Supporting Information:

"Catch and Release": A Variation of the Archetypal Nucleotidyl Transfer Reaction

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Table S1. Data collection and refinement statistics of wild type and T130K ANT4'.

	WT Neomycin	T130K Neomycin	T130K Neomycin-AMPCPP	T130K Product state (adenylated neomycin)
Resolution range[1]	50.0-1.65 (1.71-1.65)	51.07-1.90 (1.94-1.90)	45.42-2.00 (2.05-2.00)	38.58-2.50 (2.60-2.50)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_1$
Unit cell				
a, b, c (Å)	57.3, 97.7, 101.1	59.0, 97.7, 102.0	59.2, 98.0, 102.4	85.5, 59.3, 102.0
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 94.80, 90
Unique reflections	68631	45570	40016	30831
Multiplicity[1]	4.3 (4.0)	6.5 (6.0)	4.9 (4.6)	3.8 (3.2)
Completeness (%)	99.2 (99.8)	97.0 (93.7)	97.8 (95.7)	86.6 (81.9)
Mean I/sigma(I)	24.2 (1.8)	18.5 (4.4)	11.4 (2.7)	12.2 (5.1)
Wilson B-factor	22.2	24.6	27.8	25.1
R-merge	0.048 (0.481)	0.056 (0.374)	0.082 (0.602)	0.077 (0.224)
Reflections used in refinement	68569	45531	39968	30831
Reflections used for R-free	1989	2334	1984	1499
R-work	0.173 (0.237)	0.170 (0.203)	0.197 (0.264)	0.173 (0.223)
R-free	0.198 (0.265)	0.210 (0.243)	0.229 (0.284)	0.230 (0.305)
Number of non-hydrogen atoms				
macromolecules	4166	4204	4129	8110
ligands	85	87	150	428
solvent	557	387	368	217
RMS(bonds)	0.006	0.012	0.006	0.004
RMS(angles)	1.14	1.41	1.14	0.98
Ramachandran favored (%)	97.4	98.4	97.6	96.4
Ramachandran allowed (%)	2.4	1.6	2.4	3.5
Ramachandran outliers (%)	0.2	0.00	0.00	0.1
Clashscore	3.4	4.50	4.89	4.19
Average B-factor				
macromolecules	25.8	26.4	30.6	29.1
ligands	31.2	39.9	36.3	35.3
solvent	37.6	34.8	36.1	25.8
PDB Code	6UN8	6NMK	6NML	6NMM

[1]Number in parentheses represent values in the highest resolution shell.

Table S2. Data collection and refinement statistics of T130K/E52D ANT4'.

	Neomycin	Neomycin-AMPCPP	Product state (adenylated neomycin)
Resolution range[1]	49.22-2.30 (2.38-2.30)	48.98 to 2.30 (2.38-2.30)	49.65 to 2.20 (2.27-2.20)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell			
a, b, c (Å)	58.70, 98.45, 101.82	58.71, 97.95, 100.70	59.75, 99.30, 101.49
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90
Unique reflections	26344	25660	30558
Multiplicity[1]	5.2 (5.0)	3.5 (3.4)	6.8 (6.8)
Completeness (%)	98.4 (98.6)	97.3 (95.2)	97.6 (94.6)
Mean I/sigma(I)	5.5 (5.0)	5.8 (2.0)	8.8 (3.9)
Wilson B-factor	34.7	26.0	24.7
R-merge	0.162 (0.644)	0.140 (0.575)	0.124 (0.440)
Reflections used in refinement	26263	25620	30512
Reflections used for R-free	1427	1396	1549
R-work	0.196 (0.284)	0.180 (0.245)	0.194 (0.239)
R-free	0.235 (0.344)	0.221 (0.313)	0.224 (0.292)
Number of non-hydrogen atoms			
macromolecules	4167	4098	4060
ligands	85	150	150
solvent	143	199	228
RMS(bonds)	0.005	0.010	0.007
RMS(angles)	1.10	1.38	1.25
Ramachandran favored (%)	96.6	97.0	95.2
Ramachandran allowed (%)	3.4	3.0	4.6
Ramachandran outliers (%)	0	0	0.2
Clashscore	6.93	8.17	4.61
Average B-factor			
macromolecules	40.6	31.1	29.5
ligands	60.9	30.0	24.5
solvent	40.3	32.0	32.6
PDB Code	6PO4	6PO6	6PO8

[1]Number in parentheses represent values in the highest resolution shell.

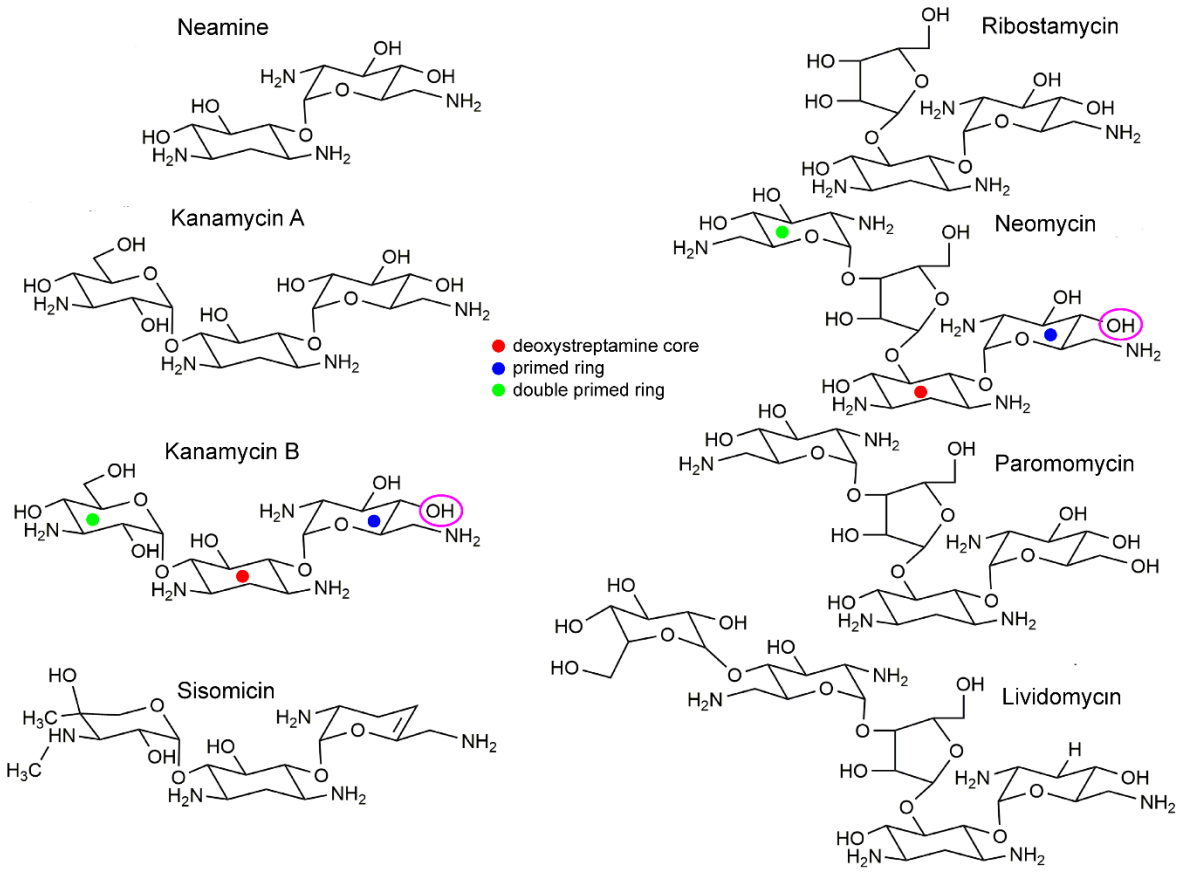

Figure S1. Two classes of aminoglycoside antibiotics. Aminoglycoside antibiotics are grouped into two classes based on the substitution pattern of a common deoxystreptamine core. Left is the kanamycin group and right is the neomycin group. The ring nomenclature is indicated for kanamycin B and neomycin, and the 4′ site of modification is circled in magenta.

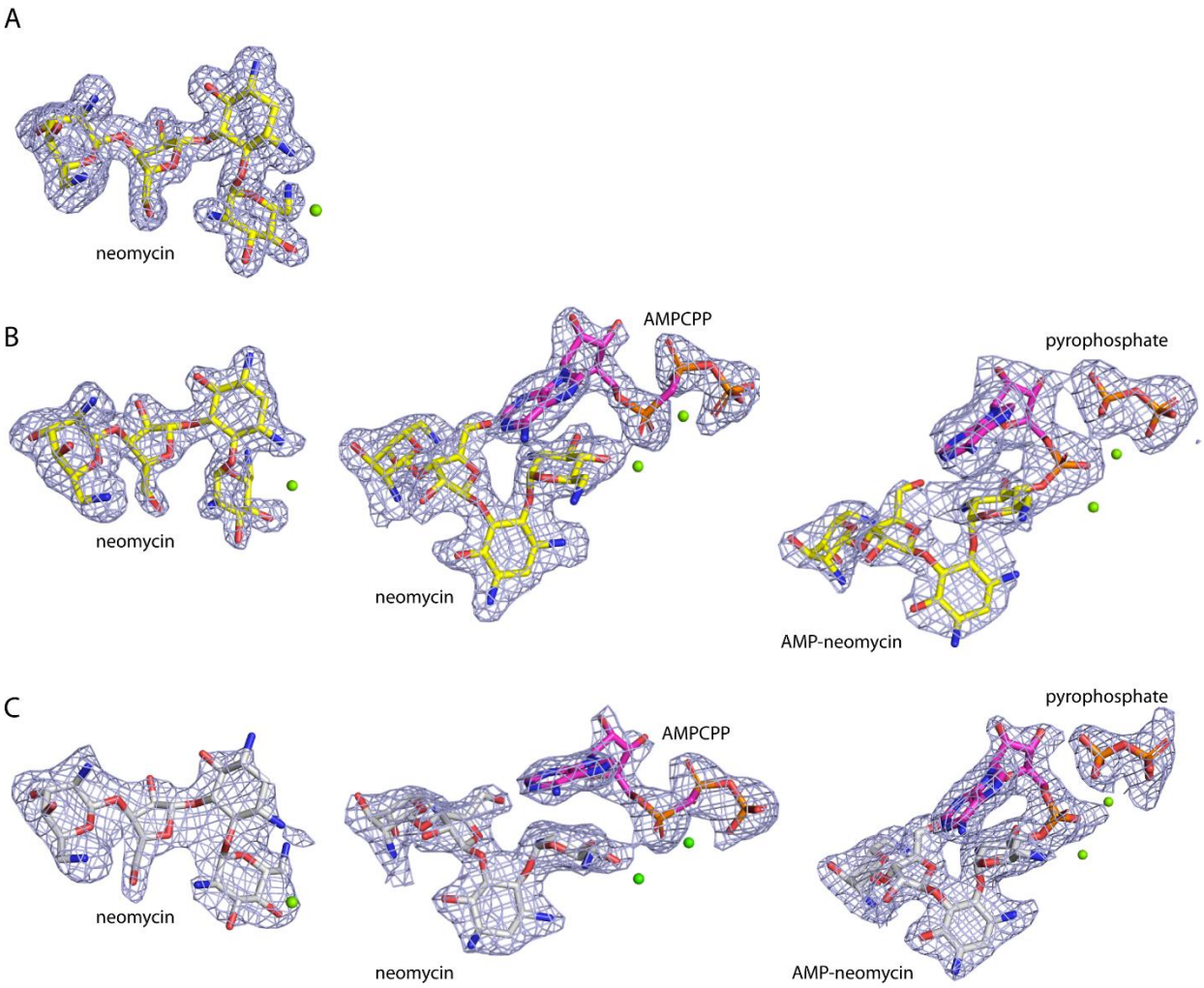

Figure S2. Polder omit maps of ANT4' ligands. (A) Wild-type ANT 4' neomycin complex. (B). T130K ANT 4' neomycin complex (left), neomycin/AMPCPP ternary complex (center) and AMP-neomycin/pyrophosphate ternary complex. (C) T130K/E52D ANT 4' neomycin complex (left), neomycin/AMPCPP ternary complex (center) and AMP-neomycin/pyrophosphate ternary complex. Polder maps are contoured at 3 sigma.

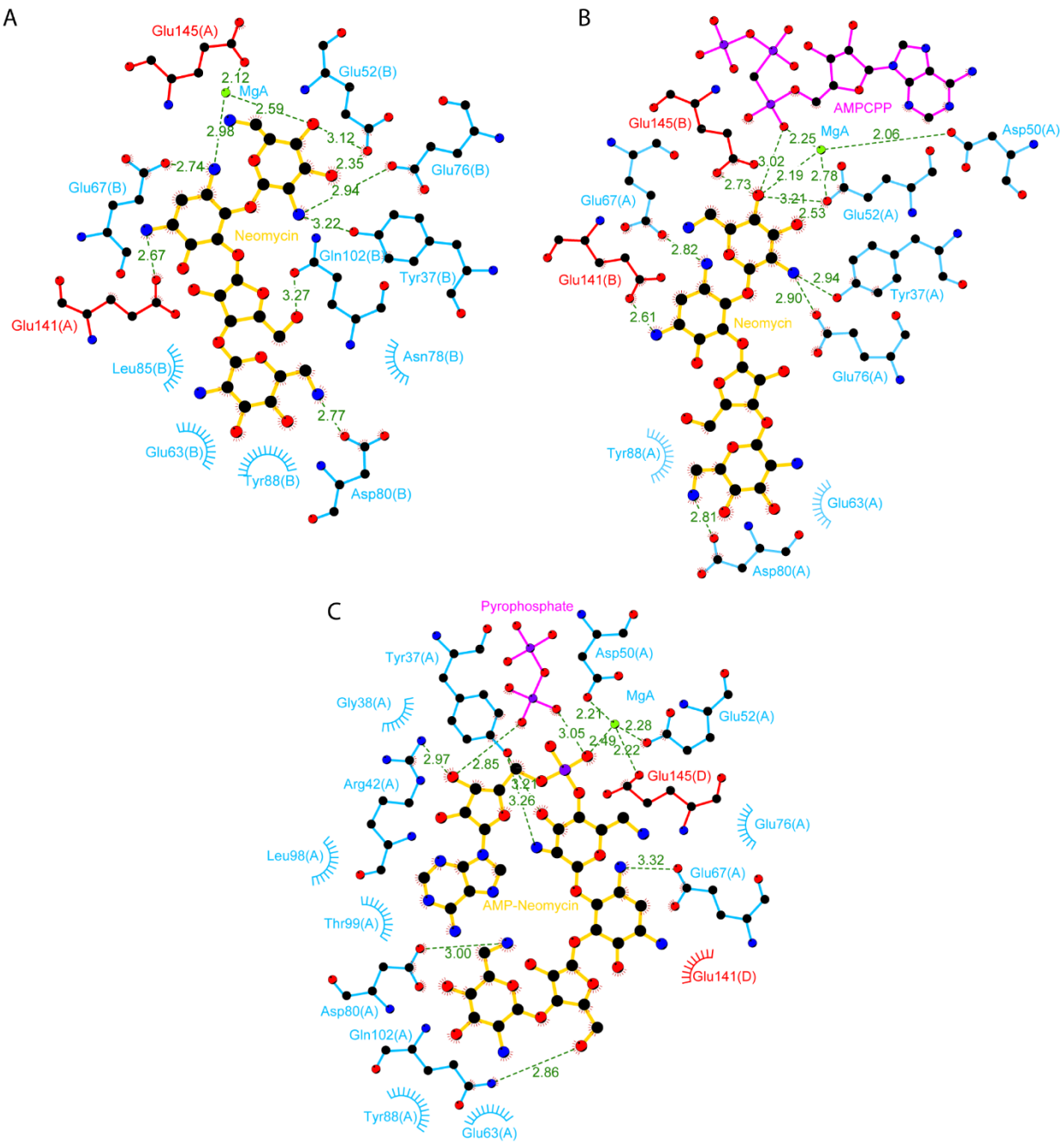

Figure S3. LigPlot+ diagram of T130K ANT4' ligands. (A) Neomycin. (B) Neomycin and AMPCPP. (C) AMP-neomycin and pyrophosphate.

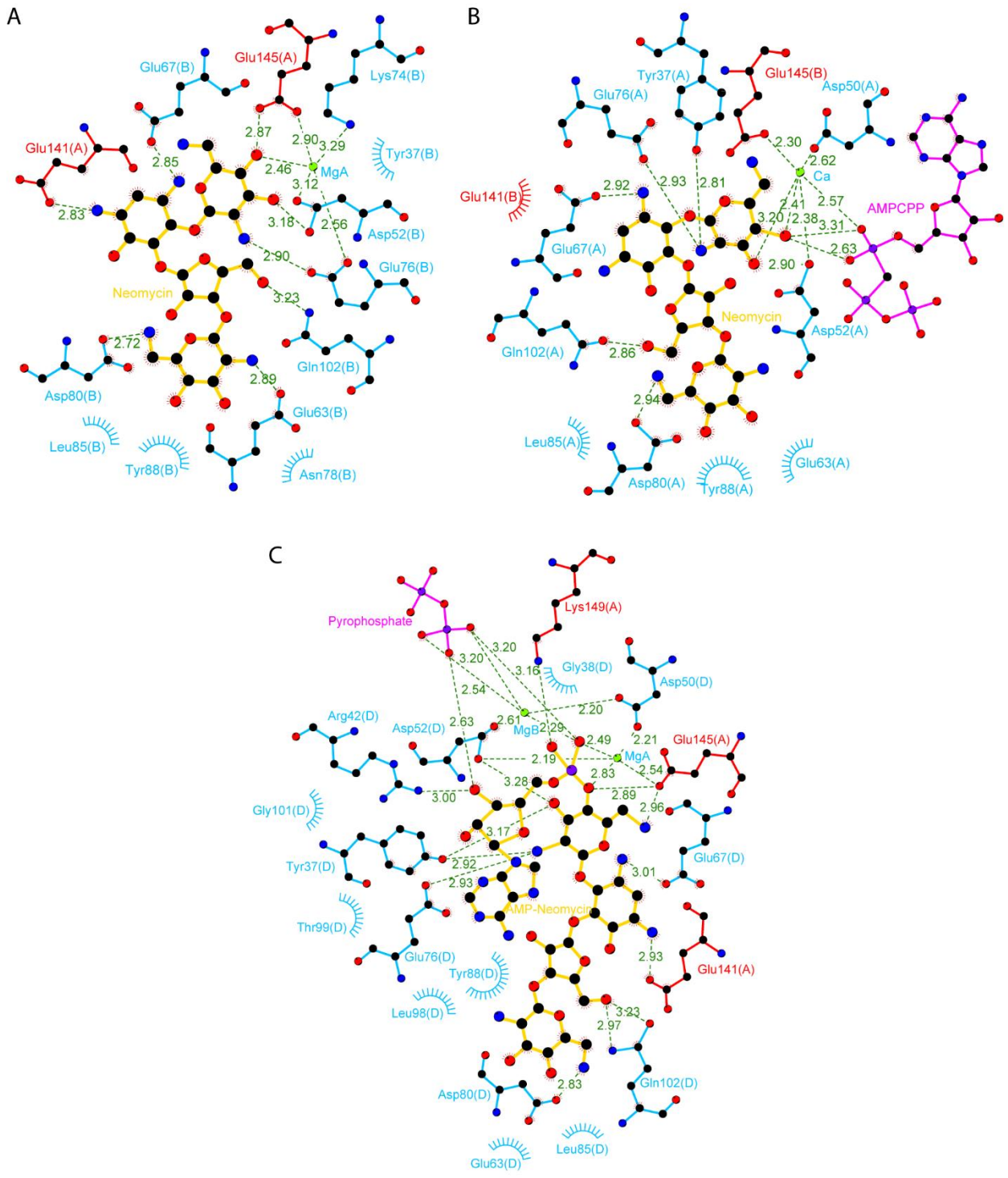

Figure S4. LigPlot+ diagram of T130K/E52D ANT4' ligands. (A) Neomycin. (B) Neomycin and AMPCPP. (C) AMP-neomycin and pyrophosphate.

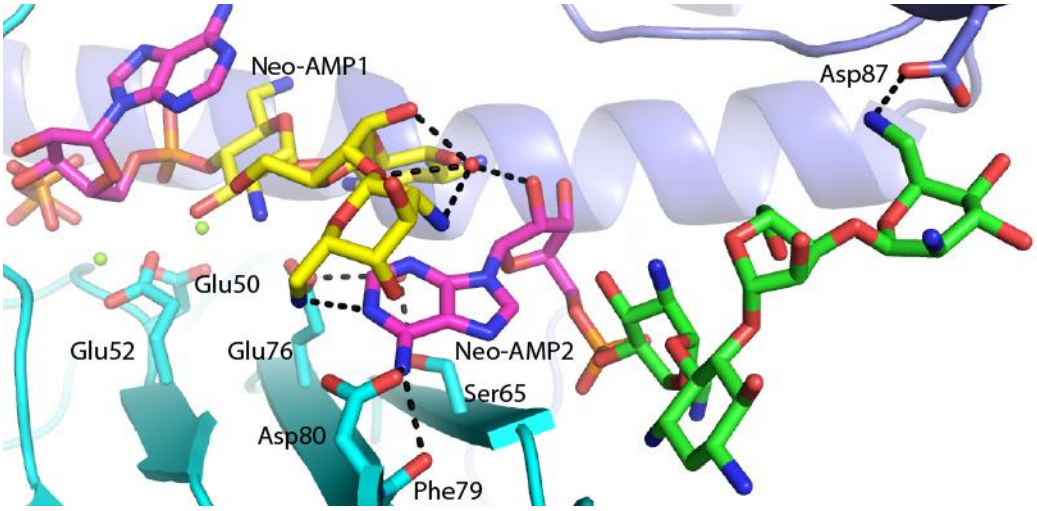

Figure S5. Additional adenylated neomycin in T130K ANT4' structure. The second adenylated neomycin product (Neo-AMP2) binds directly to the adenylated neomycin in the active site (Neo-AMP1) and both monomers of ANT4'. Hydrogen bonds are represented as black dashed lines. The protein monomers are colored blue and cyan. The adenylated neomycin in the active site is colored magenta and yellow, whereas the second product state outside of the active site is colored magenta and green.

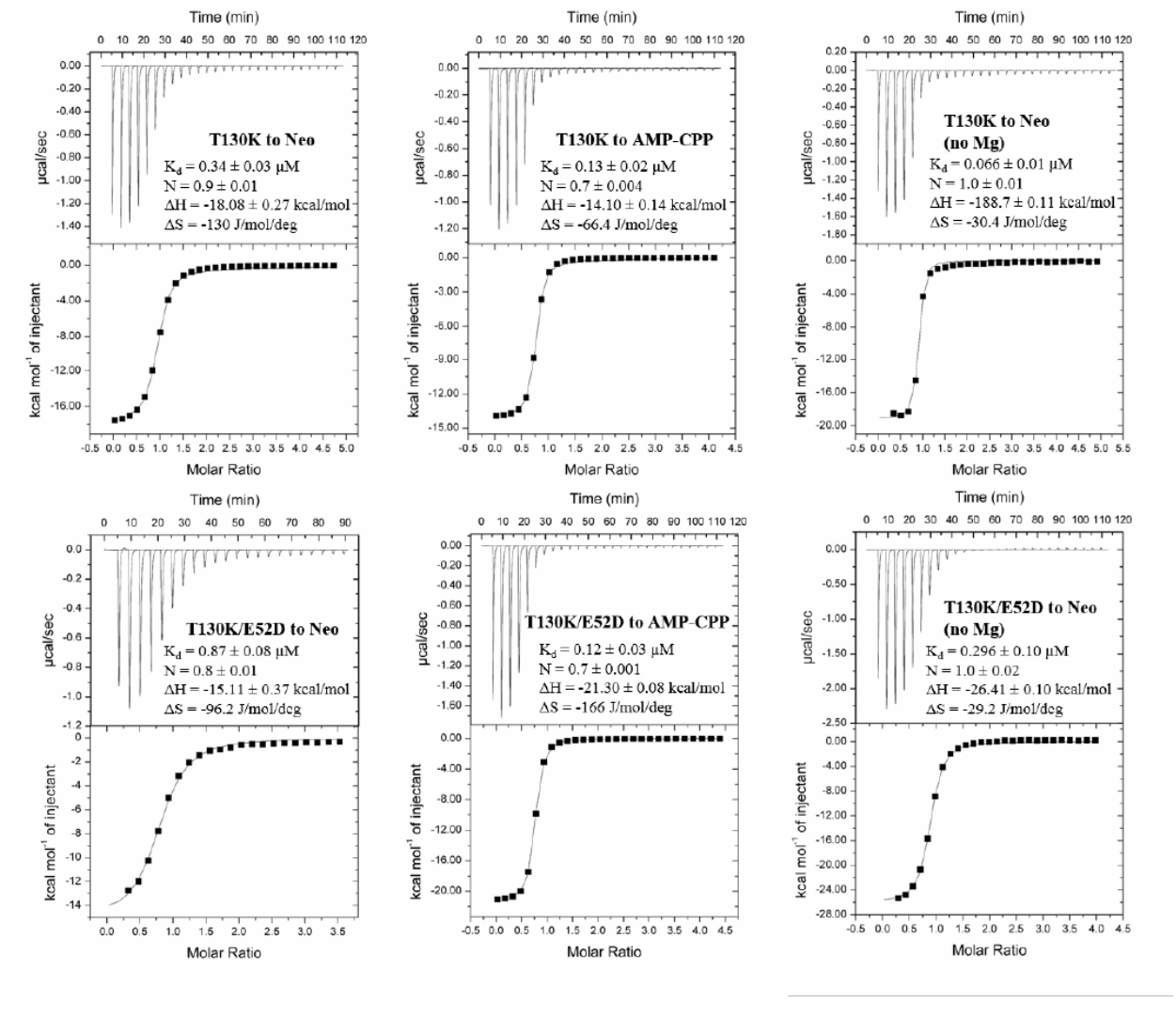

Figure S6. Thermodynamic parameters for the binding of neomycin to T130K and T130K/E52D ANT4' using ITC.