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Knowledge-based directed evolution for heat stabilization of an ω-Transaminase

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High valuable β-amino acids as parts of pharmaceuticals (e.g. Paclitaxel), can be produced by a several chemical or chemo-enzymatic reactions. Recently, a synthesis strategy was proposed to synthesize Sitagliptin via a β-amino acid [1]. All existing industrial synthesis strategies have the disadvantage that the maximal yield of an optically pure enantiomere of a β-amino acid is only 50 % (kinetic resolution). A promising approach to increase the yield is the synthesis of β-amino acids by asymmetric synthesis by enantioselective enzymes, like the ω-Transaminase (TA). However, two major challenges are accompanied with this strategy :

1) Long time thermostability of the ω-TA.
2) Stability of the reactants (figure 1).

Solution concepts

- Enzymatic cascade reaction to overcome the problem of instable reactants and to increase the optical purity.
- Using energy calculations for every amino acid position in the enzyme to increase the thermostability by site directed mutagenesis.

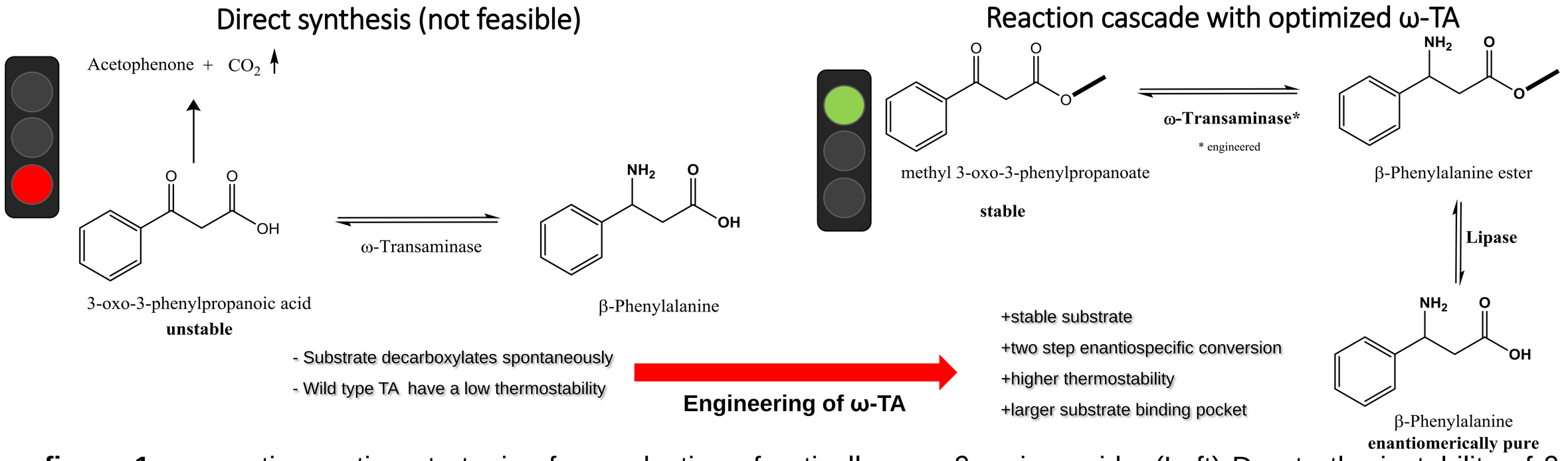

figure 1 enzymatic reaction strategies for production of optically pure β-amino acids. (Left) Due to the instability of β-keto acids, is this synthesis not feasible. (Right) enzymatic cascade reaction of a β-keto acid ester (stable) to an β-amino acid. Additional figures of the active site of ω-TA are available under this QR-code:

Stability improvement:

I. For the reduction of possible sites for mutagenesis of the ω-TA we used the force field algorithm **FoldX** [2].
This algorithm based on a linear combination of empirical terms to calculate free energy (in kcal mol^{-1}). We exchanged every proteinogenic amino acid (AA) against every AA.

$$\Delta G = a * \Delta G_{vdw} + b * \Delta G_{solvH} + \cdots$$

The result is a list of potential beneficial AA exchanges in the enzyme, which will be tested by activity tests at different temperatures for each mutation.
II. Creation of additional disulfide bonds inside of the protein by MD-simulations.
III. Combination of beneficial mutations of I and II to yield a considerably higher thermostability [3] .

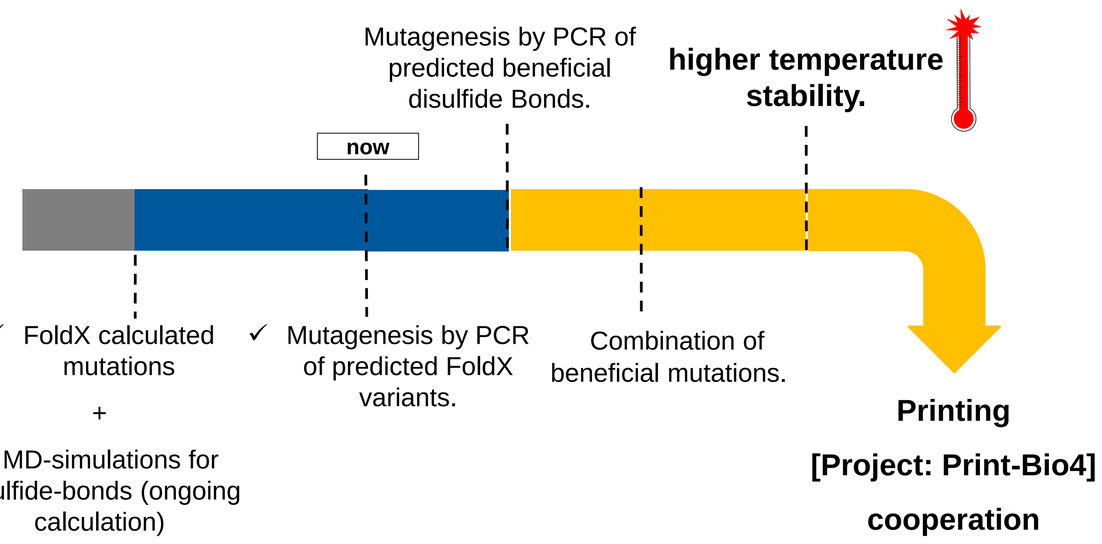

figure 2 Strategy for improvement of ω-TA.

Aims:

- ❑ **Enhance temperature- and long time stability for printing in cascade reaction systems. (PB-4)**
- ❑ **Extend substrate spectrum of the ω-TA by semi-rational protein engineering.**

References

[1] Mathew *S.et* al..Asymmetric synthesis of aromatic β-amino acids using ω-transaminase: Optimizing the lipase concentration to obtain thermodynamically unstable β-keto acids. Biotechnology Journal (accepted), 2015.

[2] Schymkowitz J, Borg J, Stricher F, Nys R, Rousseau F, Serrano L. The FoldX web server: an online force field. Nucleic Acids Research. 2005;33.

[3] Wijma, Hein J. et al. "Computationally Designed Libraries for Rapid Enzyme Stabilization." Protein Engineering, Design and Selection 27.2 (2014): 49–58. PMC. Web. 16 Nov. 2015.

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