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. Syroegin¹, Dmitrii I. Shiriaev², Alena Paleskava^{5,6}, Victor G. Kartsev⁷,

Alexey A. Bogdanov², 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: <u>i.osterman@skoltech.ru</u> (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.

Page | 2 of 7

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	P212121				
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)				
$I/\sigma I$ (outer shell with $I/\sigma I=1$)	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 Observed	14,260,035				
Used: 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				
lons (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				

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

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 $\sim 160^{\circ}$ and form a symmetric *trans* A-A Hoogsteen/Hoogsteen base pair with the residue m²A2503, 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.

		10	20	3	0	40	50	6 0	70	
E.coli	1	MEL	VLKDAQS -	ALTVSETT	FGRDF -	NEALVHQ	VVVAYAAG	RQG T <mark>RAQ</mark> K TR	AEV TG SG KKP WR	61
E.coli N282(ErvR)	1	MEL	VLKDAQS -	ALTVSETT	FGRDF -	NEALVHQ	VVVAYAAG	ROGTRAOKTE		61
B subtilis	1	- MPKVALY	NONGSTAG		EGIEP.		ALLMORASI	ROGTHKVKNE	SEVEGGERKEWE	66
B bifidum	1 MAN		DGKGOATO	SVEADAEL	EGHTAD	EVOAHVELIHO	VVVAOLAA			73
S coolicolor	1 MGN		SDACEKTO	SVEL DAEL	E CVEK		VVVAONAA			67
S.coelicolor	1 W 5 I		SPAGENIG	SVELPAET	GVER-		V V AQ NAAA			0/
Cyanobacteria	1	MVECVVK	NWQGEAVG	QATLELRV	AKETS -	ASHVVHR	ALIKQLIN	ARQG I <mark>ANT</mark> K I F	AE VRGGGRKPWR	66
Y.pestis	1		-MKDAPG	ALTVSETT	FGRDF -	NEAL VHQ	V V V A Y A A G A	ARQG T <mark>RAQ</mark> K TF	R	57
V.cholerae	1	MEL	MVKGAN	ALTVSETT	FGREF -	NEALVHQ	V V V A Y A A G /	RQG T <mark>RAQ</mark> K TF	א <mark>S</mark> EV <mark>S</mark> GGG <mark>A</mark> KPWR א	60
P.aeruginosa	1	MQL	NVNGAQ - ·	AIEVSER1	FGGEF-	NETLVHQ	AVVAYMAGO	GRQG <mark>SKAQ</mark> K <u>T</u> F	א <mark>SEVS</mark> GGG <mark>K</mark> KPWR	60
S.pneumoniae	1	MANVTLF	DQTGKEAG	GVVLSDAV	FGIEP-	N E S V V F D	VIISQRASI	RQGTHAVKNF	SAVS GGG <mark>R</mark> KPWR	66
S.aureus	1	MANYDVL	KLDGTKSG	SIELSDAV	FGIEP-	NNSVLFE	AIHLQRASI	RQGTHAVKNE	SAVSGGGRKPWK	66
M.tuberculosis	1 MAAQE	QKTLKIDVK	TPAGKVDG	AIELPAEL	DVP	ANIALMHQ	VVTAQRAA	RQGTHSTKT	GEVS GGG R KP Y R	73
T.pallidum	1	MEKTVY	SVEGVALE	SVELDESV	FGLSV-	NRGVIYY	AINSELSN		SEVHGSNTKPYK	65
T thermonhilus		VYOLPVI	SPSGRREI			NPHI I WE	VVRWOLAKE	RRGTASTKT	GEVAYSGRKIWP	66
II nan/um	1	MAKIKII	SIDGNE			- EVPHKOAMED	SVLAENAAR			66
0.parvum	1		DISCKVC							
w.gaiisepucum	1	WSKIKLF	-DLSGRVG			····	ATLAENLSC			
M.pneumoniae	1	MAKLKLI	-KIDGSFE	TEPVKLSP	GLIA	- KELKQQPVFD	AVLVEQAS	VRQGIHSILIP	GE VRGGG KKPYK	67
M.penetrans	1	MSSVKLF	KDLLGNTE	TVELKNKK	(LFISD-	- KKINHQEIFN	SVLVEEANS	S RQ S TA S T L TH	KAEVRGGGRKPYK	(
	80	90	1	00	110	120	130	140	150	
E coli		DADECELKE	DIWBSCC							426
				TEAARD O			IL SELVRQI		VEAPKTKLLAG	130
E.coll_N282(EryR)	62 QEGIG	RARSGSIKS	PTWRSGG	IFAARP-G	DHSQKV	NKKMYRGALKS	ILSELVRQI	ORLIVVEKES.	-VEAPKIKLLAG	136
B.subtilis	67 Q K G T G	IRARQG SIRS	PQWRGGG V		KSYSY <u>k</u> L	PKKVRRLAIKS	VLSSKVIDI	INTIVLEDLT	-LDTAKTKEMAA	141
B.bifidum	74 Q K G T G	RAR <mark>Q</mark> GSIR <mark>A</mark>	PQWYHGG1	IVFGPQP-F	RDYSQRT	P K K M K A A A L R Y	VLSDRANAC	BRVVVVDFGV1	- D T P S T K A - A I A	148
S.coelicolor	68 Q K G T G	RAR <mark>Q</mark> GSTR <mark>A</mark>	. P <mark>Q F A</mark> G G G V	/ <u>V H</u> G P <mark>Q P -</mark> F	R D Y SQ R T	P <mark>KK</mark> MKAAAL RH	ALTDRARH	IR I H V V TG V I E	GENP <u>STKA</u> - ART	143
Cyanobacteria	67 Q K G T G	RAR <mark>A</mark> GS <u>IR</u> S	PLWRGGG \	/ T F <u>G P</u> K P - F	R T Y N L KM	NRKEELLALRT	AFAS RAI	DMVVVEDFAE	EQ I SQ <mark>P K T K</mark> E M T A	141
Y.pestis	58 Q K G T G	RAR <mark>A</mark> GS <mark>VKS</mark>	PIWRSGG	TFAAKP-G	DHSQKV	N K K M Y R G A L K S	ILSELVRQ	ORLIIVEKFS ·	- VEAPKTKLLAQ	132
V.cholerae	61 Q K G T G	RAR <mark>A</mark> G T IRS	PIWRTGG	TFAAKP-C	DHSQKV	N K K M Y R G A M K S	ILSELVRQE	RLIVVENFS	- VEAPKTKALVA	135
P.aeruginosa	61 Q K G T G	RAR <mark>A</mark> GTIRS	PIWRGGG	TEAAKP-E	SHEQKL	NKKMYRAALRS	ILAELVRLD	RLVVVADFA	- VDAPKTKGLVA	135
S nneumoniae	67 OKGTG	RAROGSIRS	POWRGGG		SYGYKI	POKVERLALKS	VYSEKVAE	KEVAVDALS	- E TAPKTAFFAK	141
S aurous	67 OKGTG	PAPOCTIPA	POWREGE	VEGPTP	SVAVKM		ALSEKVOEN	GI TVYDAEN.		1 1 1 1
M tuboroulooio		PAROCSTRA			DYSODT		AL SDRARNO		CONDETKE ADA	140
W.IUDerculosis					DEUVAL		ALSOKARNO			149
r.pailidum		RARKGUKKS	PLLVGGG	I F G P K P - F	DENTAL	PKKVKKLAWKS	LLSLKAQGI	JALIVIEDEI	-VESGNIRDLIG	140
I.tnermophilus	67 QKHIG	RARHGDIGA	PTFVGGGV	VFGPKP-F	DYSYIL		AVADRAREC	KLLLVEAFAG	VERQDQATPAWA	143
U.parvum	67 Q K H T G	KARTGSTRN	IP H WTGGGV	/ V F G P K P N F	RNYNLKV	NAKVRLLAFKS	ALTIKLNEC	GKMLGLVANSE	0 L E T P - S T K K M V N	143
M.gallisepticum	67 Q K H T G	RARQGSIR <mark>N</mark>	IPHYVGGG I	AFGPKPNF	RNY K I KV	NKKVSSLA <u>FK</u> S		NEFLGLVDSIM	(QDKP - <u>STK</u> AIAK	(143
M.pneumoniae	68 Q K H T G	KARQGSTR <mark>N</mark>	IPHFVGGG I	V F G P K P N F	NYSLKL	NKKAHTAALHT	VWSEKLASI	DN <u>T</u> HL <u>V</u> DQNLF	NKTEG <mark>KTK</mark> VMMQ	145
M.penetrans	70 Q K H T G	RAR <mark>Q</mark> G SIR <mark>N</mark>	I P H Y V G G G F	RAFGPSPEM	(N Y T L KQ	N S <mark>K</mark> A Y K L A F Q S	AMTLKLNEG	Q G L N L L V N K I C	MKEP - STKTISK	(146
	<u> </u>									
	160) 17	70	180	190	200	210	220	230	
	1	· · · · · · · · · · · · · · · · · · ·		177 178 - 188 - 198 - 198	,					
E.coli	137 K KDM	1ALED VL	IITGELD	EN LF L A ARN		RDATGIDPVSL	IAFDKVVM.	TA DAVKQVEEN	1L A	201
E.coli_N282(EryR)	137 K KDM	1ALED VL	IITGELD	NLFLAARN		RDATGIDPVSL	IAFDKVVM	A D A V KQ V E E N	1 L A	201
B.subtilis	142 ILKGL	. S V E K K A L	IVTADAN	AVALSARN	IPGVTV	VEANGINVLDV	VNHEKLLI	KAAVEKVEE\	′ <mark>L</mark> A	207
B.bifidum	149 A 🛯 T P V	TENKF T	VVFSRDN	NEWLSVRN	I P T V H P	IFADQLNTYDV	<u>V T A Q Y V V</u> F	KEGLEAFVD#	KTKPAAKEA	221
S.coelicolor	144 LFGKI	SERKN LL	LVVDRAD	AAWLSARN	LPQIHI	LEPGQLNTYDV	LVSDDVVF	QAAFESFVSG	PNKAVDTEGSEA	219
Cyanobacteria	142 AIARW	VG I D P G T K V L	LIVAEET	NVYLSARN	IAKLTL	ISADELNVYDI	LNADTIVA	SSALNMIQE	YSA	210
Y.pestis	133 K KDM	1ALED VL	IVTGELDE		LYKVDV	RDVAGIDPVSL	LAFDKVVM	ADAVKQVEEN	1 L A	197
V.cholerae	136 KLKEL	ELND VL	IVTGEVDE			RDVTGIDPVSL	LAFDKVLM	AAAVKQVEEN	1L A	200
P aeruginosa	136 K DTL	GI KD VI				RDVOGSDRVSL	LAYDKVLV	VSAVKKEEEI		200
S nnoumoniao		SIDSK	VILEEGN			ATATTASVIDI	ANSDRULV			207
S.priounionide	142 V TT	EOBKK	VVTENER			TTAOGLNVIDI	TNADELVI			207
S.aureus	142 V 1 1 L		VVLCDED							20/
M.tuberculosis	150 FLASL		VVIGRSD	AGAKSVRN	PGVHT		LKADUVVFS		-NILISEEVSA-	223
r.pallidum	141 V RHF	AQRER TV	FILQNDDA	LLKRAGRN	IPTLSF	LSYNRLRAHDL	FYGRKVLVL	ETAVHKIADE	TRSKDAAQDGTY	216
T.thermophilus	144 KEAGL	DGSES VL				LAPEGLNVYDI	VRTERLVM	DLDAWEVFQNF	IGGEA	210
U.parvum	144 F I N N A		LVIVDNFS		ILQKVTT	KLWYQVSVRDL	MHANVVVV	\ E E <mark>A</mark> F T N Y A <u>R</u> P	(VSK	211
M.gallisepticum	144 L <mark>LK</mark> EL	. KV - NKK - VL	IVAFEKN	NLEKSSAN	IL P N V S Y	K L WNQ V S V K D L	IDANCVLAC	Q K S 🔼 I N N W V 🖻 F	RLN	209
Mnneumoniae							MIANALIVE		EK	212
w.prieumoniae	140 FLRSA		FVVNILN	LEQSIS						

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.

Khabibullina N.F. et al.

Supplementary Information



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. Highresolution 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.