

Supplementary Material

Structural and Phylogenetic Analyses of Resistance to Next-generation Aminoglycosides Conferred by AAC(2') Enzymes

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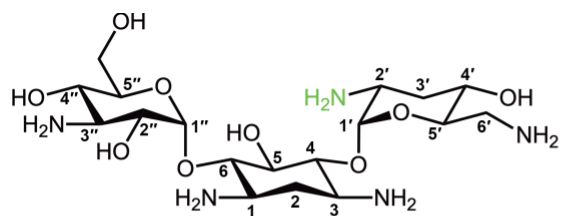
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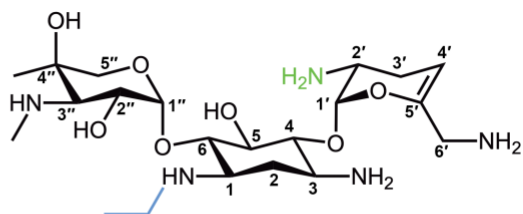
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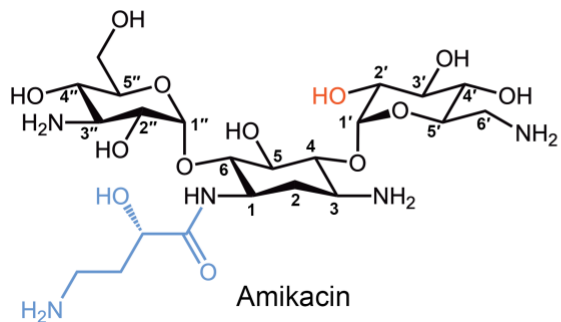
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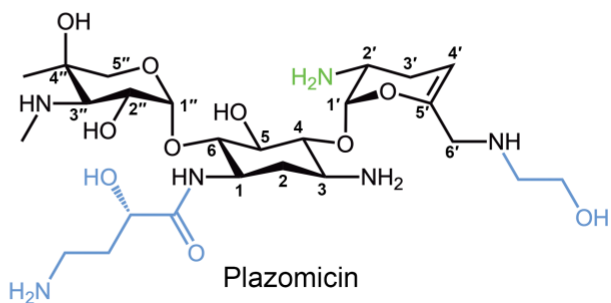
Tobramycin



Netilmicin



Amikacin



Plazomicin

Figure S1: Panel of aminoglycosides used in crystallographic studies. Next-generation aminoglycoside features are colored in blue. Site of 2'-acetylation is colored in green, while 2'-hydroxyl of non-substrate, amikacin, is colored in red. Carbons are numbered as per their aminoglycoside ring, i.e., central ring, prime ring, or double-prime ring.

				↓Asp37				
Non-binders	<i>Cellulosimicrobium cellulans</i>	1	MT	----	TTSDASYTLESVDWADPRAAALRDAMDAEMGARYASAGPMSAAAHAALTVDPAA	56		
	<i>Mycobacter senuensis</i>	1	MF	----	GQVNTARLIHTSDLDDDTRRGARQLVDRAFGASG-DAPPGHWHALGGM----	51		
Unlikely binders	<i>Chloroflexus aggregans</i>	1	MN	----	TRLTIQVVPSSAHLSDADRKTIIALCNRAY-ED-DLE--PLFDTFVGAT----	46		
	<i>Klenkia soli</i>	1		-----	-----MPTANLTAERTALRDLDGAF-AGDDPFD-EDWGHALGGV----	40		
Likely binders	<i>Vibrio cholerae</i>	1		-----	-----MLISVNLNDELDEETLHELIKLCEAF-EE--DFN--YFSLKNAK----	42		
	<i>Acinetobacter baumannii</i>	1		-----	-----MQFKIQAQDLDRNQRHQIADLCFSAF-DE--DPW--SQYAFMQKA----	42		
Binders	<i>Providencia stuartii</i> [AAC(2')-Ia]	1	MG	----	-----IEYRSLHTSOLTLSEKEALYDLLEGF-EG-DFSH-DDFAHTLGGM----	45		
	<i>Mycobacterium tuberculosis</i> [AAC(2')-Ic]	1	MH	----	-----TQVHTARLVHTADLDSETRQDIQRMVTGA-F-AG-DFTE-TDWEHTLGGM----	48		
	<i>Mycobacterium arabiense</i>	1		-----	----------MVIDAFGGGETDFTD-ADWEHALGGM----	25		
Plasmid-encoded	<i>Deinococcus wulumuqiensis</i>	1	MT	TRPGRPAQALTLRVRHTAHPGAGRAALREL	LDTVY-GG-DFSG-EDWDHALGGL----	53		
	<i>Deinococcus sp. NW-56</i>	1	MT	PPG-----	LSLRGRHTAHPGGTRAALREL	LDLVY-EG-DFSS-DDWDHALGGL----	48	
Non-binders	<i>Cellulosimicrobium cellulans</i>	57	VR	HVVLAVDHDGTP	IGHAALRDH-----GGEW-----EVKRVVVAAGQRGRGVGRAVMSEVE	108		
	<i>Mycobacter senuensis</i>	52	--	HALIW--RRGILIAHGAVRRHLLYRGRS-LRCGVVEAVAVAEEDHRGKGLATAVLDACE	107			
Unlikely binders	<i>Chloroflexus aggregans</i>	47	--	HVLAY--HKGHLVSHALWVTRYLQVSGSGLLRTAYIEAVATDPAYRNRGFATTIMKRIV	103			
	<i>Klenkia soli</i>	41	--	HVLAS--RDGELLGHAAVVGRLIAGGRT-LRTGVVEAVAVAAAARRQGIAGALMAEVE	96			
Likely binders	<i>Vibrio cholerae</i>	43	--	HVIGY--LDGKIVSHAAVVERILYTEQRS-YRAAYIEAVATHPSVQKGLSQIMSHIT	98			
	<i>Acinetobacter baumannii</i>	43	--	HVVGI--LNNQIVSHAAVVERILYTEQRS-YRAAYIEAVATHPSVQKGLSQIMSHIT	99			
Binders	<i>Providencia stuartii</i> [AAC(2')-Ia]	46	--	HVMAF--DQKLVGHALWTRRVFTINGSSDVKTAYISVGVVEAMVVEQSYRQGLIQRQLMLQTN	101			
	<i>Mycobacterium tuberculosis</i> [AAC(2')-Ic]	49	--	HALIW--HHGAILIAHAAVQRRLIYRGNALRCGVVEGVAVRADWRGRLVSKLDVAE	104			
	<i>Mycobacterium arabiense</i>	26	--	HALIF--RHGAILIAGAVVQRRLIHGGRALRCGVLEAVAVREDWRGRLAMAIMNGLE	81			
Plasmid-encoded	<i>Deinococcus wulumuqiensis</i>	54	--	HTLAF--LEGRLVGHAAALVGRALLVSGSEP-RRAGVLEAMGVHPAQRRLIGRAILRRVN	109			
	<i>Deinococcus sp. NW-56</i>	49	--	HTLAW--LDGRLVGHAAALVGRALLVSGSEP-RRAGVLEAMGVHPAQRRLIGRAILRRVN	104			
				↓Glu149				
Non-binders	<i>Cellulosimicrobium cellulans</i>	109	RVARAAGAERV	LQTGDRQPEAEALYALGWT	-----	140		
	<i>Mycobacter senuensis</i>	108	QVIRG-AFEVGLSSAE	---ISRRIYSSRGWLPWRGPTSVLSPTGPQRT-PEADGT-VFVM	162			
Unlikely binders	<i>Chloroflexus aggregans</i>	104	DEIG-DYELAAALSPSS	---VVFYERLQWELWRGPLFIRTTDVLTPAAEQ--VMIL	154			
	<i>Klenkia soli</i>	97	RLIRG-GFELGALAASA	---DGAALYDARGWTRWTGPLRCLTPDGVV---DGDG	149			
Likely binders	<i>Vibrio cholerae</i>	99	TLID-EFELGALSPSE	---HEFYARLQWLSWEGPLGYVDSLNEFSCFDEE--VMILY	149			
	<i>Acinetobacter baumannii</i>	100	ETLTHELEYELAAQPED	---EAFYEKLQWTVWKGKLSIKQDTCSYLT-DEYE--IMLY	151			
Binders	<i>Providencia stuartii</i> [AAC(2')-Ia]	102	KIIAS-CYQLGLSSASD	---DGOKLYHSVQWIKKGLFELKQGSYIRS-IEEESG-VMGW	156			
	<i>Mycobacterium tuberculosis</i> [AAC(2')-Ic]	105	QVMRG-AYQLGALSSA	---RARRLYASRGWLPWHGPTSVLAPTGPVTRT-PDDGT-VFVL	159			
	<i>Mycobacterium arabiense</i>	82	QVVRG-AYHLGALSSA	---AGRPLYARGWLPWHGPTSVLAPTGPVTRT-PDDGT-VFVL	136			
Plasmid-encoded	<i>Deinococcus wulumuqiensis</i>	110	ROVAR-GYDFGALSASD	---EGLGLYRAGWEVWRGPLAVLTPGGLVPT-PEEAGG-VLVY	164			
	<i>Deinococcus sp. NW-56</i>	105	LQVAR-GYDLGALSSD	---EGLALYRAGWAVWRGPLGVMTGGIVPT-PEEAGG-VLVY	159			
				↓Asp176	↓Trp178			
Non-binders	<i>Cellulosimicrobium cellulans</i>	141	RIP	----	TYPPTYD TALP	QSRCEYKRL	-----A	164
	<i>Mycobacter senuensis</i>	163	PIE	----	VDLDTTSD	LACDWRDGGV	-----W	184
Unlikely binders	<i>Chloroflexus aggregans</i>	155	RLP	----	KTPSLDPNAP	LSAEWRTGEKVVVRVPS	-----W	184
	<i>Klenkia soli</i>	150	PVP	----	GIDAGTGDLP	LICDWRGSL	-----W	173
Likely binders	<i>Vibrio cholerae</i>	150	QIG	----	EINDIDFKGK	LICNWRGGDV	-----W	173
	<i>Acinetobacter baumannii</i>	152	PLNIQMKDRLSNSSEEDT	ICADWRGEL	-----W	180		
Binders	<i>Providencia stuartii</i> [AAC(2')-Ia]	157	KAD	----	GEVDTAS	LYCDFRGGDQ	-----W	178
	<i>Mycobacterium tuberculosis</i> [AAC(2')-Ic]	180	PID	----	ISLDTSAE	LYCDFRGGDQ	-----W	181
	<i>Mycobacterium arabiense</i>	137	PVALP	----	DGVDTRGE	ITCDFRGGDV	-----W	181
Plasmid-encoded	<i>Deinococcus wulumuqiensis</i>	165	APA	----	GELDSQS	ITCDFRGGDV	-----W	186
	<i>Deinococcus sp. NW-56</i>	160	APA	----	GNLNLTLP	ITCDFRGGDV	-----W	181

Figure S2: Alignment of a subset of sequences based on binding classification. The MAFFT sequence alignment highlights overall sequence identity in blue. Residues important for aminoglycoside binding are denoted with an arrow. Identified residues are numbered as per AAC(2')-Ia from *Providencia stuartii*.

AAC(2')-Ia_{HIS} • CoA	
Resolution range (Å)	52.75-1.3 (1.346-1.3)
Space group	P 43 21 1
Unit cell (Å, °)	58.7 58.7 120.0
Total reflections	632665 (60458)
Unique reflections	52409(5117)
Multiplicity	12.1 (11.8)
Completeness (%)	100.0 (99.9)
Mean I/sigma(I)	28.1 (2.6)
Wilson B-factor	19.9
R-merge	0.045 (1.58)
R-meas	0.047 (1.65)
CC_{1/2}	1.00 (0.65)
CC*	1.00 (0.89)
Refinement Statistics	
R_{work}	0.148 (0.206)
R_{free}^a	0.189 (0.234)
Number of non-hydrogen atoms	1982
macromolecules	1726
ligands	65
water	191
Protein residues	182
RMS (bonds)	0.030
RMS (angles)	2.4
Ramachandran favored (%)	94.4
Ramachandran outliers (%)	1.1
Clashscore	12.63
Average B-factor	29.9
macromolecules	28.0
ligands	38.0
solvent	44.3

Table S1: Data Collection and Refinement Statistics of AAC(2')-Ia C-terminal His Complex. Statistics for the highest-resolution shell are shown in parentheses.

^a R_{free} was calculated by randomly omitting 10% of observed reflections from refinement.