COFACTOR SWITCH: DEVELOPMENT OF A NAD$^+$-DEPENDENT CASCADE FOR THE PRODUCTION OF URSODEOXYCHOLIC ACID (UDCA)

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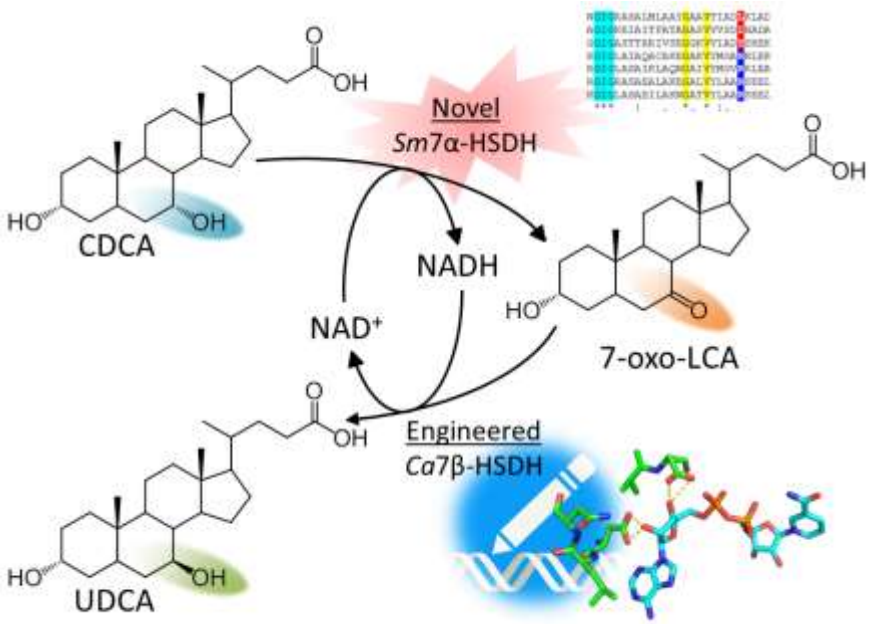
The employment of alcohol dehydrogenases in cascade reactions is often limited by the different cofactor specificity of the enzymes involved: the employment of additional cofactor regeneration systems and the excess amount of sacrificial substrates frequently increase the environmental impact and the costs of biocatalytic processes. Additionally, a NADP$^+$-dependent process is generally less desirable, inasmuch this cofactor is more expensive, unstable and less naturally available than NAD$^+$, leading to an increase of the process costs. Nowadays, protein engineering offers the possibility to switch the cofactor dependency of enzymes introducing few targeted mutation[1].
We applied this methodologies for the development of the first fully NAD$^+$ mediated cascade for the production of Ursodeoxycholic acid (UDCA), a widely used pharmaceutical ingredient for the clinical treatment of cholesterol gallstones and liver diseases[2]. The enzymatic epimerization of CDCA into UDCA, can be carried out with two specific hydroxysteroid dehydrogenases (HSDH): the 7α-OH group is firstly oxidized to the ketone by 7α-HSDH and subsequently re-reduced with opposite stereochemistry (7β-OH) by 7β-HSDH. All procedures up to now require a set of a respectively NAD$^+$ and NADP$^+$ dependent enzymes. Nevertheless, gene sequences of NAD$^+$-dependent 7β-HSDH were not reported. In order to obtain a full-NAD$^+$-dependent process, the NADP$^+$ dependent 7β-HSDH from *Clostridium absonum* was engineered. Employing a semi-rational mutagenesis approach, we obtained a variant with shifted cofactor preference. Importantly, this study allows to identify the residues responsible of the cofactor recognition in other 7β-HSDH homologues and, thus, to the identification of the gene coding for a wild-type NADH-dependent homologue from *Lactobacillus spicheri* (*Ls*7β-HSDH). These novel NAD$^+$-dependent 7β-HSDH enzymes in combination with 7α-HSDH from *Stenotrophomonas maltophilia* permitted the redox-neutral biotransformations of CA and CDCA in the presence of catalytic amounts of NAD$^+$, resulting in high yields (>90 %) of UCA and UDCA[3].
Further studies are underway to develop a flow process for this industrially relevant biotransformation.



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