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# 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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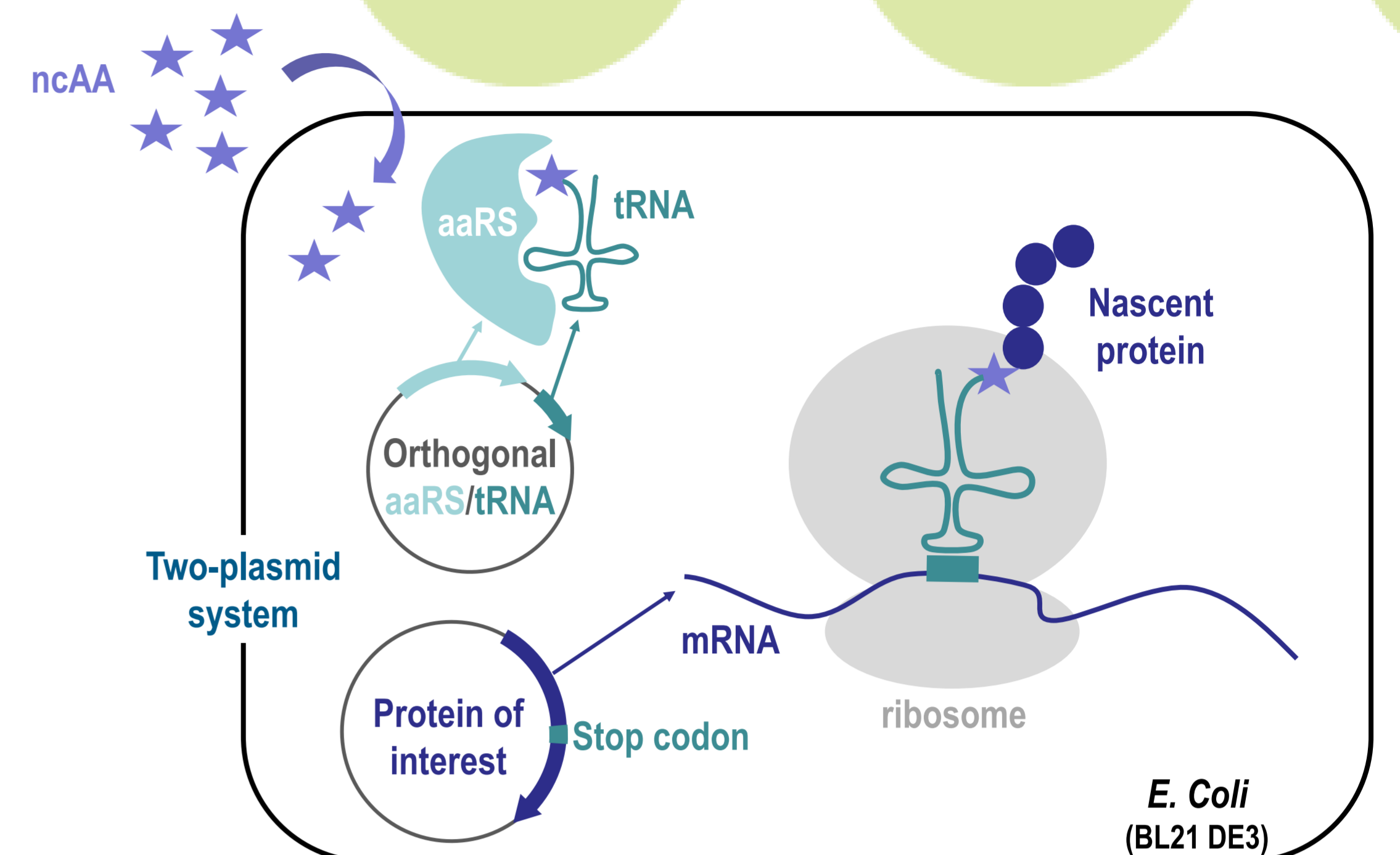
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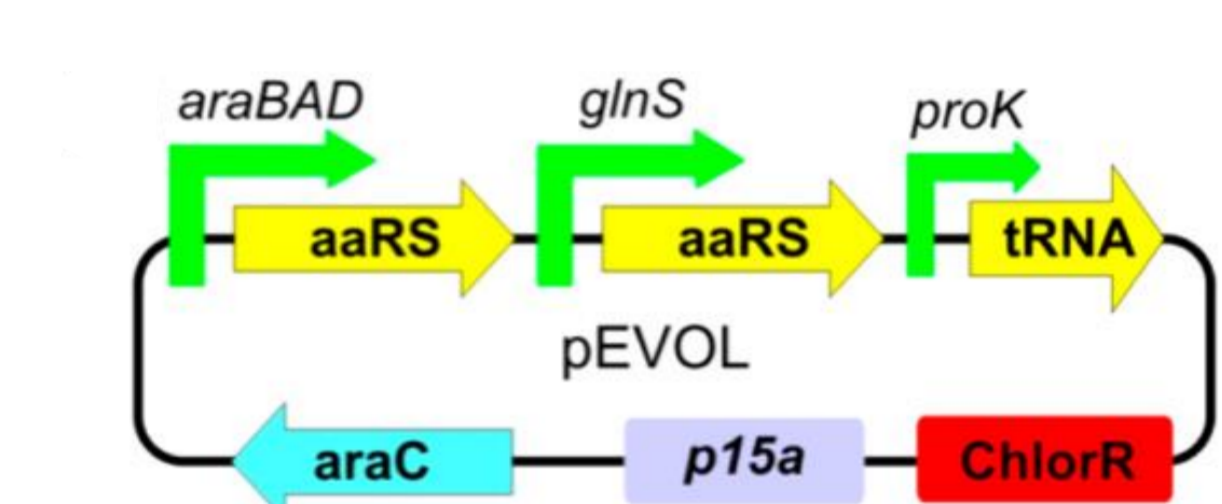


## Background

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].

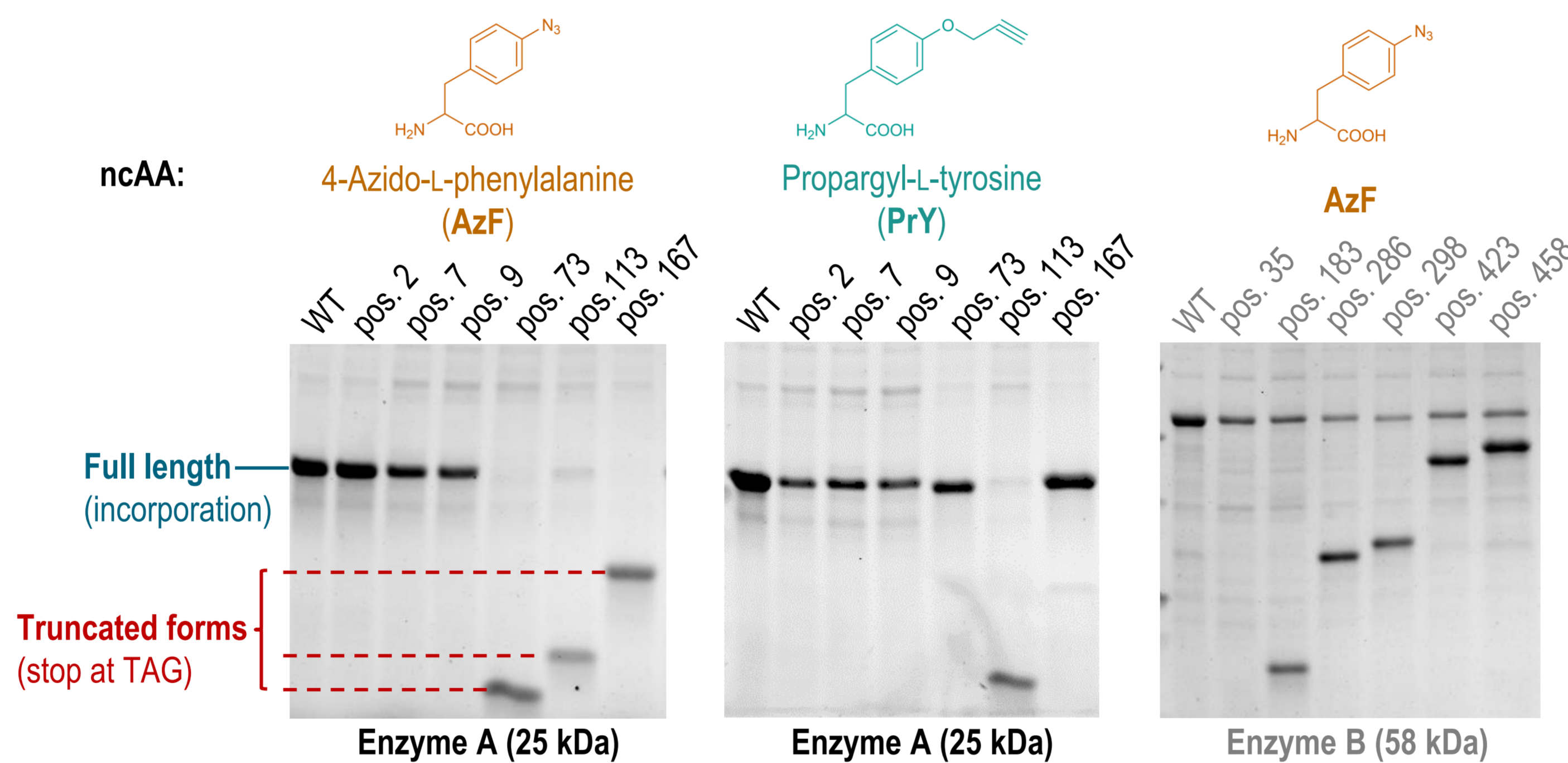


## Improving the incorporation efficiency



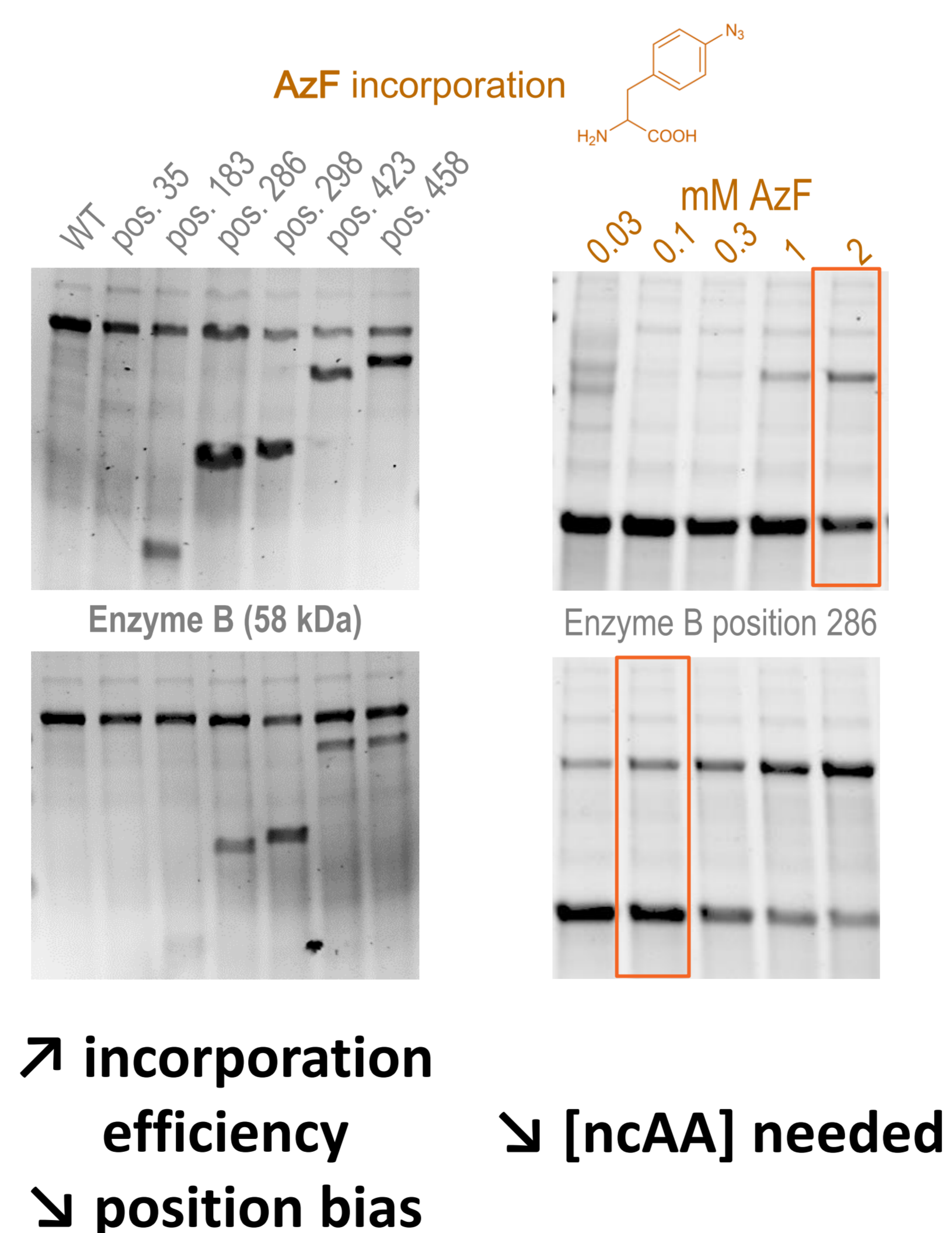
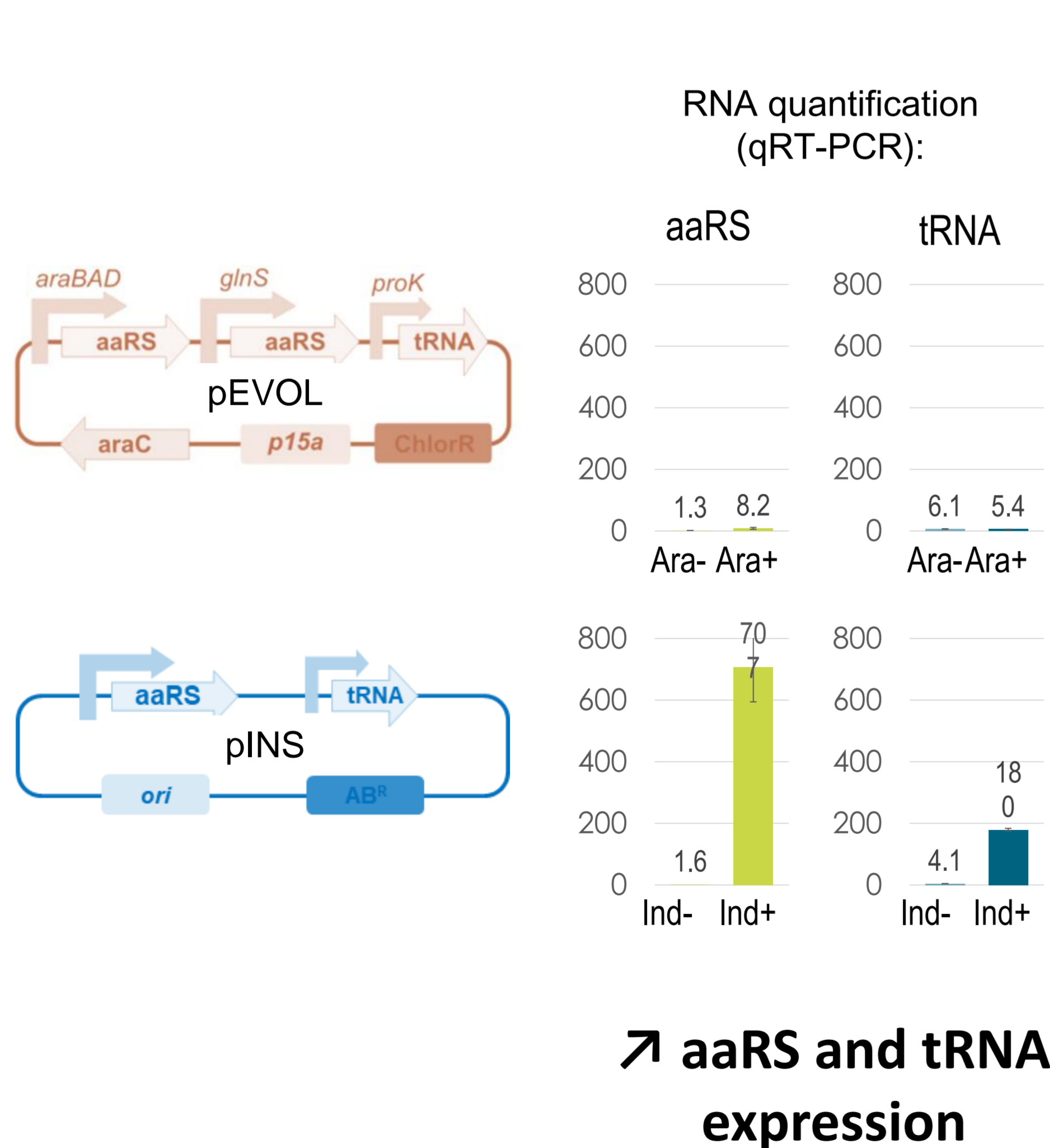
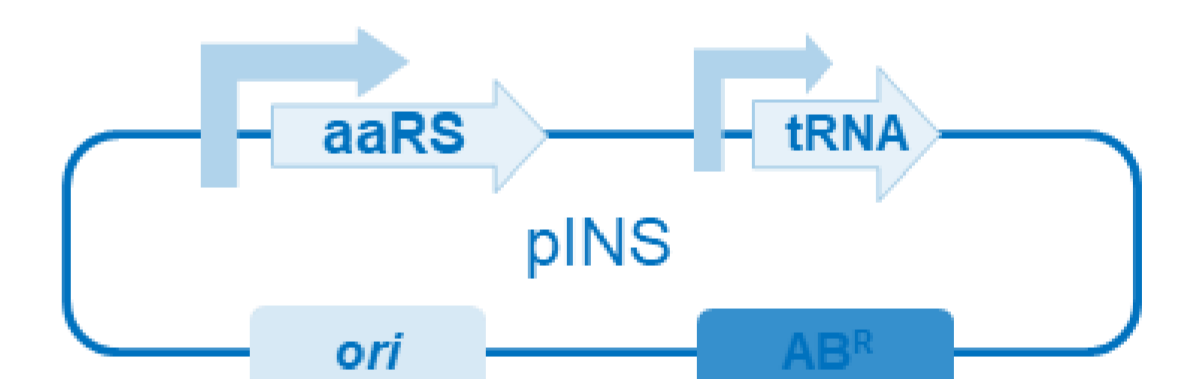
The incorporation efficiency depends on:

- the incorporation position
- the nature of the ncAA
- the protein

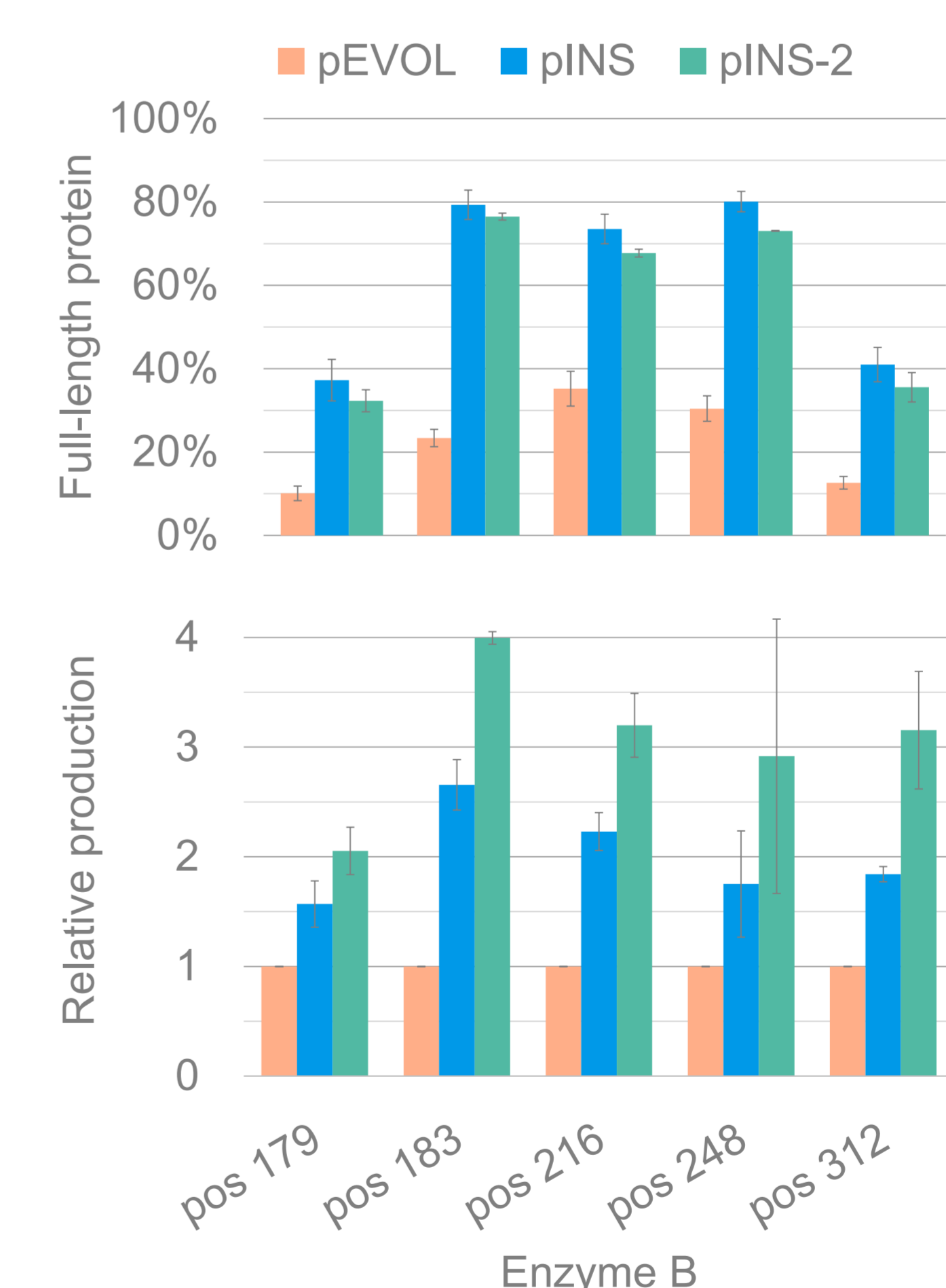


The incorporation efficiency can be improved by:

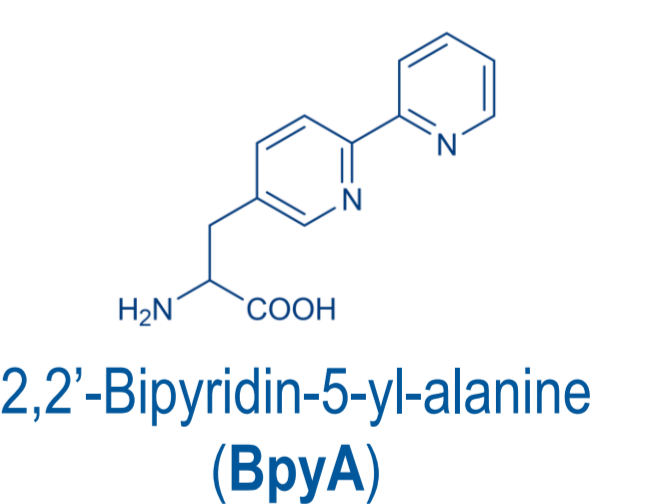
- aaRS and tRNA engineering
  - strain engineering
  - modulation of the aaRS and tRNA expression levels
- New inducible orthogonal expression system: **pINS**



## pINS-2: modification of the aaRS/tRNA ratio



Incorporation efficiency x3



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