

Supplemental Data for “INTEIN-MEDIATED CYCLIZATION OF BACTERIAL ACYL CARRIER PROTEIN STABILIZES ITS FOLDED CONFORMATION BUT DOES NOT ABOLISH FUNCTION” by Volkmann *et al*

TABLE S1. Primers used in this study

<i>Primer</i>	<i>Sequence (5'—3')</i>
IC- <i>for</i>	GGGAGCTCATGGAAGCAGTATTAATTACAATCAC
IC- <i>rev</i>	GACTAATGTATCTCCAGAAAAACAGGAAGAGCATATGCTAGCGCTGTTATGGACAAACAC
ICMUT- <i>rev</i>	GACTAATGTATCTCCAGAAAAAGCGGAAGAGCATATGCTAGCGCTTGCATGGACAAACAC
IN- <i>for</i>	GTGTTTGTCCATAACAGCGCTAGCATATGCTCTTCCTGTTTTTCTGGAGATACATTAGTC
INMUT- <i>for</i>	GTGTTTGTCCATGCAAGCGCTAGCATATGCTCTTCCGCTTTTTCTGGAGATACATTAGTC
INHIS- <i>rev</i>	GGCTGCAGTTAATGGTGATGGTGATGGTGATGACCAGAATCTTCC
ACP- <i>for</i>	GGGCTAGCAACATCGAAGAACGCGTAAAGAAAATC
ACP- <i>rev</i>	GGGGCTCTTCTACAACCCTGAGCGCTGTTTACG
GSTC- <i>for</i>	GGGTGGCCATCATAACGTTATATAGCTGACAAGC
GSTC- <i>rev</i>	CGTTCTTCGATGTTGCTAGCAGAACGACCTTCGATCAGATCC
LINL46W- <i>for</i>	GGATCTGATCGAAGGTCGTTCTGCTAGCAACATCGAAGAACG
LINL46W- <i>for</i> 2	CCCATATGTCTGCTAGCAACATCGAAGAACGCG
LINL46W- <i>rev</i>	GGGAGCGCTTCATTAACCCTGAGCAGAGTTTACGTAGTCG
LINL46W- <i>rev</i> 2	CCCTGCAGTCATTAACCCTGAGCAGAGTTTACG
LINF50A- <i>for</i>	GAGGCTGACACTGAGATTCCTGATGAAG
LINF50A- <i>rev</i>	CTCTTCCAGAGCCATTACTAGCTCTACAGTG

TABLE S2. Bacterial strains and plasmids used in this study

<i>Strain or plasmid</i>	<i>Relevant features or description</i>	<i>Source</i>
Strains		
BL21(DE3)pLysS	<i>ompT</i>	^a
CY1861	Δ <i>acpP::cat</i> pACYC/ <i>acpP</i>	This study
Plasmids		
pT _{I_C} (NS) _{I_N} H	Permuted <i>Synechocystis ssp.</i> PCC6803 GyrB split-intein sequence cloned into <i>SacI/PstI</i> sites of pT; contains <i>NheI</i> and <i>SapI</i> sites between I _C and I _N sequences, respectively, for insertion of target protein sequence for in vivo cyclization	This study
pTCYC-L46W	<i>Vibrio harvei</i> ACP L46W mutant cloned into pT _{I_C} (NS) _{I_N} H via <i>NheI</i> and <i>SapI</i> .	This study
pTCYC-F50A	As pTCYC-L46W, but with F50A mutation in place of L46W	This study
pTPRE-L46W-mut	Cyclization-deficient control construct of pTCYC-L46W, with C1A mutation in I _C and N435A mutation in I _N	This study
pMAL	Expression of <i>malE</i> from P _{tac} promoter	New England Biolabs
pMCYC-L46W	Amp ^r , L46W cyclization ORF cloned into <i>NdeI/PstI</i> sites of pMAL	This study
pMCYC-F50A	As pMCYC-L46W, but with F50A mutation in place of L46W	This study
pMPRE-L46W-mut	Cyclization-deficient control construct of pMCYC-L46W	This study
pMLIN-L46W	Encodes linear equivalent to cycl46W protein, cloned between <i>NdeI/PstI</i> sites of pMAL	This study
pMLIN-F50A	As pMLIN-L46W, but with F50A mutation in place of L46W	This study
pGEX-linL46W	Amp ^r , expression of GST-linL46W fusion protein from P _{tac} promoter	This study
pGEX-F50A	Amp ^r , expression of GST-F50A fusion protein from P _{tac} promoter	(20)

^a Studier, F.W. and Moffatt, B.A. (1986) *J. Mol. Biol.* **189**, 113-130