s1.m6.p12 **Crystal structures of human cytochrome P450 2C9 and 3A4 with bound ligands.** Dijana Matak-Vinkovic, Pamela A. Williams, Jose Cosme, Alison Ward, Philip Day, Ian J. Tickle, Harren Jhoti, *Astex Technology, 436 Cambridge Science Park, Milton Road, Cambridge CB4 0QA, UK. E-mail: d.vinkovic@astex-technology.com*

**Keywords: P450; CYP2C9; CYP3A4**

The mammalian cytochrome P450 enzymes are a family of membrane-associated haem-containing proteins which play a major role in the metabolism of numerous and diverse xenobiotics such as drug molecules. Despite significant efforts, the molecular basis of drug recognition by human cytochrome P450 proteins has remained elusive. Here we describe crystal structures of human cytochrome P450 proteins, CYP2C9 [1] and CYP3A4 both in an unliganded form and in complex with marketed drugs. CYP3A4 is the most important member of P450 family, responsible for metabolising 50 % of drugs while CYP2C9 metabolises some 15 % of all marketed therapeutics. These crystal structures provide insights into the principles of substrate binding for these promiscuous enzymes.

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s1.m6.p13 **Crystal structure of Y10F mutant of Sh28GST: insight into GSH activation mechanism and isomerisation of prostaglandin H2 to prostaglandin D2.** Adriana E. Miele,[a] Francesco Angelucci,[a] Paola Baiocco,[a] Louise Gourlay,[a] Francois Trottein,[b] Andrea Bellelli[a] and Maurizio Brunori[a], [a]*Dipartimento di Scienze Biochimiche - University of Rome "La Sapienza", Italy, and* [b]*U547 INSERM - Institute Pasteur de Lille, France. E-mail: adriana.miele@uniroma1.it*

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Parasitic diseases are a major threat on human health and Schistsomiasis is second only to malaria for the number of people affected and the gravity of pathology, although it is spread only in tropical and sub-tropical areas. A collaboration between the University of Rome "La Sapienza" and the Pasteur Institute at Lille has led to the 1.8Å X-ray crystal structure of the *Schistosoma haematobium* 28kD glutathione S-transferase (Sh28GST), which is a major antigen and a putative vaccine in fase 2 clinical trials [1]. Sh28GST is thought to protect the schistosome from xenobiotic attack via a detoxification process or simply by binding and sequestering toxic compounds. Sh28GST has also been demonstrated to have a high prostaglandin $D_2$ synthase activity. Prostaglandin D2 is able to control the human cutaneous immune response helping schistosomes to escape the host immune system [2]. The overall fold of Sh28GST is similar to that of all other GST's: GSH binds to the N-terminal thioredoxin-like domain, while substrates for GST transferase activity bind to the C-terminal alpha-helical domain. Despite extensive dialysis prior to crystallization, the active site is still 40% saturated with GSH in the crystal and shows a unique feature which has not been reported in other GST structures: the crucial active site Tyr10 side chain occupies two different positions [3]. One of those two positions is a non-canonical rotamer: the phenolic ring is found away from the active site, tilted towards the exterior of the protein, with the OH exposed to the solvent. This unusual conformer is stabilized by an on-face H-bond with the conserved Arg21. The question arose whether this new conformer was an artifact due to crystallographic contacts; hence we produced, expressed, purified, crystallized and solved the structure of the Y10F mutant of Sh28GST at 1.9.Å. The structure is superimposable to the wild type and presents the same characteristics also with respect to the residue in position 10. The on-face hydrogen bond is still maintained between the Arg21 and the benzene ring of the Phe10, confirming what it has been previously observed. Moreover the Y10F mutant is not functional either in activating glutathione or to the isomerization of prostaglandins. These results could be rationalized with our new proposed mechanisms with the flipping of the Tyr10 in the native enzyme being the key step. If our hyphotesis will be confirmed it will be possible to design new drugs for parts of the protein that were not previously taken into considered.

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