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Supplementary Materials for

Structural insight into the methyltransfer mechanism of the bifunctional Trm5

Caiyan Wang, Qian Jia, Jianhua Zeng, Ran Chen, Wei Xie

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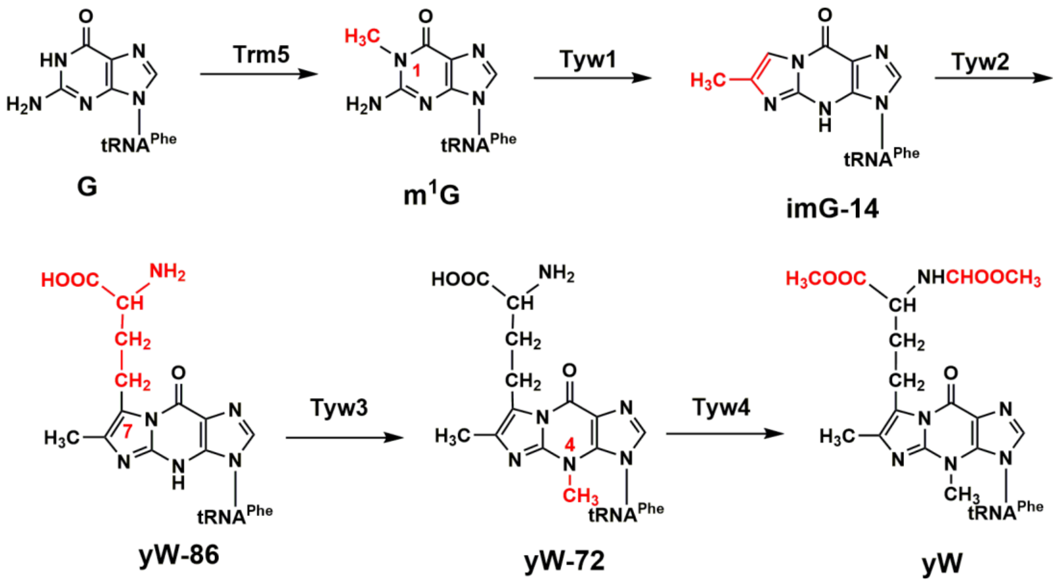

fig. S1. Biosynthesis of wyosine derivatives in eukaryotic tRNA^{Phe}s.

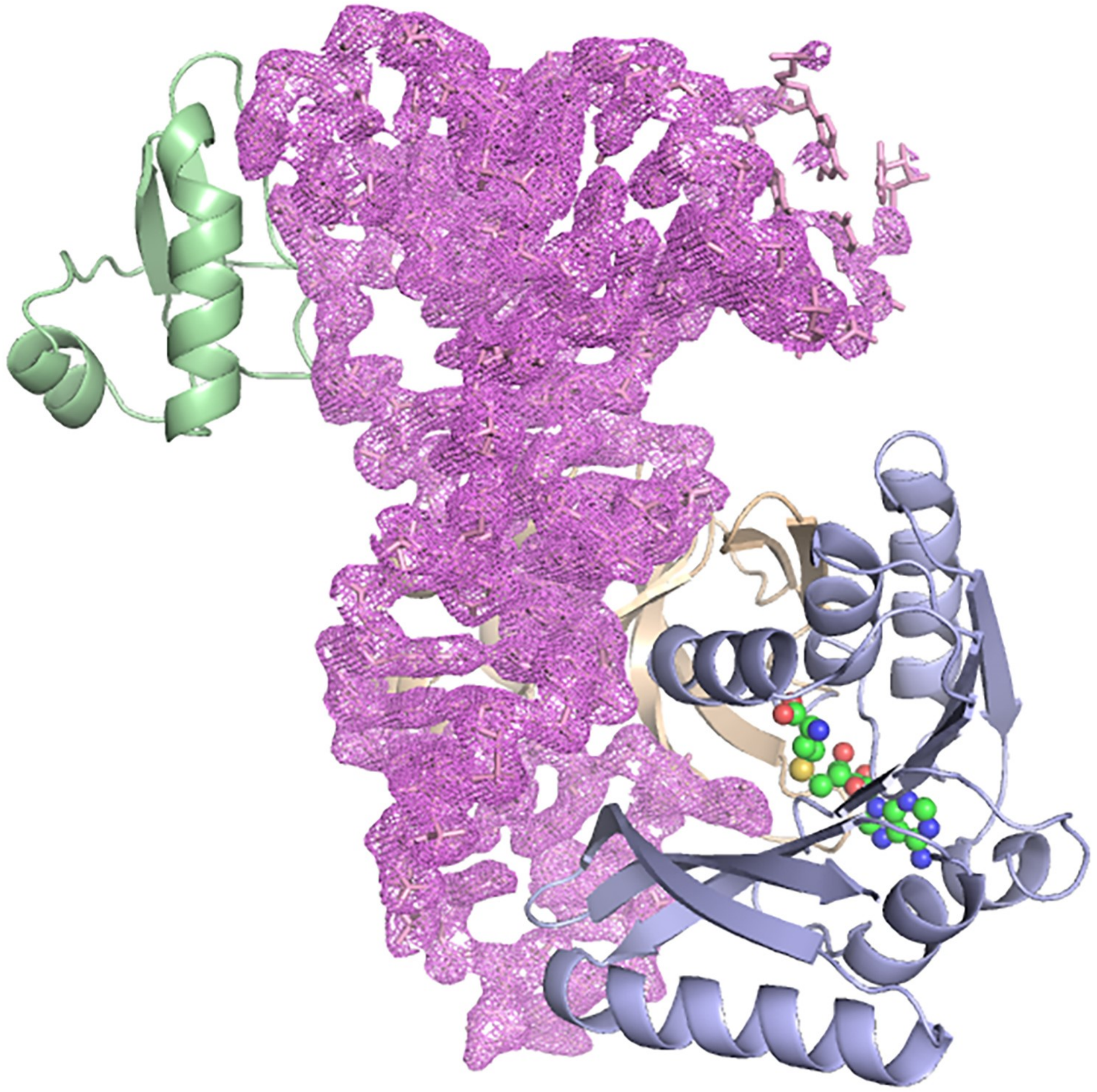

fig. S2. The overall structure of the PaTrm5a-tRNA^{Phe}-SAH ternary complex. The coloring scheme is as in Fig. 1A and the 2Fo-Fc map for tRNA is contoured at 1σ.

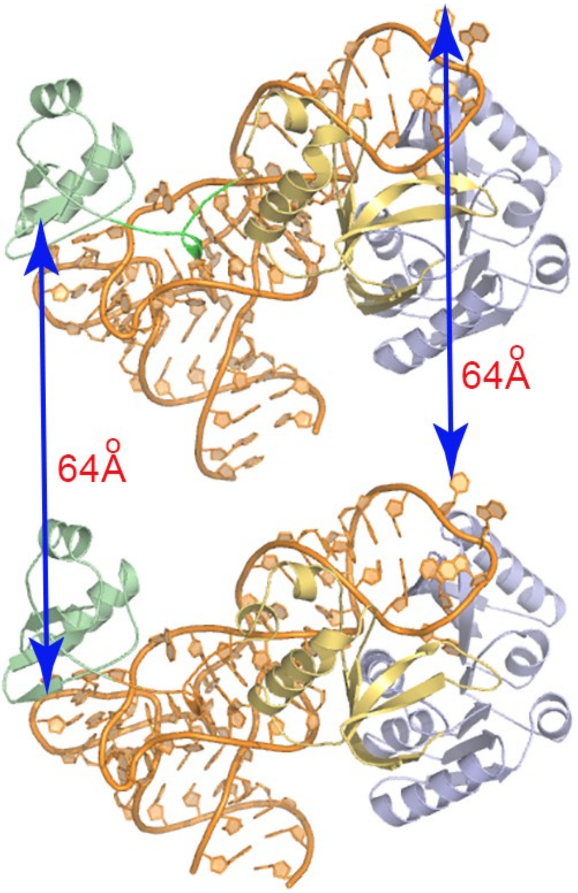

fig. S3. The asymmetric unit contents in the cocrystals of the PaTrm5a-tRNA$^{\mathrm{Phe}}$-SAH ternary complex.

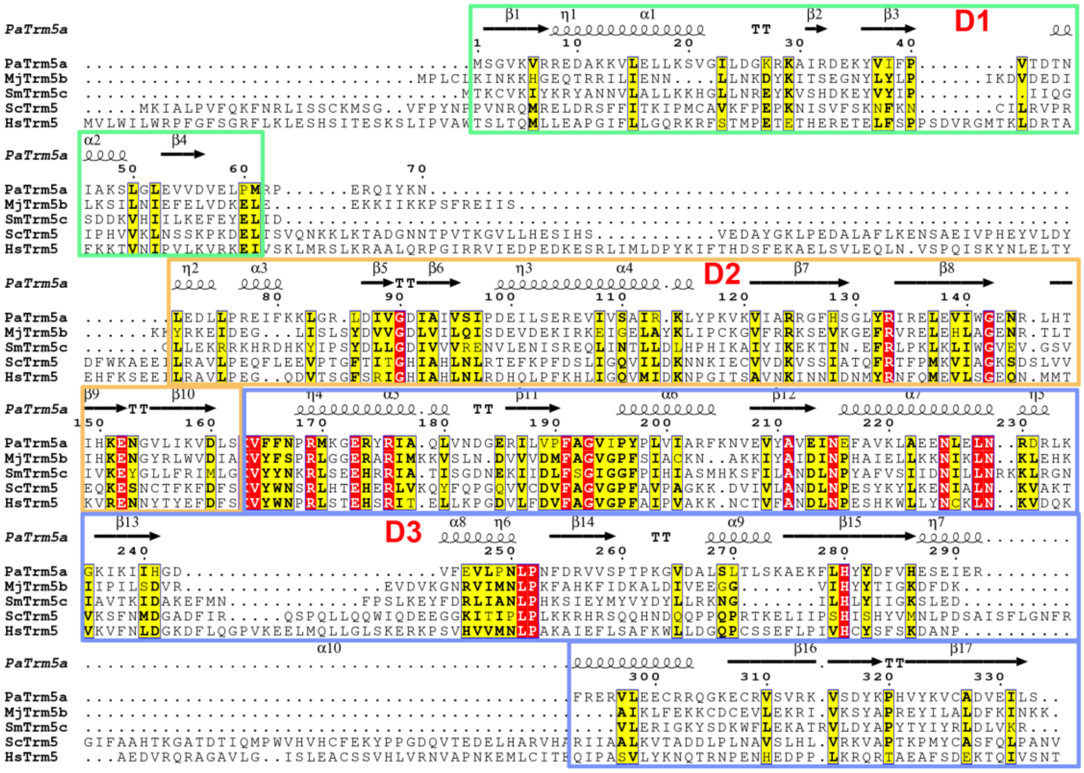

fig. S4. The multiple sequence alignment of Trm5 sequences from different model organisms. Abbreviations used: Pa, *Pyrococcus abyssi*; Mj, *Methanocaldococcus jannaschii*; Sm, *Staphylothermus marinus*; Sc, *Saccharomyces cerevisiae* and Hs, *Homo sapiens*. The secondary structure elements of PaTrm5a are labeled above the sequences. The domains are in colored boxes as described in Fig. 1A.

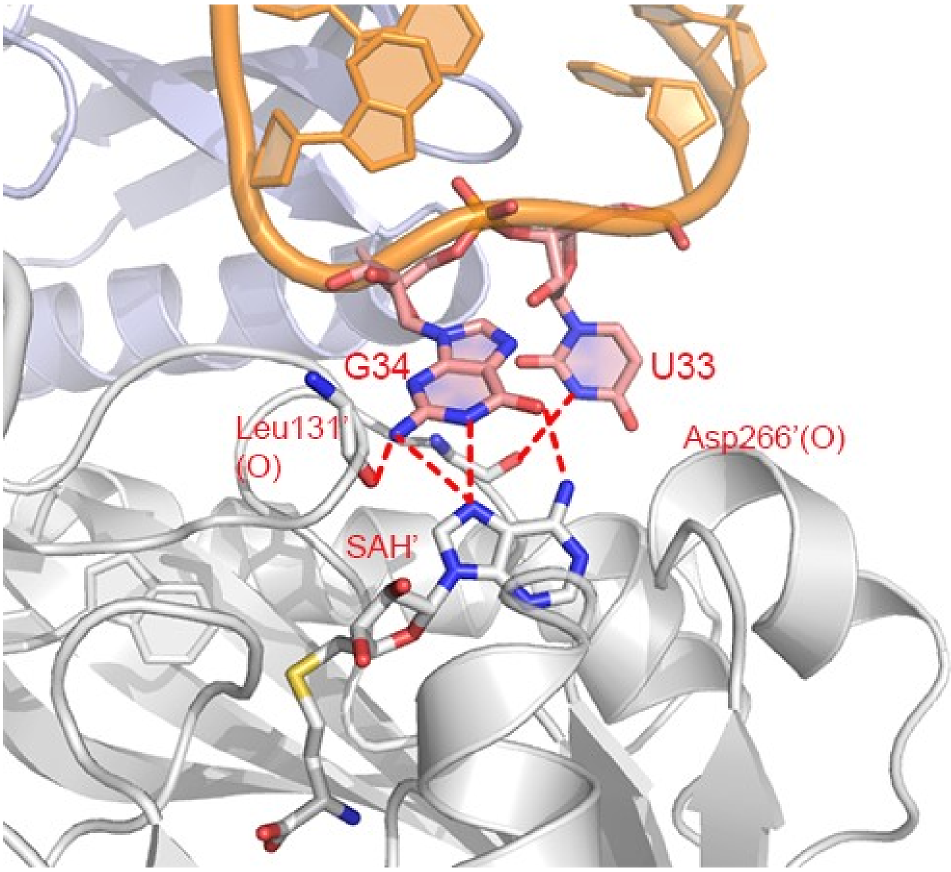

fig. S5. The interaction mode of U33/G34 in the PaTrm5a-tRNA^{Phe}-SAH ternary complex. Note that the gray color and primes indicate residues/domains from a symmetry mate.

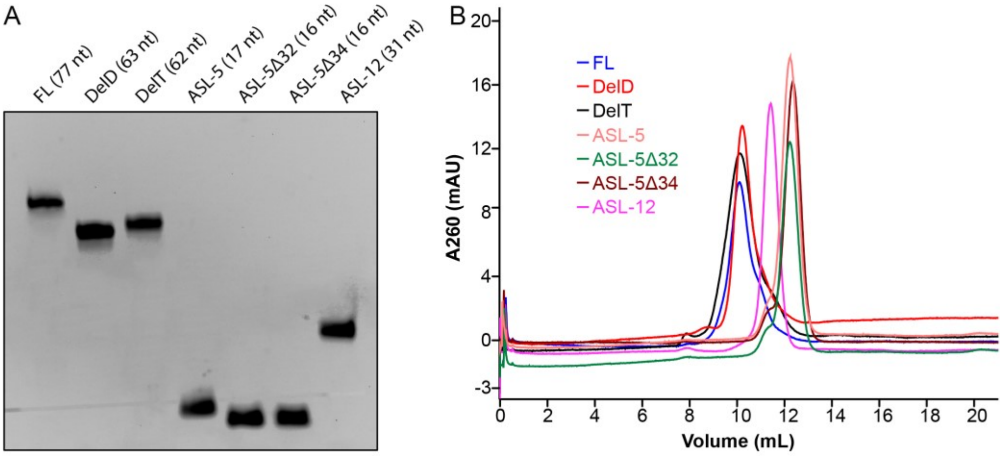

fig. S6. Purity test of tRNA truncation mutants by size exclusion chromatography and Urea-PAGE. (**A**) Purity test of the tRNA truncation mutants by Urea-PAGE. (**B**) The overlaid FPLC traces of the FL, DelD, DelT, ASL-5, ASL-5-Δ32, ASL-5-Δ34 and ASL-12 RNA variants from a Superdex 75 column.

table S1. Specific interactions between the enzyme and the tRNA substrate. We consider distances between functional groups involved in hydrogen-bonding interactions <3.6 Å.

PatRNA^{Phe}		PaTrm5a		Distance
resid	ato	resid	ato	
C11	O2	Lys1	NZ	2.97
C11	O3’	Asn1	N	3.03
C11	O2’	Asn1	N	3.31
U12	OP	Gly1	N	2.89
C13	O3’	Lys1	NZ	3.27
A14	OP	Lys1	NZ	2.60
A14	O5’	Lys1	NZ	3.13
G15	O3’	Gln6	NE	3.38
C16	OP	Gln6	NE	2.64
G19	OP	Lys2	NZ	3.41
G19	O3’	Lys1	NZ	3.57
G19	N2	Lys1	N	3.56
G19	O6	Arg3	NE	3.24
G19	N7	Arg3	NH	2.54
G20	OP	Lys1	NZ	2.54
G20	O5’	Lys1	NZ	2.64
G20	N2	Arg6	NH	3.51
G20	N1	Arg6	NH	3.43
G20	N2	Gln6	O	3.58
G20	N1	Gln6	O	2.86
G20	O6	Tyr6	N	2.98
G20	N2	Tyr6	OH	3.31
A23	O2’	Tyr1	OH	2.98
A23	N3	Tyr1	OH	2.72
G24	O4’	Tyr1	OH	2.93
G24	N9	Tyr1	OH	3.38
G24	N2	Lys1	NZ	3.54
G24	O3’	Leu8	O	3.05
G24	O2’	Leu8	O	2.88
C25	O5’	Arg8	NH	2.70
C25	OP	Arg8	NH	3. 04
C25	OP	Arg8	NE	2.54
C25	O3’	Arg1	NE	3.53
C25	O2’	Lys1	NZ	3.40
C25	O2	Lys1	NZ	2.96
A26	OP	Arg8	NH	2.82
A31	N6	Asp3	OD	3.18
C32	N3	His3	N	3.39
C32	N4	Tyr3	OH	3.31
U33	OP	His3	NE	3.18
A35	OP	Lys3	NZ	2.49
A35	O5’	Lys3	NZ	3.27
A36	OP	Lys3	NZ	2.73
A36	O5’	Tyr3	OH	3.26
A36	OP	Tyr3	OH	2.72
A36	O2’	Tyr3	O	2.47
G37	OP	His1	N	3.22
G37	O2’	His1	NE	3.06
G37	OP	Arg1	NH	2.89
G37	N7	Arg1	NH	2.75
G37	O6	Arg1	NE	2.54
G37	O6	Lys2	N	3.05
A38	OP	Arg1	NH	3.59
A38	OP	Arg1	NH	3.55
A38	O2’	Arg1	NH	3.48
A38	O3’	Arg1	NH	3.02
A38	N6	Pro3	N	3.35
A38	N9	Tyr3	O	3.40
A38	N7	Tyr3	O	3.59
A38	N7	Lys3	N	3.43
U39	OP	Arg1	NH	3.48
U39	OP	Arg1	NH	3.37
U39	OP	Arg1	NH	3.16
U39	O4	Asp3	OD	3.40
C40	OP	Leu8	O	3.51
C56	N1	Arg8	O	3.07
C56	O2	Lys1	N	3.21

table S2. Statistics on SAXS data collection, analysis, and modeling.

		apo-PaTrm5a
Data Collection Parameters		
Instrument		SSRF-BL19U2
Beam geometry		Slit
Wavelength (Å)		1.033
q range (Å $^{-1}$)		0.01-0.45
Exposure time (s)		1.0 per frame
Temperature (K)		277
Structural Parameters		
From Guinier fit	*I(0)* (cm^{-1})	16.53 ± 0.029
	Rg (Å)	28.07 ± 2.50
From P(r)	*I(0)* (cm^{-1})	16.81 ± 0.042
	Rg (Å)	29.70 ± 0.132
	D_{max} (Å)	116.63
Modeling		
DAMMIF	$\chi 2$	1.017
	NSD	0.716 ± 0.030