**Towards genetic incorporation of an Orthogonal Ribosome-tRNA pair and D-amino-acids in *E.coli*.**

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**Project Goals: Interactions between the acceptor arm of tRNA and the active-site of ribosome is characterized by a set of Watson-crick base-pairs, conserved across all three domains of life on Earth. By exploring alternative ribosome-tRNA base-pairing interactions at this conserved loci, we have begun to lay the foundations for operationalizing a fully orthogonal genetic code in *E.coli.* Finally, we have also established a robust and sensitive analytical pipeline for detection of D-amino-acids at various stages of translation such as tRNA amino-acylation and in target proteins.**

Functionally mature tRNAs across all domains of life of have a conserved terminal 3'-CCA trinucleotide in their acceptor arm. The 3'-CCA terminus of the tRNA engages in a highly-conserved set of Watson-crick base pair interactions with the Peptidyl-transferase centre (PTC) of the Ribosome, as it moves from the A-site to the P-site during the transpeptidation step [1]. Additionally, the integrity of 3'-CCA terminus is subject to surveillance by a host of tRNA processing machineries[2]. We have genetically engineered *E.coli* strains that obviate 3'-CCA tRNA surveillance and repair and have identified a subset of amino-acyl tRNA synthetases that can amino-acylate mutant tRNA acceptor ends. To further aid our efforts in screening for engineered synthetases that can act on mutant tRNA acceptor ends, we are harnessing T-box riboswitches as potential sensors[3]. Together with orthogonal ribosomes that carry compensatory mutations in their PTC to interact with variant tRNA acceptor arms, we are operationalizing a fully orthogonal genetic code in *E.coli*[4].

Finally, a number of barriers remain in the way of incorporating D-amino acids into proteins[5]. Towards addressing this, we have developed robust and sensitive analytical methods for detection of D-amino-acids at various stages of translation such as tRNA amino-acylation and incorporation into target proteins. By integrating these analytical methods with strain engineering and directed evolution of amino-acyl tRNA synthetases we are establishing strategies towards robust and efficient genetic incorporation of D-amino-acids in *E.coli.*

## References

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