Supplementary Information

Structure of the Bifunctional Aminoglycoside Resistance Enzyme AAC(6')-Ie-APH(2")-Ia Revealed by Crystallographic and Small-Angle X-ray Scattering Analysis

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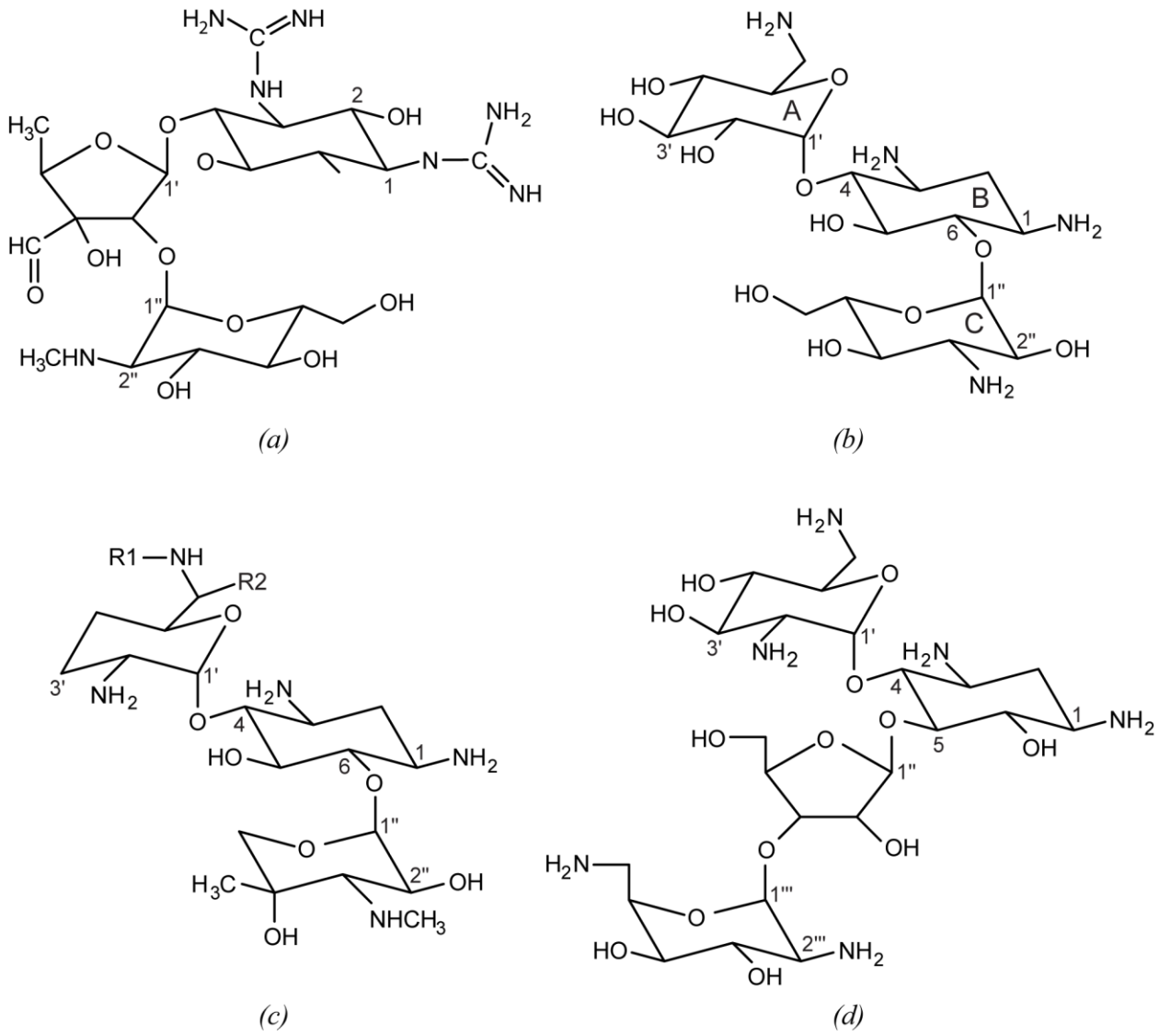

Figure S1: Aminoglycoside antibiotics. (*a*) Streptomycin (*b*) Kanamycin A, indicating the A, B and C rings. (*c*) Gentamicin C, typically a mixture of types C1 (R1 = R2 = CH_3), C1a (R1 = R2 = H) and C2 (R1 = CH_3, R2 = H). (*d*) Neomycin.

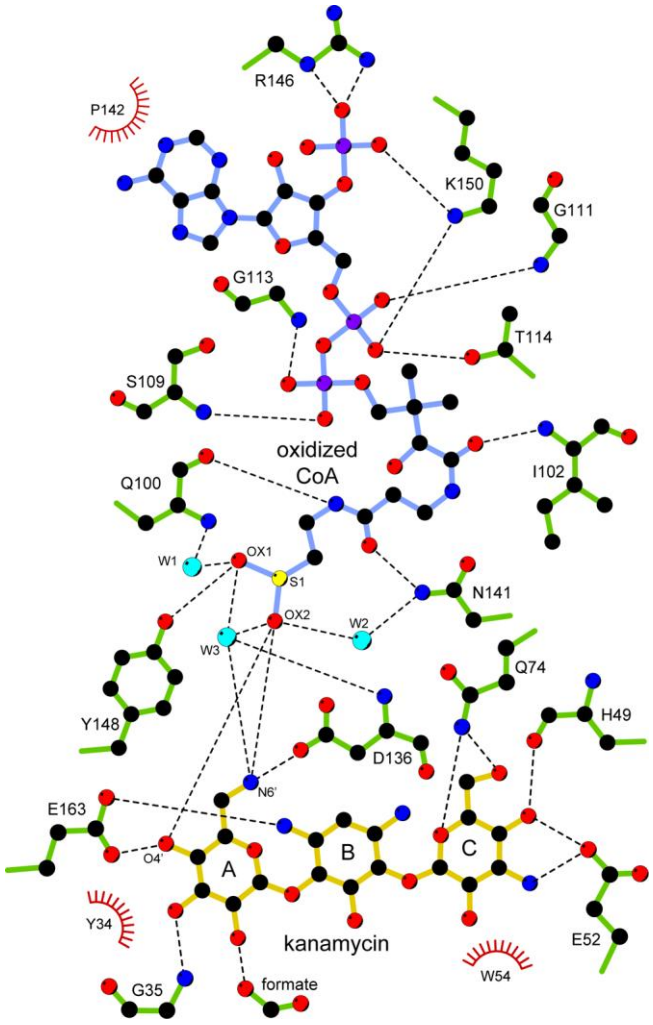

Figure S2: Schematic representation of the coenzyme A (light blue bonds) and kanamycin (yellow bonds) environments showing all the protein interactions (green bonds) and three of the water molecules associated with both substrates.

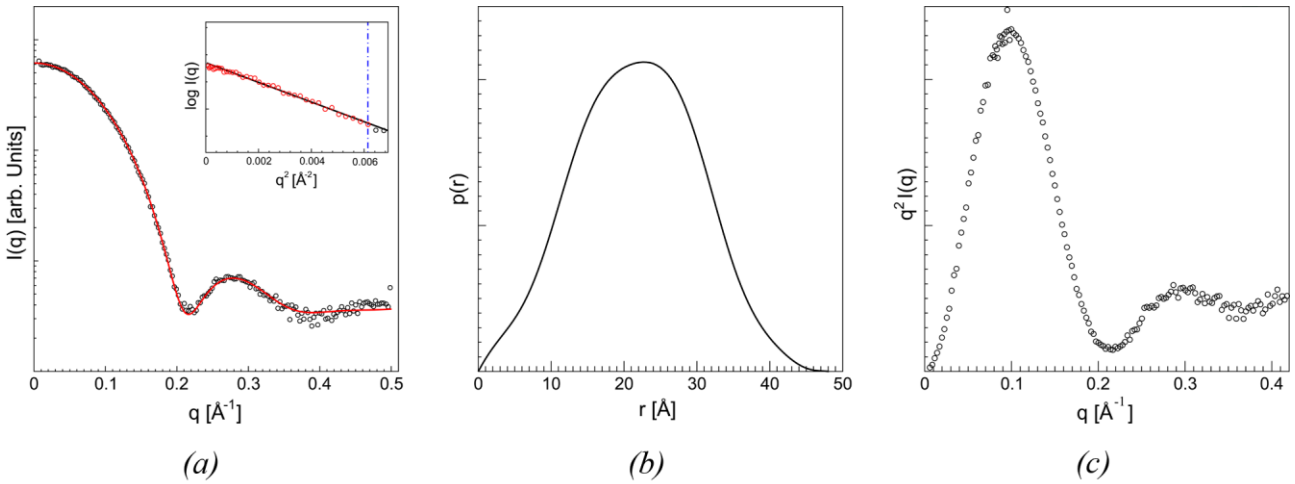

Figure S3: (*a*) Log-scale plot of the measured SAXS intensity I(q) for the AAC(6')-Ie domain (open black circles). The solid red line depicts the *CRYSOL* fit of the rigid-body calculation using the refined AAC(6')-Ie structure (chi=2.37). (*b*) Radial distance distribution function, p(r), calculated using *GNOM*. (*c*) Kratky transformation of the scattering curve for AAC(6')-Ie.

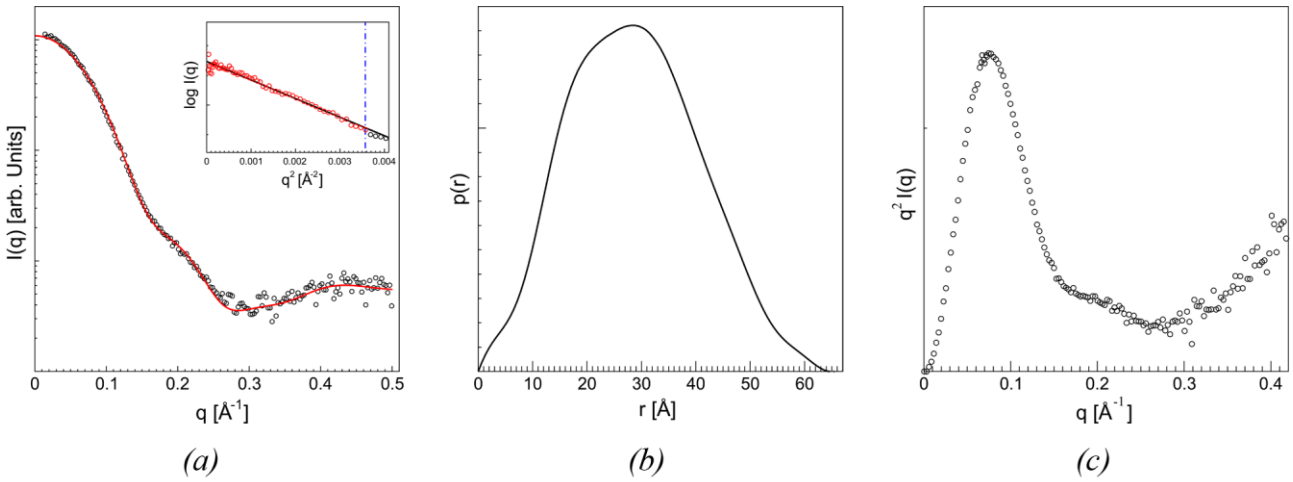

Figure S4: (*a*) Log-scale plot of the measured SAXS intensity I(q) for the APH(2”)-Ia domain (open black circles). The solid red line depicts the *CRYSOL* fit of the rigid-body calculation using the refined APH(2”)-Ia structure (chi=3.67). (*b*) Radial distance distribution function, p(r), calculated using *GNOM*. (*c*) Kratky transformation of the scattering curve for APH(2”)-Ia.

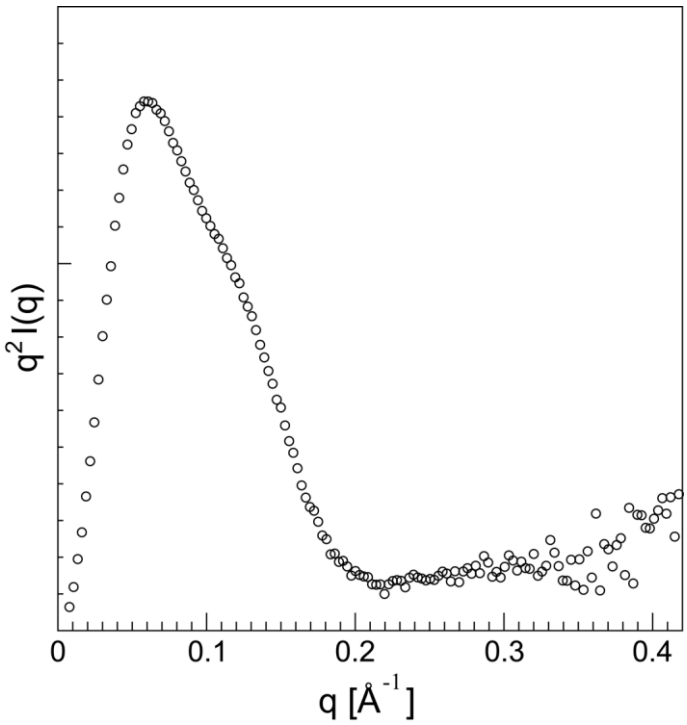

Figure S5: Kratky transformation of the scattering data for the bifunctional AAC(6')-Ie-APH(2")-Ia enzyme.

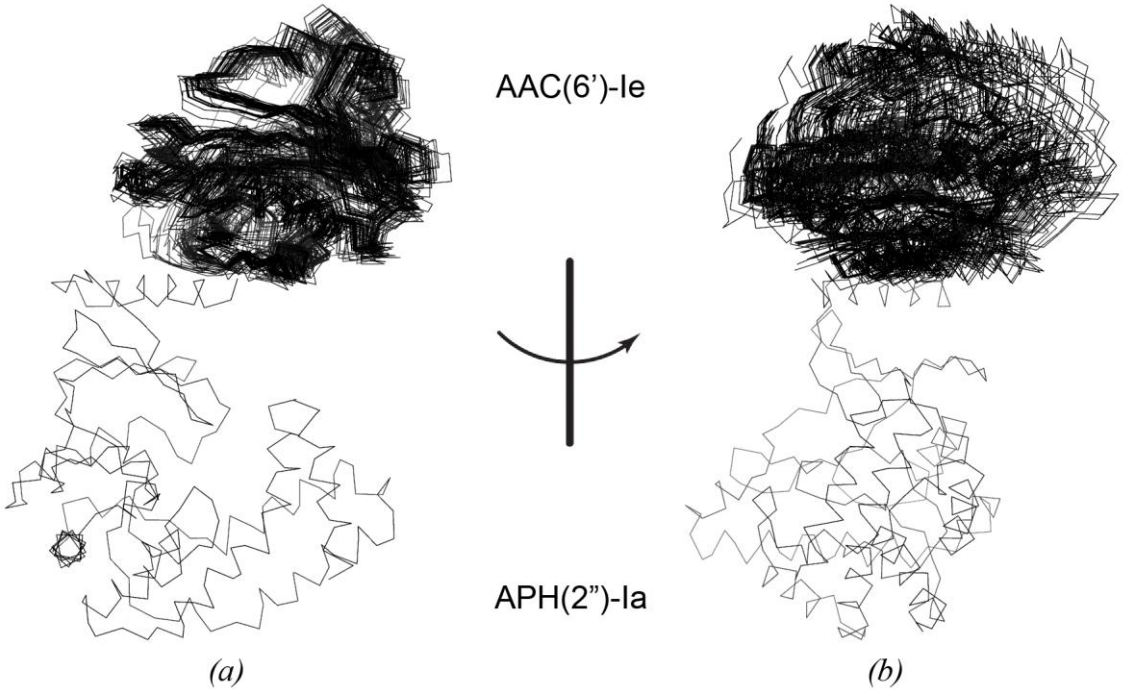

Figure S6: Ensemble of AAC(6')-Ie-APH(2")-Ia models from the rigid-body fitting to the SAXS data for the bifunctional enzyme in two orientations related by a 90° rotation about the vertical axis shown.

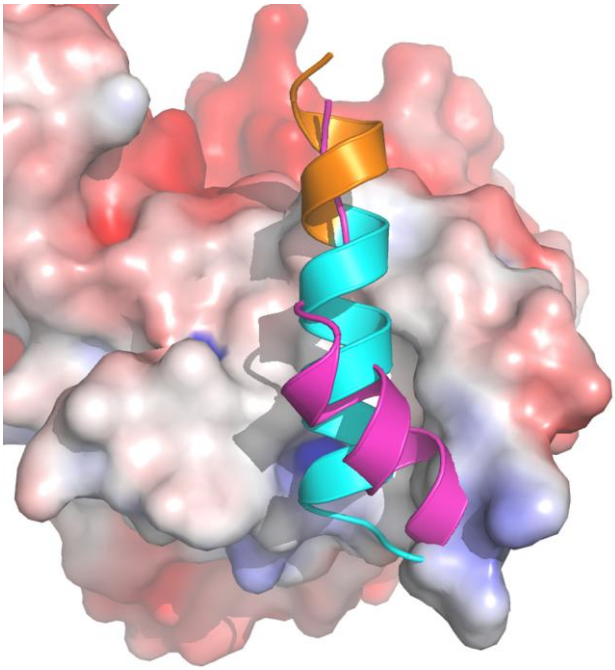

Figure S7: Electrostatic molecular surface representation of the N-terminal domain of APH(2”)-Ia with the A1 helix removed from the surface calculation and reintroduced as a ribbon (cyan and orange). The equivalent helix from APH(2”)-IIa is shown as a magenta ribbon, based upon a superposition of the whole N-terminal domain.

Table S1

Oxidized coenzyme-A hydrogen bonding interactions

Kan - kanamycin

Group	Atom	Molecule A		Molecule B	
	N1A	Wat291	2.74	-	-
	N3A	Wat272	2.94	Wat207	3.02
adenine	N6A	Wat148	2.97	Wat271	3.03
		Wat435	2.91	Wat167	2.92
	N7A	Wat148	3.02	Wat271	2.79
	O2B	Arg146 N_{ε} †	2.91	-	-
		Arg146 $N_{\eta 2}$	2.97	-	-
		Wat6	2.87	Wat525	2.88
		Wat537	3.07	-	-
ribose	O7A	Wat748	2.68	Wat205	2.69
	O8A	Wat537	2.52	-	-
	O9A	Lys150 N_{ζ}	2.58	Lys150 N_{ζ}	2.70
		Wat35	2.57	Wat254	2.73
		Wat247	2.81	-	-
	O1A	Wat35	2.86	Wat254	2.90
		Gly111 N	2.96	Gly111 N	2.88
	O2A	Lys150 N_{ζ}	3.06	Lys150 N_{ζ}	3.02
diphosphate		Thr114 $O_{\gamma 1}$	2.68	Thr114 $O_{\gamma 1}$	2.65
	O4A	Wat130	2.60	Wat136	2.60
		Gly113 N	2.74	Gly113 N	2.73
	O5A	Ser109 N	2.83	Ser109 N	2.89
	O9P	Ile102 N	2.79	Ile102 N	2.76
	N8P	Wat132	3.19	Wat134	3.16
	O5P	Wat132	2.75	Wat134	2.79
		Asn141 $N_{\delta 2}$	3.04	Asn141 $N_{\delta 2}$	2.95
	N4P	Gln100 O	3.03	Gln100 O	3.05
pantothenate	S1P	Wat3	3.15	Wat1	2.85
	OX1	Tyr148 O_{η}	2.59	Tyr148 O_{η}	2.65
		Wat4	2.75	Wat2	2.78
		Wat422	2.79	Wat340	2.92
	OX2	Wat3	2.53	Wat1	2.98
		Kan N6’	2.84	Kan N6’	3.00

† The equivalent residue in monomer B has a different conformation.

Table S2

Kanamycin hydrogen bonding interactions

FMT = formate, CAO = oxidized coenzyme-A

Ring	Kanamycin atom	Molecule A		Molecule B	
	O2' (O6)	Wat274	2.67	Wat414	3.02
		FMT1 O2	2.58	FMT2 O2	2.89
	O3' (O7)	Gly35 N	2.79	Gly35 N	2.84
		Wat135	2.69	Wat413	2.69
	O4' (O8)	CAO OX2	3.28	CAO OX2	3.30
		Glu163 $O_{\varepsilon 2}$	2.74	Glu163 $O_{\varepsilon 2}$	2.78
A		Wat131	2.94	Wat1	2.67
		Wat137	3.5†	Wat347	2.67
	N6' (N1)	CAO OX2	2.84	CAO OX2	3.00
		Asp136 $O_{\delta 1}$	3.22	Asp136 $O_{\delta 1}$	3.5†
		Wat137	2.74	Wat347	2.38
		Wat422	2.74	Wat340	2.43
	O5 (O10)	Wat142	3.04	Wat257	2.44
	N3 (N2)	Glu163 $O_{\varepsilon 1}$	2.79	Glu163 $O_{\varepsilon 1}$	2.87
		Wat133	2.87	-	-
B		Wat137	3.17	-	-
		Wat425	2.92	Wat596	2.86
	N1 (N3)	Wat146	2.91	-	-
		Wat502	2.53	-	-
		Wat565	3.19	Wat631	3.4†
	O4" (O14)	His49 O	2.72	His49 O	2.88
		Glu52 $O_{\varepsilon 2}$	3.24	Glu52 $O_{\varepsilon 2}$	3.19
	N3" (N4)	Glu52 $O_{\varepsilon 2}$	2.84	Glu52 $O_{\varepsilon 2}$	2.86
		Wat180	2.83	Wat528	2.80
C		Wat236	2.76	Wat417	2.58
	O2" (O13)	Wat145	2.81	-	-
		Wat565	3.30	Wat631	2.69
		Wat180	3.4†	Wat528	3.22
	O7" (O12)	Gln74 $N_{\varepsilon 2}$	3.01	Gln74 $N_{\varepsilon 2}$	3.04
	O6" (O15)	Gln74 $O_{\varepsilon 1}$	2.90	Gln74 $O_{\varepsilon 1}$	2.85
		Wat129	2.79	-	-
		-	-	Arg60 NH1‡	2.41
		-	-	Arg60 NH2	2.67

† These interactions are too long to be realistic hydrogen bonds but have been included in the table to show the similarity of the binding sites in the two monomers.

‡ The equivalent residue in monomer A has a different conformation.