A HYPERSTABLE GLYCOSYLTRANSFERASE FOR BLUE DENIM DYEING

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Indigo is the most used dye for blue denim worldwide[1]. Its synthesis and the dyeing process require chemical steps that are environmentally damaging, including the use of reducing agents and alkali for indigo solubilization. Different approaches aim at replacing the harmful processes with ecologically attractive alternatives, but the economic and social aspects of sustainability are often overlooked, resulting in poor implementation[2,3,4]**.** The glycosyltransferase PtUGT1 adds a glucose moiety to the reactive indigo precursor indoxyl to form indican, preventing spontaneous oxidation and keeping the dye-precursor soluble[5]. This reaction could lead to a chemoenzymatic approach to replace the use of strong reducing agents during the dyeing process. To make this strategy economically feasible, we performed a Techno-Economic Assessment (TEA), which pinpointed that the main parameters to decrease reaction costs would be to replace the buffer for water, and to increase substrate concentration above 80 mM. Unfortunately, PtUGT1 is inactive at this substrate concentration (Fig. 1B). Leveraging the structural information of PtUGT1 obtained by X-ray crystallography (PDB ID: 5nlm)[2], we therefore rationally designed 108 mutants to increase enzyme stability to tolerate the required high substrate concentration. As a result we have developed several active PtUGT1 variants with up to 15°C increase in melting temperature (T_mB) (Fig. 1A), which correlated with increased chemo-stability and a strong reduction in substrate deactivation at the required concentrations. This allowed the biocatalytic synthesis of indican from up to 100 mM indoxyl with a 65% yield (Fig. 1B). Further, Life Cycle Assessment (LCA) based on the updated TEA showed that this chemo-enzymatic approach would outperform the current process in 13 of the 18 parameters analyzed, including global warming, ionizing radiation and ozone depletion, among others (Fig. 1C). Overall, our results emphasize the power of integrating technical and sustainability aspects from the beginning of enzyme engineering efforts.

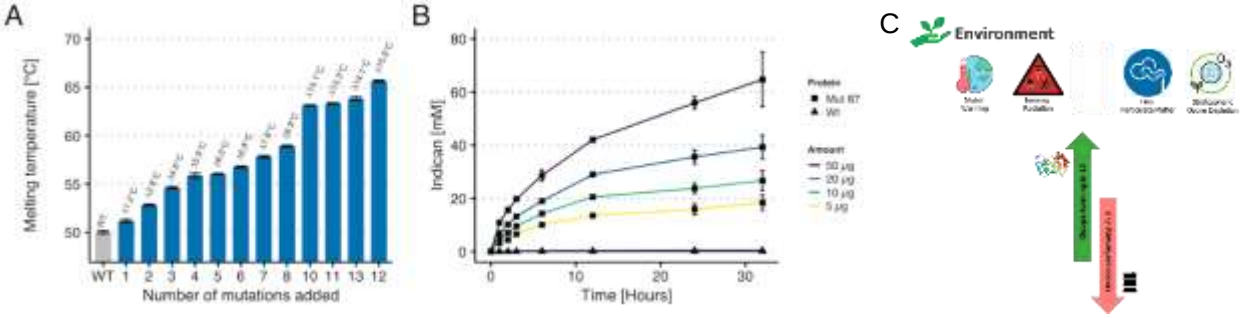

Figure 1– A) DSF comparing T*mB of PtUGT1 WT and designed mutants. B) Kinetics of indican synthesis using 100 mM indoxyl-acetate as substrate. C) Environmental impacts of chemo-enzymatic approach compared with current process.*

References:
1) Balfour-Paul, J. Indigo (Firefly Books, 2011).
2) Božič, M.; Kokol, V. Dye. Pigment. 2008, 76 (2), 299–309.
3) Berry, A *et. al*. J. Ind. Microbiol. Biotechnol. 2002, 28 (3), 127–133.
4) Rai, S.; Saremi, R.; Sharma, S.; Minko, S. Green Chem. 2021, 23 (20), 7937–7944.
5) Hsu TM *et. al*. Nat Chem Biol. 2018 Mar;14(3):256-261.