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Poster Abstract

Expression and Characterization of Cytochrome P450 2D6 Variants in *Pichia pastoris*

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Cytochrome P450s catalyze the introduction of a single atom of molecular oxygen to a nonactivated carbon atom of substrates, which is difficult to perform by standard chemical means. This turns them into very interesting candidates for industrial application in biotechnology and metabolite synthesis for pharmacological studies. Nevertheless, the lack of simple high-throughput expression and screening methods hinders the development of improved P450 biocatalysts from eukaryotes.

Here we show the use of the efficient expression host *P.pastoris* for laboratory evolution of cytochrome P450s.

The current work is focused on improving the catalytic properties of the cytochrome P450 2D6 (CYP2D6) towards testosterone, an atypical CYP2D6 substrate. Site saturation mutagenesis was performed on two positions believed to play a role in substrate specificity. Whole cell conversions of testosterone coupled with a fluorimetric assay as well as coupled with HPLC-MS constitutes a rapid screening system to evaluate the potential of the created CYP2D6 variants.