From *in silico* to *in vitro* substrate characterization of Macaca fascicularis P450 2C20

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Macaques are the most widely used nonhuman primates in preclinical studies to obtain FDA approval of new drugs but the lack of information on cytochrome P450 genes has hampered the understanding of drug metabolism in this species.

Human P450 2C8 metabolizes more than 8% of drugs cleared by Phase I reaction but today there are no data on the substrates recognized by the homologous Macaca fascicularis P450 2C20.

In this work, a 3D model of cynomolgus 2C20 was generated and optimized on the basis of the available crystal structure of the P450 2C8 by employing the YASARA program. A set of 60 known substrates of P450 2C8 were taken from the literature and docked in the active site of both the 2C20 model and the available 2C8 structure. Based on the predicted binding energies, both proteins can potentially bind the selected P450 2C8 substrates with similar affinity.

In order to validate the *in silico* docking data by *in vitro* methods, the gene encoding CYP2C20 was cloned in pCW vector and successfully expressed in *E.coli.* Furthermore, a chimeric protein consisting of P450 2C20 and the soluble reductase domain of CYP102A1 (Bacillus megaterium-BMR) was engineered. The solubility and catalytic self-sufficiency of this protein would greatly simplify the *in vitro* preclinical studies of cytochrome P450 2C20 mediated drug metabolism.

Three drugs were then selected from the energy output profile of the *in silico* docking experiments namely Paclitaxel, Torzasertib (anti-cancers) and Amodiaquine (anti-malarial) for *in vitro* testing with the purified P450 2C20 and its chimera. The turnover of the P450 2C20 and the chimera led to Km values of 1.9±0.2 μM and 5.9±2.3 μM for Paclitaxel; 1.5±0.2 μM and 2.1±0.7 μM for Tozasertib; 1.2±0.2 μM and 1.6 ±0.17 μM for Amodiaquine, respectively.

Finally, the data obtained from the *in silico* and *in vitro* analysis demonstrate the ability of the cynomolgus P450 2C20 to recognize and turnover P450 2C8 substrates, its human homologue.