

Supporting Information for

**Sequential magnesium binding facilitates lysyl-tRNA synthetase to recognize ATP**

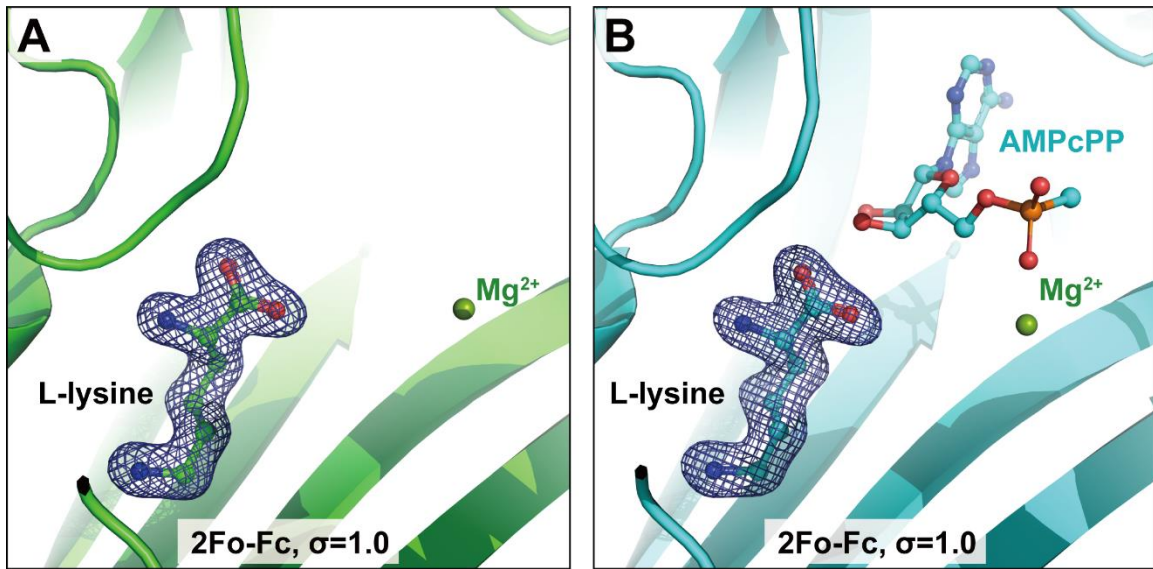
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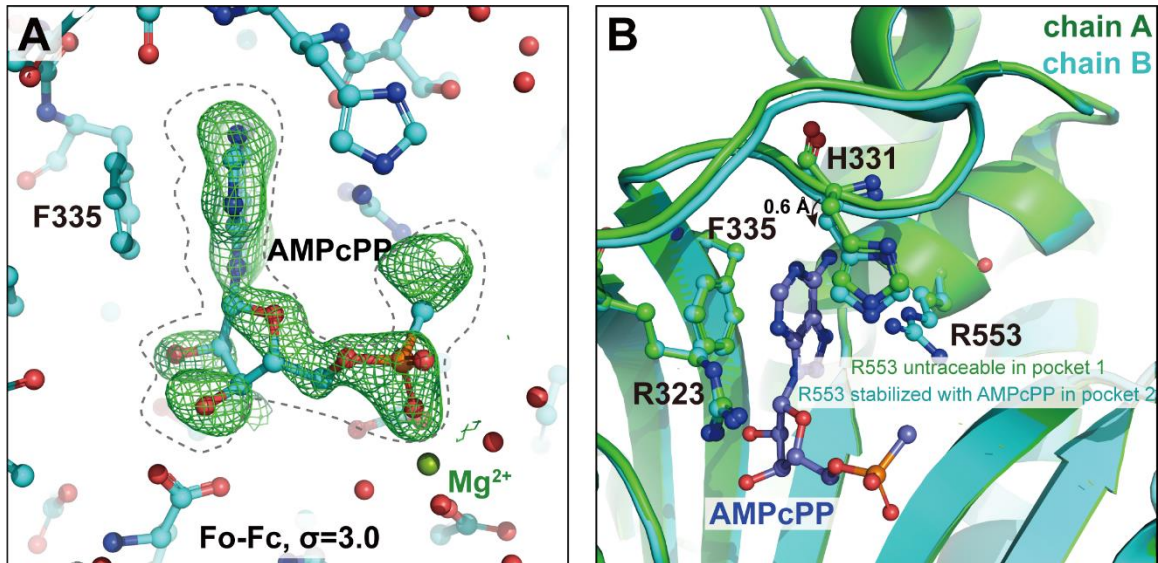
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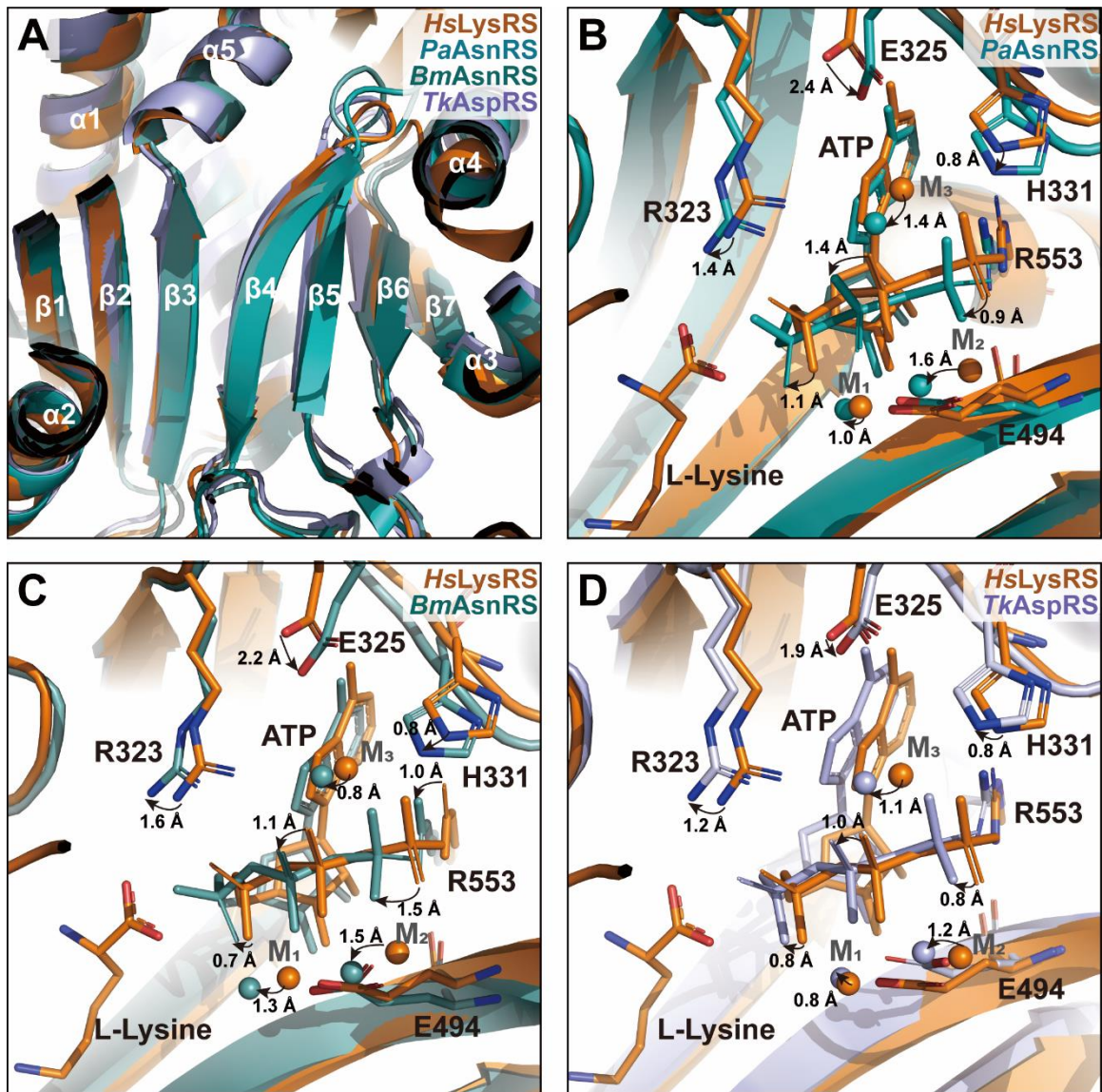
Supplementary Figures S1-S3.



**Figure S1.** The electron density maps of the L-lysine in pocket 1 (**A**) and pocket 2 (**B**).



**Figure S2.** Increased occupancy of AMPcPP in LysRS pocket 2. **(A)** The AMPcPP omitted Fo-Fc map is shown as green meshes in pocket 2. **(B)** Superimposition of chain A and chain B shows that the increased occupancy of AMPcPP in pocket 2 tightens up the loop of the class II aaRS signature motif 2.



**Figure S3.** The three metal ions are localized in conserved positions in the catalytic pocket of class II aaRSs. (A) The catalytic core in class II aaRSs is composed of a characteristic seven-stranded antiparallel  $\beta$ -sheet surrounded by a number of  $\alpha$ -helices. (B) Superimposition of the human LysRS (PDB: 3BJU, orange) with the *Pyrococcus abyssi* AsnRS (PDB: 3REU, teal). (C) Superimposition of the human LysRS (PDB: 3BJU, orange) with the *Brugia malayi* AsnRS (PDB: 2XTI, lightteal). (D) Superimposition of the human LysRS (PDB: 3BJU, orange) with the *Thermococcus kodakarensis* AspRS (PDB: 3NEM, lightblue). M: metal ions.