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Developing an Unnatural Amino Acid-Specific Aminoacyl tRNA Synthetase

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Introduction

Unnatural Amino Acids (UAAs), amino acids not present in the standard genetic code, have been synthesized to have a broad range of useful properties, particularly as probes and sensors which can be integrated directly into a protein. In this work, 3-(2-Pyridyl)-L-Alanine is the target UAA to place into bacterial proteins because it is commercially available and binds Cu^{2+} ions, which may have application in metallodrugs or sensing by protein conformation change of metal binding.[1,2] By introducing a mechanism for site-specific incorporation of metal-binding function, applications using this function could later be developed.

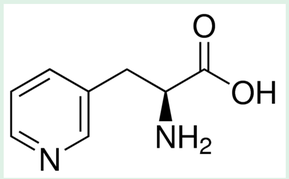
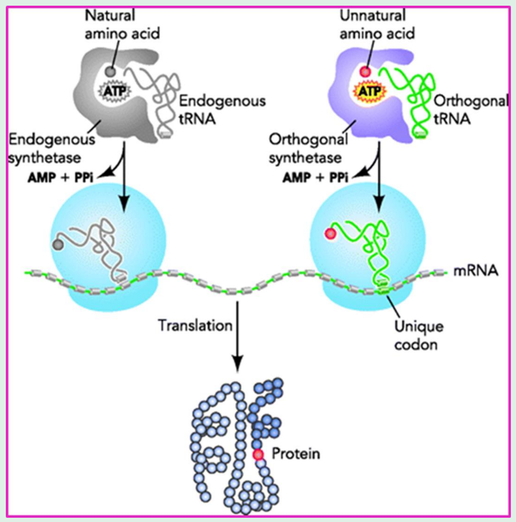


Figure 1: Structure of 3-(2-Pyridyl)-L-Alanine

Figure 2: Outcome of successful charging of a UAA to a tRNA. If an amino acid is successfully charged, inserted amber codon is suppressed and a functional protein is built with the UAA at the mutation site.



In order for the cell to place a UAA into the protein, two components, an aminoacyl tRNA synthetase (aaRS) and a corresponding tRNA must be present. A tRNA which places a UAA into a protein in response to a stop codon, in this case, the TAG codon has previously been developed.[3] In addition, many synthetases which charge a specific UAA to the tRNA have been made. In this work, the range of UAAs which can be incorporated into proteins using the E. coli's own machinery is expanded by the development of novel aminoacyl tyrosine tRNA synthetases originating from *Methanococcus jannaschii*. By making a library of synthetase-coding plasmid variants and performing positive and negative screenings using the amber suppression method, the binding pocket of the synthetase can be modified for specificity to a UAA while not allowing the tRNA to be charged with a natural amino acid.

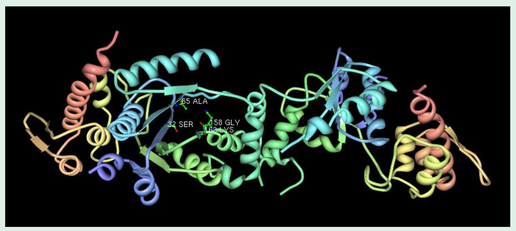


Figure 3: Structure of Tyr-aaRS with some amino acids in binding pocket labeled.

Desired Mutations and Methods

Positive and Negative Screenings	aaRS Library Creation
eGFP Positive Screening Plasmid • TAG mutation was made at position 151. This mutation stops production of green fluorescent protein, but incorporation of UAA by a tRNA-aaRS overrides this mutation. Therefore, fluorescent colonies indicate incorporation of an amino acid due to suppression of the TAG codon. **Barnase Negative Screening Plasmid** • TAG codon was made by creating a G→A mutation at position 93. This mutation inhibits the production of barnase, a toxic protein. Cells are grown with barnase production induced, but no UAA. If nonspecific incorporation occurs, the cell dies, but if the aaRS is specific for UAA, the cell does not produce barnase and lives.	A random mutant library was created using saturation mutagenesis. Using four primers, five randomized amino acid sites were incorporated into the binding pocket of an existing UAA aaRS (p-cyanophenylalanine aaRS). The first two letters of each codon were randomized. • **L32** (CTG→NNG) • **V65** (GTT→NNT) • **W108** (TGG→NNG) • **G158** (GGT→NNT) • **A159** (GCT→NNT) Each individual variant will be cotransformed with a screening plasmid, and the variant will be tested for specific incorporation of the target UAA.

Discussion

Much work remains in making this effort produce a viable aaRS for3-(2-Pyridyl)-L-Alanine. Firstly, while all mutations have been attempted, the only one confirmed by sequencing is the positive selection mutation. The rest must be produced in sufficient quantity for sequencing before they can be cotransformed into a permanent cell line (BL21(DE3)).

If all library colonies contain different mutations, there are currently ten variants being tested. More may be needed to find a variant which is selective to UAAs. Once a successful aaRS variant is found, it can be tested for permissivity to other UAAs using a pre-existing method. Other future work could include testing the metal-binding capacity of the UAA in an out of a protein.

References

[1]Zawisza, Izabella; et. Al. The impact of synthetic…. *J. Inorg. Biochem*. **2014**, *139*, 1-8.

[2]Drienovska, Ivana; et. Al. Design of an enantioselective artificial metallohydratase enzyme…. *Chem. Sci*. **2017**, *8*, 7228-7235.

[3]Xie, Jianming; Schultz, Peter. An expanding genetic code. *Methods* **2005**, *36*, 227-238.

Acknowledgements

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pMT413 (barnase/barstar) was a gift from Robert Hartley (Addgene plasmid # 8606) (*J. Mol. Biol.* 1988)

pET GFP LIC cloning vector (2GFP-T) was a gift from Scott Gradia (Addgene plasmid # 29716)

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