Supplemental Data Figure Legends

Supplemental Figure 1 – Van't Hoff and Eyring plots for the tRNA^{Tyr} aminoacylation reaction. Van't Hoff and Eyring plots are shown for the binding of tyrosine to unliganded tyrosyl-tRNA synthetase (Panel A), the binding of ATP to the TyrRS•Tyr complex (panel B), formation of the TyrRS•[Tyr-ATP]‡ complex (panel C), binding of ATP to unliganded tyrosyl-tRNA synthetase (panel D), formation of the TyrRS•Tyr-AMP•PP$_i$ complex (panel E), dissociation of pyrophosphate from the TyrRS•Tyr-AMP•PP$_i$ complex (panel F), binding of tRNA^{Tyr} to the TyrRS•Tyr-AMP intermediate (panel G), and formation of the TyrRS•[Tyr-tRNA^{Tyr}•AMP]‡ complex (panel H). Least squares analysis was used to fit the data to a nonlinear equation (model 2) using equations (7) and (9). Error bars indicate standard deviations for the experimental measurements. In most cases, the error bars are smaller than the symbols used to represent the data points and are obscured.

Supplemental Figure 2 – Van't Hoff and Eyring plots for the tRNA^{Tyr} aminoacylation reaction. Van't Hoff and Eyring plots are shown for the binding of tyrosine to unliganded tyrosyl-tRNA synthetase (Panel A), the binding of ATP to the TyrRS•Tyr complex (panel B), formation of the TyrRS•[Tyr-ATP]‡ complex (panel C), binding of ATP to unliganded tyrosyl-tRNA synthetase (panel D), formation of the TyrRS•Tyr-AMP•PP$_i$ complex (panel E), dissociation of pyrophosphate from the TyrRS•Tyr-AMP•PP$_i$ complex (panel F), binding of tRNA^{Tyr} to the TyrRS•Tyr-AMP intermediate (panel G), and formation of the TyrRS•[Tyr-tRNA^{Tyr}•AMP]‡ complex (panel H). Least squares analysis was used to fit the data to a linear equation (model 1) using equations (6) and (8). Error bars indicate standard deviations for the experimental measurements. In most cases, the error bars are smaller than the symbols used to represent the data points and are obscured.
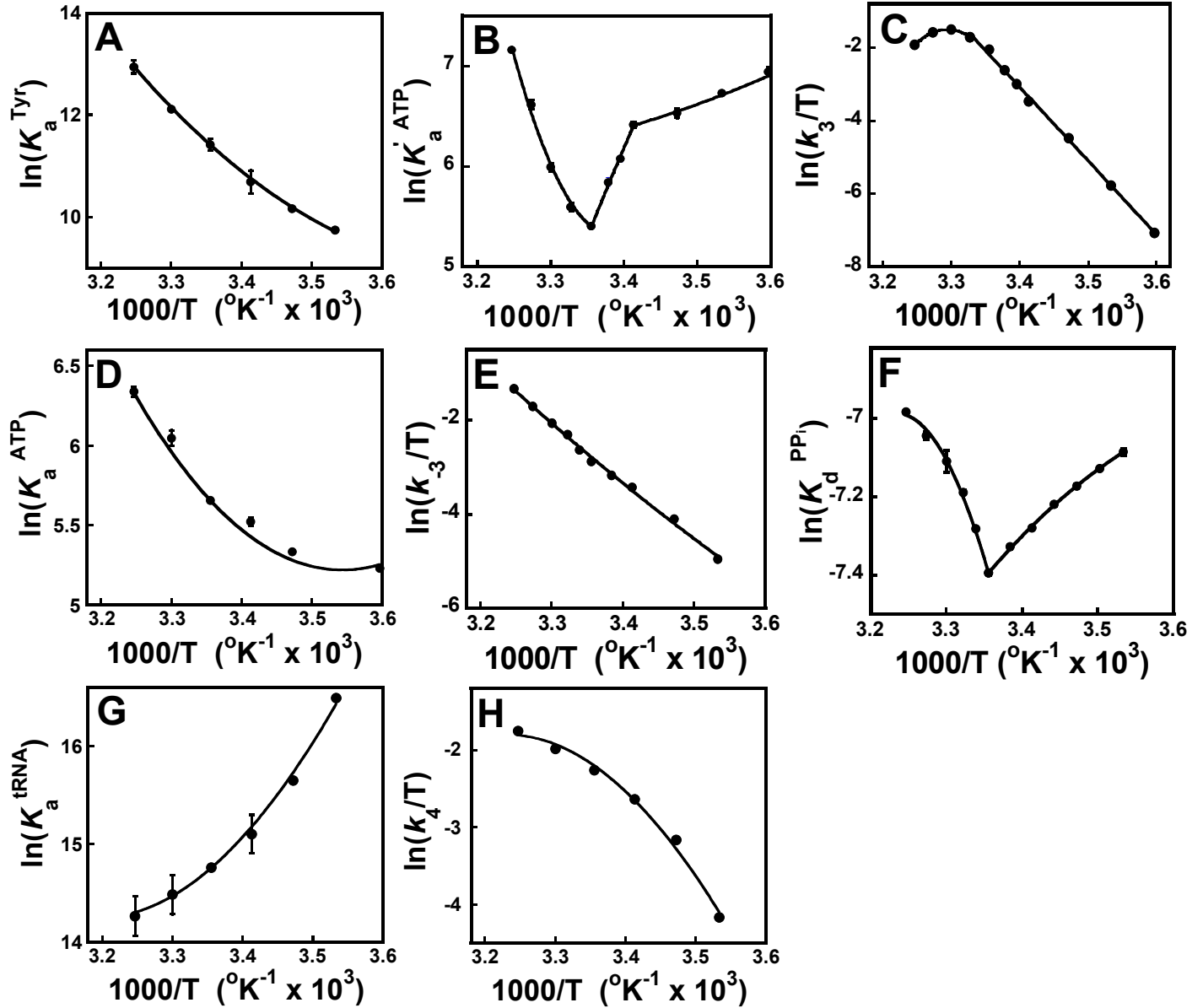
Supplementary Table I
Standard enthalpy, entropy, and free energy values for catalysis of tRNA^{Tyr} aminoacylation reaction using van't Hoff and Eyring's equations (equations 6 & 7)

Enzyme Complex	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/mol-K)	$\Delta G°$ (kJ/mol)
TyrRS + Tyr $\rightleftarrows$ TyrRS•Tyr			
5-35 °C	93.4 (±2)	408 (±4)	-28.5 (±0.1)
TyrRS + ATP $\rightleftarrows$ TyrRS•ATP			
5-20 °C	14.7 (±3)	100 (±10)	-14.1 (±0.2)
20-35 °C	43 (±2)	191 (±5)	-14.3 (±0.1)
TyrRS•Tyr + ATP $\rightleftarrows$ TyrRS•Tyr •ATP			
5-20 °C	-24 (±2)	-29 (±3)	-15.4 (±0.1)
20-25 °C	-144 (±6)	-438 (±6)	-13.3 (±0.2)
25-35 °C	138 (±8)	506 (±1)	-13.3 (±0.3)
TyrRS•Tyr •ATP $\rightleftarrows$ TyrRS•[Tyr-ATP]‡			
5-29 °C	168 (±7)	347 (±2)	64.7 (±0.2)
29-35 °C	-65 (±3)	-425 (±4)	61.5 (±0.2)
TyrRS•[Tyr-ATP]‡ $\rightleftarrows$ TyrRS•Tyr-AMP•PP$_i$			
10-35 °C	103 (±3)	125 (±3)	-65.7 (±0.5)
TyrRS•Tyr-AMP•PP$_i$ $\rightleftarrows$ TyrRS•Tyr-AMP + PP$_i$			
10-25 °C	-14.2 (±0.4)	-109 (±2)	18.3 (±0.3)
25-35 °C	30 (±2)	41 (±1)	18.3 (±0.3)
TyrRS•Tyr-AMP + tRNA^{Tyr} $\rightleftarrows$ TyrRS•Tyr-AMP•tRNA^{Tyr}			
10-20 °C	-96 (±3)	-202 (±4)	-37 (±3)
20-35 °C	-42 (±3)	18 (±1)	-47 (±3)
TyrRS•Tyr-AMP•tRNA^{Tyr} $\rightleftarrows$ TyrRS•[Tyr-tRNA^{Tyr}•AMP]‡			
10-20 °C	106 (±4)	-140 (±10)	65 (±3)
20-35 °C	44 (±3)	69 (±3)	66 (±3)

Supplementary Table II
Standard enthalpy, entropy, and free energy values for catalysis of tRNA^{Tyr} aminoacylation reaction using integrated van't Hoff and Eyring's equations (equations 8 & 9)

Enzyme Complex	$\Delta H°$ (kJ/mol)	$\Delta S°$ (J/mol-K)	$\Delta G°$ (kJ/mol)	ΔCp (kJ/mol-K)
TyrRS + Tyr $\rightleftarrows$ TyrRS•Tyr				
5-35 °C	103 (±2)	437 (±4)	-27.2[1]	3.0 (±0.3)
TyrRS + ATP $\rightleftarrows$ TyrRS•ATP				
5-35 °C	39 (±3)	180 (±10)	-14.0[1]	2.5 (±0.4)
TyrRS•Tyr + ATP $\rightleftarrows$ TyrRS•Tyr •ATP				
5-20 °C	-27 (±5)	-37 (±3)	-15.4 (±0.1)	1.1 (±0.2)
20-25 °C	-150 (±10)	-469 (±6)	-13.3[1]	3.3 (±0.5)
25-35 °C	150 (±10)	450 (±8)	-13.3[1]	18 (±3)
TyrRS•Tyr •ATP $\rightleftarrows$ TyrRS•[Tyr-ATP]‡				
5-29 °C	166 (±9)	340 (±10)	64.7 (±0.2)	0.16 (±0.09)
29-35 °C	-87 (±3)	-411 (±9)	61.5 (±0.2)	3.5 (±0.4)
TyrRS•[Tyr-ATP]‡ $\rightleftarrows$ TyrRS•Tyr-AMP•PP$_i$				
10-35 °C	105 (±3)	132 (±9)	-65.7 (±0.5)	0.9 (±0.5)
TyrRS•Tyr-AMP•PP$_i$ $\rightleftarrows$ TyrRS•Tyr-AMP + PP$_i$				
10-25 °C	-18.7 (±0.4)	-124 (±2)	18.3[1]	-0.57 (±0.06)
25-35 °C	55.8 (±0.2)	126 (±1)	18.3[1]	-5.0 (±0.6)
TyrRS•Tyr-AMP + tRNA^{Tyr} $\rightleftarrows$ TyrRS•Tyr-AMP•tRNA^{Tyr}				
10-35 °C	-52 (±3)	-50 (±10)	-36.6[2]	3.5 (±0.4)
TyrRS•Tyr-AMP•tRNA^{Tyr} $\rightleftarrows$ TyrRS•[Tyr-tRNA^{Tyr}•AMP]‡				
10-35 °C	53 (±4)	-40 (±10)	65.1[2]	4.7 (±0.9)

Supplemental Figure 1

Supplemental Figure 2