

Supporting Information for:

Structural and Functional Survey of Environmental Aminoglycoside Acetyltransferases Reveals Functionality of Resistance Enzymes

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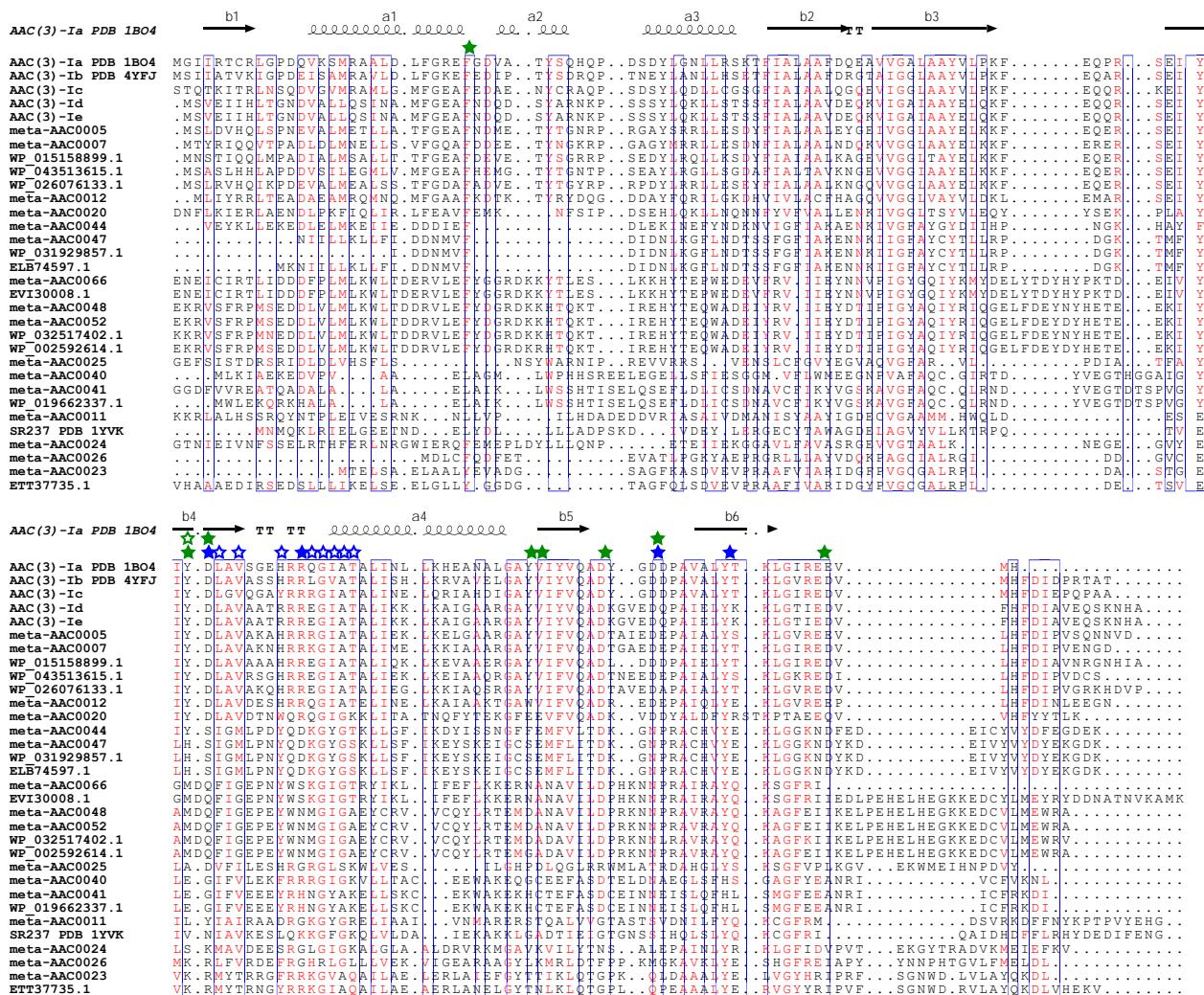
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This document is 12 pages (including this page) and contains Figure S1, Figure S2, Figure S3, Figure S4, Figure S5, Figure S6, Table S1 and Table S2.

meta-AAC enzymes and orthologs - GNAT family



meta-AAC enzymes and orthologs - Antibiotic NAT family

protein-ligand contacts

CoA

backbone

sidechain

protein-ligand contacts

CoA	
backbone	★
sidechain	★

Figure S1 (previous two pages). Sequence alignment of meta-AAC, AAC and homologous enzymes in NCBI. First alignment = GNAT family, second = Antibiotic_NAT family. Sequences from NCBI possess >70% sequence identity to a meta-AAC or AAC enzyme. Secondary structure elements from structures of AAC(3)-Ia (PDB 1BO4) and meta-AAC0038 (PDB 5HT0) are indicated at top of alignment. Legend indicates labeling of amino acids (either backbone or sidechain atoms) involved in protein-ligand (CoA or sisomicin) contacts as observed in crystal structures of meta-AAC0005•CoA complex (PDB 5HMN), meta-AAC0020•CoA complex (PDB 5F48), meta-AAC0020^{Y138A}•sisomicin complex (PDB 5UO8) or meta-AAC0038 (PDB 5HT0).

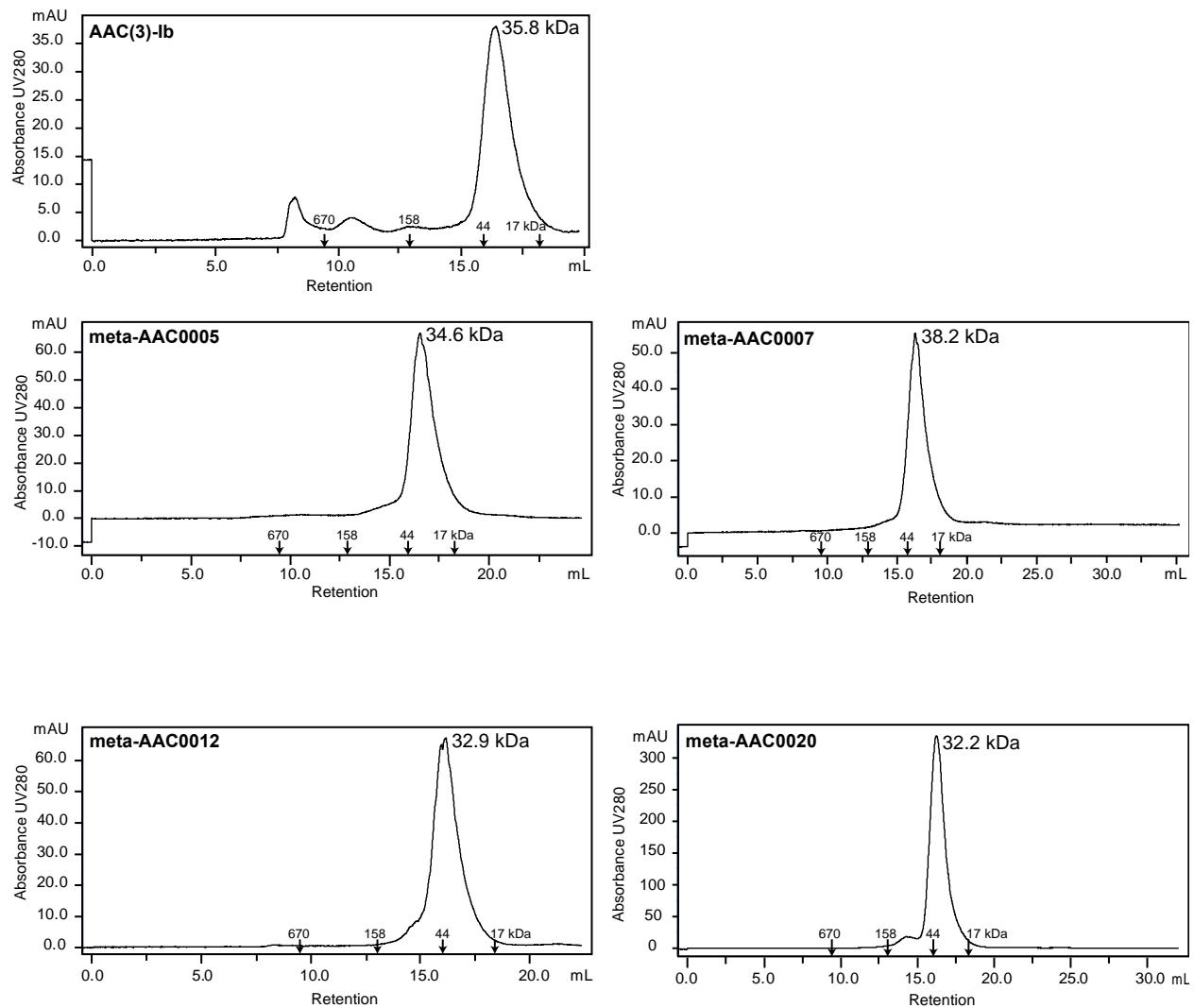
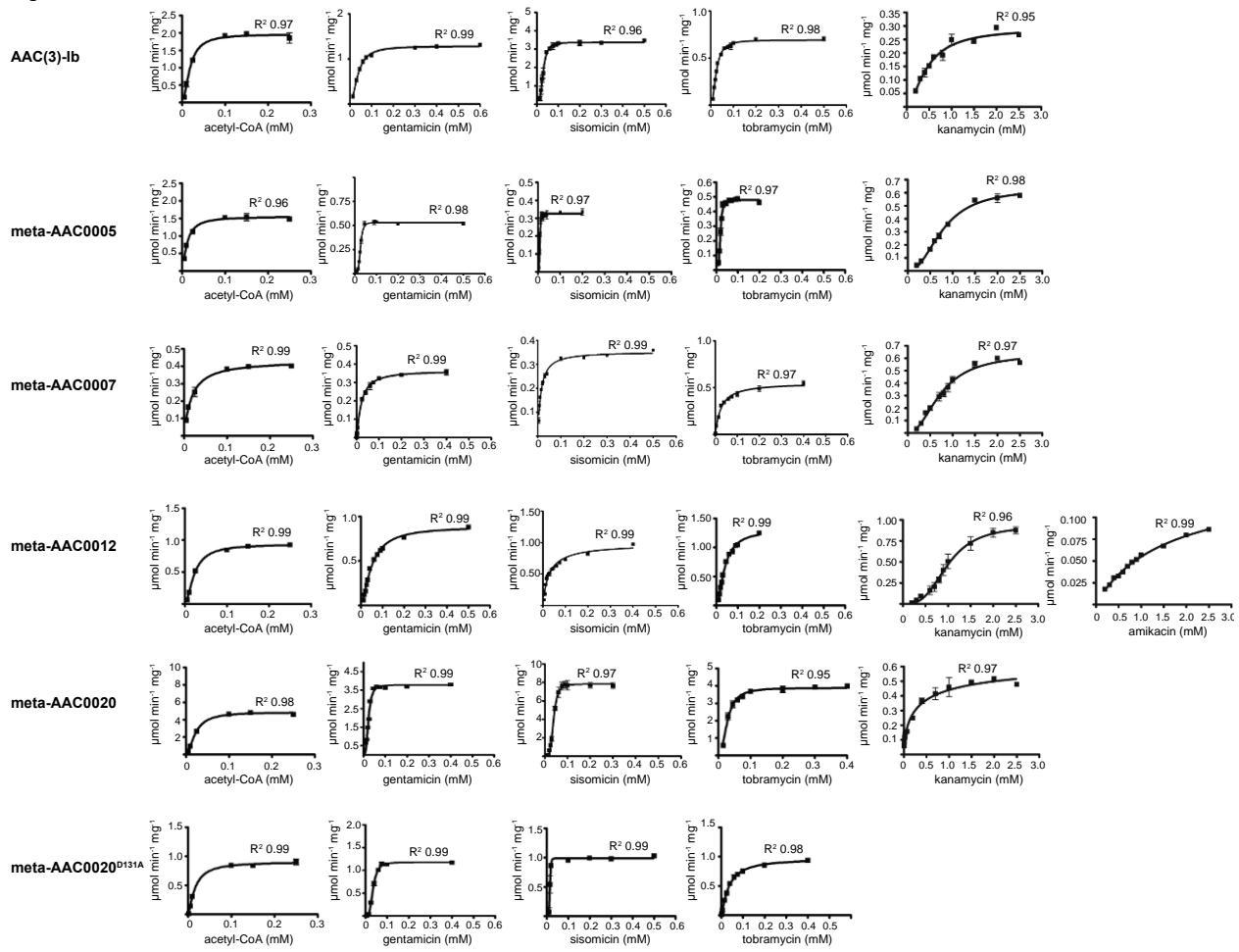


Figure S2. Size exclusion chromatography profiles of AAC(3)-Ib and meta-AAC enzymes.
Molecular weight markers are indicated with arrows.

Sigmoidal fits



Michaelis-Menten fits

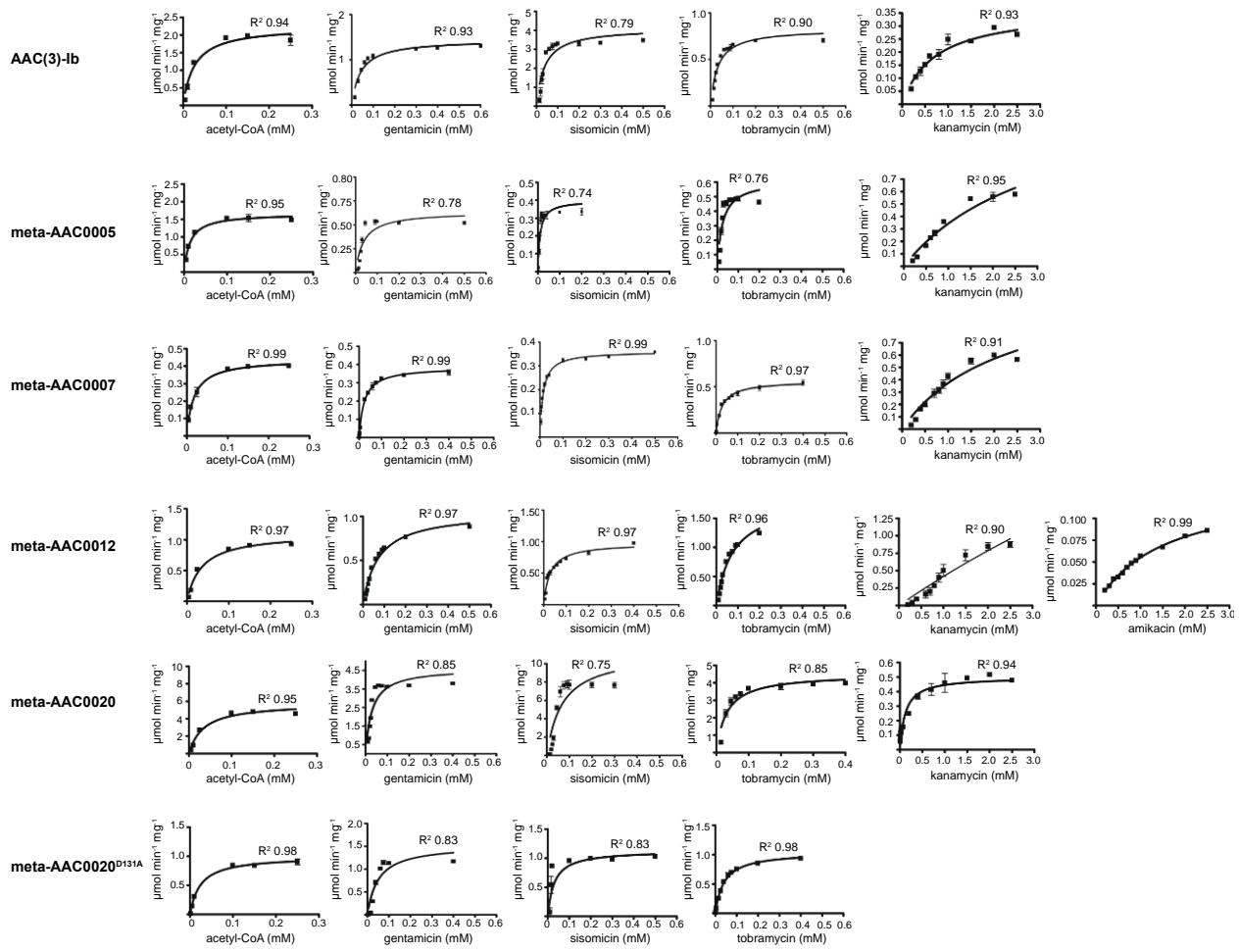


Figure S3 (two pages). Enzyme kinetics of AAC(3)-Ib and meta-AAC enzymes vs acetyl-CoA, gentamicin, sisomicin, tobramycin, kanamycin and amikacin (only versus meta-AAC0012). Shown on first and second pages are sigmoidal and Michaelis-Menten model fits, respectively.

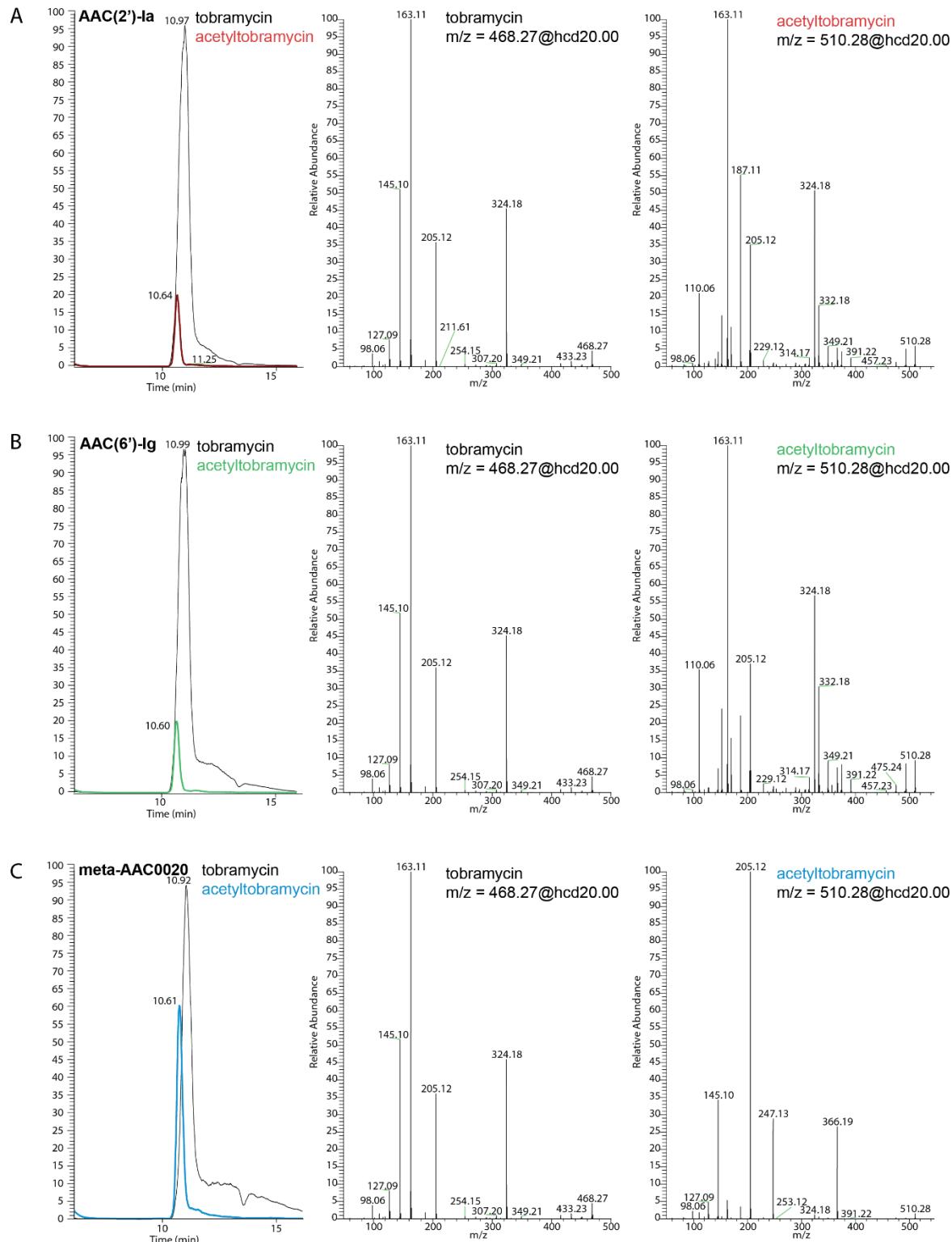


Figure S4. LC-MS/MS profiles of tobramycin and acetyl-tobramycin (post-reaction mixture of reactions catalyzed by AAC(2')-Ia (top), AAC(6')-Ig (middle) and meta-AAC0020 (bottom). Left panels = LC of tobramycin (black plot) and acetyl-tobramycin (red, green and blue plots). Middle panels = MS/MS spectrum of tobramycin. Right panels = MS/MS spectrum of acetyl-tobramycin.

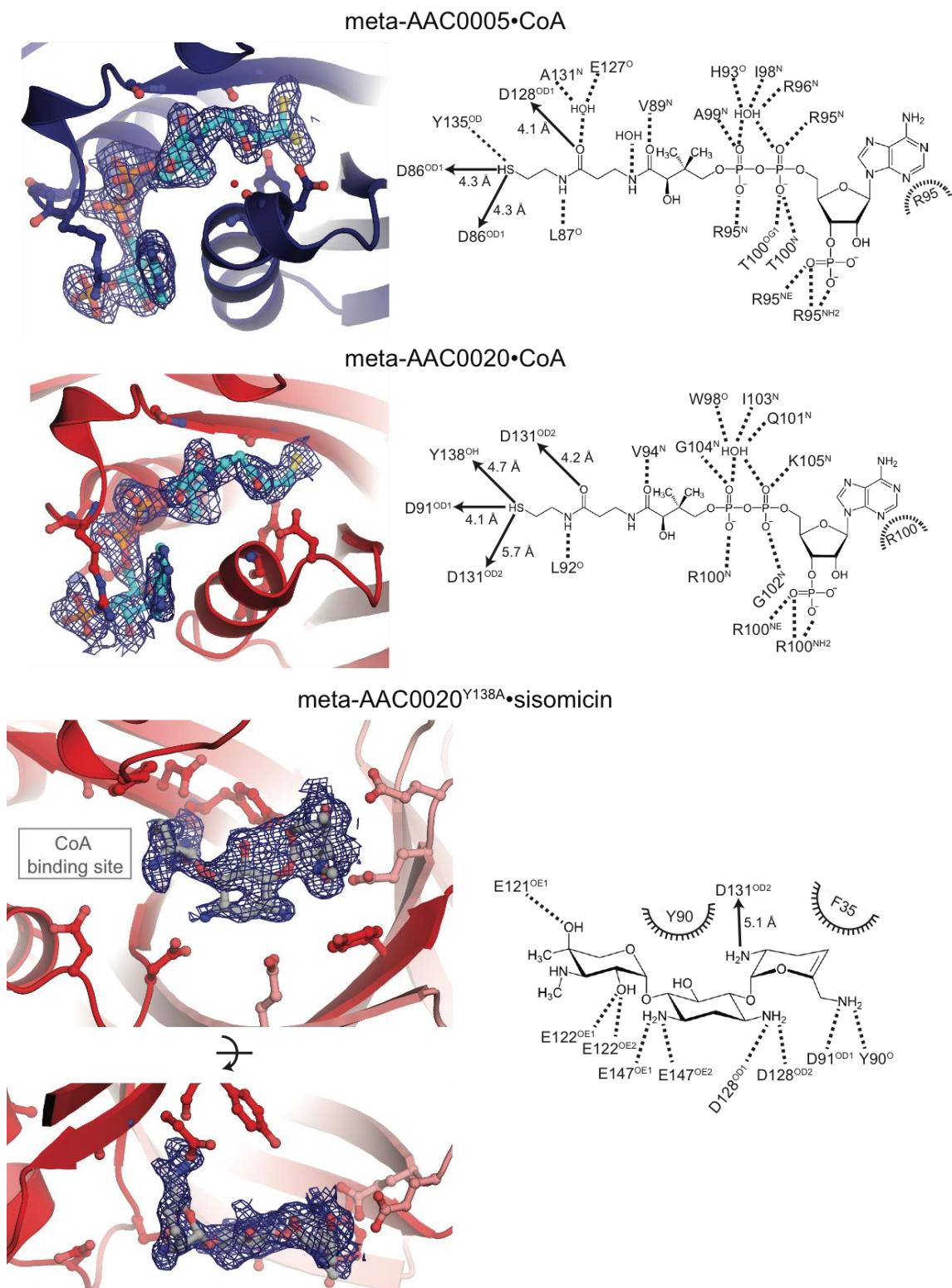


Figure S5. Electron density maps (left) and schematic representations (right) of crystal structures of meta-AAC0005•CoA, meta-AAC0020•CoA, meta-AAC0020^{Y138A}•sisomicin complexes. Electron density maps shown are simulated annealing omit maps contoured at 2.0 σ . Arrows on schematics indicate distances to key catalytic amino acids.

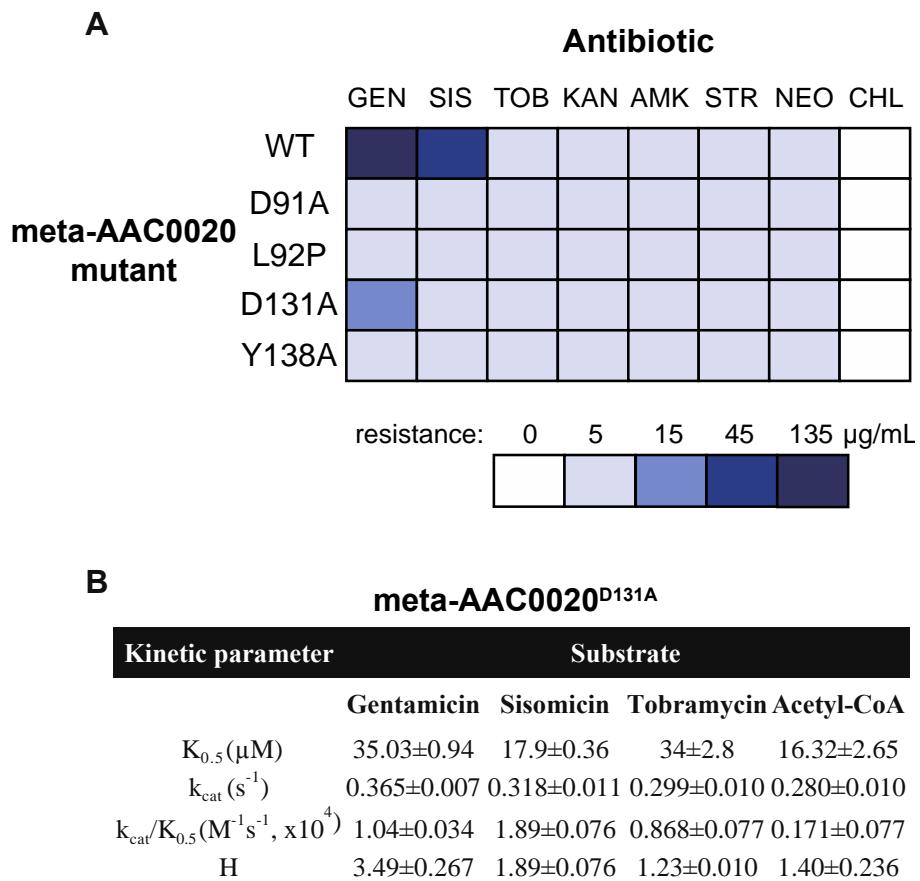


Figure S6. Functional characterization of meta-AAC0020 and its mutants. (A) Kirby-Bauer disk diffusion assay on meta-AAC0020 WT and four mutants. D91A, L92P and Y138A do not confer resistance. (B) Kinetic characterization of meta-AAC0020^{D131A} mutant shows reduced activity.

Table S1: Orthologs of meta-AAC proteins not labeled on the GNAT and the Antibiotic_NAT phylogenies (>70% sequence identity as determined by BLAST).

GNAT family			
Closest meta-AAC protein	Genbank accession #	Annotation	Organism
meta-AAC0041	WP_019662337.1	GNAT family N-acetyltransferase	<i>Eubacterium siraeum</i>
meta-AAC0066	EVI30008.1	AAC domain of bifunctional AAC/APH	<i>Staphylococcus aureus</i>
meta-AAC0048	WP_032517402.1	aminoglycoside N-acetyltransferase AAC(6')-Im	<i>Enterococcus spp.</i>
meta-AAC0023	ETT37735.1	N-acetyltransferase GCN5	<i>Paenibacillus sp.</i>
meta-AAC0047	ELB74597.1	acetyltransferase	<i>Enterococcus faecium</i>
meta-AAC0047	WP_031929857.1	N-acetyltransferase	<i>Staphylococcus aureus</i>
meta-AAC0005	WP_015158899.1	AAC(3)-I family aminoglycoside 3-N-acetyltransferase	<i>Chamaesiphon minutus</i>
meta-AAC0005	WP_043513615.1	AAC(3)-I family aminoglycoside 3-N-acetyltransferase	<i>Halomonas sp.</i>
meta-AAC0005	WP_026076133.1	AAC(3)-I family aminoglycoside 3-N-acetyltransferase	<i>Herbaspirillum massiliense</i>
Antibiotic_NAT family			
Closest meta-AAC protein	Genbank accession #	Annotation	Organism
meta-AAC0029	WP_012695485.1	AAC(3) family aminoglycoside N-acetyltransferase	<i>Gammaproteobacteria</i>
meta-AAC0029	AFN07611.1	aminoglycoside-(3)-N-acetyl-transferase	<i>Escherichia coli</i>
meta-AAC0029	ACV60580.1	aminoglycoside-(3)-N-acetyltransferase II	<i>Klebsiella pneumoniae</i>
meta-AAC0008	ADW20348.1	bla aacC3-like protein	<i>Pseudomonas aeruginosa</i>
meta-AAC0008	WP_023911614.1	aminoglycoside 3-N-acetyltransferase	<i>Gammaproteobacteria</i>
meta-AAC0033	AKF06106.1	aminoglycoside N3-acetyltransferase	<i>Sandaracinus amyloolyticus</i>
meta-AAC0033	WP_043428585.1	AAC(3) family N-acetyltransferase	<i>Cystobacter fuscus</i>
meta-AAC0033	WP_047855546.1	AAC(3) family N-acetyltransferase	<i>Archangium gephyra</i>
meta-AAC0033	WP_044985444.1	AAC(3) family N-acetyltransferase	<i>Sorangium cellulosum</i>
meta-AAC0033	WP_037583372.1	aminoglycoside 3-N-acetyltransferase	<i>Stigmatella aurantiaca</i>
meta-AAC0033	WP_046714710.1	AAC(3) family N-acetyltransferase	<i>Myxococcus fulvus</i>
meta-AAC0018	WP_029724114.1	AAC(3) family N-acetyltransferase	<i>Sphingomonas sp.</i>

Table S2: NMR chemical shift analysis of meta-AAC0020 catalyzed acetyl-tobramycin.

Ring	Position	Tobramycin		Acetyl-tobramycin		Δ ppm	
		^{13}C	Atom ^1H	^{13}C	Atom ^1H	^{13}C	Atom ^1H
central	1	50.68	2.913	50.46	3.08	-0.22	0.167
	2 _{ax}	35.72	H _{ax} :1.235, H _{eq} :1.964*	33.69	H _{ax} :1.36, H _{eq} :2.08	-2.03	H _{ax} :0.125, H _{eq} :0.116
	3	49.73	2.886	49.3	2.9	-0.43	0.014
	4	86.13	3.336	82.68	3.47	-3.45	0.134
	5	74.62	3.632	74.56	3.65	-0.06	0.018
	6	88.16	3.247	86.4	3.36	-1.76	0.113
prime	1'	99.4	5.188	96.6	5.447	-2.8	0.259
	2'	49.35	2.978	48.8	3.224	-0.55	0.246
	3'	34.85	H _a :1.615 H _b :2.031	32.71	H _a :1.76, H _b :2.14	-2.14	H _a :0.145, H _b :0.109
	4'	66.34	3.523	69.6	3.58	3.26	0.057
	5'	73.17	3.629	70.03	3.81	-3.14	0.181
	6'	41.88	H _a : 2.794 H _b : 3.053	40.67	H _a : 3.06, H _b :3.22	-1.21	H _a : 0.266, H _b :0.167
double	1''	100.2	5.036	100.1	5.045	-0.1	0.009
	2''	72.04	3.501	68.28	3.43	-3.76	-0.071
	3''	54.66	3.007	54.9	3.14	0.24	0.133
	4''	69.51	3.322	70.6	3.63	1.09	0.308
	5''	73.17	3.629	72.53	3.9	-0.64	0.271
	6''	60.5	H _a : 2.794, H _b : 3.053	3.77	60.35	-0.15	0
Acetyl	CH ₃ =O	N/A	N/A	1.91	22.45		

*H_{ax} = axial hydrogen, H_{eq} = equatorial hydrogen.