Supporting Information

Ravilious et al. 10.1073/pnas.1115772108

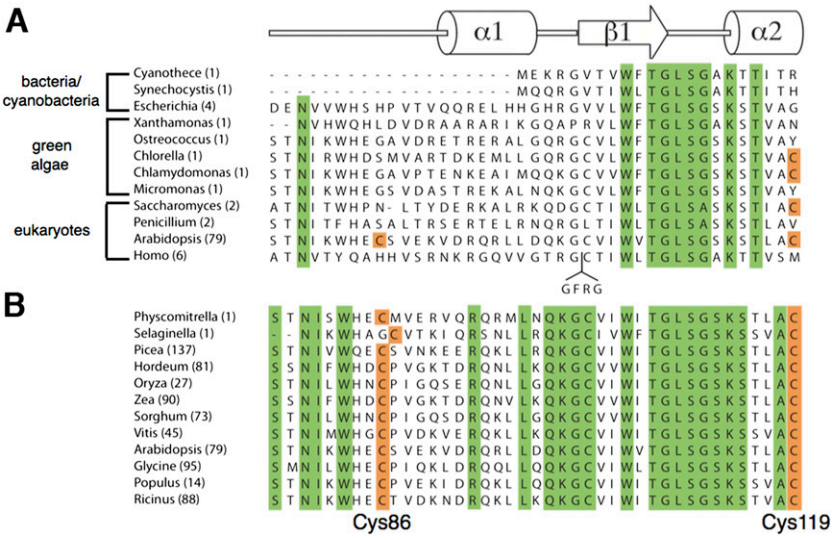

Fig. S1. Sequence alignment of representative adenosine-5′-phosphosulfate kinase (APSK) from bacteria, cyanobacteria, green algae, and eukaryotes (*A*) and plant species (*B*). The secondary structure of the APSK from *Arabidopsis thaliana* (AtAPSK) is shown above the alignment. Invariant residues are highlighted in green. Conserved cysteines corresponding to those in AtAPSK that form the disulfide bond are highlighted in orange.

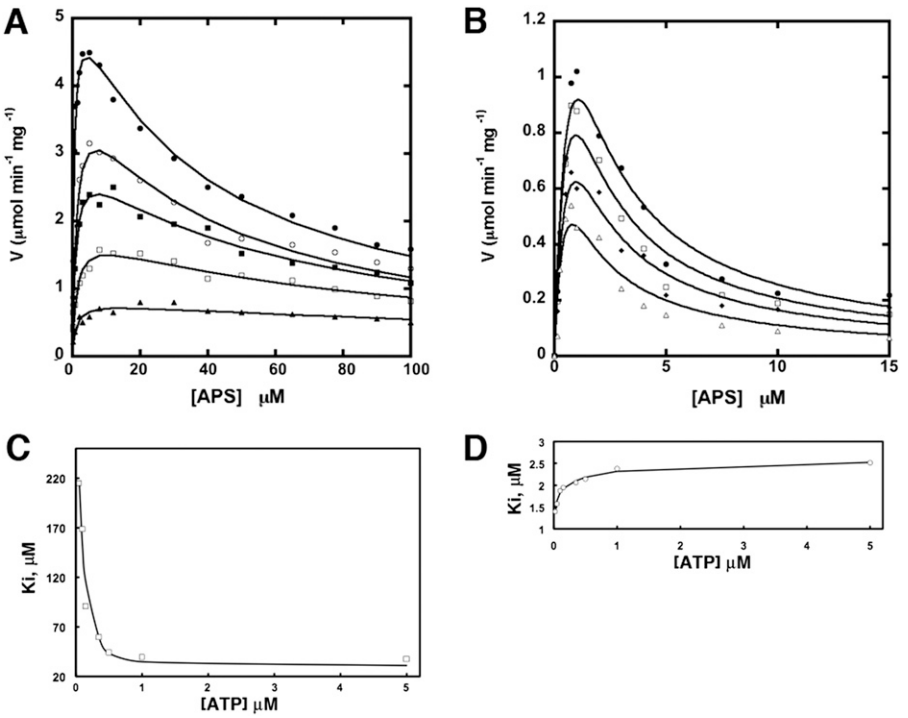

Fig. S2. Velocity curves of *v* vs. APS concentration for (*A*) $AtAPSK_{red}$, (*B*) $AtAPSK_{ox}$, and (*C* and *D*) the effect of [ATP] on K_i of $AtAPSK_{red}$ and $AtAPSK_{ox}$. (*A*) Initial velocities were measured at 5 mM (●), 1 mM (○), 0.35 mM (■), 0.15 mM (□), and 0.05 mM (▲) ATP. The curves represent best fits to the general substrate inhibition model described in *Materials and Methods*. (*B*) Initial velocities were measured at 5 mM (●), 0.5 mM (□), 0.1 mM (◆), 0.025 mM (△) ATP. (*C* and *D*) Effect of ATP concentration on K_i of $AtAPSK_{red}$ (*C*) and $AtAPSK_{ox}$ (*D*).

Table S1. Summary of crystallographic statistics

Parameter	Detail
Crystal	AtAPSK•AMP-PNP•Mg^{2+}•APS
Space group	C2
Cell dimensions, Å*	
a	121.1
b	95.31
c	73.33
Cell β,°	114.1
Data collection	
Wavelength, Å	0.979
Resolution range (highest shell resolution), Å	30.5–1.80 (1.83–1.80)
Reflections	
Total	255,905
Unique	69,476
Completeness (highest shell), %	98.0 (98.4)
$\langle I/\sigma \rangle$ (highest shell)	34.1 (1.7)
R_{sym}[a] (highest shell), %	3.2 (44.1)
Model and refinement	
R_{cryst}[b]/R_{free}[c]	17.3/20.1
No. of protein atoms	4,525
No. of water molecules	479
No. of ligand atoms	177
rmsd, bond lengths, Å	0.006
rmsd, bond angles,°	1.08
Average B-factor, $Å^2$	
Protein	39.5
Waters	50.0
Ligands	46.0
Stereochemistry, %	
Most favored	97.5
Allowed	2.3
Generously allowed	0.2

a, $R_{sym} = \Sigma|I_h - \langle I_h \rangle|/\Sigma I_h$, where $\langle I_h \rangle$ is the average intensity over symmetry; *b*, $R_{cryst} = \Sigma|F_o - \langle F_c \rangle|/\Sigma F_o$, where summation is over the data used for refinement; *c*, R_{free} is defined the same as R_{cryst}, but was calculated using 5% of data excluded from refinement.

Table S2. Oligonucleotides used for generation of the AtAPSK C86A/C119A mutant and for cloning of APSK from *Synechocystis* sp. PCC 6803

Mutant	Oligonucleotide
C86A-F	5′-dCTCGACAAATATAAAGTGGCATGA*AGC*TTCTGTTG-3′
C86A-R	5′-dCTGTCTATCAACTTTCTCAACAGAA*GCT*TCATGCC-3′
C119A-F	5′-dGGGAAGAGTACTTTGGCT*GCT*GCTTTG -3′
C119A-R	5′-dCAACATCTGATTCAAAGC*AGC*AGCCAAAG -3′
SynAPSK-F	5′-dTTTATGGCTAGC**ATG**CAACAACGTGGCGTAAC-3′
SynAPSK-R	5′-dATAGAATTCG**TTA**GCCCTCGATATATTTTAGATCTACTAGCTTCTG-3′

Mutated codons are italicized, restriction sites are underlined, and start/stop codons are shown in bold.