

## SUPPLEMENTARY MATERIAL

### **Structural and Biochemical Characterization of N<sup>5</sup>- Carboxyaminoimidazole Ribonucleotide Synthetase and N<sup>5</sup>- Carboxyaminoimidazole Ribonucleotide Mutase from *Staphylococcus aureus***

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**CAPTIONS:**

**Scheme 1.** *De novo* purine biosynthesis pathway

**Scheme 2.** Enzymatic assays used. **A:** Enzymatic assay for PurK. **B:** Enzymatic assay for PurE

**Chart 1.** Plot  $V_0$  vs.  $[\text{HCO}_3^-]$  for PurK. Fit to a hyperbola using Origin.

**Figure 1.** Gel filtration chromatography for PurK, PurE and PurC

**Figure 2.A:** *S. aureus* PurK colored by B-factors. Blue indicates low B-factors, yellow indicates high B-factors. **B:** Superposition of the apo-PurK (grey) and PurK complexed with ADP structure (yellow). The RMSD for the A- and C- domains is 0.19 Å while the RMSD for domain B is 3.2 Å.

**Figure 3.** Sequence alignment of *S. aureus* PurK with *E. coli* PurK

**Figure 4.** Sequence alignment of *S. aureus* PurE with human PurE.

**Figure 5.** Overlay of *S. aureus* PurE (orange) and human bifunctional enzyme PurC-PurE. The PurE domain of the human enzyme is in the center and the PurC domain in the outside.

The diagram illustrates the purine biosynthesis pathway, showing the conversion of PRPP to IMP and the subsequent conversion of IMP to various purine derivatives. The pathway is divided into two main branches: the de novo pathway (top) and the salvage pathway (bottom).

**De novo pathway (top):**

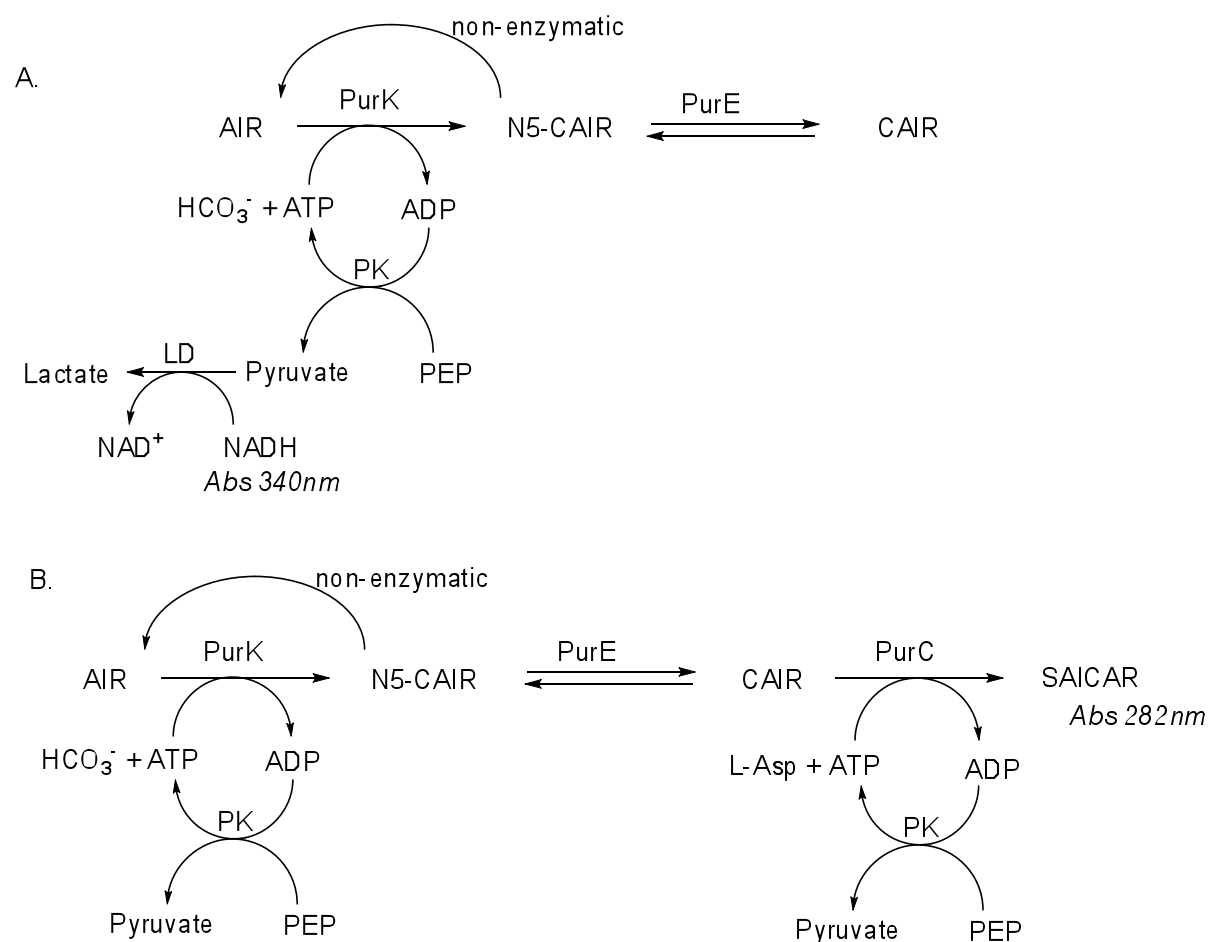
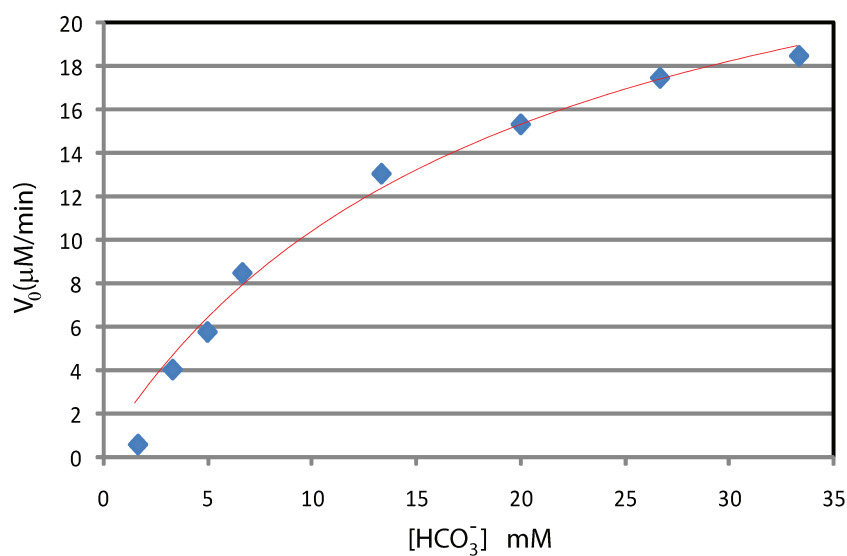
- PRPP is converted to PRA by PurF, using L-Gln and releasing PP<sub>i</sub>.
- PRA is converted to AIR by PurD, using ATP, Gly, and releasing ADP and P<sub>i</sub>.
- AIR is converted to N<sup>5</sup>-CAIR by PurK, using ATP and HCO<sub>3</sub><sup>-</sup>, and releasing ADP and P<sub>i</sub>.
- N<sup>5</sup>-CAIR is converted to CAIR by PurE (class II), releasing CO<sub>2</sub>.
- CAIR is converted to IMP by PurC, using ATP and L-Asp, and releasing ADP and P<sub>i</sub>.
- IMP is converted to FAICAR by PurJ and PurO, releasing H<sub>2</sub>O.
- FAICAR is converted to AICAR by PurH and PurP, using ATP and formate, and releasing ADP and P<sub>i</sub>.
- AICAR is converted to SAICAR by PurB, releasing fumarate.
- SAICAR is converted to FGAM by PurL or PurSLQ, using ATP and L-Gln, and releasing ADP+P<sub>i</sub> and L-Glu.
- FGAM is converted to FGAR by PurM, using ATP, and releasing ADP and P<sub>i</sub>.
- FGAR is converted to GAR by PurN and PurT, using N<sup>10</sup>-formyl-THF, ATP, and formate, and releasing THF, ADP, and P<sub>i</sub>.
- GAR is converted to FGAR by PurN and PurT, releasing N<sup>10</sup>-formyl-THF, ADP, and P<sub>i</sub>.
- FGAR is converted to FGAM by PurL or PurSLQ, releasing ATP, L-Gln, and ADP+P<sub>i</sub>, and L-Glu.

**Salvage pathway (bottom):**

- IMP is converted to AICAR by PurH and PurP, using ATP and formate, and releasing ADP and P<sub>i</sub>.
- AICAR is converted to SAICAR by PurB, releasing fumarate.
- SAICAR is converted to FGAM by PurL or PurSLQ, using ATP and L-Gln, and releasing ADP+P<sub>i</sub> and L-Glu.
- FGAM is converted to FGAR by PurM, using ATP, and releasing ADP and P<sub>i</sub>.
- FGAR is converted to GAR by PurN and PurT, using N<sup>10</sup>-formyl-THF, ATP, and formate, and releasing THF, ADP, and P<sub>i</sub>.
- GAR is converted to FGAR by PurN and PurT, releasing N<sup>10</sup>-formyl-THF, ADP, and P<sub>i</sub>.
- FGAR is converted to FGAM by PurL or PurSLQ, releasing ATP, L-Gln, and ADP+P<sub>i</sub>, and L-Glu.

**Legend:**


- R = ribose 5'-phosphate
- ATP = Adenosine Triphosphate
- ADP = Adenosine Diphosphate
- P<sub>i</sub> = Inorganic Phosphate
- PP<sub>i</sub> = Pyrophosphate
- THF = Tetrahydrofolate
- N<sup>10</sup>-formyl-THF = N<sup>10</sup>-Methylen-THF
- formate = Formate
- L-Gln = L-Glutamine
- L-Glu = L-Glutamate
- L-Asp = L-Aspartate
- fumarate = Fumarate

**Scheme 2.** Enzymatic assays used. **A:** Enzymatic assay for PurK. **B:** Enzymatic assay for PurE**Chart 1.** Plot  $V_0$  vs.  $[\text{HCO}_3^-]$  for PurK. Fit to a hyperbola using Origin.

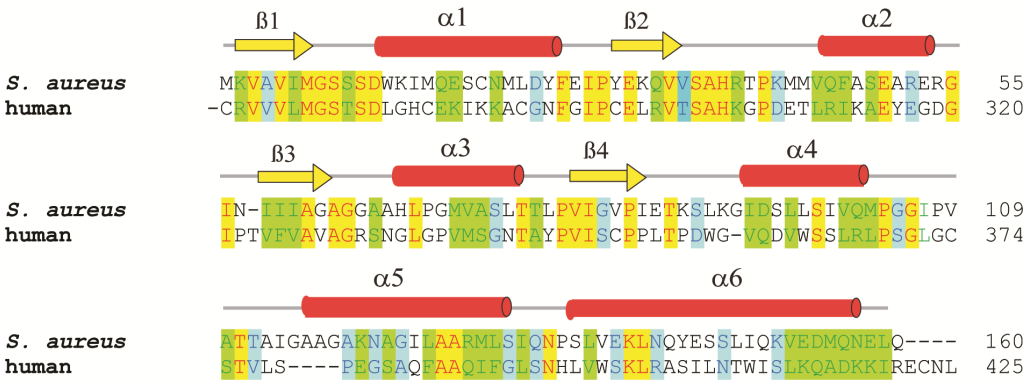
$$V_0 = \frac{V_{\max} [S]}{K_m + [S]} \quad \begin{array}{l} V_{\max} = 29.6 \pm 3 \mu\text{M}/\text{min} \\ K_m = 18.8 \pm 3.9 \text{ mM} \end{array}$$

SEC chromatogram of GFPurECK081110:1\_UV. The x-axis represents elution volume in ml (60.0 to 90.0), and the y-axis represents absorbance in mAU (0.0 to 50.0). The chromatogram shows three distinct peaks corresponding to the proteins PurE, PurK, and PurC. The peak for PurE is at approximately 72.5 ml (138.7 kDa), PurK is at approximately 78.5 ml (85.5 kDa), and PurC is at approximately 83.5 ml (53.9 kDa). The legend indicates the data source as GFPurECK081110:1\_Logbook.

Protein	Elution Volume (ml)	Molecular Weight (kDa)
PurE	~72.5	138.7
PurK	~78.5	85.5
PurC	~83.5	53.9

[illegible]

**Figure 4.**Sequence alignment of *S. aureus* PurE with human PurE.



**Figure 5.**Overlay of *S. aureus* PurE (orange) and human bifunctional enzyme PurC-PurE (PDB ID: 2H31). The PurE domain of the human enzyme is in the center and the PurC domain in the outside.

