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Article Title:	**Chemoenzymatic exchange of phosphopantetheine on protein and peptide**
Corresponding Author:	Michael Burkart (mburkart@ucsd.edu)

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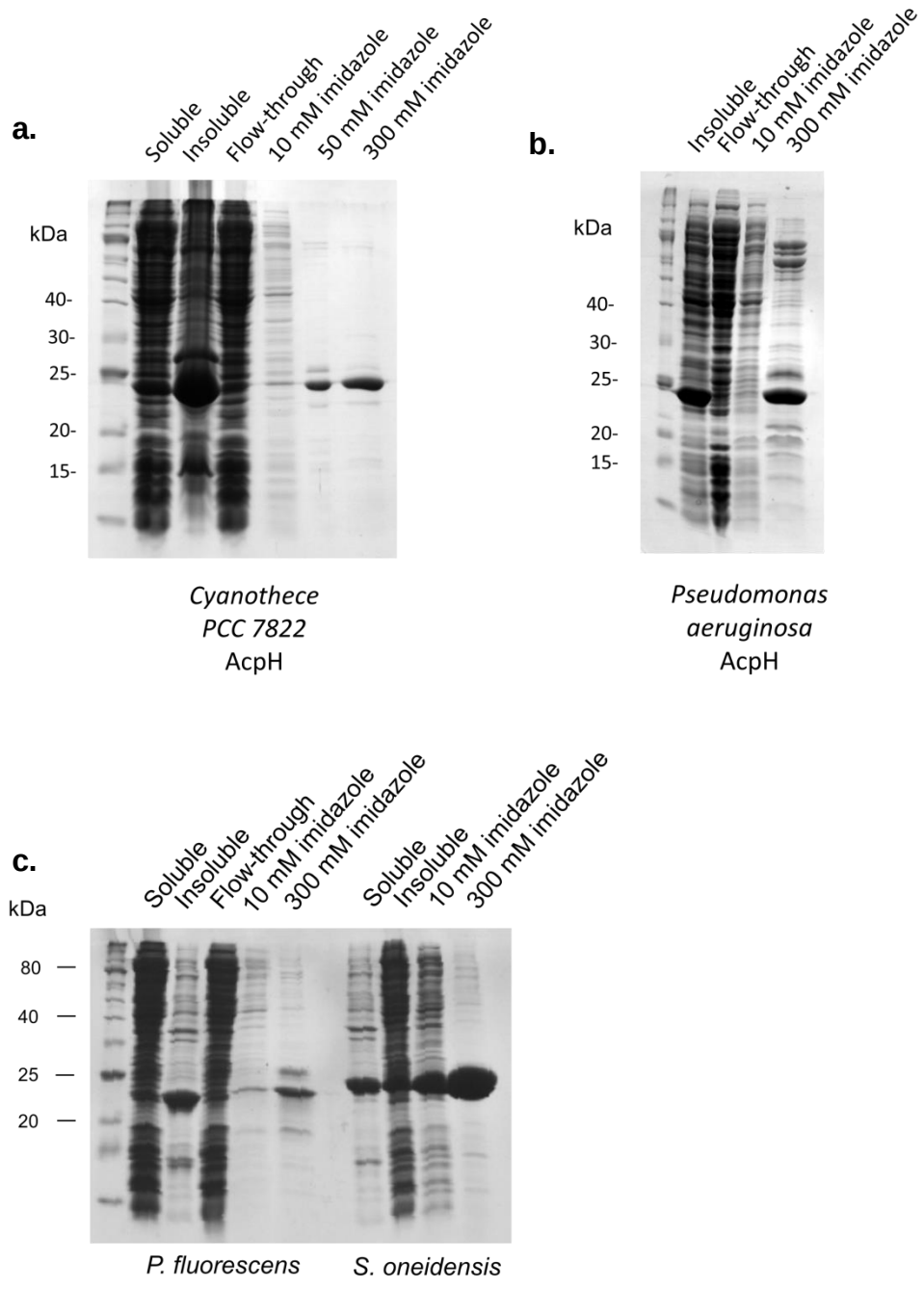
Supplementary Figure 1

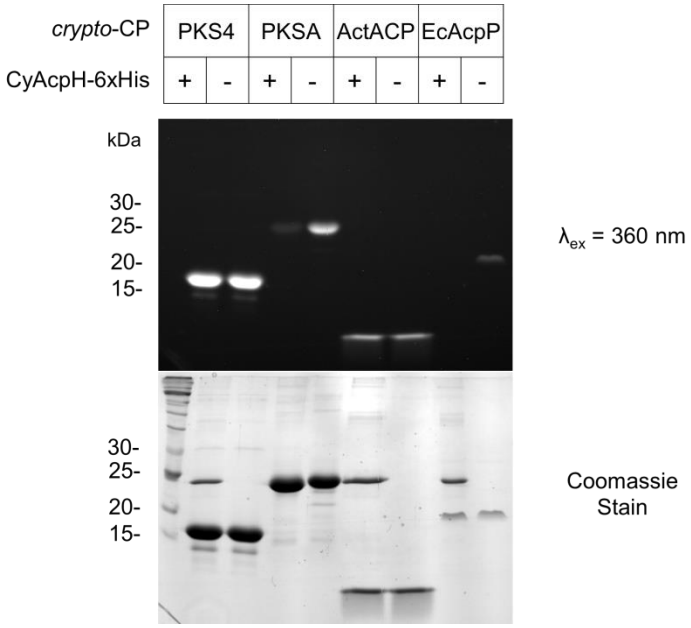
Purification of AcpH protein homologs



AcpH homolog protein from *Cyanothece* PCC7822 (**a**), *P. aeruginosa* (***b***), *P. fluorescens* (**c**), and *S. oneidensis* (**c**) was expressed recombinantly and purified using Ni-NTA chromatography.

Supplementary Figure 2

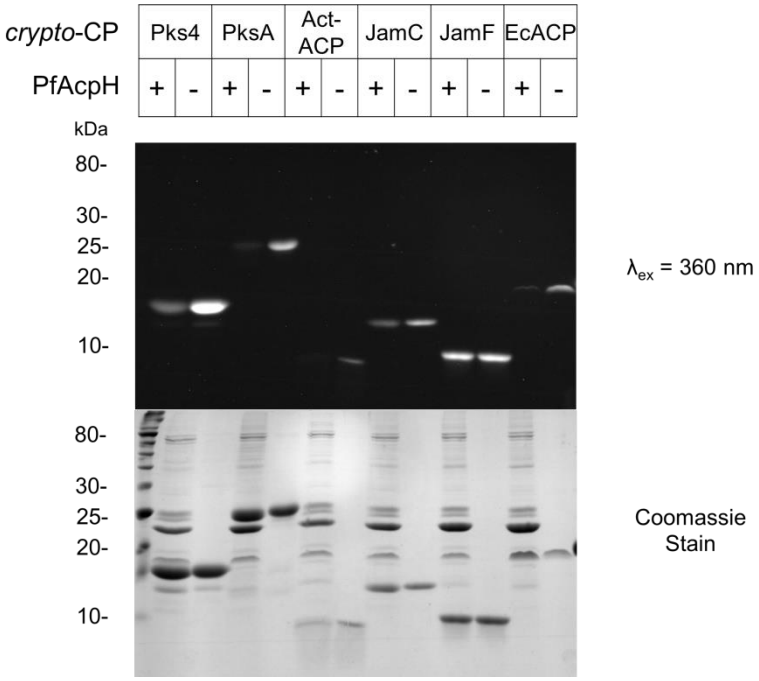
Analysis of CyAcpH activity with Pks4, PksA, ActACP (PKS) and *E. coli* AcpP (FAS)



CyAcpH is evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 3

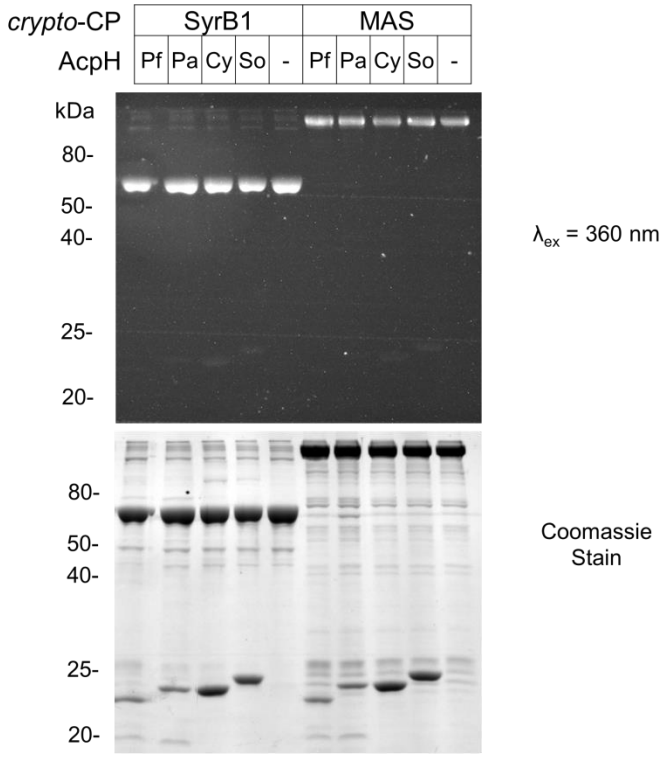
P. fluorescens AcpH activity vs. Pks4, PksA, ActACP, JamC, JamF (PKS) and EcAcpP (FAS)



PfAcpH is evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 4

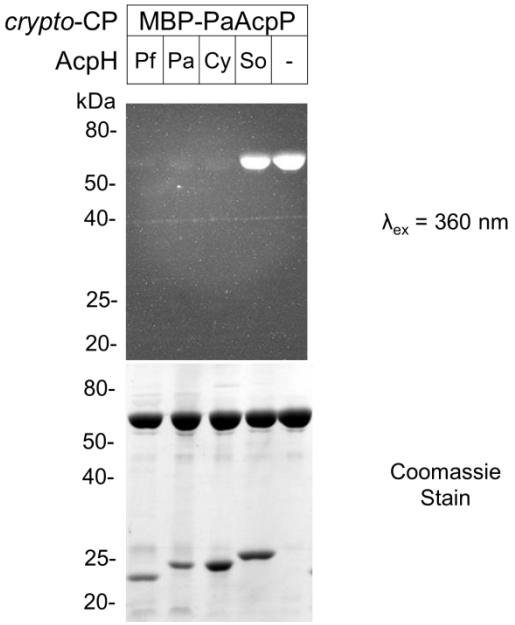
Analysis of AcpH homolog activity with SyrB1 (NRPS) and MAS (FAS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 5

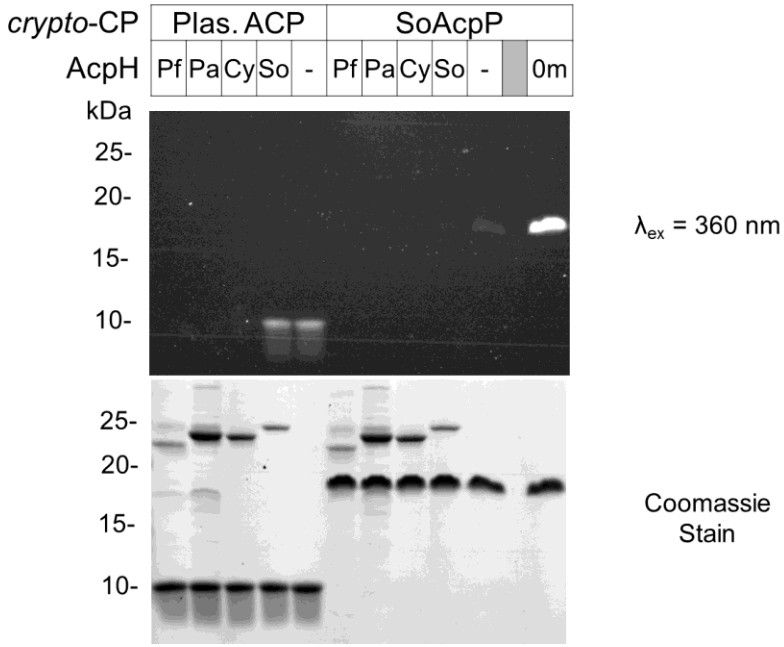
Analysis of AcpH homolog activity with MBP-PaAcpP (FAS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 6

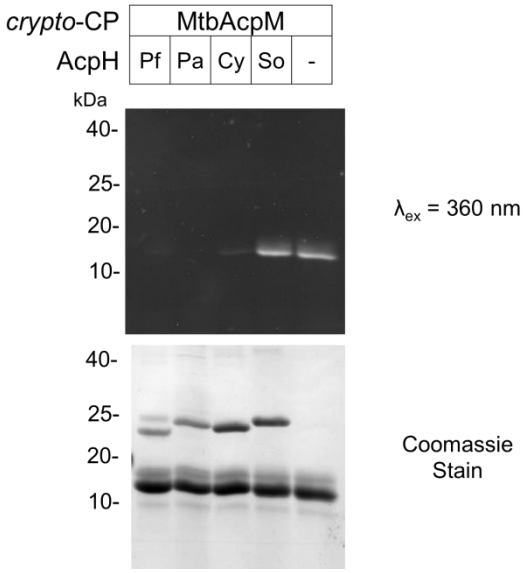
Analysis of AcpH homolog activity with *Plasmodium falciparum* ACP *and SoAcpP* (FAS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C. Coumarin-PPant from SoAcpP appeared to hydrolyze overnight in incubated samples compared to non-incubated sample “0m”.

Supplementary Figure 7

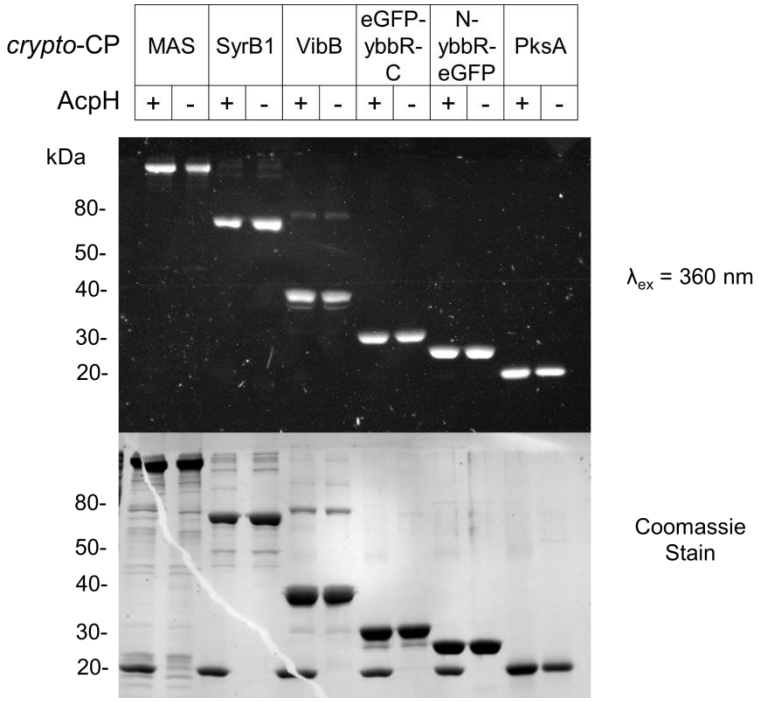
Analysis of AcpH homolog activity with MtbAcpM (FAS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 8

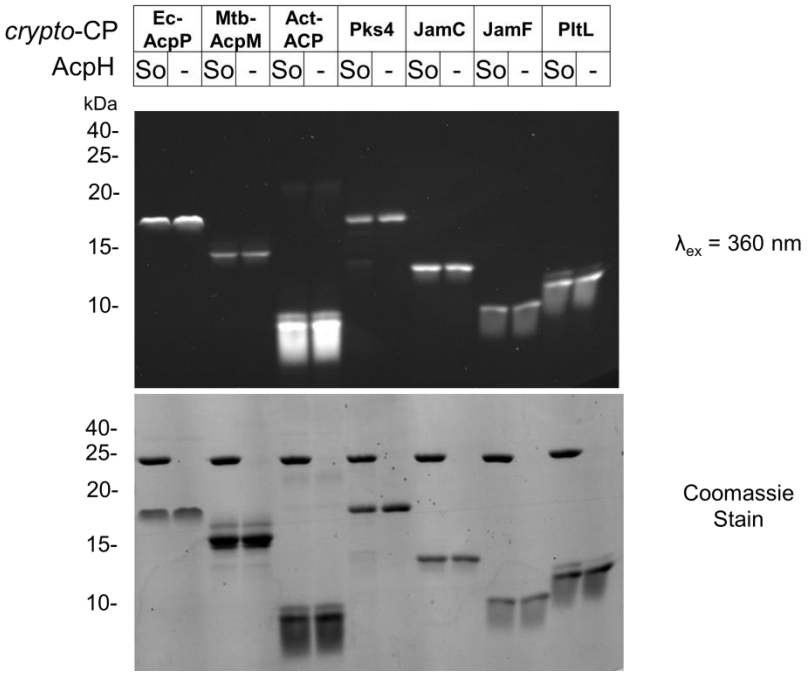
Analysis of SoAcpH activity with MAS (FAS), SyrB1 and VibB (NRPS), PksA (PKS), eGFP-YbbR C- and N-terminal fusions (peptide)



AcpH homolog from *S. oneidensis* (So) is evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 9

Analysis of SoAcpH activity with EcAcpP, MtbAcpM (FAS), ActACP, Pks4, JamC, JamF (PKS), and PltL (NRPS)

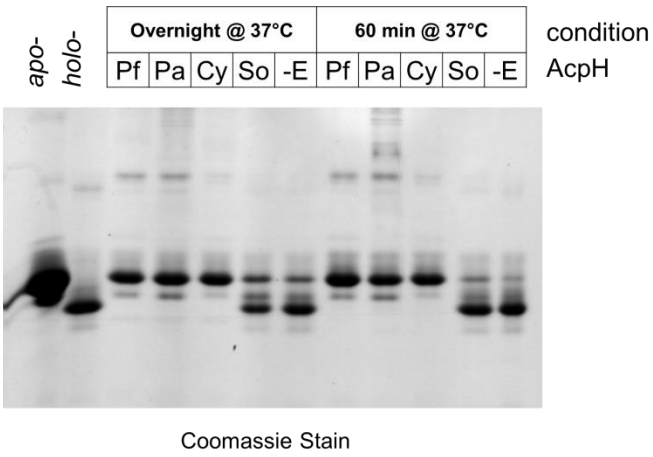


AcpH homolog from *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 10

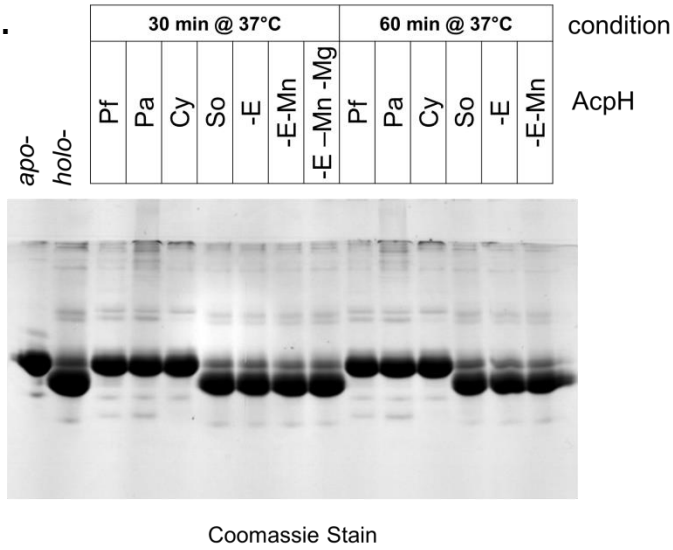
Analysis of AcpH activity with *holo*-SoAcpP (FAS) at various time-points

a.



Coomassie Stain

b.

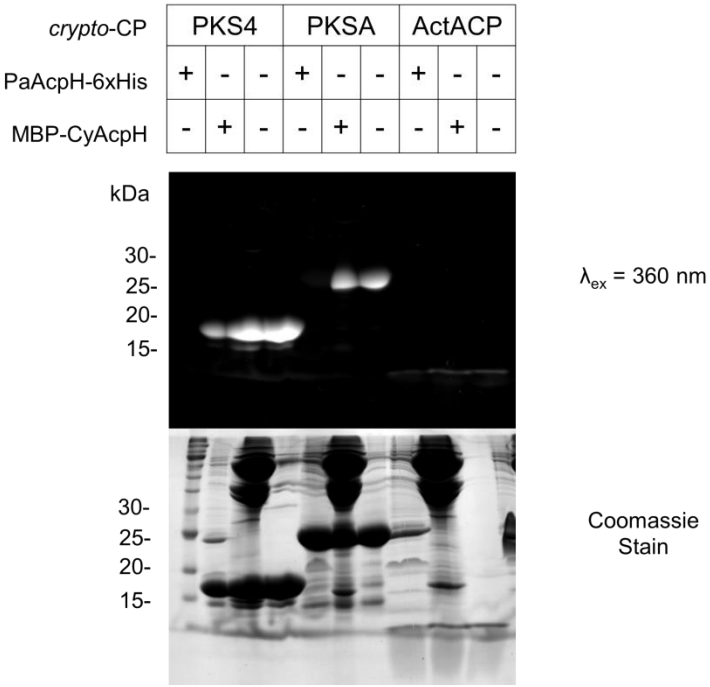


Coomassie Stain

AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by Urea-PAGE with *holo*-CP (+) compared to buffer blanks (-E) after various incubation times at 37°C. Samples were quenched with EDTA at listed times. Blank samples were also prepared without Mn^{2+} (-E-Mn) and without Mn^{2+}/Mg^{2+} in order to evaluate non-enzymatic PPant hydrolysis.

Supplementary Figure 11

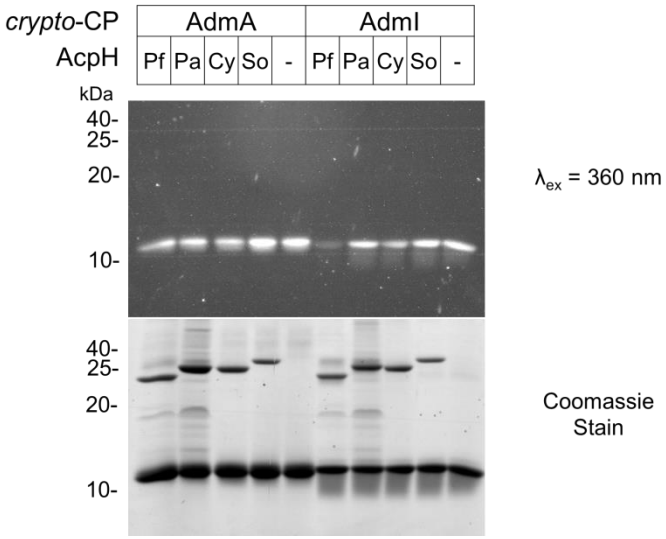
Analysis of PaAcpH and MBP-CyAcpH with Pks4, PksA, ActACP (PKS) activity.



AcpH homologs from *P. aeruginosa* (Pa) and MBP- fusion AcpH homolog from *Cyanothece* PCC7822 (MBP-CyAcpH) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 12

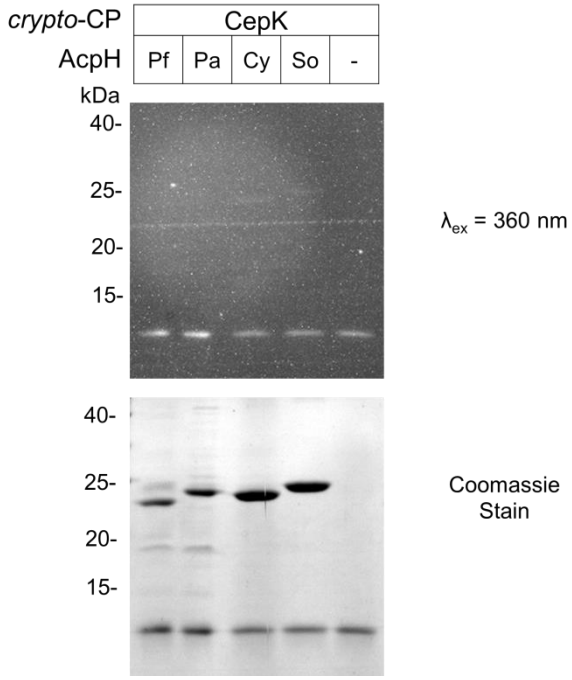
Analysis of AcpH homolog activity with AdmA (PKS) and AdmI (NRPS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 13

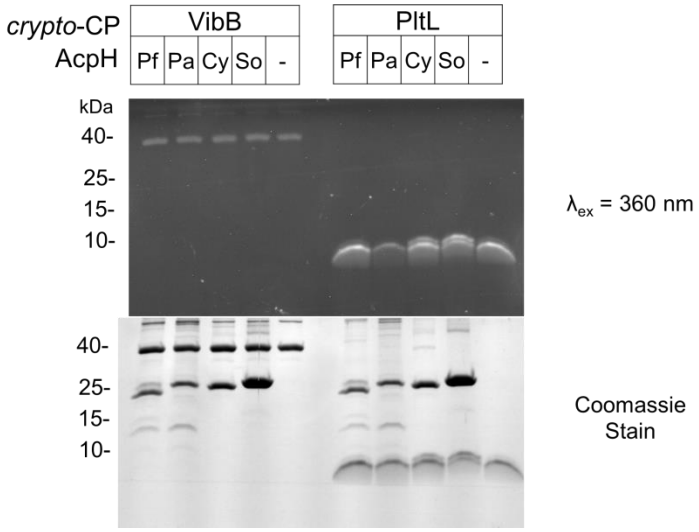
Analysis of AcpH homolog activity with CepK (NRPS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 14

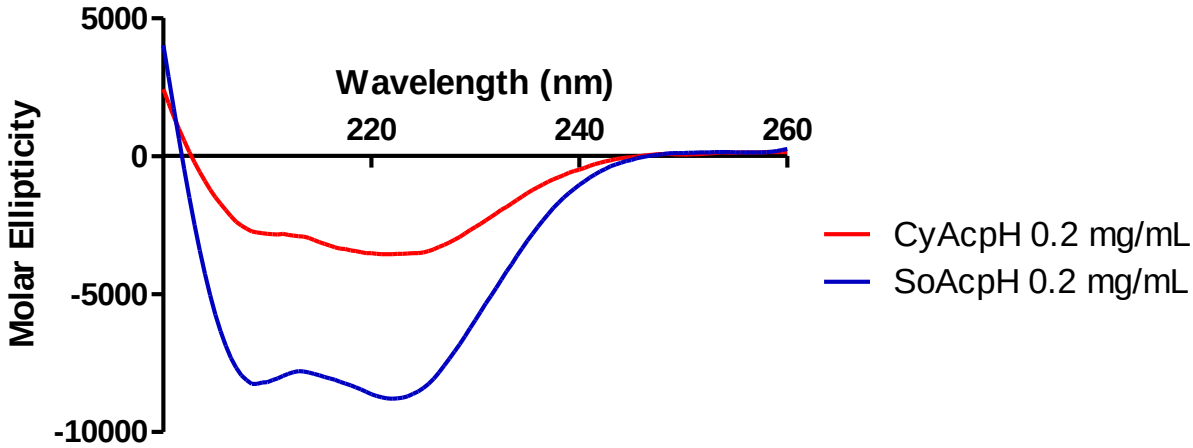
Analysis of AcpH homolog activity with VibB and PltL (NRPS)



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with various *crypto*-CP (+) compared to no enzyme blanks (-) after overnight incubation at 37°C.

Supplementary Figure 15

Circular dichroism analysis of CyAcpH and SoAcpH



Circular dichroism analysis of suspected inactive *S. oneidensis* AcpH (SoAcpH) compared to known active *Cyanothece sp.* PCC7822 (CyAcpH) reveals strong alpha-helical character in SoAcpH at similar protein concentrations. This suggests that SoAcpH maintains consistent secondary structure.

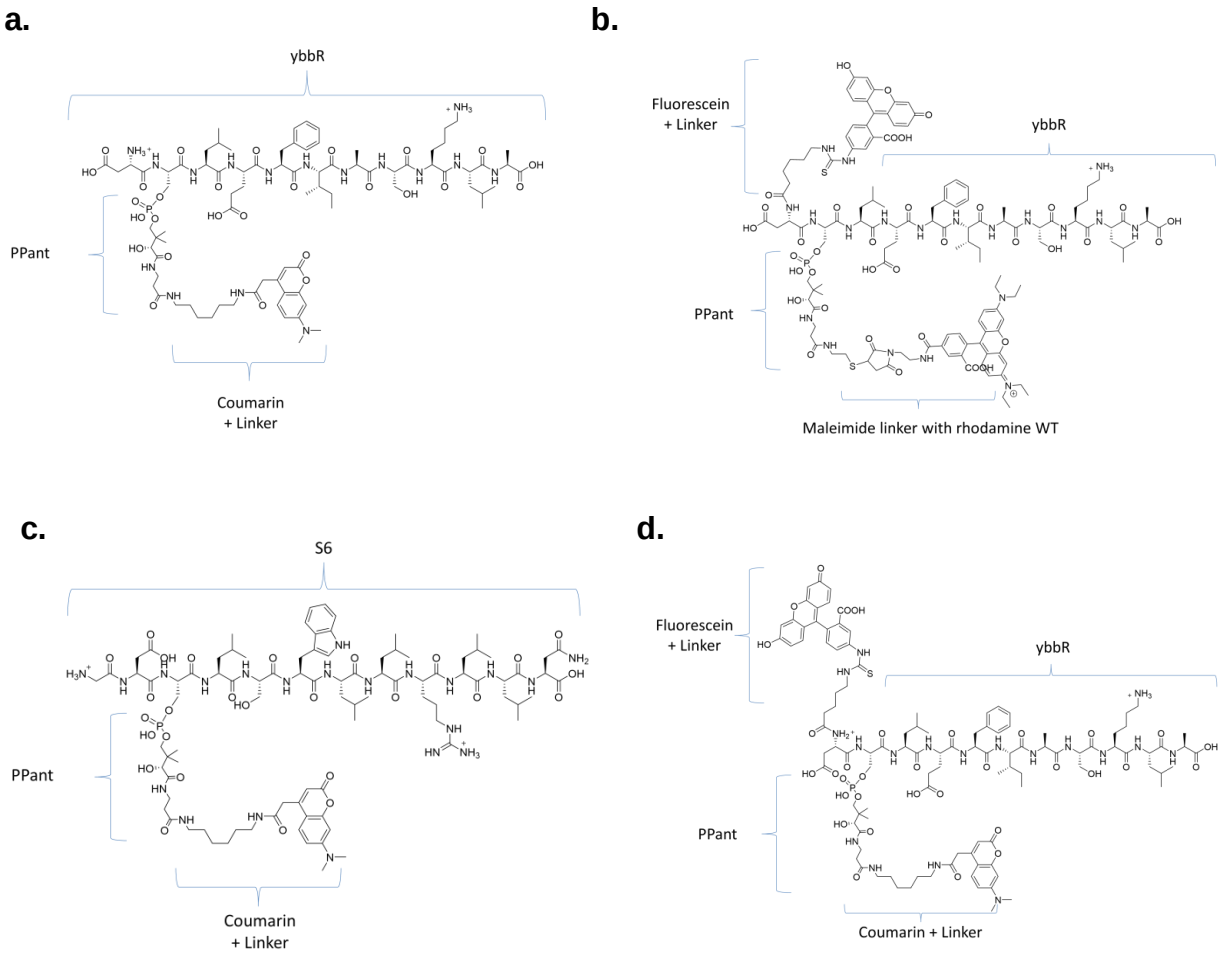
Supplementary Figure 16

Sequence alignment of known active AcpH homologs to SoAcpH

Known active AcpH homologs from *E. coli* (EcAcpH),*(1, 2) Cyanothece* PCC7822 (CyAcpH), *P. aeruginosa* (PaAcpH),*(3, 4)* and *P. fluorescens* (PfAcpH) are aligned to the annotated hypothetical AcpH homolog from *S. oneidensis* (SoAcpH). The alignment reveals that only the SoAcpH lacks two out of three suspected active-site aspartate residues corresponding to D24, D78, D82 in EcAcpH predicted by Cronan for Mn^{2+} binding and previously mutated for activity impact.*(1)*

Supplementary Figure 17

Chemical structures of non-recombinant YbbR and S6 peptide



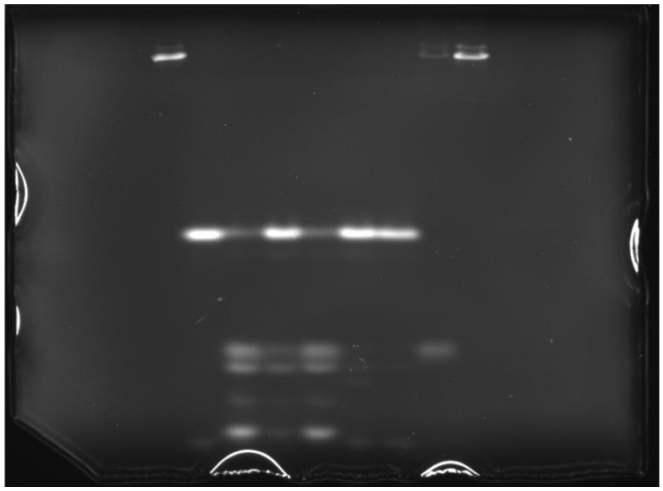
Variations of YbbR subjected to coumarin-PPant labeling AcpH activity Urea-PAGE analysis include free peptide (**a**), and FITC-conjugated peptide (**b**). Additionally, S6 peptide was labeled chemoenzymatically for AcpH reaction analysis (**c**). A rhodamine-PPant conjugate of YbbR was synthesized for FRET kinetics evaluation (**d**).

Supplementary Figure 18

Analysis of AcpH activity with coumarin-YbbR

coumarin-CP	P	Ybbr					P	P
AcpH	-	-	Pf	Pa	Cy	So	-	Pf	-
OvN/37°C	-	-	+	+	+	+	+	+	+

"P" = PksA



λ_{ex} = 360 nm

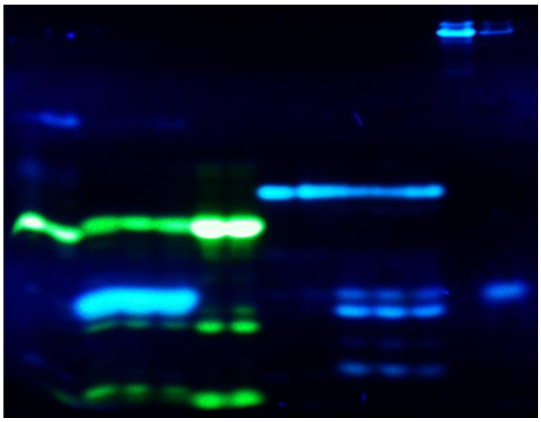
AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by Urea-PAGE with *crypto*-YbbR and *crypto*-PksA (P) compared to no enzyme blanks (-) after overnight incubation at 37°C (+) or non-incubation (-).

Supplementary Figure 19

Analysis of AcpH activity with coumarin-labeled YbbR and FITC-YbbR

coumarin-CP	Coum-FITC-YbbR					FITC-YbbR		Coum-YbbR					Coum-PksA	
AcpH	-	-	Cy	Pf	Pf*	-	Pf	-	-	Cy	Pf	Pf*	-	Pf
OvN/37°C	-	+	+	+	+	+	+	-	+	+	+	+	+	+

* = PMSF pre-treatment

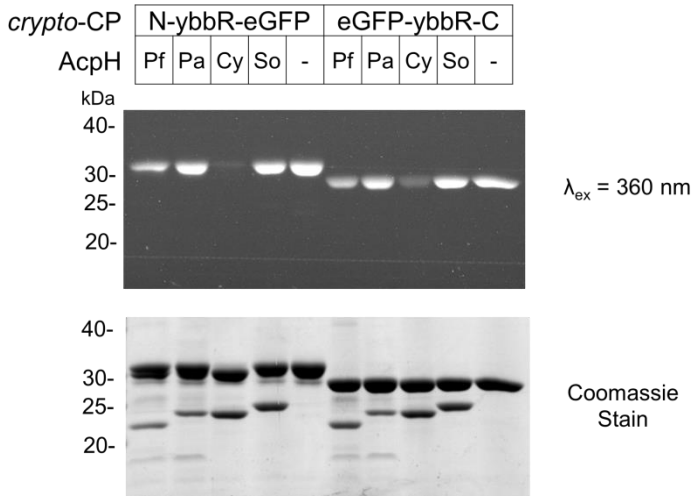


λ_{ex} = 360 nm

AcpH homologs from *P. fluorescens* (Pf), and *Cyanothece* PCC 7822 (Cy) are evaluated by Urea-PAGE with *crypto*-YbbR and *crypto*-PksA compared to no enzyme blanks (-) after overnight incubation at 37°C (+) or non-incubation (-). PMSF is also used to pretreat a PfAcpH sample to ensure that probe hydrolysis is not due to serine-protease.

Supplementary Figure 20

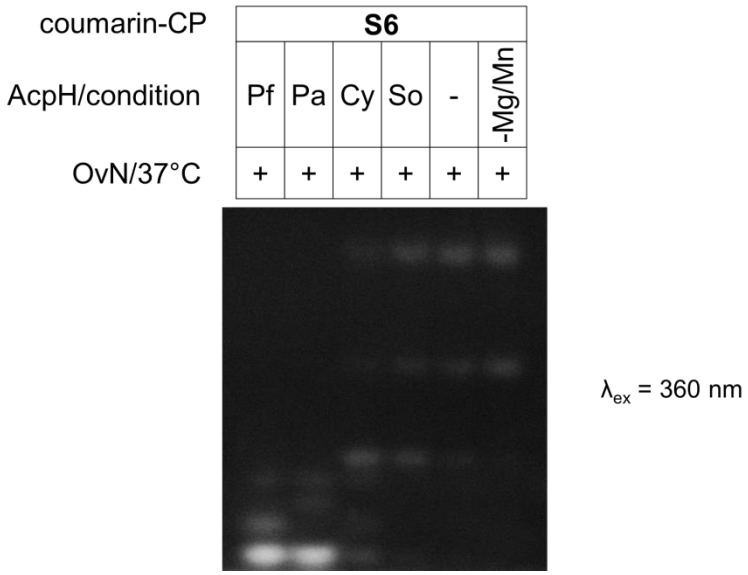
Analysis of AcpH homolog activity with YbbR-eGFP fusions



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by SDS-PAGE with *crypto*- YbbR-eGFP N- and C-terminal fusions compared to no enzyme blanks (-) after overnight incubation at 37°C

Supplementary Figure 21

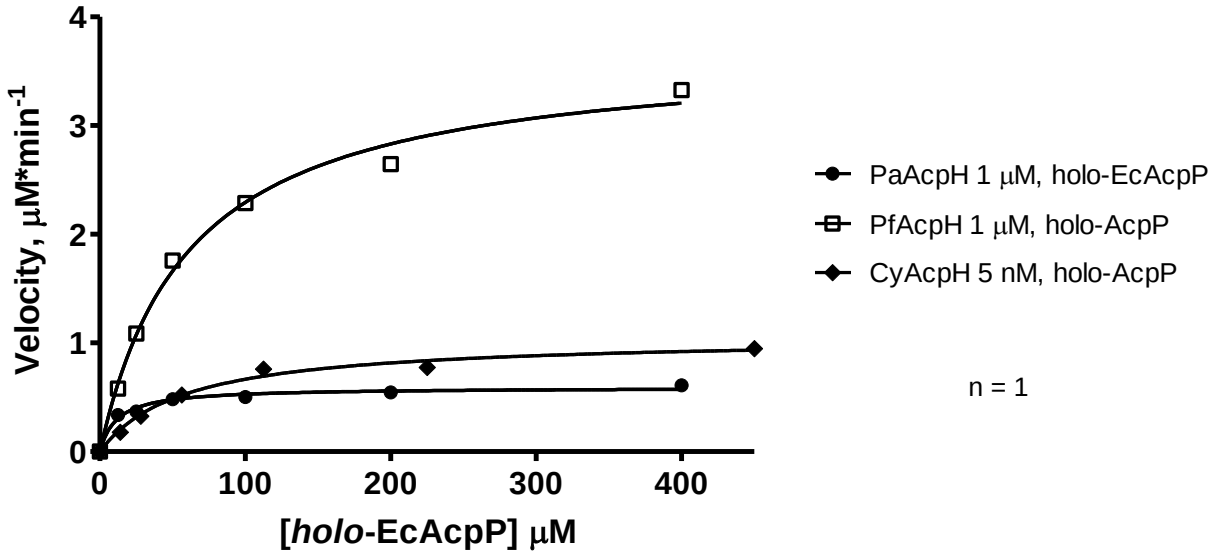
Analysis of AcpH homolog activity with S6 peptide



AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) and *S. oneidensis* (So) are evaluated by Urea-PAGE with *crypto*-S6 compared to no enzyme blanks (-), and no enzyme/Mg/Mn blank (-Mg/Mn) after overnight incubation at 37°C (+).

Supplementary Figure 22

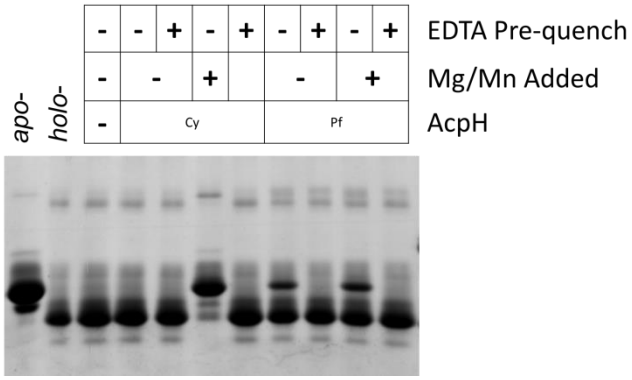
Analysis of AcpH homolog kinetics with *holo*-EcAcpP (FAS)



Reaction of AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa), *Cyanothece* PCC 7822 (Cy) with *holo*-EcAcpP, and subsequent EDTA-quenching allow derivation of HPLC kinetic values. CyAcpH demonstrated significantly higher turnover, and required a lower enzyme concentration of 5 nM, compared to 1 □M utilized for other AcpH homologs. SoAcpH did not demonstrate activity in the qualitative assays, so was not evaluated for kinetics.

Supplementary Figure 23

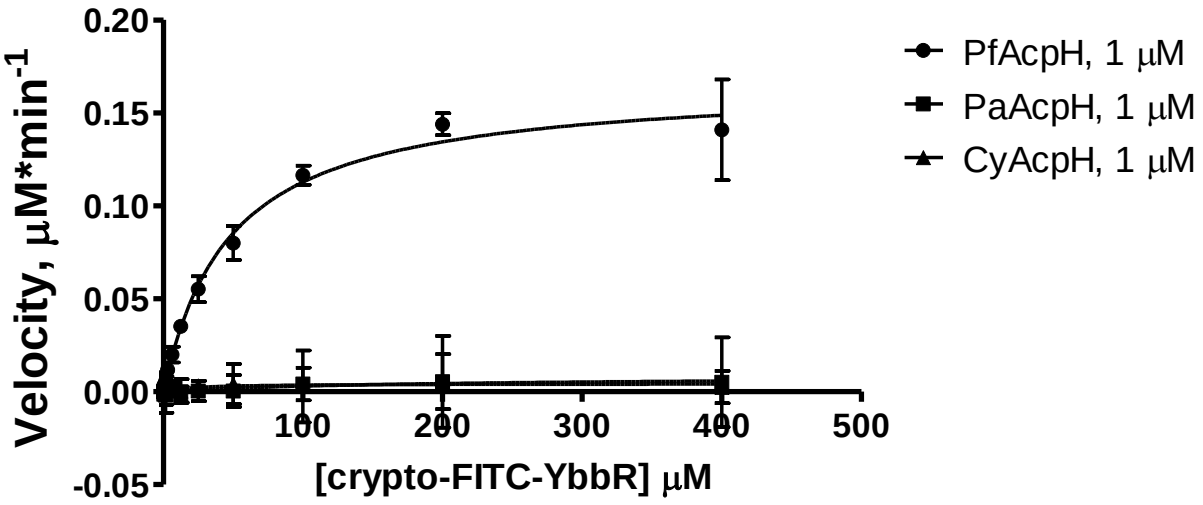
Verification of EDTA quench method



AcpH homologs from *P. fluorescens* (Pf), and *Cyanothece* PCC 7822 (Cy) are evaluated to verify the *holo*- *E. coli* AcpP reaction termination expected by EDTA addition prior to evaluating HPLC kinetic samples. No conversion of *holo*- to *apo*- AcpP was observed following a10 minute incubation at 37°C, followed by overnight incubation at room temperature.

Supplementary Figure 24

Analysis of AcpH homolog kinetics with *crypto*-FITC-YbbR

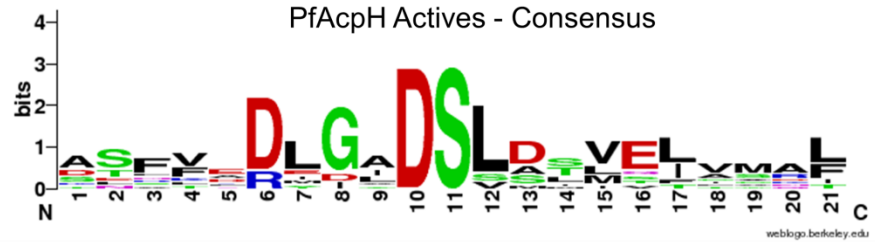


Reaction of AcpH homologs from *P. fluorescens* (Pf), *P. aeruginosa* (Pa),and *Cyanothece* PCC 7822 (Cy) in microwell format with *crypto*-FITC-YbbR allows derivation of HPLC kinetic values. While PaAcpH and CyAcpH appeared to demonstrate signal above background, PfAcpH demonstrated clear signal indicating substrate turnover.

Supplementary Figure 25

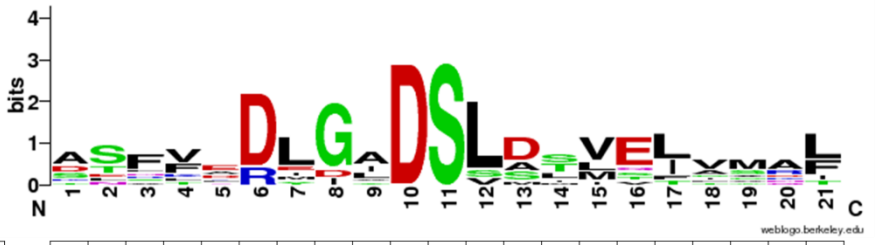
PfAcpH consensus substrate sequence and CP alignments

a.



ID																				# matching consensus
EcAcpP	A	S	F	V	E	D	L	G	A	D	S	L	D	T	V	E	L	V	M	A	L	18
PaAcpP	A	S	F	V	E	D	L	G	A	D	S	L	D	T	V	E	L	V	M	A	L	18
SoAcpP	A	S	F	V	D	D	L	G	A	D	S	L	D	T	V	E	L	V	M	A	L	18
Plas. ACP	S	N	F	T	K	D	L	G	A	D	S	L	D	L	V	E	L	I	M	A	L	15
MtbAcpM	K	S	F	V	D	D	L	D	I	D	S	L	S	M	V	E	I	A	V	Q	T	9
PksA	D	S	N	F	A	D	M	G	I	D	S	L	S	S	M	V	I	G	S	R	F	8
Pks4	T	T	L	L	A	D	L	G	V	D	S	L	M	S	L	T	I	L	G	N	F	7
JamC	S	T	S	F	N	R	Y	G	L	D	S	S	A	S	I	S	L	T	S	D	F	6
ActACP	D	L	R	F	E	D	I	G	Y	D	S	L	A	L	M	E	T	A	A	R	L	8
AdmI	A	E	V	K	L	R	E	I	L	D	S	V	D	I	L	Q	F	V	Y	K	I	4
Ybbr13										D	S	L	E	F	I	A	S	K	L	A		4
S6									G	D	S	L	S	W	L	L	R	L	L	N		3
										*	*				:							

b.

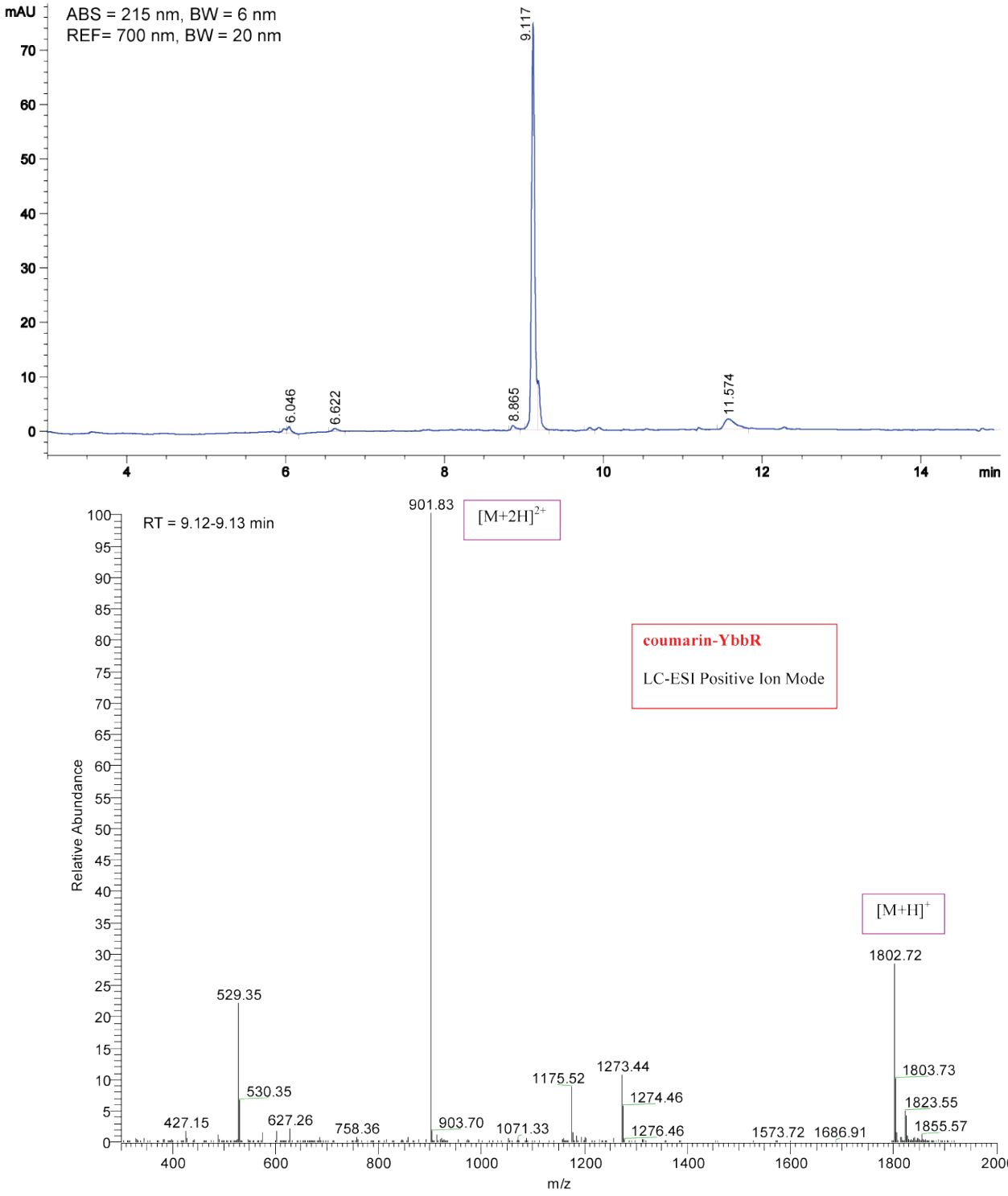


ID																				# matching consensus
JamF	T	D	S	L	K	N	L	G	I	D	S	V	N	R	A	E	I	I	M	M	V	5
VibB	Q	E	N	L	L	F	L	G	L	D	S	I	R	A	I	Q	L	L	E	K	W	5
CepK	D	D	S	F	F	D	I	G	G	H	S	L	L	A	T	R	L	V	A	R	I	6
PltL	T	P	L	L	E	W	G	I	L	N	S	M	N	I	V	K	L	M	V	Y	I	3
SyrB1	N	D	D	F	F	D	S	G	G	T	S	S	L	A	L	I	R	S	L	S	K	4
MAS	D	R	S	F	I	E	Y	G	L	D	S	L	G	M	L	E	M	R	T	H	V	6
AdmA	L	L	A	E	E	G	L	A	L	D	S	V	D	V	L	D	I	F	V	G	I	4

The consensus sequence of all PfAcpH active carrier protein sequences was generated using WebLogo (http://weblogo.berkeley.edu). The consensus demonstrates several residues matching those of the type II FAS ACPs as being particularly pervasive across active substrates (**a**). Inactive substrates contain 6 or fewer residues matching the active consensus (**b**), while only one active substrate, YbbR, contains fewer than 6 residues matching the consensus.

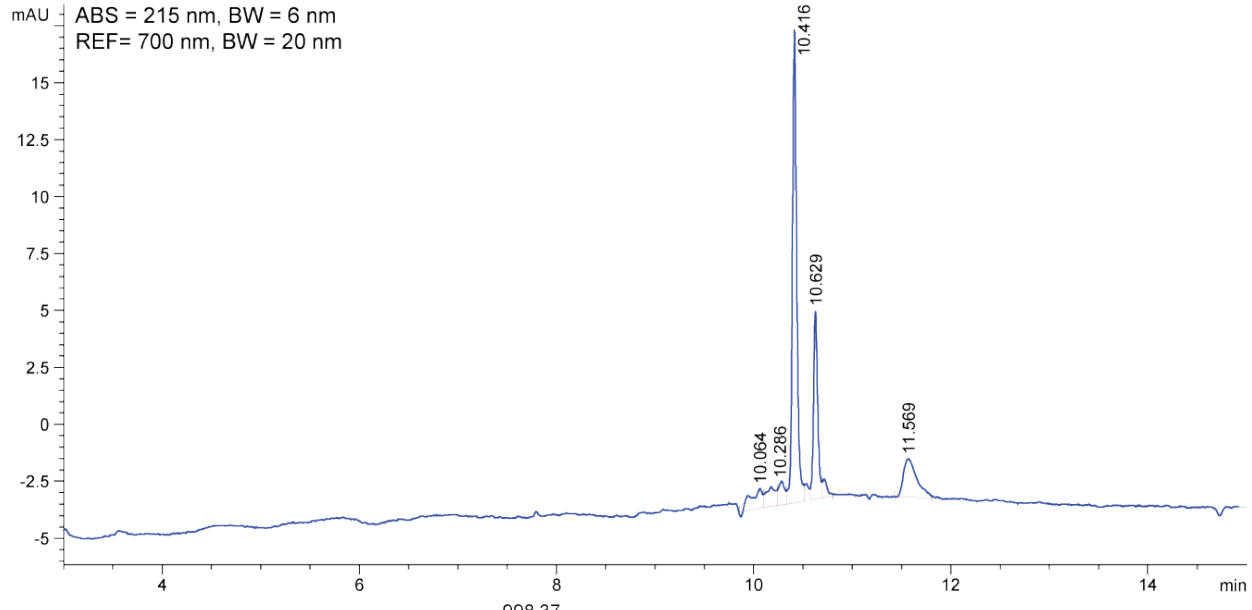
Supplementary Figure 26

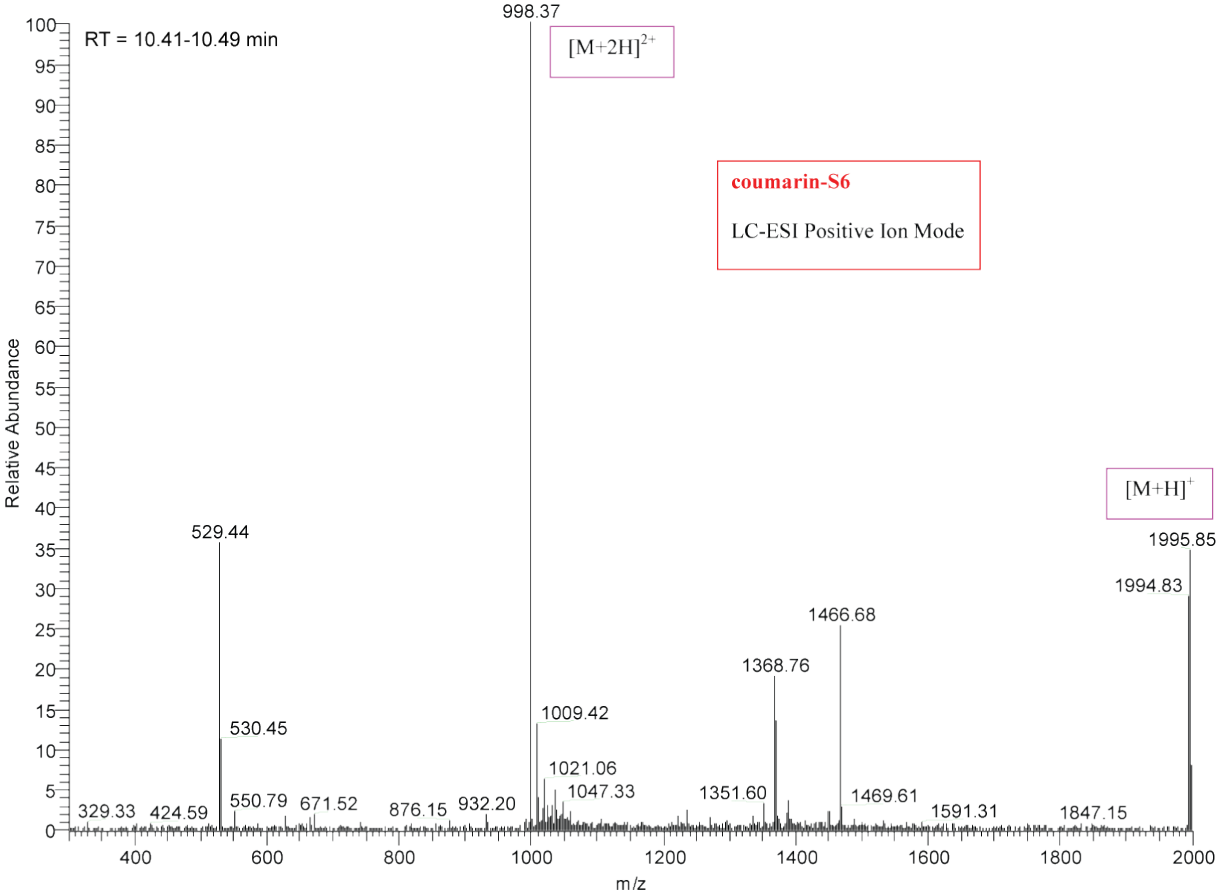
Coumarin-YbbR: LC-MS analysis



Supplementary Figure 27

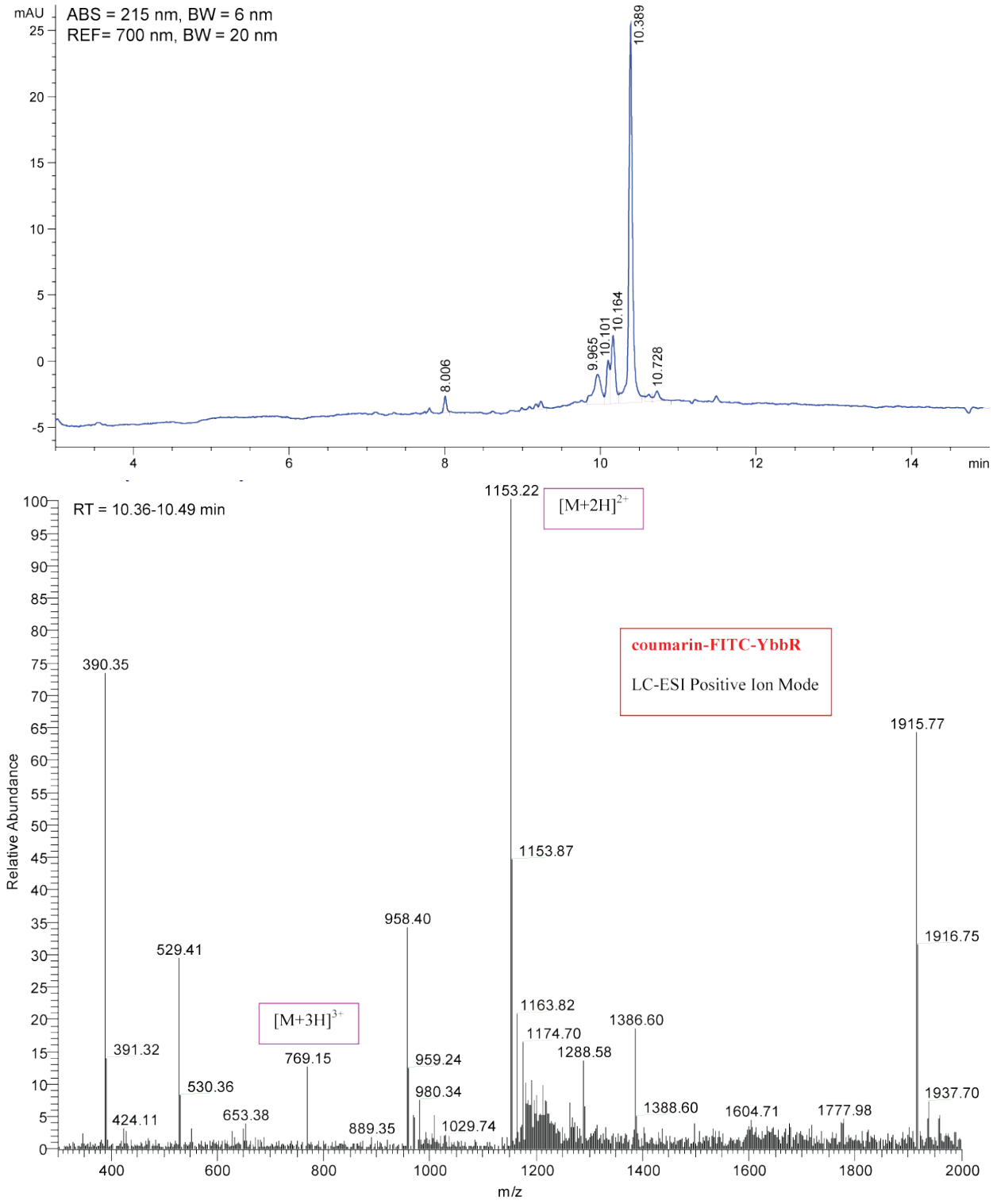
Coumarin-S6: LC-MS analysis





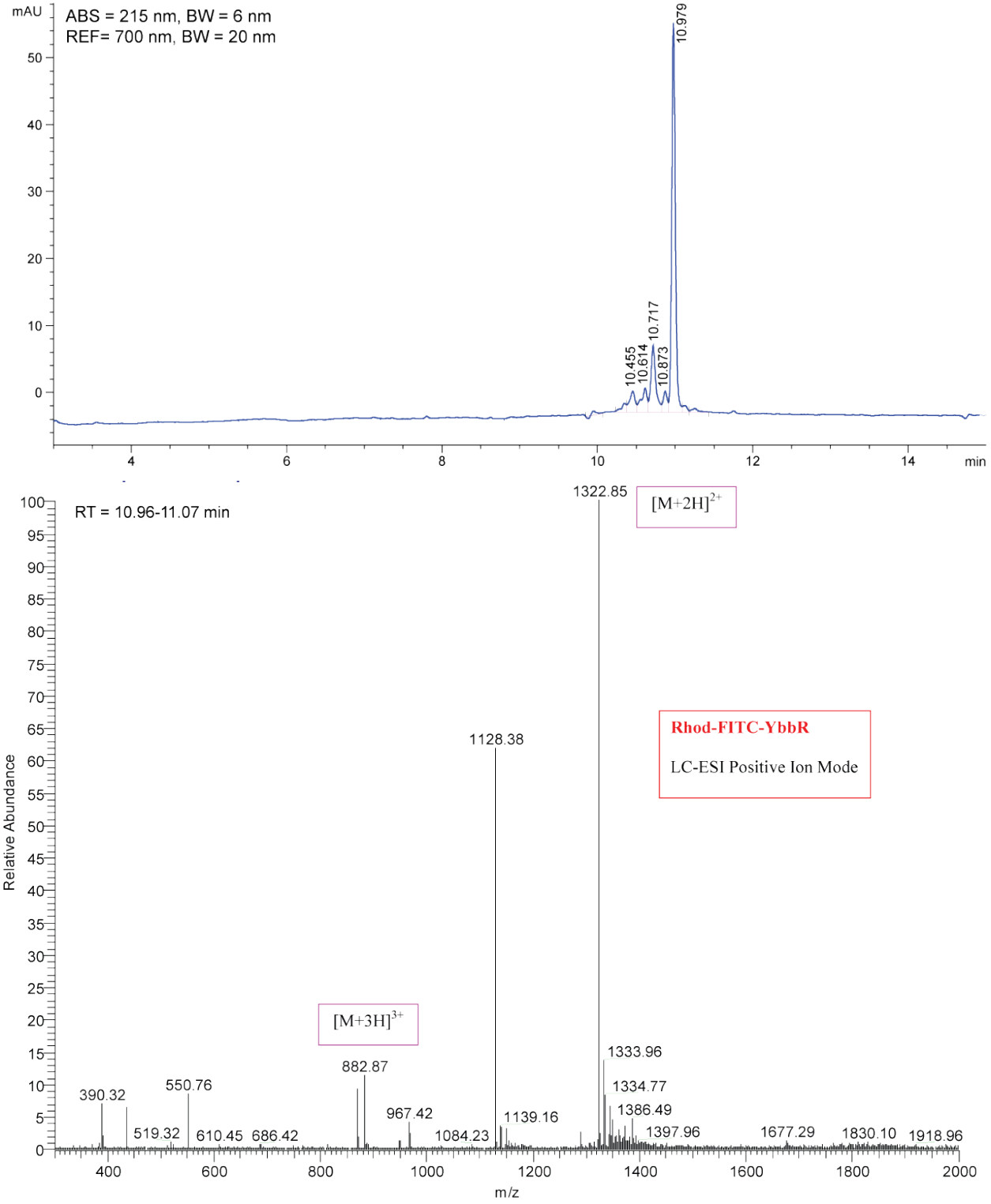
Supplementary Figure 28

Coumarin-FITC-YbbR: LC-MS analysis



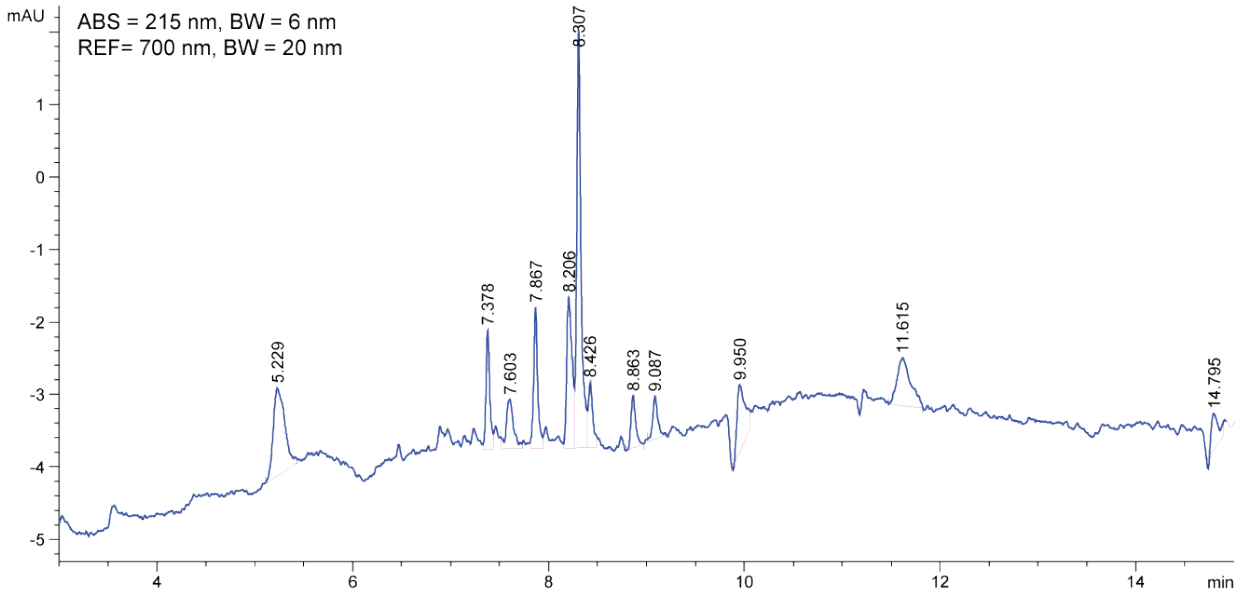
Supplementary Figure 29

Rhodamine-FITC-YbbR: LC-MS analysis



Supplementary Figure 30

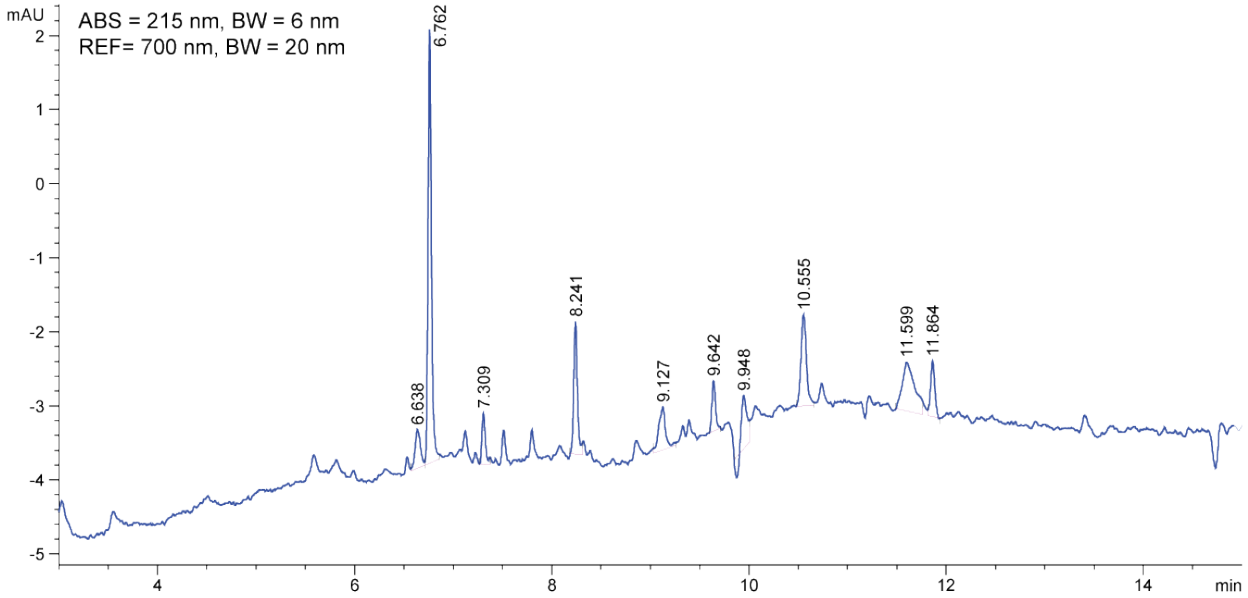
Coumarin-YbbR PfAcpH reaction: LC analysis



Treatment of coumarin-YbbR with PfAcpH results in the depletion of the coumarin-YbbR peak at a retention time of 9.2 minutes (Supplementary Figure 26).

Supplementary Figure 31

Coumarin-S6 PfAcpH reaction: LC analysis



Treatment of coumarin-S6 with PfAcpH results in the depletion of the coumarin-YbbR peak at a retention time of 10.4 minutes (Supplementary Figure 27).

Supplementary Table 1

Carrier proteins studied for AcpH activity

	Protein Name	Source Organism	Accession Number
FAS	AcpP	*E. coli*	NP_287228
	AcpP	*P. aeruginosa*	NP_251656
	AcpP	*S. oneidensis*	NP_718356
	PfACP	*P. falciparum*	3GZL_A
	AcpM	*M. tuberculosis*	NP_216760
	MAS	*M. tuberculosis*	YP_006516394
PKS	ActACP	*S. coelicolor*	NP_629239
	PksA	*A. parasiticus*	2KR5_A
	Pks4	*G. fujikuroi*	CAB92399
	JamC	*L. majuscula*	AAS98798
	JamF	*L. majuscula*	CAB46501
	AdmA	*P. agglomerans*	AAO39095
NRPS	AdmI	*P. agglomerans*	AAO39103
	VibB	*V. cholerae*	AAC45926
	CepK	*A. orientalis*	KF672793
	PltL	*P. protogens*	AAD24885
	SyrB1	*P. syringae*	AAZ99831

Carrier proteins used in this study used for PPant labeling and subsequent analysis of AcpH homolog activity via PPant hydrolysis.

Supplementary Table 2

Primers used for cloning

Primer Name	Primer Sequence (5' → 3')
PfAcpH F1	AAAACATATGAATTATCTCGCACATCTGCACC
PfAcpH R1	AAAACTCGAGTGCAAAGGCCTGCAACTCTGG
PfAcpH R2	AAAACTCGAGTTAAAATTGGAGTGCAAAGGCCTGC
PfAcpH R3	AAAACTCGAGAAATTGGAGTGCAAAGGCCTGCAAC
CyAcpH F1	AAAACATATGAATTATCTGGCTCATTTATTTTTAGC
CyAcpH R1	AAAACTCGAGAGCCAAGTTAACATAATCAATCAGTTG
SoAcpH F1	AAAACATATGAACATTCTTACACACTTACATCTGG
SoAcpH R1	AAAACTCGAGCTCGGGTAAGTAGTCAATTGGAG

Primers used in cloning/sub-cloning for gene products used in this manuscript.

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1. J. Thomas, D. J. Rigden, and J. E. Cronan, *Biochemistry*, 2007, **46**, 129-136.

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3. E. Murugan, R. Kong, H. Sun, F. Rao, and Z. X. Liang, *Protein Expres. Purif.*, 2010, 71, 132-138.

4. N. M. Kosa, R. W. Haushalter, A. R. Smith, and M. D. Burkart, *Nat. Meth.*, 2012, 9, 981-984.