Supporting Information

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SI Text

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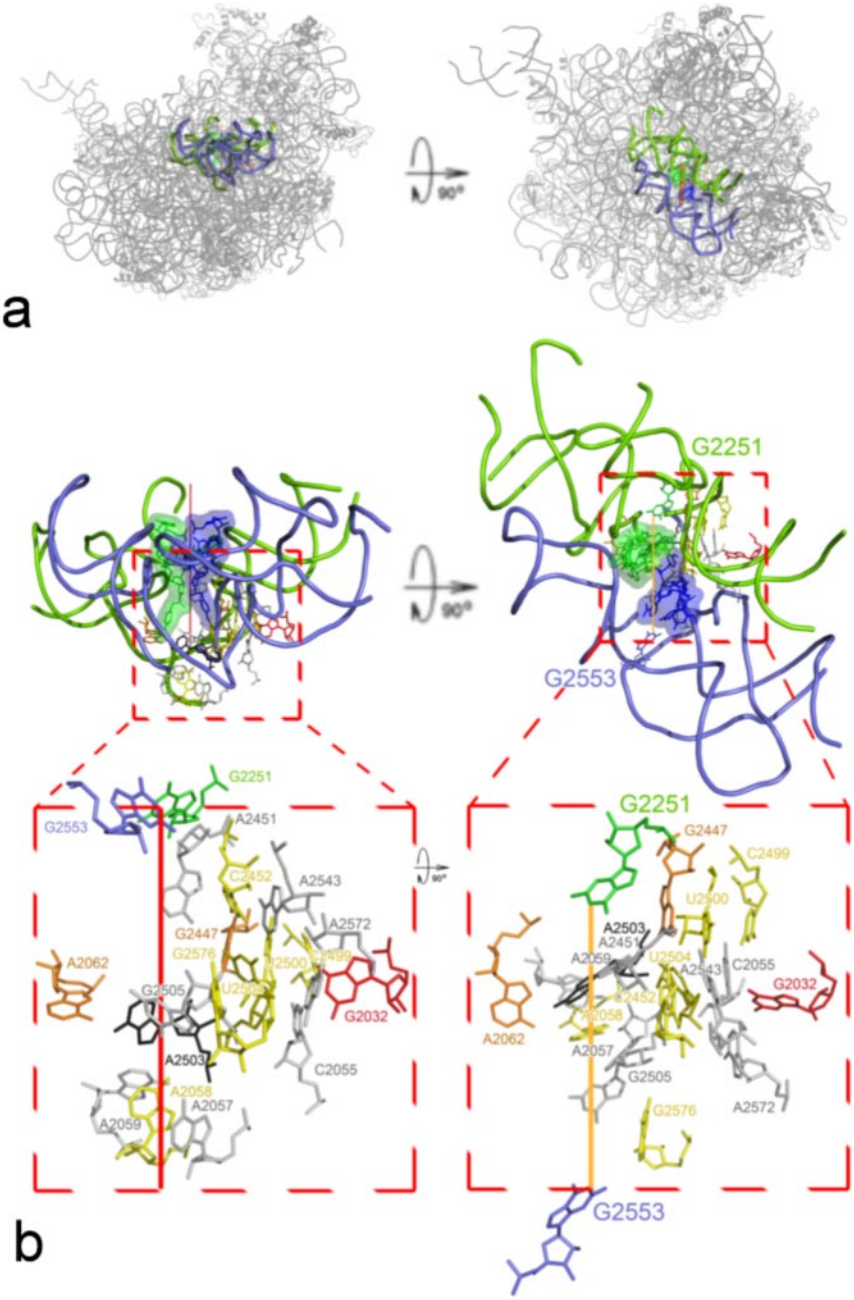

Fig. S1. The centrality of the symmetrical region and the 2 perpendicular axes that define the planes confining the PTC regions that participate in antibiotic resistance. In all: images were captured perpendicular to the 2-fold symmetry axes (*Right*) and to the axes defined by the line connecting G2553 to G2251 (*Left*). The rRNA backbone is shown in gray, except for the symmetrical region, where the subregion containing the A loop (called A region), is colored blue and that containing the P-loop (the P-region) is shown in green. The imaginary 2-fold rotation axis is shown in red. The color code for substrate mimics and nucleotides involved in resistance is as in Fig. 2. (*a*) Two views of the large ribosomal subunit showing the centrality of the internal 2-fold symmetry region. The left side shows the large subunit as seen from its interface surface, and the right side is a view taken into the PTC. (*b*) The symmetrical region with the two imaginary axes relevant to cross-resistance. (*Left*) The 2-fold symmetry axis (in red). (*Right*) The line connecting G2553 to G2251, which divides the front and back walls of the PTC (in orange). The 2 pseudo-symmetry regions of the PTC, including the A loop and the P loop, are pale blue and green, respectively. Zooms into these views, in which the nucleotides involved in resistance or reduced susceptibility are depicted, are shown in *Lower*.

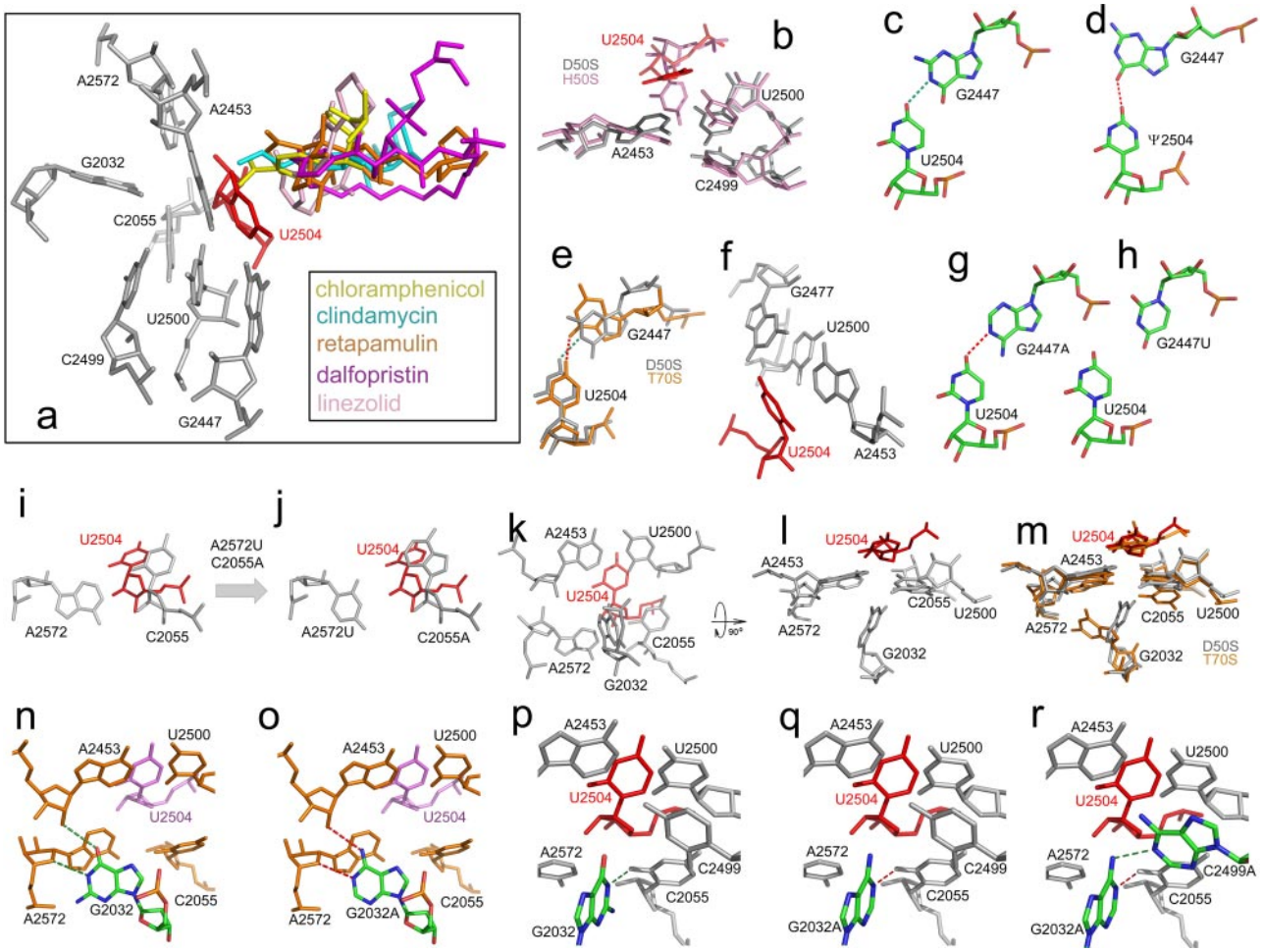

Fig. S2. Remote mutations that affect the conformation and/or the flexibility of U2504 by a network of interactions. (*a*) PTC antibiotics chloramphenicol (yellow), clindamycin (cyan), retapamulin (orange), dalfopristin (magenta), and linezolid (pink) bind in close proximity to U2504 (red). (*b*–*r*) The interactions network around U2504. rRNA is gray, pink, or orange for D50S, H50S, or T70S, respectively. Wherever indicated U2504 is red for D50S or purple for T70S. When applicable, different atoms are colored according to CPK color code to emphasize the chemical differences occurring by the mutagenesis. CPK colored nucleotides in n and o are of T70S, *d* shows E70S nucleotides after *in silico* modification from U to pseudo uracil at position 2504. All other images colored according to CPK are of D50S. Images of D50S, T70S, E70S, and H50S were generated from their coordinates (PDB ID codes 1NKW, 2J01, 2AWD, and 1S72, respectively).

Table S1. Nucleotides mediating PTC antibiotic resistance

Nucleotide	Appeared clinically (Y/N)	Antibiotic (family)	Bacterial strain	Ref.
2032	N	Chloramphenicol (phenicol)	*E. coli*	1
			B. hyodysenteriae	2
		Clindamycin (lincosamide)	*E. coli*	1
		Linezolid (oxazolidinine)	*E. coli*	3
		Tiamulin (pleuromutilin)	*B. hyodysenteriae*	2
2055	N	Tiamulin (pleuromutilin)	*B. pilosicoli*	2
2057	N	Chloramphenicol (phenicol)	*E. coli*	1, 4
2058	Y	Chloramphenicol (phenicol)	*E .coli*	1
		Clindamycin (lincosamide)	*E. coli*	1
2059	N	Virginiamycin M1 and Pristinamycin II (streptogramins A)	*H. halobium*	5
2062	Y	Chloramphenicol (phenicol)	*H. halobium*	6
		Linezolid (oxazolidinone)	*H. halobium*	7
		Pristinamycin II (streptogramins A)	*S. pneumoniae*	8
		Pristinamycin I with pristinamycin II or dalfopristin with quinupristin (streptogramins A and B)	*S. pneumoniae*	8
2447	N	Linezolid (oxazolidinone)	*E. coli*	3
			M. smegmatis	9
		Tiamulin (pleuromutilin)	*B. hyodysenteriae*	2
			E. coli	10
		Chloramphenicol (phenicol)	*E. coli* and *B. stearothermophilus*	11
2451	N	Chloramphenicol (phenicol)	*E. coli* and *B. stearothermophilus*	11
2452	N	Chloramphenicol (phenicol)	*H. halobium*	6
		Linezolid (oxazolidinone)	*H. halobium*	7
2453	N	Linezolid (oxazolidinone)	*H. halobium*	7
2499	N	Linezolid (oxazolidinone)	*H. halobium*	7
		Tiamulin (pleuromutilin)	*B. hyodysenteriae*	2
2500	Y	Linezolid (oxazolidinone)	*S. aureus* (MRSA)	12
			H. halobium	7
			E. coli	10
		Tiamulin (pleuromutilin)	*E. coli*	10
2503	Y	Chloramphenicol and florfenicol (phenicols)	*E. coli*	13
			S. aureus and *E. coli*	14, 15
			S. aureus	16
		Clindamycin (lincosamide)	*S. aureus* and *E. coli*	14,15
			S. aureus	16
		Linezolid (oxazolidinones)	*S. aureus* and *E. coli*	14, 15
			S. aureus	16
		Tiamulin and Valnemulin (pleuromutilins)	*S. aureus* and *E. coli*	14, 15
		virginamycin M_1 (streptogramins A) and	*S. aureus* and *E. coli*	14, 15
		dalfopristin/quinupristin (streptogramins A and B)	*H. halobium*	5
2504	N	Linezolid (oxazolidinone)	*H. halobium*	7
		Tiamulin (pleuromutilin)	*Brachyspira pilosicoli*	2
			B. hyodysenteriae	2
2505	N	Linezolid (oxazolidinone)	Enterococcus	17
			E. faecalis	18
2572	N	Tiamulin (pleuromutilin)	*B. hyodysenteriae*	2
2576	Y	Linezolid (oxazolidinone)	*Enterococcus*	17
			Enterococcus	19
			S. aureus	20
			E. faecium	21
			S. aureus	22
			S. aureus	23
			E. faecium	24
		Tiamulin (pleuromutilin)	*S. aureus*	10