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Supporting information for article:

**Octameric structure of *Staphylococcus aureus* enolase in
complex with phosphoenolpyruvate**

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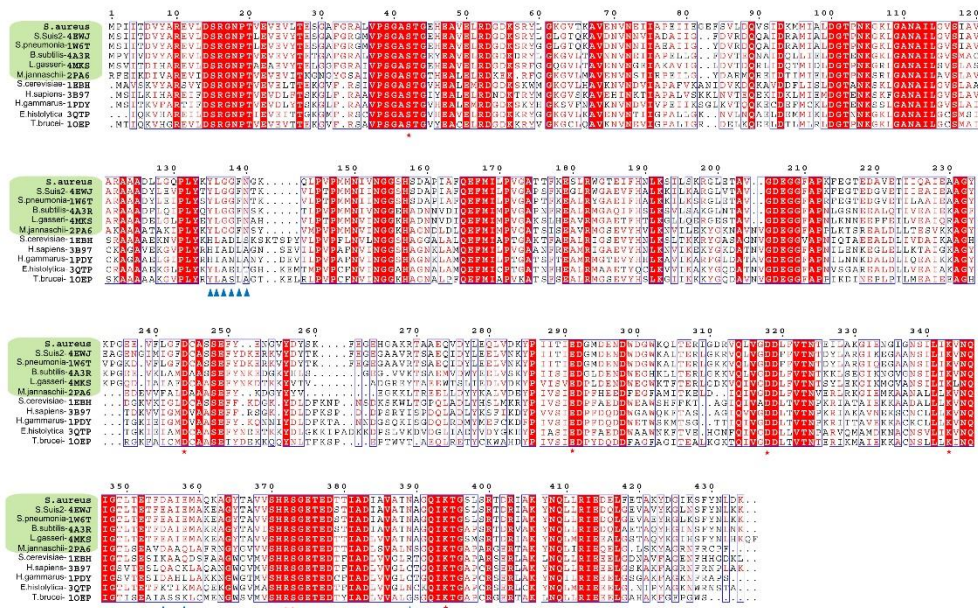


Figure S1 Multiple sequences alignment of enolase from representative species. Enolases from *S. suis* 2, *S. pneumoniae*, *B. subtilis*, *L. gasseri*, *M. jannaschii*, *S. cerevisiae*, *H. sapiens*, *H. gammarus*, *E. histolytica*, and *T. brucei* are used for sequences alignment. Active site residues are marked with red star, conserved dimer-dimer interface residues are marked with blue triangle. The octameric enolases are highlighted with green background

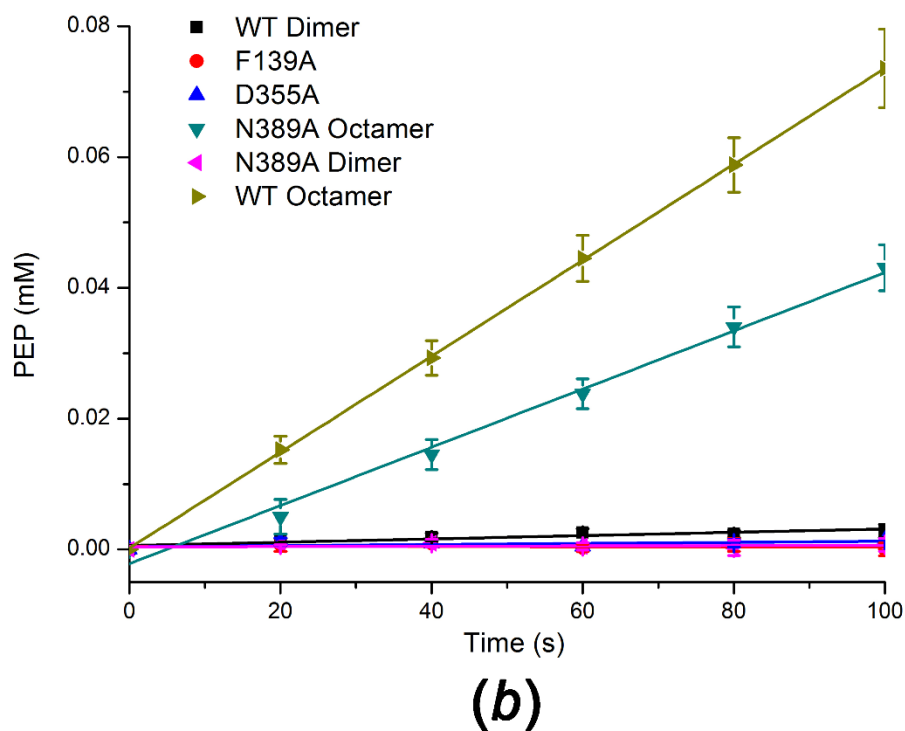
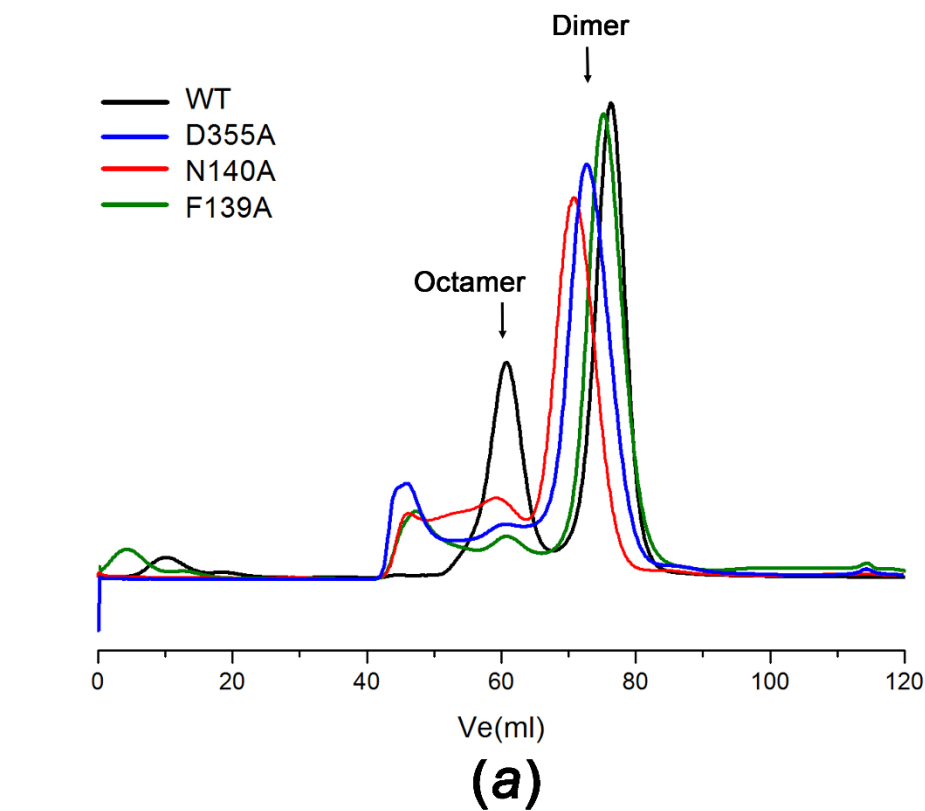


Figure S2 Oligomeric states analysis and enzymatic activity assays of WT *Sa_enolase* and its mutants. (a) Oligomeric states analysis of wild type *Sa_enolase* and its mutants using size exclusion chromatography with column Superdex200 16/60 (GE). (b) Activity assays were

performed in 20 mM IMD/HCl, 400 mM KCl, 1 mM Mg(Ac)₂, pH 7.0 buffer using 30 nM enzyme and 1 mM 2-PG to a final volume of 100 μL. N389A octamer had partial enzymatic activity comparing to WT octamer, while WT dimer, F139A, D355A, and N389A dimer are inactive. Each reaction was repeated for three times.

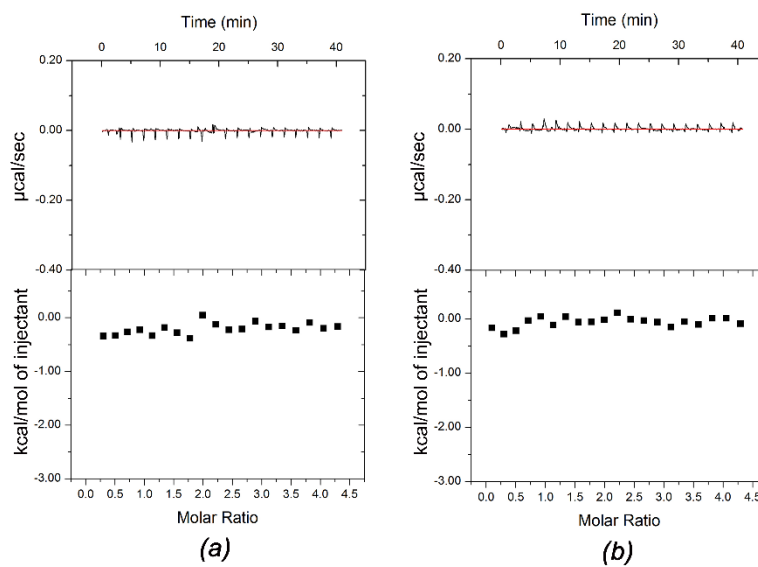


Figure S3 ITC assays for *Sa*_enolase mutants F139A (a) and D355A (b) binding to 2-PG.