Supplementary Information for

Structure of dirithromycin bound to the bacterial ribosome suggests new ways for rational improvement of macrolides.

Nelli F. Khabibullina[1,*], Andrey G. Tereshchenkov[2,*], Ekaterina S. Komarova[3,4], Egor A. Syroegin[1], Dmitrii I. Shiriaev[2], Alena Paleskava[5,6], Victor G. Kartsev[7], Alexey A. Bogdanov[2], Andrey L. Konevega[5,6,8], Olga A. Dontsova[2,4,9], Petr V. Sergiev[2,4], Ilya A. Osterman[2,4,#], and Yury S. Polikanov[1,10,11,#]

[1] Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA
[2] Department of Chemistry and A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119992, Russia
[3] Department of Bioengineering and Bioinformatics and A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, 119992, Russia
[4] Skolkovo Institute of Science and Technology, Skolkovo, Moscow region, 143025, Russia
[5] Petersburg Nuclear Physics Institute, NRC "Kurchatov Institute", Gatchina, 188300, Russia
[6] Peter the Great St.Petersburg Polytechnic University, Saint Petersburg, 195251, Russia
[7] Interbioscreen Ltd, Chernogolovka, Moscow Region, 142432, Russia
[8] NRC "Kurchatov Institute", Moscow, 123182, Russia
[9] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, 117997, Russia
[10] Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL 60607, USA
[11] Center for Biomolecular Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA

* Authors contributed equally to this work
[#] To whom correspondence should be addressed:
E-mail: i.osterman@skoltech.ru (I.A.O.)
yuryp@uic.edu (Y.S.P.)

This file includes:

I. Supplementary Table S1;
II. Supplementary Figures S1 to S4 with legends;
III. Supplementary References.

I. SUPPLEMENTARY TABLES

Table S1. X-ray data collection and refinement statistics.

Crystals		**70S complex with A-, P- and E-tRNAs and Dirithromycin**
Diffraction data		
Space Group		$P2_12_12_1$
Unit Cell Dimensions, Å (a x b x c)		208.15 x 446.4 x 615.53
Wavelength, Å		0.9795
Resolution range (outer shell), Å		361-2.80 (2.87-2.80)
I/σI (outer shell with I/σI=1)		10.18 (0.93)
Resolution at which I/σI=1, Å		2.80
Resolution at which I/σI=2, Å		2.97
CC(1/2) at which I/σI=1, %		43.4
CC(1/2) at which I/σI=2, %		65.2
Completeness (outer shell), %		99.8 (99.3)
R_{merge} (outer shell)%		27.9 (309.3)
No. of crystals used		1
No. of Reflections Used:	Observed	14,260,035
	Unique	1,386,444
Redundancy (outer shell)		10.29 (9.85)
Wilson B-factor, Å^2		57.3
Refinement		
R_{work}/R_{free}, %		21.5/26.3
No. of Non-Hydrogen Atoms		
RNA		200,295
Protein		90,976
Ions (Mg, K, Zn, Fe)		2,813
Waters		4,377
Ramachandran Plot		
Favored regions, %		93.59
Allowed regions, %		5.55
Outliers, %		0.86
Deviations from ideal values (RMSD)		
Bond, Å		0.004
Angle, degrees		0.846
Chirality		0.041
Planarity		0.005
Dihedral, degrees		15.047
Average B-factor (overall), Å^2		60.0

$R_{merge} = \Sigma |I - \langle I \rangle| / \Sigma I$, 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.

II. SUPPLEMENTARY FIGURES

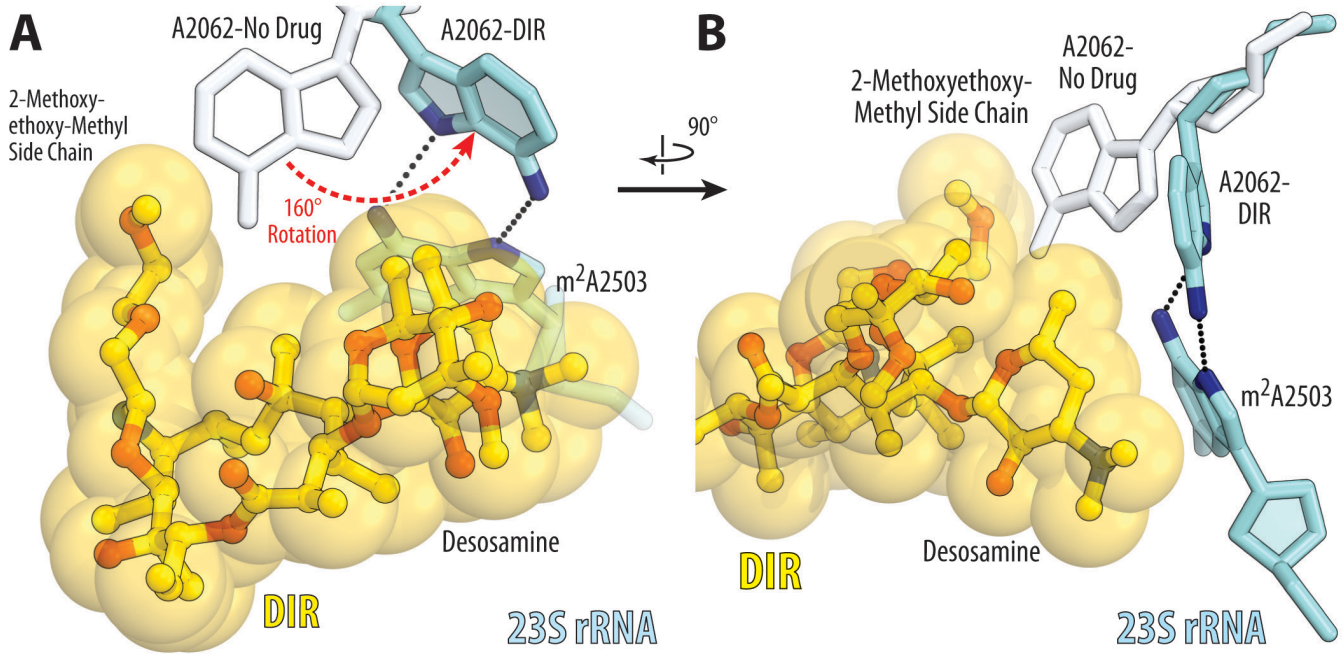

Figure S1. Re-orientation of the A2062 residue upon dirithromycin binding. Interaction of DIR (and other macrolides) with the ribosome causes nucleotide A2062 of the 23S rRNA to rotate by ~160° and form a symmetric *trans* A-A Hoogsteen/Hoogsteen base pair with the residue m^2A2503, which is favorable for the drug interaction with the wall of NPET. Carbon atoms of DIR are colored yellow, nitrogens are blue, and oxygens are red.

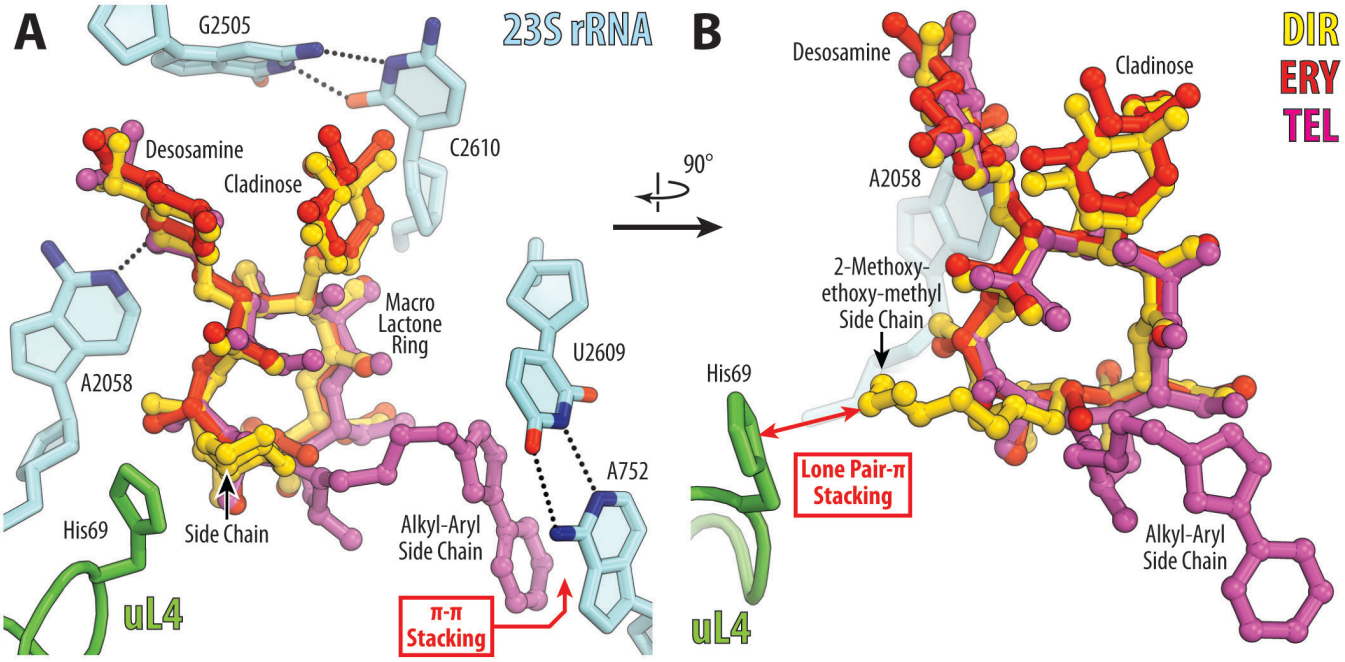

Figure S2. Side-chain-mediated contacts of dirithromycin and telithromycin with the 70S ribosome are principally different. Shown is the superposition of ribosome-bound DIR (yellow) with ERY (red, PDB entry 6ND6 (1)) and TEL (magenta, PDB entry 4V7Z (2)) viewed from two different perspectives. All structures were aligned based on the domain V of the 23S rRNA. Note that alkyl-aryl side chain of TEL establishes standard π-π stacking interaction with the A752-U2609 base-pair of the 23S rRNA, while the side chain of DIR forms lone pair-π stacking interaction with the aromatic imidazole ring of the His69 residue in the ribosomal protein uL4.

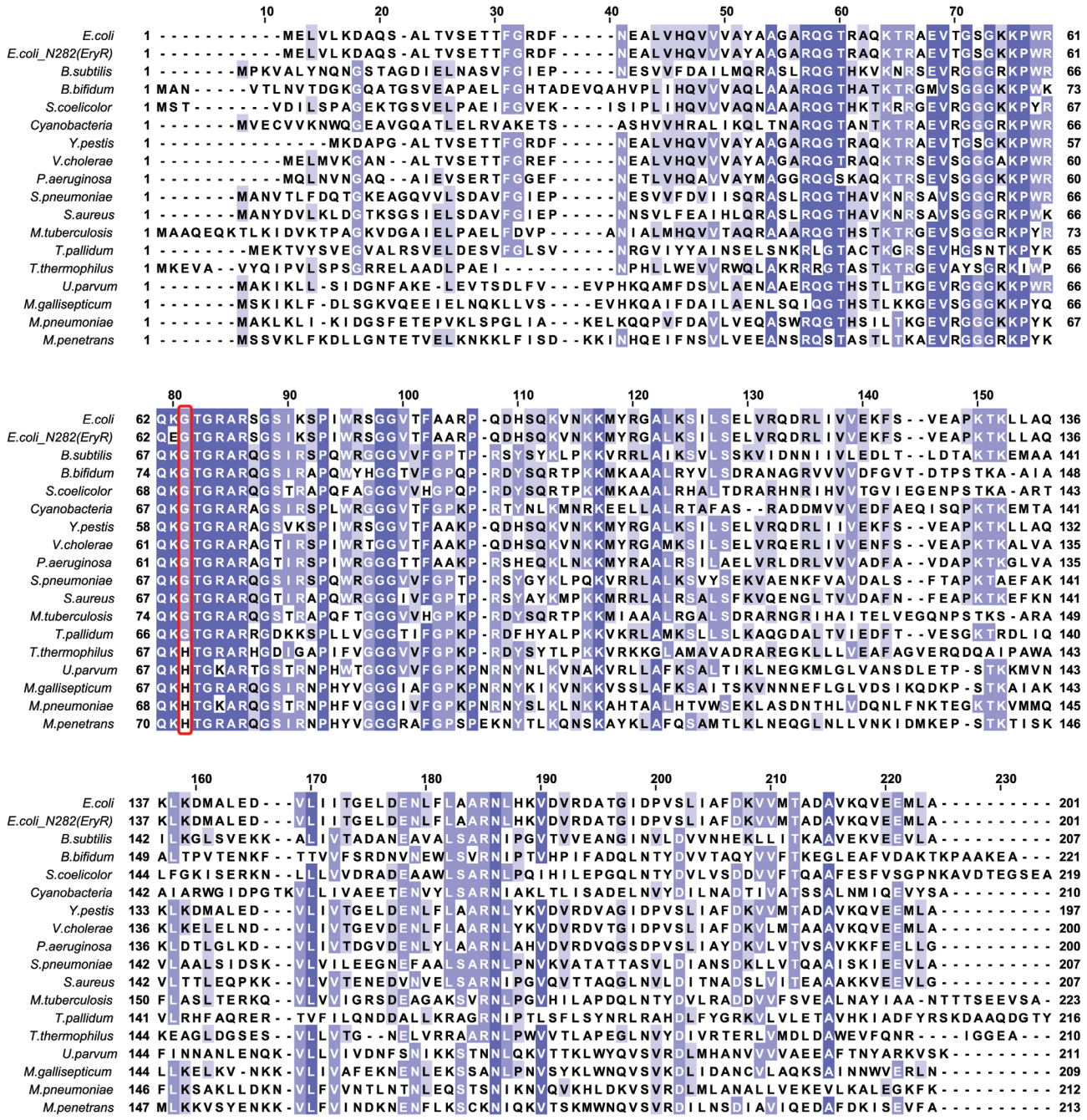

Figure S3. Multiple sequence alignment of the ribosomal protein L4 from several bacterial species. Red box highlights the amino acid residue of uL4, which contacts the side chain of DIR in our structure. Note that the preceding conserved lysine residue is mutated to glutamic acid in one of the *E. coli* strains resistant to erythromycin.

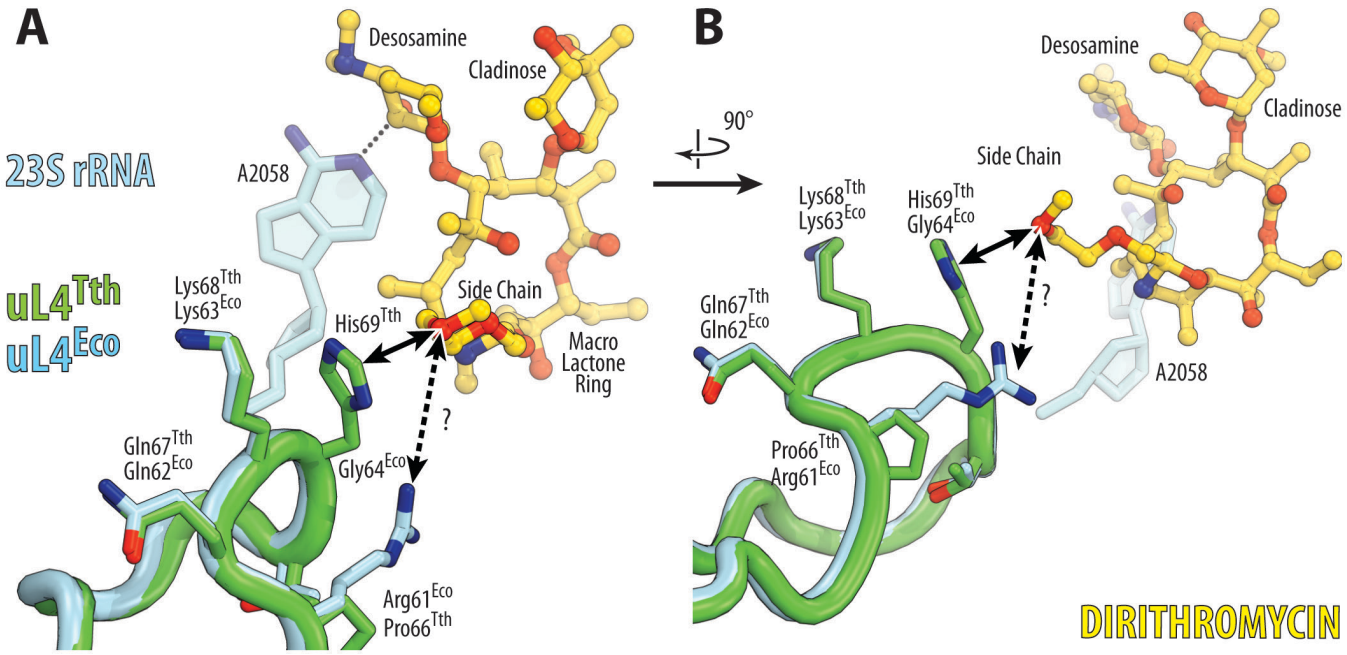

Figure S4. The side-chain of dirithromycin could potentially form a contact with the loop of *E. coli* protein uL4. Shown is the superposition of the ribosomal protein uL4 from *T. thermophilus* ribosome (green) in complex with DIR (yellow) with the same protein from drug-free *E. coli* ribosome (light blue, PDB entry 4YBB (3)) viewed from two different perspectives. The two structures were aligned based on the domain V of the 23S rRNA. Note that the side chain of DIR could reorient and form a contact with the residue Arg61 of the ribosomal protein uL4 in the *E. coli* ribosome (dashed arrow). The observed lone pair-π stacking interaction of DIR side chain and the residue His69 of uL4 protein in *T. thermophilus* ribosome is shown by solid arrow.

III. SUPPLEMENTARY REFERENCES

1. **Svetlov MS, Plessa E, Chen CW, Bougas A, Krokidis MG, Dinos GP, Polikanov YS.** 2019. High-resolution crystal structures of ribosome-bound chloramphenicol and erythromycin provide the ultimate basis for their competition. RNA Journal doi:10.1261/rna.069260.118.

2. **Bulkley D, Innis CA, Blaha G, Steitz TA.** 2010. Revisiting the structures of several antibiotics bound to the bacterial ribosome. Proc Natl Acad Sci USA **107:**17158-17163.

3. **Noeske J, Wasserman MR, Terry DS, Altman RB, Blanchard SC, Cate JH.** 2015. High-resolution structure of the *Escherichia coli* ribosome. Nat Struct Mol Biol **22:**336-341.