Prolyl-tRNA synthetase (ProRS) is class II synthetases that catalyze covalent attachment of proline to the 3’-end of the tRNA\(^{34}\). ProRSs from all three kingdoms of life have shown to misactivate non-cognate alanine and cysteine, and form mischarged aminoacyl-tRNA\(^{34}\). The insertion domain (I–I0 amino acids) of Escherichia coli ProRS is the post-transfer editing active site that hydrolyses specifically mischarged alanyl-tRNA\(^{34}\). The highly conserved lysine 279 (K279) in the insertion domain is critical for the post-transfer editing reaction and previous studies have shown that mutation of this lysine to alanine is detrimental to the post-transfer editing function of the enzyme. The exact mechanism through which K279 catalyzes the post-transfer editing reaction has remained poorly understood. In an attempt to gain insight into the mechanism of post-transfer editing reaction of Escherichia coli ProRS, the \(p_K\) calculations of the K279 have been performed using combined quantum mechanical and molecular mechanical (QM/MM) simulations. Herein, we report the effect of charged residues on the \(p_K\) of both ProRS and K279 and thereby, on the post-transfer editing function of Escherichia coli prolyl-tRNA synthetase. These computational results are also validated through site-directed mutagenesis.

### Methods

**QM/MM Setup**

1. 30 Å water sphere added around the editing active site center: Lys 279
2. Deleting all atoms beyond the 30 Å sphere: Quantum mechanics
3. Stochastic boundary condition
4. Explicitly treated water molecules are modeled by TIP3P
5. The charge of the 30 Å solvated enzyme was made 0 by putting counter ions

**Quantum Mechanical/Molecular Mechanical (QM/MM) Simulations**

- **Thermodynamic Integrations**
- **Thermodynamic diagram for computing \(p_K\) of K279**
  - \(\text{Lys}^-\text{H2O}\)
  - \(\text{Lys}^+\text{H2O}\)
  - \(\text{Lys}^-\text{H2O} + \text{H}^+\)
  - \(\text{Lys}^+\text{H2O} - \text{H}^+\)

**Results**

- **Linear Variation of \(\Delta G(A)\)**
- **Free Energy Changes**

### Conclusions

- The computed \(p_K\) of K279 of Ef ProRS is \(-36\), which is five units higher than the free lysine (\(p_K\)=10.3). The protonated state of the lysine is important for the interaction with the phosphate group of the tRNA\(^{34}\).
- The protonated state of the lysine is stabilized by the surrounding charged residues like D299, H298, and D347, whereas destabilized by the charged residues like R335 and E265.
- The preliminary mutational data supports the theoretical findings that the salt-bridge interaction between D299 and K279 is critical for the post-transfer editing reaction by the E. coli ProRS.
- Previous mutational study by Wang et al.\(^{11}\) has shown that D350 (D347 of Ef ProRS) has profound effect on post-transfer editing reaction by E. coli ProRS which is in agreement with the computed results.

### Future Directions

- Compute the \(p_K\) of K279 (Ef ProRS) by mutating the H298 and D299 to alanine.
- To determine the kinetic parameters for the amino acid activation and post-transfer editing reaction by Ef ProRS using active-site concentrations of enzymes.
- Examine the post-transfer editing reaction of the double mutant K279D and D299K in order to probe the exact role of K279.
- To explore the effects of R352A mutation on the post-transfer editing function.