Supplementary Materials for:

A structural analysis of base substitutions in *Thermus thermophilus* 16S ribosomal RNA conferring streptomycin resistance

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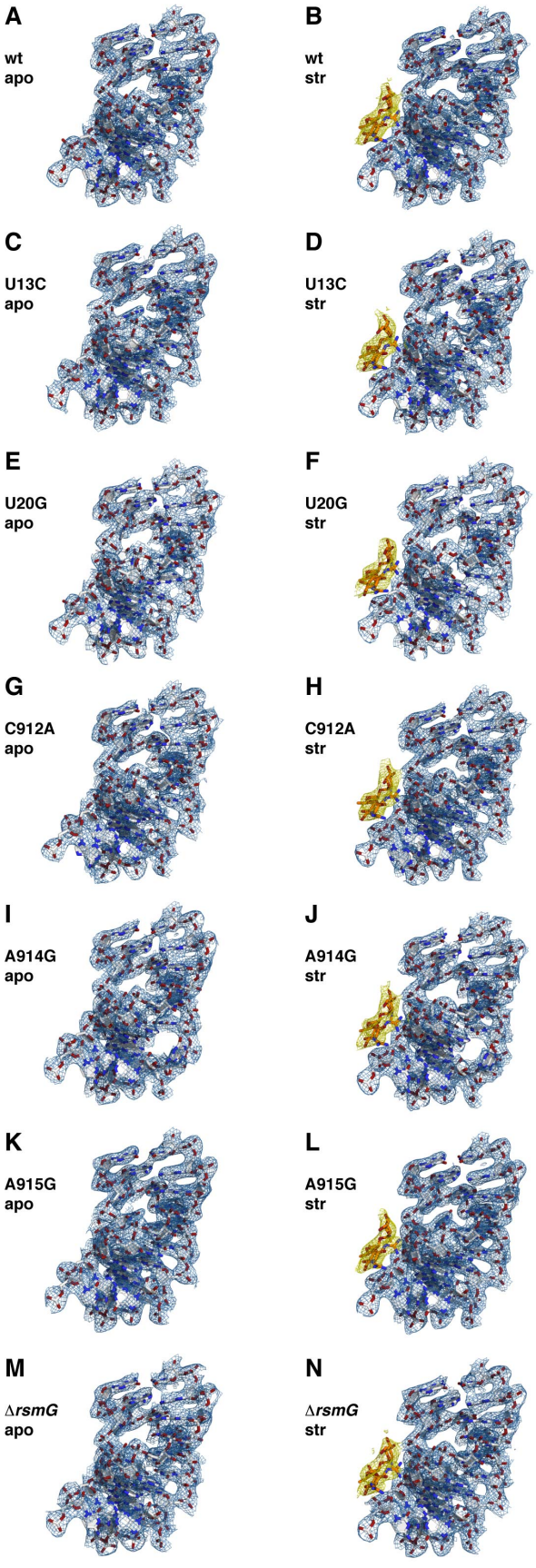
A
wt
apo
B
wt
str
C
U13C
apo
D
U13C
str
E
U20G
apo
F
U20G
str
G
C912A
apo
H
C912A
str
I
A914G
apo
J
A914G
str
K
A915G
apo
L
A915G
str
M
ΔrsmG
apo
N
ΔrsmG
str

FIG S1. (previous page) Composite omit $2mF_o$-DF_c electron density maps of the central pseudoknot. Shown are composite omit maps of the central pseudoknot structures from the wild-type 30S subunit and each of the 30S subunit structures from streptomycin-resistant mutants. Included in these maps are 16S rRNA residues A10-U24, G885, and C912-U920. Streptomycin is shown as yellow sticks.

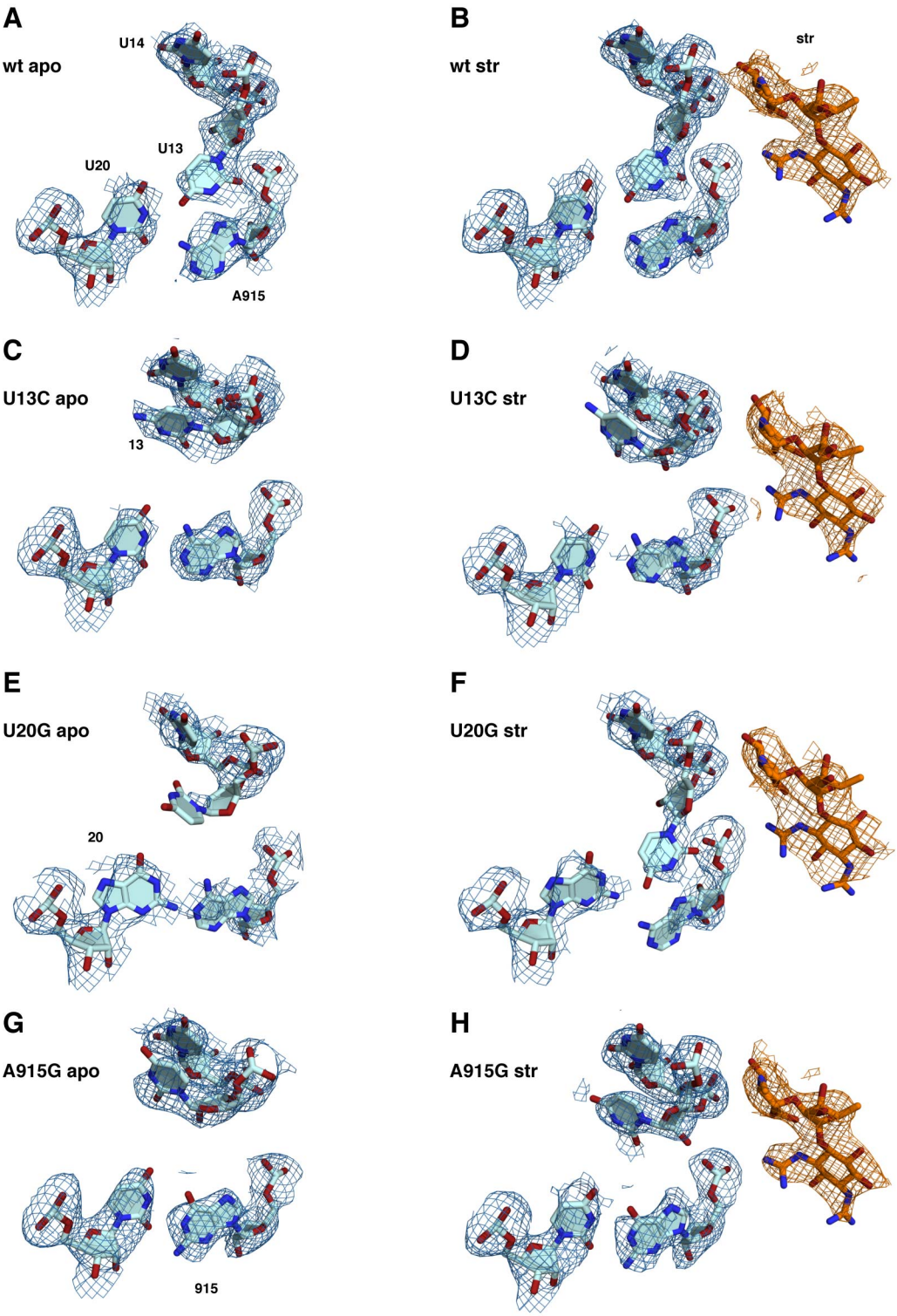

FIG S2. Composite omit $2mF_o$-DF_c electron density maps showing the secondary structure rearrangements in the U13C, U20G and A915G mutants.

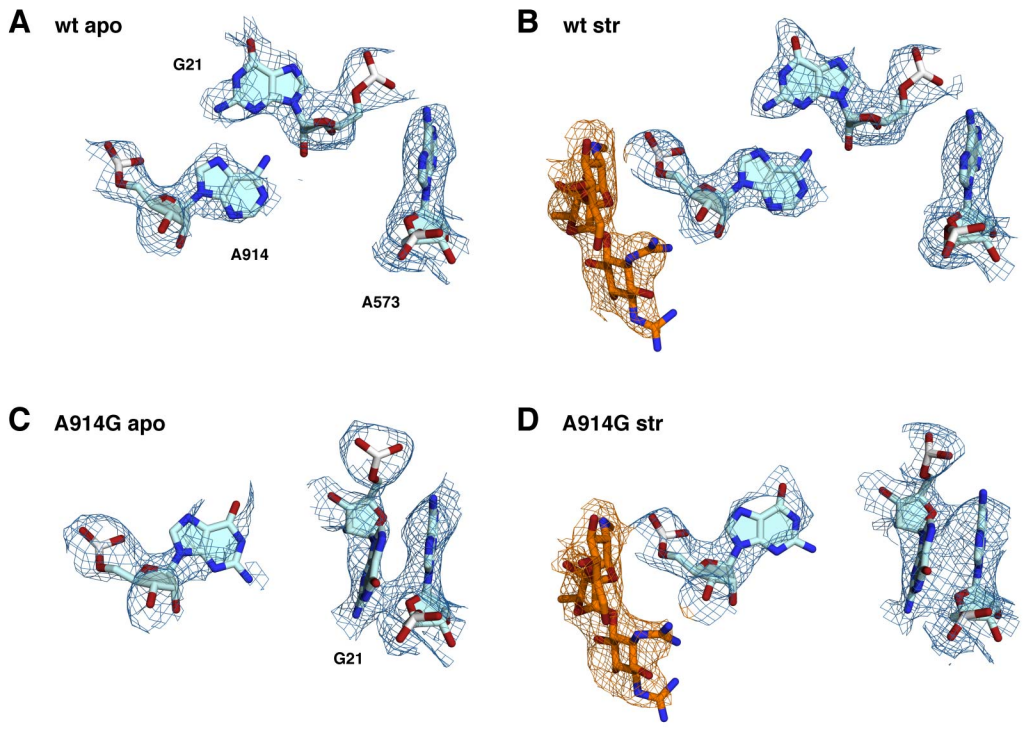

FIG S3. Composite omit $2mF_o$-DF_c electron density maps showing secondary structure rearrangements of the A914G mutant.

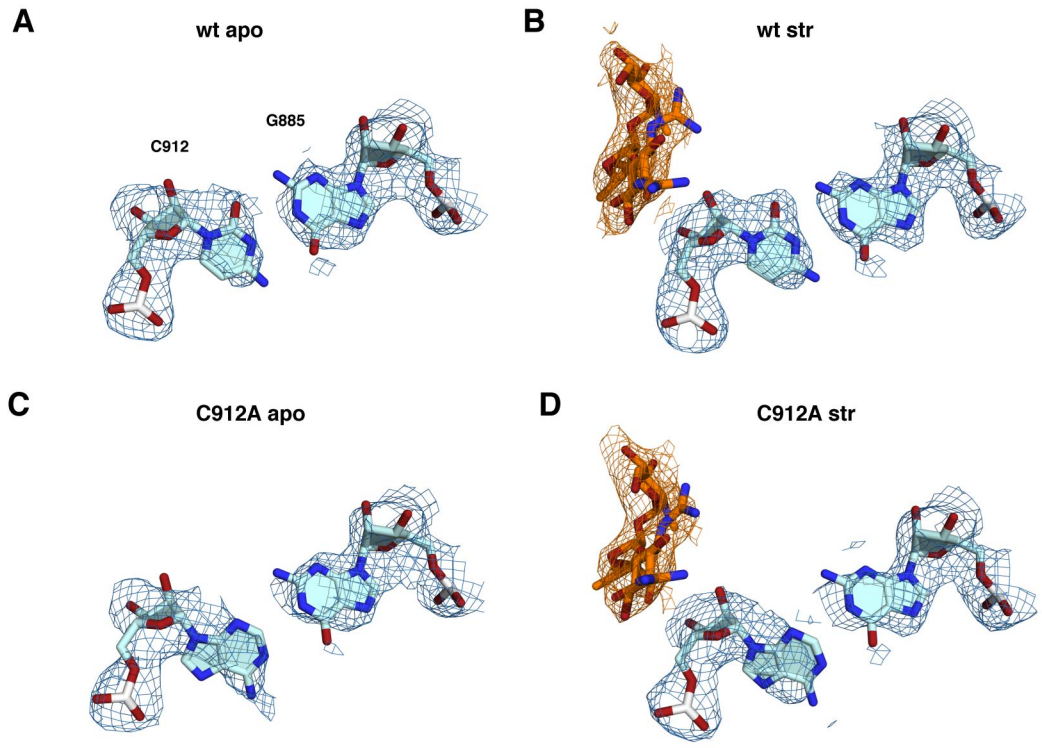

FIG S4. Composite omit $2mF_o$-DF_c electron density maps of the G885-C912 base pair and the effect of the C912A base substitution.

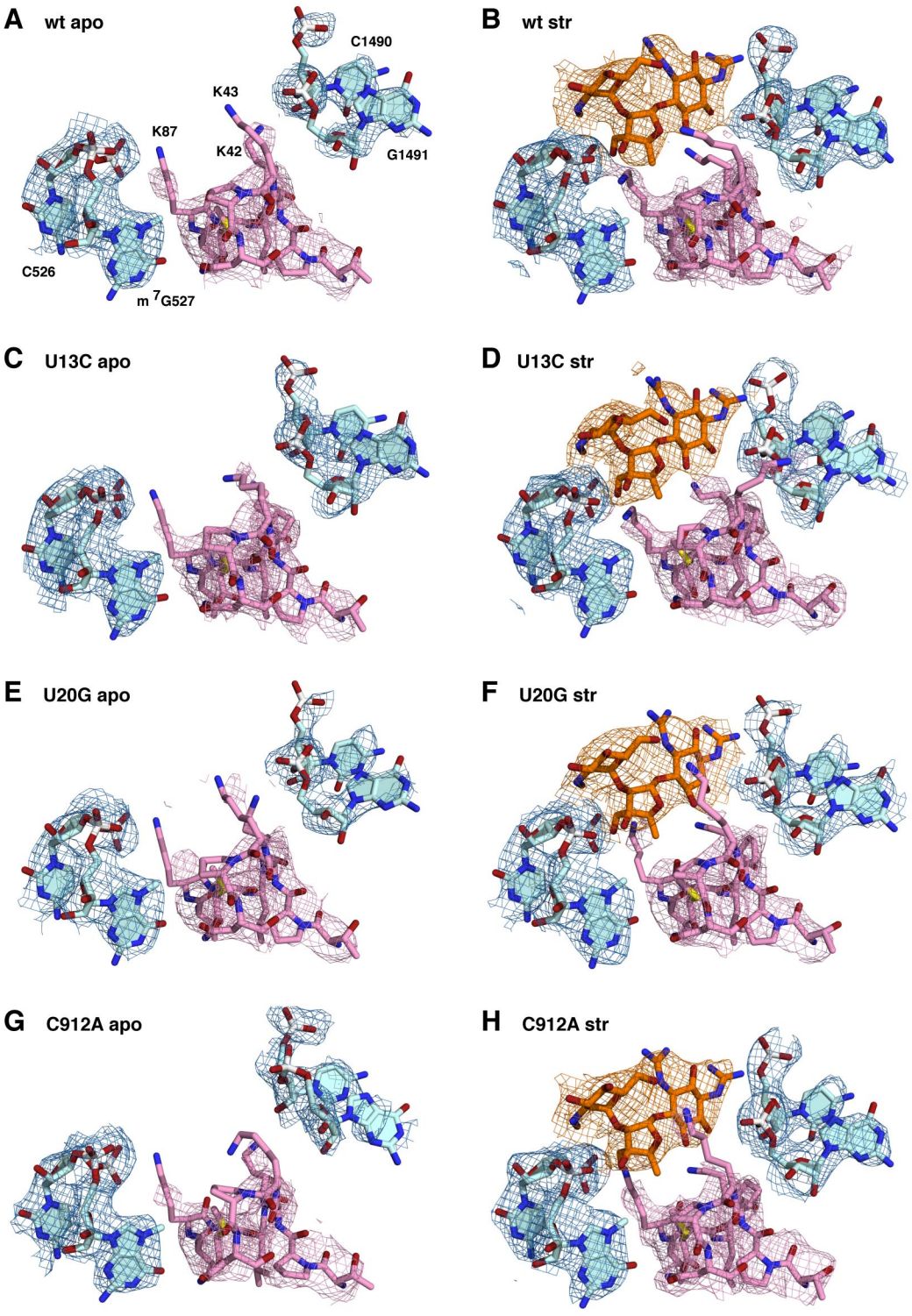

FIG S5. Composite omit $2mF_o$-DF_c electron density maps showing ribosomal protein S12 interactions with 16S rRNA and streptomycin. Ribosomal protein S12 is shown as pink sticks.

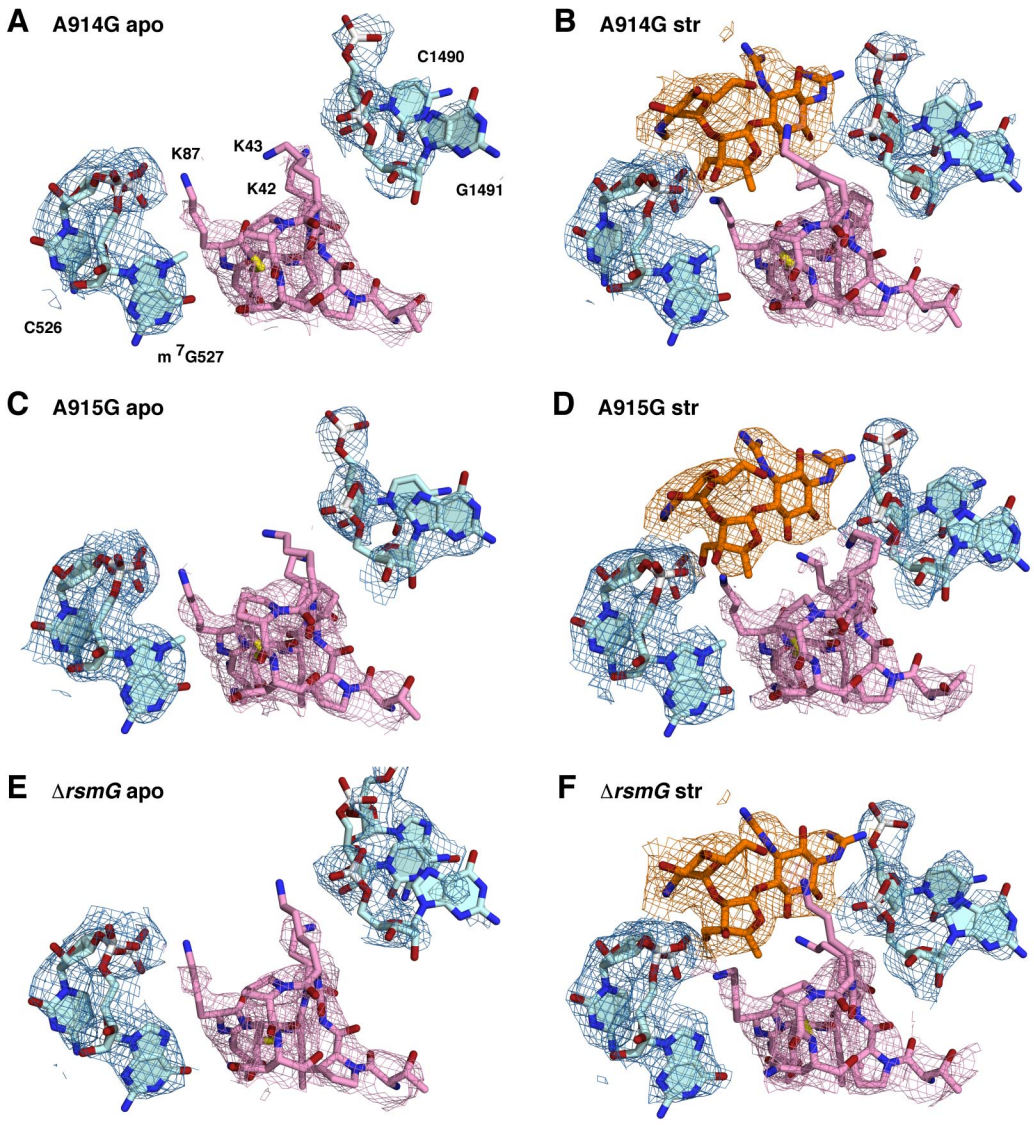

FIG S6. Composite omit $2mF_o$-DF_c electron density maps showing ribosomal protein S12 interactions with 16S rRNA and streptomycin (cont'd). Ribosomal protein S12 is shown as pink sticks.

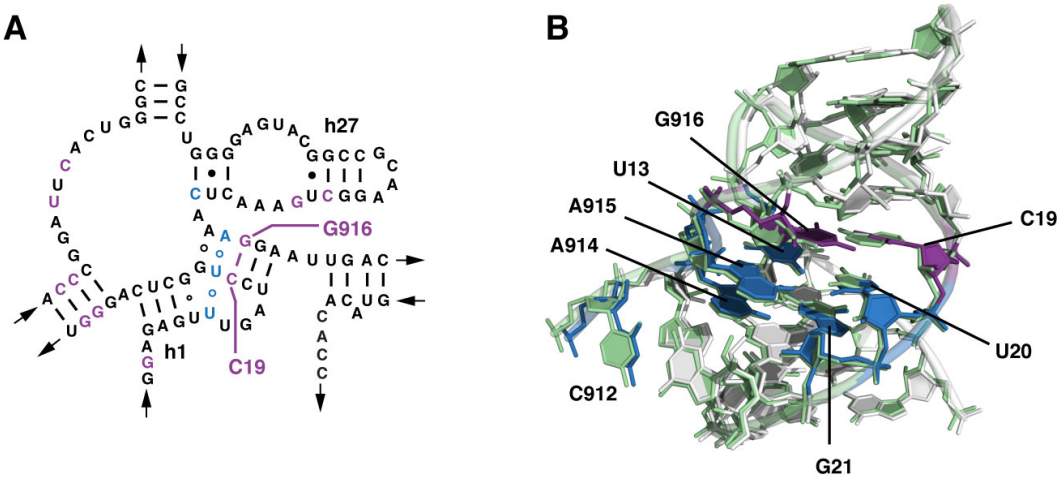

FIG S7. Comparison of the 16S rRNA central pseudoknot of *T. thermophilus* and *E. coli*. (A) Secondary structure model of the central pseudoknot of *T. thermophilus* 16S rRNA (modified from accession number M26923; ref. 1), with sites of Str^r mutations in blue and residues differing between *T. thermophilus* and *E. coli* in purple. (B) Structure of the central pseudoknot in the 30S ribosomal subunit crystal structure of *T. thermophilus* (white, pdb entry 4DR1; ref 2) aligned with the 30S subunit in the *E. coli* 70S crystal structure (pale green, pdb entry 2AVY; ref 3). Structures were aligned with the alignment algorithm of PyMol (4) using 16S rRNA phosphate atoms of residues 1-925, with the default 2σ rejection criterion and 50 iterative alignment cycles. Residues mutated in Str^r ribosomes are shown in blue, and residues differing between *T. thermophilus* and *E. coli* are shown in purple.

TABLE S1 Data collection and refinement statistics.

	U13C apo	U13C str	U20G apo	U20G str
PDB ID	4DUY	4DUZ	4DV0	4DV1
Data collection[1]				
Beamline	APS (ID-24-C)	APS (ID-24-C)	APS (ID-24-C)	APS (ID-24-C)
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions				
a, *b*, *c* (Å)	402.5, 402.5, 175.9	402.5, 402.5, 174.0	402.1, 402.1, 174.0	403.5, 403.5, 173.4
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)[2]	35.0-3.4 (3.52-3.4)	35.0-3.65 (3.78-3.65)	35.0-3.85 (3.99-3.85)	35-3.85 (3.99-3.85)
R_{merge}	0.064 (0.647)	0.080 (0.519)	0.07(0.817)	0.075 (0.724)
$I / \sigma I$	14.17 (1.79)	11.37 (1.69)	14.77 (1.73)	13.74 (1.70)
Completeness (%)	97.5 (98.2)	95.8 (97.6)	97.6 (99.5)	97.3 (99.0)
Redundancy	3.1 (3.0)	3.0 (2.8)	3.2 (3.2)	3.0 (3.0)
Refinement				
Resolution (Å)	35-3.4 (3.42-3.40)	35-3.65 (3.69-3.65)	35-3.85 (3.90-3.85)	35-3.85 (3.89-3.85)
No. reflections	192822 (5152)	150379 (4606)	130643 (4098)	130989 (4119)
R_{work} / R_{free}	0.159/0.202 (0.289/0.316)	0.156/0.216 (0.268/0.330)	0.148/0.206 (0.273/0.299)	0.150/0.212 (0.269/0.308)
No. atoms				
Protein	19090	19090	19090	19090
RNA	32644	32507	32647	32510
Ligand/Ion/Water	850	692	716	697
B-factors				
Protein	147.3	178.3	205.2	208.2
RNA	138.9	169.7	192.1	194.2
Ligand/Ion/Water	139.5	144.4	175.2	168.6
Coordinate errors	0.34	0.30	0.31	0.33
R.m.s deviations				
Bond lengths (Å)	0.006	0.014	0.011	0.012
Bond angles (°)	0.93	1.70	1.43	1.52
Ramachandran plot				
Favored (%)	1836 (89.6)	1788 (87.2)	1800 (87.8)	1754 (85.6)
Allowed (%)	190 (9.3)	235 (11.5)	228 (11.1)	272 (13.3)
Generously allowed (%)	23 (1.1)	25 (1.2)	22 (1.1)	23 (1.1)
Disallowed (%)	1 (0.0)	2 (0.1)	0 (0.0)	1 (0.0)

[1]One crystal used for each dataset (two crystals used for A915G str dataset).

[2]Highest resolution shell is shown in parenthesis.

TABLE S1 Data collection and refinement statistics (cont'd).

	C912A apo	C912A str	A914G apo	A914G str
PDB ID	4DV2	4DV3	4DV4	4DV5
Data collection[1]				
Beamline	APS (ID-24-C)	APS (ID-24-C)	APS (ID-24-C)	APS (ID-24-C)
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions				
a, *b*, *c* (Å)	403.7, 403.7, 173.2	403.0, 403.0, 172.6	402.6, 402.6, 174.7	402.1, 402.1, 172.6
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)[2]	30.0-3.65 (3.78-3.65)	30.0-3.55 (3.68-55)	35.0-3.65 (3.78-3.65)	35-3.70 (3.83-3.70)
R_{merge}	0.120 (0.759)	0.156 (0.770)	0.080(0.686)	0.102 (0.608)
$I / \sigma I$	12.32 (1.80)	10.79 (1.75)	12.50 (1.65)	9.18 (1.73)
Completeness (%)	97.2 (97.5)	96.6 (90.9)	98.3 (99.5)	98.3 (98.5)
Redundancy	3.1 (3.1)	2.8 (2.6)	3.2 (3.2)	2.9 (2.7)
Refinement				
Resolution (Å)	30-3.65 (3.69-3.65)	30-3.55 (3.59-3.55)	35-3.65 (3.69-3.65)	35-3.70 (3.73-3.70)
No. reflections	153299 (4479)	164335 (4746)	155190 (4708)	148962 (4453)
R_{work} / R_{free}	0.165/0.223 (0.299/0.336)	0.166/0.220 (0.280/0.329)	0.153/0.208 (0.291/0.348)	0.159/0.212 (0.274/0.328)
No. atoms				
Protein	19090	19090	19090	19090
RNA	32646	32509	32645	32508
Ligand/Ion/Water	705	703	699	702
B-factors				
Protein	177.9	157.3	173.9	153.5
RNA	172.2	150.3	166.2	146.3
Ligand/Ion/Water	146.5	127.6	148.8	124.4
Coordinate errors	0.35	0.30	0.28	0.33
R.m.s deviations				
Bond lengths (Å)	0.013	0.013	0.013	0.015
Bond angles (°)	1.59	1.60	1.62	1.82
Ramachandran plot				
Favored (%)	1791 (87.4)	1793 (87.5)	1773 (86.5)	1777 (86.7)
Allowed (%)	235 (11.5)	225 (11.0)	257 (12.5)	248 (12.1)
Generously allowed (%)	22 (1.1)	30 (1.5)	18 (0.9)	24 (1.2)
Disallowed (%)	2 (0.1)	2 (0.1)	2 (0.1)	1 (0.0)

[1]One crystal used for each dataset (two crystals used for A915G str dataset).

[2]Highest resolution shell is shown in parenthesis.

TABLE S1 Data collection and refinement statistics (cont'd).

	A915G apo	A915G str	Δ*rsmG* apo	Δ*rsmG* str
PDB ID	4DV6	4DV7	4NXM	4NXN
Data collection[1]				
Beamline	APS (ID-24-C)	APS (ID-24-C)	APS (ID-24-C)	APS (ID-24-C)
Space group	$P4_12_12$	$P4_12_12$	$P4_12_12$	$P4_12_12$
Cell dimensions				
a, *b*, *c* (Å)	401.3, 401.3, 176.0	403.4, 403.4, 173.6	402.9, 402.9, 174.3	403.1, 403.1, 173.5
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)[2]	35.0-3.3 (3.42-3.30)	35.0-3.3 (3.42-3.30)	35.0-3.65 (3.78-3.65)	35.0-3.55 (3.68-3.55)
R_{merge}	0.097 (0.731)	0.126 (0.950)	0.081 (0.738)	0.092 (0.691)
$I / \sigma I$	12.73 (1.69)	13.43 (1.78)	15.50 (1.74)	11.42 (1.76)
Completeness (%)	98.9 (99.3)	98.8 (99.9)	98.5 (98.8)	99.2 (99.1)
Redundancy	3.8 (3.8)	4.8 (4.6)	3.7 (3.6)	3.3 (3.3)
Refinement				
Resolution (Å)	35-3.3 (3.33-3.30)	35-3.3 (3.33-3.30)	35-3.65 (3.69-3.65)	35-3.55 (3.58-3.55)
No. reflections	210495 (5518)	210877 (6240)	155250 (4508)	170277 (5219)
R_{work} / R_{free}	0.163/0.208 (0.290/0.330)	0.168/0.210 (0.307/0.356)	0.180/0.226 (0.304/0.359)	0.193/0.231 (0.314/0.357)
No. atoms				
Protein	19090	19090	19090	19090
RNA	32645	32508	32643	32506
Ligand/Ion/Water	888	739	461	541
B-factors				
Protein	136.7	168.6	149.7	153.1
RNA	128.7	157.3	145.2	145.8
Ligand/Ion/Water	130.8	144.4	99.1	103.1
Coordinate errors	0.31	0.29	0.37	0.37
R.m.s deviations				
Bond lengths (Å)	0.006	0.007	0.008	0.005
Bond angles (°)	0.98	1.02	0.83	0.76
Ramachandran plot				
Favored (%)	1845 (90.0)	1817 (88.6)	1819 (88.7)	1821 (88.8)
Allowed (%)	178 (8.7)	209 (10.2)	209 (10.2)	209 (10.2)
Generously allowed (%)	25 (1.2)	24 (1.2)	21 (1.0)	20 (1.0)
Disallowed (%)	2 (0.1)	0 (0.0)	1 (0.0)	0 (0.0)

[1]One crystal used for each dataset (two crystals used for A915G str dataset).

[2]Highest resolution shell is shown in parenthesis.

REFERENCES

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