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# Engineering next-generation biocatalysts by in vivo selections

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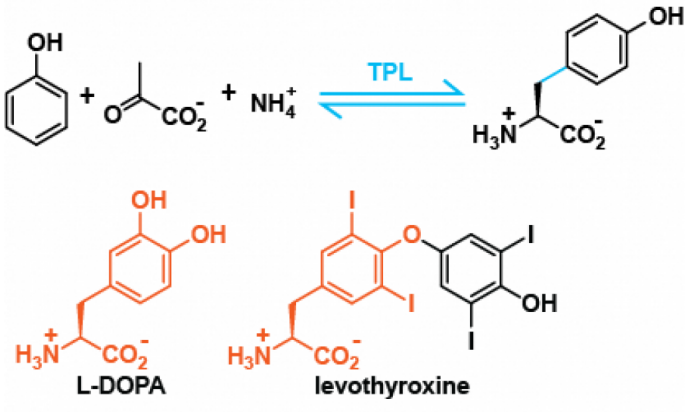
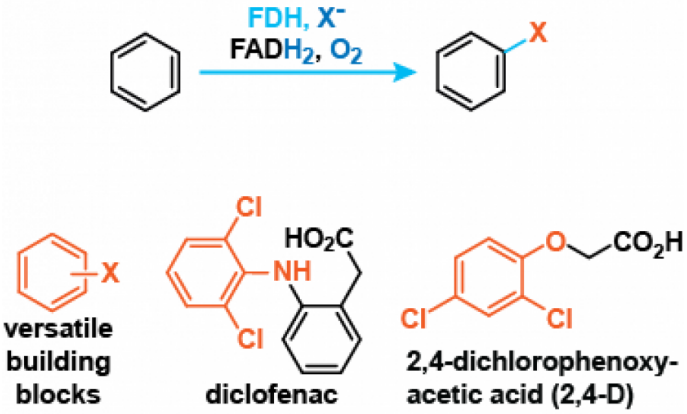
## PURPOSE OF THE ABSTRACT
Enzymes are impressive catalysts that accelerate reactions with unmatched rates and selectivity. While biocatalysts found in nature are rarely suitable for industrial applications, directed evolution can tailor enzyme properties to a user's need. Although powerful, enzyme engineering campaigns are notoriously labor- and time-consuming, which derives from the need to assess the activities of enzyme variants one-by-one. To overcome this bottleneck, we have recently provided proof-of-principle for a directed evolution strategy, in which the activity of a model enzyme could be linked to bacterial survival.[1] Critically, this strategy allows us to assess millions of enzyme variants all at once, removing much of the time, cost and technical challenges associated with enzyme evolution.
Here, we apply such in vivo selections to the directed evolution of mechanistically diverse enzymes, which are underexploited, yet promising candidates for the sustainable synthesis of valuable compounds. As target enzymes, we selected tyrosine phenol lyases, TPLs, that promote enantioselective C-C bond formations (Fig. 1), and flavin-dependent halogenases, FDHs, that install halogens at aromatic C-H bonds (Fig. 2). To engineer these biocatalysts, we leverage recoded organisms that are addicted to non-canonical amino acids and select improved TPLs and FDHs based on their ability to provide these ncAAs from externally-supplied, synthetic precursors. The resulting next-generation biocatalysts and the designer microbes that produce them will be employed for the synthesis of valuable halogenated building blocks, as well as tyrosine analogs and blockbuster drugs, such as L-DOPA and levothyroxine.

## FIGURES




### FIGURE 1

Tyrosine phenol lyase

General reactivity of TPLs, and their potential industrial targets.

### FIGURE 2

Flavin-dependent halogenases

General reactivity of FDHs, and their potential industrial targets.


## KEYWORDS

Direct evolution | In vivo selections | Biocatalysis


## BIBLIOGRAPHY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