Tata Institute of Fundamental Research
Survey No. 36/P, Gopanpally Village, Serilingampally, Ranga Reddy Dist., Hyderabad – 500 046

Internal Webinar

Expansion of the Genetic Code: Development of Fluorescent Triazolyl Unnatural Amino Acids Encoded T7 RNA Polymerase, Superoxide Dismutase A, and β-Galactosidase, and Generation of Orthogonal Aminoacyl tRNA Synthetase Library

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Genetic code expansion (GCE) enables the site-specific incorporation of unnatural amino acids (uAAs) into proteins, thereby expanding their chemical and functional diversity. This thesis investigates the incorporation of fluorescent triazolyl unnatural amino acids (FTUAAs) into superfolder green fluorescent protein (sfGFP), T7 RNA polymerase, manganese-dependent superoxide dismutase (Mn-SodA), and β-galactosidase. Computational modelling and experimental studies demonstrated successful incorporation of FTUAAs while largely preserving protein structure and expression. The engineered proteins exhibited distinctive fluorescence properties and altered biological functions, including enhanced transcriptional activity, improved SodA catalytic performance, and site-dependent modulation of β-galactosidase activity. Metal-substitution studies further revealed the potential of FTUAA incorporation to fine-tune metalloprotein function. Additionally, a focused orthogonal aminoacyl-tRNA synthetase library was developed to improve the incorporation efficiency of bulky FTUAAs. Overall, this work establishes FTUAAs as versatile genetically encodable fluorophores for introducing optical and functional properties into proteins.

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14:30 Hrs