Supporting Information: Materials and Methods

Construction of vector pKLD66nCBP. Two complementary synthetic oligonucleotides (Primers 07 and 08; Table S2) were purchased from Integrated DNA Technologies (Coralville, IA) which, when annealed together, encoded the complete 26 amino acid calmodulin binding peptide sequence from muscle myosin light chain kinase (Zheng *et al.*, 1997) with NdeI and SacI equivalent overhangs at the 5'- and 3'-ends, respectively. The annealed oligonucleotide was treated with T4 polynucleotide kinase to phosphorylate the 5' end. The phosphorylated oligonucleotide was ligated with vector pKLD66 (Rocco *et al.*, 2008), previously digested with NdeI and SacI and treated with calf intestinal alkaline phosphatase. The resulting expression vector, pKLD66nCBP, encodes an N-terminal 26 amino acid calmodulin binding peptide followed by a consensus sequence for the tobacco etch virus protease upstream of the multiple cloning region. The GenBank accession number for this plasmid is KT020828.

Cloning of expression vectors and constructs. The cloning of N-terminally $(His)_8$-tagged and non $(His)_8$-tagged *Ps*AH and *Ps*UC has been previously described (Lin & St Maurice, 2013) . Urea amidolyase associated proteins 1 and 2 (UAAP1 and UAAP2) were amplified by PCR from *P. syringae* genomic DNA. The *P. syringae* UAAP1 gene (PSPTO_4241, GenBank ID=NP_794003) was amplified by PCR using primer 5'-AGC TGG TAC CTC GAT GAC TGA CTC GA-3' and 5'-ATC CCT GCA GGT CAA GCG AAC AGG CG-3', digested by KpnI and SbfI and ligated into a KpnI/SbfI digested pKLD66nCBP vector, a modified version of the pKLD66 vector (Rocco *et al.*, 2008) containing an N-terminal calmodulin binding peptide (construction of the pKLD66nCBP vector is described above). The *P. syringae* UAAP2 gene (PSPTO_2, GenBank accession number = NP_794003) was amplified using primers 5'- CCC TGC AGA TGA ACT CAC ATA ATC -3' and 5'- GGC TCG AGT CAT CAG TCG CGA ATC AGC -3', digested by NcoI and XhoI and ligated into the pET28a-$(His)_8$-TEV vector, downstream of the T7 promoter, N-terminal $(His)_8$ tag, and rTEV protease cleavage sequence (Lietzan *et al.*, 2011) .

The gene encoding the *Gb*AH-UC fusion protein was cloned as follows: first, the genes encoding AH and UC from *Granulibacter bethesdensis* (strain ATCC BAA-1260/CGDN1H1) were separately PCR amplified from genomic DNA and separately cloned into the pET28a-$(His)_8$-TEV vector, downstream of the T7 promoter, N-terminal $(His)_8$ tag, and rTEV protease recognition sequence. The cloning of pET28a-$(His)_8$-TEV-*Gb*AH has previously been described (Lin & St Maurice, 2013) . To clone the pET28a-$(His)_8$-TEV-*Gb*UC, the *Gb*UC gene was amplified by PCR from *G. bethesdensis* genomic DNA using primers 5'-GGG GCT GCA GAT GTT CGA TCA TGT TCT GG-3' and 5'-CAG CTC GAG GTC AGG CTT CCT CCT CG-3'. The PCR product encoding *Gb*UC and pET28a-$(His)_8$-TEV were digested by PstI and XhoI prior to ligation. Next, a HindIII site was introduced in place of the TAA stop codon in the pET28a-$(His)_8$-TEV-*Gb*AH vector by Quikchange mutagenesis. Subsequently, the *Gb*UC gene in pET28a-$(His)_8$-TEV was PCR amplified using the reverse primer 5'-GGC CGA AAT CGG CAA AAT CCC-3', complementary to the pET28a vector backbone downstream of the PsiI restriction site, and the forward primer 5'-G TAT TTT CAA GGC CTT CTG CAG **GAA GCT TTG CGT GCC TCC AAA AAA CCA** ATG TTC GAT CAT GTT CTG-3' which harbors a HindIII

site and includes the linker region adapted from *Ca*UAL (EALRASKKP; bold). The pET28a-$(His)_8$-TEV-*Gb*AH vector and *Gb*UC PCR product was digested by HindIII and PsiI. The two fragments, once ligated, generated the ~10.7 kb pET28a-$(His)_8$-TEV-*Gb*AHUC vector, which encodes an N-terminal *G. bethesdensis* AH and a C-terminal *G. bethesdensis* UC, linked by the short sequence EALRASKKP that connects AH and UC in *C. albicans* UAL. Note that the native His residue at position 2 (underlined) of the *C. albicans* linker was mutated to Ala for cloning purposes.

To clone $(His)_8$-tagged UAL from *S. cerevisiae* and *C. albicans*, the *DUR1,2* genes were separately cloned into the pESC-URA vector with an N-terminal myc-tag, or into pET28a-$(His)_8$-TEV vector, with an N-terminal $(His)_8$ tag. The *S. cerevisiae DUR1,2* gene was cloned from yeast strain MLY40α (MATα ura3-52) by PCR amplification using primers 5'-ACG CGT CGA CAT GAC AGT TAG TTC CG-3' and 5'-CCG GCT AGC TCA TCA TGC CAA TGT CTC TAT G-3'. The PCR product was digested with SalI and NheI and ligated into the pESC-URA vector digested with XhoI and NheI. The resulting plasmid, *ScDUR1,2*-pESC-URA, encodes *S. cerevisiae* UAL with an N-terminal myc tag under the inducible control of the GAL promoter. The *S. cerevisiae DUR1,2* gene from yeast strain MLY40α (*MATα ura3-52*) was cloned into the pET28a-$(His)_8$-TEV vector by PCR amplification using primers 5'-GAG AGT CGA CAA ACA GTT AGT TCC GAT ACA ACT GC-3' and 5'-GAG AGC GGC CGC TCA TGC CAA TGT CTC TAT G-3'. The resulting PCR product was inserted into the pET28a-$(His)_8$-TEV vector by digestion of the PCR product and vector with SalI and NotI, followed by ligation to produce plasmid *ScDUR1,2*-pET28a-$(His)_8$-TEV. The *C. albicans DUR1,2* gene was cloned into the pESC-URA vector by PCR amplification with primers 5'-GAG AGC GGC CGC ATG GGC AGC AGC CAT CAT C-3' and 5'-GAG AAG ATC TTT ATT CTA AAA CAA CCA CTA AAT CAC CG-3'. The resulting PCR product was inserted into the pESC-URA vector by digestion of the PCR product and vector with NotI and BglII, followed by ligation. The resulting plasmid, *CaDUR1,2*-pESC-URA, encodes *C. albicans* UAL with an N-terminal myc tag under the inducible control of the GAL promoter. The *C. albicans DUR1,2* gene was cloned into the pET28a-$(His)_8$-TEV expression vector by PCR amplification with primers 5'-GAG AGT CGA CAA TCA TAC TTA GGT TGG TCA GTA G-3' and 5'-GAG ACT CGA GTT ATT CTA AAA CAA CCA CTA AAT CAC C-3'. The resulting PCR product was inserted into the pET28a-$(His)_8$-TEV vector by digestion of both the PCR product and vector with SalI and *Pst*I, followed by ligation to produce vector *CaDUR1,2*-pET28a-$(His)_8$-TEV. The N-terminal myc tag on vectors *ScDUR1,2*-pESC-URA and *CaDUR1,2*-pESC-URA were replaced with an N-terminal $(His)_8$ tags as follows: A SmaI site was introduced downstream of the T7 promoter and u*Ps*tream of the open reading frame in the pET28a-$(His)_8$-TEV-*DUR1,2* vectors by Quikchange site-directed mutagenesis (Stratagene, La Jolla, CA) using primers 5'- GAA ATA ATT TTG TTT AAC TTT ACC CGG GGA GAT ATA CCA TGG GCA GC -3' and 5'- GCT GCC CAT GGT ATA TCT CCC CGG GTA AAG TTA AAC AAA ATT ATT TC -3' (the introduced SmaI restriction site is underlined). Subsequently, the *DUR1,2*-pESC-URA vectors and the pET28a-$(His)_8$-TEV-SmaI-*DUR1,2* vectors were digested by SmaI and HindIII. The digested products were separated by agarose gel electrophoresis. The >10,000bp fragment from digestion of pESC-URA-myc-*DUR1,2* vectors and the ~1800 bp fragment from digestion of the pET28a-$(His)_8$-TEV-SmaI-*DUR1,2* vectors were ligated to generate *ScDUR1,2*-pESC-URA-$(His)_8$ and *CaDUR1,2*-pESC-URA-$(His)_8$.

The *Ps*AH$_{S179A}$ mutant was generated by Quikchange site-directed mutagenesis (Stratagene, La Jolla, CA) using Pfu Turbo polymerase. The complete sequences of all clones were confirmed by complete gene sequencing by Functional Biosciences (Madison, WI).

Supporting Information: Materials and Methods - References

Lietzan, A. D., Menefee, A. L., Zeczycki, T. N., Kumar, S., Attwood, P. V., Wallace, J. C., Cleland, W. W. and St Maurice, M. (2011). Interaction between the biotin carboxyl carrier domain and the biotin carboxylase domain in pyruvate carboxylase from rhizobium etli. *Biochemistry, 50*, 9708-9723.

Lin, Y. and St Maurice, M. (2013). The structure of allophanate hydrolase from *Granulibacter bethesdensis* provides insights into substrate specificity in the amidase signature family. *Biochemistry, 52*, 690-700.

Rocco, C. J., Dennison, K. L., Klenchin, V. A., Rayment, I. and Escalante-Semerena, J. C. (2008). Construction and use of new cloning vectors for the rapid isolation of recombinant proteins from escherichia coli. *Plasmid, 59*, 231-237.

Zheng, C. F., Simcox, T., Xu, L. and Vaillancourt, P. (1997). A new expression vector for high level protein production, one step purification and direct isotopic labeling of calmodulin-binding peptide fusion proteins. *Gene, 186*, 55-60.

Supporting Information Tables

Table SI. Cloning vectors and strategies employed in this study.

Name	Insert	Vector	Primers	Cloning strategies	Reference	Notes
pET28a-(His)8-TEV -*Ps*AH	*Ps*AH	pET28a-$(His)_8$-TEV [1]			[2]	
pET28a-$(His)_8$-TEV -*Ps*UC	*Ps*UC	pET28a-$(His)_8$-TEV			[2]	
pET28a-*Ps*AH	*Ps*AH	pET28a			[2]	
pET28a-*Ps*AH	*Ps*UC	pET28a			[2]	
pET28a-$(His)_8$-TEV-*Gb*AH	*Gb*AH	pET28a-$(His)_8$-TEV			[2]	
pET28a-$(His)_8$-TEV-*Gb*UC	*Gb*UC	pET28a-$(His)_8$-TEV	01, 02	PstI and XhoI digestion and ligation	This study	
pET28a-$(His)_8$-TEV-*Gb*AH * to Ala		pET28a-$(His)_8$-TEV-*Gb*AH	03, 04	Quikchange site-directed mutagenesis	This study	
pET28a-$(His)_8$-TEV-*Gb*AH-UC	*Gb*UC	pET28a-$(His)_8$-TEV-*Gb*AH * to Ala	05, 06	*Gb*UC was PCR amplified, digested with HindIII and PsiI, ligated to pYL7 digested with HindIII and PsiI.	This study	A linker region adapted from *Ca*UAL (EALRASKKP) was inserted between AH and UC
pKLD66nCBP		pKLD66 [3]	07, 08		This study	
pKLD66nCBP-*UAAP1*	UAAP1 (NP_794 002.1)	pKLD66nCBP	09, 10	KpnI and SbfI digestion and ligation	This study	
pET28a-$(His)_8$-TEV-*UAAP2*	UAAP2 (NP_794 003.1)	pET28a-$(His)_8$-TEV	11, 12	NcoI and XhoI digestion and ligation	This study	
pESC-URA-Myc-*Sc*UAL	*Sc*UAL	pESC-URA	13, 14	*Sc*UAL PCR amplified, digested with SalI and NheI and ligated into the pESC-URA vector digested with XhoI and NheI	This study	

pET28a-$(His)_8$-TEV-*ScDUR1,2*	*Sc*UAL	pET28a-$(His)_8$-TEV	15, 16	SalI and NotI digested and ligated	This study	
pESC-URA-Myc-CAUAL	*Ca*UAL	pESC-URA	17, 18	NotI and BglII digested and ligated	This study	
pET28a-$(His)_8$-TEV-*CaDUR1,2*	*Ca*UAL	pET28a-$(His)_8$-TEV	19, 20	SalI and PstI digested and ligated	This study	
pET28a-SmaI-$(His)_8$-TEV-*ScDUR1,2*		pET28a-$(His)_8$-TEV-*ScDUR1,2*	21, 22	Quikchange site-directed mutagenesis	This study	A SmaI site was introduced downstream of the T7 promoter and upstream of the open reading frame in the pET28a-$(His)_8$-TEV-*ScDUR1,2* vectors
pET28a-SmaI-$(His)_8$-TEV-*CaDUR1,2*		pET28a-$(His)_8$-TEV-*CaDUR1,2*	21, 22	Quikchange site-directed mutagenesis	This study	A SmaI site was introduced downstream of the T7 promoter and upstream of the open reading frame in the pET28a-(His)8-TEV-*CaDUR1,2* vectors
pESC-URA-$(His)_8$-*Sc*UAL	pET28a-SmaI-$(His)_8$-TEV-*ScDUR1,2*	pESC-URA-Myc-*Sc*UAL		Both vectors were digested by SmaI and HindIII, the >10,000bp fragment from pESC-URA-myc-*DUR1,2* and the ~1800 bp fragment from the pET28a-(His)8-TEV-SmaI-*DUR1,2* were ligated	This study	
pESC-URA-$(His)_8$-*Ca*UAL	pET28a-SmaI-$(His)_8$-TEV-*CaDUR1,2*	pESC-URA-Myc-*Ca*UAL		Both vectors were digested by SmaI and HindIII. The >10,000bp fragment from pESC-URA-myc-*DUR1,2* and the ~1800 bp fragment from the pET28a-(His)8-TEV-SmaI-*DUR1,2* were ligated	This study	

pET28a-$(His)_8$-TEV-*Ps*AH S179A		pET28a-$(His)_8$-TEV-*Ps*AH	23, 24	Quikchange site-directed mutagenesis	This study	

[1] Lietzan, A. D., Menefee, A. L., Zeczycki, T. N., Kumar, S., Attwood, P. V., Wallace, J. C., Cleland, W. W. and St Maurice, M. (2011). Interaction between the biotin carboxyl carrier domain and the biotin carboxylase domain in pyruvate carboxylase from rhizobium etli. *Biochemistry, 50*, 9708-9723.

[2] Lin, Y. and St Maurice, M. (2013). The structure of allophanate hydrolase from Granulibacter bethesdensis provides insights into substrate specificity in the amidase signature family. *Biochemistry*, *52*, 690-700.

[3] Rocco, C. J., Dennison, K. L., Klenchin, V. A., Rayment, I. and Escalante-Semerena, J. C. (2008). Construction and use of new cloning vectors for the rapid isolation of recombinant proteins from escherichia coli. *Plasmid, 59*, 231-237.

Table SII. Primers employed in this study.

Primer	Sequence (5' to 3')
01	GGG GCT GCA GAT GTT CGA TCA TGT TCT GG
02	CAG CTC GAG GTC AGG CTT CCT CCT CG
03	CGC AGG AAG CTT GAG CAC CAC CAC CAC
04	GTG GTG GTG GTG CTC AAG CTT CCT GCG
05	G TAT TTT CAA GGC CTT CTG CAG GAA GCT TTG CGT GCC TCC AAA AAA CCA ATG TTC GAT CAT GTT CTG
06	GGC CGA AAT CGG CAA AAT CCC
07	TAT GAA GCG ACG ATG GAA AAA GAA TTT CAT AGC CGT CTC AGC AGC CAA CCG CTT TAA GAA AAT CTC ATC CTC CGG GGC ACT TAG CT
08	AAG TGC CCC GGA GGA TGA GAT TTT CTT AAA GCG GTT GGC TGC TGA GAC GGC TAT GAA ATT CTT TTT CCA TCG TCG CTT CA
09	AGC TGG TAC CTC GAT GAC TGA CTC GA
10	ATC CCT GCA GGT CAA GCG AAC AGG CG
11	CCC TGC AGA TGA ACT CAC ATA ATC
12	GGC TCG AGT CAT CAG TCG CGA ATC AGC
13	ACG CGT CGA CAT GAC AGT TAG TTC CG
14	CCG GCT AGC TCA TCA TGC CAA TGT CTC TAT G
15	GAG AGT CGA CAA ACA GTT AGT TCC GAT ACA ACT GC
16	GAG AGC GGC CGC TCA TGC CAA TGT CTC TAT G
17	GAG AGC GGC CGC ATG GGC AGC AGC CAT CAT C
18	GAG AAG ATC TTT ATT CTA AAA CAA CCA CTA AAT CAC CG
19	GAG AGT CGA CAA TCA TAC TTA GGT TGG TCA GTA G
20	GAG ACT CGA GTT ATT CTA AAA CAA CCA CTA AAT CAC C
21	GAA ATA ATT TTG TTT AAC TTT ACC CGG GGA GAT ATA CCA TGG GCA GC
22	GCT GCC CAT GGT ATA TCT CCC CGG GTA AAG TTA AAC AAA ATT ATT TC
23	GAC ACC GCA GGC GCG GGG CGA GTG
24	CAC TCG CCC CGC GCC TGC GGT GTC
25	GAA ATA TCT TTT TTA TAG TCA CAA TAA ATT TCA GTT TTG ATT AAA AAC GTA CGC TGC AGG TCG AC
26	GAC TAA ATG AGT GCT CCT TTG ATT TTA GAT GCC AAC AAA AGG TAA TAT CGA TGA ATT CGA GCT CG
27	GGC TCA AGT TTT TAT AAG C
28	CTT TTC ATA CAT TTT ACA G

Supporting Information Figures

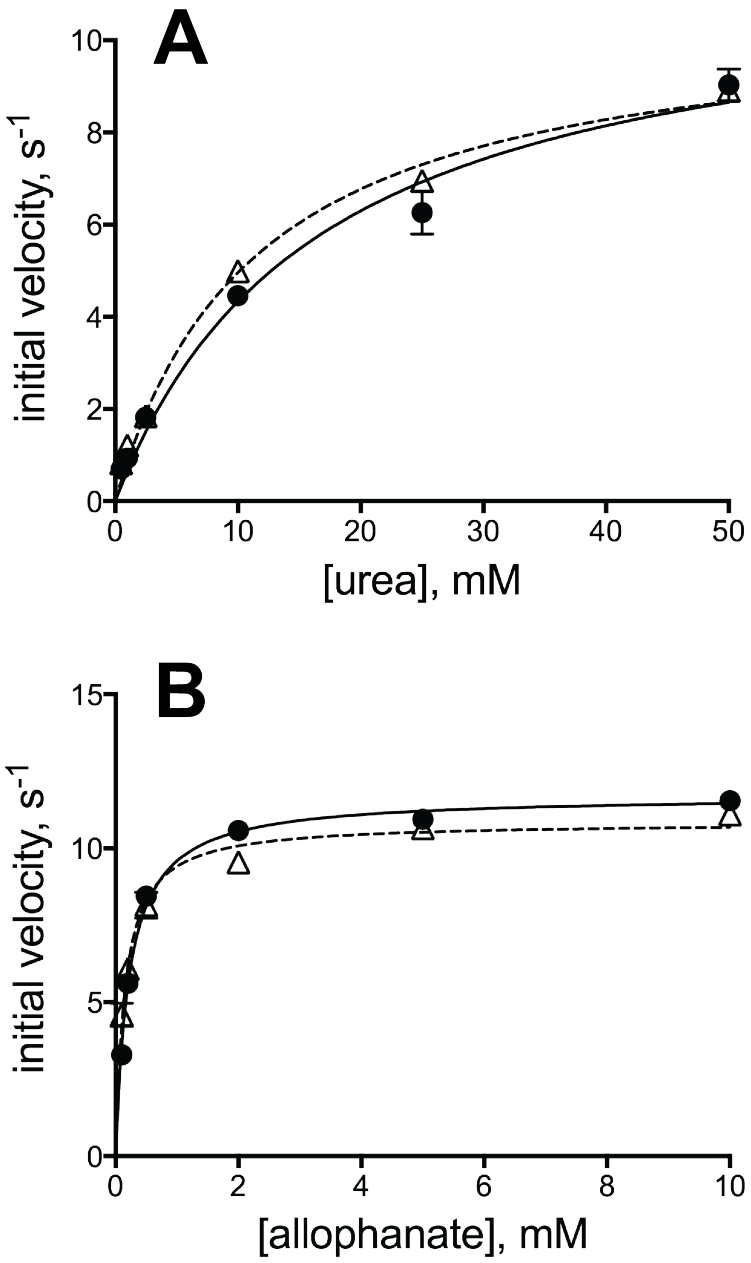

Figure S1. The enzyme activities of *Ps*AH and *Ps*UC are fully independent of one another. Initial velocity vs. [substrate] plots for *Ps*AH and *Ps*UC, in the presence and absence of the other enzyme. (**A**) Initial velocity measurements of ATP cleavage by *Ps*UC, in the presence (open triangles; dashed line) and absence (closed circles; solid line) of a molar equivalent of *Ps*AH. (**B**) Initial velocity measurements of allophanate hydrolysis activity by *Ps*AH, in the presence (open triangles; dashed line) and absence (closed circles; solid line) of a molar equivalent of *Ps*UC. Data were fit by non-linear regression analysis to the Michaelis-Menten equation.

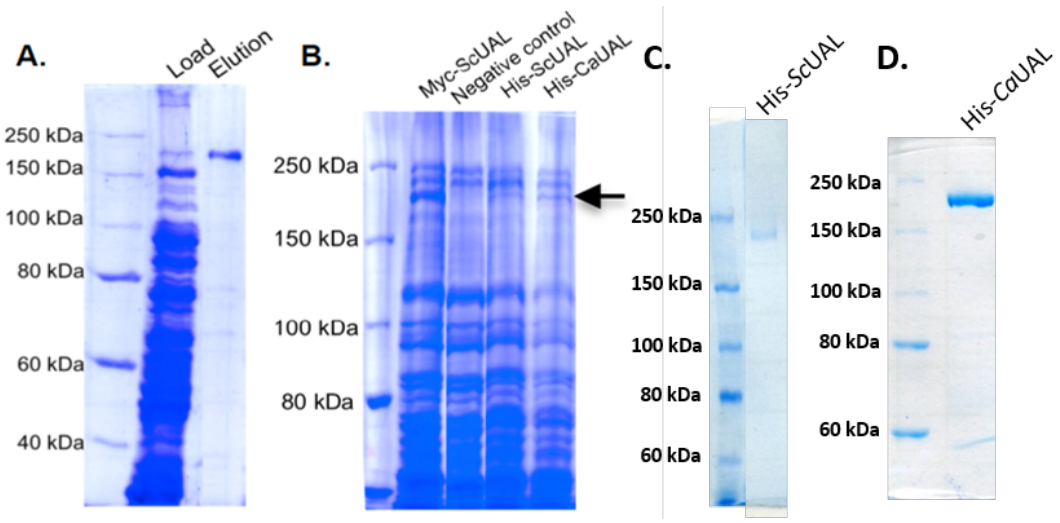

Figure S2. Protein overexpression and purification. SDS-PAGE showing the result of (**A**) Purification of *Gb*AH-UC protein. Cell lysate loaded onto Ni^{2+} column and elution from the Ni^{2+} column was resolved on the SDS-PAGE gel. (**B**) Overexpression of UAL from *S. cerevisiae* and *C. albicans* in *S. cerevisiae dur1,2*Δ strain. The total protein extract were prepared from corresponding yeast strains using the TCA method. The *S. cerevisiae dur1,2*Δ strain transformed with pESC-URA vector was used as negative control. The overexpression of myc-tagged *Sc*UAL, $(His)_8$-tagged *Sc*UAL and *Ca*UAL is shown. The arrow indicates the position of UAL on the gel. (**C-D**) Purified protein of $(His)_8$-tagged *Sc*UAL (C) and $(His)_8$-tagged *Ca*UAL (D) from *S. cerevisiae.*

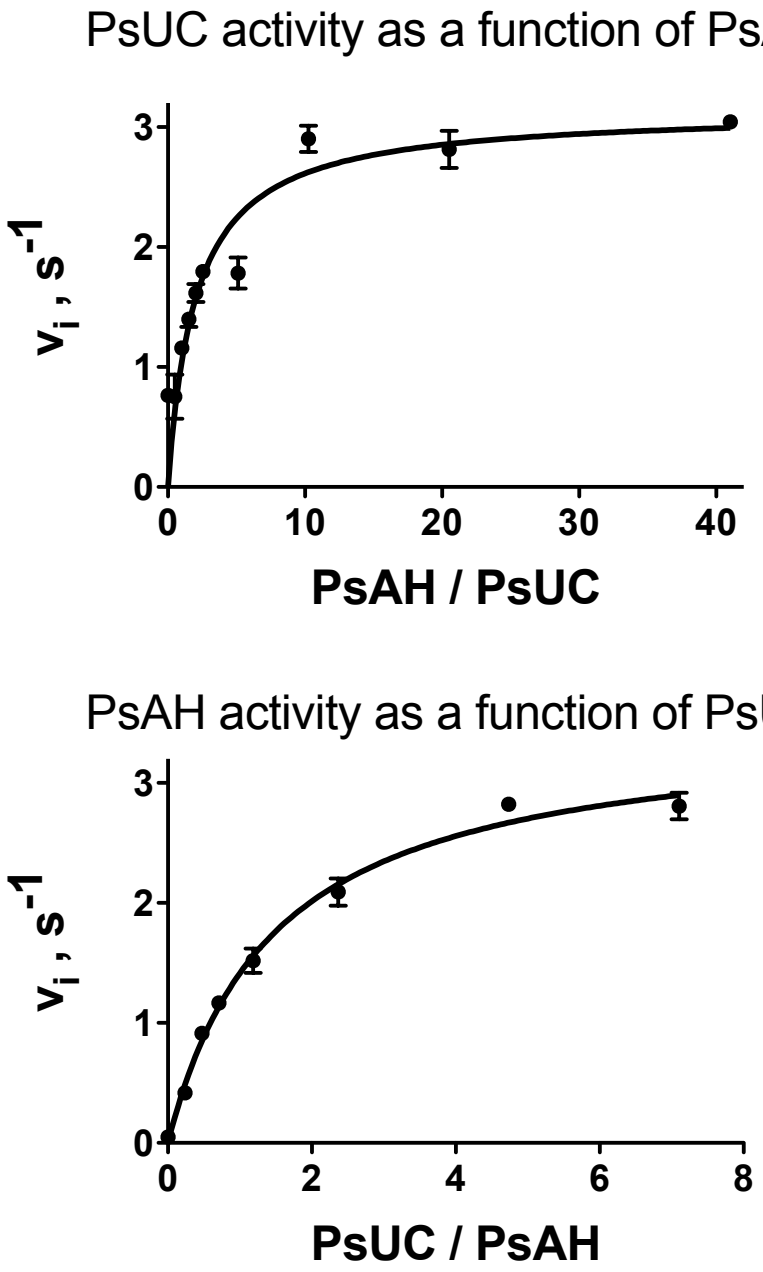

Figure S3. Assay of the *Ps*UC and *Ps*AH overall reaction (production of NH_3 from HCO_3, ATP and urea), titrating with increasing concentrations of *Ps*AH or *Ps*UC. (**A**) Titrating *Ps*AH into *Ps*UC, from 0 to 40 fold molar excess. (**B**) Titrating *Ps*UC into *Ps*AH from 0 to 7 fold molar excess. The lines represent the non-linear regression fit to the standard Michaelis-Menten equation.

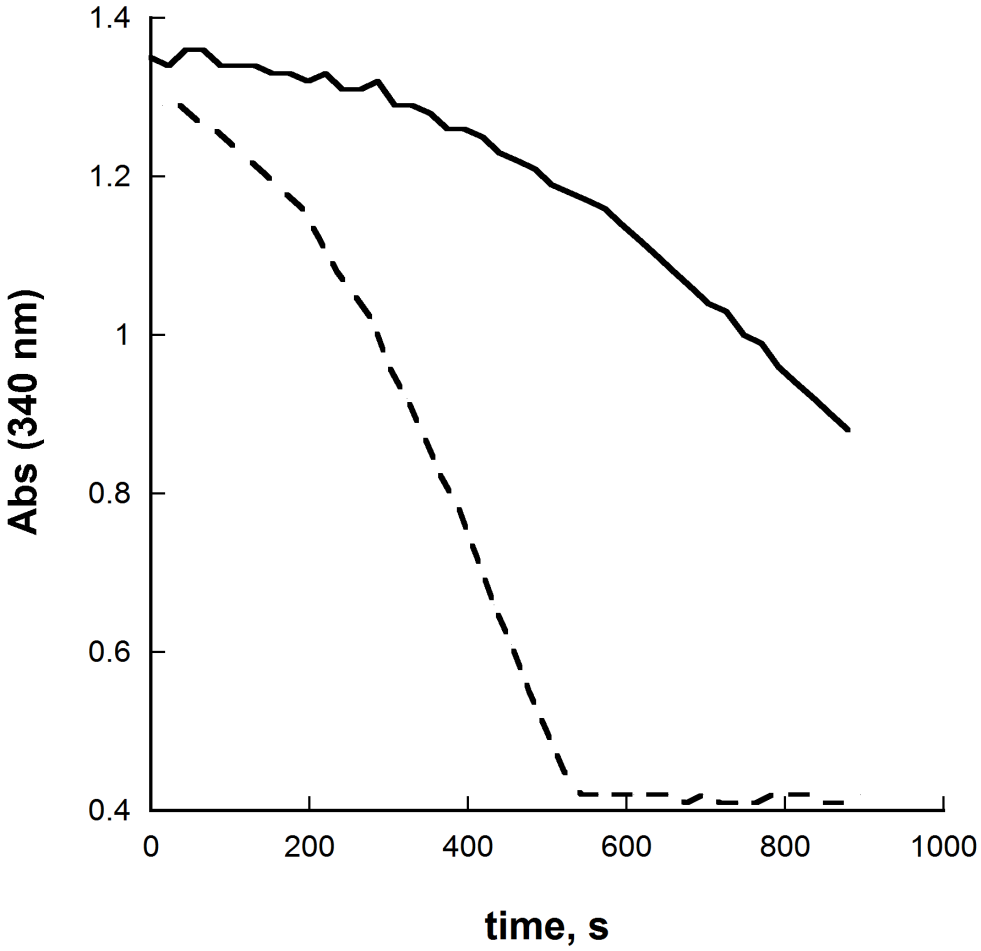

Figure S4. Representative traces for the enzyme-catalyzed conversion of HCO_3^-, urea and ATP to NH_4^+ and CO_2 by the combined actions of *Ps*AH and *Ps*UC. The reaction was followed using the glutamate dehydrogenase coupled assay in a buffer containing 50 mM HEPES (pH 7.3) and 50 mM NaCl and in the presence of 0.15 mM NADH, 8 mM $MgSO_4$, 50 mM Urea, 100 μM ATP, 8 mM $NaHCO_3$, 10 mM 2-oxoglutarate and 20 U/ml of glutamate dehydrogenase. The solid line represents a 1:1 molar ratio of PsUC:PsAH, while the dashed line represents a 1:10 molar ratio of PsUC:PsAH. A lag is observed in both cases, proportional to the *Ps*AH concentration, indicating that the lag results from a rate-limiting step in the reaction catalyzed by *Ps*AH and not by glutamate dehydrogenase.