SUPPORTING INFORMATION for

Smith and Jackman "Kinetic analysis of 3'-5' nucleotide addition catalyzed by eukaryotic $tRNA^{His}$ guanylyltransferase"

Supporting information figure legends

Figure S1: Single turnover enzyme dependence of adenylylation at pH 7.5. (A) Reactions were carried out with 4 mM ATP and limiting 5'-[^{32}P]$tRNA^{His}$ in the presence of 0.6 (▲), 1 (+), 3 (×), 5 (♦), 10 (■), and 15 (●) μM yThg1. Product formation at each concentration of enzyme was plotted as a function of time and fit to a single exponential equation (eq. 1) to yield the observed rate (k_{obs}). (B) Observed rates of adenylylation were plotted as a function of [E] and fit to $k_{obs} = k_{on}*[E]$ to give an estimate of $k_{on} = 60\ M^{-1}s^{-1}$.

Figure S2: Single turnover ATP dependence of adenylylation at pH 6. (A) Reactions were performed with 15 μM yThg1 and limiting 5'-[^{32}P]$tRNA^{His}$ in the presence of 0.1 (●), 0.25 (□), 0.5 (▲), 1 (×), 1.5 (♦), 2 (Δ), and 3 (○), mM ATP. Product formation at each concentration of ATP was plotted as a function of time and fit to a single exponential equation (eq. 1) to yield the observed rate (k_{obs}). (B) Observed rates of adenylylation were plotted as a function of [ATP] and fit to eq. 2 to determine the first-order adenylylation rate constant (k_{aden}) and apparent equilibrium dissociation constant (K_{Dapp}) for p-$tRNA^{His}$.

Figure S3: Single turnover GTP dependence of nucleotidyl transfer at pH 7.5. (A) Reactions were carried out with 15 μM yThg1 and limiting p*pp-$tRNA^{His}$ in the presence of 0.4 (×), 1.0 (■), 10 (+), 25 (●), 50 (◊), 100 (▲), 200 (○), and 500 (□) μM GTP. Product formation at each concentration of GTP was plotted as a function of time and fit to a single exponential equation (eq. 1) to yield the observed rate (k_{obs}). (B) Observed rates of nucleotidyl transfer were

plotted as a function of [GTP] and fit to eq. 2 to determine the first-order nucleotidyl transfer rate constant (k_{ntrans}) and apparent equilibrium dissociation constant (K_{Dapp}) for GTP.

Figure S4: Single turnover analysis of adenylylation and nucleotidyl transfer catalyzed by S76A yThg1. (A) Adenylylation reactions were performed with 15 μM yS76A and limiting 5'-[^{32}P]tRNA^{His} in the presence of 0.075 (▲), 0.25 (■), 0.5 (♦), 1 (+), 3 (□), and 5 (●) mM ATP. Product formation at each concentration of ATP was plotted as a function of time and fit to a single exponential equation (eq. 1) to yield the observed rate (k_{obs}). (B) Nucleotidyl transfer reactions were carried out with 15 μM yS76A and limiting p*pp-tRNA^{His} in the presence of 5 (●), 15 (□), 75 (▲), and 250 (◊) μM GTP. Product formation at each concentration of GTP was plotted as a function of time and fit to a single exponential equation (eq. 1) to yield the observed rate (k_{obs}).

Figure S1

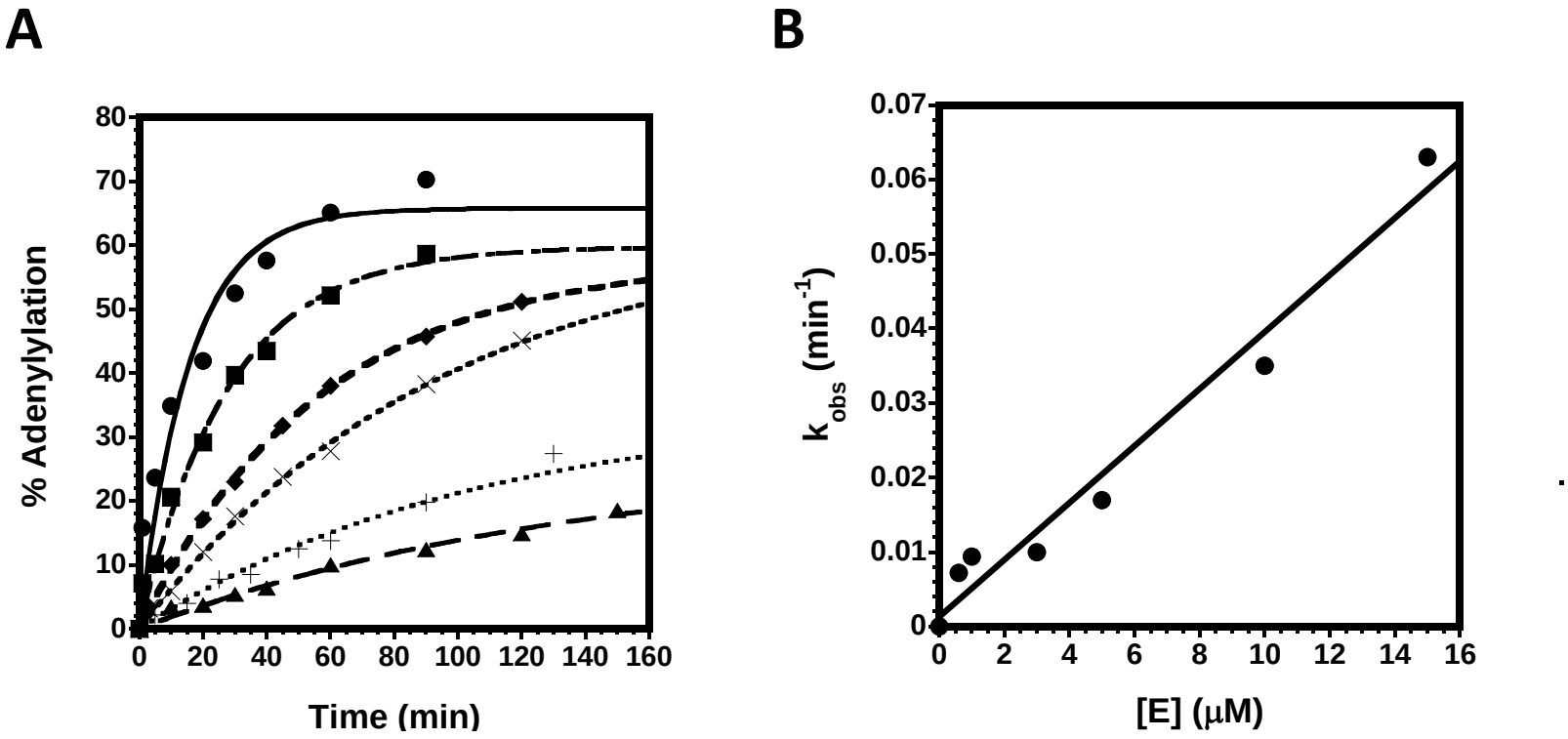

A
% Adenylylation
Time (min)
0
10
20
30
40
50
60
70
80
0
20
40
60
80
100
120
140
160
B
k_{obs} (min^{-1})
[E] (μM)
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0
2
4
6
8
10
12
14
16

Figure S2

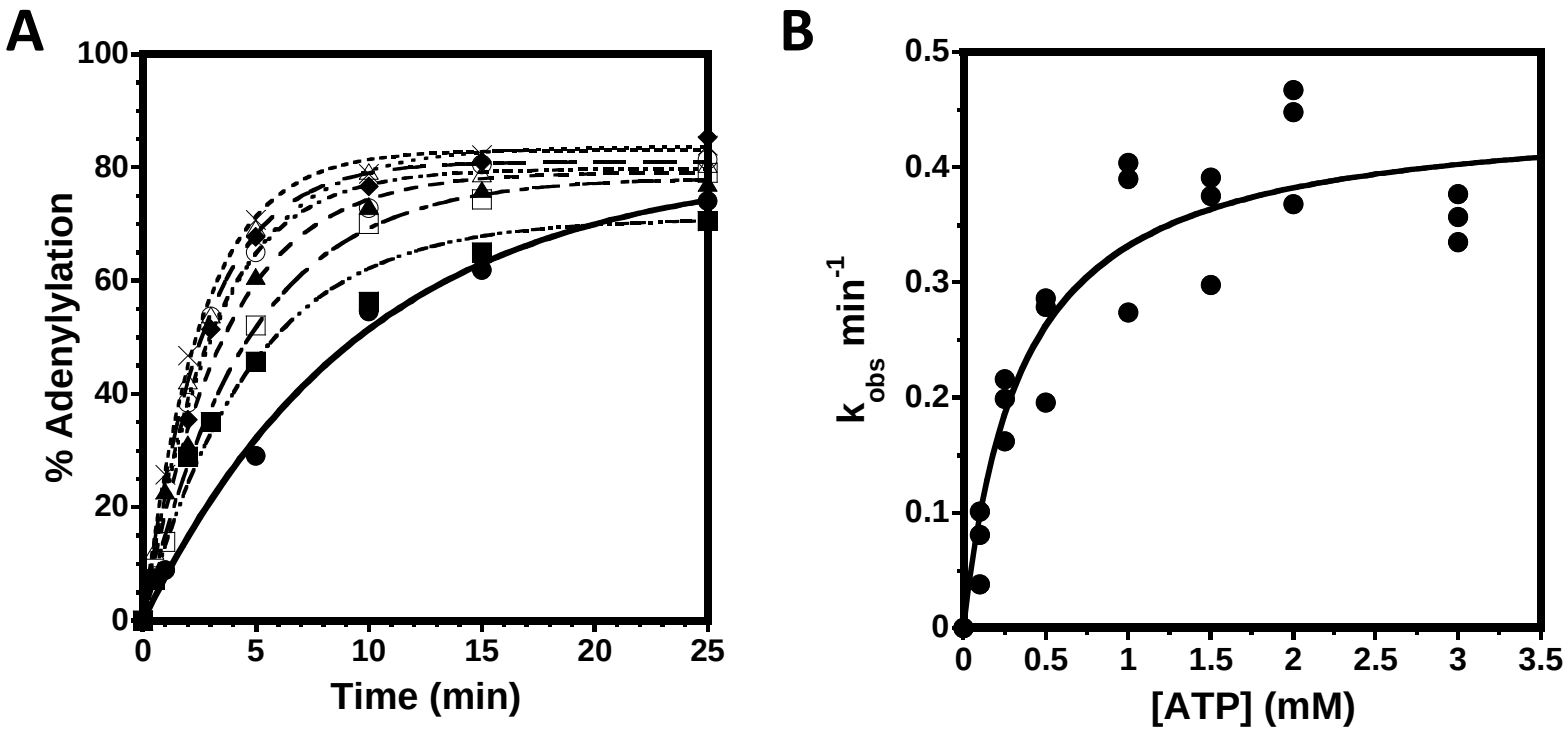

A
% Adenylylation
Time (min)
0
20
40
60
80
100
0
5
10
15
20
25
B
k_{obs} min^{-1}
[ATP] (mM)
0
0.1
0.2
0.3
0.4
0.5
0
0.5
1
1.5
2
2.5
3
3.5

Figure S3

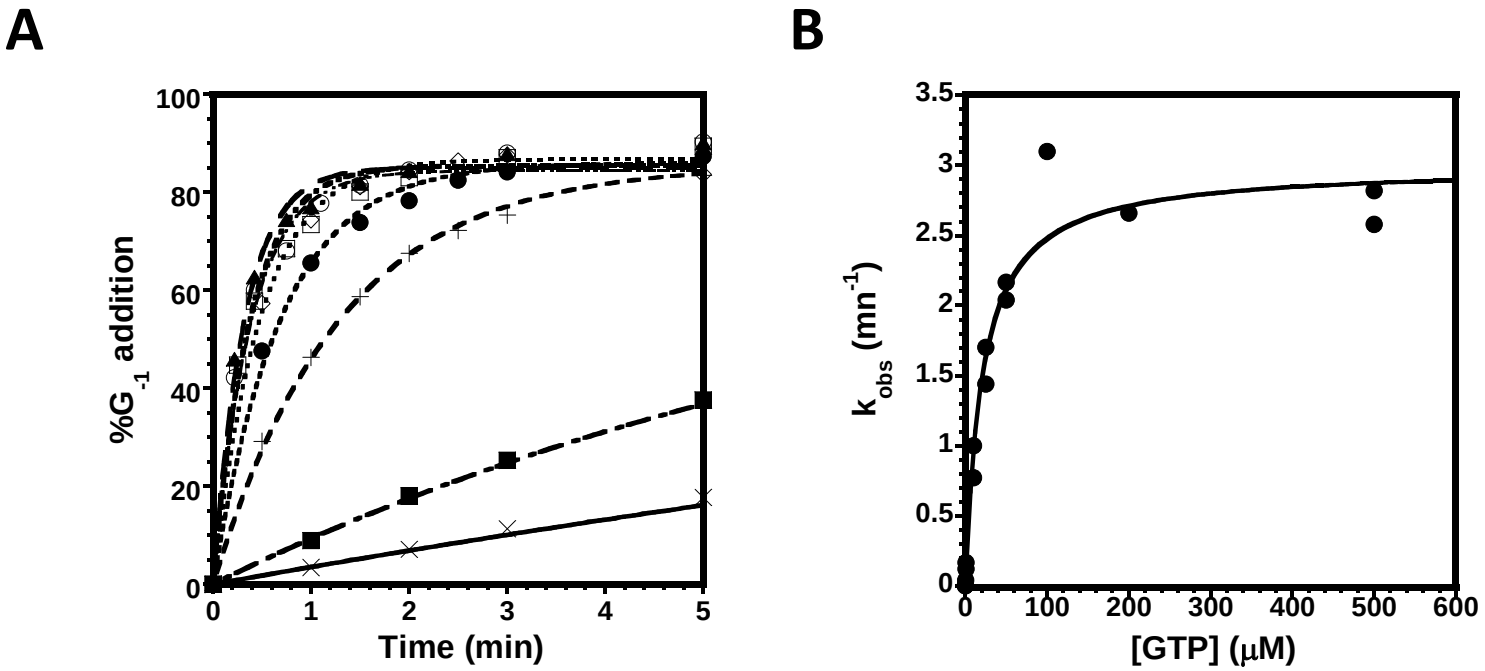
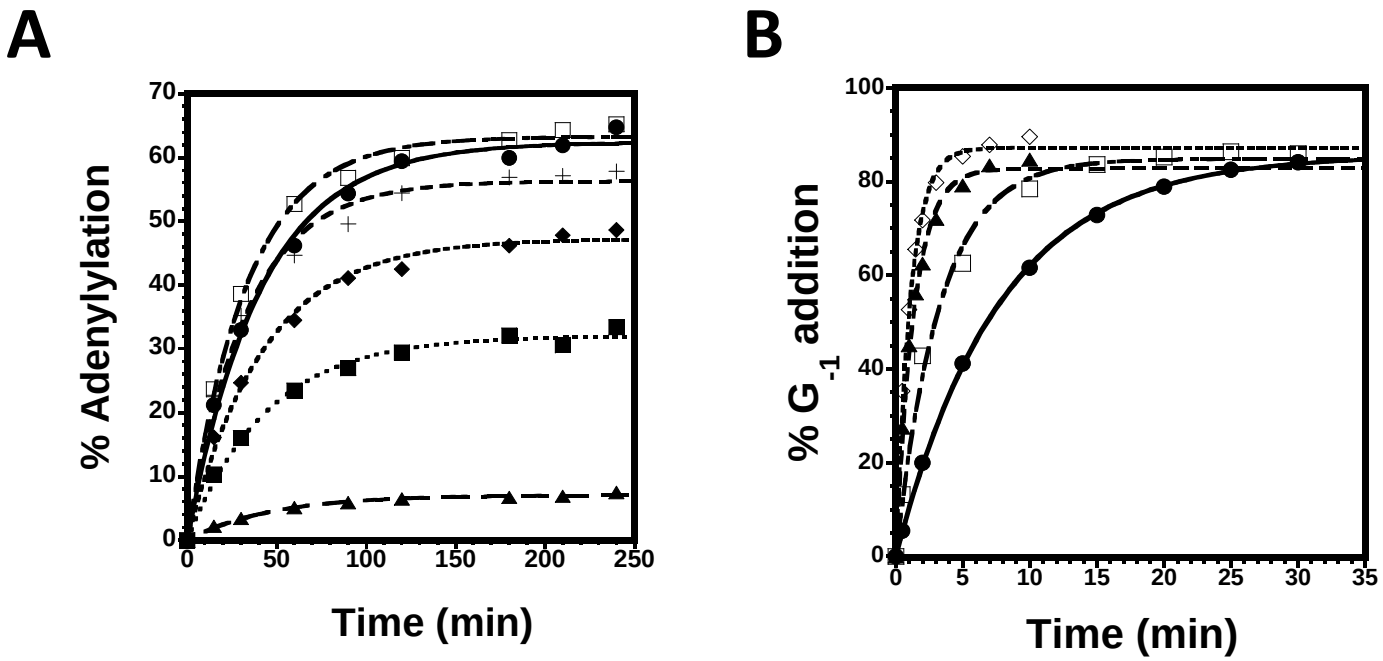

A
%G-1 addition
Time (min)
0
20
40
60
80
100
0
1
2
3
4
5
B
kobs (mn-1)
[GTP] (μM)
0
0.5
1
1.5
2
2.5
3
3.5
0
100
200
300
400
500
600

Figure S4


A
% Adenylylation
Time (min)
0
10
20
30
40
50
60
70
0
50
100
150
200
250
B
% G-1 addition
Time (min)
0
20
40
60
80
100
0
5
10
15
20
25
30
35