Towards improved catalytic efficiency in engineered human cytochrome P450 3A4-BMR

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Cytochrome P450 enzymes form a family of b-type haem-thiolate proteins that carry out the monooxygenation of a large number of endogenous and exogenous compounds using NADPH as the electron donor. The electron equivalents that are not utilized to oxidize the substrate in the P450 reaction cycle lead to the uncoupling process and production of reactive oxygen species. Therefore, understanding the uncoupling at the haem active site and/or at the level of multi-domain electron transfer is an important element in cytochrome P450 chemistry.

In this work, a chimeric model system consisting of human cytochrome P450 3A4 and the soluble reductase domain of CYP102A1 from *Bacillus megaterium* (BMR) is used to study the relationship between electron transfer and the coupling efficiency in substrate monooxygenation. Several regulatory features were considered including pH, ionic strength, concentration of exogenous cofactors (FAD and FMN) together with the length of the linker joining the two domains of the 3A4-BMR chimeric enzyme.

This linker has been proposed to function as a flexible hinge between the two domains and could provide the chimeric enzyme with the degree of freedom required to fold correctly. The relevance of the linker and the importance of the flexibility of this region are considered as a critical and crucial parameter to affect the uncoupling of the electron flow. The ability of the reductase and haem domains of the 3A4-BMR chimera to form an active complex was evaluated by altering the length of the linker region through introduction of a chain of 3 or alternatively 5 glycine residues.