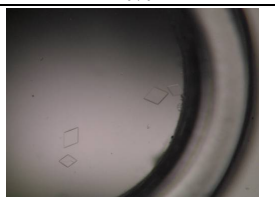
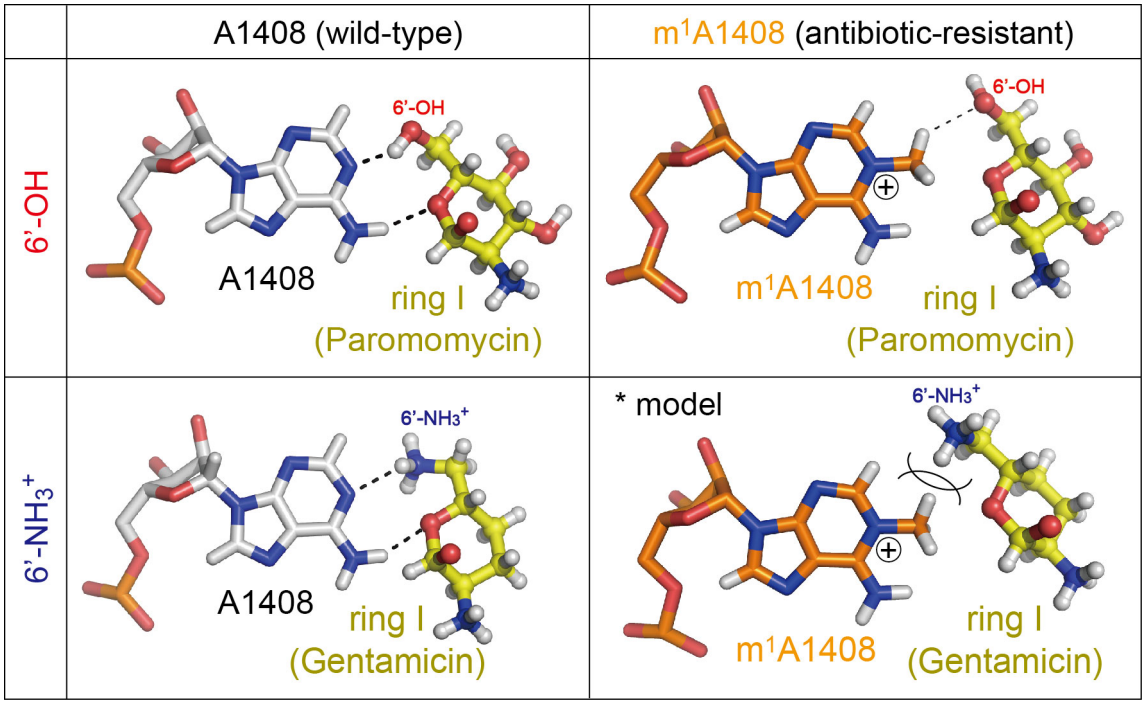
Supplementary Table 1. Crystallization conditions

Crystal code	A1408m^{1}A	A1408m^{1}A-G418
Temperature	20 °C	20 °C
Sample solution (1 µl)		
RNA	1 mM	1 mM
Aminoglycoside	-	2 mM (G418)
Sodium cacodylate (pH = 7.0)	50 mM	50 mM
Crystallization solution (1µl)		
Sodium cacodylate (pH = 7.0)	50 mM	50 mM
Spermine tetrahydrochloride	10 mM	10 mM
Ammonium chloride	250 mM	400 mM
Potassium chloride	-	-
2-Methyl-2,4-pentanediol	10%	10%
Reservoir solution (250 µl)		
2-Methyl-2,4-pentanediol	40%	40%
Crystals		
Size of crystal (mm^3)	0.10 × 0.07 × 0.01	0.07 × 0.07 × 0.01

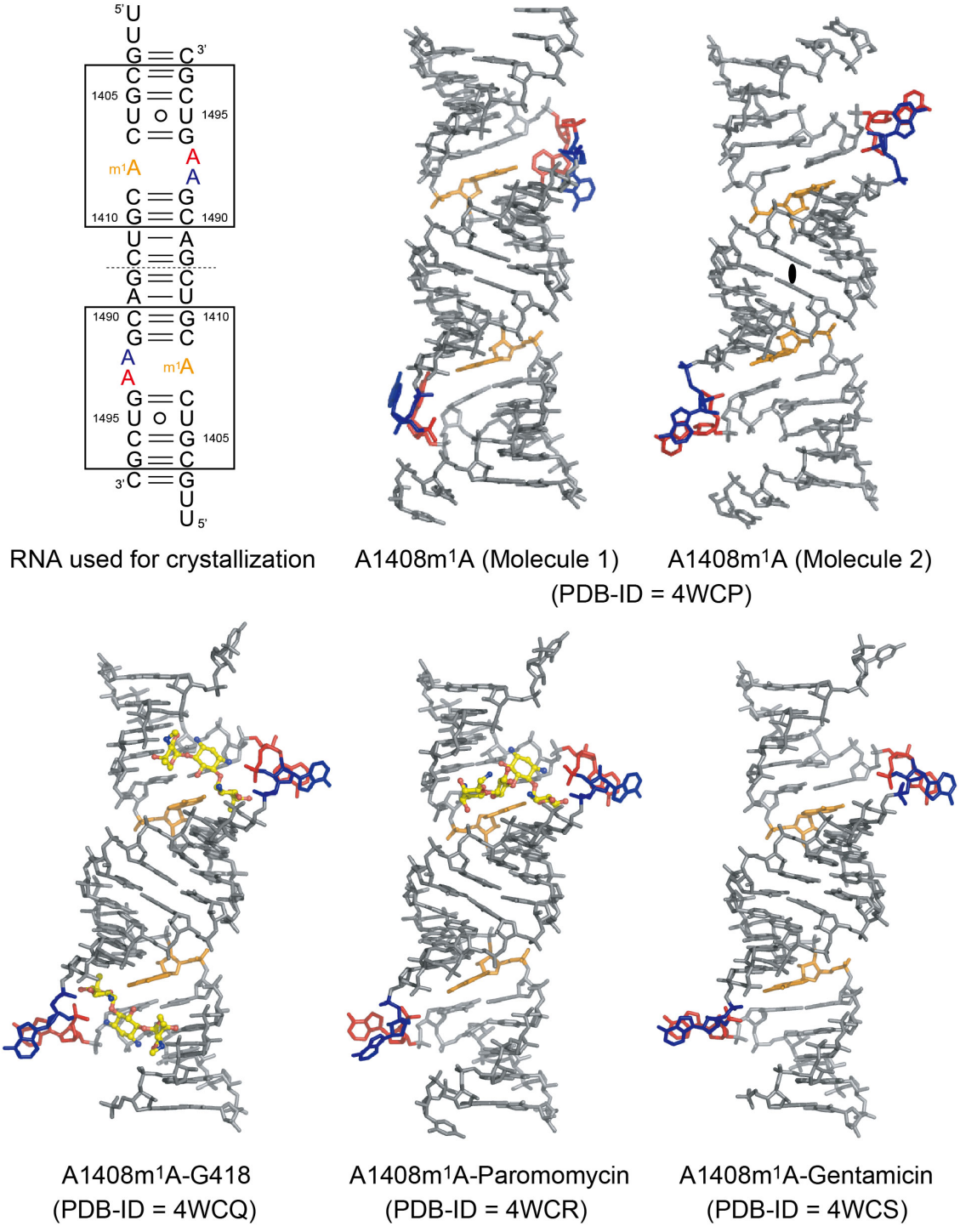
Crystal code	A1408m^{1}A-Paromomycin	A1408m^{1}A-Gentamicin
Temperature	20 °C	20 °C
Sample solution (1 µl)		
RNA	1 mM	1 mM
Aminoglycoside	2 mM (Paromomycin)	2 mM (Gentamicin)
Sodium cacodylate (pH = 7.0)	50 mM	50 mM
Crystallization solution (1µl)		
Sodium cacodylate (pH = 7.0)	50 mM	50 mM
Spermine tetrahydrochloride	10 mM	10 mM
Ammonium chloride	400 mM	-
Potassium chloride	-	250 mM
2-Methyl-2,4-pentanediol	10%	10%
Reservoir solution (250 µl)		
2-Methyl-2,4-pentanediol	40%	40%
Crystals		
Size of crystal (mm^3)	0.05 × 0.05 × 0.01	0.07 × 0.07 × 0.01

Supplementary Table 2. Susceptibilities of aminoglycosides reported in Wachino *et al*., 2007.

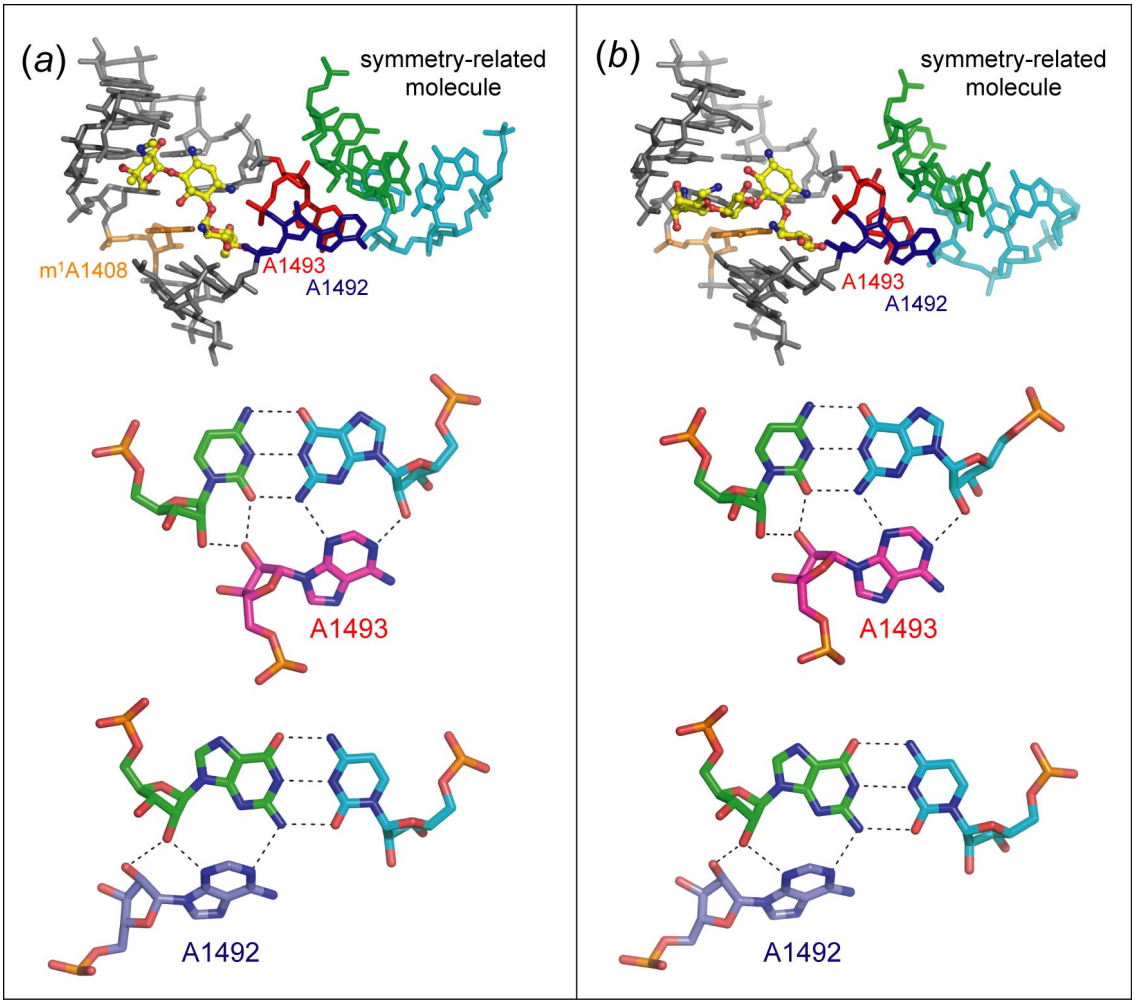
Aminoglycosides	Minimum inhibitory concentration (μg/ml)		
	E. coli CSH-2	*E. coli* ARS3 (Clinical isolate)	*E. coli* CSH-2 (pARS3) (Transconjugant)
4,6-Disubstituted aminoglycosides			
(6'-OH subtype)			
Arbekacin	0.13	64	4
(6'-NH_3^+ subtype)			
Amikacin	0.13	256	16
Dibekacin	0.13	> 256	128
Kanamycin	0.25	> 256	> 256
Tobramycin	≤ 0.06	> 256	128
Gentamicin	≤ 0.06	> 256	128
Isepamicin	0.13	> 256	64
Netilmicin	≤ 0.06	> 256	> 256
Sisomicin	≤ 0.06	> 256	> 256
4,5-Disubstituted aminoglycosides			
(6'-OH subtype)			
Paromomycin	0.5	64	4
(6'-NH_3^+ subtype)			
Lividomycin A	0.5	256	16
Neomycin	0.13	> 256	64
Ribostamycin	0.25	> 256	> 256



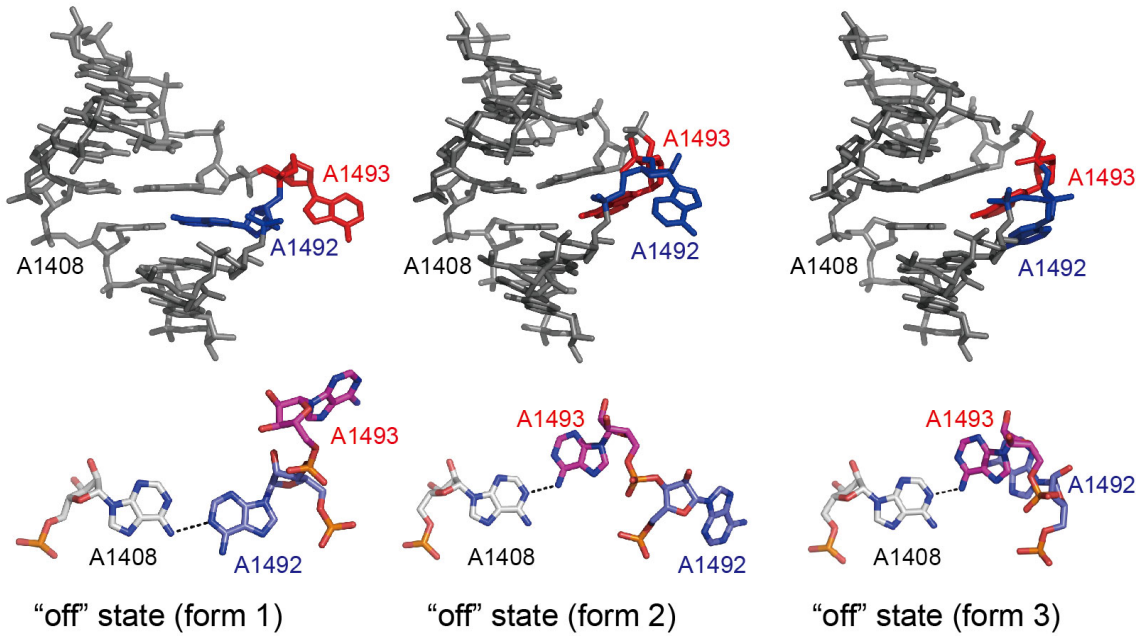
Supplementary Figure 1. Pseudo pairs between A/m^{1}A1408 and ring I with the 6'-OH or 6'-NH_3^+ groups. An impossible pseudo pair between m^{1}A1408 and ring I of gentamicin was constructed by molecular modeling. Other pseudo pairs were observed in crystal structures (PDB-ID = 1J7T, 2ET3 and 4WCR).



Supplementary Figure 2. Secondary structure of RNA used for crystallization and overall structures obtained in this study. The m^1A1408, A1492 and A1493 residues are colored in orange, blue and red, respectively. Aminoglycosides G418 and paromomycin are represented by yellow ball-and-stick model. A crystallographic two-fold axis located at the center of the molecule 2 in the A1408m^1A crystal is indicated by black oval.



Supplementary Figure 3. The "on" state of the m^{1}A1408-modified A site in complex with G418 (*a*) and paromomycin (*b*). In crystals, a symmetry-related molecule (green and cyan) mimics the tRNA-mRNA complex (top), and two Watson-Crick G=C base pairs of the molecule are recognized by A1492 and A1493 through the A-minor interactions (middle and bottom).



Supplementary Figure 4. Three different “off” states of the bacterial wild-type A site observed in crystal structures (PDB-ID = 1T0E and 3BNL). Overviews (above) and A1408oA1492 and A1408oA1493 base pairs (below) are shown. Hydrogen bonds are represented by dashed lines.