Supporting Information

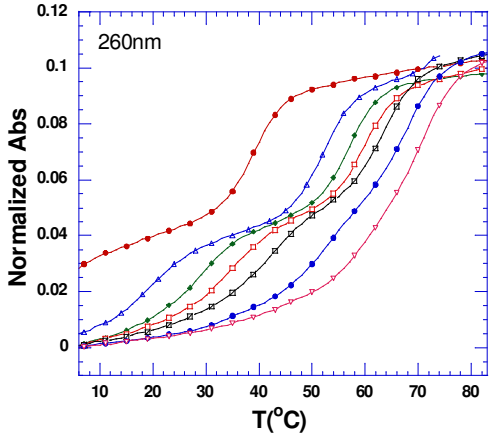

Figure 1: UV melting profiles of the 5'-dA12-x-dT12-x-dT12-3' triplex in the absence of drug at 260 nm. From left to right, the salt concentration was 0, 50, 100, 150, 250, 500, 800 mM, pH 7.2. Buffer solutions contain 10 mM sodium Cacodylate, and 0.5 mM EDTA.

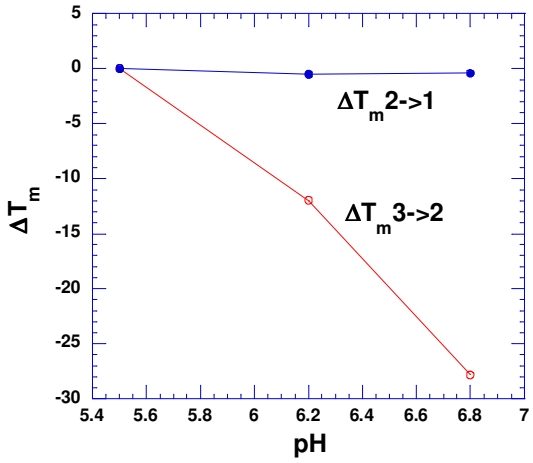

Figure 2: Plots of the $\Delta T_{m3\rightarrow2}$ and $\Delta T_{m2\rightarrow1}$ of poly(dA)•2poly(dT) triplex as a function of increasing pH value. Buffer solutions contain 10 mM sodium Cacodylate, 0.5 mM EDTA and 100 mM KCl.

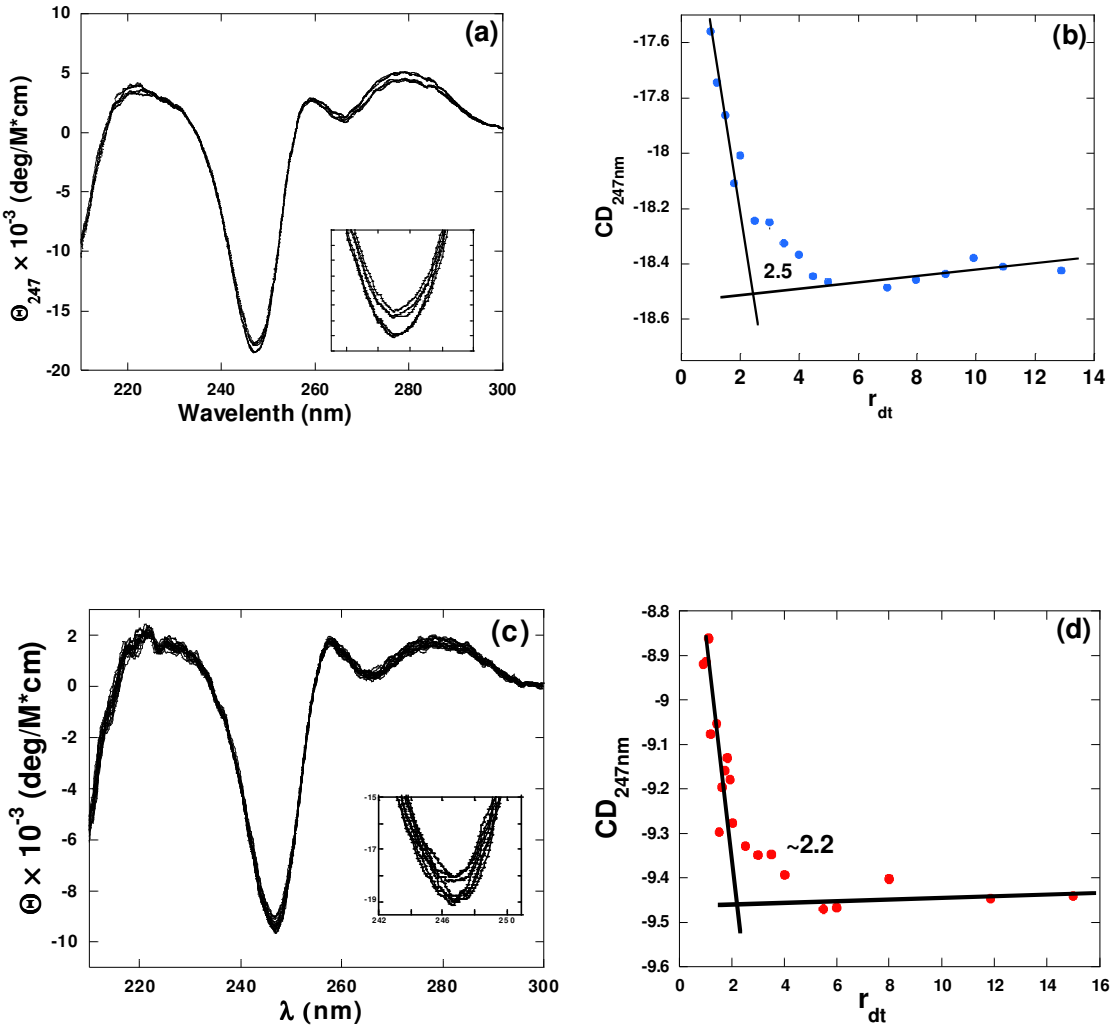

Figure 3: CD titration curves of paromomycin(a) and neomycin (c) with 5′-dA_{12}-x-dT_{12}-x-dT_{12}-3′ triplex. The insert is an expanded spectrum from 242 nm to 252 nm. For clarity, not all the CD spectra are shown. Plots of normalized molar ellipticity vs $1/r_{db}$ for CD titration of 5′-dA_{12}-x-dT_{12}-x-dT_{12}-3′ triplex with paromomycin (b) and neomycin (d) were drawn. The continuous lines in the right plot reflect the linear least-squares fits of each apparent linear domain of the experimental data. Buffer conditions: 10 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl, and pH 6.8. T=10 °C. DNA triplex concentration was 4 μM in strand.

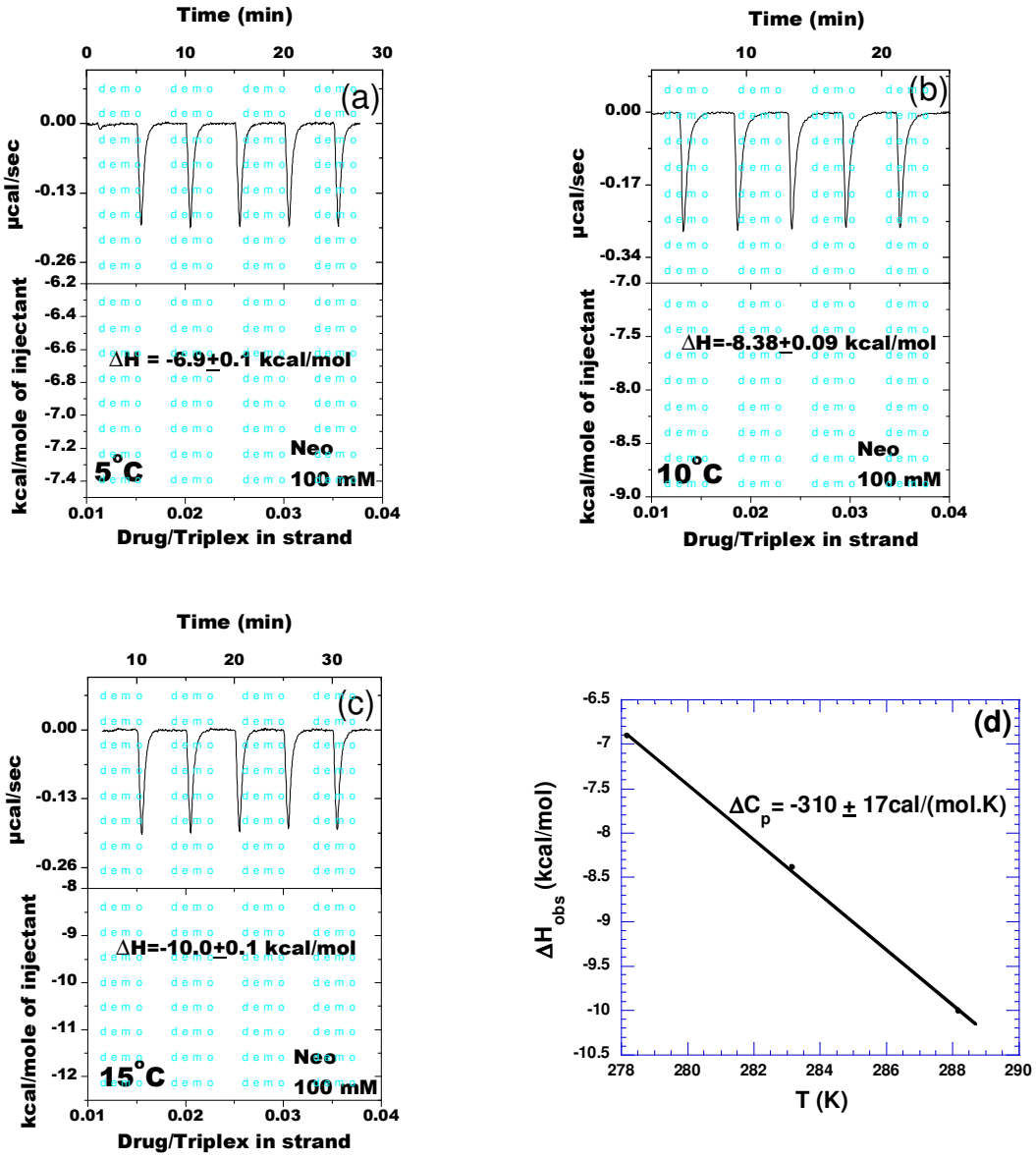

Figure 4: Sample ITC excess site titration of neomycin with 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' at 5°C (a), 10 °C (b) and 15 °C (c). (d) A plot of observed binding enthalpies *vs* corresponding temperatures. The slope reflects the heat capacity change ΔC_p. Buffer condition: 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl, and pH 6.8.

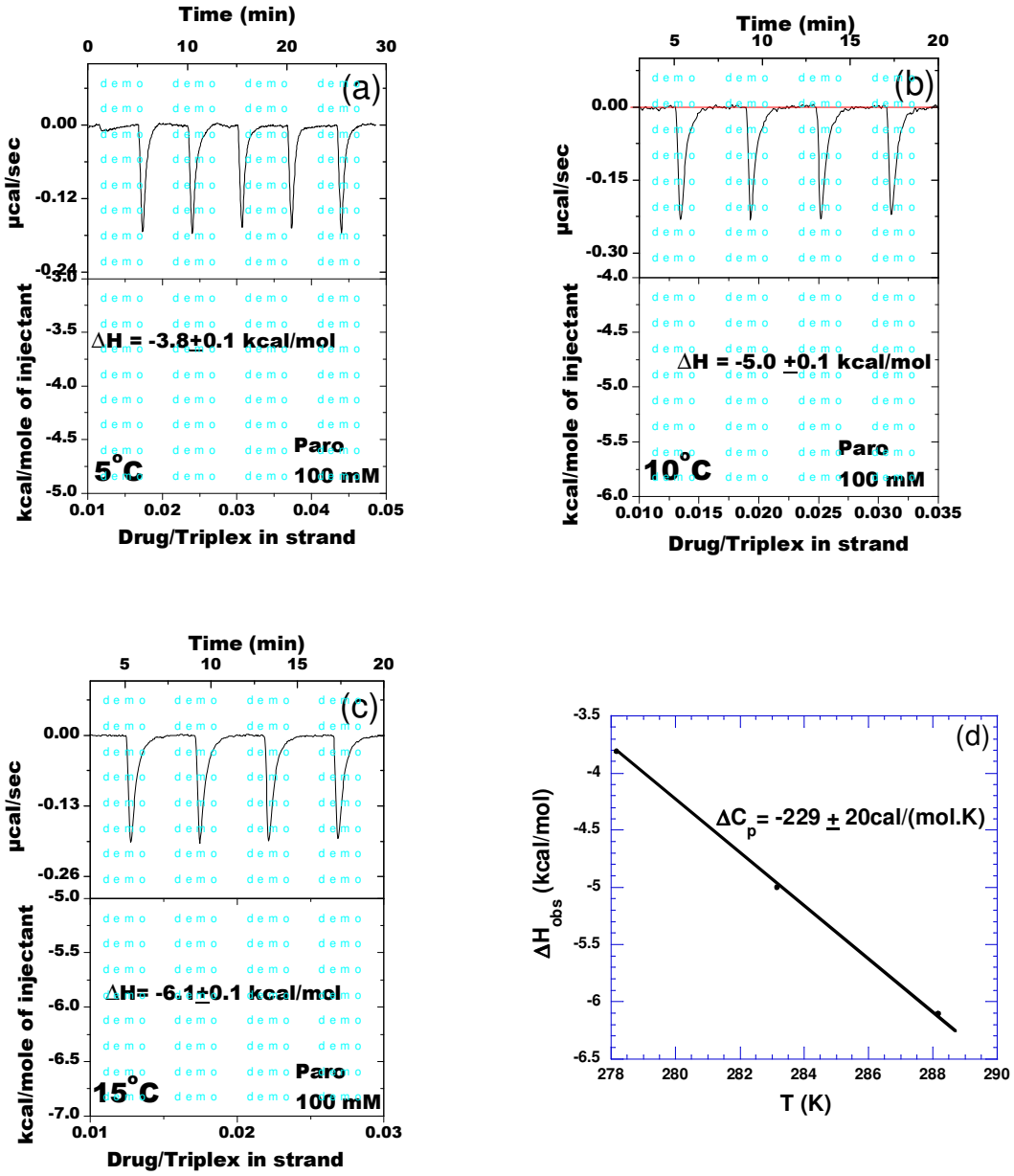

Figure 5: Sample ITC excess site titration of paromomycin with 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' at 5°C (a), 10 °C (b) and 15 °C (c). (d) A plot of observed binding enthalpies *vs* corresponding temperatures. The slope reflects the heat capacity change ΔC_p. Buffer condition: 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl, and pH 6.8.

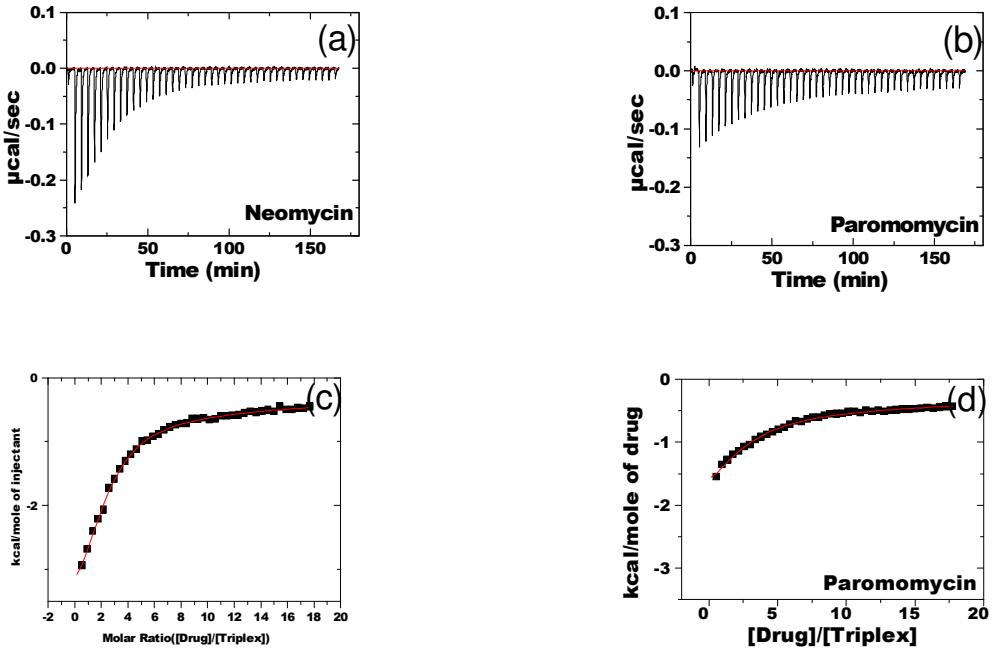

Figure 6: ITC studies of intramolecular triplex 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' with neomycin (a) and paromomycin (b) in 10 mM MOPS, 0.5 mM EDTA, 150 mM KCl, pH 6.8; T=10 ºC. [DNA] = 6 µM/strand, [Drug] = 700 µM. (c-d) Corrected injection heat as a function of [drug]/[triplex] ratio. The data points represent the experimental injection heat and the solid lines correspond to the calculated fits of the data by using a model with two sets of binding sites (Origin 5.0).

Table 1: Heat capacity changes of neomycin and paromomycin binding with 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' triplex at four salt concentrations in 10 mM sodium cacodylate, 0.5 mM EDTA, and pH 6.8.

[KCl] (mM)	Drug	ΔH_b(5°C) (kcal/mol)	ΔH_b(10 °C) (kcal/mol)	ΔH_b(15 °C) (kcal/mol)	ΔC_p (cal/mol·K)
100	Neomycin	-6.9±0.1	-8.4±0.1	-10.0±0.3	-310±43
150	Neomycin	-4.8±0.1	-5.5±0.1	-6.3±0.2	-158±30
200	Neomycin	-3.6±0.0	-4.1±0.1	-4.5±0.0	-84±12
250	Neomycin	-	-2.7±0.0	-2.8±0.1	-17±22
100	Paromomycin	-3.8±0.1	-5.0±0.10	-6.1±0.1	-229±36
150	Paromomycin	-3.2±0.0	-3.7±0.1	-4.1±0.1	-96±24
200	Paromomycin	-	-2.2±0.0	-2.3±0.1	-26±18
250	Paromomycin	-	-1.2±0.0	-1.2±0.0	-13±6
100	Ribostamycin	-	-3.0±0.1	-3.4±0.1	-76±30

ΔH_b were obtained from the ITC excess sites binding experiments.

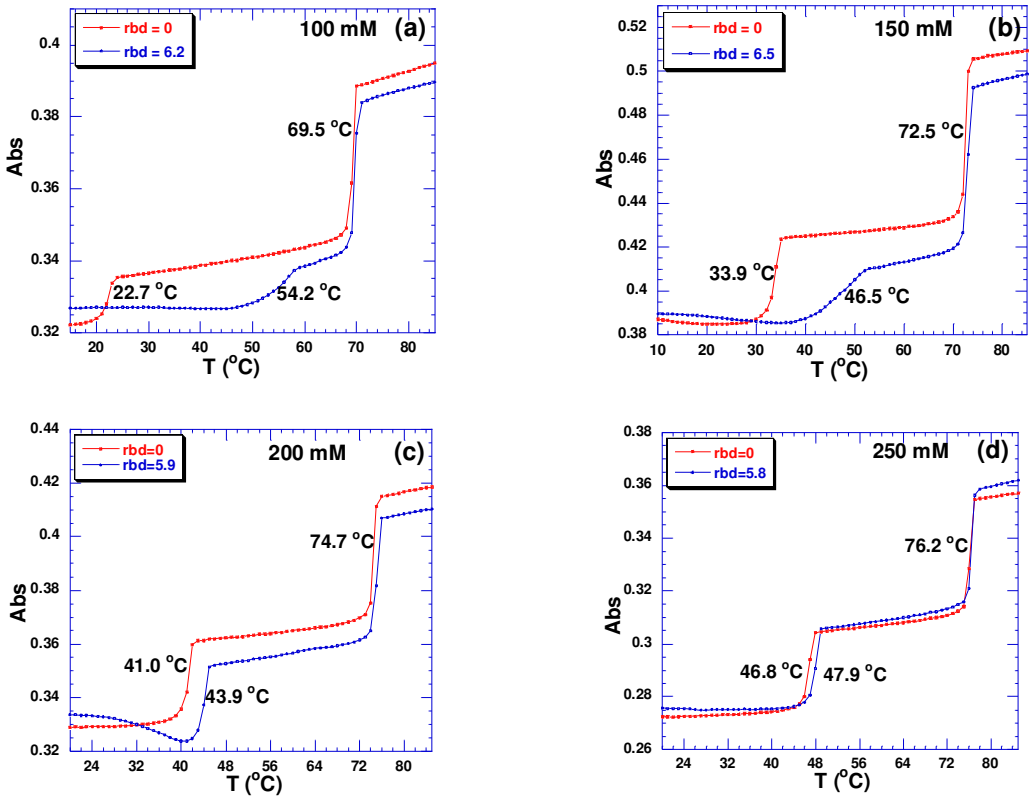

Figure 7: UV melting profiles of poly(dA)•2poly(dT) triplex at absence and saturated amount of neomycin in 10 mM sodium cacodylate, 0.5 mM EDTA, pH 6.8, and various KCl concentrations as indicated in profiles.

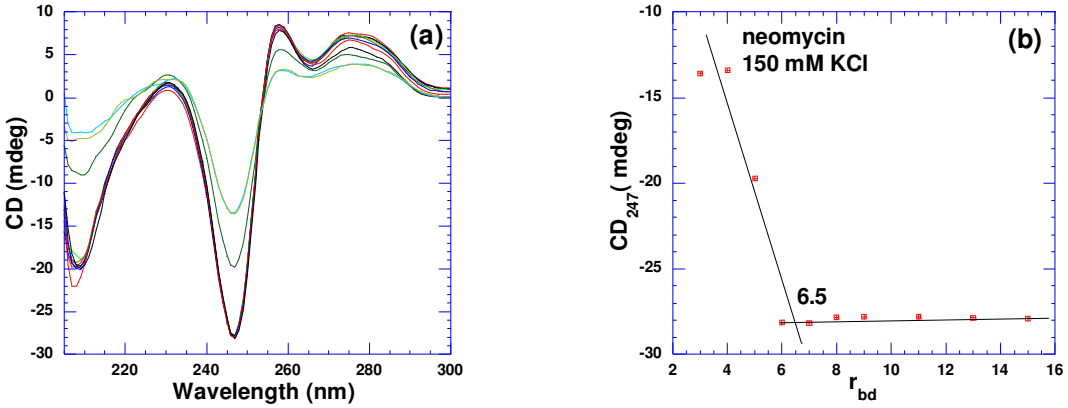

Figure 8: CD spectra of poly(dA)•2poly(dT) triplex with neomycin (a). CD signals at 247nm plotted *vs* corresponding r_{bd} values were shown at right panel corresponding to the left titration scans (b). The continuous lines in the right plot reflect the linear least-squares fits of each apparent linear domain of the experimental data. Buffer conditions: 10 mM sodium cacodylate, 0.5 mM EDTA, 150 mM KCl and pH 6.8. T=10 °C. [DNA]= 50 µM/bp.

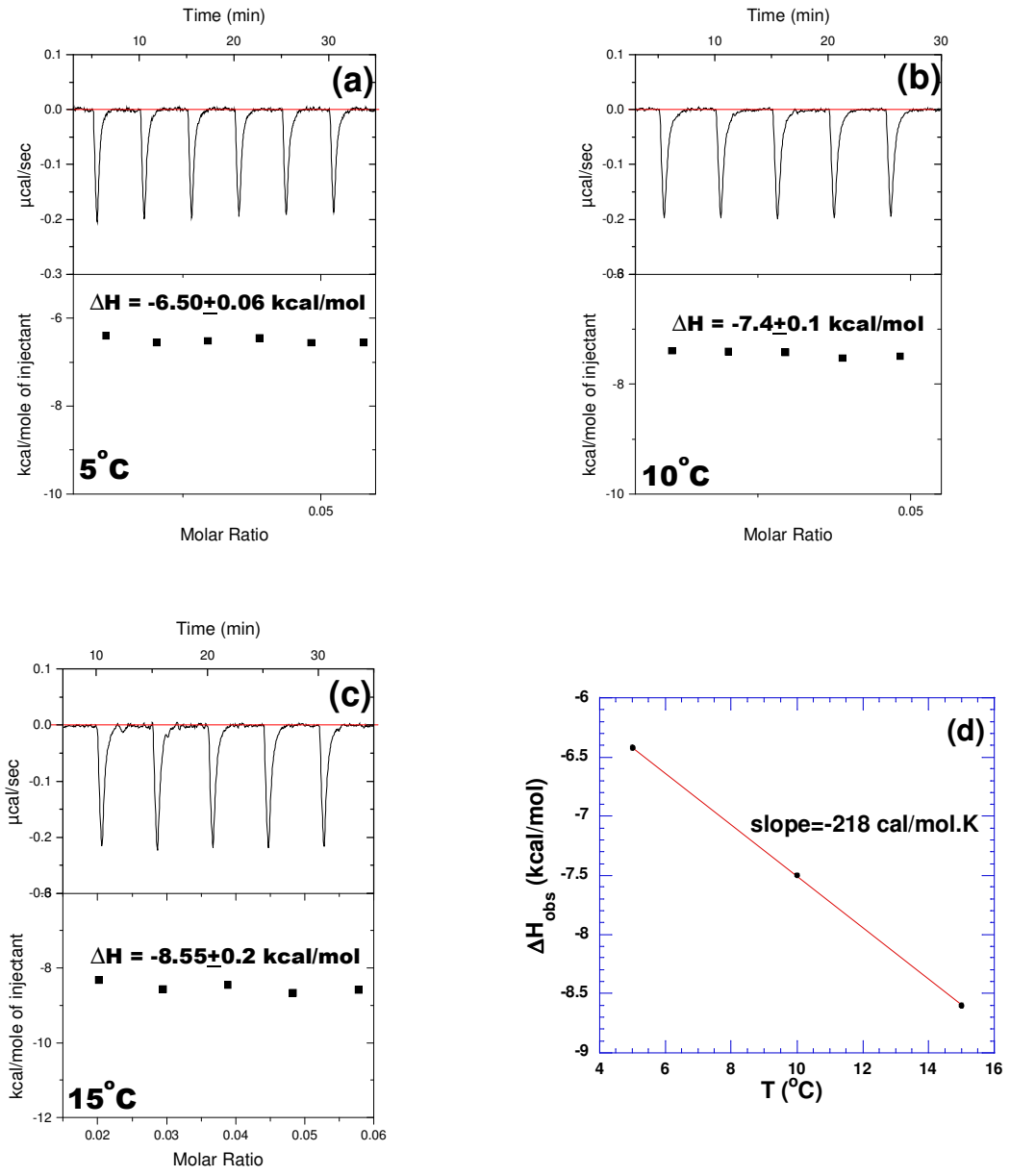

Figure 9: ITC excess site titration of neomycin with poly(dA)•2poly(dT) at 5°C (a), 10 °C (b) and 15 °C (c). (d) A plot of observed binding enthalpies vs corresponding temperatures. The slope reflects the heat capacity change ΔCp. Buffer condition: 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl, and pH 6.8.

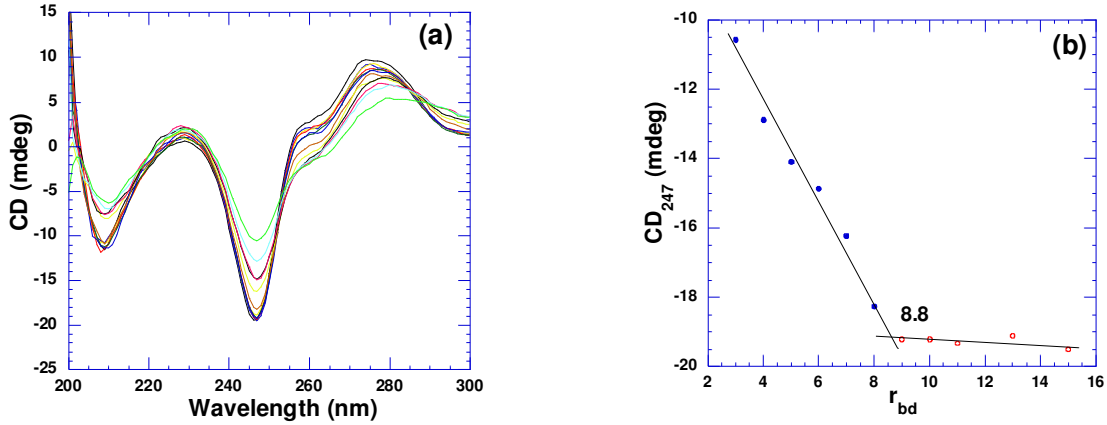

Figure 10: CD titration of neomycin into poly(dA)•2poly(dT). (a) CD spectra of poly(dA)•2poly(dT) triplex with increasing neomycin. (b) CD signals at 247nm plotted *vs.* corresponding r_{bd} values. The continuous lines in the right plot reflect the linear least-squares fits of each apparent linear domain of the experimental data. Buffer condition: 10 mM sodium cacodylate, 0.5 mM EDTA, 100 mM KCl, and pH 5.5.

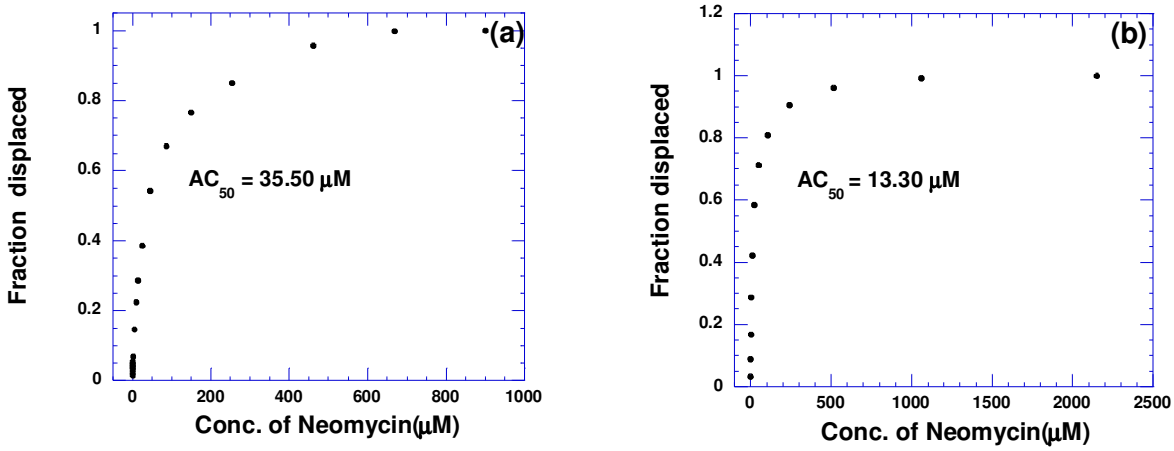

Figure 11: FID titration of neomycin in 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' triplex. A plot between fraction of thiazole orange displaced with increase in the concentration of neomycin at pH 6.8 (A) and at pH 5.5 (B). The solution was incubated for 1 hr. at 10^0C before titrating it with neomycin. The solution was equilibrated for 5 min. before taking the fluorescence emission scan. Buffer conditions: 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [5'-dA_{12}-x-dT_{12}-x-dT_{12}-3'] triplex = 100 nm/strand. [TO] = 650 nM.

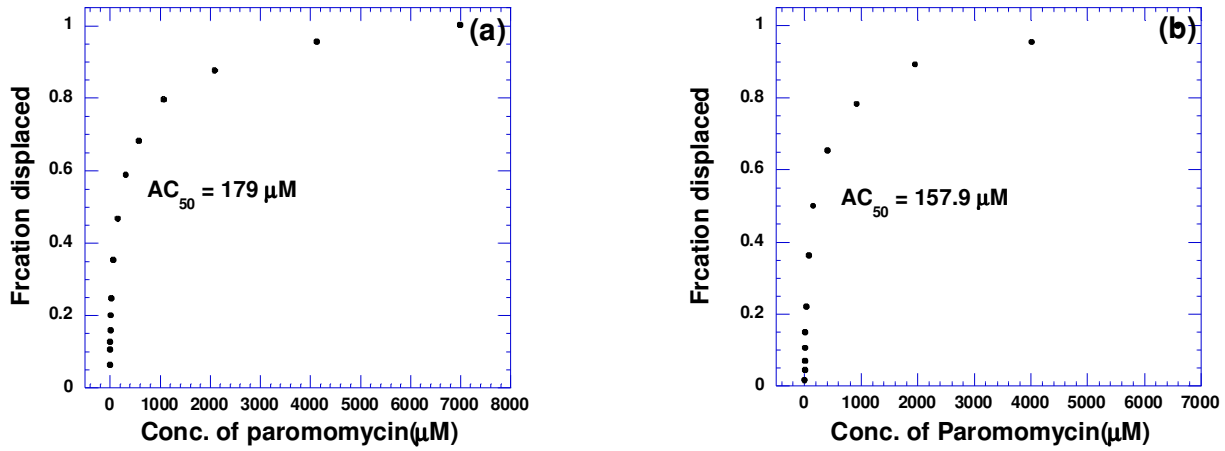

Figure 12: FID titration of paromomycin in 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' triplex. A plot between fraction of thiazole orange displaced with increase in the concentration of paromomycin at pH 6.8 (A) and at pH 5.5 (B). The solution was incubated for 1 hr. at 10^0C before titrating it with paromomycin. The solution was equilibrated for 5 min. before taking the fluorescence emission scan. Buffer conditions: 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [5'-dA_{12}-x-dT_{12}-x-dT_{12}-3'] triplex = 100 nm/strand. [TO] = 650 nM.

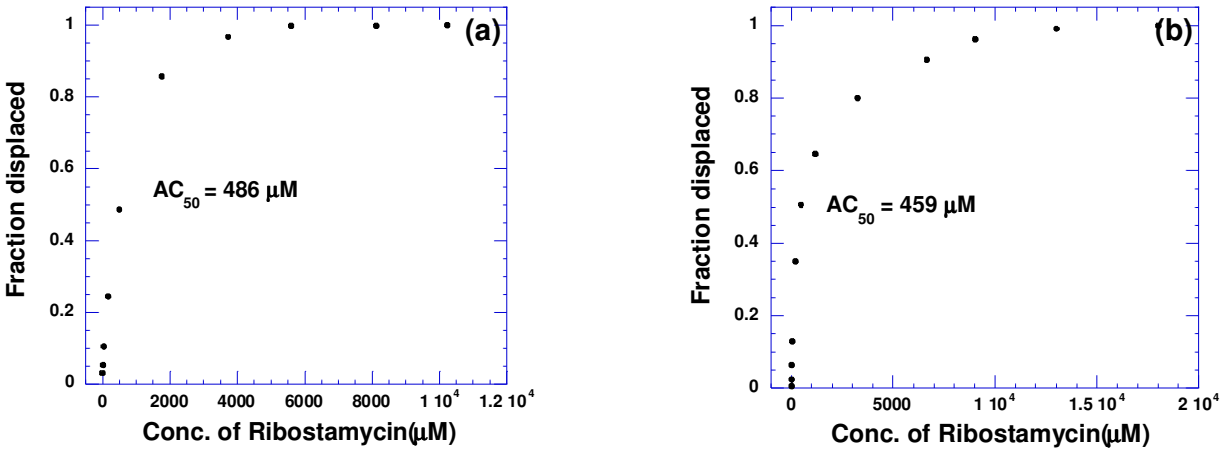

Figure 13: FID titration of ribostamycin in 5'-dA_{12}-x-dT_{12}-x-dT_{12}-3' triplex. A plot between fraction of thiazole orange displaced with increase in the concentration of ribostamycin at pH 6.8 (A) and at pH 5.5 (B). The solution was incubated for 1 hr. at 10^0C before titrating it with ribostamycin. The solution was equilibrated for 5 min. before taking the fluorescence emission scan. Buffer conditions: 150 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8. [5'-dA_{12}-x-dT_{12}-x-dT_{12}-3'] triplex = 100 nm/strand. [TO] = 650 nM.