The methyltransferase TrmA facilitates tRNA folding through interaction with its RNA-binding domain

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Supplemental Materials and Methods

Table S1. Primers used for TrmA site-directed mutagenesis and PCR co-culture competition assay.

Primer Name	Sequence
TrmAQ190A sense	5'-GTAGAAAACAGCTTTACGGCGCCGAACGCGGCGATG-3'
TrmAQ190A antisense	5'-CATCGCCGCGTTCGGCGCCGTAAAGCTGTTTTCTAC-3'
TrmAG220D sense	5'-GGCGATTTACTCGAGCTGTACTGCGACAACGGTAAC-3'
TrmAG220D antisense	5'-GTTACCGTTGTCGCAGTACAGCTCGAGTAAATCGCC-3'
TrmAC324A sense	5'-GTATCCGCGTATTTTGTACATCAGCGCTAACCCGGAAACGTTATGCAAG-3′
TrmAC324A antisense	5'-CTTGCATAACGTTTCCGGGTTAGCGCTGATGTACAAAATACGCGGATAC-3'
TrmAE358Q sense	5'-CCTACACGCACCACATGCAGTGCGGCGTATTAC-3′
TrmAE358Q antisense	5'-GTAATACGCCGCACTGCATGTGGTGCGTGTAGG-3'
TrmAR51A sense	5′-GGAGTTCGCCATATGGCACGATGGCGATG-3′
TrmAR51A antisense	5′-GTGCCATATGGCGAACTCCGCGCGCATCC-3′
TrmA F106A sense	5′-CACAAGCTTGCCCAGATTGATTACCTCAC-3′
TrmA F106A antisense	5′-AATCTGGGCAAGCTTGTGGCGCAGAACGG-3′
TrmA H125A sense	5′-CTATACGCTAAGAAGCTTGATGATGAGTGG-3′
TrmA H125A antisense	5′-TCAAGCTTCTTAGCGTATAGCAGGGAAACC-3′
TrmA pTrc99a sense	5′-ACTAATCCCATGGATGACCCCCGAACACCTT-3′
TrmA pTrc99a antisense	5′-AATGTTATAAGCTTTTACTTCGCGGTCAGTAATACG-3′

Table S2: PCR conditions for engineering TrmA variants.

Step	Temperature (°C)	Time, Cycles
Initial denaturation	98	30 seconds, 1 cycle
Denaturation	98	10 seconds, 20 cycle*
Annealing and Extension	72	9 minutes, 20 cycles*
Final extension	72	9 minutes, 1 cycle*
* For variant TrmA R51A denaturation, annealing and extension were completed for 30 cycles. Additionally, the annealing and extension step was 3 minutes and 30 seconds.		

Table S3: PCR conditions for amplification of the trmA gene in the co-culture competition assay.

Step	Temperature (°C)	Time, Cycles
Initial denaturation	95	5 minutes, 1 cycle
Denaturation	95	20 seconds, 16 cycles
Annealing	55	20 seconds, 16 cycles
Extension	72	2 minutes, 16 cycles
Final extension	72	10 minutes, 1 cycle

Table S4: PCR protocol for amplification of $tRNA^{Phe}$ gene.

Step	Temperature (°C)	Time, Cycles
Initial denaturation	95	5 minutes, 1 cycle
Denaturation and annealing	1. 95 2. 45 (increase 1°C each repeat) 3. 72	1. 30 seconds, 6 cycles 2. 30 seconds, 6 cycles 3. 20 seconds, 6 cycles
Extension	1. 95 2. 50 3. 72	1. 30 seconds, 29 cycles 2. 30 seconds, 29 cycles 3. 20 seconds, 29 cycles
Final extension	72	11 minutes, 1 cycle

Table S5: Dissociation constants for tRNA and/or SAM binding by TrmA and variants.

TrmA variant	K_D, µM			
	[^{3}H]-tRNA	[^{3}H]-tRNA + SAM	[^{3}H]-SAM	[^{3}H]-SAM + tRNA
Wild-type	0.02 ± 0.01	0.66 ± 0.11*	17 ± 2	27 ± 6
C324A	1.08 ± 0.16	0.03 ± 0.02	24 ± 11	0.15 ± 0.06
E358Q	0.07 ± 0.04	0.011 ± 0.007	14 ± 3	0.38 ± 0.21
Q190A	0.28 ± 0.10	1.5 ± 0.2*	75 ± 52	0.39 ± 0.15
G220D	0.22 ± 0.05	0.06 ± 0.03	no binding	no binding
R51A	0.14 ± 0.07	1.15 ± 0.34*	9 ± 3	6 ± 3
R51AC324A	2.8 ± 0.7	0.16 ± 0.06	17 ± 5	0.78 ± 0.70
F106A	1.0 ± 0.4	2 ± 1*	6 ± 2	2 ± 1
F106AC324A	18 ± 7	6 ± 2	56 ± 34	10 ± 7
H125A	4 ± 3	6 ± 2*	20 ± 5	2 ± 1
H125AC324A	47 ± 27	3 ± 1	13 ± 9	5 ± 2

*TrmA variants that are active in tRNA methylation will form methylated tRNA when incubated with both tRNA and an excess of SAM. In these cases, the binding of methylated tRNA, i.e. the product of TrmA, is observed rather than binding of unmodified substrate tRNA.

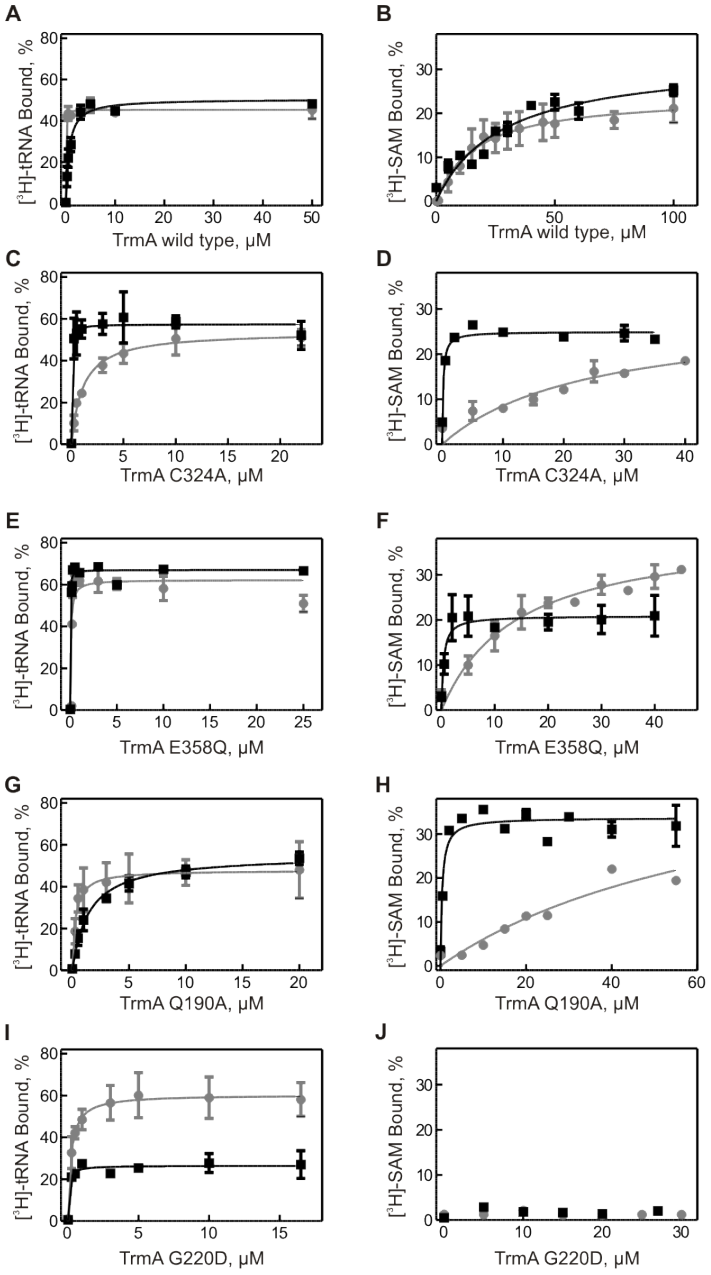


Figure S1. Ligand binding analysis of TrmA wild-type and active site variants.
Nitrocellulose filter binding assays were completed with 50 nM ^{3}H- tRNA (left panels: A, C, E, G, I) or 50 nM ^{3}H-SAM (right panels: B, D, F, H, J). Increasing concentrations of enzyme were titrated into the radioactive ligand either in the presence (black squares) or absence (grey circles) of the other ligand. Different TrmA variants were tested: TrmA wild-type (A & B), TrmA C324A (C & D), TrmA E358Q (E & F), TrmA Q190A (G & H), and TrmA G220D (I & J). Following a short incubation, reaction mixtures were filtered and washed with cold buffer. The retained radioactivity was quantified via scintillation counting. The percentage of bound substrate was plotted against enzyme concentration and fitted with a hyperbolic curve (smooth lines). The dissociation constant for each enzyme is reported in Table 4.4.

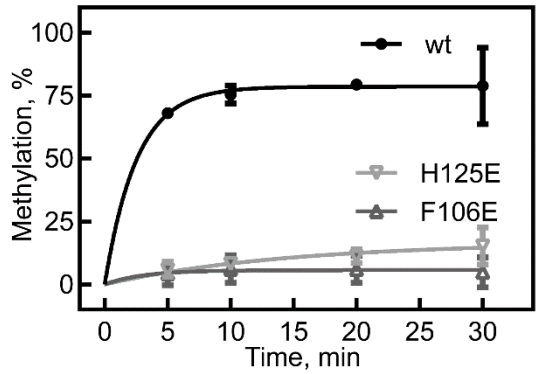


Figure S2: Methylation activity of TrmA wt and variants under high protein concentrations. Single turn-over methylation with increased concentrations of TrmA wt and TrmA tRNA binding variants (20 μM for all TrmA wt and F106E, 8.8 μM for TrmA H125E), 50 μM SAM and 600 nM tRNA: TrmA wt (black circles), H125E (open inverted light grey triangles), and F106E (open grey triangles).

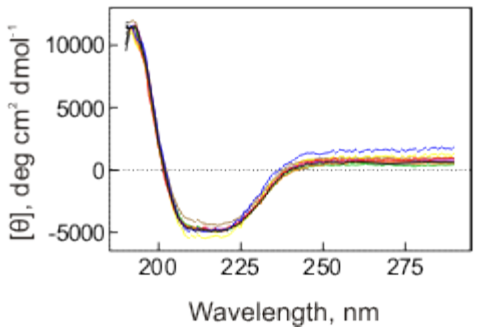
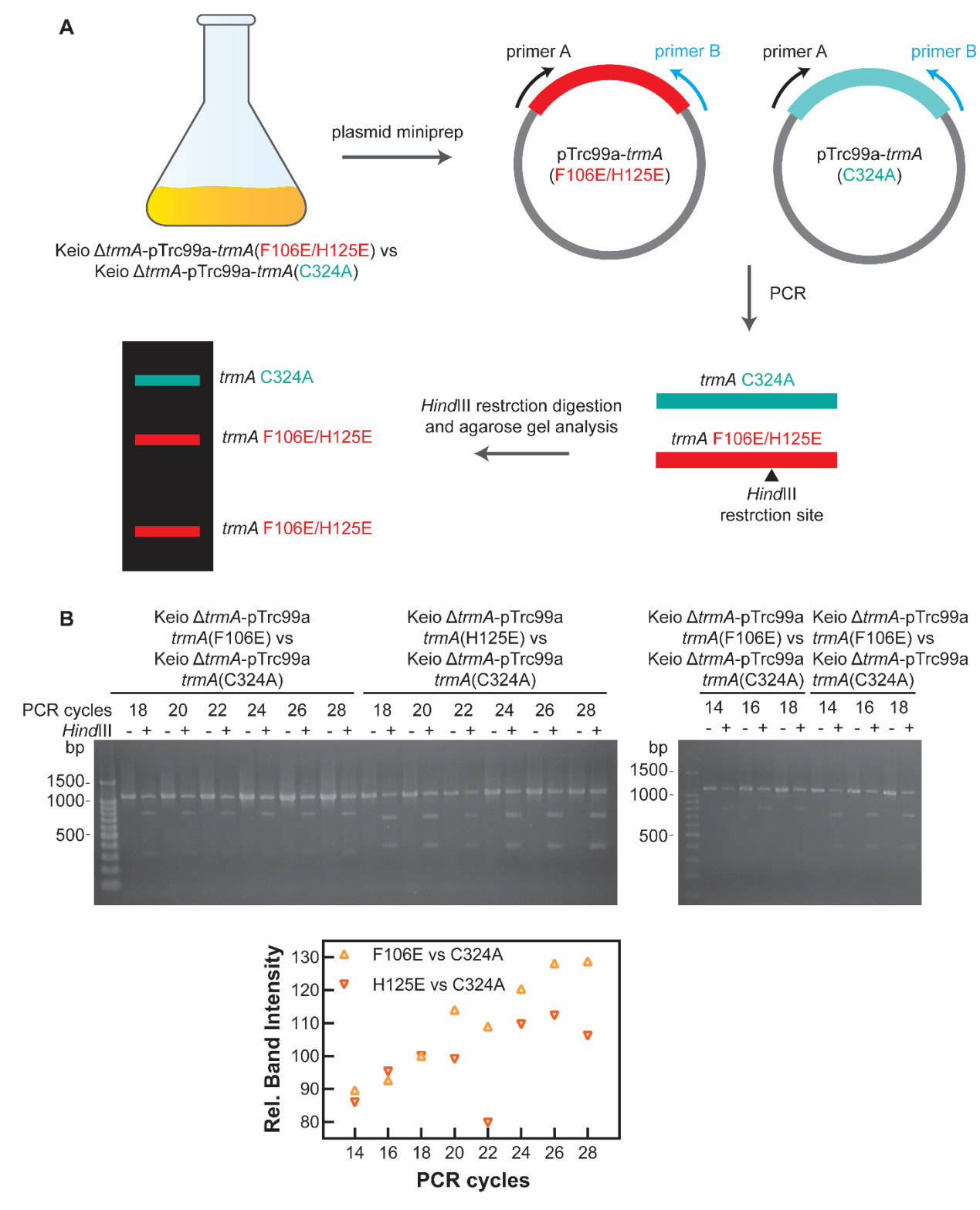


Figure S3. Circular dichroism spectroscopy of TrmA variants with substitutions near the active site. No substantial changes in structure for the TrmA variants compared to wild-type were revealed as ellipticity remained relatively unaffected between wild type and all variants. TrmA wild-type (solid black line), C324A (blue), E358Q (orange), Q190A (green), G220D (red), R51A (purple), F106A (yellow), and H125A (brown) were diluted in 50 mM sodium phosphate buffer, pH 7.5, to 1 μM prior to being scanned.


A
plasmid miniprep
Keio ΔtrmA-pTrc99a-trmA(F106E/H125E) vs
Keio ΔtrmA-pTrc99a-trmA(C324A)
primer A
primer B
pTrc99a-trmA
(F106E/H125E)
pTrc99a-trmA
(C324A)
PCR
trmA C324A
trmA F106E/H125E
HindIII restrction digestion
and agarose gel analysis
HindIII
restrction site
trmA C324A
trmA F106E/H125E
trmA F106E/H125E
B
Keio ΔtrmA-pTrc99a
trmA(F106E) vs
Keio ΔtrmA-pTrc99a
trmA(C324A)
Keio ΔtrmA-pTrc99a
trmA(H125E) vs
Keio ΔtrmA-pTrc99a
trmA(C324A)
PCR cycles 18 20 22 24 26 28 18 20 22 24 26 28
HindIII - + - + - + - + - + - + - + - + - + - + - +
bp
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1000-
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Keio ΔtrmA-pTrc99a
trmA(F106E) vs
Keio ΔtrmA-pTrc99a
trmA(C324A)
Keio ΔtrmA-pTrc99a
trmA(F106E) vs
Keio ΔtrmA-pTrc99a
trmA(C324A)
14 16 18 14 16 18
- + - + - + - + - + - +
bp
1500-
1000-
500-
Rel. Band Intensity
130
120
110
100
90
80
F106E vs C324A
H125E vs C324A
14 16 18 20 22 24 26 28
PCR cycles

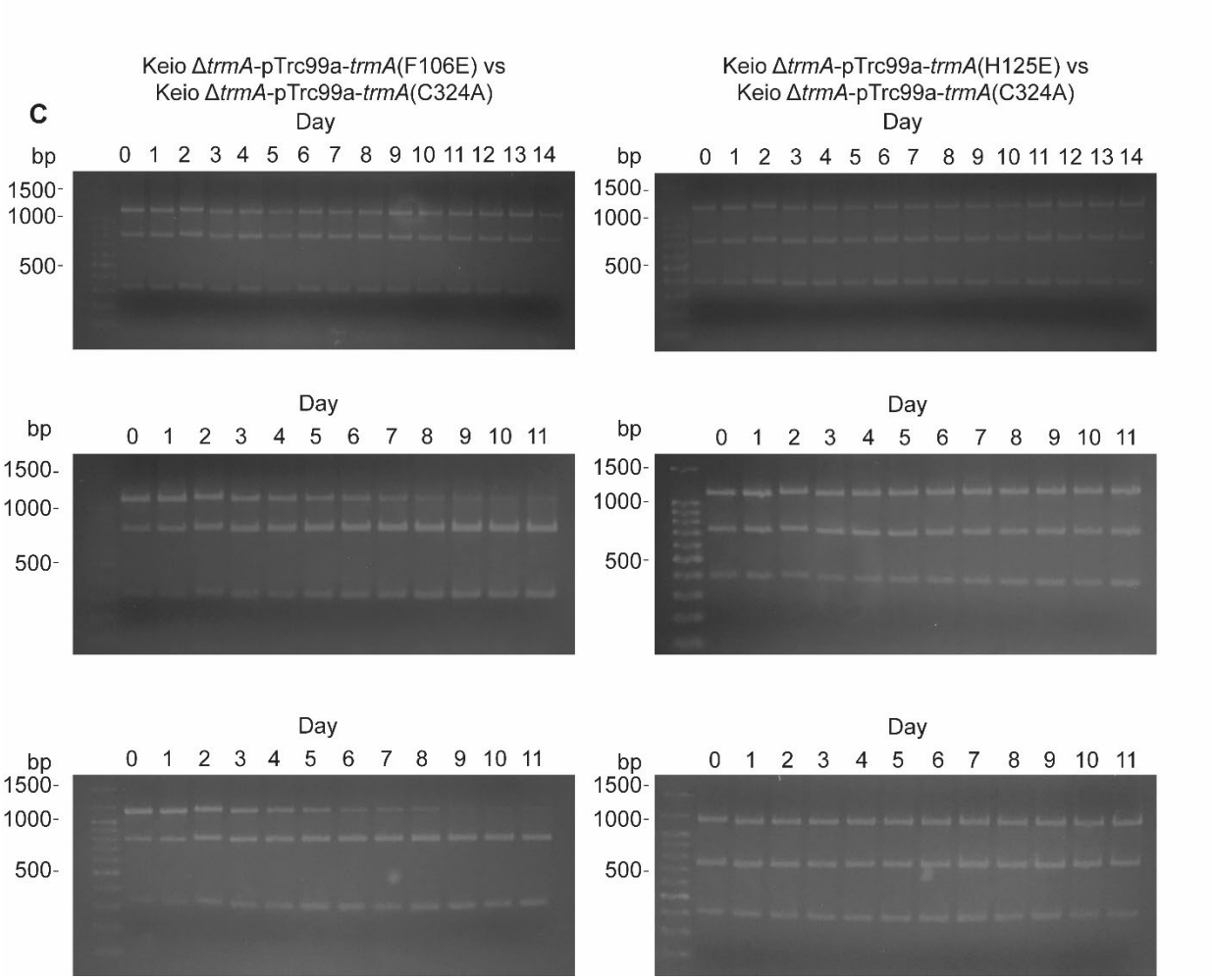

Figure S4. PCR-based co-culture competition assay to assess the relative contribution of tRNA binding and catalytic activity by TrmA to bacterial fitness. A Schematic depiction of PCR-based analysis of co-culture competition assays. The plasmids bearing either the TrmA binding variants (F106E or H125E) or the catalytically inactive variant (C324A) were isolated from the co-culture of *E. coli* strains expressing TrmA C324A and TrmA F106E or H125E. The same primer set is used to PCR amplify *trmA* from the plasmids. Subsequently, the PCR products are subjected to *Hind*III digestion and agarose gel analysis to distinguish between *trmA* C324A (no *Hind*III site) and *trmA* F106E/H125E (one *Hind*III site). Ratios of band intensities between the catalytically inactive and binding variant are determined by ImageJ analysis. **B** Assessing the number of PCR cycles on product yield. The band intensities of the PCR products were normalized relative to the band intensity observed for 18 cycles in each gel and plotted against the PCR cycle number showing that the PCR is not saturated at 16 cycles used in the further analysis. **C** PCR and restriction-based analysis of the bacterial co-culture competition between *E. coli* Δ*trmA* strains expressing TrmA C324A and TrmA F106E (left panels) or H125E (right panels). The samples were processed as described in **A** and the band intensity ratios of the *trmA* C324A:*trmA* F106E/H125E bands are depicted in Figure 4B.