

## RATIONAL ENGINEERING OF A HYPERSTABLE GLYCOSYLTRANSFERASE FOR BLUE DENIM DYEING

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Key Words: Rational engineering, Thermostability, Glycosyltransferases

Indigo is one of the most used dyes to produce the textile blue denim worldwide<sup>1</sup>. Its synthesis and the dyeing process require chemical steps that are environmentally damaging, including the use of reducing agents for indigo solubilization. The glycosyltransferase PtUGT1 is able to add a glucose moiety to the reactive indigo precursor indoxyl to form indican, preventing spontaneous oxidation and keeping it soluble<sup>2</sup>. This strategy could be used in a chemoenzymatic approach to replace the use of reducing agents, but in order to do this it's necessary that PtUGT1 resist, and to be active under the harsh conditions used in the industrial process, including high pH and high temperature. We have characterized the activity profile of PtUGT1 at different pH values and temperatures and have determined the enzyme stability by differential scanning fluorometry (DSF) and residual activity. Leveraging the structure information of PtUGT1 obtained by X-ray crystallography (PDB ID: 5nlm)<sup>2</sup>, we have rationally designed different mutants to develop a variant adapted to higher temperatures and pH values, including hypothetical residue pair mutants that could lead to the formation of intramolecular disulfide bridges, and mutants that could either improve the hydrophobic packing, lead to formation of polar interactions or improve Pro/Gly ratio, consequently increasing the rigidity/stability of PtUGT1. As a result we have developed several active PtUGT1 variants with up to 15°C increase in their melting temperature (T<sub>m</sub>B) (Fig. 1), the highest ever reported for an UDP-dependent glycosyltransferase.

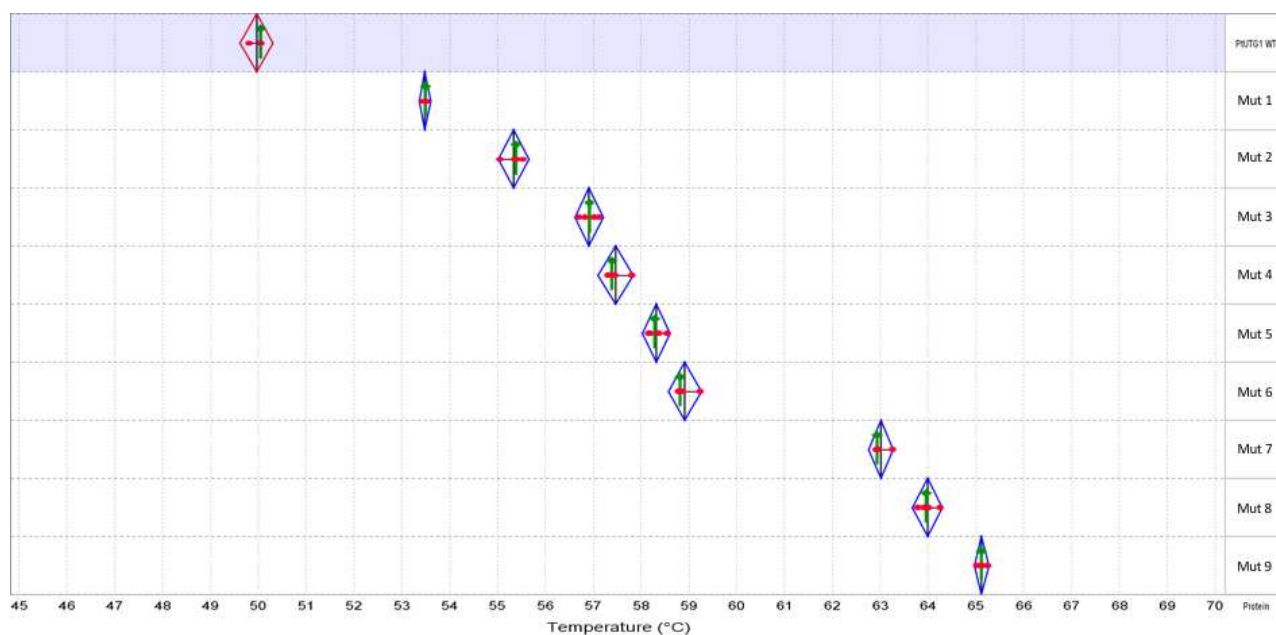


Figure 1 – DSF comparing T<sub>m</sub>B of PtUGT1 WT and Mutants designed.

References: 1) Balfour-Paul, J. Indigo (Firefly Books, 2011).  
2) Hsu TM, Welner DH, Russ ZN, Cervantes B, Prathuri RL, Adams PD, Dueber JE. (2018) Employing a biochemical protecting group for a sustainable indigo dyeing strategy. Nat Chem Biol. 2018 Mar;14(3):256-261