Supporting Information

Matching protein interfaces for improved medium-chain fatty acid production

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Materials and Methods

Materials. Chemical standards were purchased from: C8:0 (A10991, Alfa Aesar); C10:0 (A15658, Alfa Aesar); C12:0 (A12492, Alfa Aesar); C14:0 (A10257, Alfa Aesar); C16:0 (P0006, TCI); C18:0 (S0080, TCI).

Homology Model and Docking. AbTE homology mode was generated using Phyre2[1] intensive mode. The mode for 181 out of the 183 residues (99%) was modeled at >90% confidence. For docking *Escherichia coli* acyl-carrier protein (ACP, PDB ID: 2FAE, chain A) onto *E. coli* tesA (PDB ID: 1U8U) ClusPro was used[2]. The 9 balanced models were analyzed using PyMol used to deduce the most likely ACP-tesA interactions. Structural alignment of TesA with AbTE was used to identify the ACP-AbTE interactions.

AbTE mutant generation. Site-directed mutagenesis was performed using the QuikChange protocol with some modifications. PCR reaction: 0.8 ng/µL of template, 2.5 ng/µL of each primer, 1X iProof HF polymerase buffer, 0.02 U/µL iProof polymerase (BioRad), 0.5 mM dNTPs to 50 µL final volume. Thermocycler protocol: 95°C 1min, 17 cycles: [95°C 50sec, 60°C 50sec, 72°C 2min 30sec], 72°C 7 min. DpnI (1 µL) was added to PCR reaction and incubated at 37°C for 1 hour and heat inactivated at 65°C for 20 min. 10 µL of reaction was transformed into competent DH10B *E. coli* cells.

SDS-PAGE gel of AbTE:WT and AbTE variants. Overnight cultures of PPY1331, PPY1333, PPY1340 were diluted 1:50 in 1 mL of M9 media (0.5% glucose, amp^{100}) and grown at 37˚C, 250 r.p.m., until reaching OD_{600} = 0.3-0.4. The cells were then induced with 500 µM of IPTG (500 mM stock) and grown at 30˚C, 250 rpm for 24 hours. A 1 mL of sample was removed from the culture medium and centrifuged for 5 min at 7354*g*. The pellet was resuspended in 200 µL PBS (Teknova cat # P0195), sonicated twice for 20 sec each, and centrifuged at 7354*g* for 5 min. The A_{280} of the resulting supernatant was measured using the NanoDrop Lite (Thermo) to measure protein concentration The supernatants were diluted to a concentration of 2 mg/mL of total protein to a final volume of 20 µL. After addition of 4 µL of 6X SDS loading dye to the 20 µL of the supernatant, the samples were then heated at 95°C for 15 min. 20 µL of each sample was loaded to the SDS-PAGE gel, which was run at 200 V for 50 min at 4°C, and stained with Coomassie Blue.

Table SI1. Table of strains

Strain #	Description	Reference
PPY11	W303: MATa, leu2−3, trp1−1, can1−100, ura3−1, ade2−1, his3−11	ATCC 208352
PPY140	PPY11 Δ*far1*, Δ*sst2*, Δ*ste2*	Mukherjee, 2015
PPY643	PPY140, pESC-His3-P_{TEF1}-OR1G1, pRS415-Leu2-P_{FIG1}-GFP	Mukherjee, 2015
PPY252	DH10B	Invitrogen
PPY251	MG1655	ATCC 47076
PPY260	DH5α	Invitrogen
PPY1151	BW25113 Δ*fadE*739::kan	Keio collection
PPY1236	PPY252, pMB1-Amp^r-P_{TRC}-AbTE:WT	This study
PPY1331	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:WT	This study
PPY1332	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:S11A	This study
PPY1333	PPY251, pMB1-Ampr-P_{TRC}-AbTE:G17R	This study
PPY1334	PPY251, pMB1-Ampr-P_{TRC}-AbTE:A165R	This study
PPY1335	PPY251, pMB1-Ampr-P_{TRC}-AbTE:A121R	This study
PPY1336	PPY251, pMB1-Ampr-P_{TRC}-AbTE:T120R	This study
PPY1337	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/A121R	This study
PPY1338	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/T120R	This study

PPY1339	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:T120R/A121R	This study
PPY1340	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/A165R	This study
PPY1341	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:A121R/A165R	This study
PPY1342	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:T120R/A165R	This study
PPY1396	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/A165R/T120R	This study
PPY1397	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/A165R/A121R	This study
PPY1398	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/A165R/N158D	This study
PPY1403	PPY251, pMB1-Amp^r-P_{TRC}-CnTE	This study
PPY1404	PPY251, pMB1-Amp^r-P_{TRC}-CpTE	This study
PPY1405	PPY251, pMB1-Amp^r-P_{TRC}-UcTE	This study
PPY1406	BL21	NEB
PPY1407	PPY260, pMB1-Amp^r-P_{TRC}-AbTE:WT	This study
PPY1408	PPY1152, pMB1-Amp^r-P_{TRC}-AbTE:WT	This study
PPY1409	PPY1461, pMB1-Amp^r-P_{TRC}-AbTE:WT	This study
PPY1503	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17E	This study
PPY1504	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17E/A165E	This study
PPY1505	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17E/A165R	This study
PPY1506	PPY251, pMB1-Amp^r-P_{TRC}-AbTE:G17R/A165E	This study

Table SI2. Table of plasmids

Strain #	**Plasmid name**	**Description**	**Reference**
PPY269	pKM260	pESC-His3-P_{TEF1}-OR1G1- P_{ADH1}	Mukherjee, 2015
PPY586	pKM586	pRS415-Leu2-P_{FIG1}-GFP	Mukherjee, 2015
PPY1023	pSS185	pMB1-Amp^r-P_{TRC}-AgGPPS-$(GSG)_2$-AgPS	Sarria, 2014
PPY1090	pSS174	pMB1-Amp^r-P_{TRC}-CnTE	This study
PPY1148	pSS183	pMB1-Amp^r-P_{TRC}-CpTE	This study
PPY1236	pSS192	pMB1-Amp^r-P_{TRC}-AbTE:WT	This study
PPY1237	pSS193	pMB1-Amp^r-P_{TRC}-UcTE	This study
PPY1310	pSS196	pMB1-Amp^r-P_{TRC}-AbTE:G17R	This study
PPY1311	pSS197	pMB1-Amp^r-P_{TRC}-AbTE:T120R	This study
PPY1312	pSS198	pMB1-Amp^r-P_{TRC}-AbTE:A121R	This study
PPY1320	pSS199	pMB1-Amp^r-P_{TRC}-AbTE:A165R	This study
PPY1321	pSS200	pMB1-Amp^r-P_{TRC}-AbTE:G17R/A165R	This study
PPY1322	pSS201	pMB1-Amp^r-P_{TRC}-AbTE:A121R/A165R	This study
PPY1323	pSS202	pMB1-Amp^r-P_{TRC}-AbTE:T120R/A165R	This study

PPY1326	pSS203	pMB1-Amp^r-P$_{TRC}$-AbTE:G17R/A121R	This study
PPY1327	pSS204	pMB1-Amp^r-P$_{TRC}$-AbTE:G17R/T120R	This study
PPY1328	pSS205	pMB1-Amp^r-P$_{TRC}$-AbTE:T120R/A121R	This study
PPY1329	pSS206	pMB1-Amp^r-P$_{TRC}$-AbTE:S11A	This study
PPY1393	pSS208	pMB1-Amp^r-P$_{TRC}$-AbTE:G17R/A165R/T120R	This study
PPY1394	pSS209	pMB1-Amp^r-P$_{TRC}$-AbTE:G17R/A165R/A121R	This study
PPY1395	pSS210	pMB1-Amp^r-P$_{TRC}$-AbTE:G17R/A165R/N158D	This study
PPY1499	pSS251	pMB1-Amp^r-P$_{TRC}$-AbTE:G17E	This study
PPY1500	pSS252	pMB1-Amp^r-P$_{TRC}$-AbTE:G17E/A165E	This study
PPY1501	pSS253	pMB1-Amp^r-P$_{TRC}$-AbTE:G17E/A165R	This study
PPY1502	pSS254	pMB1-Amp^r-P$_{TRC}$-AbTE:G17R/A165E	This study
BL136	pTB1	pET-28b -AbTE:WT	This study
BL137	pTB2	pET-28b –AbTE:G17R	This study
BL138	pTB3	pET-28b -AbTE:G17R/A165R	This study

Table SI3. Table of primers

Name	**Sequence (codon change)**
SS455	TAACAATTTCACACAGGAAACAGACCATGGATGTTGCCAGATTGGTCTATG
SS456	CCTGCAGGTCGACTCTAGAGGATCCCCGGGTTATTTAGATTCAGTTGGATGCAAACCC
SS547	GAAAATACCACCAAATTATGGCACT**AGA**TATAGTCAGGCATTTG
SS541	CGACAGTCTGAGTGCGGGTTAT**AGA**ATTAACCCCGAACAGGGCTGG
SS542	CCAGCCCTGTTCGGGGTTAAT**TCT**ATAACCCGCACTCAGACTGTCG
SS545	GCAAAATGACCAGATCCATCCAAAT**CGC**AAAGCCCAGTCAATCTTGCTAAATAACG
SS546	CGTTATTTAGCAAGATTGACTGGGCTTT**GCG**ATTTGGATGGATCTGGTCATTTTGC
SS547	GAAAATACCACCAAATTATGGCACT**AGA**TATAGTCAGGCATTTG
SS548	CAAATGCCTGACTATA**TCT**AGTGCCATAATTTGGTGGTATTTTC
SS549	GAAAATACCACCAAATTATGGC**AGG**GCCTATAGTCAGGCATTTG
SS550	CAAATGCCTGACTATAGGC**CCT**GCCATAATTTGGTGGTATTTTC
SS583	GAAAATACCACCAAATTATGGCAGG**AGA**TATAGTCAGGCATTTG
SS584	CAAATGCCTGACTATA**TCT**CCTGCCATAATTTGGTGGTATTTTC
SS601	CAAAACCATTCTTATCTTAGGCGAC**GCT**CTGAGTGCGGGTTATGGCATTAACC
SS602	GGTTAATGCCATAACCCGCACTCAG**AGC**GTCGCCTAAGATAAGAATGGTTTTG
SS608	GGCTGGACACAAAAGTCTAATGCAA**GAT**GACCAGATCCATCCAAATGC
SS609	GCATTTGGATGGATCTGGTC**ATC**TTGCATTAGACTTTTGTGTCCAGCC

SS617	CGACAGTCTGAGTGCGGGTTAT**GAA**ATTAACCCCGAACAGGGCTGG
SS618	CCAGCCCTGTTCGGGGTTAAT**TTC**ATAACCCGCACTCAGACTGTCG
SS619	GCAAAATGACCAGATCCATCCAAAT**GAA**AAAGCCCAGTCAATCTTGCTAAATAACG
SS620	CGTTATTTAGCAAGATTGACTGGGCTTT**TTC**ATTTGGATGGATCTGGTCATTTTGC
TB1	TGTTTAACTTTAAGAAGGAGATATACCatgggcaaaaccattcttatcttag
TB2	CTTTCGGGCTTTGTTAGCAGCCGGATCttaatggtgatggtgatggtgtaaag
TB3	ctttacaccatcaccatcaccattaaGATCCGGCTGCTAACAAAGCCCGAAAG
TB4	ctaagataagaatggttttgcccatGGTATATCTCCTTCTTAAAGTTAAACA

Table SI4. Site-directed mutagenesis primers and templates

Strain #	Plasmid name	Mutation	Template	Mutagenesis primers
PPY1310	pSS196	G17R	PPY1236 (pSS192)	SS541/542
PPY1311	pSS197	T120R	PPY1236 (pSS192)	SS549/550
PPY1312	pSS198	A121R	PPY1236 (pSS192)	SS547/548
PPY1320	pSS199	A165R	PPY1236 (pSS192)	SS545/546
PPY1321	pSS200	G17R/A165R	PPY1310 (pSS196)	SS545/546
PPY1322	pSS201	A121R/A165R	PPY1312 (pSS198)	SS545/546
PPY1323	pSS202	T120R/A165R	PPY1320 (pSS199)	SS549/550
PPY1326	pSS203	G17R/A121R	PPY1312 (pSS198)	SS541/542
PPY1327	pSS204	G17R/T120R	PPY1311 (pSS197)	SS541/542
PPY1328	pSS205	T120R/A121R	PPY1311 (pSS197)	SS583/584
PPY1329	pSS206	S11A	PPY1236 (pSS192)	SS601/602
PPY1393	pSS208	G17R/A165R/T120R	PPY1321 (pSS200)	SS549/550
PPY1394	pSS209	G17R/A165R/A121R	PPY1321 (pSS200)	SS547/548
PPY1395	pSS210	G17R/A165R/N158D	PPY1321 (pSS200)	SS608/609
PPY1499	pSS251	G17E	PPY1310 (pSS196)	SS617/618
PPY1500	pSS252	G17E/A165E	PPY1499 (pSS251)	SS619/620
PPY1501	pSS253	G17E/A165R	PPY1321 (pSS200)	SS617/618
PPY1502	pSS254	G17R/A165E	PPY1310 (pSS196)	SS619/620

	Escherichia coli TesA	*Acinetobacter baylyi* TE
Escherichia coli TesA		38.3%
Acinetobacter baylyi TE	38.3%	
Cocos nucifera TE	19.0%	16.9%
Umbellularia californica TE	21.1%	14.7%
Cuphea palustris TE	16.7	16.9%

Figure SI1. Percent sequence identity of thioesterases uses in this work. Bacterial thioesterases (blue boxes); plant thioesterases (green boxes).

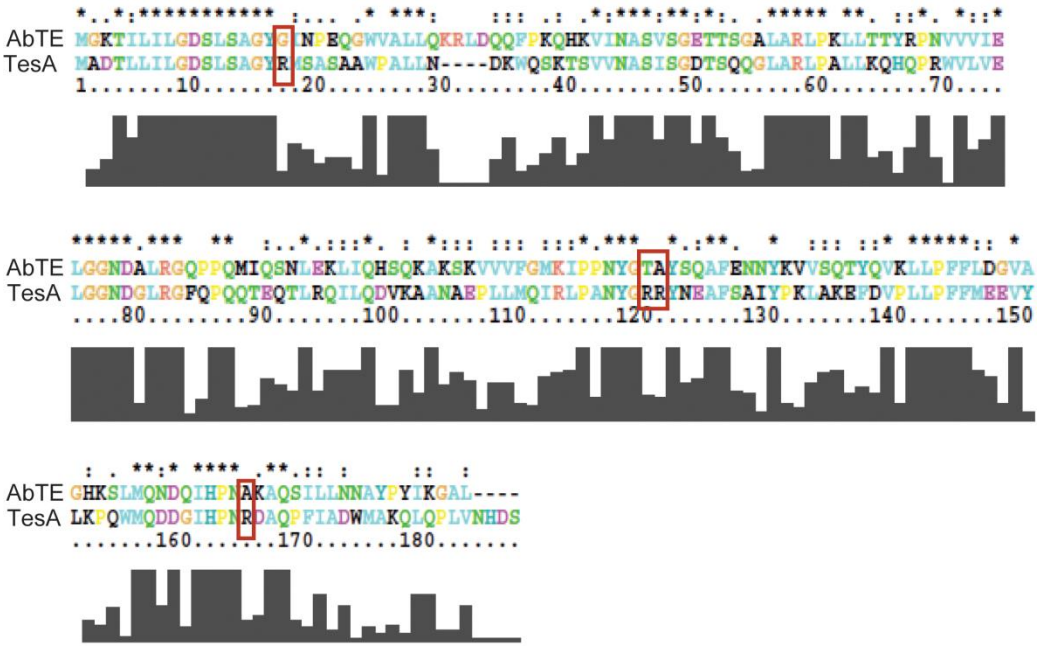

Figure SI2. Amino acid sequence alignment of AbTE wt and *E. coli* TesA with the signal peptide removed. Red boxes indicate amino acids targeted for mutagenesis.

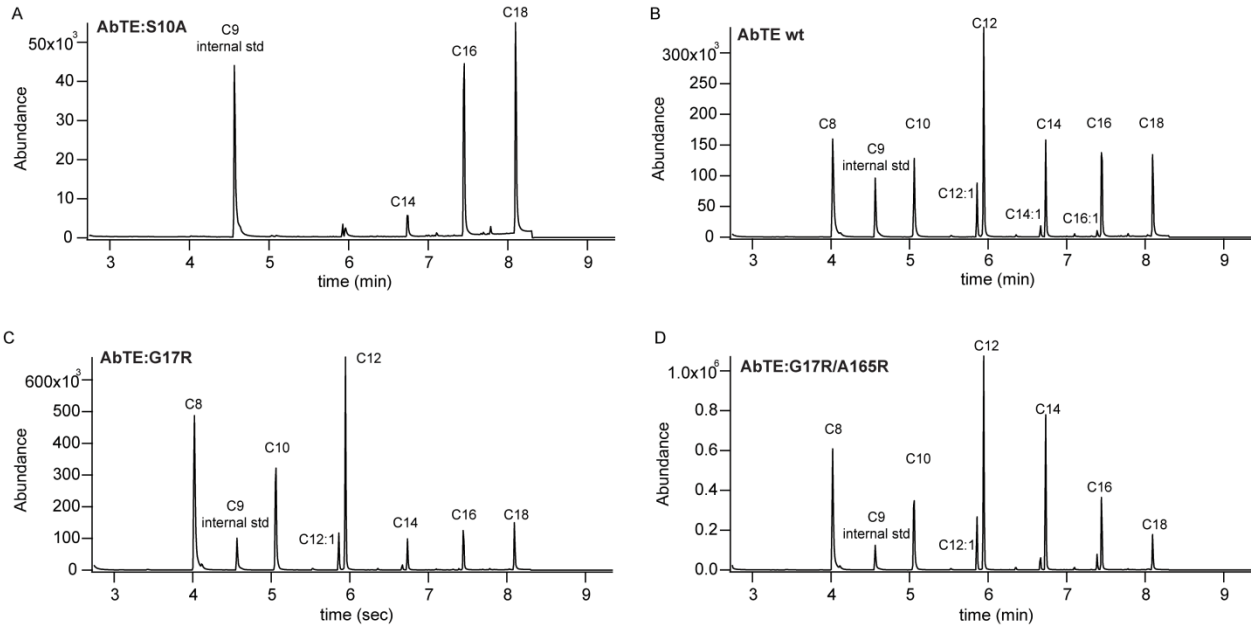

Figure SI3. Gas chromatograms of secreted fatty acids produced by *E. coli* expressing AbTE and AbTE variants. A) AbTE:S11A (inactive enzyme), B) Wild-type AbTE) C) AbTE:G17R D) AbTE:G17R/A165R. Single Ion Monitoring: 74 and 87.

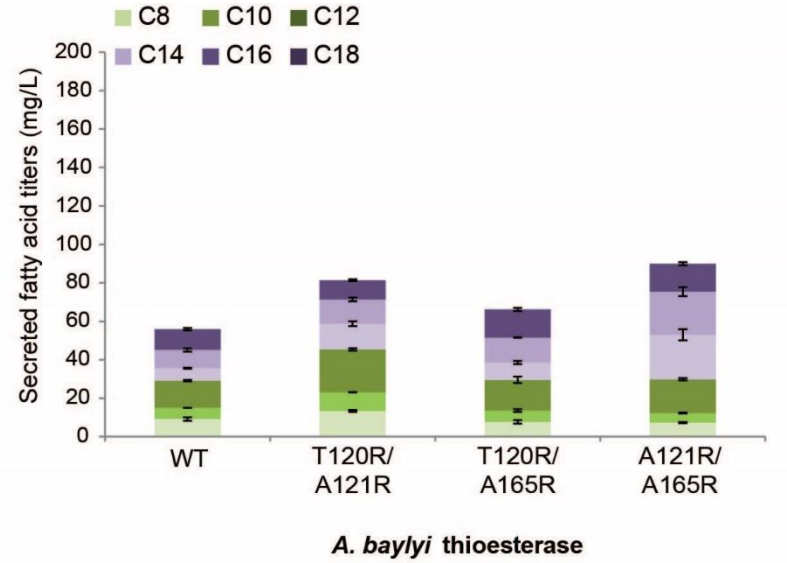

Figure SI4. Secreted fatty acid production of *E. coli* expressing AbTE:WT, AbTE: T120R/A121R, AbTE: T120R/A165R, AbTE: A121R/A165R.

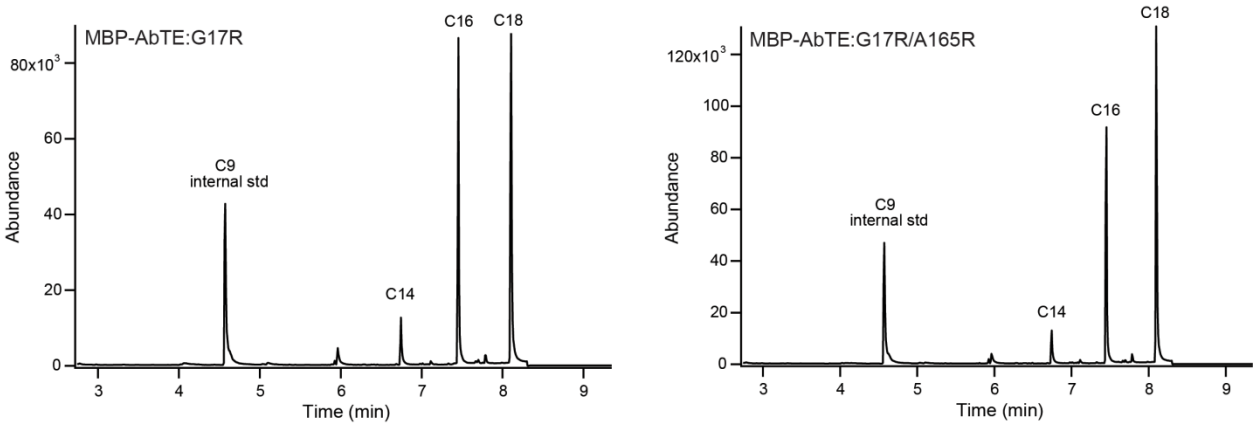

Figure SI5: Effect of fusing maltose binding protein (MBP) to AbTE mutants. Gas chromatograms of MBP-AbTE:G17R (left) and MBP-AbTE:G17R/A165R (right). Single Ion Monitoring: 74 and 87.

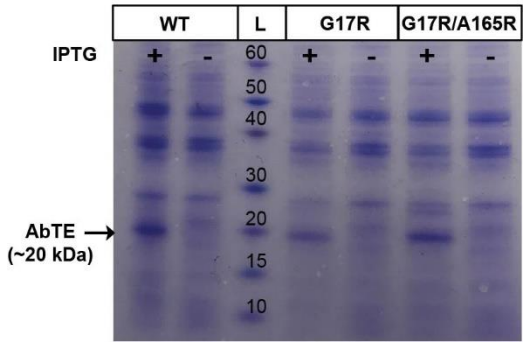

Figure SI6: Protein levels of Acitenobacter baylyi TE expressed in *E. coli.* Coomassie stained SDS-PAGE gel of induced (+IPTG) and uninduced (-IPTG) *E. coli* cultures expressing AbTE:WT, AbTE:G17R and AbTE:G17R/A165R.

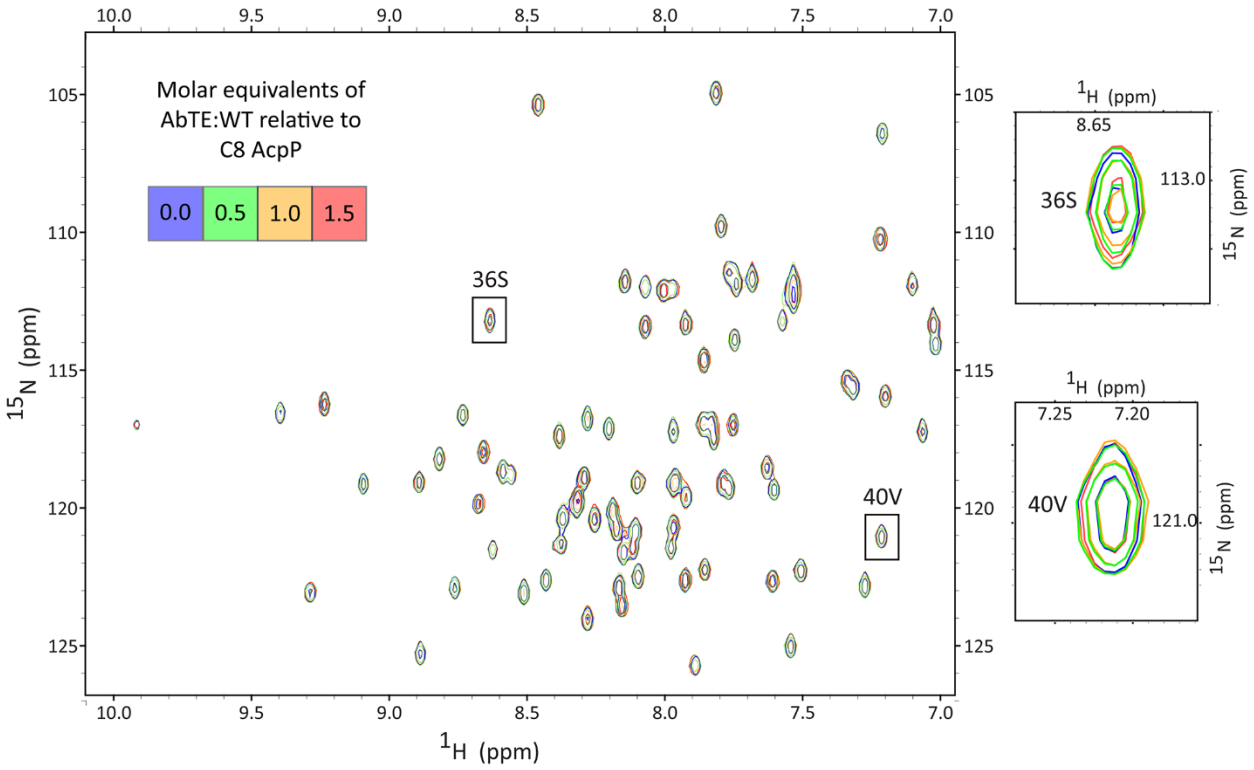

Figure SI7: HSQC titration of wild type AbTE with *E. coli* ^{15}N-octanoyl-AcpP. The titration was performed up to 1.5 molar equivalents of AbTE:WT to confirm lack of interactions.

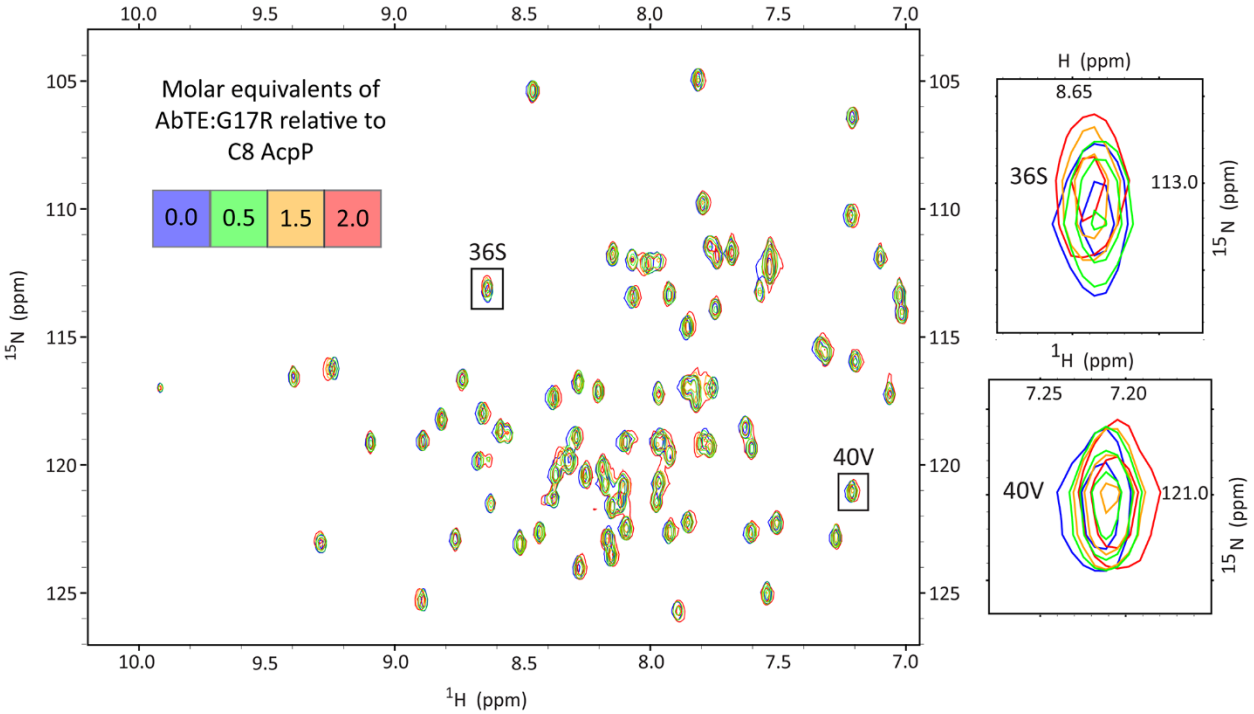

Figure SI8: HSQC titration of AbTE:G17R with *E. coli* ^{15}N-octanoyl-AcpP. The titration was performed up to 2 molar equivalents of AbTE:G17R to confirm small interactions.

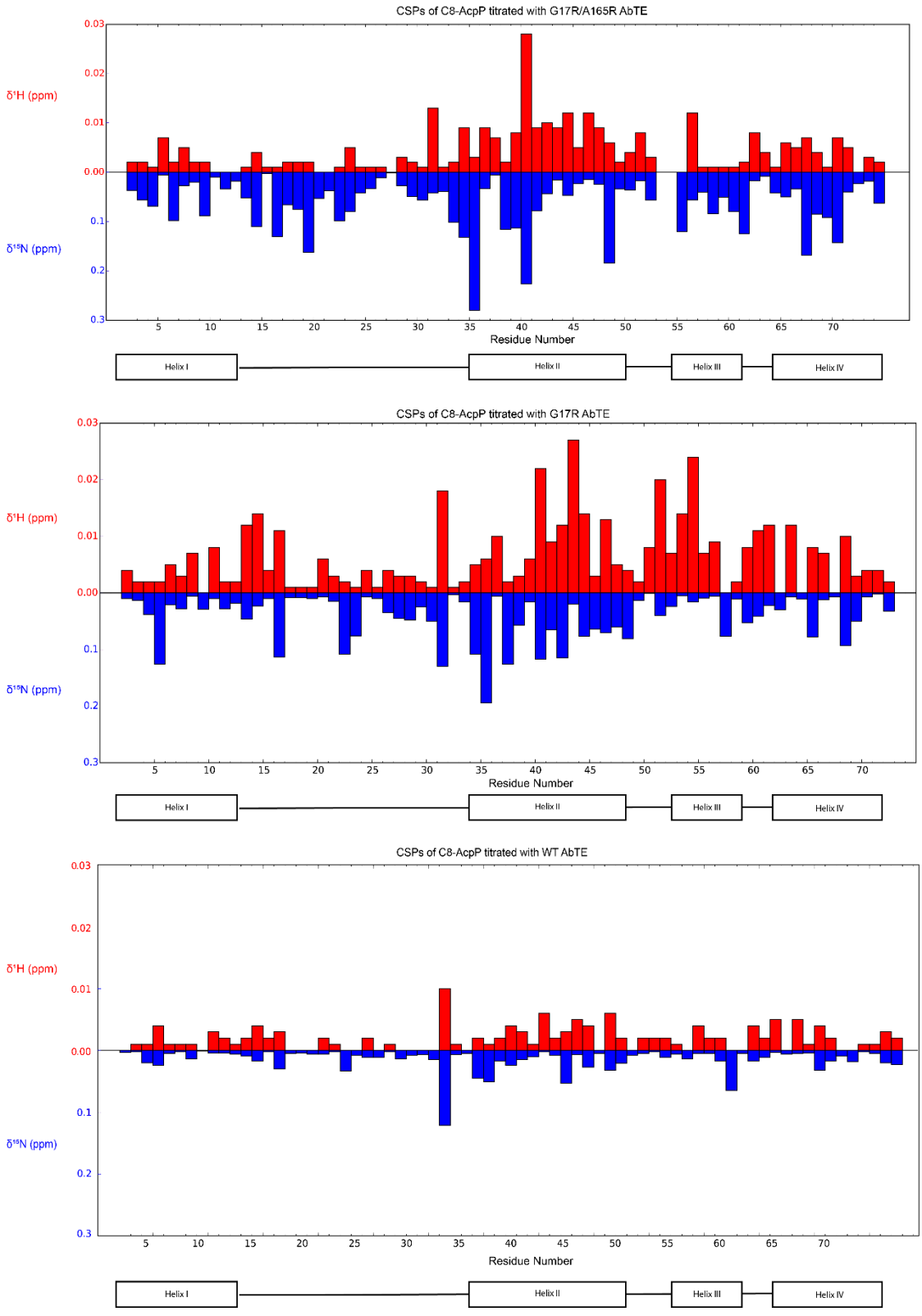

Figure SI9: HSQC titrations of AbTE:WT, AbTE:G17R, and AbTE:G17R/A165R with *E. coli* ^{15}N-octanoyl-AcpP. Here perturbations are separated by resonances in the ^{1}H and ^{15}N dimensions

to highlight the respective CSPs. This representation illustrates the magnitude of each chemical shift before averaging by the CSP equation: $CSP = \sqrt{\left(\frac{1}{2}\right)[\delta_H^2 + (\alpha x \delta_N)^2}$ where α=0.2.

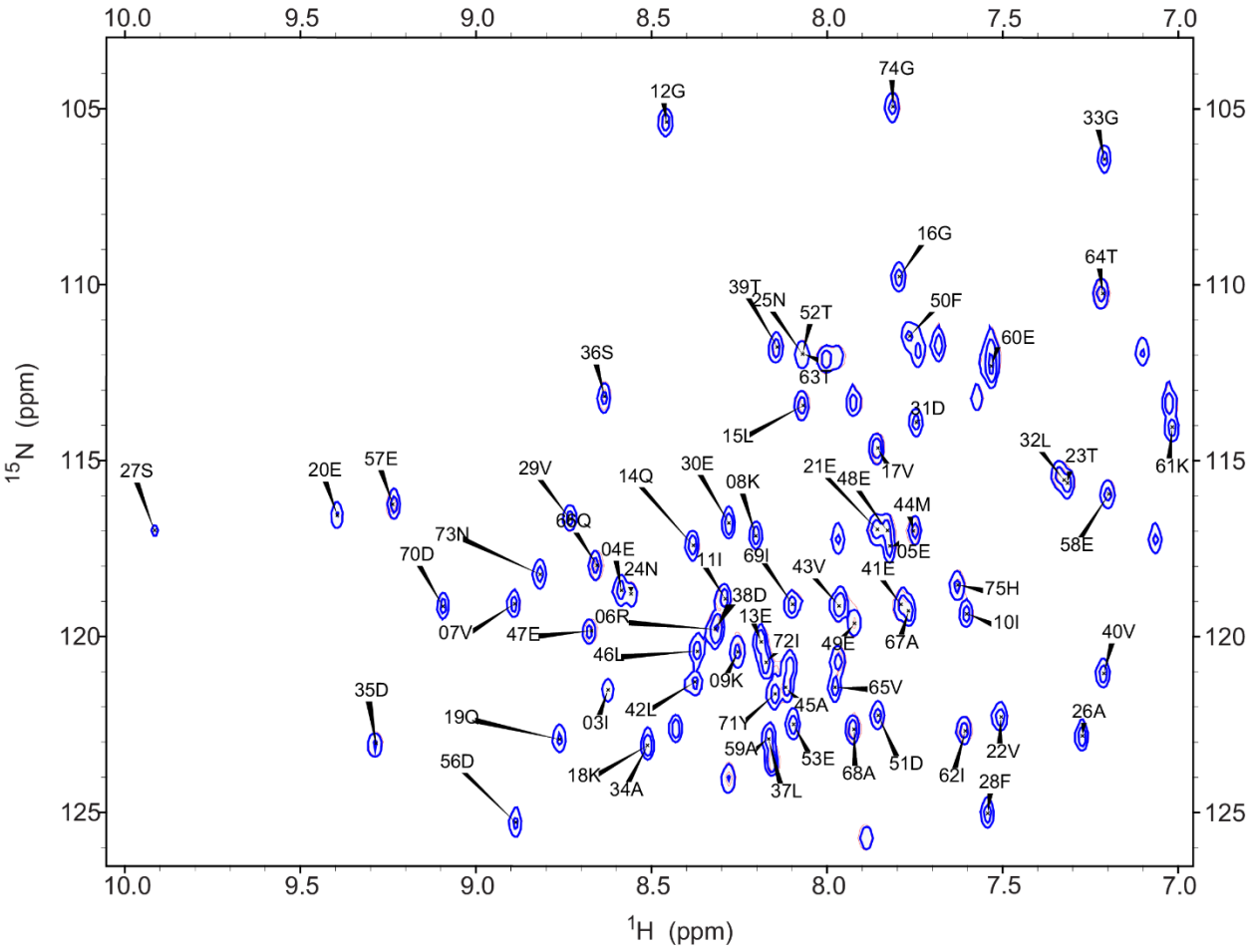

Figure SI10: The HSQC spectrum of ^{15}N-octanoyl-AcpP alone. Peak assignments from prior work with C8-AcpP [3].

Sequences

Acetobacter baylyi TE (non-codon optimized, N-terminal signal peptide removed)

ATGGGCAAAACCATTCTTATCTTAGGCGACAGTCTGAGTGCGGGTTATGGCATTAACCCCGAACAGG
GCTGGGTCGCTTTATTACAAAAACGTCTGGATCAACAATTTCCCAAGCAGCATAAAGTCATTAATGCC
AGTGTAAGTGGGGAAACCACCAGTGGTGCTTTAGCTCGTTTACCCAAACTACTTACTACTTATCGACC
TAATGTGGTGGTCATTGAGCTTGGTGGTAATGATGCATTAAGAGGACAACCGCCTCAAATGATTCAA
AGTAATCTGGAAAAATTAATCCAGCACAGCCAAAAGGCAAAATCTAAAGTCGTGGTGTTTGGAATGAA
AATACCACCAAATTATGGCACTGCCTATAGTCAGGCATTTGAAAATAATTATAAGGTAGTGAGTCAAA
CATATCAGGTTAAGTTGTTGCCATTTTTTCTTGATGGTGTGGCTGGACACAAAAGTCTAATGCAAAAT
GACCAGATCCATCCAAATGCCAAAGCCCAGTCAATCTTGCTAAATAACGCATACCCATATATTAAAGG
CGCTTTATAA

***Cocos nucifera* thioesterase FatB3 (*S. cerevisiae* codon-optimized, N-term signal peptide removed):**

ATGTTGCCAGATTGGTCTATGTTGTTGGCTGCTATTAGAACCATTTTCTCCGCTGCTGAGAAGCAATG
GACTTTGCTCGATTCTAAGAAGCGAGGTGCTGATGCTGTTGCTGATGCTTCTGGTGTTGGTAAGATG
GTTAAGAATGGCTTGGTCTACAGACAGAACTTCTCCATTAGATCCTACGAAATTGGTGTTGATAAGAG
AGCTTCCGTTGAGGCTTTGATGAATCATTTCCAAGAAACTTCTTTGAATCATTGTAAGTGTATTGGTTT
GATGCATGGTGGTTTCGGTTGTACTCCAGAAATGACTAGAAGAAATTTGATTTGGGTTGTTGCTAAGA
TGTTGGTTCATGTTGAAAGATACCCCTGGTGGGGTGATGTTGTTCAAATTAATACTTGGATTTCTTCTT
CTGGTAAGAATGGTATGGGTAGAGATTGGCATGTTCATGATTGTCAAACTGGTTTGCCAATTATGAGA
GGTACTTCTGTTTGGGTTATGATGGATAAGCATACTAGAAGATTGTCTAAGTTGCCAGAAGAAGTTAG
AGCTGAAATTACTCCATTCTTCTCTGAAAGAGATGCTGTTTTGGATGATAATGGTAGAAAGTTGCCAA
AGTTCGATGACGATTCTGCTGCTCATGTTAGAAGAGGTTTGACTCCAAGATGGCATGATTTCGATGTT
AATCAACATGTTAATAATGTTAAGTACGTTGGTTGGATTTTGGAATCTGTTCCAGTTTGGATGTTGGAT
GGTTACGAGGTTGCTACTATGTCTTTGGAGTACAGAAGAGAGTGTAGAATGGATTCTGTTGTTCAATC
TTTGACTGCTGTTTCTTCTGATCATGCTGATGGTTCTCCAATTGTTTGTCAACATTTGTTGAGATTGGA
AGATGGTACTGAAATTGTTAGAGGTCAAACTGAATGGAGACCAAAGCAACAAGCTAGAGATTTGGGT
AATATGGGTTTGCATCCAACTGAATCTAAATAA

***Cuphea palustris* thioesterase FatB1 (*S. cerevisiae* codon-optimized, N-term signal peptide removed):**

ATGAGGCCAAACATGTTGATGGATTCCTTCGGCTTGGAAAGAGTCGTCCAAGATGGTTTGGTCTTCA
GACAATCCTTCTCCATTAGATCCTATGAAATTTGTGCTGATAGAACTGCTTCCATTGAAACTGTCATGA
ACCATGTCCAAGAAACTTCCTTGAACCAATGTAAGTCCATTGGTTTGTTGGATGATGGTTTCGGTAGA
TCCCCAGAAATGTGTAAGAGAGATTTGATTTGGGTCGTCACTAGAATGAAGATTATGGTCAACAGATA
CCCAACTTGGGGTGATACTATTGAAGTCTCCACTTGGTTGTCTCAATCTGGTAAGATTGGTATGGGTA
GAGATTGGTTGATTTCTGATTGTAACACTGGTGAAATTTTGGTCAGAGCTACTTCCGTCTACGCTATG
ATGAACCAGAAGACGAGAAGATTCTCCAAGTTGCCACATGAAGTCAGACAAGAATTTGCTCCACATTT
CTTGGATTCCCCACCAGCTATTGAAGATAACGATGGTAAGTTGCAAAAGTTCGATGTCAAGACTGGT
GATTCCATTAGAAAGGGTTTGACTCCAGGTTGGTACGATTTGGATGTCAACCAACATGTCTCTAACGT
CAAGTACATTGGTTGGATTTTGGAATCTATGCCAACTGAAGTCTTGGAAACTCAAGAATTGTGTTCTT
TGACTTTGGAATACAGAAGAGAATGTGGTAGAGATTCTGTCTTGGAATCCGTCACTTCTATGGACCCA
TCTAAGGTCGGTGATAGATTCCAATACAGACATTTGTTGAGATTGGAAGATGGTGCTGATATTATGAA
GGGTAGAACTGAATGGAGACCAAAGAACGCTGGTACTAACGGTGCTATTTCTACTGGTAAGACTTAA

***Umbellularia californica* thioesterase FatB2 (*E. coli* codon-optimized, N-term signalpeptide removed):**

ATGACTCTAGAGTGGAAACCGAAACCAAAACTGCCTCAACTGCTGGATGATCACTTCGGTCTGCACG
GTCTGGTGTTTCGTCGTACTTTCGCAATTCGTTCTTATGAAGTGGGTCCAGATCGTTCTACCTCCATC
CTGGCCGTCATGAACCACATGCAGGAAGCCACCCTGAATCACGCGAAATCTGTTGGTATCCTGGGT
GATGGTTTCGGCACTACTCTGGAAATGTCTAAACGTGACCTGATGTGGGTAGTGCGTCGCACCCAC
GTAGCAGTAGAGCGCTACCCTACTTGGGGTGACACTGTGGAAGTCGAGTGTTGGATTGGCGCGTCC
GGTAACAATGGTATGCGTCGCGATTTTCTGGTCCGTGACTGTAAAACGGGCGAAATCCTGACGCGTT
GCACCTCCCTGAGCGTTCTGATGAACACCCGCACTCGTCGCCTGTCTACCATCCCGGACGAAGTGC
GCGGTGAGATCGGTCCTGCTTTCATCGATAACGTGGCAGTTAAAGACGACGAAATCAAGAAACTGCA
AAAACTGAACGACTCCACCGCGGACTACATCCAGGGCGGTCTGACTCCGCGCTGGAACGACCTGGA

TGTTAATCAGCATGTGAACAACCTGAAATACGTTGCTTGGGTCTTCGAGACTGTGCCGGACAGCATT
TTCGAAAGCCATCACATTTCCTCTTTTACTCTGGAGTACCGTCGCGAATGTACTCGCGACTCCGTTCT
GCGCAGCCTGACCACCGTAAGCGGCGGTTCTAGCGAGGCAGGTCTGGTCTGCGACCATCTGCTGC
AACTGGAAGGCGGCTCCGAAGTCCTGCGTGCGCGTACGGAGTGGCGTCCAAAGCTGACGGATTCTT
TCCGCGGCATCTCCGTAATTCCGGCGGAACCTCGTGTTTAA

References

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