Structure of the bifunctional methyltransferase YcbY (RlmKL) that adds the m^7G2069 and m^2G2445 modifications in *Escherichia coli* 23S rRNA.

Supplementary Information

Kai-Tuo Wang, Benoit Desmolaize, Jie Nan, Xiao-Wei Zhang, Lan-Fen Li

Stephen Douthwaite, & Xiao-Dong Su

Figure S1: Bioinformatics analysis of YcbY fusion orthologs in Gammaproteobacteria and the two separate orthologs in other bacteria. (*A*) Gram-negative Gammaproteobacteria, where 213 out of 238 sequenced species have orthologs of the YcbY fusion. (*B*) in the Beta- and Deltaproteobacteria, and the Bacilli branch of Firmicutes, most of the sequenced species have two separate genes. These are exemplified by the *B. subtilis* genes *ypsC* and *ywbD* and the *S. mutans* genes *smu472* and *smu776*. Gene fusion data were analyzed in StringDB (http://string.embl.de/).

Only two of the genomes of Gammaproteobacteria, *Psychrobacter cryohalolentis* and *Psychrobacter arcticum*, have a separate ortholog comprising the N-terminus without a corresponding separate ortholog of the C-terminus of YcbY (and this is possibly a misannotation, as these species also have a complete YcbY fusion). Three Gram-negative genomes have separate YcbY-N and YcbY-C orthologs, including *Vibrio vulnificus* CMCP6, although here again the genes are possibly incorrectly annotated. *Pseudomonas aeruginosa* PA14 and *Acinetobacter baumannii* ATCC 17978 have separate genes for YcbY-N and YcbY-C. Sixteen genomes in the Gammaproteobacteria do not have any ortholog of YcbY.

In other groups, only six bacteria have YcbY as a fusion protein. Three of these are also Gram-negative bacteria (*Magnetococcus* sp MC-1, *Desulfotalea psychrophila*, and *Pelobacter curbinolicus*), while the other three are Gram-positives (*Eggerthella lenta*, *Slackia heliotrinireducens* and *Atopobium parvulum*). Alphaproteobacteria do not possess orthologs of YcbY, while Beta- and Deltaproteobacteria have the same gene arrangement as in the Gram-positive Firmicutes branch of Bacilli. The NMB0445 and NMB1367 orthologs of *Neisseria meningitides* were analyzed in a contemporary study (1) and shown to have function methyltransferase functions identical to YcbY-N and YcbY-C, respectively.


A
Gamma-proteobacteria
Escherichia coli K12_MG1655
Escherichia coli K12_DH10B
Escherichia coli BW2952
Escherichia coli K12_W3110
Escherichia coli EC4115
Escherichia coli TW14359
Escherichia coli Sakai
Escherichia coli UMN026
Escherichia coli CFT073
Escherichia coli 11128
Escherichia coli 0739
Escherichia coli ED1a
Escherichia coli E0L933
Escherichia coli SMS35
Escherichia coli HS
Escherichia coli S88
Escherichia coli SE11
Escherichia coli 55989
Escherichia coli E2348
Escherichia coli IAI39
Escherichia coli IAI1
Escherichia coli 11368
Escherichia coli UTI89
Escherichia coli APEC01
Escherichia coli BL21
Escherichia coli 536
Escherichia coli 12009
Escherichia coli REL606
Escherichia coli E24377A
Escherichia fergusonii
Salmonella enterica AH1L2601
Salmonella enterica Paratyphi
Salmonella enterica CT18
Salmonella enterica Typ
Salmonella enterica Heidelberg
Salmonella enterica Agona
Salmonella enterica CVM19633
Salmonella enterica RKS4594
Salmonella enterica Gallinarium
Salmonella enterica Enteritidis
Salmonella enterica LT2
Salmonella enterica Dublin
Salmonella enterica Newport
Salmonella enterica Choleraesuis
Salmonella enterica SPB7
Salmonella enterica Arizonae
Yersinia pestis CO92
Yersinia pestis Pestoides
Yersinia pestis Angola
Yersinia pestis Microtus
Yersinia pestis KIM10
Yersinia pestis Antiqua
Yersinia pestis Nepal516
Yersinia pseudotuberculosis PB1
Yersinia pseudotuberculosis 32953
Yersinia pseudotuberculosis YPIII
Yersinia pseudotuberculosis 31758
Yersinia enterocolitica
Shigella flexneri 2457T
Shigella flexneri 301
Shigella flexneri 8401
Shigella boydii 3083
Shigella boydii Sb227
Shigella dysenteriae
Shigella sonnei
Buchnera aphidicola BpAPS
Buchnera aphidicola Ap5A
Buchnera aphidicola ApTuc7
Buchnera aphidicola Cc
Buchnera aphidicola Sg
Buchnera aphidicola Bp
Klebsiella pneumoniae K2044
Klebsiella pneumoniae 342
Klebsiella pneumoniae 78578
Dickeya dadantii Ech586
Dickeya dadantii Ech703
Dickeya zeae
Blochmannia floridanus
Blochmannia pennsylvanicus
Hamiltonella defensa
Pectobacterium carotovorum
Pectobacterium atrosepticum
Pectobacterium wasabiae
Cronobacter sakazakii
Cronobacter turicensis
Edwardsiella tarda
Edwardsiella ictaluri
Photorhabdus asymbiotica
Photorhabdus luminescens
Erwinia pyrifoliae
Erwinia tasmaniensis
Sodalis glossinidius
Wigglesworthia glossinidia
Citrobacter koseri
Serratia proteamaculans
Proteus mirabilis
Enterobacter sp. 638
Pseudomonas aeruginosa PA14
Pseudomonas aeruginosa PA7
Pseudomonas aeruginosa LESB58
Pseudomonas aeruginosa PAO1
Pseudomonas mendocina
Pseudomonas putida F1
Pseudomonas putida W619
Pseudomonas putida KT2440
Pseudomonas putida GB1
Pseudomonas fluorescens SBW25
Pseudomonas fluorescens PF5
Pseudomonas fluorescens PFO1
Pseudomonas syringae DC3000
Pseudomonas syringae 1448A
Pseudomonas syringae B728a
Pseudomonas entomophila
Pseudomonas stutzeri
Azotobacter vinelandii
Cellvibrio japonicus
Acinetobacter baumannii ACICU
Acinetobacter baumannii AYE
Acinetobacter baumannii 17978
Acinetobacter baumannii SDF
Acinetobacter baumannii AB307
Acinetobacter baumannii AB0057
Acinetobacter sp. ADP1
Psychrobacter sp. PRwf
Psychrobacter cryohalolentis
Psychrobacter arcticus
Shewanella baltica OS223
Shewanella baltica OS195
Shewanella baltica OS185
Shewanella baltica OS155
Shewanella oneidensis MR1
Shewanella piezotolerans
Shewanella sp. ANA3
Shewanella pealeana
Shewanella denitrificans
Shewanella woodyi
Shewanella sp. W3181
Shewanella sediminis
Shewanella putrefaciens
Shewanella loihica
Shewanella halifaxensis
Shewanella frigidimarina
Shewanella amazonensis
Shewanella sp. MR4
Shewanella sp. MR7
Saccharophagus degradans
Alteromonas macleodii
Marinobacter aquaeolei
Pseudoalteromonas atlantica
Pseudoalteromonas haloplanktis
Teredinibacter turnerae
Colwellia psychrerythraea
Idiomarina loihiensis
Psychromonas ingrahamii
Haemophilus influenzae 02BNP
Haemophilus influenzae KW20
Haemophilus influenzae PittGG
Haemophilus influenzae PittEE
Haemophilus ducreyi
Haemophilus parasuis SH0165
Actinobacillus pleuropneumoniae JL03
Actinobacillus pleuropneumoniae AP76
Actinobacillus pleuropneumoniae L20
Actinobacillus succinogenes
Haemophilus somnus 129PT
Haemophilus somnus 2336
Aggregatibacter actinomycetemcomitans
Aggregatibacter aphrophilus
Pasteurella multocida
Mannheimia succiniciproducens
Xanthomonas oryzae 10331
Xanthomonas oryzae PXO99A
Xanthomonas oryzae 311018
Xanthomonas campestris 8004
Xanthomonas campestris 33913
Xanthomonas campestris B100
Xanthomonas axonopodis 306
Xanthomonas campestris 8510
Xylella fastidiosa 9a5c
Xylella fastidiosa M23
Xylella fastidiosa Temecula1
Xylella fastidiosa M12
Stenotrophomonas maltophilia K279a
Stenotrophomonas maltophilia R551
Vibrio cholerae MJ1236
Vibrio cholerae N16961
Vibrio cholerae M66Z
Vibrio cholerae O395
Vibrio vulnificus CMCP6
Vibrio vulnificus YJ016
Vibrio parahaemolyticus
Vibrio harveyi
Vibrio sp. Ex25
Vibrio splendidus
Vibrio fischeri MJ11
Vibrio fischeri ES114
Aliivibrio salmonicida
Photobacterium profundum
Francisella tularensis FSC198
Francisella tularensis WY96
Francisella tularensis SCHU54
Francisella tularensis holarctica
Francisella tularensis FTNF002
Francisella tularensis OSU18
Francisella tularensis FSC147
Francisella novicida
Francisella philomiragia
Thiomicrospira crunogena
Coxiella burnetii Q212
Coxiella burnetii Dugway
Coxiella burnetii 331
Coxiella burnetii Q154
Coxiella burnetii 493
Legionella pneumophila Lens
Legionella pneumophila Corby
Legionella pneumophila Philadelphia
Legionella pneumophila Paris
Vesicomyosocius okutanii
Ruthia magnifica
Carsonella ruddii
Kangiella koreensis
Bawumia cicadellilincola
Halorhodospira halophila
Alkalilimnicola ehrlichii
Thioalkalivibrio sp. HLEbGR7
Halothiobacillus neapolitanus
Nitrosococcus oceani
Marinomonas sp. MWYL1
Chromohalobacter salexigens
Hahella chejuensis
Alcanivorax borkumensis
Aeromonas hydrophila
Aeromonas salmonicida
Tolumonas auensis
Acidithiobacillus ferrooxidans 23270
Acidithiobacillus ferrooxidans 53993
Dichelobacter nodosus
Methylococcus capsulatus
B
Beta-proteobacteria
Burkholderia pseudomallei 1710b
Burkholderia pseudomallei 1106a
Burkholderia pseudomallei K96243
Burkholderia pseudomallei 668
Burkholderia pseudomallei MSHR346
Burkholderia mallei 10247
Burkholderia mallei 3344
Burkholderia mallei 10229
Burkholderia mallei 5WY1
Burkholderia thailandensis
Burkholderia cenocepacia J2315
Burkholderia cenocepacia 2424
Burkholderia cenocepacia 1054
Burkholderia cenocepacia MC0-3
Burkholderia ambifaria MC40
Burkholderia ambifaria AMMD
Burkholderia vietnamiensis
Burkholderia sp. 383
Burkholderia multivorans 17616
Burkholderia glumae
Burkholderia phytofirmans PsJN
Burkholderia xenovorans
Burkholderia phymatum STM815
Ralstonia eutropha H16
Cupriavidus taiwanensis
Ralstonia metallidurans
Ralstonia eutropha JMP134
Ralstonia pickettii 12D
Ralstonia pickettii 12J
Ralstonia solanacearum
Polynucleobacter necessarius QLW
Polynucleobacter necessarius STIR1
Polaromonas sp. JS666
Polaromonas naphthalenivorans
Acidovorax citrulli
Acidovorax sp. JS42
Variovorax paradoxus
Delftia acidovorans
Diaphorobacter sp. TPSY
Comamonas testosteroni
Verminephrobacter eiseniae
RhodoFerax ferrireducens
Bordetella pertussis
Bordetella avium
Bordetella parapertussis
Bordetella petrii
Bordetella bronchiseptica
Herminiimonas arsenicoxydans
Janthinobacterium sp. Marseille
Methylibium petroleiphilum
Leptothrix cholodnii
Neisseria meningitidis alpha14
Neisseria meningitidis Z2491
Neisseria meningitidis MC58
Neisseria meningitidis FAM18
Neisseria meningitidis 053442
Neisseria gonorrhoeae 11945
Neisseria gonorrhoeae 1090
Lariobacter hongkongensis
Chromobacterium violaceum
Thauera sp. MZ1T
Dechloromonas aromatica
Azoarcus sp. BH72
Aromatoleum aromaticum
Nitrosomonas eutropha
Nitrosomonas europaea
Nitrosospira multiformis
Methylotenera mobilis
Methylovorus sp. SIP3-4
Methylobacillus flagellatus
Thiobacillus denitrificans
Accumulibacter phosphatis
Delta-proteobacteria
DesulFovibrio vulgaris Miyazaki
DesulFovibrio vulgaris DP4
DesulFovibrio vulgaris Hildenborough
DesulFovibrio desulfuricans 27774
DesulFovibrio desulfuricans G20
DesulFovibrio salexigens
DesulFovibrio magneticus
Lawsonia intracellularis
DesulFohalobium retbaense
DesulFomicrobium baculatum
Geobacter sp. FRC32
Geobacter metallireducens
Geobacter sulfurreducens
Geobacter uraniireducens
Geobacter lovleyi
Geobacter sp. M21
Geobacter bemidjiensis
Pelobacter propionicus
Pelobacter carbinolicus
Anaeromyxobacter dehalogenans 2CPC
Anaeromyxobacter dehalogenans 2CP1
Anaeromyxobacter sp. Fw109-5
Anaeromyxobacter sp. K
Myxococcus xanthus
Haliangium ochraceum
Sorangium cellulosum
DesulFobacterium autotrophicum
DesulFatibacillum alkenivorans
DesulFococcus oleovorans
DesulFotalea psychrophila
Syntrophobacter fumaroxidans
Syntrophus aciditrophicus
Bdellovibrio bacteriovorus
Bacilli branch of Firmicutes
Streptococcus pyogenes MGAS5005
Streptococcus pyogenes M1GAS
Streptococcus pyogenes SSI1
Streptococcus pyogenes MGAS315
Streptococcus pyogenes MGAS2096
Streptococcus pyogenes MGAS9429
Streptococcus pyogenes MGAS10270
Streptococcus pyogenes MGAS10394
Streptococcus pyogenes Manfredo
Streptococcus pyogenes MGAS8232
Streptococcus pyogenes NZ131
Streptococcus pyogenes MGAS6180
Streptococcus pyogenes MGAS10750
Streptococcus pneumoniae G54
Streptococcus pneumoniae 19F14
Streptococcus pneumoniae 70585
Streptococcus pneumoniae TIGR4
Streptococcus pneumoniae CGSP14
Streptococcus pneumoniae D39
Streptococcus pneumoniae 1SN6
Streptococcus suis 05ZYH33
Streptococcus suis P17
Streptococcus suis BM407
Streptococcus suis SC84
Streptococcus suis 98HH33
Streptococcus equi MGCS10565
Streptococcus equi H70
Streptococcus equi 4047
Streptococcus dysgalactiae
Streptococcus thermophilus LMD9
Streptococcus thermophilus Z1066
Streptococcus thermophilus 18311
Streptococcus agalactiae 2603V/R
Streptococcus agalactiae A909
Streptococcus agalactiae NEM316
Streptococcus uberis
Streptococcus mutans
Streptococcus sanguinis
Streptococcus gordonii
Lactococcus lactis MG1363
Lactococcus lactis SK11
Lactococcus lactis KF147
Lactococcus lactis IL1403
Lactobacillus rhamnosus 705
Lactobacillus rhamnosus GG
Lactobacillus reuteri 1112
Lactobacillus reuteri 20016
Lactobacillus delbrueckii 11842
Lactobacillus delbrueckii BAA365
Lactobacillus johnsonii 533
Lactobacillus johnsonii FI9785
Lactobacillus plantarum JDM1
Lactobacillus plantarum WCFS1
Lactobacillus casei BL23
Lactobacillus casei 334
Lactobacillus brevis
Lactobacillus sakei
Lactobacillus helveticus
Lactobacillus acidophilus
Lactobacillus salivarius
Lactobacillus fermentum
Lactobacillus gasseri
Pediococcus pentosaceus
Leuconostoc citreum
Leuconostoc mesenteroides
Oenococcus oeni
Enterococcus faecalis
Bacillus cereus E33L
Bacillus cereus B4264
Bacillus cereus Q1
Bacillus cereus 03A579
Bacillus cereus 03BB102
Bacillus cereus AH820
Bacillus cereus G9842
Bacillus cereus 10987
Bacillus cereus AH187
Bacillus anthracis Sterne
Bacillus anthracis A0248
Bacillus anthracis Ames
Bacillus anthracis 684
Bacillus anthracis AmesA
Bacillus thuringiensis Hakam
Bacillus thuringiensis Konkukian
Bacillus cereus 3910B
Bacillus weihenstephanensis
Bacillus licheniformis 14580
Bacillus subtilis
Bacillus amyloliquefaciens
Bacillus clausii
Bacillus pumilus
Bacillus halodurans
Geobacillus sp. Y412MC10
Geobacillus kaustophilus
Geobacillus thermodenitrificans
Geobacillus sp. Y412MC61
Geobacillus sp. WCH70
Oceanobacillus iheyensis
Anoxybacillus flavithermus
Staphylococcus aureus TCH1516
Staphylococcus aureus FPR3757
Staphylococcus aureus 8325
Staphylococcus aureus MW2
Staphylococcus aureus MSSA476
Staphylococcus aureus NM2
Staphylococcus aureus ED98
Staphylococcus aureus JH1
Staphylococcus aureus MW50
Staphylococcus aureus COL
Staphylococcus aureus JH9
Staphylococcus aureus N315
Staphylococcus aureus Newman
Staphylococcus aureus MRSA252
Staphylococcus aureus RF122
Staphylococcus epidermidis 12228
Staphylococcus epidermidis RP62A
Staphylococcus haemolyticus
Staphylococcus carnosus
Staphylococcus saprophyticus
Macrococcus caseolyticus
Listeria monocytogenes EGD
Listeria monocytogenes HCC23
Listeria monocytogenes 80459
Listeria monocytogenes F2365
Listeria welshimeri
Listeria innocua
Brevibacillus brevis 100599
Paenibacillus sp. JDR2
Exiguobacterium sp. AT1b
Exiguobacterium sibiricum
Lysinibacillus sphaericus
Alicyclobacillus acidocaldarius

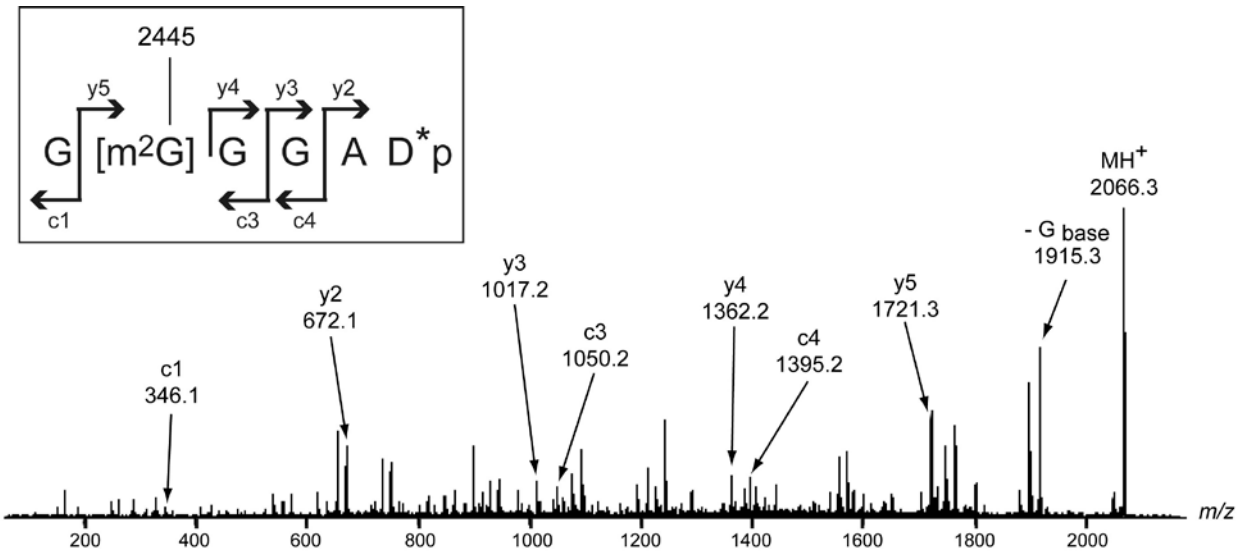

Figure S2: MALDI tandem mass spectrum of the 23S rRNA region containing m^2G2445

Prior to analysis, the 23S rRNA sequence 2422-2467 containing m^2G2445 was dried under vacuum and RNase A was dried separately. Each was resuspended in 2 µl of heavy water (>95% ^{18}O) and mixed. Digestion produced the fragment GGGGADp (G2444 to D2449, boxed) containing the m^2G2445 and a second modification with an initial mass of 2066.3 *m/z* (MH^+). This was an additional 2 *m/z* greater than the fragment produced without heavy water, and helped to discriminate the *y* ions in the fragmentation pattern. D* indicates that the *y2* ion carried 14 Da of extra mass that was possibly another methyl group attached to D2449; the location of this extra mass was confirmed by tandem MS analysis of an overlapping fragment (Figure S3). The ions are annotated according to McLuckey *et al*.(2).

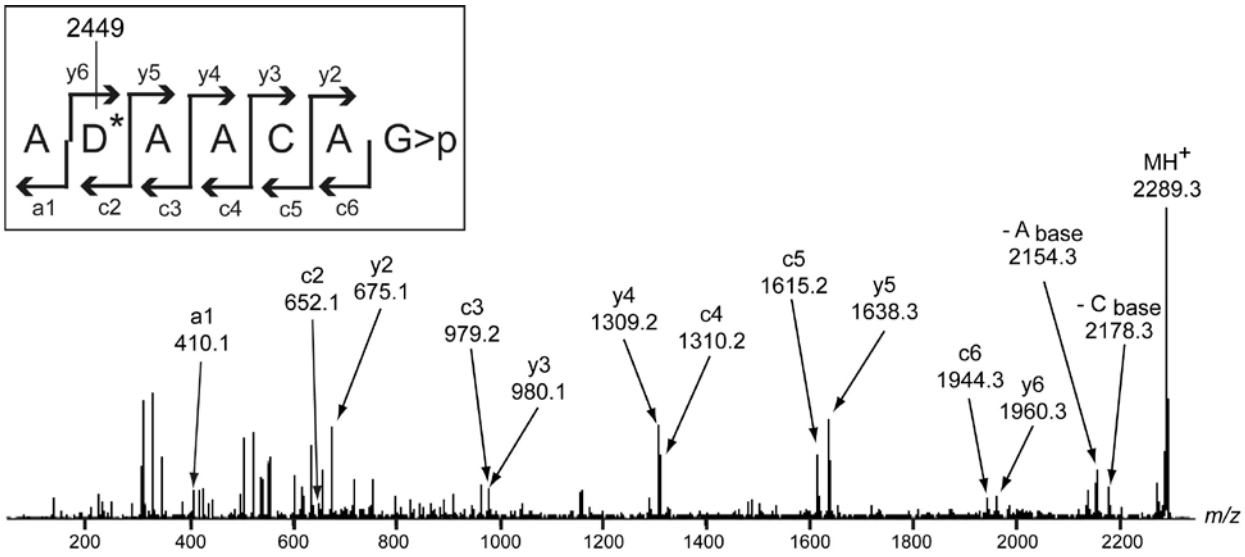

Figure S3: MALDI tandem mass spectrum of the 23S rRNA fragment containing D2449

The nucleotide was analyzed after generation of RNase T1 digestion products from the C2422 to C2467 region of rRNA from the Δ*ycbY* strain. The RNase T1 oligo ADAACAG>p corresponds to nucleotides 2448 to 2454 (box). D2449 was shown to carry the 14 Da of extra mass, which is presumed to be a methylation, and this modification is unconnected with the function of YcbY. The ion fragments are annotated as above (2).

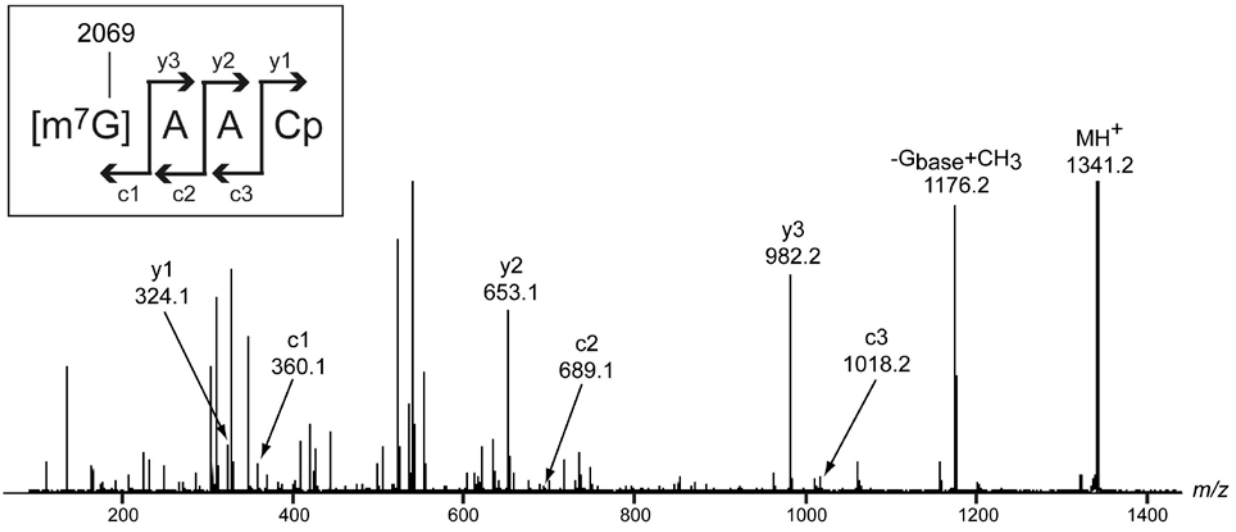

Figure S4: MALDI tandem mass spectrum of 23S rRNA containing m^7G2069

Nucleotide m^7G2069 was analyzed after generation of RNase A digestion products from the C2044 to C2093 region from the wild-type rRNA strain; m^7G2069 is contained in the GAACp fragment (nucleotides 2069 to 2072, box). The initial mass of the fragment was 1341.2 *m/z* (MH^+). The ion fragmentation pattern is annotated as above (2).

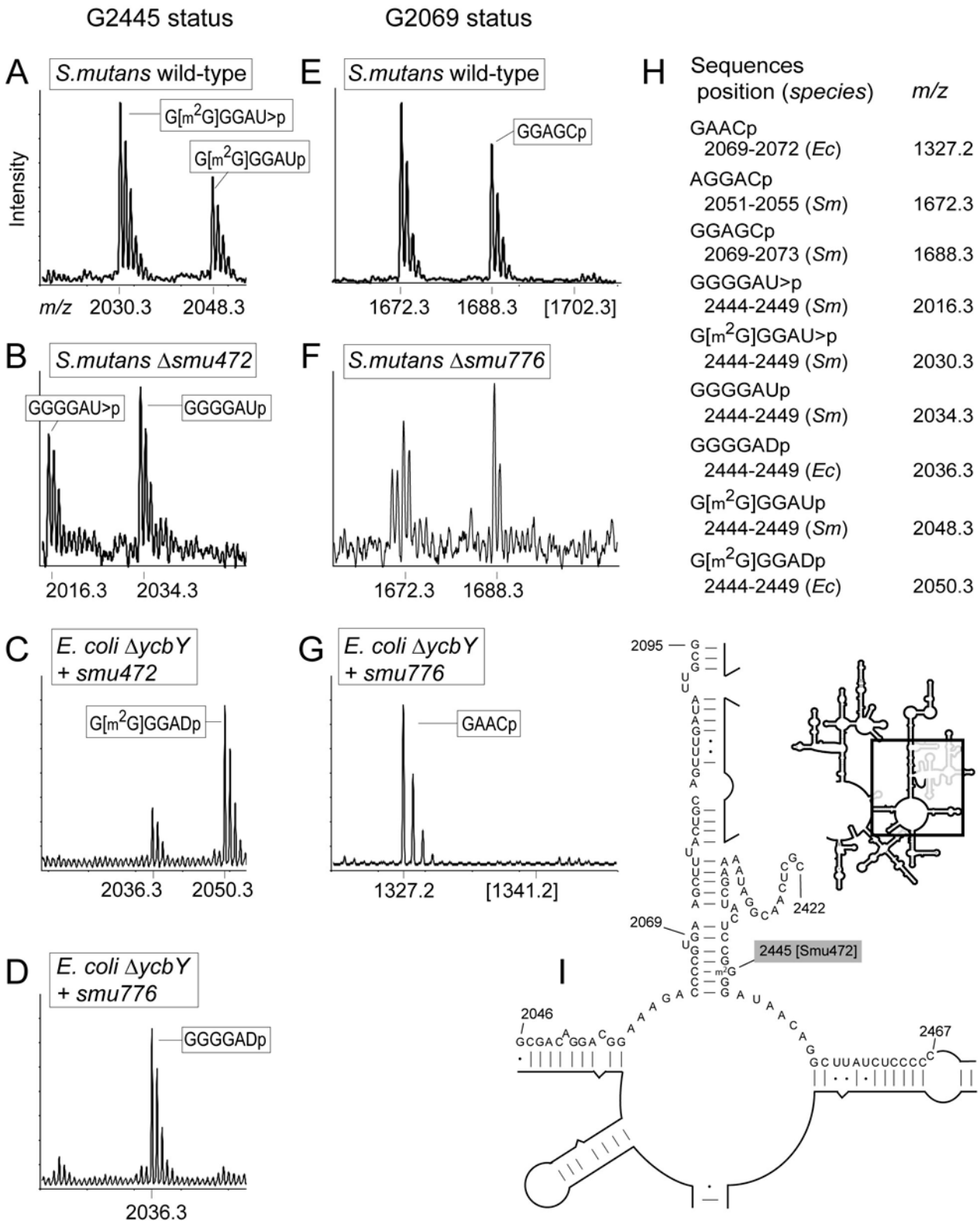

Figure S5: MALDI-MS analysis of rRNA modification by Smu472 and Smu776.

(*A*-*B*) Isolation of the C2422 to C2467 region from *S. mutans* 23S rRNA followed by RNase A digestion generates GGGGAU fragments containing nucleotide G2445. In these spectra, digestion

gave rise to a mixture of linear phosphates (p) and 2´-3´-cyclic phosphates (>p); their sequences are identical, although the latter is missing the equivalent of a water molecule and is 18 Da lighter. The m^2G2445 modification is present and stoichiometric in the *S. mutans* wild-type strain (*A*). The modification is lost in the *smu472*-null strain (*B*). (*E-F*) Isolation of the G2046 to G2095 region of *S. mutans* followed by RNase A digestion generate a fragment GGAGC with the nucleotide G2069. There is no m^7G2069 modification in the *S. mutans* wild-type strain (*E*) or the *smu776*-null strain (*F*). Complementation of the *E. coli ycbY*-null strain with active copies of *smu472* partially restored methylation at m^2G2445 (*C*); whereas *smu776* had no effect on G2445 (*D*) or G2069 (*G*). Sequences and theoretical mass/charges (*m/z*) of all the RNase fragments are shown in (*H*). The enlarged secondary structure (boxed in the outline) of the *S. mutans* 23S rRNA is showed (*I*). Nucleotides are numbered according to the *E. coli* rRNA structure (3-4).

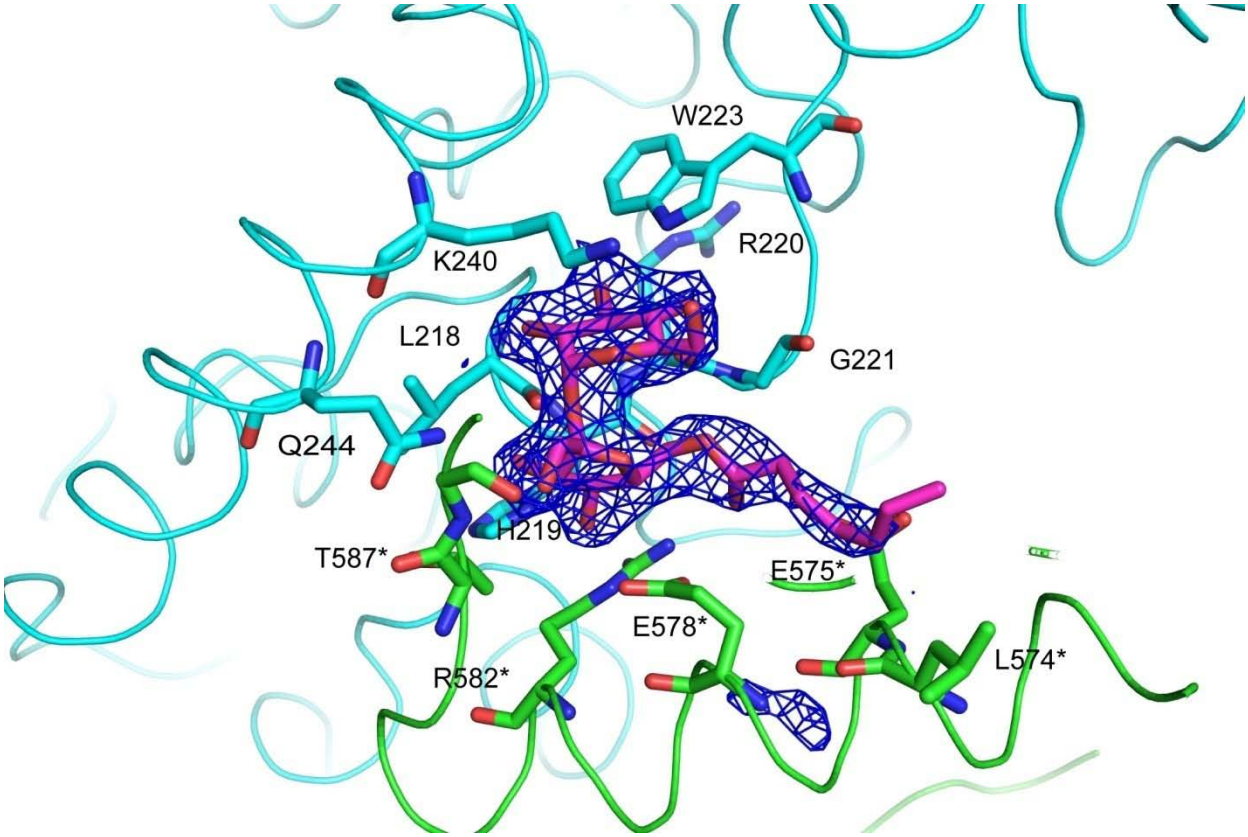

Figure S6: NCS related YcbY monomers

Monomers in the crystal are shown in ribbon and colored cyan and green. The interaction residues

are shown in stick mode and labeled. The asterisk (*) indicates the NCS related monomer. |*Fo-Fc*| electron density map was calculated with the protein model excluding n-octanoylsucrose and contoured at 4.0 σ.

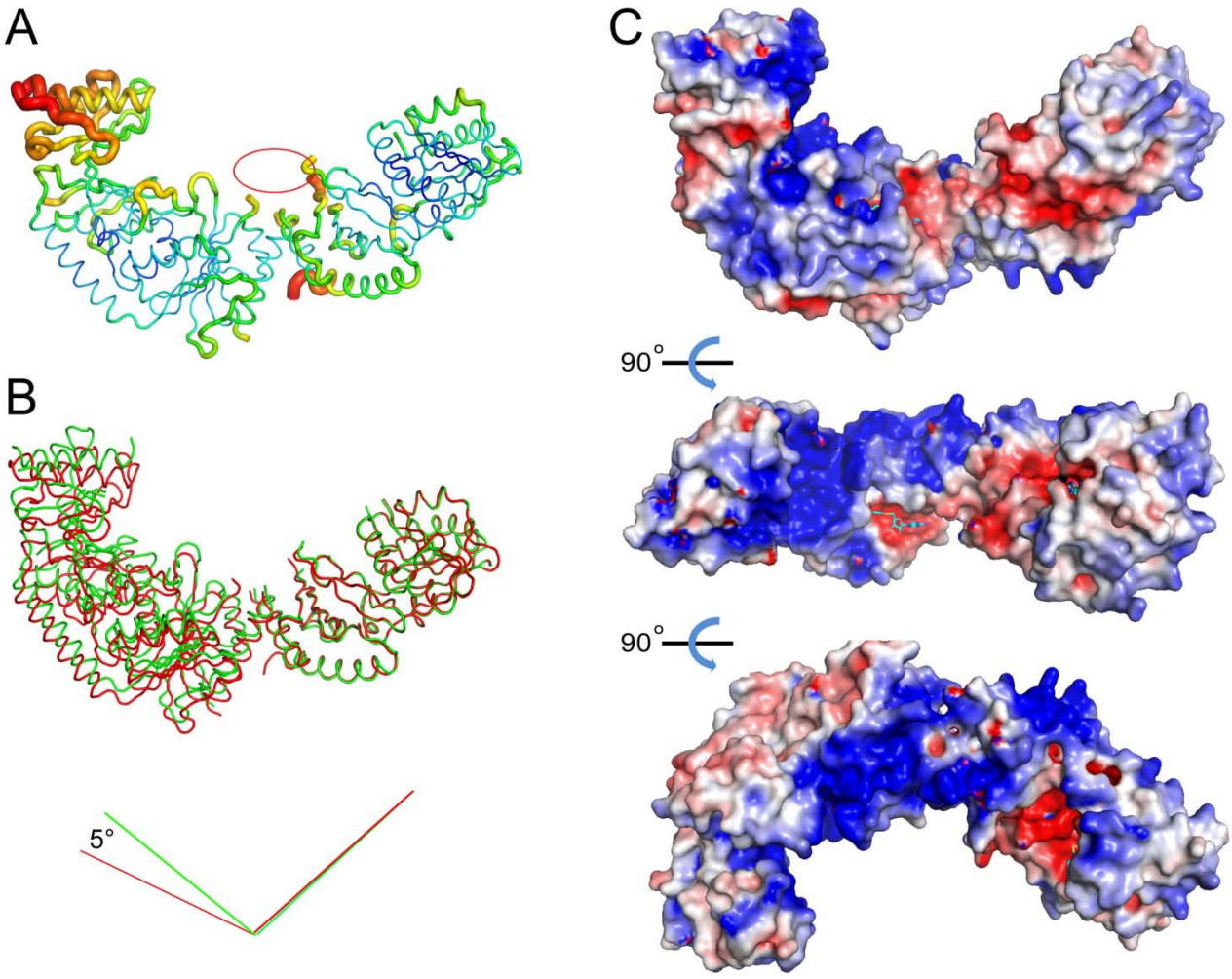

Figure S7: Surface potential on the YcbY structure

(*A*) B-factor presentation of the YcbY monomer showing unstable regions in the THUMP and EEHEE domains. The loops encircle in red could not be modeled. (*B*) Open angle difference between NCS related monomers aligned over the C-terminal halves. (*C*) Surface potential representation of YcbY in three 90 degree steps with SAH molecules shown as sticks. The YcbY structure has an overall concaved shape with a central cleft that is large enough to accommodate double-stranded RNA. It is feasible that the enzyme could bind in minor groove of helix 74 and flip

the two target bases into their respective active sites during one binding event (see main text). The protein structure is highly flexible and the angle between YcbY-N and YcbY-C differs by 5 degrees in the NCS related monomers. Several other features complicate building of an unambiguous model for RNA-protein interaction. For instance, the THUMP domain has high B-factors in the one monomer, and is barely visible in the other where no supporting interactions occur during crystal packing. Furthermore, the EEHEE domain and two loop regions (residues 446~452 and residues 484~495) could not be modeled in either monomer. These domains would feasibly become more stably structured upon RNA binding, and better resolution must therefore await YcbY-RNA co-crystal studies.

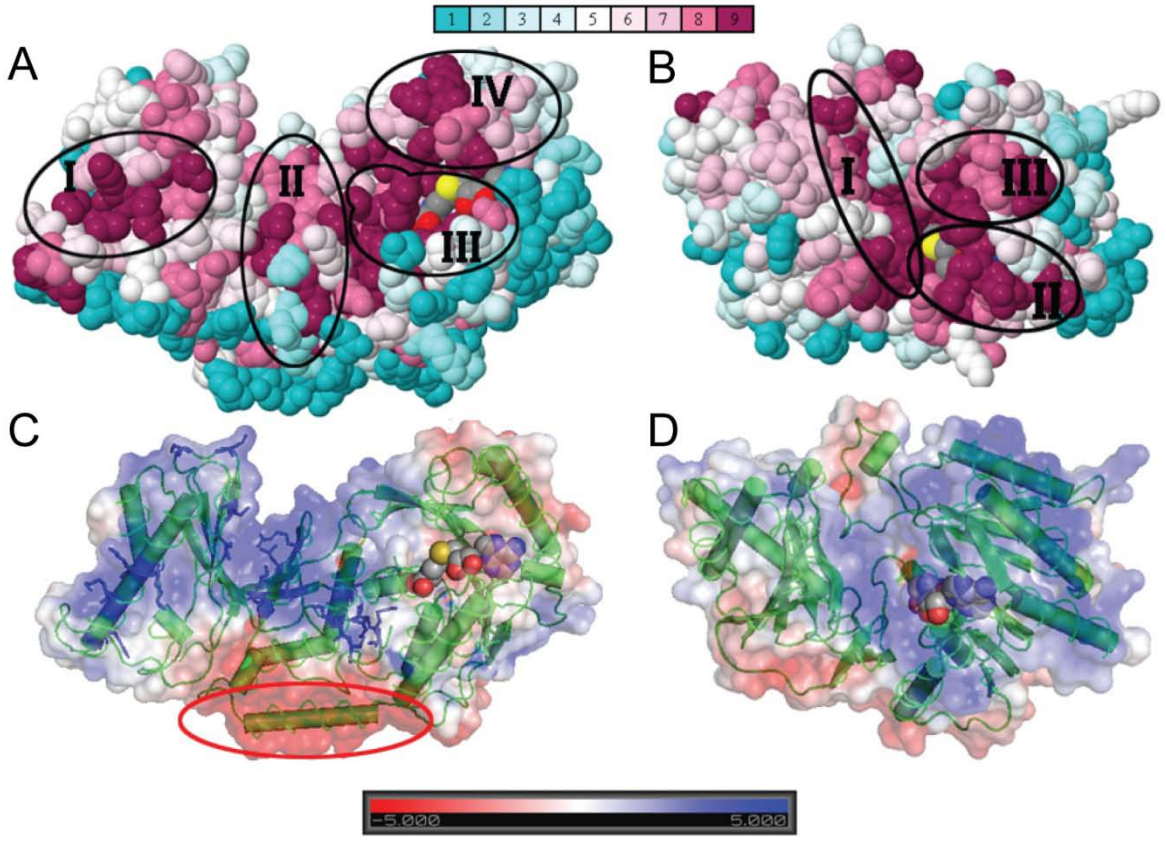

Figure S8: Residue conservation and surface potentials of Smu472 and Smu776.

The relative conservation of residues in (*A*) Smu472 and (*B*) Smu776 without the PUA domain is

shown with surface residues coloured according to their conservation levels calculated in ConSurf Server (5). (*A*) The encircled regions in the THUMP domain (I) and in the central groove (II) have concentrated positive charged residues that could interact with phosphates in the RNA backbone. Region III represents the conserved residues that directly bind SAH. Residues 307NPPYGERL314 in region IV constitute the most conserved motif of Smu472, and are situated in the loop between β4-N and αD-N of the N-terminal methyltransferase fold. (*B*) Conserved residues in the cleft between EEHEE and the methyltansferase domain (I) on the surface of Smu776. Region II binds the cofactor. Region III represents a conserved insertion motif between β4-C and αD-C in the C-terminal methyltansferase fold where a β-hairpin interacts directly with the base moiety of SAH forming a lid-like structure over the cofactor; this region appears to be important for catalysis. Surface potential representation of (*C*) Smu472 and (*D*) Smu776 without the PUA domain showing positively charged regions (blue) that could serve to bind the RNA backbone. The red circle indicates insertion sequences that are highly acidic in Smu472 but not in YcbY-N.

Table S1: Crystal growth, data collection, structure determination and refinement statistics

	Smu472	Smu776	YcbY-SAH	YcbY-SAM
Reservoir conditions	0.1 M Bicine pH9.0, 2% v/v Dioxane, 10% w/v PEG20000	0.1 M Na cacodylate pH 6.5, 0.2M $Mg(OAc)_2$ 20% PEG8000	0.1 M HEPES pH 7.0, 12% (w/v) PEG 8000	0.1 M HEPES pH 7.0, 12% (w/v) PEG 8000
Additive	none	none	6% (w/v) n-Octanoylsuc	6% (w/v) n-Octanoylsuc
Data collection	Photon Factory Japan	Home X-ray source Cu anode	SSRF, Shanghai China	SSRF, Shanghai China
Structure determination methods	Se-SAD	SIRAS	MR	MR
Space group	$P2_12_12_1$	C2	$P2_1$	$P2_1$
Unit cell dimensions				
a, *b*, *c*, Å	65.25 67.35 91.85	165.68 50.823 59.57	73.646 140.834 102.866	73.724 140.586 102.097
α,β,γ,°	90.0 90.0 90.0	90.00 107.14 90.00	90.00 102.30 90.00	90.00 101.88 90.00
Resolution, Å	50-1.97	50-2.20 (2.30-2.20)	50-2.20 (2.24-2.20)	50-2.60 (2.66-2.60)
R_{merge}[a], %	11.0 (27.0)	3.99(14.93)	12.1 (68.0)	8.8 (29.3)
I/σ	26.3 (4.9)	10.73(2.68)	16.4 (1.6)	23.7 (4.84)
completeness, %	99.6 (96.6)	90.8 (71.9)	96.8 (88.2)	94.7 (76.5)
Refinement resolution, Å	50-1.97 (2.02-1.97)	50-2.20 (2.33-2.20)	20-2.20 (2.26-2.20)	20-2.60 (2.67-2.60)
R_{cryst}[b], %	18.1 (28.0)	17.3 (18.7)	21.1 (35.0)	18.2 (21.9)
R_{free}[c], %	23.8 (40.7)	23.2 (24.9)	25.8 (39.0)	24.7 (31.0)

Table S2: Deoxyoligonucleotide primers used for rRNA isolation and for PCR analyses.

Primer name	Sequence [5´- 3´]	Application
p87	GCCCTCAACTTTATGACATG	Checking the integrity of *ybcY* gene
p88	GCGCTACAATCAAATCTTGC	Checking the integrity of *ybcY* gene
p90	GATATGAATTCTCTGTTTGCCAGTAC-GGCCCGTGGGCTGGAAGAGCTGTTA-AAAACTGAA**GTGTAGGCTGGAGC-TGCTTCG**	Knock-out of *ybcY* gene; complementary sequence for hybridization to kanamycin resistance cassette (bold)
p91	CTTTCAGGCTGCGGTAATCAGCCA-GCAGTTGTGGATCTGGCGGTTACG-GGCGAAATCCTG**CATATGAATATC-CTCCTTAGTTCC**	Knock-out of *ybcY* gene; complementary sequence to kanamycin cassette in bold
Ec2069rt	GCTCCCACCTATCCTACACATC	Reverse transcriptase extension to investigate methylation at G2069
Ec2445rt	CTTGGGCGGTATCAGCCTG	Reverse transcriptase extension to investigate methylation at G2445
Ec2069	CAGTGTCAAGCTATAGTAAAGGTTC-ACGGGGTCTTTCCGTCTTGCCGCGG	Isolation of *E. coli* 23S rRNA around position G2069 for MS
Ec2445	GGGCGGTATCAGCCTGTTATCCCC-GGAGTACCTTTTATCCGTTGAGCG	Isolation of *E. coli* 23S rRNA around position G2445 for MS
Sm2069	GCAATATCAAACTGCAGTAAAGCT-CCATGGGGTCTTTCCGTCCTGTCGC	Isolation of *S. mutans* 23S rRNA around position G2069 for MS
Sm2445	GGGGGAGATAAGCCTGTTATCCCC-AGGGTAGCTTTTATCCGTTGAGCG	Isolation of *S. mutans* 23S rRNA around position G2445 for MS

References

1. Kimura, S., Ikeuchi, Y., Kitahara, K., Sakaguchi, Y., Suzuki, T., and Suzuki, T. (2012) Base methylations in the double-stranded RNA by a fused methyltransferase bearing unwinding activity, *Nucleic Acids Res* **in press**.
2. McLuckey, S. A., Van Berkel, G. J., and Glish, G. L. (1992) Tandem mass spectrometry of small multiply charged oligonucleotides, *J. Am. Soc. Mass Spectrom* **3**, 60-70.
3. Cannone, J. J., Subramanian, S., Schnare, M. N., Collett, J. R., D'Souza, L. M., Du, Y., Feng, B., Lin, N., Madabusi, L. V., Müller, K. M., Pande, N., Shang, Z., Yu, N., and Gutell, R. R. (2002) The Comparative RNA Web (CRW) Site: an online database of comparative sequence and structure information for ribosomal, intron, and other RNAs, *BMC Bioinformatics* **3**, 2.
4. Noller, H. F. (1984) Structure of ribosomal RNA., *Ann. Rev. Biochem.* **53**, 119-162.
5. Ashkenazy, H., Erez, E., Martz, E., Pupko, T., and Ben-Tal, N. (2010) ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids, *Nucleic Acids Res* **38**, W529.