

EF-Tu (elongation factor thermo unstable)

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Introduction

The central dogma of biology is the pathway that starts from a DNA sequence and leads to the formation of a protein; therefore, DNA directs the synthesis of proteins. The process of protein synthesis, also known as translation, can be divided into three steps: initiation, elongation, and termination. The central step in translation is elongation, which entails the one-at-a-time addition of amino acids to a growing polypeptide chain in accordance to the sequence of codons on the mRNA at the ribosome. In prokaryotes, EF-Tu is an elongation factor which catalyzes this elongation process. EF-Tu catalyzes the elongation process by transferring aminoacyl-tRNAs (aa-tRNAs) to the A site of the ribosome. If the anticodon on the tRNA matches the corresponding codon on the mRNA at the ribosomal A site it is released by EF-Tu. Once tRNA binds to the A site, the growing polypeptide chain is moved from the tRNA occupying the adjacent P site to the amino acid attached to the tRNA in the A site. The ribosome then translocates to the next codon, moving the deacyl-tRNA from the P site to the E site and the tRNA containing the growing polypeptide chain from the A site to the P site. The deacyl-tRNA in the E site then exits the ribosome and EF-Tu transfers another aminoacyl-tRNA to the A site to restart the process.

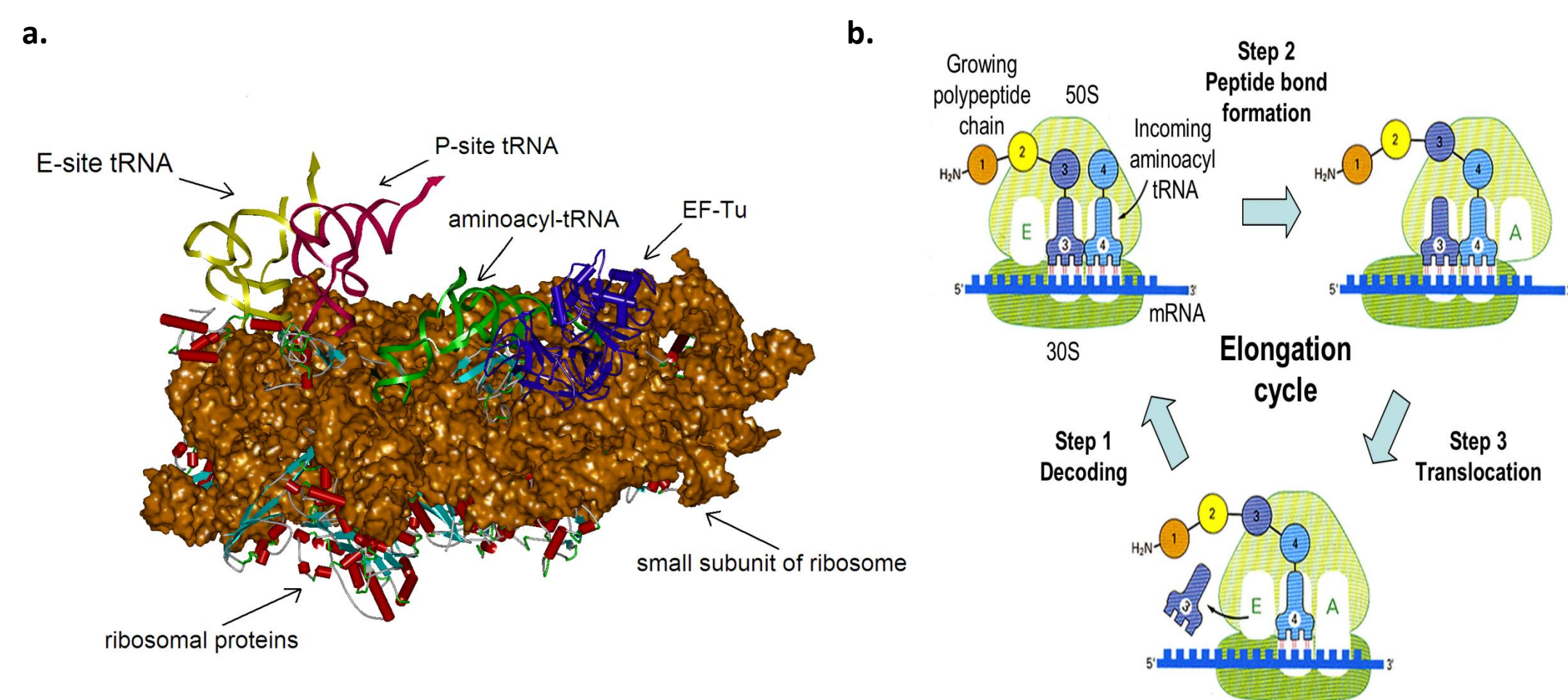


Figure 1. a.) This image portrays the ribosome complex undergoing the elongation of a polypeptide chain. The small ribosomal subunit is displayed with a solid surface. The tRNAs are displayed in ribbon form and the EF-Tu and ribosomal proteins in the tube form. **b.)** A diagram is used to help portray the elongation process at the ribosome.

Function

EF-Tu is a G protein, also known as a GTP binding protein, because it binds to GTP while in the active state and to GDP while in the inactive state.¹ In the active state, EF-Tu forms complexes with aminoacyl-tRNAs. Aminoacyl-tRNAs are tRNA molecules with an attached amino acid that corresponds to the anticodon on the tRNA. EF-Tu can form complexes with all aa-tRNAs, but cannot with uncharged tRNAs or initiator tRNAs. The tight association of the amino acid and EF-Tu increases the affinity of EF-Tu for aa-tRNAs. These tRNAs are able to bind to the ribosomal A site without EF-Tu but at a rate too slow to support cell growth. EF-Tu catalyzes this process, allowing for protein synthesis to be possible at the necessary rate for proper growth. EF-Tu is the most abundant protein in *E. Coli*, with about 100,000 copies per cell. This is a convenient number because this is about the number of tRNA molecules in each cell. EF-Tu does not only move tRNA to the ribosome but it also monitors and ensures the proper translation of the proteins.¹ When the EF-Tu-tRNA complex binds to the ribosomal A site and the codon-anticodon pairing between the mRNA and tRNA is correct, GTP hydrolyzes to GDP and the aa-tRNA is released into the ribosome. If the codon-anticodon pairing is incorrect, the EF-Tu-tRNA complex dissociates from the ribosome and takes the incorrect aa-tRNA with it.

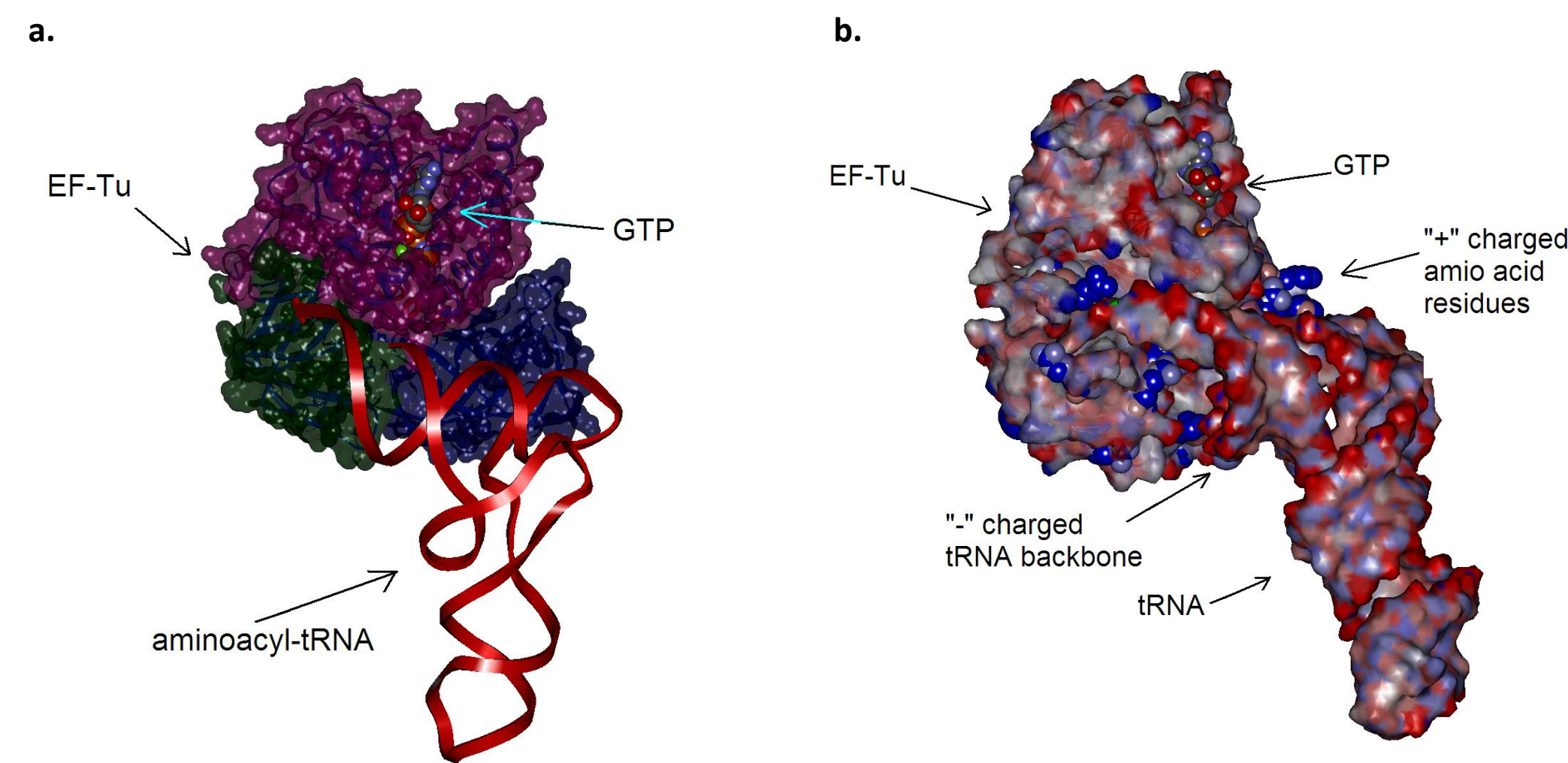


Figure 2. a.) An EF-Tu-GTP bound to an aminoacyl-tRNA complex. EF-Tu is shown with a surface and with each of its domains represented by a different color. **b.)** This image shows the electrostatic interactions between the positive amino acid residues (lysine and arginine) of EF-Tu and the negatively charged phosphate backbone of the aminoacyl-tRNA.

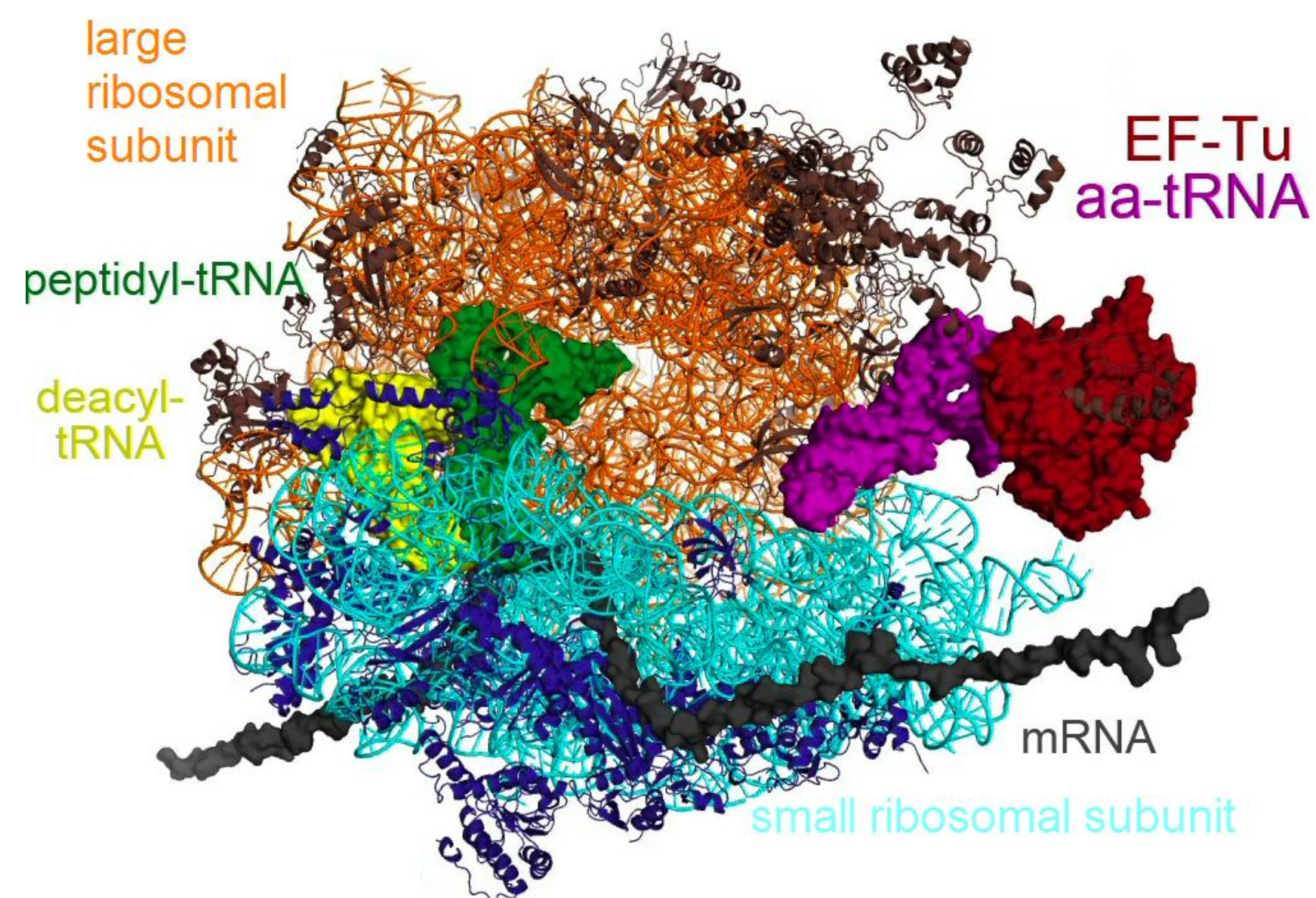


Figure 3. A complete structural model of the elongation process at the ribosome. The EF-Tu and tRNAs are displayed with solid surfaces. The rRNAs are displayed as ladders and the ribosomal proteins as ribbons (Image credit: Rebecca Voorhees and Martin Schmeing).³

Structure

EF-Tu consists of three different domains, regions of each of these domains contribute to the tRNA binding site. These regions consist of amino acids that electrostatically interact with the negative phosphates of the tRNA backbone. The amino acids Lys90, Arg91, and Arg300 form a groove that specifically interacts with the G1 base on tRNA. The side chain of Lys376 also makes contact with the phosphate backbone at C67. Electrostatic interactions between the tRNA backbone and Arg389 are also present. Glu56, Asp87, and Asp227 are very important amino acids on EF-Tu located at the interface between the aa-tRNA backbone and EF-Tu.¹ These amino acids ensure that the binding affinity of the tRNA to EF-Tu is balanced electrostatically so that the tRNA is able to release from the protein at a reasonable rate when GTP is hydrolyzed. The interface between the aa-tRNA and EF-Tu consists of approximately the same amount of repulsive interactions as attractive, but the attractive interactions dominate. The attractions at this interface between the tRNA and EF-Tu are mainly conducted by the positively charged amino acids on EF-Tu directly contacting the negative backbone of the tRNA. Repulsion interactions between the negatively charged amino acids and the negative phosphate backbone are present but are less powerful. To help coordinate the binding of the EF-Tu and tRNA, the repulsive interactions are conveniently located near the most attractive electrostatic interactions. When GTP is hydrolyzed to release the aa-tRNA, a switch helix in EF-Tu rearranges, leading to a major structural conformation change which disrupts the tRNA binding site releasing the tRNA.²

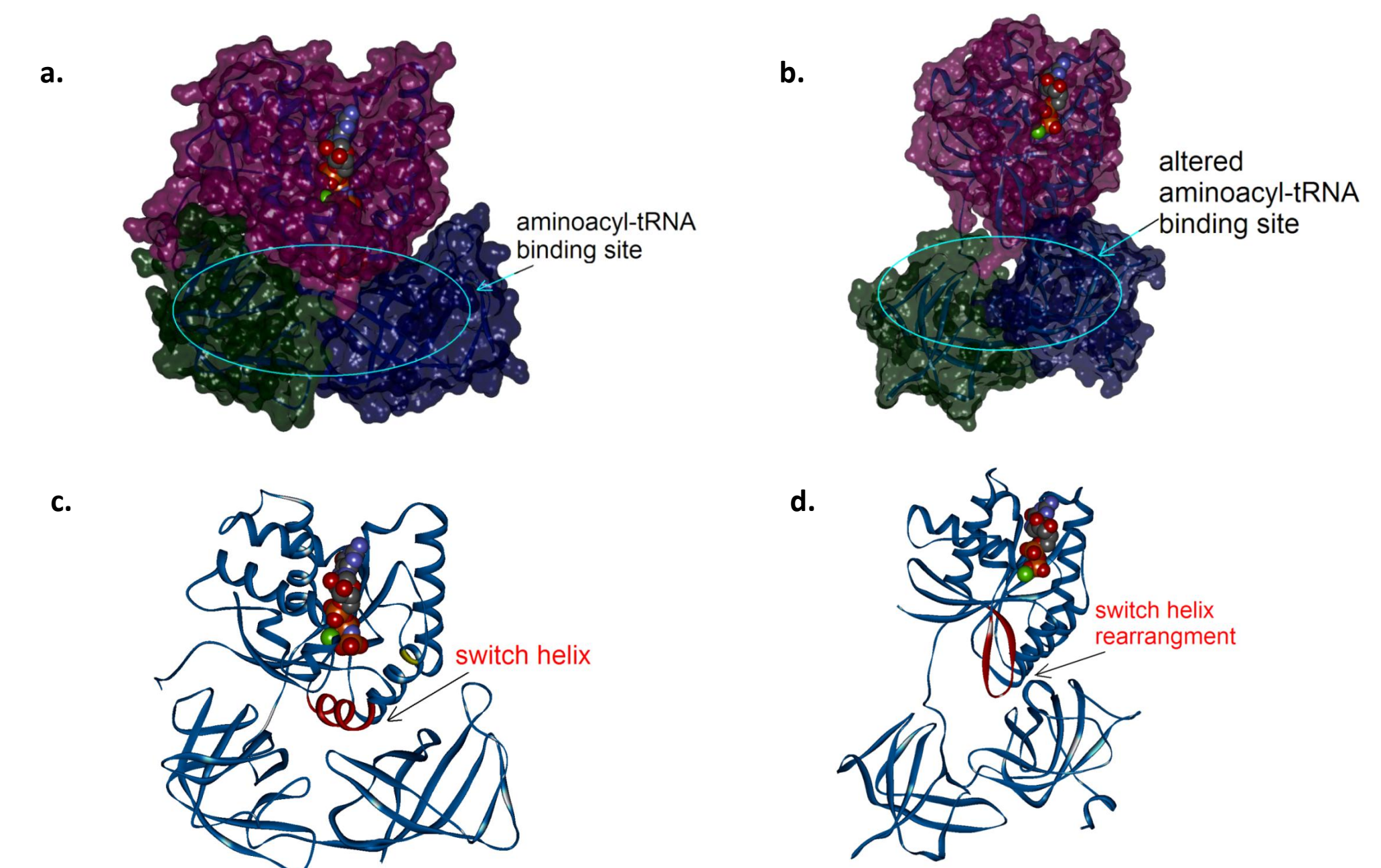


Figure 4. a.) EF-Tu with solid surface in GTP bound state. No aa-tRNA is displayed to better illustrate the tRNA binding site. **b.)** EF-Tu with solid surface in GDP bound state. Conformation change of protein after hydrolysis of GTP to GDP can be seen. **c.)** Ribbon structure of EF-Tu displaying the region of rearrangement, the switch helix, during the hydrolysis of GTP **d.)** Ribbon structure of EF-Tu displaying switch helix rearrangement.

Activity

The active site of the protein is the site of the hydrolysis of GTP to GDP, which occurs to release the aa-tRNA molecule. The protein and its interacting amino acids along with the Mg^{2+} metal stabilize this active site. Without stability in the active site the hydrolysis of GTP to GDP would not be able to be induced properly and the function of the protein would be hindered. Electrostatic attractions occur between the negative R groups and the positively charged magnesium ion. The negative R groups that are associated with these electrostatic attractions are ASP 81, 51, and 21. More precisely the negative charge is found on the carboxylate within each of these aspartic acids. The Mg^{2+} also contributes to the tight association between the protein and the tRNA by counter-reacting the negative repulsions associated with the negative phosphates in the backbone. This is known because complexes with low Mg^{2+} concentrations show more motion in the tRNA when bound to the EF-Tu because they are not associated as tightly as they should be.¹

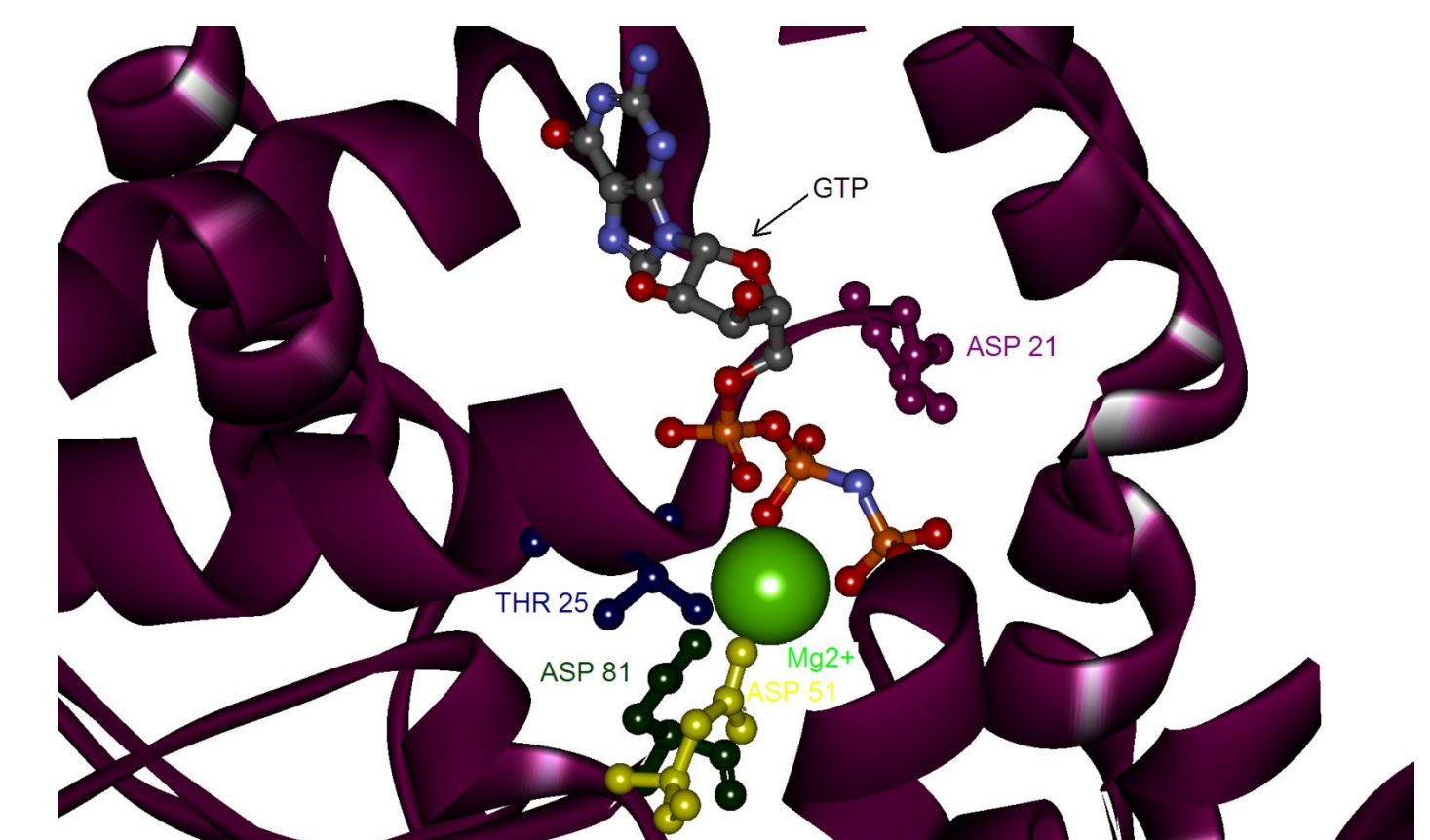


Figure 5. The active site of EF-Tu stabilized by the Mg^{2+} ion and amino acid residues.

References

1. Eargle, J., Black, A., Sethi, A., Trabuco, L., and Luthey-Schulten, Z. Dynamics of Recognition between tRNA and elongation factor Tu, *J Mol Biol*, **377(5)** 1382–1405 (2008).
2. Kulczycka, K., Dlugosz, K., and Trylska, J. Molecular dynamics of ribosomal elongation factors G and Tu, *Eur Science* **40(3)**: 289–303. (2010).
3. Schmeing, T.M., Voorhees, R., Kelly, A., Gao, Y., Murphy, F., Weir, J.R., and Ramakrishnan, V. The Crystal Structure of the Ribosome Bound to EF-Tu and Aminoacyl-tRNA, *Science* **326**, 688-694 (2009).