Amino Acid purification and Single-Molecule FRET study of tRNA translocation

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Background

Proteins are molecular machines that carry out vital tasks within cells. The cell utilizes ribosomes, a combination of RNA and protein, to manufacture these important machines. The ribosome reads an mRNA transcript in 3-nucleotide pairs which correspond to amino acids, the building blocks of proteins. Inhibiting such processes from occurring in deadly pathogens gives rise to the possibility of new anti-biotics. To do this, prior research must first be conducted to understand this process of translation and translocation which can be done through smFRET techniques. However, such techniques requires pure samples of amino acids for clear results. Below, we outline our procedure to purify Glutamic Acid and study its translocation through smFRET.

Methodology

tRNA Purification The $tRNA^{fmet}$ and $tRNA^{Phe}$ can be expressed in E. Coli cells and purified through size exclusion and ion exchange chromatography. $tRNA^{Glu}$ can be overexpressed in vivo by inserting the sequence of interest in a vector and transform into E. Coli cells.

Kinetics of peptide bond formation involving Glutamic acid

Mechanism of peptidyl transferase reaction with different status of tRNAs. Pre-complex and Post-complex are shown in ribosome A, P and E sites.

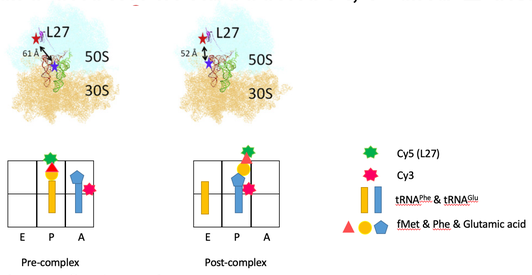


Diagram Created by Ran Lin

smFRET Principle Two laser beams, 532 nm and 640 nm are shot into the sample compartment at an angle producing total internal reflection (TIRF). The beams excite specific fluorophores, either Cy3 or Cy5, which then emit waves at a specific frequency exciting nearby different fluorophores producing FRET. Illumination signifies proximity between fluorophores and labeling sites.

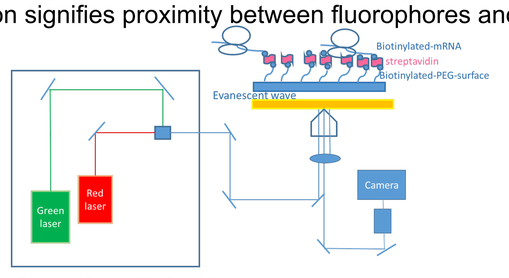
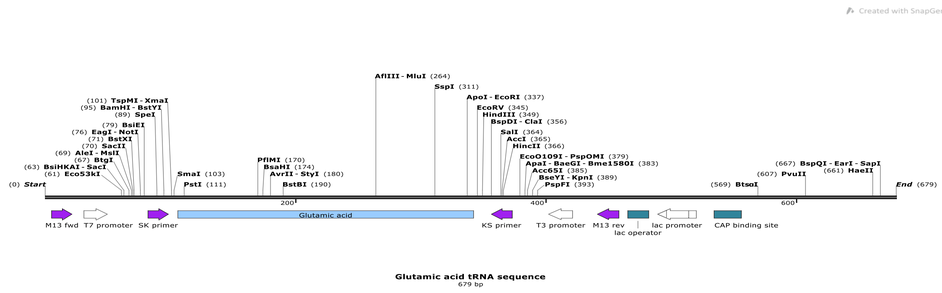


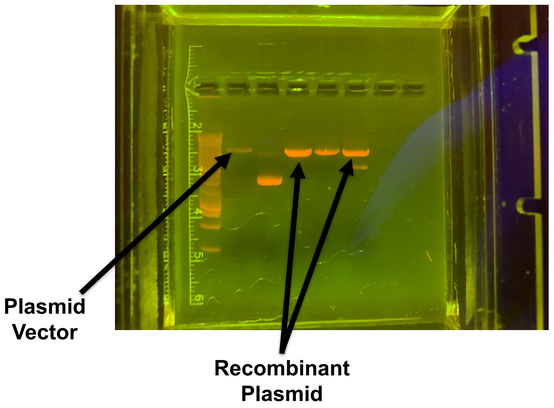
Diagram Created by Ran Lin

Results

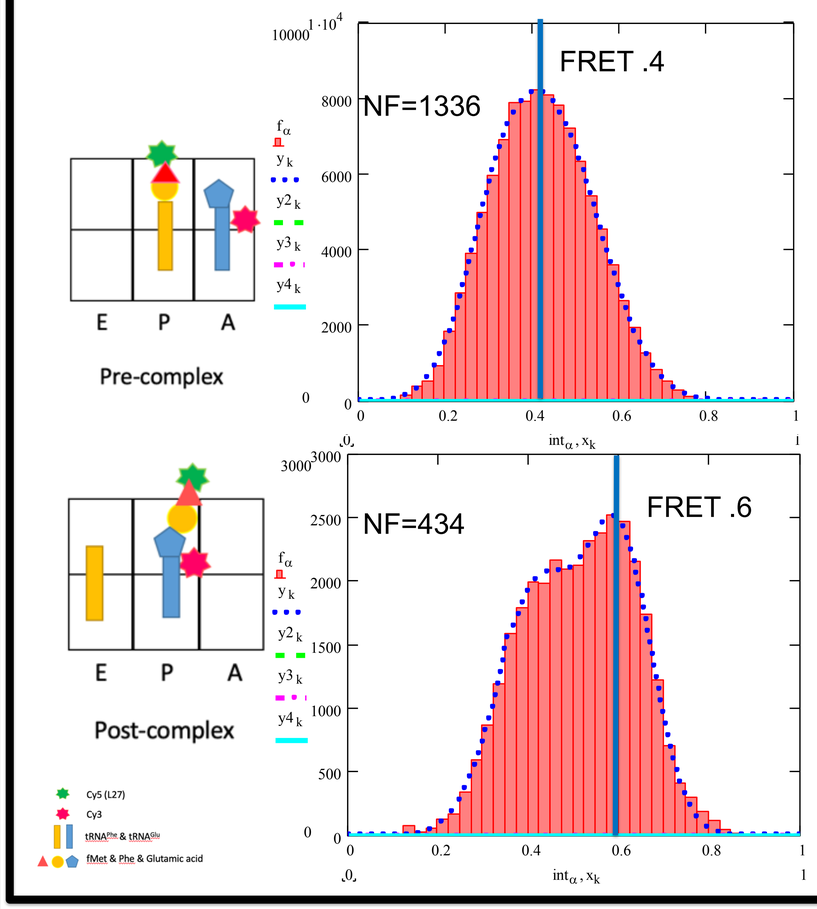
Recombinant Plasmid for $tRNA^{Glu}$

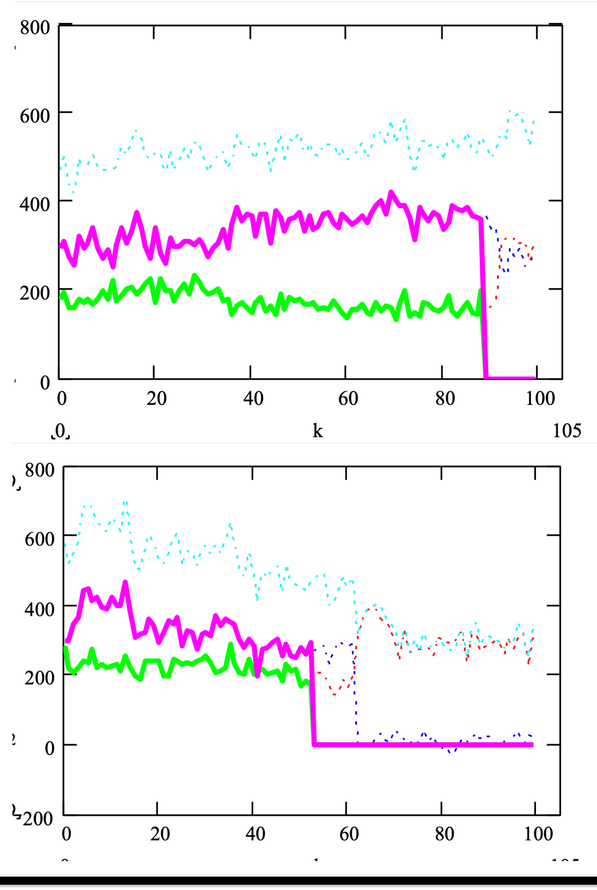


Example of a recombinant plasmid on an Agarose Gel (1.5%)



smFRET Results (Pre and Post)





Conclusions

Glutamic Acid tRNA was successfully purified through recombination and overexpression in E. Coli cells which allowed for clear smFRET results showing its translocation from the A site to the P site in the ribosome. Future studies should focus on the proteins and various motifs that can be targeted to inhibit translocation of the amino acid in the ribosome.

References

1. Dual DNA rulers reveal an 'mRNA looping' intermediate state during ribosome translocation Heng Yin, Shoujun Xu & Yuhong Wang (2018), RNA Biology, 15:11, 1392 1398, DOI: 10.1080/15476286.2018.1536590
2. High-Efficiency "–1" and "–2" Ribosomal Frameshiftings Revealed by Force Spectroscopy Te-Wei Tsai, Haopeng Yang, Heng Yin, Shoujun Xu, and Yuhong Wang *ACS Chemical Biology* **2017** *12* (6), 1629-1635 DOI: 10.1021/acschembio.7b00028

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