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Regioselective oxidation of bulky substrates by expression of new unspecific peroxygenases (UPOs) with broader tunnels and automated in silico screening

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PURPOSE OF THE ABSTRACT

Unspecific peroxygenases (UPOs) are versatile biocatalysts capable of oxidizing various organic compounds. However, the small tunnels and binding pockets of currently expressed UPOs limit their use in converting bulkier substrates that are readily transformed by P450 monooxygenases. 1-3 Achieving selective conversions of bulky substrates typically involves medium to high-throughput screening, which can be a tedious and time-consuming laboratory process.

This study aims to express new UPOs with broader tunnels and substrate pockets that can convert bulkier substrates as well as design an automated program for screening substrate/product libraries with a set of selected UPOs to predict regioselective oxidation products. To achieve this, we successfully expressed 9 out of 13 new UPOs using signal peptide shuffling4 in Saccharomyces cerevisiae and developed an automated in silico screening program based on docking and the concept of near-attack conformation states. Docking poses that meet the restrictions of the near-attack conformation state are assumed as productive and are further filtered for the desired regioselectivity. The method was first validated by a set of standard substrates. In the upcoming months, the program will be applied to a list of ~50 bulky substrates with industrial relevance to be oxidized selectively. Ten substrates that were predicted to be converted with high regioselectivity and which docking poses are in a productive conformation will be tested in the lab by a set of new UPOs. Our approach will enable efficient identification of the optimal UPO for each desired substrate-product pair, thus facilitating the development of more efficient and sustainable biocatalytic processes for a wide range of industrial applications.

FIGURES

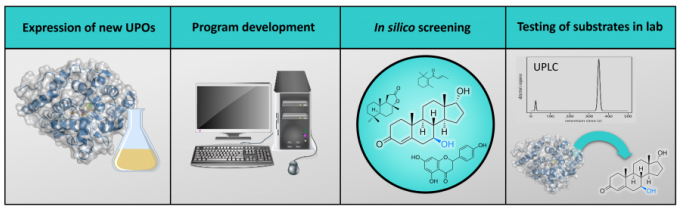

FIGURE 1

Process of the project

Starting with the expression of new UPOs with broader substrate tunnels, followed by the program development for automated docking and docking evaluation, which is applied to library of bulky substrates to finally test the best enzyme-substrate pairs.

FIGURE 2

KEYWORDS

unspecific peroxygenase | computational | regioselectivity | oxidation

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