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Next-generation of enzyme engineering toward improved terminal hydroxylations

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PURPOSE OF THE ABSTRACT
Terminal hydroxylation represents an attractive biocatalytic route toward terminal alcohols, aldehydes, and acids, which are of special interest as building blocks for the sustainable production of polymers. To customize biocatalysts for this purpose, several described alkane degradation pathways offer valuable starting points. Particularly interesting are alkane monooxygenases displaying a broad substrate spectrum efficiently ω-oxyfunctionalising alkanes, cycloalkanes, thioesters and fatty acid esters [1, 2]. In-depth knowledge of protein sequence-function relationship would enable the generation of tailored alkane monooxygenases as biocatalysts for stereo- and regioselective biotransformations and further industrial applications. However, due to the lack of crystal structures, enzyme engineering of membrane-bound alkane monooxygenases proved to be challenging. Therefore, we aim to elucidate sequence-function relationships of alkane monooxygenases using simple high-throughput screening (HTS) or selection assays. For the development of the HTS assay, the activity of Pseudomonas oleovorans AlkB alkane hydroxylase system was coupled with alcohol dehydrogenase activity of AlkJ and the LuxAB biosensor system [3]. The activity of AlkB was successfully detected with the established HTS assay starting from different alkane substrates. Additionally, the versatility of the developed HTS assay was corroborated by detecting the activity of four AlkB homologs. The HTS assay will be further applied to obtain datasets for machine learning, predicting mutational hotspots toward improved hydroxylating activities. Overall, this would result in enzyme variants showing desired activities, subsequently, accelerating the engineering of alkane monooxygenases.

FIGURES

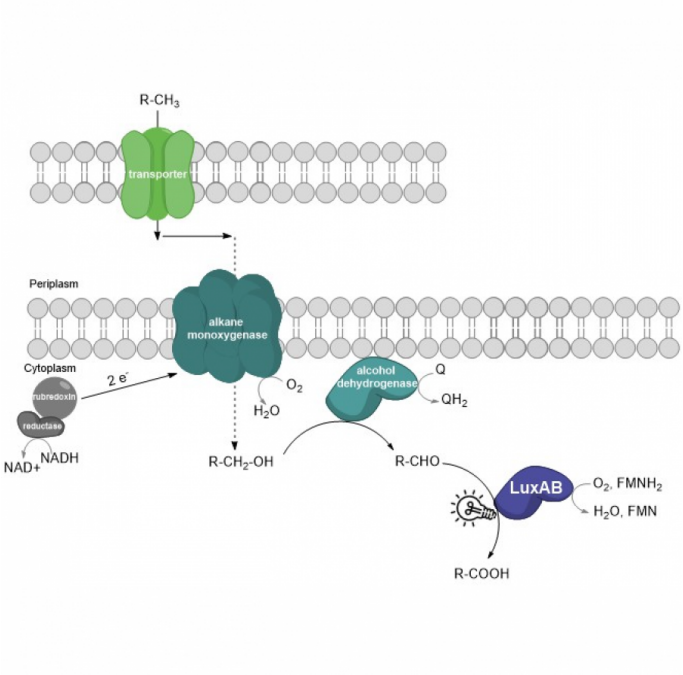

FIGURE 1

Figure 1.

LuxAB-based high-throughput assay for the detection of the activity of alkane hydroxylase system.

FIGURE 2

KEYWORDS

protein engineering | hydroxylation | alkane monooxygenase | high-throughput assay

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