Supplementary Material

The Crystal Structure of Yeast Mitochondrial ThrRS in Complex With the Canonical Threonine tRNA

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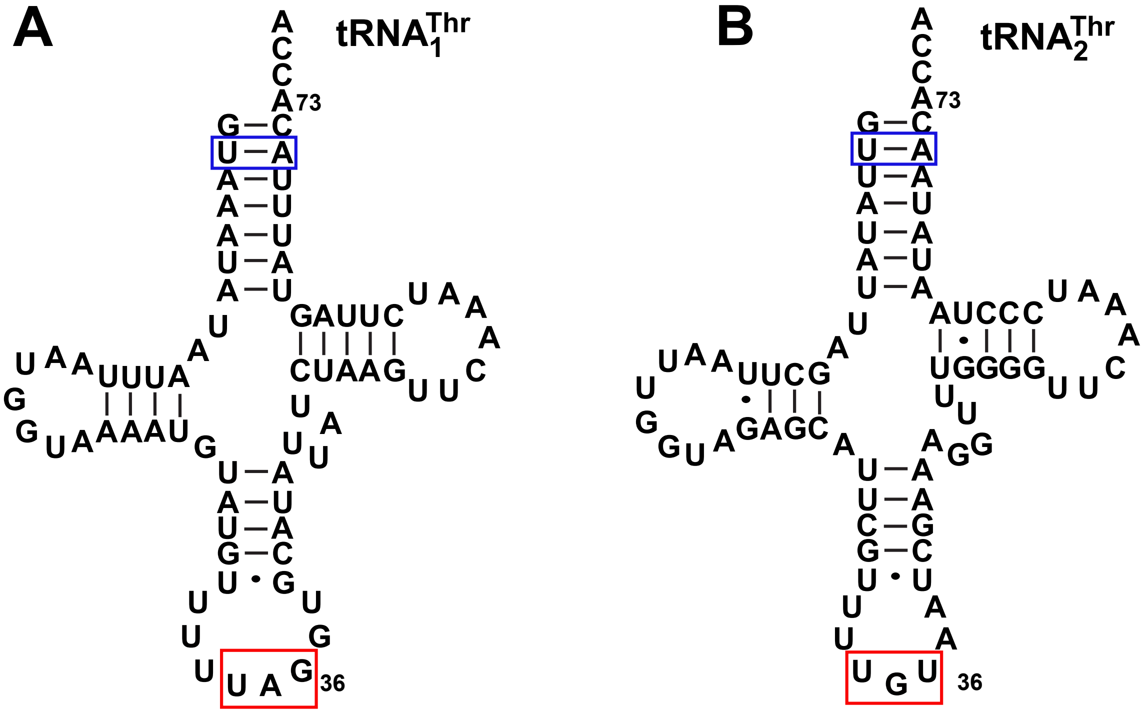
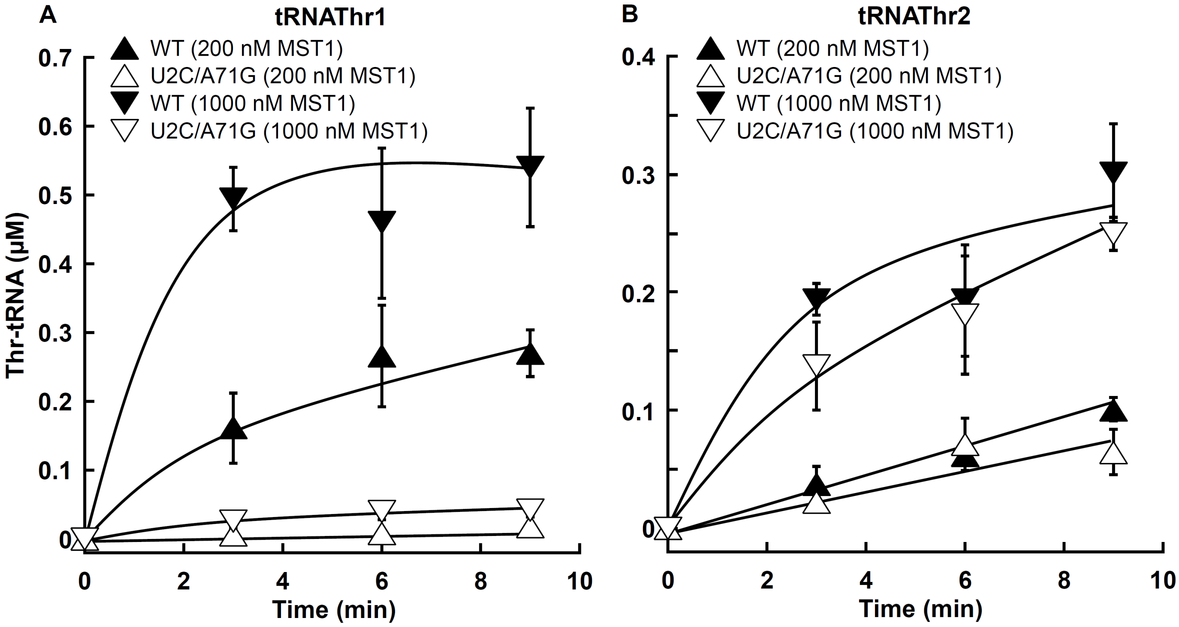
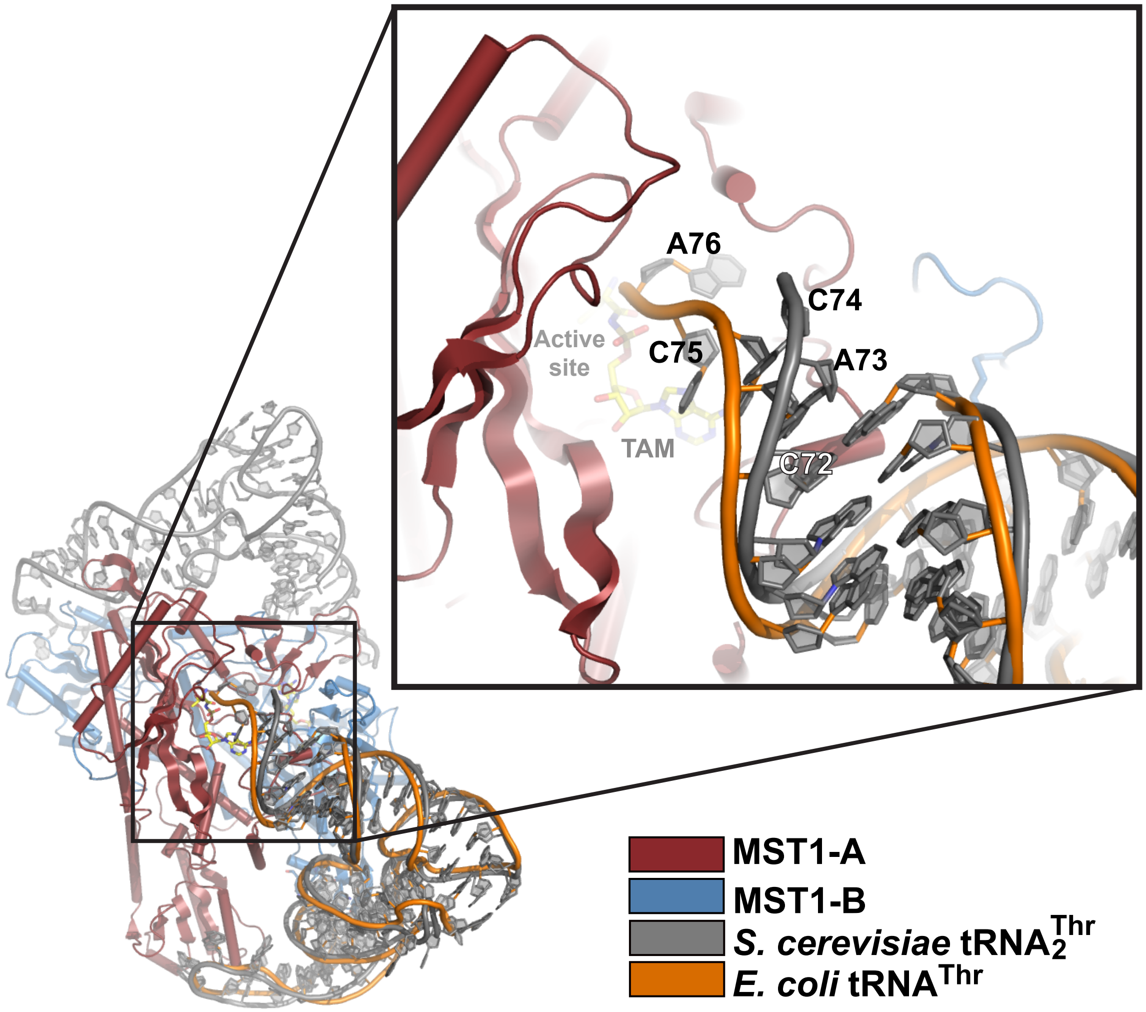


Supplementary Figure 1. Nucleotide sequences of *S. cerevisiae* mitochondrial tRNAs. (A) tRNA$_1^{Thr}$ contains an enlarged 8-nucleotide anticodon loop with the UAG anticodon sequence (red box). **(B)** tRNA$_2^{Thr}$ contains the canonical 7-nucleotide anticodon loop and the canonical UGU anticodon (red box). The second base pair in the acceptor stem is highlighted with blue box. The structure of the MST1- tRNA$_2^{Thr}$ complex suggests that MST1 recognizes the sequence of this conserved base pair.



Supplementary Figure 2. Aminoacylation of *S. cerevisiae* mitochondrial tRNA variants by MST1. The experiment was repeated three times with error boars indicating standard deviations.



Supplementary Figure 3. The 3’ end of tRNA$_2^{Thr}$ remains flexible after binding to MST1. Superimpositioning of the acceptor stem of *E. coli* tRNA^{Thr} onto tRNA$_2^{Thr}$ bound to MST1 shows the flexibility of the 3’ end of tRNA$_2^{Thr}$. C75 and A76 were not visible in our crystal structure.

Supplementary Table 1. Aminoacylation kinetics of $tRNA_2^{Thr}$ variants

Scmt $tRNA_2^{Thr}$	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m (min^{-1} $μM^{-1}$)	Fold change
WT	0.28 ± 0.03	0.23 ± 0.08	1.29 ± 0.32	1
U2C/A71G	0.10 ± 0.008	0.26 ± 0.19	0.56 ± 0.31	2.3