Enhancing non-canonical amino acid incorporation towards enzyme engineering upgrading Genetic code expansion tool improvement towards biocatalytic reprogramming

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Genetic code expansion tool improvement towards biocatalytic reprogramming

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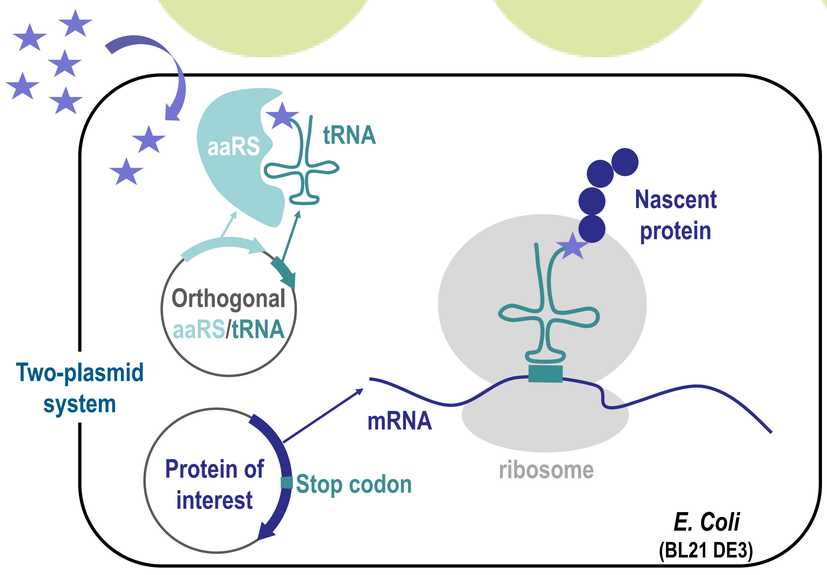
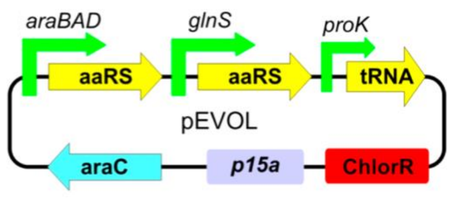
Background

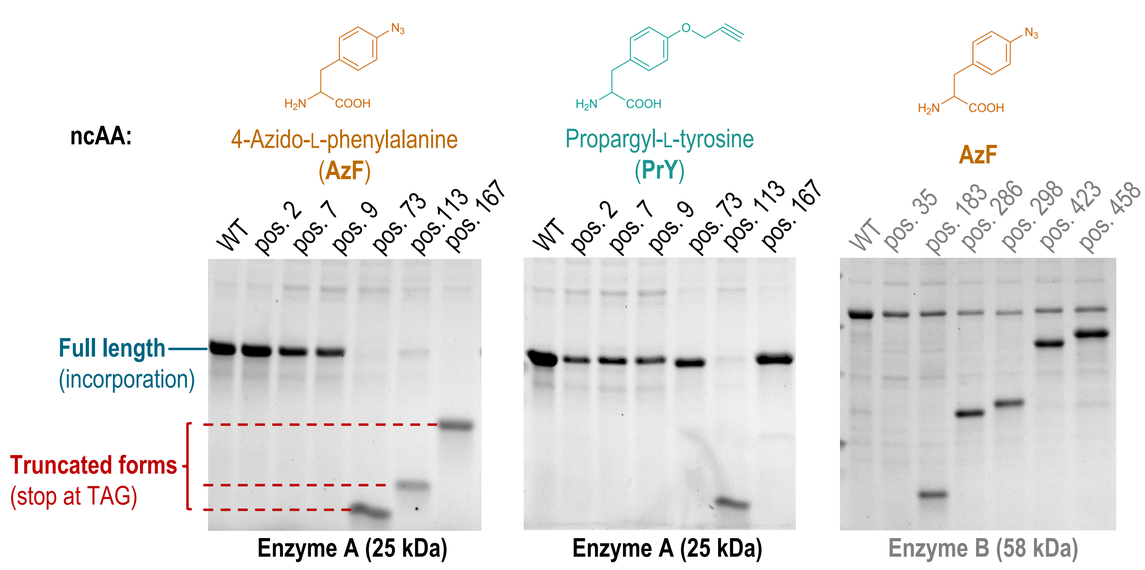
Enzyme engineering benefits from the > 150 non-canonical amino acids (ncAAs) available for the introduction of non-naturally encountered chemical functions [1]. NcAAs may be incorporated at a target position of a protein of interest by genetic code expansion. This method is based on the reassignment of a nonsense codon to an ncAA by introducing an orthogonal amino-acyl tRNA synthetase (aaRS)/tRNA pair. In *E. coli*, the pEVOL system is the historical and most widely used [2]. The pUltra system allows improved incorporation efficiencies in some conditions and can be combined with the pEVOL system for the incorporation of two different ncAAs [3].



Improving the incorporation efficiency

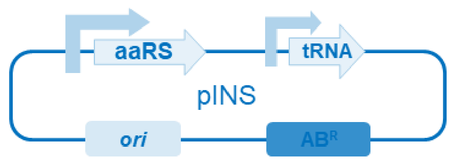


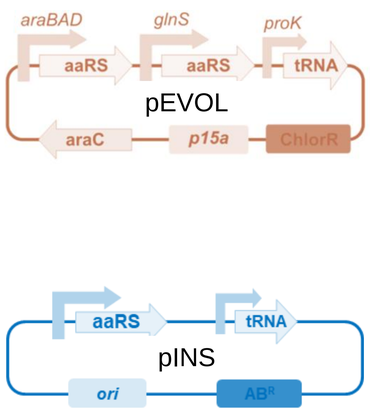
The incorporation efficiency depends on:
- the incorporation position
- the nature of the ncAA
- the protein

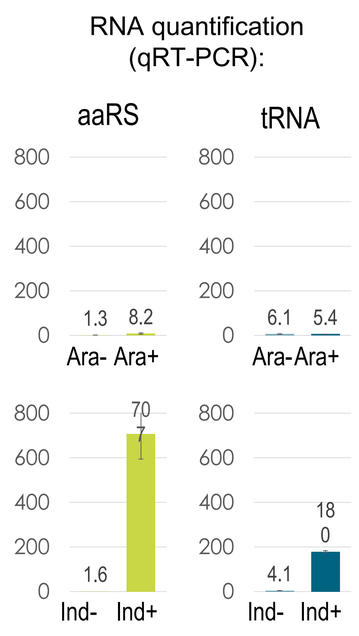


The incorporation efficiency can be improved by:
- aaRS and tRNA engineering
- strain engineering
- modulation of the aaRS and tRNA expression levels

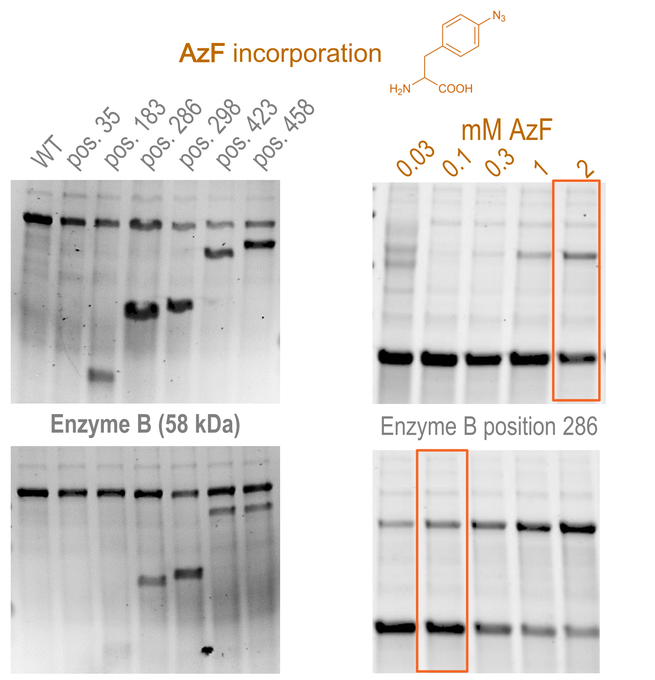
→ New inducible orthogonal expression system: **pINS**





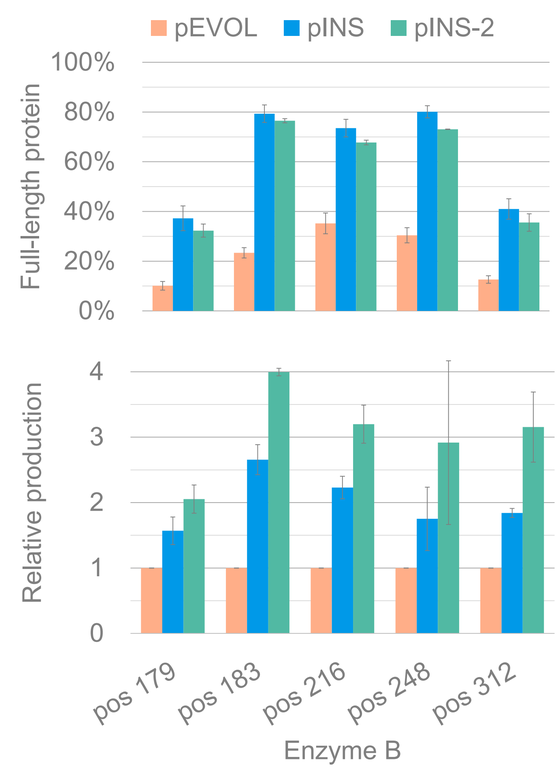


↗ aaRS and tRNA expression

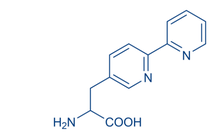


↗ incorporation efficiency
↘ position bias
↘ [ncAA] needed

pINS-2: modification of the aaRS/tRNA ratio



Incorporation efficiency x3



2,2'-Bipyridin-5-yl-alanine (**BpyA**)

Production up to x4

References

[1] Agostini *et al.* (2017). Biocatalysis with Unnatural Amino Acids. *Angew Chem Int Ed* **56**(33):9680-9703
[2] Young *et al.* (2010). An Enhanced System for Unnatural Amino Acid Mutagenesis in *E. coli*. *J Mol Biol* **395**(2):361-374
[3] Chatterjee *et al.* (2013). A Versatile Platform for Single- and Multiple-Unnatural Amino Acid Mutagenesis in *Escherichia coli*. *Biochemistry* **52**(10):1828-1837

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