CHARACTERISATION, METAGENOMIC SCREENING AND ENGINEERING OF BACTERIAL NITROREDUCTASES FOR BIOMEDICAL RESEARCH APPLICATIONS

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Bacterial nitroreductases are NAD(P)H-dependent oxidoreductases (generally homodimeric and FMN-binding) that can catalyse the 4- or 6-electron reduction of nitro groups on aromatic rings. This results in a profound electronic shift that can dramatically alter the properties of the molecule as a whole, e.g. activating latent cytotoxins, or detoxifying certain pollutants or antibiotics. We have exploited these properties and the characteristic promiscuity of these enzymes to develop useful tools for biomedical research and therapy, in particular the anticancer strategy gene-directed enzyme prodrug therapy, and targeted cellular ablation in zebrafish models of degenerative disease. We have used directed evolution to improve desirable activities and are also investigating the use of dual positive and negative selection strategies to tailor reaction specificity and to better understand how the evolution of promiscuous enzymes is modulated by *in vivo* constraints. A further application of our positive selection capabilities has been the recovery of novel nitroreductases from libraries of uncharacterised environmental DNA.