Engineering *Luminiphilus syltensis* (*R*)-selective amine transaminase for the acceptance of bulky substrates

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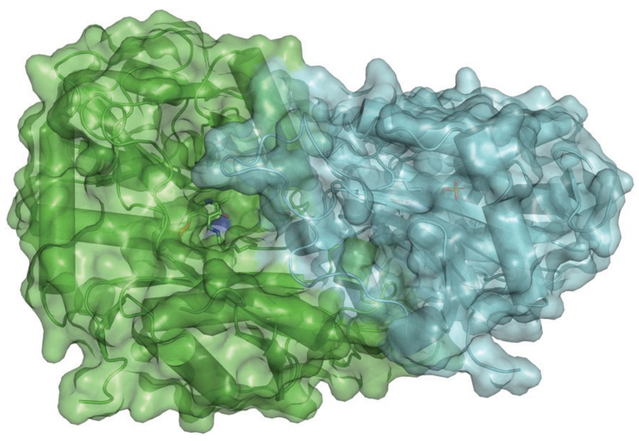
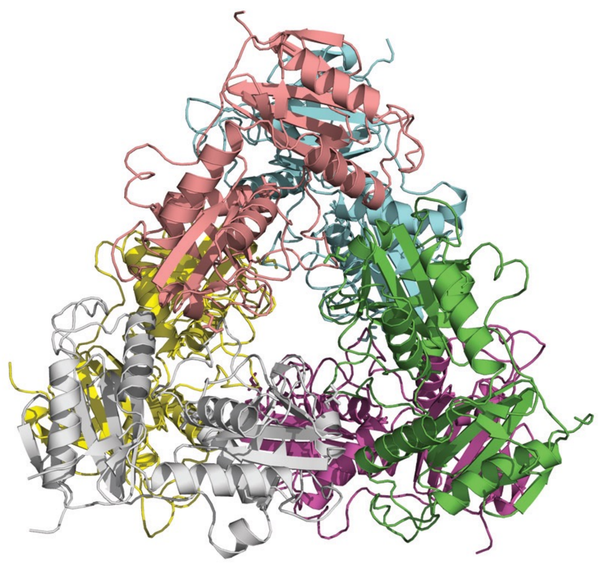
Background

Despite having a good understanding of the (*S*)-selective amine transaminases (ATAs) and ways to evolve them, the (*R*)-selective ATAs are not as well-studied. The ATA from *Luminiphilus syltensis* (LS_ATA) was one of the first (*R*)-selective ATAs identified,[1] but its application is still hindered from the lack of structural insight. Herein, we present the structure elucidation on this ATA, which guided our engineering efforts towards variants active on bulkier substrates. Via bioinformatic analysis, we identified positions of interest in the small binding pocket and the variants were prepared, expressed, purified and subsequently characterized.

The tertiary structure of (*R*)-selective ATA from *Luminiphilus syltensis*

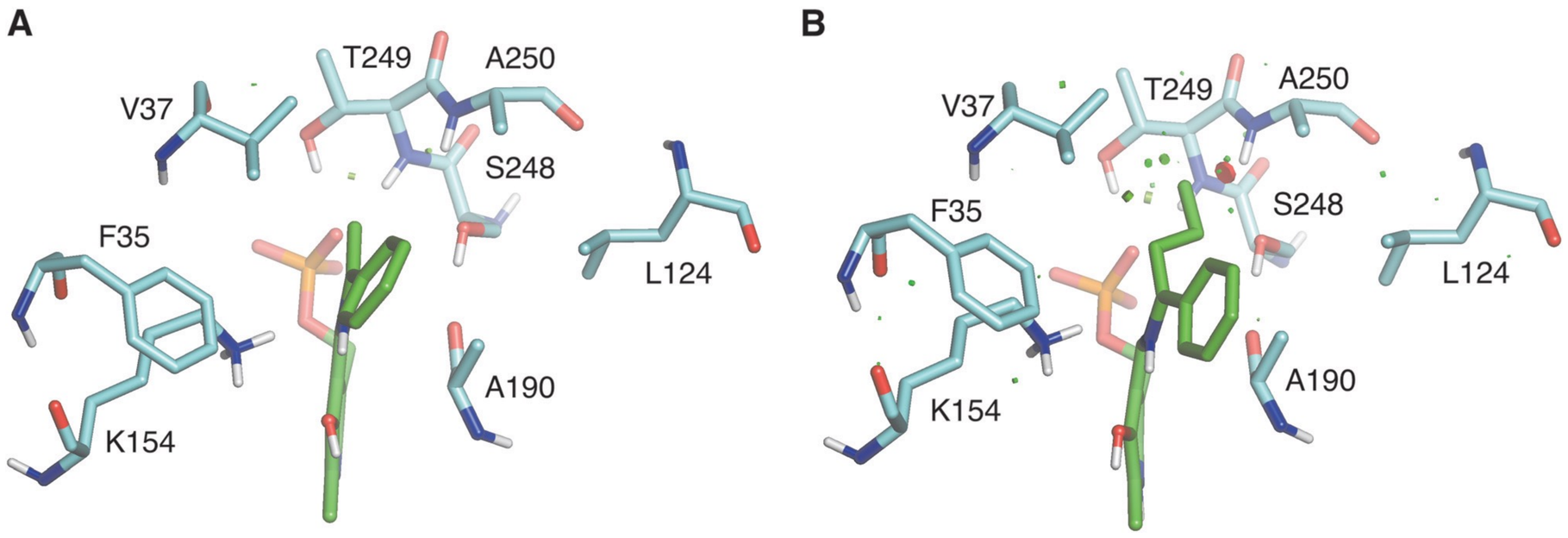
LS_ATA crystallized in hexameric form, with three homodimers, forming the active site on the interface between the two chains (PDB code: **7P3T**). No other multimer was identified with gel filtration and native gel, supporting the crystallographic results. Other known (*R*)-ATAs exist in tetrameric or hexameric form.[2,3]





Examination of the active site

- The quinonoid intermediate of (*R*)-1-phenylethylamine (**A**) is firmly held in the active site by polar contacts.
- The methyl group of (*R*)-1-phenylethylamine is oriented in the “small” binding pocket, without any significant clashes.
- The propyl (**B**) group of (*R*)-1-phenylbutylamine has unfavorable contacts with V37, S248, T249, A250
- Positions V37, S248 and T249 were selected for mutagenesis, to provide more space in the small binding pocket.

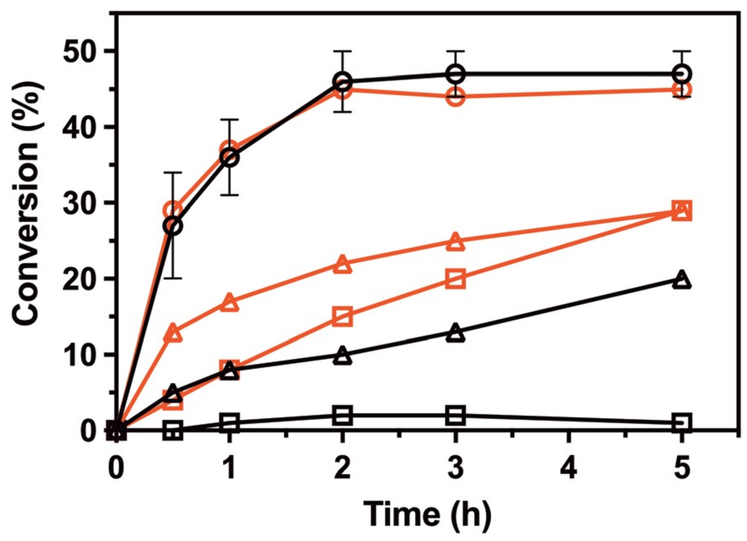


Alanine screening was performed in positions 37, 248, 249, and the variants were characterized in kinetic resolution mode against (*R*)-1-phenylethylamine (**circle**), (*R*)-1-phenylpropylamine (**triangle**) and (*R*)-1-phenylbutylamine (**square**).[4]

S248A and T249A variants, positions located on the β-turn of the P-pocket, lost their activity, as they are not able to even form PMP (probably due to loss of PLP coordination[5]).

V37A (red lines) improved the minimal activity the wild-type (black) has against (*R*)-1-phenylpropylamine, and even enabled the acceptance of (*R*)-1-phenylbutylamine.

Experimental proof



Conclusion

- The structure of LS_ATA, a unique enzyme in terms of sequence, was elucidated.
- Mutations on the *β*-turn of the P-pocket are not tolerated and lead to inactive variants.
- V37 seems an interesting (*R*)-ATA for accessing variants active towards bulky substrates.

References

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