

## ***“New Engineered enzymes for sulfate ester hydrolysis to improve doping control”***

**Dr. M. McLeod, Dr. B. Stevenson, Dr. D. Ollis** (Australian National University, Australia)

### **Project aims:**

When an athlete dopes the drug is changed by the body and excreted in the urine. These changed drugs or drug metabolites must be processed by anti-doping laboratories to enable detection using a range of sophisticated techniques. A protein or enzyme called beta-glucuronidase, isolated from *Escherichia coli* (*E. coli*) bacteria, is routinely used by anti-doping labs to process samples prior to analysis. It has become an important basic tool used by analysts in the fight against doping. Unfortunately this *E. coli* beta-glucuronidase enzyme only works on some drug metabolites called glucuronides leaving others such as sulfates unprocessed, and this may mean that the doping goes undetected. Creating a new method to process sulfate metabolites would significantly improve anti-doping analysis.

In this project we will develop a separate bacterial protein isolated from *Pseudomonas aeruginosa* (*P. aeruginosa*) called a sulfatase that we have shown is able to process the sulfate metabolites that *E. coli* beta-glucuronidase cannot. However, the activity for some drug metabolites is low leading to inefficient hydrolysis. We will employ laboratory-based methods of rapid evolution to enhance the activity of the *P. aeruginosa* sulfatase enzyme for anti-doping applications.

The outcome of the project will be an improved sulfatase enzyme for processing drug metabolites that will complement *E. coli* beta-glucuronidase. Including the improved enzyme in the methods used to process drug metabolites will increase the sensitivity of analysis and allow doping to be detected for a longer period after an athlete takes a banned drug. We expect this improved *P. aeruginosa* sulfatase will join *E. coli* beta-glucuronidase and also become an indispensable tool used by anti-doping laboratories in the fight against doping.

### **Results and Conclusions:**

Previously, we had identified a bacterial sulfatase, *Pseudomonas aeruginosa* arylsulfatase (PaS), and found it to compare favourably with commercially available sulfatase preparations (Drug Test. Anal. 2015, 7, 903–911). However, the activity towards  $\beta$ -configured anabolic androgenic steroid sulfates (testosterone sulfate, TS, for example) was several orders of magnitude lower than aryl sulfates such as estrone sulfate. Furthermore, the activity towards  $\alpha$ -configured steroid sulfates, such as etiocholanolone 3-

sulfate (ECS) or epitestosterone 17sulfate (ETS) and androsterone 3-sulfate (AS) was undetectable. Thus at the outset of our project our goals were to achieve:

- A 10-50-fold improvement for testosterone 17-sulfate and significant activity for other targeted steroid sulfates.
- An improved pH profile with maximum activity closer to neutral pH.

These enhancements in activity and scope will deliver new enzyme preparations suitable for anti-doping applications.

We took two approaches to improve PaS in parallel. The first approach made use of the published structure and our modelling to predict how steroid sulfates bind with the enzyme and identify nearby residues for targeted mutagenesis and screening. The second used directed evolution with random mutagenesis and subsequent selection for improved activity. In both cases we selected for variants with greater affinity and catalytic activity for TS hydrolysis (measured as  $V_{max}/K_m$  or initial rates).

In summary the major aims of the project have been met with a 270-fold improvement ( $V_{max}/K_m$ ) for TS hydrolysis, and an improvement in substrate scope with PaS mutants derived from the project capable of hydrolysing ECS and ETS at significant rates where the parent PaS enzyme could not. Further mutants developed by the project show significantly increased absolute and relative activity at pH 7.

## **Publications**

Uduwela D, Pabis A, Stevenson B, Kamerlin S, McLeod M. Enhancing the Steroid Sulfatase Activity of the Arylsulfatase from *Pseudomonas aeruginosa*. ACS Catal. 2018, 8, 8902–8914