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SEMI-RATIONAL ENGINEERING OF Adh2 FOR IMPROVED METHANOL UTILIZATION IN *KOMAGATAELLA PHAFFII*

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Komagataella phaffii is a yeast species of high biotechnological interest in part due to its ability to utilize methanol to generate both biomass (assimilation) and energy (dissimilation). The first reaction of the methanol utilization pathway in *K. phaffii* is carried out by the alcohol oxidase enzymes AOX1 and AOX2, which catalyze the oxidation of methanol to formaldehyde in the peroxisome (Cregg 1989). It was recently demonstrated that in the absence of AOX1 and AOX2, *K. phaffii* remains able to utilize methanol through the native alcohol dehydrogenase 2 (Adh2) enzyme, significantly increasing in the amount of cellular energy (NADH) harvested through the dissimilatory pathway (Zavec 2021). However, due to low activity towards methanol, Adh2 is unable to support a growth phenotype on methanol in the absence the AOX genes. We therefore aim to use semi-rational enzyme engineering to produce variants of Adh2 with improved in vivo activity towards methanol. The selection of targets for site saturation mutagenesis (SSM) is informed by molecular dynamics simulations of the substrates and products of the reaction with the crystal structure of *K. phaffii* Adh2 (Zhang 2018). A high-throughput screen (HTS), enabled by a novel in vivo biosensor coupled with flow cytometry, is used to assess the performance of the enzyme variants in the native environment. Here we present an iterative, computer-aided enzyme engineering workflow, demonstrating a combination of state-of-the-art technologies for the improvement of methanol utilization in *Komagataella phaffii*.

Artistic rendition of Alcohol Dehydrogenase 2 from K. phaffii. (Produced by Diethard Mattanovich 2021)

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