Jason Micklefield – A Chemical Biology Approach for Re-engineering Riboswitches, Enzymes and Biosynthetic pathways.

Research in the Micklefield lab is focused on challenges at the *chemistry-biology interface* utilising techniques and knowledge from organic chemistry through to biochemistry and molecular genetics. Current themes in the lab include: small molecule control of gene expression; enzyme mechanism and directed evolution; and biosynthetic engineering. For example we have been exploring how riboswitches, present within mRNA, can control gene expression in response to specific metabolites present in cells. Based on these insights, we succeeded in re-engineering (or rewiring) riboswitches so that they are no longer triggered by the natural metabolites, but instead can be controlled by the addition of various synthetic molecules [1] (Fig 1). Using our new orthogonally selective synthetic riboswitches we were able to demonstrate the simultaneous and differential control of multiple genes, in bacteria, which is very difficult to achieve using existing methods. We envisage that our riboswitches can be used to study fundamental biological pathways and processes *in vivo* and could also be used in pharmaceutical target validation, drug discovery and the emerging field of synthetic biology [1].

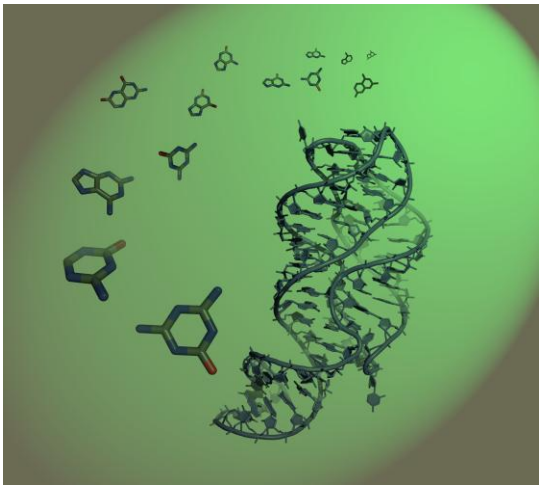

Figure 1: 1.7Å resolution structure of a re-engineered orthogonally selective riboswitch in complex with a synthetic ligand (image designed by N. Dixon & M. Dunstan) [1].

In the area of enzymology and biocatalysis, we recently elucidated the first structure and mechanism of the malonate decarboxylases (AMDases) [2]. This revealed a “dioxyanion hole” motif, not previously described, which can stabilize a putative high-energy enediolate intermediate (Fig. 2). Based on these mechanistic and structural insights we were able to develop directed evolution approaches to extend the biocatalytic repertoire of the decarboxylases and also to select improved enzymes with over 50 fold increased catalytic activity. The new decarboxylases are of industrial importance because they can catalyse the asymmetric decarboxylation of pro-chiral disubstituted malonates to give valuable homochiral carboxylic acids (patented with BASF). We also, introduced the first biocatalytic method for the site-selective covalent protein immobilization [3]. The method uses a phosphopantetheinyl transferase enzyme to catalyse the ligation of tagged protein of interest, to CoA molecules covalently attached to solid surfaces (*e.g.* glass). This efficient single step method allows tagged proteins to be immobilised directly from crude lysates, thus alleviating the need for protein purification. This method has many applications, most notably in the highly sought after production of stable functional protein arrays [3].

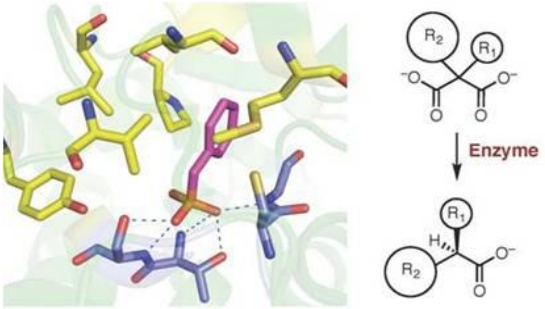

Figure 2: Structure of the AMDase with a mechanism based inhibitor bound.

Finally our lab has been investigating the biosynthesis of nonribosomal peptide natural products, which include several important therapeutic agents. For example, we elucidated the biosynthetic origins of the calcium dependant lipopeptide antibiotics (CDA) from *Streptomyces coelicolor* [4]. This involved a combination of gene knockouts and product analysis (NMR and MS-MS). In addition, many key biosynthetic enzymes were overproduced and characterised by *in vitro* assays with synthetic substrates and intermediates. Using this knowledge, we were able to develop a wide range of biosynthetic engineering approaches (combinatorial biosynthesis), altering the specificity of the biosynthetic enzymes, which enabled us to generate many “non-natural” lipopeptide

variants. The lipopeptides share a similar structure and mechanism of action to daptomycin, which is one of the most potent intravenous antibiotics in the clinic. It is envisaged that the biosynthetic engineering approaches we have developed could be used to generate the second generation of lipopeptide antibiotics which are urgently required to combat the emerging drug-resistant pathogens. We are grateful to all our collaborators and coworkers.

[1] **Riboswitches** *Proc. Natl. Acad. Sci. USA* **2010**, *107, 2830-2835* *Paper*
[2] **AMDase mechanism and directed evolution** *Angew. Chem. Int. Ed.* **2009**, *48,* 7691-7694 *Paper*
[3] **Site-selective protein immobilisation** *J. Am. Chem. Soc*. **2008**, *130*, 12456-12464 *Paper*
Chem. Rev. **2009**, *109,* 4025-4053 *Paper*
[4] **Lipopeptide biosynthesis** *J. Am. Chem. Soc* **2007**, *129*, 15182-15191 *Paper*
J. Am. Chem. Soc. **2006,** *128*, 11250-11259 *Paper*
J. Am. Chem. Soc **2007,** *129*, 12011-12018 *Paper*