

Supplementary information for:

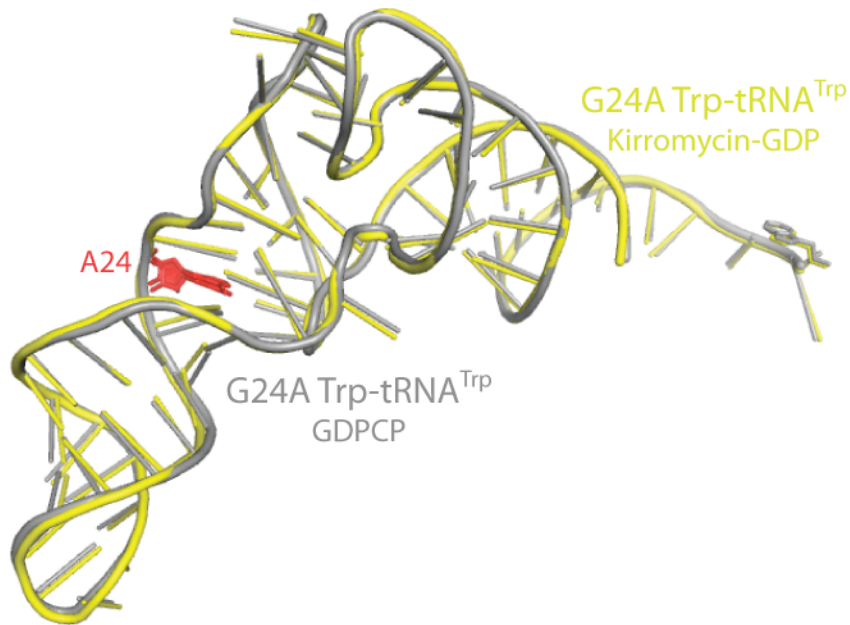
**How mutations in tRNA distant from the anticodon affect the fidelity of decoding**

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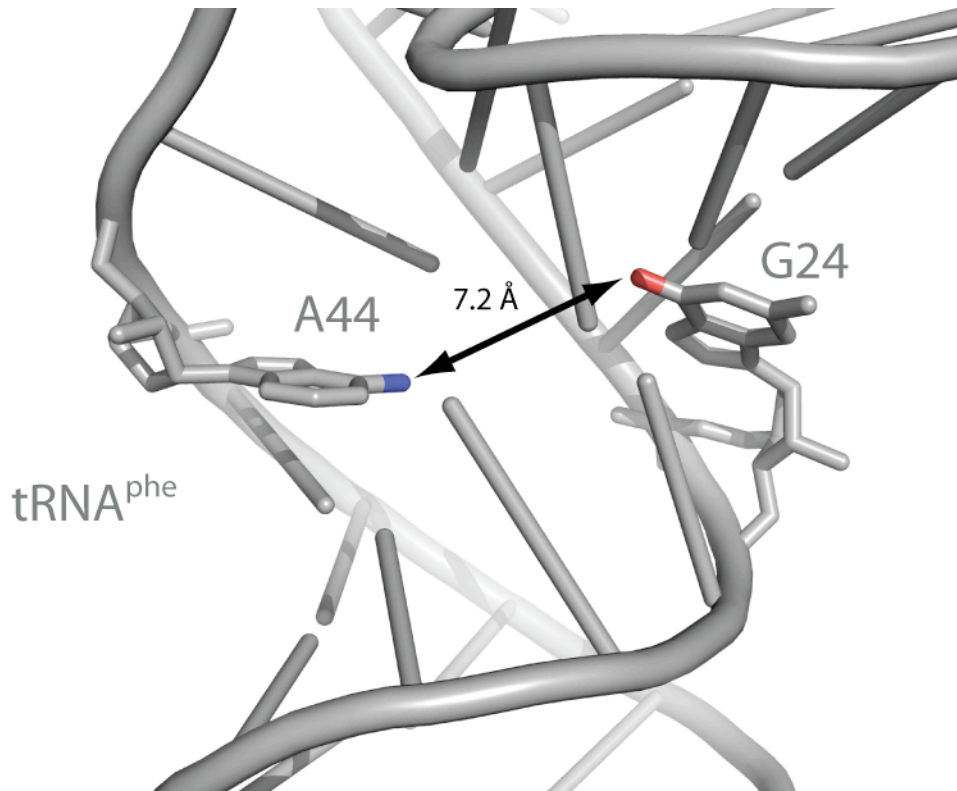
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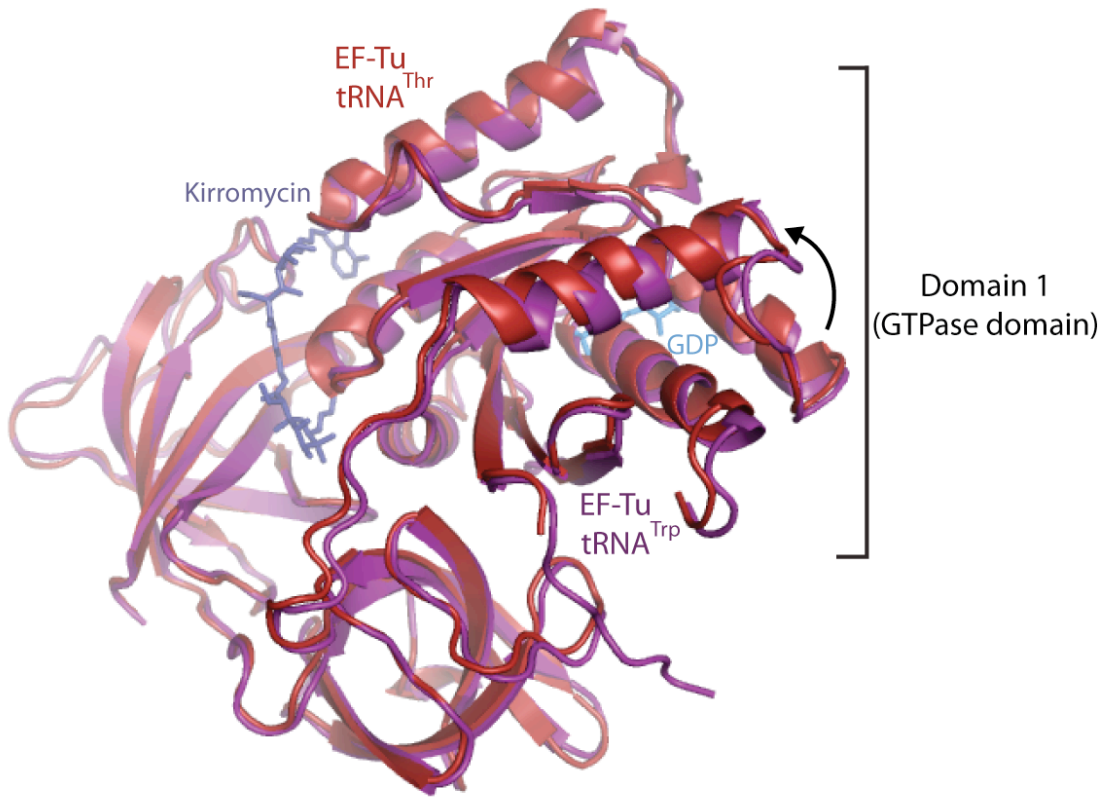
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**Supplemental Figure 1:** Comparison of the conformations of G24A Trp-tRNA<sup>Trp</sup> in structures of the ribosome complexed with G24A Trp-tRNA<sup>Trp</sup> (yellow)- EF-Tu-GDP – kirromycin and with G24A Trp-tRNA – EF-Tu-GDPCP (grey)<sup>1</sup>. The conformations of the ribosome, EF-Tu and in particular the tRNA body are exceedingly similar. That tRNA conformation does not change between the activated pre-GTP hydrolysis state and the post-GTP hydrolysis kirromycin-stalled structures, indicates that this is the conformation of the tRNA at the point of GTP hydrolysis, when tRNAs are selected. Nucleotide A24 is shown in red sticks.



**Supplemental Figure 2:** Residues 24 and 44 are further apart in unbent tRNA. In yeast tRNA<sup>Phe</sup>, N6 of A44 and O6 of G24 are separated by 7.2 Å<sup>2</sup>. In *E. coli* tRNA<sup>Trp</sup>, residue 44 is a guanosine.



**Supplemental Figure 3:** The conformation of EF-Tu is affected by tRNA identity. Superposition of domain 3 of EF-Tu from the structures of tRNA<sup>Thr</sup> (red) <sup>3</sup> and tRNA<sup>Trp</sup> (purple) bound to the ribosome demonstrates that tRNA identity can affect EF-Tu conformation. In particular a small rotation of domain 1 relative to domains 2 and 3 is observed between the two structures.

## References

1. Voorhees, R. M., Schmeing, T. M., Kelley, A. C. & Ramakrishnan, V. The mechanism for activation of GTP hydrolysis on the ribosome. *Science* **330**, 835-838 (2010).
2. Shi, H. & Moore, P. B. The crystal structure of yeast phenylalanine tRNA at 1.93 Å resolution: a classic structure revisited. *RNA* **6**, 1091-105. (2000).
3. Schmeing, T. M. et al. The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* **326**, 688-694 (2009).