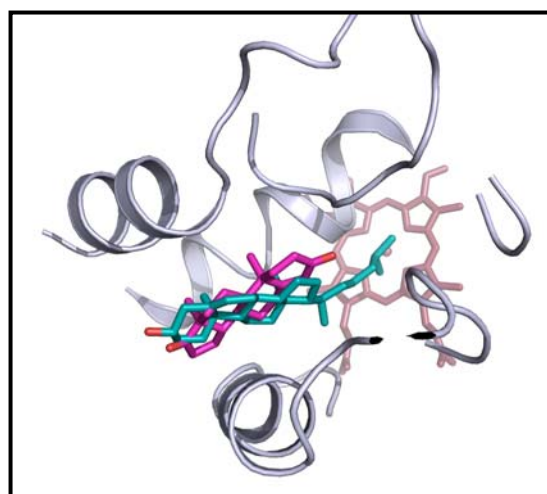


Andrew Munro – Cytochrome P450 redox systems in the pathogen *Mycobacterium tuberculosis*

The chronic disease tuberculosis (TB) is caused by human pathogen *Mycobacterium tuberculosis* (Mtb), and drug and multidrug resistant strains of Mtb are now prevalent across the globe – leading to resurgence in infection and disease, and the WHO predicting a “global catastrophe” in absence of development of novel therapeutics. The Mtb genome sequence revealed several genes of unknown function, along with a huge array of enzymes involved in lipid metabolism. Of particular note was the unexpectedly large number of enzymes encoding cytochrome P450 (P450 or CYP) enzymes – with 20 Mtb P450s. These are heme-containing monooxygenase enzymes that bind and reductively activate and cleave dioxygen – generally inserting a single atom of oxygen into a hydrophobic substrate bound close to the heme. Bacterial P450s have typically been assigned roles in catabolic pathways leading to use of unusual carbon sources for growth. However, studies in recent years have revealed several biosynthetic roles for bacterial P450s, including functions in antibiotic, toxin and signaling molecule production. There is substantial amino acid sequence divergence both among the 20 Mtb P450s and, in most cases, between individual Mtb P450s and other functionally characterized members of the P450 enzyme superfamily. This has made prediction of function from sequence near impossible for most of the Mtb P450 isoforms, and genetic context also has been relatively uninformative to date. However, the importance of the P450s to Mtb biology has been reinforced by the findings that at least two are essential for growth, and by the demonstration that azole-based antibiotics that bind tightly to essential Mtb P450s are also potent antitubercular drugs that can clear infection in a mouse model.

An important part of our group’s studies on structure and function of redox enzymes has been the characterization of the Mtb P450s and their accessory redox partner systems. In work funded by both BBSRC and an EU network project, we have characterized a number of Mtb P450s and redox partners, including investigations of electron transfer mechanism and coenzyme binding in the adrenodoxin reductase homologue FprA, analysis of heme ligation state and its stabilization by substrate binding in a Mtb sterol demethylase P450 (CYP51B1), and determination of crystal structures of the essential CYP121 P450 in both ligand-free and fluconazole-bound forms. For



CYP121, our collaborations with the Leys group produced the highest resolution of all P450 structures for ligand-free CYP121 (1.06 Å), while the azole-bound structure revealed a novel and unexpected mode of enzyme inhibition, with the drug bridging to the heme iron via an interstitial water molecule. In recent work, we have also solved the structure of the Mtb P450 CYP125, which is located in a gene cluster conserved between *Rhodococci* and Mtb, and involved in cholesterol metabolism. Cholesterol is required for Mtb entry into macrophages, where the bacterium is able to survive and to establish an infection. CYP125 and its associated genes are induced on macrophage entry, and are required for Mtb survival in a mouse model. In recent work, we have expressed and characterized the Mtb CYP125 protein, and determined its crystal structure in ligand-free and inhibitor/steroid-bound forms. These data reveal a “letter box” shaped entry port that fits well the dimensions of cholesterol, and indicate that the cholesterol side chain is orientated towards the P450 heme to favour 26-hydroxylation, as predicted by activity studies (see

accompanying figure showing androstenedione [magenta] and cholesterol [cyan] in the CYP125 active site). Cholesterol oxidation at this position is expected to enable degradation of cholesterol as a fuel source for Mtb engulfed in the macrophage, but oxysterols also have important regulatory functions and thus the product may also have roles in cellular signaling, possibly in evasion of Mtb cell destruction in the lysosome. Ongoing studies with other Mtb P450 systems have also recently led to establishment of the functions of the essential Mtb P450 CYP121 in the oxidative modification of a cyclic dipeptide, and in the predicted function of Mtb CYP128 (which we have shown to be membrane associated) in the hydroxylation of menaquinone, and as a step towards its sulfation in respiratory regulation. Thus, the “unique” roles predicted for many of the P450s are starting to emerge, and our studies in this area continue with the aim of establishing novel structure and function of Mtb P450s, and in targeting these enzymes for development of novel anti-Mtb drugs.