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TOPIC(s) : Synthetic biology, metabolic engineering / Enzyme production, immobilization

Co-encapsulation of P450 enzymes and their redox partner, in virus-like particles as nano-scale bioreactors

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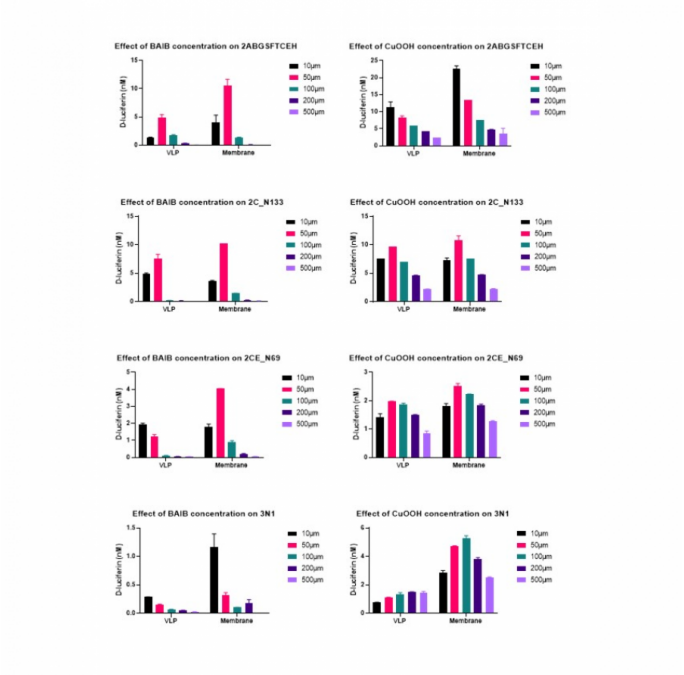
PURPOSE OF THE ABSTRACT
The superfamily of monooxygenase enzymes, the cytochromes P450, catalyze a large diversity of reactions making them highly interesting biocatalysts for diverse biotechnological industries. However, to be profitable, biocatalysts need to be both stable and easily recovered from biocatalytic reactions to reduce the costs involved in their use.
The Gillam lab has used ancestral reconstruction (ASR) to engineer thermostable, yet catalytically promiscuous P450s, based on xenobiotic-metabolizing P450s. Ancestral P450s have shown increases of up to ~ 30 degrees in thermostability compared to their extant counterparts.[1-4] However, the need to recover biocatalysts from bioreactors, and re-use them if possible, remains a challenge, since P450s are embedded in a membrane or in cells for most biocatalytic applications, complicating the recovery and work-up of product. As a biotechnological solution, encapsulating P450s in virus-like particles (VLPs) would facilitate their recovery and re-use, since the separation of the biocatalyst from the process bioreactor could be achieved using centrifugation. At the same time, immobilizing one or multiple enzymes within a VLP has been shown to improve biocatalysts' stability against harsh process conditions.[5]
This presentation will focus on co-encapsulating ancestral P450s with their redox partner, cytochrome P450 reductase (CPR) into VLPs derived from the P22 bacteriophage. P22 VLP is composed of an assembly of ~420 coat proteins in a non-infectious spherical structure (~50nm diameter) that do not contain genetic material. With a simple linker between the native scaffold protein and the biocatalyst, co-expression in Escherichia.coli gives an effective delivery system where the VLPs encapsulate the biocatalysts of interest.
In the current project, the activity of ancestral P450s towards a luminescent substrate (MultiCYP) was compared when encapsulated into VLPs compared to embedded in a membrane. The activity could be supported using different concentrations of oxygen surrogates, but activity was ~20% lower for P450s in VLPs compared to P450s in bacterial membranes, a difference that may be related to either greater sensitivity to the damaging effect of oxygen surrogates or limited transfer of substrate or product across the VLP capsid. The reusability of ancestral P450s encapsulated into VLPs was assessed using ultracentrifugation to separate the P450s in VLPs from the reaction mixture containing oxygen surrogates. While specific activity (i.e. activity per mg protein) was maintained, roughly 85% loss of total protein was observed over 5 recovery cycles and total activity declined in parallel. Co-encapsulation of the CPR that naturally supports the P450 activity would avoid the protein-degrading, use of oxygen surrogates. Two main methods to obtain VLPs co-encapsulating P450s and CPR have been pursued: firstly, in vitro assembly by reconstitution from pure proteins; and secondly, in vivo assembly realized directly within the transformed E.coli expressing the coat protein, P450 fused to the-scaffold protein and CPR fuse to the scaffold protein. Successful co-encapsulation of ancestral P450s and CPR will enable prolonged reactions at process-type temperatures with a high recycling capacity.

FIGURES



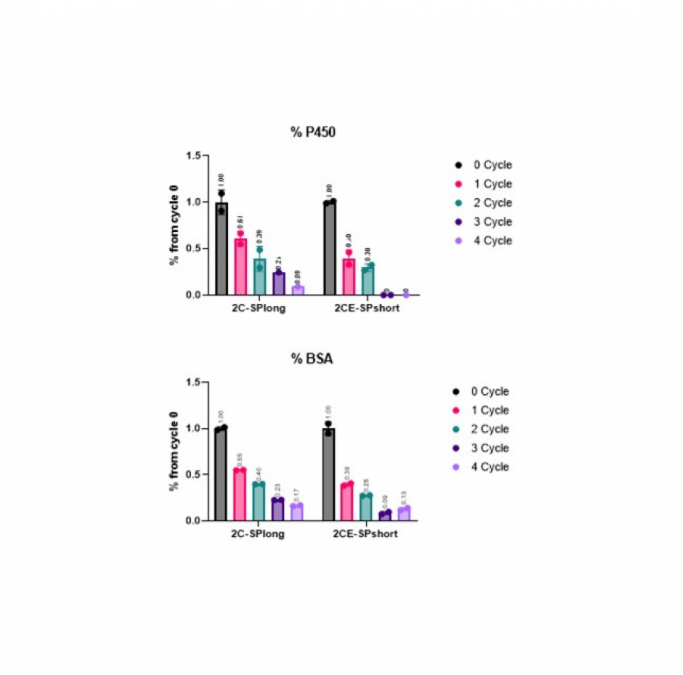


FIGURE 1

Effect of Oxygen surrogates on P450-Glo? luciferin reactions

P450-Glo? luciferin reactions were carried out with 40nM P450 (Membrane: bacterial membrane preparation / VLP: Encapsulated in VLP (P22) by expression with long scaffold protein) and 50 ?M luciferin-MultiCYP. Catalysis was supported using a range of oxyge

FIGURE 2

Recyclability of P450 encapsulated in VLP

Measure of the relative concentration of P450 and total soluble protein during a cycle of recyclability assay, P450 sample incubate for 30min at 37?C in presence of 40 ?M Cumene Hydroperoxide (to reproduce reactions conditions). Recyclability was done by

KEYWORDS

Cytochromes P450 | Virus-Like-Particles | Co-encapsulation

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