**Supporting information**

# Role for a conserved structural motif in assembly of a class I aminoacyl-tRNA synthetase active site

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## EXPERIMENTAL PROCEDURES

*In silico mutagenesis of E. coli MetRS.* To determine whether the $^{363}$LSSRID$^{368}$→IGVTKQ peptide swap could be accommodated within the MetRS structural framework, *in silico* mutagenesis followed by energy minimization was carried out. Using the MetRS 1QQT coordinates, atoms of LSSRID not held in common with IGVTKQ were removed and the amino acids were reassigned to IGVTKQ. Missing atoms (including hydrogens) were added using the standard CHARMM package (1). An energy minimization step was first performed to allow the swapped IGVTKQ peptide to relax, reducing any steric overlaps and optimizing the position of new atoms. All atoms except for the swapped peptide were constrained using a harmonic position restraint force constant of 60.0 kcal mol$^{-1}$Å$^{-2}$ for 500

cycles of conjugate gradient minimization. Then, to allow side chains to relax, all backbone atoms were constrained at the same force constant for another 500 cycles of conjugate gradient minimization. The harmonic restraint force constant was reduced by 20.0 kcal $mol^{-1}Å^{-2}$ after every 1000 such cycles of minimization (500 cycles constraining all but the swapped peptide, followed by 500 cycles constraining the heavy atoms) until a constant of 0 kcal $mol^{-1}Å^{-2}$ was reached. Lastly, all heavy atoms were constrained, to allow hydrogens to relax, using a harmonic restraint force constant of 30 kcal $mol^{-1}Å^{-2}$ and decreasing by 10.0 kcal $mol^{-1}Å^{-2}$ after every 500 cycles of conjugate gradient minimization (again until a constant of 0 kcal $mol^{-1}Å^{-2}$ was reached). A modified CHARMM22 parameter set (2) was used throughout, with modified cysteine charges incorporated (as the Cys residues complexing the $Zn^{2+}$ ion are deprotonated at the sulfur atom) to accommodate the protein's $Zn(Cys)_4$ motif (2-3).

*Alignment of energy minimized structures.* The final minimized MetRS peptide swapped structure was compared to the wild-type MetRS structure (PDB 1QQT) in which missing hydrogen atoms were added and energy minimization carried out using CHARMM22 parameter set with modified cysteine charges as above. Structural alignment was carried out using the MultiProt server (4).

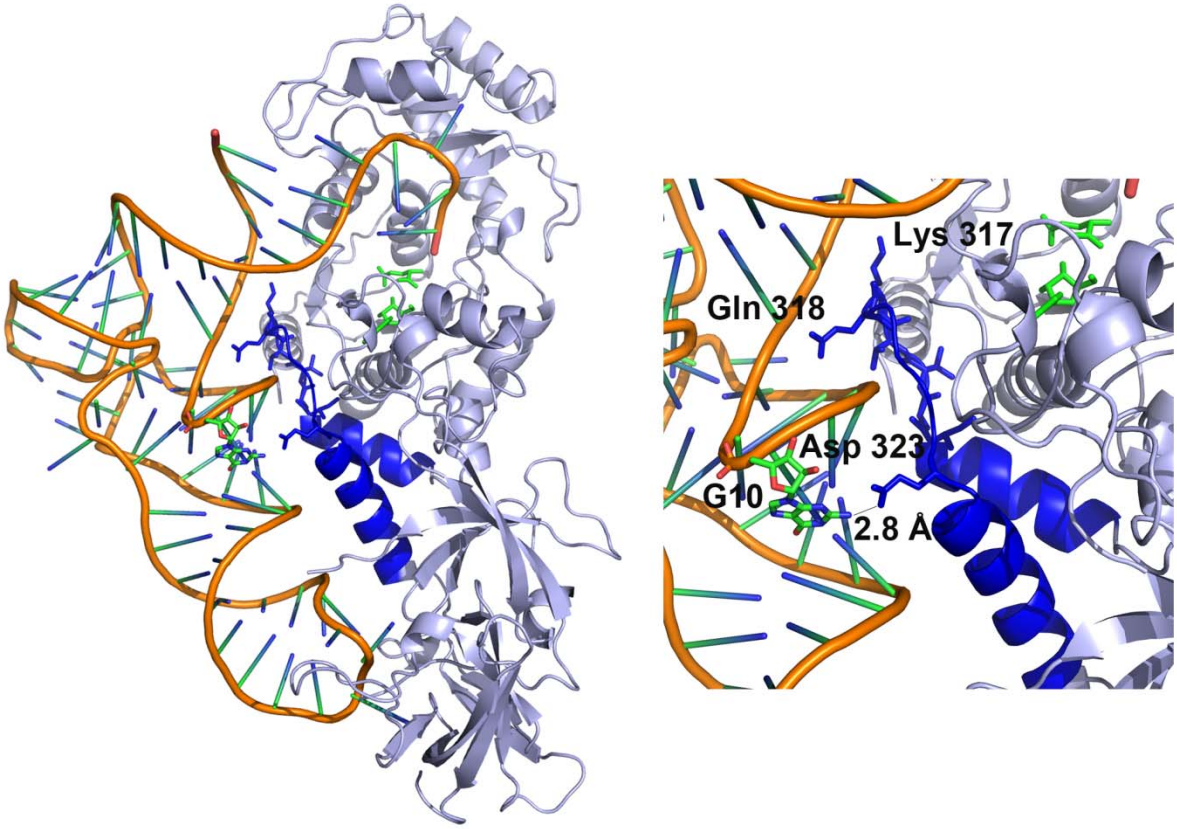


**Figure S1. Interaction of GlnRS with $tRNA^{Gln}$. Left panel**, the *E. coli* GlnRS:$tRNA^{Gln}$:Gln:AMPCPP crystal structure (PDB 1O0B) (5) is shown in cartoon representation, with glutamine and AMPCPP in green (β and γ phosphates disordered) and residues of the SCF helix-turn-helix motif in blue. Residues $^{313}$IGVTKQ$^{318}$ and Glu-323 are shown as sticks. **Right panel**, close-up of the protein:tRNA interface. The carboxylate side chain of Glu-323 is in hydrogen bond distance to the exocyclic amine of $tRNA^{Gln}$ G10; this is the only hydrogen bond contact made between residues of the SCF and $tRNA^{Gln}$ in this cocrystal structure.

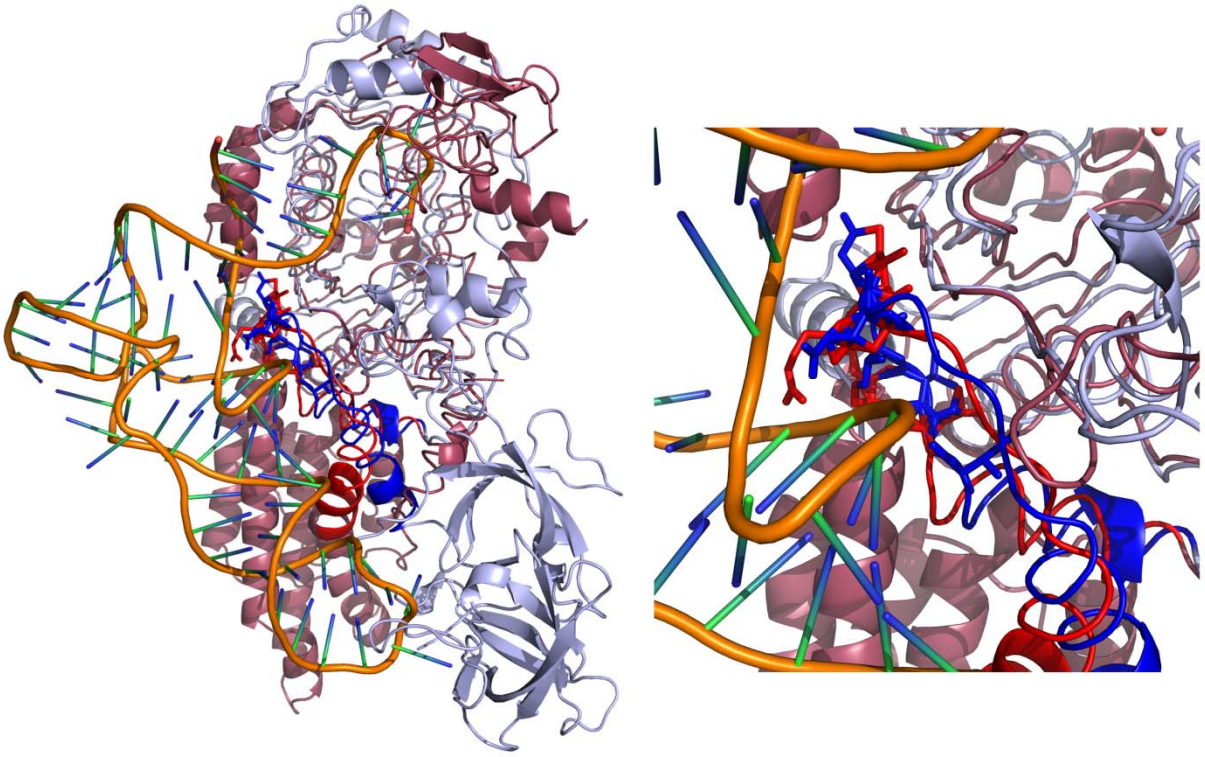

**Figure S2. Alignment of GlnRS and MetRS crystal structures.** The *E. coli* GlnRS:tRNA$^{Gln}$:Gln:AMPCPP cocrystal structure (PDB 1O0B) (5) was aligned with the *E. coli* MetRS structure (PDB 1QQT) (6) using the MultiProt server (4). The RMSD over 195 residues encompassing the active site Rossmann fold and SCF motifs was 1.76 Å. **Left panel**, proteins are shown as cartoons, with GlnRS in light blue and MetRS in raspberry. SCF motifs for GlnRS and MetRS are shown in blue and red, respectively, with IGVTKQ and LSSRID motifs shown as sticks. **Right panel**, close-up of the SCF loop region. The long-chain amino acids directed toward the tRNA surface are $^{317}$KQ$^{318}$ of GlnRS and $^{364}$RI$^{365}$ of MetRS.

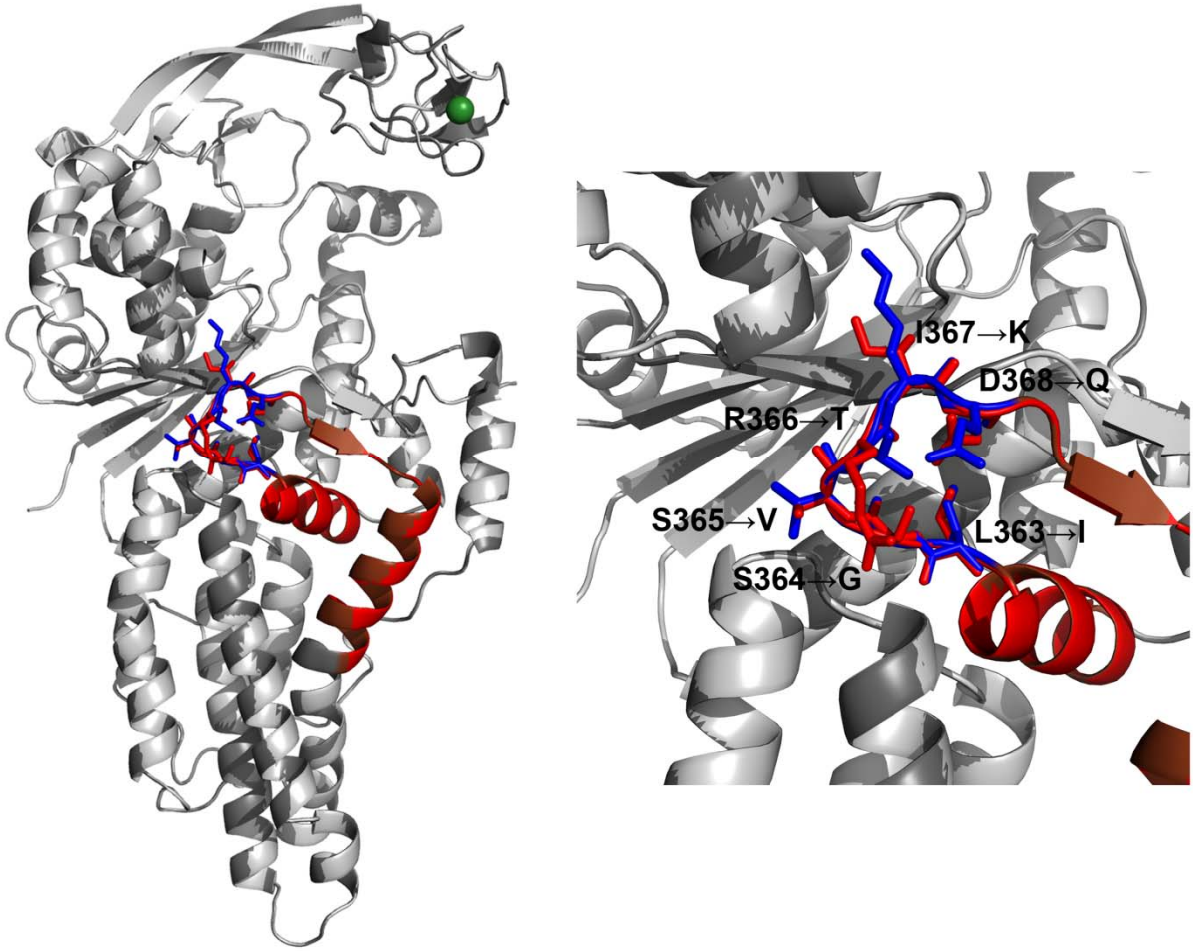


**Figure S3. Alignment of wild-type MetRS and LSSRID→IGVTKQ variant.** Residues of *E. coli* GlnRS corresponding to the *E. coli* MetRS (PDB 1QQT) SCF loop $^{363}$LSSRID$^{368}$ residues were replaced *in silico* using the CHARMM package (1) and energy minimized to system equilibration. Wild-type and variant coordinates were aligned using the MultiProt server (4). The RMSD of all 546 Cα atoms was 0.04 Å. **Left panel**, full protein showing wild-type MetRS in light gray, with SCF motif in red and LSSRID residues as sticks. The LSSRID→IGVTKQ variant is shown in darker gray, with SCF residues in dark red and IGVTKQ residues as blue sticks. The $Cys_4$-coordinated $Zn^{2+}$ is shown as a green sphere. **Right panel**, view of the SCF loop region showing alignment of the swapped peptide.

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