA, which could help in the prevention of outbreaks. An antigen (UDP-MurNAc-L-Ala- γ -D-Glu-L-Lys(ϵ -NH₂-Gly)₅-D-Ala-D-Ala) that resembles the outer surface of the *Staphylococcus aureus* (contains a Gly₅ moiety that is specific for *S. aureus*) cell wall peptidoglycan has been prepared, and attached to a carrier protein. Sheep antibodies raised against this antigen were screened using ELISA assays. The results showed that antibodies did have specificity for the antigen. Cell walls were prepared from several different bacteria, including two MRSA and one methicillin-sensitive *Staphylococcus aureus* (MSSA) strain. ELISA assays using these cell walls showed that the antibodies had specificity for cell walls containing (Gly)₅ in the order of *S. aureus* (Gly₅) > *S. simulans* (less Gly₅) > *S. pneumoniae* (no Gly_n) > *E. coli* (no Gly_n, Gram-negative strain). A particularly high response was observed for one of the two MRSA strains, detectable at 0.1 μ g of cell wall. An HPLC-based UV-Vis assay was developed to monitor the activity of peptidoglycan polymerisation enzymes from *S. aureus*, while preparing polymeric peptidoglycan as an antigen for immunisation. Several intermediates in peptidoglycan polymerisation were detected using *S. aureus* monofunctional glycosyltransferase (MGT), which allowed us to propose a new hypothesis for the early steps of peptidoglycan transglycosylation.

Publication: Sandeep Sandhu, James A. Schouten, Julie Thompson, Mark Davis and Timothy D. H. Bugg (2012), Detection of *Staphylococcus aureus* cell walls by ELISA using antibodies prepared from semi-synthetic peptidoglycan precursor. *Analyst*, 2012, 137,

1130-1136.

We prepare UDPMurNac-decapeptide peptidoglycan precursor found in *S. aureus* (MRSA), and report the detection of the staphylococcal peptidoglycan monomer and staphylococcal cell walls using antibodies raised to this antigen.



Fusion protein production in yeast for Enhanced ADEPT Project

PhD student: William Humphries. Oct 2008 to Mar 2012

University of Kent (Canterbury), Dr Peter Nicholls. Mologic

supervisors: Prof Paul Davis & Sandra Hemmington

PhD title: Characterising fusion proteins for an Antibody Directed Enzyme/pro-Drug Therapy (ADEPT) approach to colorectal and gastric cancer (submitted March 2013).

Glycopeptide recognition

PhD student: Matthew Donaldson. Oct 2009

John Innes Centre & University of East Anglia Norwich, Prof Robert

Field, David Russell (UEA) & Nathalie Guge (IFR). Mologic

supervisors: Prof Paul Davis & Mark Davis.

PhD title: Glycopeptide recognition.

MMPs as biomarkers of pulmonary disease (COPD)

PhD student: Jennifer Cane. October 2010 to October 2013.

University of Nottingham, Prof Alan Knox, Dr Charlotte Bolton, Dr Simon Johnson & Dr Doug Forrester (Division of Therapeutics & Molecular Medicine).

Mologic supervisors Prof. Paul Davis & Gita Parekh.

Advanced NMR studies of antibody/peptide interactions

PhD student: Dhadchayini Jeyaharan. March 2011 to March 2014.

University of Warwick, Dr Ann Dixon, Assistant Professor of Chemistry.

Mologic supervisors: Prof. Paul Davis & Dr James Schouten & Dr Joannah Towler

Translatable biotechnology for detecting oxidative amino acid modifications and understanding of the role of radicals in inflammation

PhD student: Stuart Meredith. September 2012 to June 2015.

Aston University, Dr Corrine Spickett & Prof Helen Griffiths

Mologic supervisors: Prof. Paul Davis & Gita Parekh