Constructing catalytically proficient and functionally diverse enzymes from simple, de novo designed proteins

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Project description:

Bespoke protein catalysts compatible with the natural biomolecular components of living cells are key to realising the ambitious goals of synthetic biology and to the provision of cheap, green catalysts for industrial biotechnology. To this end, the manmade protein maquettes designed in our lab have proved particularly adaptable to a tractable de novo enzyme design process, as highlighted through our successful design of a hyperthermostable and catalytically proficient de novo peroxidase, recently published in Nature Communications. Since then, we have atomistically designed a new series of heme-containing de novo proteins that allow for precision engineering of the protein structure and active site. We have done so using a powerful combination of Rosetta protein design and Molecular Dynamics simulations, allowing for both computational design and rapid structural assessment in silicon prior to protein expression biophysical characterisation.

The aims of this project are to implement a powerful synthesis of computational and experimental methods in the de novo design of functional, catalytically active heme-containing proteins and enzymes. These proteins will be designed with particular emphasis on addressing challenging chemistries pertinent to industry, including catalytic monooxygenation and carbene transfer activities. Initially, they will be designed and assessed using computational methods (e.g. Rosetta protein design suite & Molecular Dynamics software), and proteins that are subsequently selected for expression and purification will be subjected to a comprehensive biophysical analysis (e.g. circular dichroism, EPR and NMR spectroscopies, redox potentiometry, X-ray crystallography). Catalytic activity will be determined through a variety of steady-state and pre-steady-state kinetic methods (e.g. plate reader assays, stopped-flow spectrophotometry), and products will be indentured using a variety of techniques (e.g. NMR spectroscopy, HPLC, LC-MS). QM/MM calculations will be employed to examine the catalytic cycles in detail, informing future protein designs. Directed evolution will also be employed to improve and hone nascent activity.