SUPPLEMENTARY INFORMATION

Substrate and Enzyme Functional Groups Contribute to Translational Quality Control by Bacterial Prolyl-tRNA Synthetase

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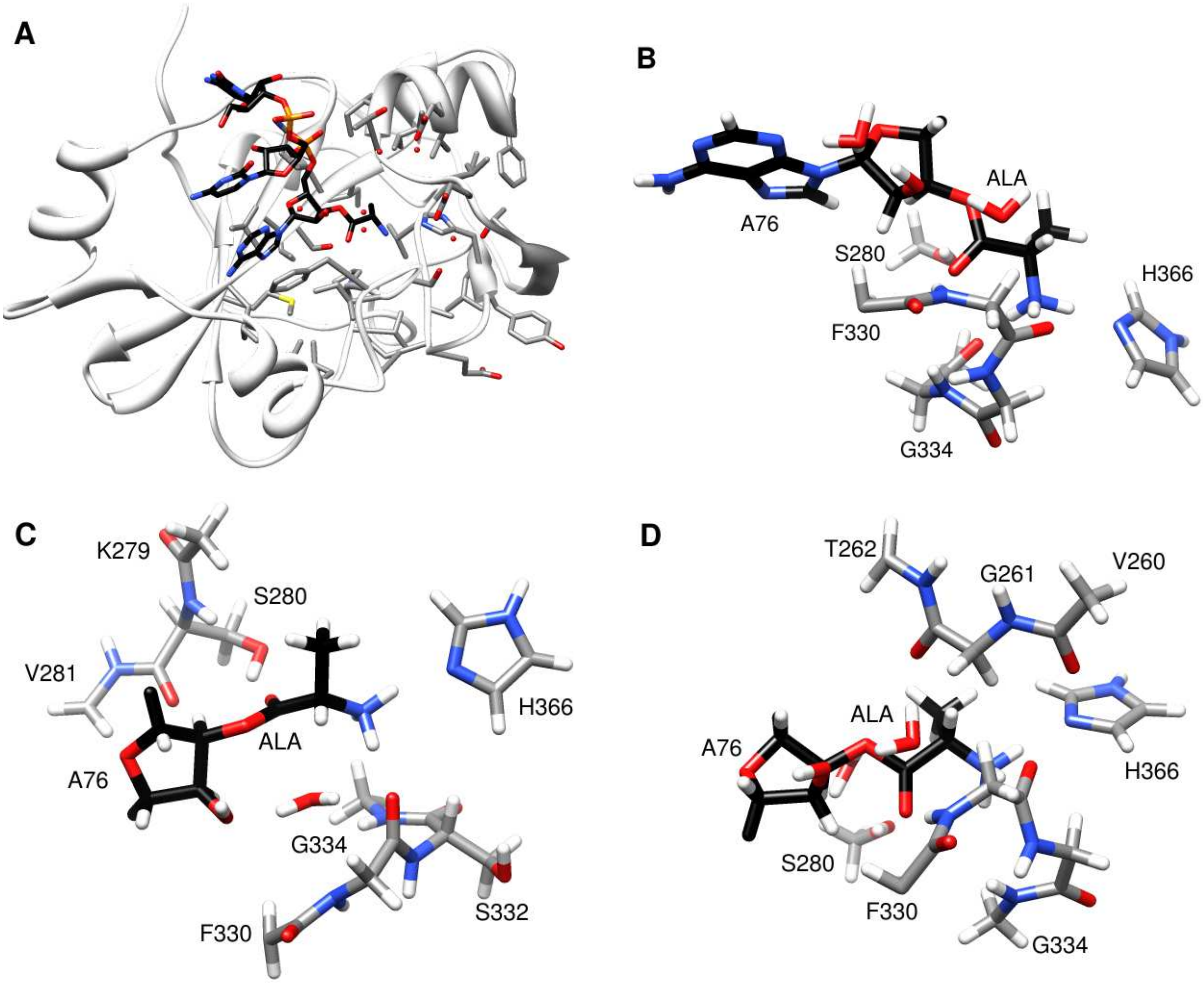
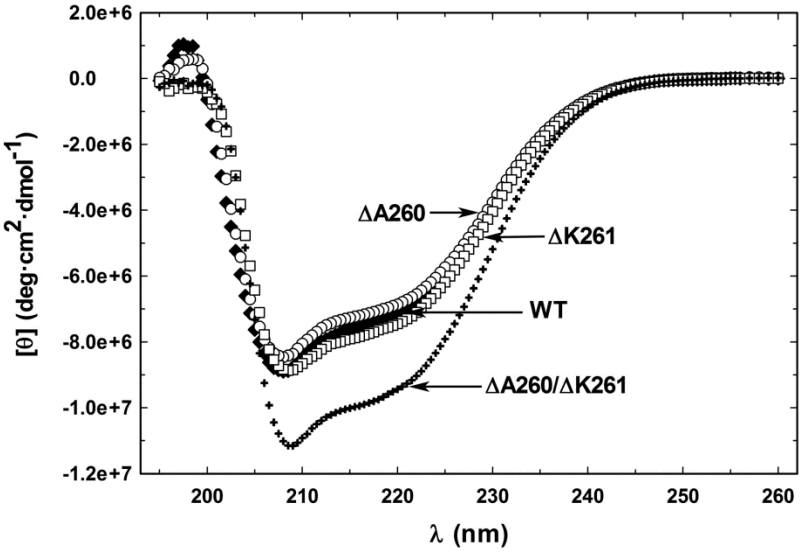
Supplementary Figure 1. System for QM/MM studies. (A) Structural model of *E. faecalis* ProRS INS domain bound to Ala-tRNA^{Pro} substrate analog 5'-CCA-Ala (shown as stick model). The enzyme-substrate complex generated by computational modeling[1] was solvated in a 5Å shell of water molecules and optimized using the QM/MM method. For initial optimization and subsequent calculations, all residues with any atom within 8Å of substrate Ala were active while other residues were frozen. Some structural waters are not shown for clarity. **(B)** Mechanism involving N3 of A76 as general base. Atoms treated with QM included substrate Ala, adenine base, ribose ring, His366 and Ser280 side chain, Phe330 – Gly334 backbone atoms, and catalytic and structural water molecules. **(C)** Mechanism involving 2'-OH as general base and carbonyl stabilization by Ser280 residue. Atoms treated with QM included substrate Ala, ribose ring, H366 and S280 side chain, Lys279 – Val281 and Phe330 – Gly334 backbone atoms, and catalytic and structural water molecules. **(D)** Mechanism involving Gly261 carbonyl in proton relay. Atoms treated with QM included substrate Ala, ribose ring, His366 and Ser280 side chain, Val260 – Thr262 and Phe330 – Gly334 backbone atoms, and catalytic and structural water molecules.


A
B
ALA
A76
S280
H366
F330
G334
C
K279
S280
V281
H366
ALA
A76
G334
S332
F330
D
T262
G261
V260
ALA
A76
H366
S280
F330
G334

Supplementary Figure 2. Circular Dichroism Spectroscopy of WT and deletion variants of *E coli* ProRS. CD spectra of WT (♦), ΔA260 (○), ΔK261 (□) and ΔA260/K261 (+) ProRS is shown. CD spectra were measured using a J-815 spectropolarimeter (Jasco) and ~ 0.5 mg/ml protein in buffer containing 25 mM HEPES (pH 7.5) and 75 mM NaCl at room temperature with a 1 mm path-length cuvette. A background spectrum of buffer alone was also measured and subtracted from each of the protein spectra.



(1) Kumar, S.; Das, M.; Hadad, C. M.; Musier-Forsyth, K. *J. Biol. Chem.* **2011**, DOI: 10.1074/jbc.M111.313619.