Supplementary Data for "Kinetic Discrimination of tRNA Identity by the Conserved motif 2 loop of a Class II Aminoacyl-tRNA Synthetase"

Ethan Guth & Christopher Francklyn

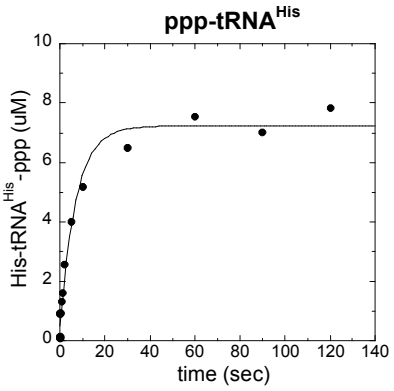
Figure Legends

Figure S1. Single turnover Progress curves for mutant $tRNA^{His}$ and mutant HisRS. HisRS with preformed adenylate was rapidly mixed with $tRNA^{His}$ mutants at 37 ^{o}C, pH 7.5 in the RQF-3 quench flow apparatus. The accumulation of product was monitored as described in Experimental Procedures. **A**., 5'-ppp $tRNA^{His}$/wt HisRS. **B**, wt $tRNA^{His}$/ E115A HisRS. **C**, wt $tRNA^{His}$/ Q118E HisRS. **D**, wt $tRNA^{His}$/R116A HisRS. **E**, wt $tRNA^{His}$/ R123A HisRS.

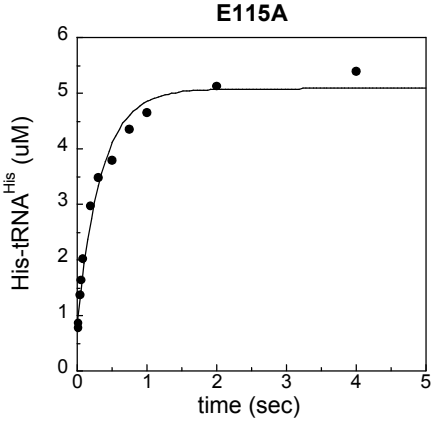
Figure S2. Multiple turnover progress curves for mutant HisRS and mutant $tRNA^{His}$ comparing the production of [^{32}P]-AMP and [^{14}C]-His~$tRNA^{His}$ at pH 7.5 and 37^{o}C. Multiple turnover progress curves for wild type $tRNA^{His}$ and mutant HisRS comparing the production of AMP and His-tRNA at pH 7.5 and 37^{o}C. **A**, **B**, Representative enzyme-tRNA combinations in which k_{trans} was greater than k_{cat}, and progress curves for the two products were linear. The plots for wt $tRNA^{His}$/E115A, and wt $tRNA^{His}$/Q127A HisRS are depicted here. **C, D, E**, Enzyme-tRNA combinations in which there was a burst of AMP formation in the first turnover. The plots for wt $tRNA^{His}$/R123A HisRS, and wt $tRNA^{His}$/Q118A HisRS and wt $tRNA^{His}$/R116A HisRS are depicted here.

Figure S3. Rate of decay curve for histidyl-adenylate preformed on HisRS in the absence of tRNA. The adenylate complex was formed in the presence of excess histidine and ATP, purified by spin chromatography, and then incubated at 37 ^{o}C. The amount of adenylate remaining was quantitated by subsequent re-chromatography of aliquots through G25 Sepahade spin columns. Similar kinetics were obtained by enzyme histidyl:adenylate capture using nitrocellulose filtration.

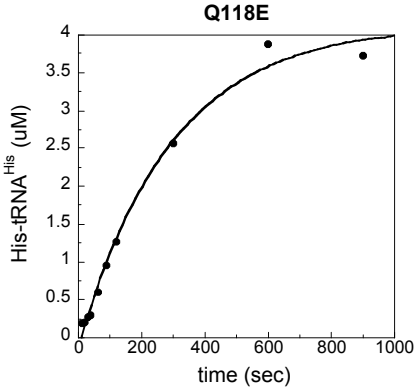
A



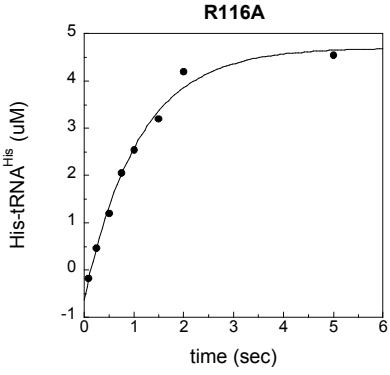
B



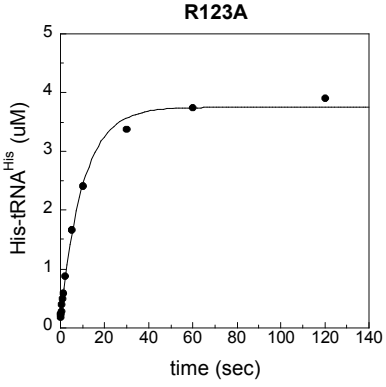
C



D

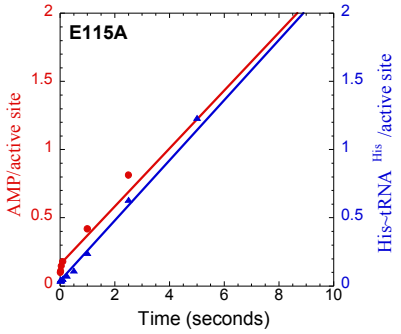


E

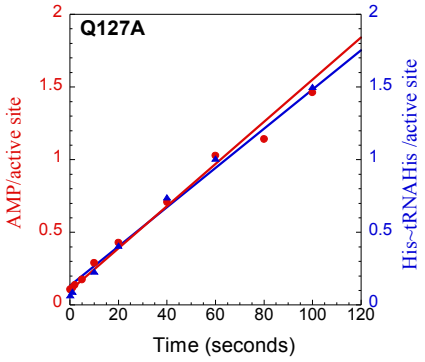


Guth &Francklyn Figure S1

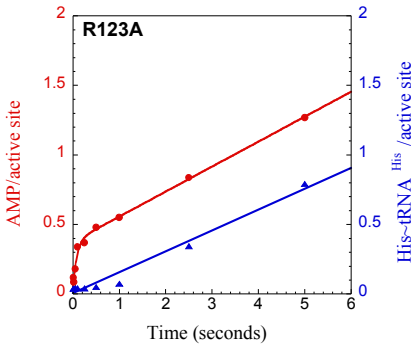
A



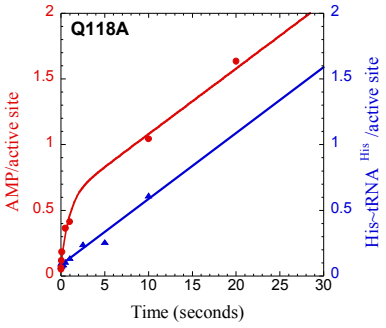
B



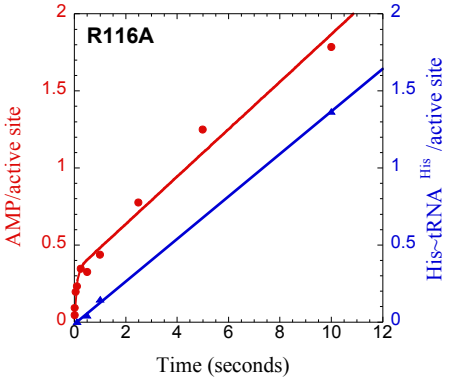
C



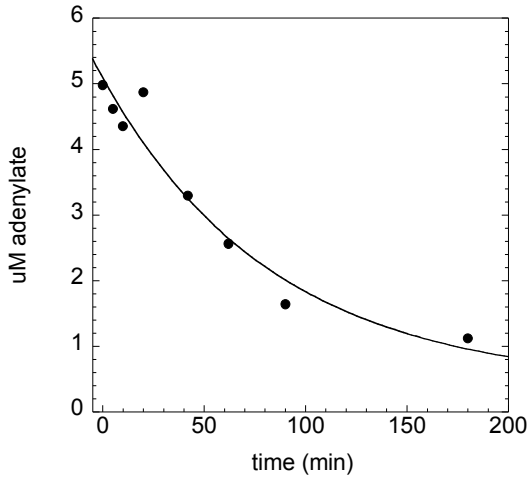
D



E



Guth & Francklyn Figure S2



Guth & Francklyn Figure S3