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Accelerating Discovery of Substrate Promiscuity in Cytochrome P450 BM3

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PURPOSE OF THE ABSTRACT
Enzymes collectively display a great breadth of catalytic properties yet are individually confined to one or a few specific catalytic tasks. Despite key advances in enzyme engineering, our capacity to predict the effects of mutations on function remains nebulous. Here we present advances in engineering non-native substrate recognition for biocatalyzed transformation into useful products. We examine cytochrome P450 oxidase from Bacillus megaterium (P450 BM3) in its capacity to functionalize C-H bonds. Cost-effective, high-throughput colorimetric screening at the whole-cell level had previously suggested a correlation between the production of indigo and increased substrate promiscuity, in a small number of P450 BM3 variants. We greatly expand the diversity of indigo-producing P450 BM3 variants and demonstrate a correlation with promiscuous aromatic hydroxylation reactions. We look ahead to the potential for large experimental datasets to train smarter design algorithms for enzyme engineering.

FIGURES

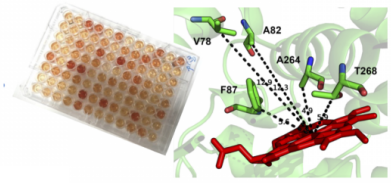

FIGURE 1

High-throughput colorimetric screening

P450 BM3 in cell lysate was screened for hydroxylation of non-native substrates

FIGURE 2

KEYWORDS

BIBLIOGRAPHY