Combining Sense and Nonsense Codon Reassignment for Site-Selective Protein Modification with Unnatural Amino Acids

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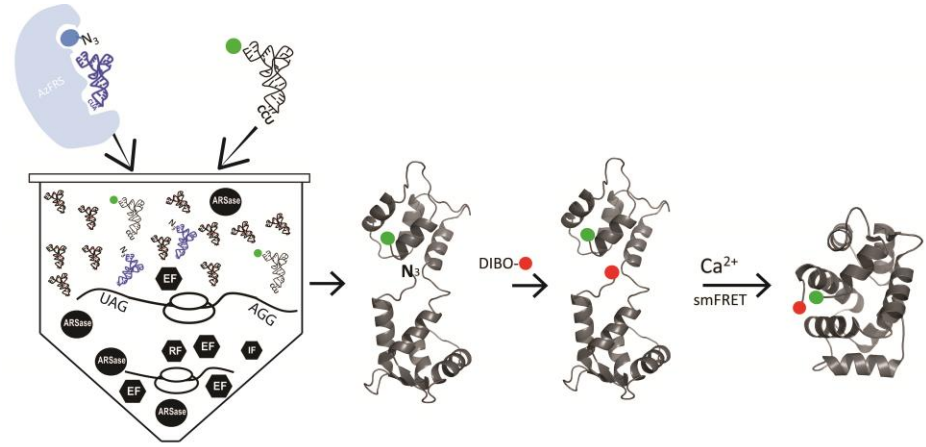
Incorporation of unnatural amino acids (uAAs) via codon reassignment is a powerful approach for introducing novel chemical and biological properties to recombinant polypeptides. However, the site-selective incorporation of multiple uAAs into polypeptides is hampered by the limited number of reassignable nonsense codons. To address this limitation we set out to orthogonalize several of the sense codons. We established a quantitative in vitro peptide translation assay that allows us to test the ability of synthetic tRNAs to decode any of 61 sense codons. The majority of synthetic *Escherichia coli* tRNAs could support protein translation. By formulating a semisynthetic tRNA complement or by using DNA-hybridization chromatography, we obtained an *E.coli in vitro* translation system devoid of specifc tRNA isoacceptors. Such system is dependent on the addition of synthetic tRNAs for the chosen sense codon to restore its translational activity. This allows multisite-selective uAAs incorporation that can be combined with amber-suppression. We successfully applied this approach to production of the calmodulin protein with FRET-forming fluorescent probes. The presented approach is universal and can be expanded to other sense codons and cell-free systems.



Cell free reaction without native tRNA isoacceptors for AGG codon