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Expanding the N-terminal tag toolbox for functional expression of Cytochrome P450s in cell factories

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Membrane-associated Cytochrome P450s represent one of the most important enzyme families for biosynthesis of plant-derived medicinal compounds such as terpenoids. Unfortunately terpenoids are often produced in tiny amounts *in planta* and despite they may represent an effective treatment for many diseases such as cancer or malaria, direct extraction from feedstocks is far from sustainable. High and stable expression of these enzymes in microbial hosts is a must for fast development of new cell factories. However, the hydrophobic nature of P450s related to their biological environment makes their expression the main bottleneck. Despite several strategies have been applied to overcome this problem such as N-terminal tags or truncations, the modification toolbox is still limited to a very few examples. Here we have created a small library of N-terminal tag chimeras of the model P450 *Sb*CYP79A1 to facilitate expression in *Escherichia coli* (Fig.1). Using a high-throughput screening technology consisting of C-terminal GFP fusions we were able to identify highly expressed and properly folded chimeras. Three N-terminal tags were subsequentially selected for assembling a heterologous pathway consisting of two plant P450s to test their activity in a meaningful cell factory scenario. Based on these, we chose a leader sequence tag to extend the expression analysis to a larger library of 49 different P450s, some of them uncharacterized, belonging to insect and medicinal plants. With this approach we expanded the toolbox of N-terminal tags thereby improving expression of chimeras to different degree with respect to the native *Sb*CYP79A1. The combination of differently tagged P450s resulted in high turn-over of the metabolic pathway compared to the native enzymatic forms (Fig.2). Additionally, the leader sequence tag was responsible for improving the expression of ~50% of the plant P450 library by more than 2 fold. Our data suggest that N-terminal tag-based cell factory design provides a new powerful toolbox for biotechnological production of plant medicinal compounds.

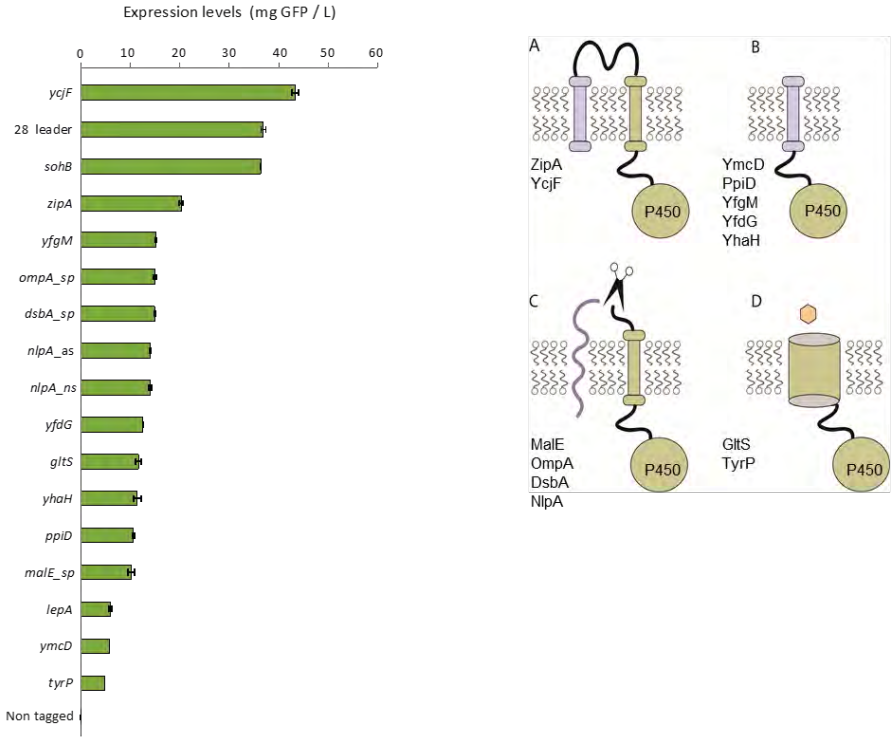

Fig. 1. Summary of the N-terminal tag library of the cytochrome P450 *Sb*CYP79A1. Right; the plot shows expression levels of the different tag fusions of the *Sb*CYP79A1 in *E.coli* after 24h induction time. Left; Illustration of the different tag topologies based on transmembrane and signal peptide prediction tools.

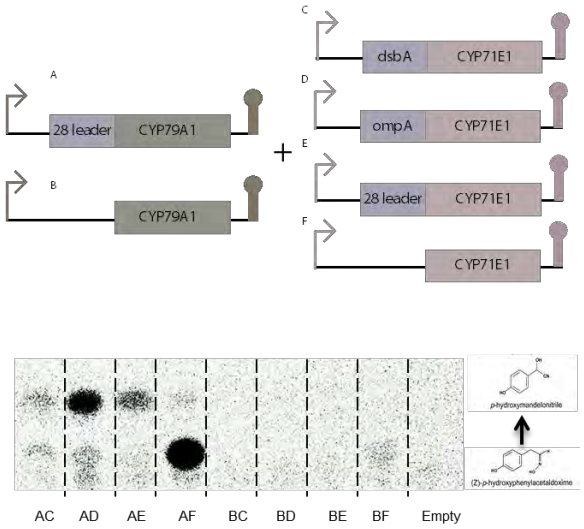

Fig. 2. DNA Assembly and activity of a plant metabolic pathway consisting of two cytochrome P450s (CYP7A1 and CYP71E1) in *E.coli*. Above; depiction of different combinations of the two N-terminally tagged and native P450s assembled together by uracil-excision cloning. Below; metabolic pathway activity assay analyzed by Thin-Layer Chromatography (TLC). Intact *E.coli* cells expressing the different cytochrome combinations were fed with radioactive labelled substrate, the two products of the pathway extracted from the supernatant and eluted in a TLC silica plate.