Using E.Coli to Incorporate Fluorescent Unnatural Amino Acids into Proteins

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The goal of this work is to identify unnatural amino acids (UAAs) that can be incorporated into proteins by E. coli using four orthogonal aminoacyl-tRNA synthetase/suppressor tRNA (aaRS/tRNA) pairs. A fluorescence screening assay was used to test the incorporation of thirteen UAAs into proteins using four aaRS/tRNA pairs that had been previously shown to possess varying degrees of promiscuity. The screen utilized a mutant green fluorescent protein (GFP) that included the amber stop codon within the open reading frame. Multiple results demonstrated that aaRS/tRNA pairs are able to bind other UAAs to a level greater than the UAA designed to match that synthetase, yet all UAA mutants had significantly lower incorporation than the wild type GFP control (UAA absent). Correlation of the relative incorporation levels versus the UAA structures provides a basis for understanding the shape and flexibility of the synthetase binding pocket and thus provides experimental data that can be used to evaluate molecular modeling results.

**Screening Assay**

*E. coli* contains two plasmids: 1) an aminoacyl tRNA synthetase (aaRS) plasmid and 2) either a wild type or 151 mutated green fluorescent protein (GFP) plasmid. The role of the aaRS is to catalyze the reaction shown in equation 1.

\[
\text{UAA} + \text{aminoacyl tRNA} \rightarrow \text{AA-tRNA} + \text{aminoacyl synthetase}
\]

If the reaction shown in eq 1 is successful, then the UAA will be incorporated into fully functional mutant GFP. If eq 1 fails, then the mutation will result in a truncated, non-fluorescent GFP (eq 2).

\[
\text{mRNA for GFP} \rightarrow \text{growing GFP chain} \rightarrow \text{release factor} \rightarrow \text{ribosome} = \text{protein manufacture factory} \rightarrow \text{truncated GFP (non-fluorescent)}
\]

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**Abstract**

**Fluorescence Data**

**Discussion and Conclusion**

- UAA incorporation efficiency not simply related to molecular weight.
- Abu and p-CN-F synthetases incorporated multiple UAAs more efficiently than the “matching” UAA.
- Both P-CN-F and Abu synthetases incorporated UAAs 3, 4 & 7 better than the “matching” UAA.
- Previous research showed UAA 3 incorporation at greater than the wild type control. Subtracting background fluorescence decreased incorporation to less than 50% of the wild type control, suggesting a significant influence of background fluorescence.
- None of the UAAs incorporated at 50% the wild type control (except UAA 2 with its matching synthetase). Although some UAAs incorporated more efficiently than the “matching” UAA, the absolute incorporation levels were low.

**Future Work**

- Screen additional UAAs.
- Use incorporation efficiency to map the binding pocket.

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**References**

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