Supplementary Material (ESI) for Molecular BioSystems
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Activity-Guided Engineering of Natural Product Carrier Proteins

Andrew S. Worthington, Gene H. Hur and Michael D. Burkart*

Electronic Supplementary Information

Cloning of parent CP genes into pET22b

The vector ΔNEntB/pET22b was supplied by the Walsh laboratory at Harvard University.[1] AcpP and FrnN were generated using 0.5 μM each primer for and reverse – NdeI and XhoI restriction sites (underlined) with 1 ng of alternate vector containing the gene of interest.
PCR reaction mixture contains primers at 0.1 μM each, 50 mM KCl, 10 mM Tris pH 8.3, 2 mM $MgCl_2$, 200 μM each dNTP, and 1 unit Pfu.

Thermocycling conditions were as follows:
95°C for 2 minutes
35 cycles of: 95°C for 30 seconds, 59°C for 30 seconds, 72°C for 30 seconds
72°C for 10 minutes

Forward and Reverse Primers

AcpPforward: ATTATATCATATGAGCACTATCGAAGAACGCG
AcpPreverse: TGATGTCCTCGAGCGCCTGGTGGCCGTTG
FrnNforward: ATTATATCATATGAGCGCACTGACCGTCGACG
FrnNreverse: TGATGTCCTCGAGGGCGGTGGCCGGGGTGG
EntBforward: ATTATATCATATGTCCCTGAAATATGTGGCCG
EntBreverse: TGATGTCCTCGAGTTTCACCTCGCGGG

PCR Ligation

Stocks of each primer set with a concentration of 2.5 μM per primer were made. One μL of each primer set (e.g. A3, F2F3) and 5 total primer sets were used per 25 μL reaction (0.1 μM for each primer in the final reaction) – see Supplementary Table 1.
The PCR reaction mixture contained primers at 0.1 μM each, 50 mM KCl, 10 mM Tris pH 8.3, 2 mM $MgCl_2$, 200 μM each dNTP, and 1 unit Pfu.

Thermocycling conditions were as follows:
95°C for 2 minutes
30 cycles of: 95ºC for 30 seconds, 64ºC for 30 seconds, 72ºC for 30 seconds
72°C for 10 minutes
Holding at 4ºC

A second round of PCR reactions was performed, with 1 μL of this reaction with 0.5 μM (final concentration) of each CP specific primer (e.g. AcpPforward and FrnNreverse) with same reaction conditions (25 μL), except a different program was used (we had to vary the annealing temperature for each construct)

Thermocycling conditions were as follows:
95°C for 2 minutes
40 cycles of: 95ºC for 30 seconds, 58-72ºC for 30 seconds, 72ºC for 30 seconds
72°C for 10 minutes
Holding at 4ºC

The second PCR reaction was run on 2% agarose gel stained with 1 % ethidium bromide. Each properly sized band was excised and purified with the Qiaquick Gel Extraction Kit (Qiagen). Inserts were then digested with 20 units each of XhoI and NdeI (NEB) at 37ºC overnight, followed by purification with the AccuPrep PCR Purification Kit (Bioneer, Inc.). Inserts were ligated to pET22b vector (Novagen) that had been digested with XhoI and NdeI and reacted with Calf Intestinal Alkaline Phosphatase (NEB). The resulting constructs were transformed into Subcloning Efficiency DH5a competent cells (Invitrogen) and plated on LB/AMP plates. Colonies were screened through sequencing of purified plasmid (Qiagen kit), which was performed by Eton Biosciences (San Diego, CA).

Chimeric CP Panel Primers

<u>A1 Primers</u>
AcpPforward
ACPA1rev:
CATTGTTGGTAACTTCTTCCTGCTTAACGCCCAGCTGTTCGCCGATAATTTTCT
TAACGCGTTCTTCGAT

<u>A2 Primer</u>
ACPA2rev:
CTCTTCTTCCAGAGCCATTACCAGCTCAACGGTGTCAAGAGAATCCGCGCCC
AGGTCTTC

<u>A3 Primers</u>
ACPA3revA: GCCTGAACGGTGGTGATTTTCTCAGCTTCTTCGTCCGGAATC
ACPA3forB: CACCACCGTTCAGGCTGCCATTGATTACATCAACGGCCACCAGG
AcpPreverse

<u>F1 Primers</u>
FrnNforward
FrnNF1revA: CGGCGAGCAGTTTCTTGAGGTCGTCGACGGTCAGTG
FrnNF1forB: GAAACTGCTCGCCGAGACCGCCGGGGAGGACG
FrnNF1revC: CGTGTCGAGTTCTCCGGCGAGGTCGACGCTGTCGTCCTCCCCG

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F2 Primer
FrnNF2rev:
CCGCTGCTGGAGCACGGCGGCCGTCTCCAGCAGCGCGAGGGAGTCGTAGCCG
AGGTCC

F3 Primers
FrnNF3revA:
CAGTTCGCGCGGGGTGCCCAGCCGGCCGACCGTCTCGTCAGTCAG
FrnNF3forB:
CCCCGCGCGAACTGCTCGACGAGGTCAACACCACCCCGGCCACCG
FrnNreverse

E1 Primers
EntBforward
EntBE1revA: CTTCAGTCATCACCACCCGGCCAGAACGTCCGGCCACATATTTC
EntBE1forB: GTGATGACTGAAGAATTACTGCCAGCACCTATCCCCGCCAGC
EntBE1revC:
CATCGGACTCGTCCAGCAACGGCAGGATCACCTCACGCAGCGCCGCTTTGCT
GGCGGGGATAG

E2 Primer
EntBE2rev:
GCACTTTGCGCCAGCGCGCCGCCAGCGCCATCATGCGCACCGAATCCAGACC
GTAGTCGATCAGGTTGTC

E3 Primers
EntBE3revA: CCACCAGGCGTCGATGGTCGGGTTTTTCGCCAGCATGACAAAG
EntBE3forB : CGACGCCTGGTGGAAGCTACTCTCCCGCGAGGTGAAA
EntBreverse

Junction Primers
A1A2FOR : GTTACCAACAATGCTTCTTTCGTTGAAGACCTGGGCGCGG
A1F2FOR : GTTACCAACAATGCTTCTTTCGTGGACCTCGGCTACGAC
A1E2FOR : GTTACCAACAATGCTTCTTTCGATGACGACAACCTGATCG
F1F2FOR : GGAGAACTCGACACGCCCTTCGTGGACCTCGGCTACGAC
F1E2FOR : GGAGAACTCGACACGCCCTTCGATGACGACAACCTGATCG
F1A2FOR : GGAGAACTCGACACGCCCTTCGTTGAAGACCTGGGCGCGG
E1E2FOR : GACGAGTCCGATGAACCGTTCGATGACGACAACCTGATCG
E1A2FOR : GACGAGTCCGATGAACCGTTCGTTGAAGACCTGGGCGCGG
E1F2FOR : GACGAGTCCGATGAACCGTTCGTGGACCTCGGCTACGAC
A2A3FOR : GCTCTGGAAGAAGAGTTTGATACTGAGATTCCGGACGAAG
A2F3FOR : GCTCTGGAAGAAGAGTTTGATATCGCGCTGACTGACGAGACG
A2E3FOR : GCTCTGGAAGAAGAGTTTGATATCGACTTTGTCATGCTGGC
F2F3FOR : GTGCTCCAGCAGCGGTACGGCATCGCGCTGACTGACGAGACG
F2E3FOR : GTGCTCCAGCAGCGGTACGGCATCGACTTTGTCATGCTGGC

F2A3FOR : GTGCTCCAGCAGCGGTACGGCACTGAGATTCCGGACGAAG
E2E3FOR : GGCGCAAAGTGCATGGTGATATCGACTTTGTCATGCTGGC
E2A3FOR : GGCGCAAAGTGCATGGTGATACTGAGATTCCGGACGAAG
E2F3FOR : GGCGCAAAGTGCATGGTGATATCGCGCTGACTGACGAGACG

Supplementary Table 1. Primer combinations used in the construction of the chimeric carrier proteins

	A2	A3	A4	A5	A6	A7	A8	A9	F2	F3	F4	F5	F6	F7	F8	F9	E2	E3	E4	E5	E6	E7	E8	E9
A1	X	X										X			X	X			X		X	X		
A1A2	X	X																						
A1F2												X			X	X								
A1E2																			X		X	X		
F1			X		X	X			X	X										X			X	X
F1F2									X	X														
F1E2																				X			X	X
F1A2			X		X	X																		
E1				X			X	X			X		X	X			X	X						
E1E2																	X	X						
E1A2				X			X	X																
E1F2											X		X	X										
A2	X	X	X	X	X	X	X	X																
A2A3			X	X																				
A2F3	X				X			X																
A2E3		X				X	X																	
F2									X	X	X	X	X	X	X	X								
F2F3											X	X												
F2E3									X				X			X								
F2A3										X				X	X									
E2																	X	X	X	X	X	X	X	X
E2E3																			X	X				
E2A3																	X				X			X
E2F3																		X				X	X	
A3			X	X						X				X	X		X				X			X
F3	X				X			X			X	X						X				X	X	
E3		X				X	X		X				X			X			X	X				

Site-directed Mutagenesis (Quickchange)

Site-directed mutagenesis was performed on constructs as necessary (many clones contained mutations from the PCR ligation, and some required two rounds of mutagenesis). PCR conditions were as follows: 100 ng template, 1 µM each primer, 1x Pfu Ultra Buffer, 200 µM each dNTP, and 2.5 units Pfu Ultra (Stratagene).

Thermocycling conditions were as follows:
95°C for 2 minutes
16 cycles of: 95ºC for 1 minute, 52ºC for 1 minute, 68ºC for 12 minutes
Holding at 4ºC

PCR reactions were incubated with 1 μL DpnI (NEB) overnight at 37ºC. 5 μL each reaction was transformed into Subcloning Efficiency DH5a competent cells (Invitrogen) and plated on LB/AMP plates.

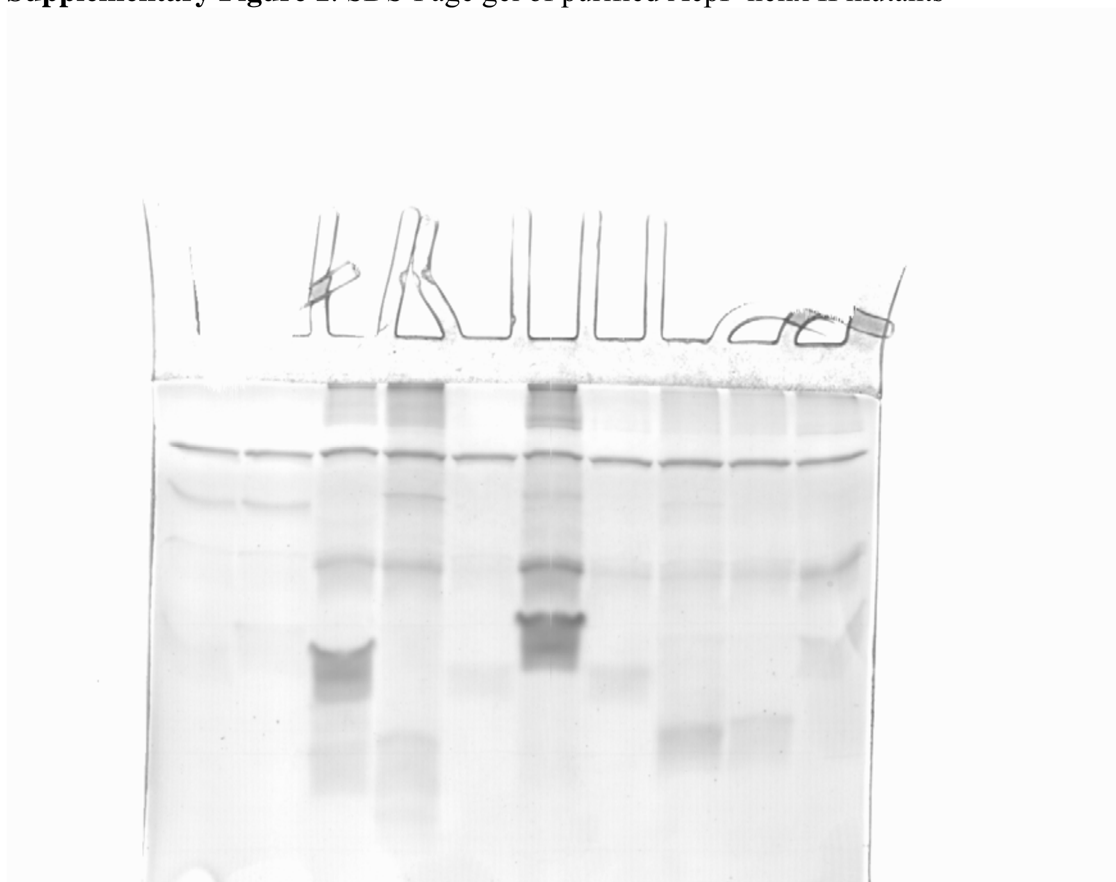
Mutagenesis Primers

EntBqc1: GCGCATGATGGAGCTGGCGGCGCGCTGGCG
EntBqc2: GCCGCCAGCTCCATCATGCGCACCGAATCC

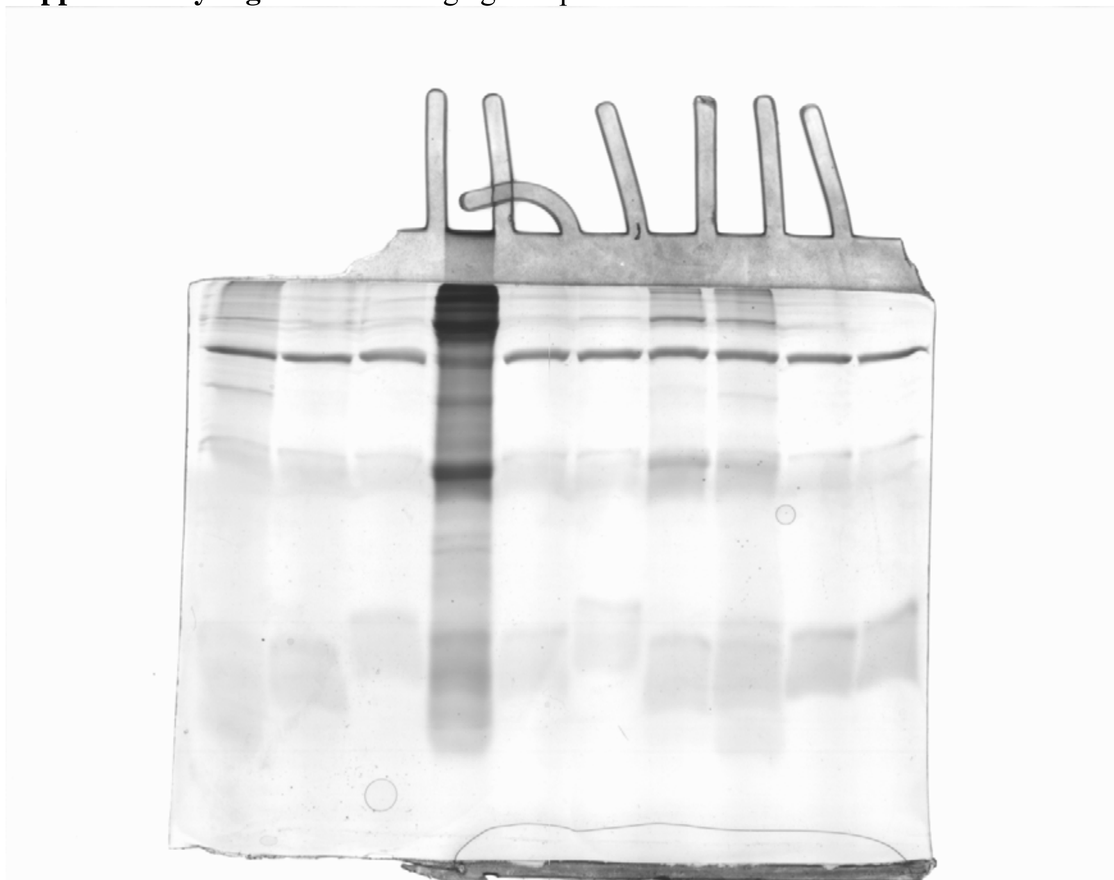
For constructs: A2, A6
A2QC1 : GACACCGTTGAGCTGGTAATGGCTCTGGAAG 3'
A2QC2 : CCATTACCAGCTCAACGGTGTCAAGAGAATC 3'
For construct: A3
QCA3A: CCAACAATGCTTCTTTCGTTGAAGACCTGG
QCA3B: CAACGAAAGAAGCATTGTTGGTAACTTCTTC
For construct: A4
QC16A: GTCGACCTCGCCGGAGAACTCGACACGCCCTTCGTTGAAG
QC16B: GGCGTGTCGAGTTCTCCGGCGAGGTCGACGCTGTCGTCC
For constructs: A4, E2, E9, F3
F1QC1 : CATATGAGCGCACTGACCGTCGACGACCTC 3'
F1QC2 : CGACGGTCAGTGCGCTCATATGTATATCTC 3'
For construct: E2
F1QC3: CGACCTCAAGAAACTGCTCGCCGAGACCGC
F1QC4: GAGCAGTTTCTTGAGGTCGTCGACGGTCAG
For construct: E3
F3QC3 : CGGCCACCGCCCTCGAGCACCACCACCACC
F3QC4: GGTGCTCGAGGGCGGTGGCCGGGGTGGTG
For construct: E4
QCE4A: CAATGCTTCTTTCGATGACGACAACCTGATC
QCE4B: GTCGTCATCGAAAGAAGCATTGTTGGTAAC
For constructs: E4, E7
E2QC1 : CGCATGATGGCGCTGGCGGCGCGCTGGCGC 3'
E2QC2 : CGCCGCCAGCGCCATCATGCGCACCGAATC 3'
For construct: E5
E3QC1: GCGAAAAACCCGACCATCGACGCCTGGTGGAAG
E3QC2: GGCGTCGATGGTCGGGTTTTTCGCCAGCATGAC
For construct: E8
QCE8A: GGTGATATCGCGCTGACTGACGAGACGGTC
QCE8B: CAGTCAGCGCGATATCACCATGCACTTTGC
For construct: F4
F3QC1 : CGCGAACTGCTCGACGAGGTCAACACCACC 3'
F3QC2 : CCTCGTCGAGCAGTTCGCGCGGGGTGCCC
For constructs: F5, F8
F2QC1 : CTCGCGCTGCTGGAGACGGCCGCCGTGCTC 3'
F2QC2 : GGCCGTCTCCAGCAGCGCGAGGGAGTCGTAG 3'

For construct: F6
F2QC3B: CTACGACTCCCTCGCGCTGCTGGAGACGGCCGCCGTGCTCCAG
F2QC4B: GGCGGCCGTCTCCAGCAGCGCGAGGGAGTCGTAGCCGAGGTCC

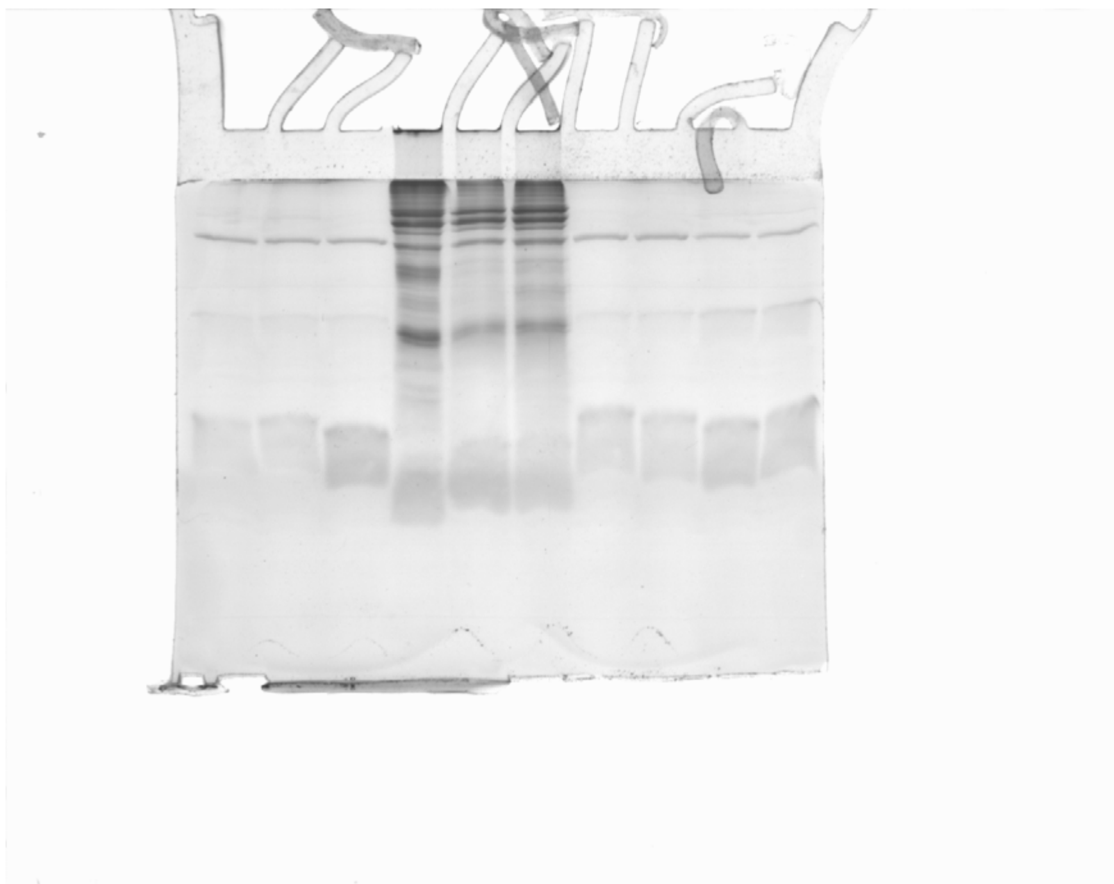
Supplementary Figure 1. SDS-Page gel of purified AcpP-helix II mutants



Supplementary Figure 2. SDS-Page gel of purified FrnN-helix II mutants



Supplementary Figure 3. SDS-Page gel of purified ΔEntB-helix II mutants



References

1. A. M. Gehring, K. A. Bradley, C. T. Walsh, *Biochemistry*, 1997, **36**, 8495.