Structural and functional characterisation of natural and engineered catalytically self-sufficient cytochromes P450

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Cytochromes P450 are a superfamily of heme-thiolate monooxigenases present in all animal kingdoms. Every organism synthetizes a huge number of P450 (1), all of them able to catalyse different class of molecules. Examples of reactions performed by CYP P450 are hydroxylation of aliphatic and aromatic compounds, epoxide formation and aromatization of steroids ring. These reactions are quite attractive for chemical synthesis where often high regio- and stereo-selectivity is required.

P450 enzymes are particularly valuable for applications in biotechnology, however, at the present time, practical applications for the bioconversion process as biocatalysts are limited due to the low stability, low activity, and cofactors dependency of most P450s.

In this project we have deeply characterized CYP116B5 a bacterial P450 from Acinetobacter *radioresistens* (2), a self-sufficient class VII P450 which was proven to be a valid alternative to the conventional route of P450 catalysis: it shows interesting activity towards aromatic compounds in absence of a reductase but using efficiently the "peroxide shunt" mechanism (*figure 1*), in which hydrogen peroxide (H_2O_2) or an organic hydroperoxide convert a substrate-bound resting state of the P450 to compound I (via compound 0). The results demonstrated interesting peculiarities in comparison with other well studied P450s such as an unusual high redox potential and the high resistance to H_2O_2. Moreover, the ability to recognise different type of substrates such as drugs, waste water pollutants and endocrine disruptors, makes this enzyme a valuable candidate as biocatalyst obviating the need for the expensive NADPH cofactor. Some of the possible applications are proposed.

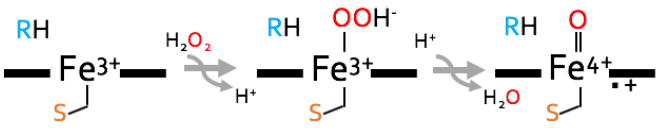

Figure 1: Oxidation of the ferric-substrate complex of the heme with oxygen-atom donors such as peroxides and hypochlorites.

Furthermore, we focused our attention on the generation of new human P450 chimeras fusing in a single polypeptide chain the reductase domain of a bacterial P450 (BMR) and a human heme domain. This method called "molecular Lego approach" (3), developed in our laboratory makes use of electron transfer proteins and redox enzymes to build multidomain systems (*figure 2*) with many advantages such as increase stability of the enzymes for biocatalysis (4). The generation of the new aromatase-BMR chimera has been compared with the already studied 3A4BMR and reveals new insights into domain interaction.

Moreover, the 3A4BMR was studied with high pressure spectroscopy following CO binding and high to low spin transitions. The results were compared with 3A4 in solution and interpreted as a different sensitivity in protein's conformers.

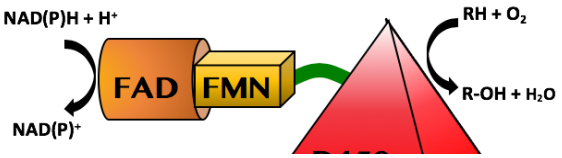

Figure 2: Artificial self-sufficient enzyme architecture with the bacterial reductase (FAD-FMN as cofactors) fused in a single polypeptide chain with a human heme domain (in red).

1) Ciaramella, A., Minerdi, D. & Gilardi, G. Catalytically self-sufficient cytochromes P450 for green production of fine chemicals. *Rendiconti Lincei* **28,** 169–181 (2017).

2) Minerdi, D. *et al.* CYP116B5: a new class VII catalytically self-sufficient cytochrome P450 from *A cinetobacter radioresistens* that enables growth on alkanes: Oxidation of *n* -alkanes by a novel self-suffcient bacterial cytochrome P450. *Molecular Microbiology* **95,** 539–554 (2015).

3) Gilardi G, Meharenna YT, Tsotsou GE, Sadeghi SJ, Fairhead M, Giannini S (2002) Molecular Lego: design of molecular assemblies of P450 enzymes for nanobiotechnology. Biosens Bioelectron 17:133–145

4) Castrignanò, S., D'Avino, S., Di Nardo, G., Catucci, G., Sadeghi, S.J., Gilardi, G., 2018. Modulation of the interaction between human P450 3A4 and B. megaterium reductase via engineered loops. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1866, 116–125.