

SUPPLEMENTAL MATERIAL

KINETIC MECHANISM OF FULLY ACTIVATED S6K1 PROTEIN KINASE*

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Running Title: Kinetic Mechanism of S6K1 Kinase

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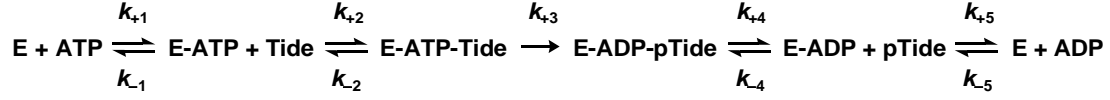
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1. Experimental Procedures

1.1. Formulation of an Ordered Bi Bi Mechanism

The kinetic formulation is described for an Ordered Bi Bi system, taking into account an irreversible chemical phosphorylation step in the ternary complex (Scheme 1).



Scheme 1

The schematic method of King and Altman (1) was used to derive the complete rate equation given by Equation S1,

$$\frac{v(\text{M s}^{-1})}{[\text{E}](\text{M})} = k(\text{s}^{-1}) = \frac{n_1 [\text{A}][\text{B}]}{d_1 + d_A [\text{A}] + d_B [\text{B}] + d_{AB} [\text{A}][\text{B}] + d_Q [\text{Q}] + d_{BQ} [\text{B}][\text{Q}] + d_{QP} [\text{Q}][\text{P}] + d_{BQP} [\text{B}][\text{Q}][\text{P}] + d_{ABP} [\text{A}][\text{B}][\text{P}]} \quad (\text{S1})$$

whereby [A], [B], [P], and [Q] represent the concentrations of the first binding substrate (ATP), the second binding substrate (Tide), the product of the second binding substrate (pTide), and the product of the first binding substrate (ADP), respectively; and the coefficients are defined by the microscopic rate constants (Scheme 1) as

$$n_1 = k_{+1} k_{+2} k_{+3} k_{+4} k_{+5}$$

$$d_1 = k_{-1} k_{-2} k_{+4} k_{+5} + k_{-1} k_{+3} k_{+4} k_{+5}$$

$$d_A = k_{+1} k_{-2} k_{+4} k_{+5} + k_{+1} k_{+3} k_{+4} k_{+5}$$

$$d_B = k_{+2} k_{+3} k_{+4} k_{+5}$$

$$d_{AB} = k_{+1} k_{+2} k_{+4} k_{+5} + k_{+1} k_{+2} k_{+3} k_{+5} + k_{+1} k_{+2} k_{+3} k_{+4}$$

$$d_Q = k_{-1} k_{-2} k_{+4} k_{-5} + k_{-1} k_{+3} k_{+4} k_{-5}$$

$$d_{BQ} = k_{+2} k_{+3} k_{+4} k_{-5}$$

$$d_{QP} = k_{-1} k_{-2} k_{-4} k_{-5} + k_{-1} k_{+3} k_{-4} k_{-5}$$

$$d_{BPQ} = k_{+2} k_{+3} k_{-4} k_{-5}$$

$$d_{ABP} = k_{+1} k_{+2} k_{+3} k_{-4}$$

According to the general rules of Cleland (2–4), the coefficient terms are converted to coefficient forms composed entirely of kinetic constants by first dividing each coefficient term by the coefficient of all reactants, d_{AB} , as shown by Equation S2.

$$k = \frac{\frac{n_1}{d_{AB}} [\text{A}][\text{B}]}{\frac{d_1}{d_{AB}} + \frac{d_A}{d_{AB}} [\text{A}] + \frac{d_B}{d_{AB}} [\text{B}] + \frac{d_{AB}}{d_{AB}} [\text{A}][\text{B}] + \frac{d_Q}{d_{AB}} [\text{Q}] + \frac{d_{BQ}}{d_{AB}} [\text{B}][\text{Q}] + \frac{d_{QP}}{d_{AB}} [\text{Q}][\text{P}] + \frac{d_{BPQ}}{d_{AB}} [\text{B}][\text{Q}][\text{P}] + \frac{d_{ABP}}{d_{AB}} [\text{A}][\text{B}][\text{P}]} \quad (\text{S2})$$

1.1.1. Two-Substrate Steady-State Kinetics

In the absence of any added products (i.e., $[Q] = [P] = 0$), Equation S2 reduces to Equation S3,

$$k = \frac{\frac{n_1}{d_{AB}}[A][B]}{\frac{d_1}{d_{AB}} + \frac{d_A}{d_{AB}}[A] + \frac{d_B}{d_{AB}}[B] + [A][B]} \quad (\text{S3})$$

whereby the ratios of the coefficient terms are related to kinetic constants as defined by Equations S4–S7 to yield the final form of the steady-state kinetic expression given by Equation S8.

$$k_{\text{cat}} = \frac{n_1}{d_{AB}} = \frac{k_{+1}k_{+2}k_{+3}k_{+4}k_{+5}}{k_{+1}k_{+2}k_{+4}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+4}} = \frac{k_{+3}k_{+4}k_{+5}}{k_{+4}k_{+5} + k_{+3}k_{+5} + k_{+3}k_{+4}} \quad (\text{S4})$$

$$K_m^B = \frac{d_A}{d_{AB}} = \frac{k_{+1}k_{-2}k_{+4}k_{+5} + k_{+1}k_{+3}k_{+4}k_{+5}}{k_{+1}k_{+2}k_{+4}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+4}} = \frac{k_{+4}k_{+5}(k_{-2} + k_{+3})}{k_{+2}(k_{+4}k_{+5} + k_{+3}k_{+5} + k_{+3}k_{+4})} \quad (\text{S5})$$

$$K_m^A = \frac{d_B}{d_{AB}} = \frac{k_{+2}k_{+3}k_{+4}k_{+5}}{k_{+1}k_{+2}k_{+4}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+4}} = \frac{k_{+3}k_{+4}k_{+5}}{k_{+1}(k_{+4}k_{+5} + k_{+3}k_{+5} + k_{+3}k_{+4})} \quad (\text{S6})$$

$$K_m^B K_d^A = \frac{d_1}{d_{AB}} \times \frac{d_A}{d_A} = K_m^B \frac{k_{-1}k_{-2}k_{+4}k_{+5} + k_{-1}k_{+3}k_{+4}k_{+5}}{k_{+1}k_{-2}k_{+4}k_{+5} + k_{+1}k_{+3}k_{+4}k_{+5}} = K_m^B \frac{k_{-1}k_{+4}k_{+5}(k_{-2} + k_{+3})}{k_{+1}k_{+4}k_{+5}(k_{-2} + k_{+3})} = K_m^B \frac{k_{-1}}{k_{+1}} \quad (\text{S7})$$

$$k = \frac{k_{\text{cat}} [A][B]}{K_d^A K_m^B + K_m^B [A] + K_m^A [B] + [A][B]} \quad (\text{S8})$$

Dividing by $[B]$ and collecting like terms, Equation S8 is rearranged to Equation S9,

$$k = \frac{k_{\text{cat}} [A]}{K_m^A \left(1 + \frac{K_d^A K_m^B}{K_m^A [B]} \right) + [A] \left(1 + \frac{K_m^B}{[B]} \right)} \quad (\text{S9})$$

which is further rearranged to the Michaelis-Menten form given by Equation S10 for when $[A]$ is the varied substrate at different fixed $[B]$.

$$k = \frac{k_{\text{cat(app)}} [A]}{K_{m(\text{app})}^A + [A]} = \frac{\frac{k_{\text{cat}}}{\left(1 + \frac{K_m^B}{[B]} \right)} [A]}{K_m^A \frac{\left(1 + \frac{K_d^A K_m^B}{K_m^A [B]} \right)}{\left(1 + \frac{K_m^B}{[B]} \right)} + [A]} \quad (\text{S10})$$

Thus, Equation S10 yields expressions for the apparent values of $k_{\text{cat(app)}}$ and $K_{m(\text{app})}^A$ at different fixed $[B]$ given by equations S11 and S12, respectively.

$$k_{\text{cat(app)}} = \frac{k_{\text{cat}}}{\left(1 + \frac{K_m^B}{[B]}\right)} = \frac{k_{\text{cat}} [B]}{K_m^B + [B]} \quad (\text{S11})$$

$$K_{m(\text{app})}^A = \frac{K_m^A \left(1 + \frac{K_d^A K_m^B}{K_m^A [B]}\right)}{\left(1 + \frac{K_m^B}{[B]}\right)} \quad (\text{S12})$$

From Equation S12, it can be seen that when $K_m^A = K_d^A$, there will be no dependence of K_m^A on $[B]$. For generation of double reciprocal plots, the reciprocal of Equation S9 is given by Equation S13.

$$\frac{1}{k} = \frac{K_m^A}{k_{\text{cat}}} \left(1 + \frac{K_d^A K_m^B}{K_m^A [B]}\right) \frac{1}{[A]} + \frac{1}{k_{\text{cat}}} \left(1 + \frac{K_m^B}{[B]}\right) \quad (\text{S13})$$

When $[B]$ is the varied substrate,

$$k = \frac{k_{\text{cat}} [B]}{K_m^B \left(1 + \frac{K_d^A}{[A]}\right) + [B] \left(1 + \frac{K_m^A}{[A]}\right)} \quad (\text{S14})$$

Rearranging Equation S14 to its Michaelis-Menten form yields expressions for the apparent values of $k_{\text{cat(app)}}$ and $K_{m(\text{app})}^B$ at different fixed $[A]$ given by equations S15 and S16, respectively.

$$k_{\text{cat(app)}} = \frac{k_{\text{cat}}}{\left(1 + \frac{K_m^A}{[A]}\right)} = \frac{k_{\text{cat}} [A]}{K_m^A + [A]} \quad (\text{S15})$$

$$K_{m(\text{app})}^B = \frac{K_m^B \left(1 + \frac{K_d^A}{[A]}\right)}{\left(1 + \frac{K_m^A}{[A]}\right)} \quad (\text{S16})$$

From Equation S16, it can be seen that when $K_m^A = K_d^A$, there will be no dependence of K_m^B on $[A]$. For generation of double reciprocal plots, the reciprocal of Equation S14 is given by Equation S17.

$$\frac{1}{k} = \frac{K_m^B}{k_{\text{cat}}} \left(1 + \frac{K_d^A}{[A]}\right) \frac{1}{[B]} + \frac{1}{k_{\text{cat}}} \left(1 + \frac{K_m^A}{[A]}\right) \quad (\text{S17})$$

1.1.2. ADP Product Inhibition Steady-State Kinetics

In the presence of $[Q]$ (i.e., $[P] = 0$), Equation S2 reduces to Equation S18,

$$k = \frac{k_{\text{cat}} [A][B]}{K_d^A K_m^B + K_m^B [A] + K_m^A [B] + [A][B] + \frac{d_Q}{d_{AB}} [Q] + \frac{d_{BQ}}{d_{AB}} [B][Q]} \quad (\text{S18})$$

whereby the two additional coefficient terms are related to kinetic constants as defined by Equations S19 and S20 to yield the final form of the steady-state kinetic expression given by Equation S21.

$$\frac{K_d^A K_m^B}{K_d^Q} = \frac{d_Q}{d_{AB}} \times \frac{d_1}{d_1} = K_d^A K_m^B \frac{k_{-1}k_{-2}k_{+4}k_{-5} + k_{-1}k_{+3}k_{+4}k_{-5}}{k_{-1}k_{-2}k_{+4}k_{+5} + k_{-1}k_{+3}k_{+4}k_{+5}} = K_d^A K_m^B \frac{k_{-1}k_{+4}k_{-5}(k_{-2} + k_{+3})}{k_{-1}k_{+4}k_{+5}(k_{-2} + k_{+3})} = K_d^A K_m^B \frac{k_{-5}}{k_{+5}} \quad (\text{S19})$$

$$\frac{K_m^A}{K_d^Q} = \frac{d_{BQ}}{d_{AB}} \times \frac{d_B}{d_B} = K_m^A \frac{k_{+2}k_{+3}k_{+4}k_{-5}}{k_{+2}k_{+3}k_{+4}k_{+5}} = K_m^A \frac{k_{-5}}{k_{+5}} \quad (\text{S20})$$

$$k = \frac{k_{\text{cat}} [A][B]}{K_d^A K_m^B + K_m^B [A] + K_m^A [B] + [A][B] + \frac{K_d^A K_m^B}{K_d^Q} [Q] + \frac{K_m^A}{K_d^Q} [B][Q]} \quad (\text{S21})$$

When [A] is the varied substrate,

$$k = \frac{k_{\text{cat}} [A]}{K_m^A \left(1 + \frac{[Q]}{K_d^Q} \right) \left(1 + \frac{K_d^A K_m^B}{K_m^A [B]} \right) + [A] \left(1 + \frac{K_m^B}{[B]} \right)} \quad (\text{S22})$$

Under conditions where $[B] \gg K_m^B$, Equation S22 reduces to Equation S23,

$$k = \frac{k_{\text{cat}} [A]}{K_m^A \left(1 + \frac{[Q]}{K_d^Q} \right) + [A]} \quad (\text{S23})$$

whereby Q displays competitive inhibition with respect to the varied A substrate; and Equation S23 shows apparent values of $k_{\text{cat(app)}}$ and $K_m^A(\text{app})$ to be given by Equations S24 and S25, respectively.

$$k_{\text{cat(app)}} = k_{\text{cat}} \quad (\text{S24})$$

$$K_m^A(\text{app}) = K_m^A \left(1 + \frac{[Q]}{K_d^Q} \right) = \frac{K_m^A}{K_d^Q} [Q] + K_m^A \quad (\text{S25})$$

In this case, apparent values of $k_{\text{cat(app)}}$ will be independent of [Q] and equal to the true k_{cat} ; and apparent values of $K_m^A(\text{app})$ will show a linear dependence on [Q]. For generation of double reciprocal plots, the reciprocal of Equation S23 is given by Equation S26.

$$\frac{1}{k} = \frac{K_m^A}{k_{\text{cat}}} \left(1 + \frac{[Q]}{K_d^Q} \right) \frac{1}{[A]} + \frac{1}{k_{\text{cat}}} \quad (\text{S26})$$

1.1.3. *pTide Product Inhibition Steady-State Kinetics*

In the presence of [P] (i.e., [Q] = 0), Equation S2 reduces to Equation S27,

$$k = \frac{k_{\text{cat}} [A][B]}{K_d^A K_m^B + K_m^B [A] + K_m^A [B] + [A][B] + \frac{d_{ABP}}{d_{AB}} [A][B][P]} \quad (\text{S27})$$

whereby the one additional coefficient term is defined as the reciprocal of the inhibition constant of P as defined by Equation S28 to yield the final form of the steady-state kinetic expression given by Equation S29.

$$\frac{1}{K_i^P} = \frac{d_{ABP}}{d_{AB}} = \frac{k_{+1}k_{+2}k_{+4}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+5} + k_{+1}k_{+2}k_{+3}k_{+4}}{k_{+1}k_{+2}k_{+3}k_{-4}} = \frac{k_{+4}k_{+5} + k_{+3}k_{+5} + k_{+3}k_{+4}}{k_{+3}k_{-4}} \quad (\text{S28})$$

$$k = \frac{k_{\text{cat}} [A][B]}{K_d^A K_m^B + K_m^B [A] + K_m^A [B] + [A][B] + \frac{[A][B][P]}{K_i^P}} \quad (\text{S29})$$

When [A] is the varied substrate,

$$k = \frac{k_{\text{cat}} [A]}{K_m^A \left(1 + \frac{K_d^A K_m^B}{[B]} \right) + [A] \left(1 + \frac{K_m^B}{[B]} + \frac{[P]}{K_i^P} \right)} \quad (\text{S30})$$

Under conditions where $[B] \gg K_m^B$, Equation S30 reduces to Equation S31,

$$k = \frac{k_{\text{cat}} [A]}{K_m^A + [A] \left(1 + \frac{[P]}{K_i^P} \right)} \quad (\text{S31})$$

whereby P displays uncompetitive inhibition with respect to the varied A substrate. Rearranging Equation S31 to its Michaelis-Menten form yields expressions for the apparent values of $k_{\text{cat}(\text{app})}$ and $K_{m(\text{app})}^A$, which show similar hyperbolic decreasing dependences on [P] according to Equations S32 and S33, respectively.

$$k_{\text{cat}(\text{app})} = \frac{k_{\text{cat}}}{\left(1 + \frac{[P]}{K_i^P} \right)} \quad (\text{S32})$$

$$K_{m(\text{app})}^A = \frac{K_m^A}{\left(1 + \frac{[P]}{K_i^P} \right)} \quad (\text{S33})$$

For generation of double reciprocal plots, the reciprocal of Equation S31 is given by Equation S34.

$$\frac{1}{k} = \frac{K_m^A}{k_{\text{cat}}} \frac{1}{[A]} + \frac{1}{k_{\text{cat}}} \left(1 + \frac{[P]}{K_i^P} \right) \quad (\text{S34})$$

1.2. Computational Scripts

1.2.1. King-Altman Derivation

The King-Altman computer algorithm (BioKin, Ltd., Pullman, WA) generated Equation S1 using the following script:

```
[reaction]
A + B <=> P + Q
[mechanism]
E + A <=> EA
EA + B <=> EAB
EAB --> AQP
EQP <=> EQ + P
EQ <=> E + Q
[end]
```

1.2.2. Pre-Steady State Kinetic Analysis

For global fitting of pre-steady state kinetic data (Fig. 3) to the Steady-State Ordered Bi Bi system (Scheme 1), the following computer script was used for the DynaFit 3.28 software (BioKin, Ltd., Pullman, WA):

```
[task]
data = progress
task = fit
[mechanism]
E + A <=> EA      : k+1 k-1
EA + B <=> EAB    : k+2 k-2
EAB --> EQP      : k+3
EQP <=> EQ + P   : k+4 k-4
EQ <=> E + Q     : k+5 k-5
[constants]
k+1 = 0.04 ?, k-1 = 0.22   ; first-order rate constants k-1, k-2, k+3, k+4, and k+5 are given in s-1
k+2 = 1 , k-2 = 180 ?     ; bimolecular rate constants k+1, k+2, k-4, and k-5 are given in uM-1 s-1
k+3 = 8.6 ?               ; ? indicates fitted parameters; otherwise fixed
k+4 = 180 , k-4 = 1
k+5 = 0.23 ?, k-5 = 0.04 ?
[concentrations]
E = 2
A = 200
B = 200
[responses]
EQP = 1                   ; this analyzes total product EQP + P required for pre-steady state kinetic data
P = 1
[progress]
directory ./s6k1_kinase/data
extension txt
files a005, a010, a020, a050, a200
vary conc. A = 5, 10, 20, 50, 200
files b005, b010, b020, b050, b200
vary conc. B = 5, 10, 20, 50, 200
files q0000, q0100, q0200, q0500, q1000
vary conc. Q = 0, 100, 200, 500, 1000
[output]
directory ./s6k1_kinase/output
[end]
```

2. Results

Table S1 summarizes the steady-state kinetic constants determined for phosphorylation of Tide substrate by fully active His₆-S6K1 α II(Δ AID)-T389E. Values of k_{cat} , $K_{\text{m}}^{\text{ATP}}$, $K_{\text{m}}^{\text{Tide}}$, $K_{\text{d}}^{\text{ATP}}$, $K_{\text{d}}^{\text{ADP}}$, and $K_{\text{i}}^{\text{pTide}}$ were independently acquired by both global and direct secondary fitting of data obtained in (i) two-substrate, (ii) ADP product inhibition, and (iii) pTide product inhibition steady-state kinetics.

2.1. Two-Substrate Steady-State Kinetics

Fig. S1A shows individual direct plots of all steady-state kinetic data for titration of active His₆-S6K1 α II(Δ AID)-T389E with varying [ATP] substrate at different fixed [Tide] substrate. Data collection in this manner provided all information necessary to construct companion individual direct plots for titration of enzyme with varying [Tide] substrate at different fixed [ATP] (Fig. S1B). Global fitting of the data to Equation S8 yielded values of $k_{\text{cat}} = 0.192 \pm 0.002 \text{ s}^{-1}$, $K_{\text{m}}^{\text{ATP}} = 5.1 \pm 0.2 \text{ }\mu\text{M}$, $K_{\text{m}}^{\text{Tide}} = 4.1 \pm 0.1 \text{ }\mu\text{M}$, and $K_{\text{d}}^{\text{ATP}} = 5.0 \pm 0.4 \text{ }\mu\text{M}$ (Table S1). To better illustrate this analysis, each individual plot was directly fitted to the Michaelis-Menten equation to obtain values of $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}$ (Table S2). Figs. S1C and S1D show secondary plots of $k_{\text{cat}(\text{app})}$ versus either fixed [Tide] or fixed [ATP], respectively. The results of direct fitting of $k_{\text{cat}(\text{app})}$ versus [Tide] yielded values of $k_{\text{cat}} = 0.192 \pm 0.001 \text{ s}^{-1}$ and $K_{\text{m}}^{\text{Tide}} = 4.1 \pm 0.1 \text{ }\mu\text{M}$ (Table S1). The results of direct fitting of $k_{\text{cat}(\text{app})}$ versus [ATP] yielded values of $k_{\text{cat}} = 0.192 \pm 0.002 \text{ s}^{-1}$ and $K_{\text{m}}^{\text{ATP}} = 5.1 \pm 0.1 \text{ }\mu\text{M}$ (Table S1). Figs. S1C and S1D also show that secondary plots of $K_{\text{m}(\text{app})}^{\text{ATP}}$ and $K_{\text{m}(\text{app})}^{\text{Tide}}$ were independent of fixed [Tide] and fixed [ATP], respectively, and were not further analyzed.

2.2. ADP and pTide Product Inhibition Steady-State Kinetics

Figs. S2A S2B show individual direct plots of all steady-state kinetic data for titration of active His₆-S6K1 α II(Δ AID)-T389E with varying [ATP] at different fixed [ADP] and [pTide], respectively. Global fitting of the data in Fig. S2A to Equation S23 for competitive inhibition yielded values of $k_{\text{cat}} = 0.190 \pm 0.003 \text{ s}^{-1}$, $K_{\text{m}}^{\text{ATP}} = 5.2 \pm 0.4 \text{ }\mu\text{M}$, and the inhibition constant $K_{\text{i}}^{\text{ADP}} = 5.5 \pm 0.5 \text{ }\mu\text{M}$; and global fitting of the data to Equation S31 (uncompetitive inhibition) yielded values of $k_{\text{cat}} = 0.184 \pm 0.004 \text{ s}^{-1}$, $K_{\text{m}}^{\text{ATP}} = 4.8 \pm 0.3 \text{ }\mu\text{M}$, and $K_{\text{i}}^{\text{pTide}} = 5.5 \pm 0.5 \text{ }\mu\text{M}$ (Table S1). To better illustrate this analysis, each individual plot was directly fitted to the Michaelis-Menten equation to obtain values of $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}$ (Table S3). Fig. S2C shows secondary plots of $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}^{\text{ATP}}$ versus fixed [ADP]. As expected for competitive inhibition with respect to varied [ATP], $k_{\text{cat}(\text{app})}$ was independent of [ADP], yielding an average value of the true $k_{\text{cat}} = 0.191 \pm 0.003 \text{ s}^{-1}$; and linear fitting of values of $K_{\text{m}(\text{app})}^{\text{ATP}}$ versus [ADP] to Equation S25 yielded values of $K_{\text{m}}^{\text{ATP}} = 5.5 \pm 0.5 \text{ }\mu\text{M}$ and $K_{\text{i}}^{\text{ADP}} = K_{\text{d}}^{\text{ADP}} = 6.0 \text{ }\mu\text{M}$ (Table S1). Fig. S2D shows secondary plots of $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}^{\text{ATP}}$ versus fixed [pTide]. As expected for uncompetitive inhibition with respect to varied [ATP], both $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}^{\text{ATP}}$ showed similar hyperbolic decreases with increasing [pTide]. Direct fitting of $k_{\text{cat}(\text{app})}$ to Equation S32 yielded values of $k_{\text{cat}} = 0.184 \pm 0.005 \text{ s}^{-1}$ and $K_{\text{i}}^{\text{pTide}} = 180 \pm 15 \text{ }\mu\text{M}$; while direct fitting of $K_{\text{m}(\text{app})}^{\text{ATP}}$ to Equation S33 yielded values of $K_{\text{m}}^{\text{ATP}} = 4.9 \pm 0.1 \text{ }\mu\text{M}$ and $K_{\text{i}}^{\text{pTide}} = 170 \pm 4 \text{ }\mu\text{M}$.

References

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3. Tables

Table S1: Summary of Steady-State Kinetic Constants

parameter	data analyzed	figure	equation	value
k_{cat} (s^{-1})	global 2-substrate	S1A	S08	0.192 ± 0.002
	$^{\text{app}}k_{\text{cat}}$ vs [Tide]	S1C	S11	0.192 ± 0.001
	$^{\text{app}}k_{\text{cat}}$ vs [ATP]	S1D	S15	0.192 ± 0.002
	global competitive	S2A	S23	0.190 ± 0.003
	$^{\text{app}}k_{\text{cat}}$ vs [ADP]	S1C	S24	0.191 ± 0.003
	global uncompetitive	S2B	S31	0.184 ± 0.004
	$^{\text{app}}k_{\text{cat}}$ vs [pTide]	S2D	S32	0.184 ± 0.005
$K_{\text{m}}^{\text{ATP}}$ (μM)	global 2-substrate	S1A	S08	5.1 ± 0.2
	$^{\text{app}}k_{\text{cat}}$ vs [ATP]	S1D	S15	5.1 ± 0.1
	global competitive	S2A	S23	5.5 ± 0.5
	$^{\text{app}}K_{\text{m}}^{\text{ATP}}$ vs [ADP]	S2C	S25	5.5 ± 0.5
	global uncompetitive	S2B	S31	4.9 ± 0.1
	$^{\text{app}}K_{\text{m}}^{\text{ATP}}$ vs [pTide]	S2D	S33	4.9 ± 0.1
$K_{\text{m}}^{\text{Tide}}$ (μM)	global 2-substrate	S1A	S08	4.1 ± 0.1
	$^{\text{app}}k_{\text{cat}}$ vs [Tide]	S1C	S11	4.1 ± 0.1
$K_{\text{d}}^{\text{ATP}}$ (μM)	global 2-substrate	S1A	S08	5.0 ± 0.4
$K_{\text{i}}^{\text{ADP}}$ (μM)	global competitive	S2A	S23	5.5 ± 0.5
	$^{\text{app}}K_{\text{m}}^{\text{ATP}}$ vs [ADP]	S2C	S25	6.0 ± 0.6
$K_{\text{i}}^{\text{pTide}}$ (μM)	global uncompetitive	S2B	S31	180 ± 10
	$^{\text{app}}k_{\text{cat}}$ vs [pTide]	S2D	S32	180 ± 15
	$^{\text{app}}K_{\text{m}}^{\text{ATP}}$ vs [pTide]	S2D	S33	170 ± 4

Table S2: Summary of $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}$ from Two-Substrate Kinetic Data

figure	fixed [Tide] (μM)	$k_{\text{cat}(\text{app})}$ (s^{-1})	$K_{\text{m}}^{\text{ATP}}(\text{app})$ (μM)
S1A	1	0.032 ± 0.001	4.0 ± 0.5
	2	0.052 ± 0.001	3.9 ± 0.4
	3	0.072 ± 0.001	4.1 ± 0.3
	5	0.095 ± 0.002	3.9 ± 0.3
	10	0.128 ± 0.002	4.3 ± 0.2
	25	0.161 ± 0.002	4.0 ± 0.2
	50	0.173 ± 0.002	4.1 ± 0.2
figure	fixed [ATP] (μM)	$k_{\text{cat}(\text{app})}$ (s^{-1})	$K_{\text{m}}^{\text{Tide}}(\text{app})$ (μM)
S1B	1	0.038 ± 0.001	4.6 ± 0.4
	2	0.063 ± 0.002	4.9 ± 0.5
	3	0.084 ± 0.002	5.4 ± 0.4
	5	0.105 ± 0.002	4.9 ± 0.3
	10	0.136 ± 0.002	5.1 ± 0.2
	25	0.164 ± 0.004	4.9 ± 0.3
	50	0.179 ± 0.002	5.2 ± 0.2

Table S3: Summary of $k_{\text{cat}(\text{app})}$ and $K_{\text{m}(\text{app})}$ from Product Inhibition Kinetic Data

figure	fixed [ADP] (μM)	$k_{\text{cat}(\text{app})}$ (s^{-1})	$K_{\text{m}}^{\text{ATP}}(\text{app})$ (μM)
S2A	0	0.190 ± 0.005	5.1 ± 0.6
	5	0.189 ± 0.007	10.3 ± 1.3
	10	0.196 ± 0.008	15.8 ± 1.9
	15	0.189 ± 0.009	19.1 ± 2.4
	20	0.189 ± 0.011	23.9 ± 3.4
figure	fixed [pTide] (μM)	$k_{\text{cat}(\text{app})}$ (s^{-1})	$K_{\text{m}}^{\text{ATP}}(\text{app})$ (μM)
S2B	0	0.182 ± 0.003	5.0 ± 0.2
	100	0.125 ± 0.002	3.1 ± 0.1
	200	0.089 ± 0.001	2.2 ± 0.1
	300	0.064 ± 0.001	1.8 ± 0.1
	400	0.053 ± 0.001	1.5 ± 0.1

4. Figure Legends

Figure S1: Two-Substrate Steady-State Kinetics

Individual direct plots of A , k versus $[ATP]$; and B , k versus $[Tide]$. The concentration of one given substrate was varied at seven different concentrations of the other given fixed substrate [$1 \mu\text{M}$ (●), $2 \mu\text{M}$ (○), $3 \mu\text{M}$ (■), $5 \mu\text{M}$ (□), $10 \mu\text{M}$ (▲), $25 \mu\text{M}$ (△), and $50 \mu\text{M}$ (▼)]. Solid lines indicate the global fit of these data to Equation S8, which describes a Steady-State Ordered Bi Bi system, whereby binding of ATP precedes binding of Tide (Scheme 1). Dashed lines indicate direct fits of the individual data sets to the Michaelis-Menten equation, which yielded values of ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{ATP}}$ for varying $[ATP]$ and values of ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{Tide}}$ for varying $[Tide]$. In addition, values of C , ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{ATP}}$ from varying $[ATP]$ and D , ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{Tide}}$ from varying $[Tide]$ were plotted against the given fixed substrate concentrations. Solid lines were generated by secondary equations using the kinetic constants determined from the global fit of the data in A and B ; and dashed lines represent direct fits of the secondary plots to the secondary equations (Table S1).

Figure S2: Product Inhibition Steady-State Kinetics

Direct plots of k versus $[ATP]$ using either A , ADP or B , pTide. A , ADP product inhibition was measured using (i) varying $[ATP] = 5, 10, 15, 20, 30, 50,$ and $100 \mu\text{M}$, (ii) saturating fixed $[Tide] = 200 \mu\text{M}$, and (iii) $[ADP] = 0 \mu\text{M}$ (●), $5 \mu\text{M}$ (○), $10 \mu\text{M}$ (▲), $15 \mu\text{M}$ (△), and $20 \mu\text{M}$ (▼). Solid lines were generated using values of the kinetic parameters obtained from global fitting to Equation S23. Dashed lines indicate direct fits of the individual data sets to the Michaelis-Menten equation, which yielded values of ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{ATP}}$ for varying $[ATP]$. B , pTide product inhibition was measured using (i) varying $[ATP] = 0.5, 1, 2, 3, 5, 10, 15,$ and $20 \mu\text{M}$, (ii) saturating fixed $[Tide] = 200 \mu\text{M}$, and (iii) $[pTide] = 0 \mu\text{M}$ (●), $100 \mu\text{M}$ (○), $200 \mu\text{M}$ (▲), $300 \mu\text{M}$ (△), and $400 \mu\text{M}$ (▼). Solid lines were generated using values of the kinetic parameters obtained from global fitting to Equation S31. Dashed lines indicate direct fits of the individual data sets to the Michaelis-Menten equation, which yielded values of ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{ATP}}$ for varying $[ATP]$. In addition, values of C , ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{ATP}}$ from varying $[ADP]$ and D , ${}^{\text{app}}k_{\text{cat}}$ and ${}^{\text{app}}K_{\text{m}}^{\text{ATP}}$ from varying $[pTide]$ were plotted against the given fixed product concentrations. Solid lines were generated by secondary equations using the kinetic constants determined from the global fit of the data in A and B ; and dashed lines represent direct fits of the secondary plots to the secondary equations (Table S1).

FIGURE S1

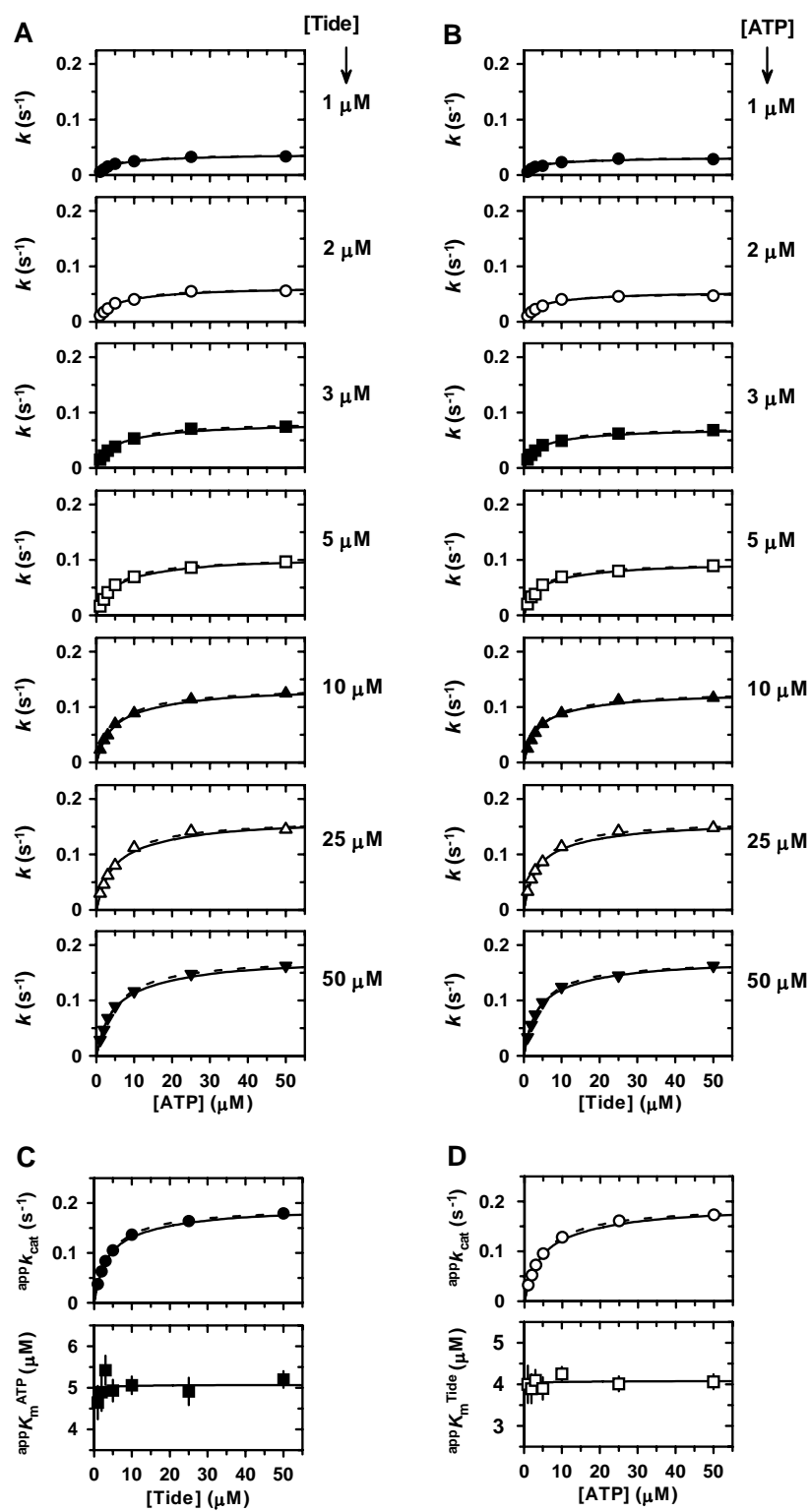


FIGURE S2

