Supplementary Data for

Insights into RNA binding by the anticancer drug cisplatin from the crystal structure of cisplatin-modified ribosome

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This file includes:

I. SUPPLEMENTARY TABLES

Table S1. Data collection and refinement statistics.

Crystals		**70S-CPT Co-Crystallization**	**70S-CPT Soaking**
Diffraction data			
Space Group		$P2_12_12_1$	$P2_12_12_1$
Unit Cell Dimensions, Å (a x b x c)		210.24, 450.36, 625.28	208.98, 446.99, 621.07
Wavelength, Å		0.9792	0.9792
Resolution range (outer shell), Å		365-2.60 (2.67-2.60)	162-2.80 (2.87-2.80)
I/σI (outer shell with I/σI=1)		8.80 (1.00)	6.43 (0.91)
Resolution at which I/σI=1, Å		2.60	2.80
Resolution at which I/σI=2, Å		2.80	3.00
CC(1/2) at which I/σI=1, %		27.7	21.8
Completeness (outer shell), %		99.3 (99.1)	98.3 (99.5)
R_{merge} (outer shell)%		12.0 (125.6)	17.5 (146.5)
No. of crystals used		1	1
No. of Reflections Used:	Observed	6,961,850	4,796,689
	Unique	1,781,049	1,384,376
Redundancy (outer shell)		3.91 (3.61)	3.46 (3.51)
Wilson B-factor, Å^2		56.3	56.5
Refinement			
R_{work}/R_{free}, %		21.7/26.4	21.9/26.8
No. of Non-Hydrogen Atoms			
RNA		199,886	200,093
Protein		90,942	90,976
Ions (Mg, K, Zn, Fe)		3,047	3,101
Waters		5,229	5,188
Ramachandran Plot			
Favored regions, %		94.33	94.69
Allowed regions, %		4.85	4.48
Outliers, %		0.83	0.84
Deviations from ideal values (RMSD)			
Bond, Å		0.004	0.005
Angle, degrees		0.917	0.889
Chirality		0.039	0.038
Planarity		0.005	0.004
Dihedral, degrees		14.967	14.870
Average B-factor (overall), Å^2		60.6	60.3

$R_{merge} = \Sigma |I - \langle I \rangle| / \Sigma I$, where I is the observed intensity and <I> is the average intensity from multiple measurements.
$R_{work} = \Sigma|F_{obs} - F_{calc}| / \Sigma F_{obs}$. For calculation of R_{free}, 5% of the truncated dataset was excluded from the refinement.

Table S2. Cisplatin modification sites in the 70S ribosome. All nucleotide residues listed in the table are modified at the N7-position of the purine base. The "Interactions" column summarizes hydrogen bonds, polar and van-der-Waals interactions.

Cisplatin modification sites in the 16S rRNA:

T. thermophilus	***E. coli***	***S. cerevisiae***	***H. sapiens***	**I/ σI**	**Nearby ribosome functional center**	**Interactions**
A774	A790	A1001	A1058	25.9	**mRNA channel**	**16S rRNA:** Hoogsteen edge of G791 2'-OH of U789 **mRNA:**2'-OH of A(-1) **P-site tRNA:** 2'-OH of A38
G1282	G1300	G1537	A1601	13.0	30S head, subunit interface	**16S rRNA:** OP2 of GG1315 OP1, OP2, N7 of G1316

Cisplatin modification sites in the 23S rRNA and ribosomal proteins:

T. thermophilus	***E. coli***	***S. cerevisiae***	***H. sapiens***	**I/ σI**	**Nearby ribosome functional center**	**Interactions**
G26	G27	G16 (5.8S)	G16 (5.8S)	16.2	**Peptide exit tunnel**	**23S rRNA:** OP1 of G533 OP2, O6 of G27 N7 of G26
G451	G425	G320	G331	13.8	50S body, cytoplasm side	**23S rRNA:** OP1 of A499 OP2, O6 of G451
G1651	G1606	G1838	C2786	19.6	50S body, cytoplasm side	**23S rRNA:** OP2 of C1650 OP2 of C1350
A1878	A1848	–	–	16.3	**Intersubunit bridge B7a**	**23S rRNA:** Hoogsteen edge of G1849 **16S rRNA:** A686
G2231/ G2232	U2220/ G2221	U2589/ G2589	C4165/ G4166	11.7	Base of the L1-stalk	**23S rRNA:** OP2 of G2231
A2542	A2531	A2900	A4477	20.8	**GTPase center, Sarcin-Ricin Loop**	**23S rRNA:** Hoogsteen edge of G2532 OP2 of A2531 **Ribosomal Protein L6:** C-terminal segment
L9-Met1	L9-Met1	–	–	13.6		

II. SUPPLEMENTARY FIGURES

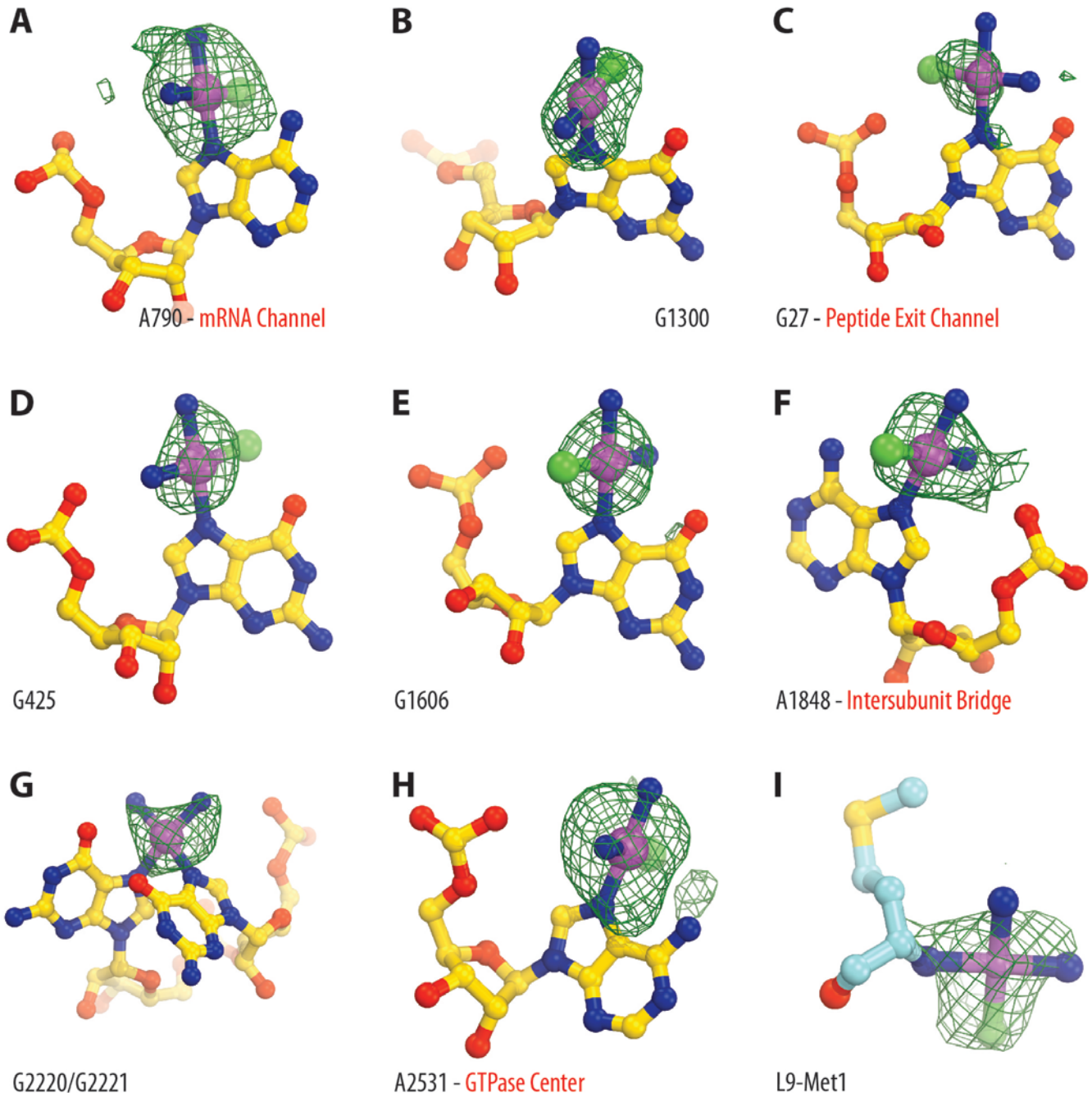

Figure S1. Anomalous difference Fourier map facilitates identification of the cisplatin modification sites within 70S ribosome. Sites of cisplatin modification are shown for 16S rRNA (**A**, **B**), 23S rRNA (**C**-**H**), and ribosomal protein L9 (**I**). The anomalous difference electron density maps are contoured at ~3σ. In panels (**A**-**H**), carbon atoms are colored yellow, nitrogens – blue, oxygens – red, platinums – magenta, chlorines – green.

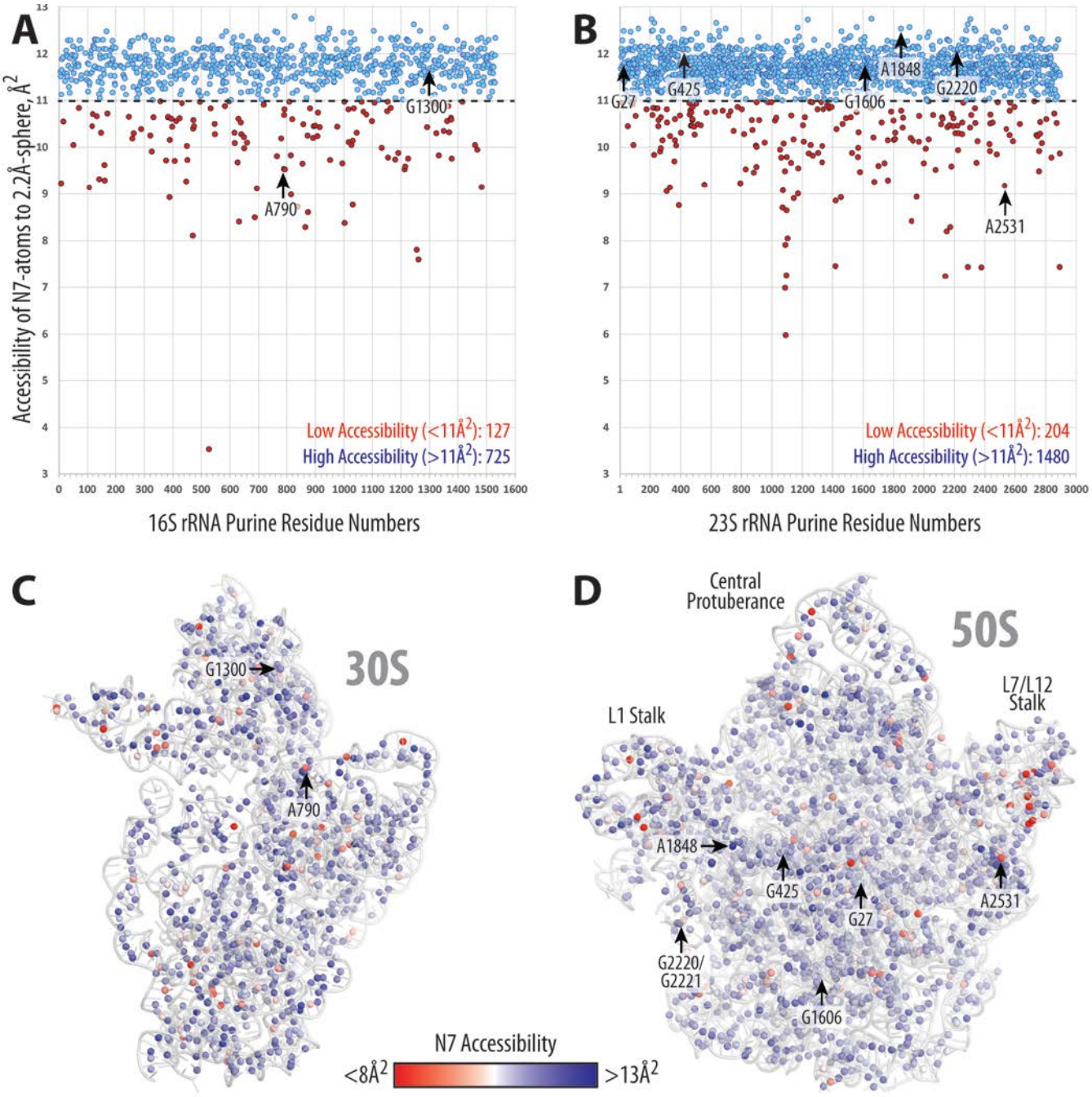

Figure S2. Solvent accessibility of the N7-atoms of purine bases in the rRNAs. Solvent accessibility plots calculated for the 16S (**A**) and 23S (**B**) rRNAs of the non-modified 70S ribosome (PDB code: 4Y4O (1)) lacking ions and water molecules. Calculations were performed using Area-Imol program from the CCP4 suite (2) using a cisplatin-size sphere of 2.2Å-radius. Red dashed line indicates arbitrary border between N7-atoms with high and low solvent accessibility. Total quantities of purine residues with high and low solvent accessibilities are indicated in the insets. Note that in the 70S ribosome the majority (>2,205) of the N7-atoms of purine residues are solvent-exposed. Also, note that cisplatin modifies not only the residues with high solvent-accessibility, but also partially buried residues, such as

A790 in the 16S rRNA or A2531 in the 23S rRNA, which points to the extreme specificity of modifications by cisplatin. (**C**, **D**) Spatial distribution of the N7-atoms of purine bases in the 30S (**C**) and 50S (**D**) subunits of the 70S ribosome. Ribosomal proteins are not shown for clarity. Locations of the cisplatin modification sites are shown by arrows. N7-atoms are shown as spheres and colored according to their accessibility to the 2.2Å-radius probe.

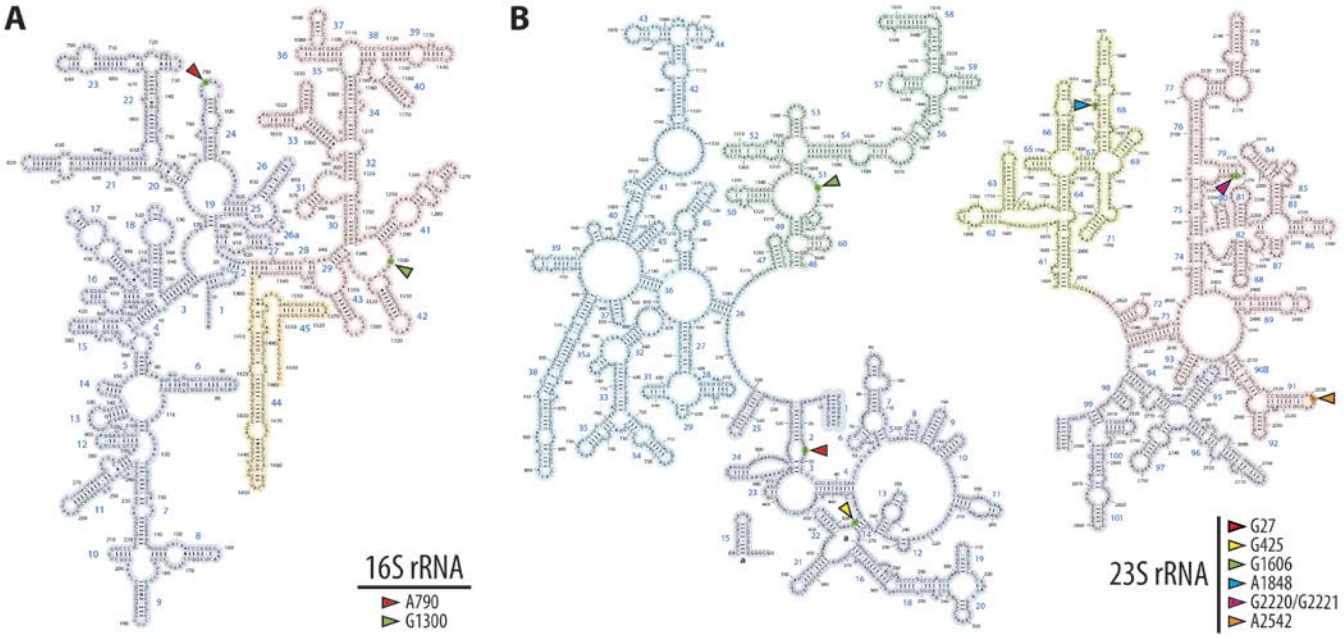

Figure S3. Positions of cisplatin-binding sites in the secondary structure diagrams of *T. thermophilus* 16S (A) and 23S (B) rRNAs (3). The cisplatin-modified nucleotides are indicated by black triangles and highlighted by green (16S) and orange (23S) circles.

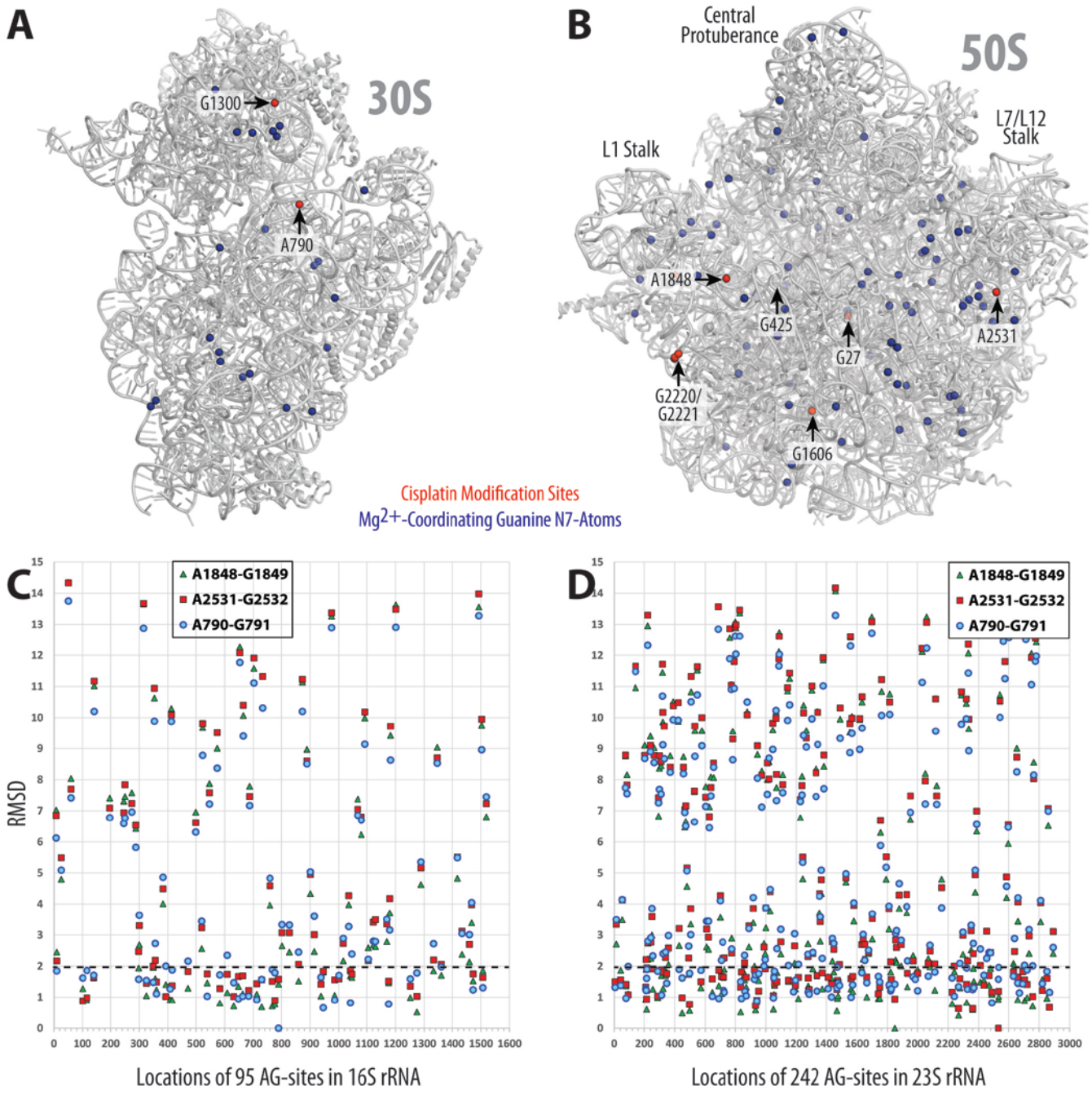

Figure S4. Differential cisplatin modifications of Mg^{2+}-coordinating guanines and of the AG-sites with specific geometry. (**A**, **B**) Locations of N7-atoms of guanine bases that coordinate Mg^{2+} ions (blue spheres) in the 16S (**A**) or 23S (**B**) rRNAs of the non-modified ribosome (PDB code: 4Y4P (1)). To identify all the guanine residues, whose N7-atoms directly coordinate Mg^{2+} ions, we used a custom-made software that searches for Mg^{2+} ions within 3.5Å from every N7-atom in a given structure. Cisplatin modification sites are shown by arrows and highlighted as red spheres. (**C**, **D**) RMS deviations of the geometry of every AG-site in the 16S (**C**) or 23S (**D**) rRNAs from the geometry of A790-G791 (blue circles), A1848-G1849 (red squares) or A2531-G2532 (green triangles) cisplatin modification

sites. Rather than use simple RMSD calculations between all pairs of AG-sites, we used a custom-made software that aligns all the AG-sites based only on their adenines and then calculates RMSDs for only the guanines. This approach is more sensitive and gives more accurate assessment of AG-site similarity. Specifically, our algorithm (i) identifies all the AG-sites in the ribosome; (ii) superimposes all the AG-sites onto either A790/G791, A1848/G1849 or A2531/G2532 based on alignment of only adenine bases; (iii) calculates RMSDs for guanines only. Black dashed line indicates arbitrary border between high and low similarity of AG-sites with those modified by cisplatin.

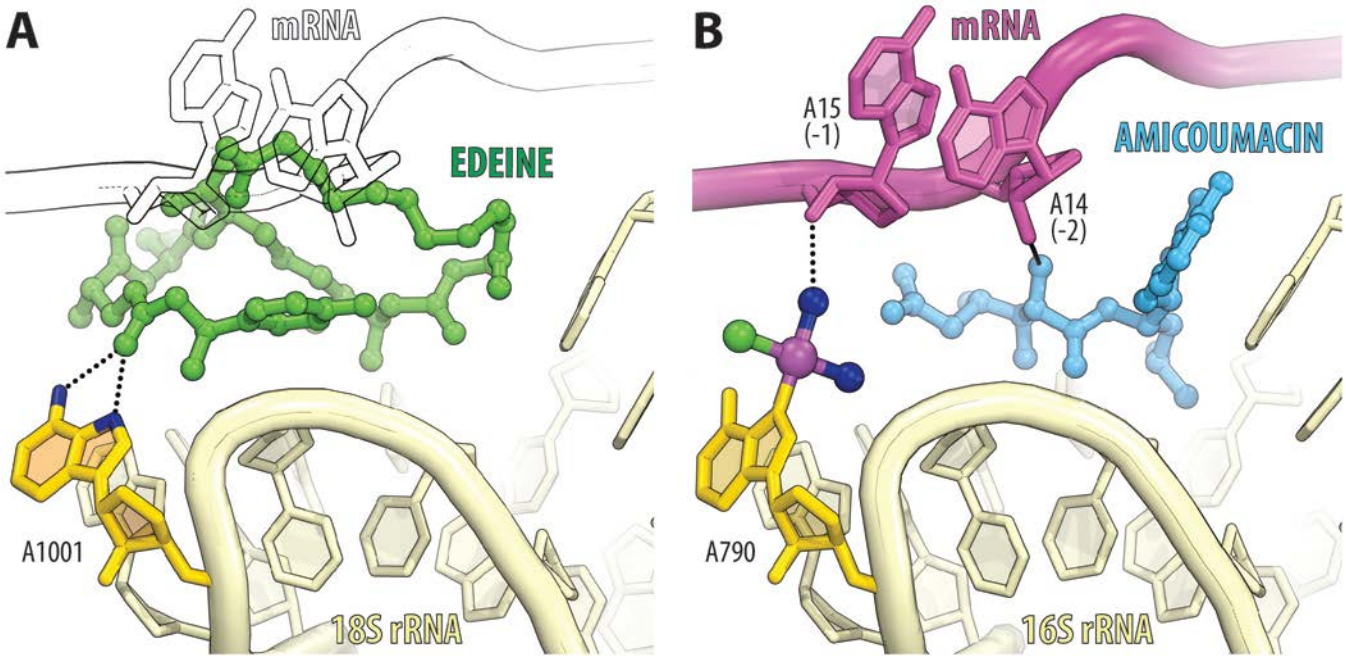

Figure S5. Similarity between cisplatin and ribosome-targeting antibiotics. (**A**) Binding site of antibiotic edeine (green) in the mRNA channel of the yeast small ribosomal subunit (PDB code: 4U4N (4)). 18S rRNA is shown in light yellow. Nucleotide A1001 of the 18S rRNA (homologous to A790 in 16S rRNA), that is important for edeine binding, is highlighted in yellow. The approximate location of mRNA is shown as transparent contour to emphasize that edeine prevents binding of mRNA. (**B**) Comparison of ribosome interactions with cisplatin (yellow, current structure) and the ribosome-targeting antibiotic amicoumacin A (blue, PDB code: 4W2F (5)). mRNA is shown in magenta. Although, cisplatin and amicoumacin A bind to slightly different but spatially close sites on the 70S ribosome, they both act in a conceptually similar manner and tether mRNA to the 16S rRNA.

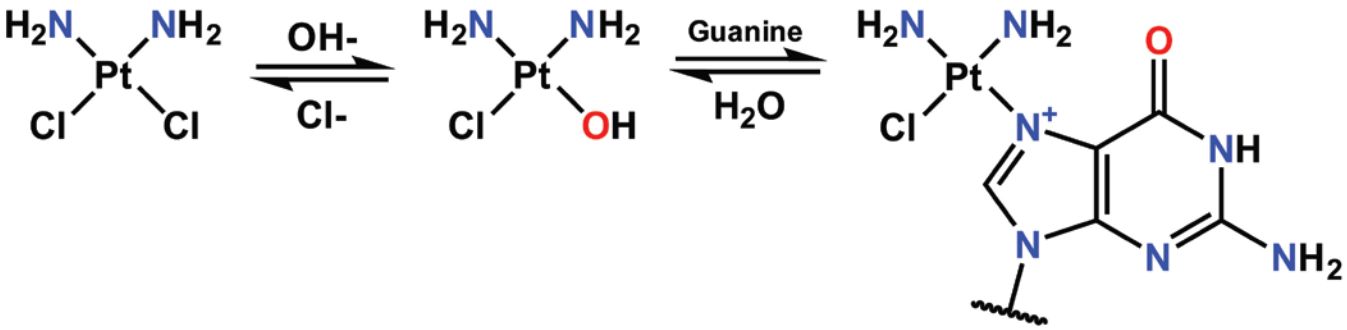

Figure S6. Scheme of cisplatin activation and its reaction with the purine nucleotides. Chemical reaction of guanine modification by cisplatin is shown as an example.

III.SUPPLEMENTARY REFERENCES

1. Polikanov, Y.S., Melnikov, S.V., Soll, D. and Steitz, T.A. (2015) Structural insights into the role of rRNA modifications in protein synthesis and ribosome assembly. *Nat. Struct. Mol. Biol.*, **22**, 342-344.

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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., Muller, K.M. *et al.* (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. Garreau de Loubresse, N., Prokhorova, I., Holtkamp, W., Rodnina, M.V., Yusupova, G. and Yusupov, M. (2014) Structural basis for the inhibition of the eukaryotic ribosome. *Nature*, **513**, 517-522.

5. Polikanov, Y.S., Osterman, I.A., Szal, T., Tashlitsky, V.N., Serebryakova, M.V., Kusochek, P., Bulkley, D., Malanicheva, I.A., Efimenko, T.A., Efremenkova, O.V. *et al.* (2014) Amicoumacin A inhibits translation by stabilizing mRNA interaction with the ribosome. *Mol. Cell*, **56**, 531-540.