Supplementary Information

Aminoglycoside binding to *Oxytricha nova* Telomeric DNA

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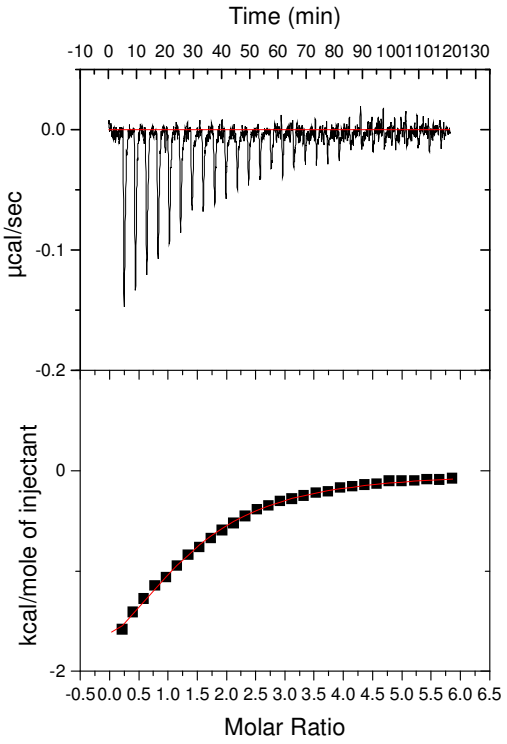
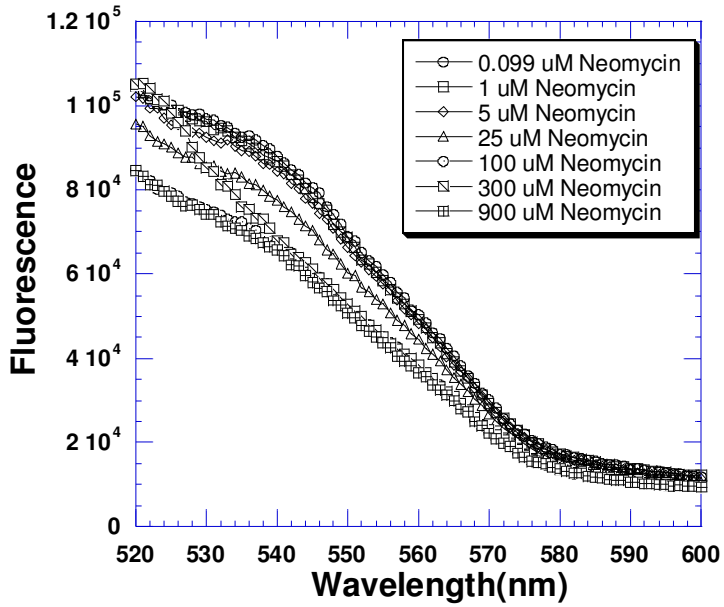


Figure S1: ITC profiles for the titration of *oxytricha nova* quadruplex $(GGGGTTTTGGGG)_2$ with Paromomycin in buffer 10 mM sodium cacodylate, 0.5 mM EDTA, and 90 mM KCl at pH 7.0. The heat burst curves (top panel in the figures) are the result of 10µL injections of a concentrated ligand solution into the DNA solution in buffer conditions as described earlier. The data points (lower panel in the figures) reflect the corrected injection heats, which were obtained by subtracting the dilution heats obtained from separate control experiments in which ligand was titrated with buffer only. The red line represents the calculated fits of the data using one binding site model. The data fitting was carried out using Origin 5.0 software.

A



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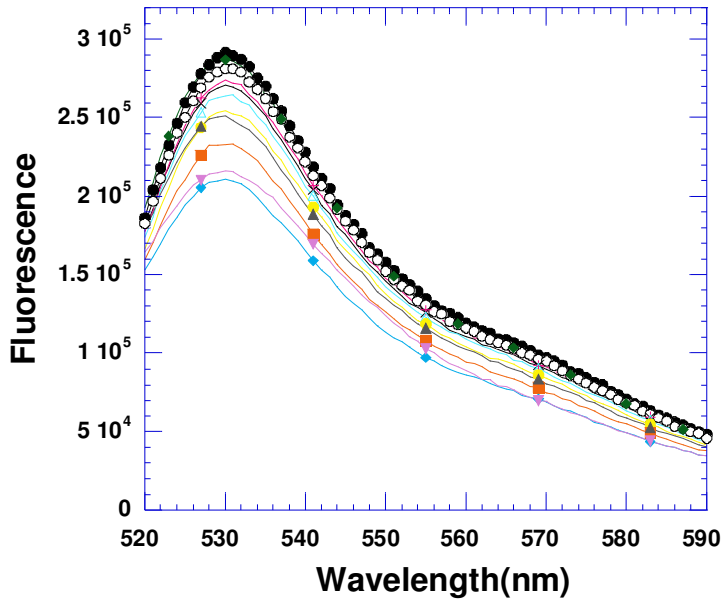

Figure S2: Determination of fluorescence quenching (A) titration of thiazole orange with neomycin in buffer 10 mM Sodium cacodylate, 100 mM Sodium Cacodylate at pH 7.0. (T=20^{0}C) (B) FID experiment of thiazole orange bound to *Oxytricha Nova* telomeric quadruplex using spermine in in buffer 10mM Sodium cacodylate, 100 mM Sodium Cacodylate at pH 7.0. (T=20^{0}C)

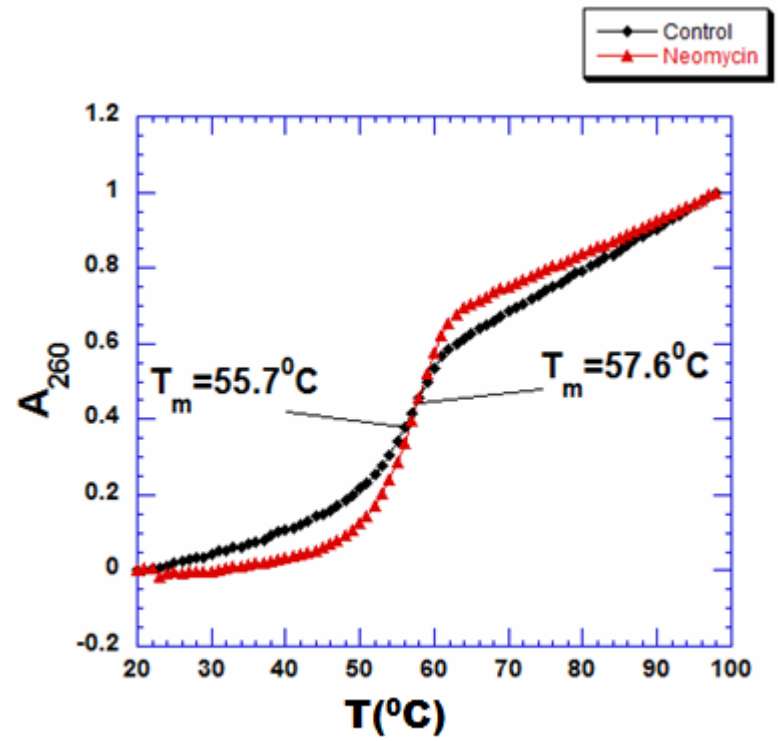

Figure S3: UV melting profile of quadruplex with and without neomycin. The oligonucleotide concentration was 10 µM/ str while the ligand concentrations were 1:1 quadruplex/ ligand ratio in buffer 10mM Sodium Cacoldylate, 0.5 mM EDTA and 100 mM NaCl at pH 7.0.The nucleic acid samples were heated at the rate of 0.2 deg/min. The melting temperatures were determined by the first derivative analysis.

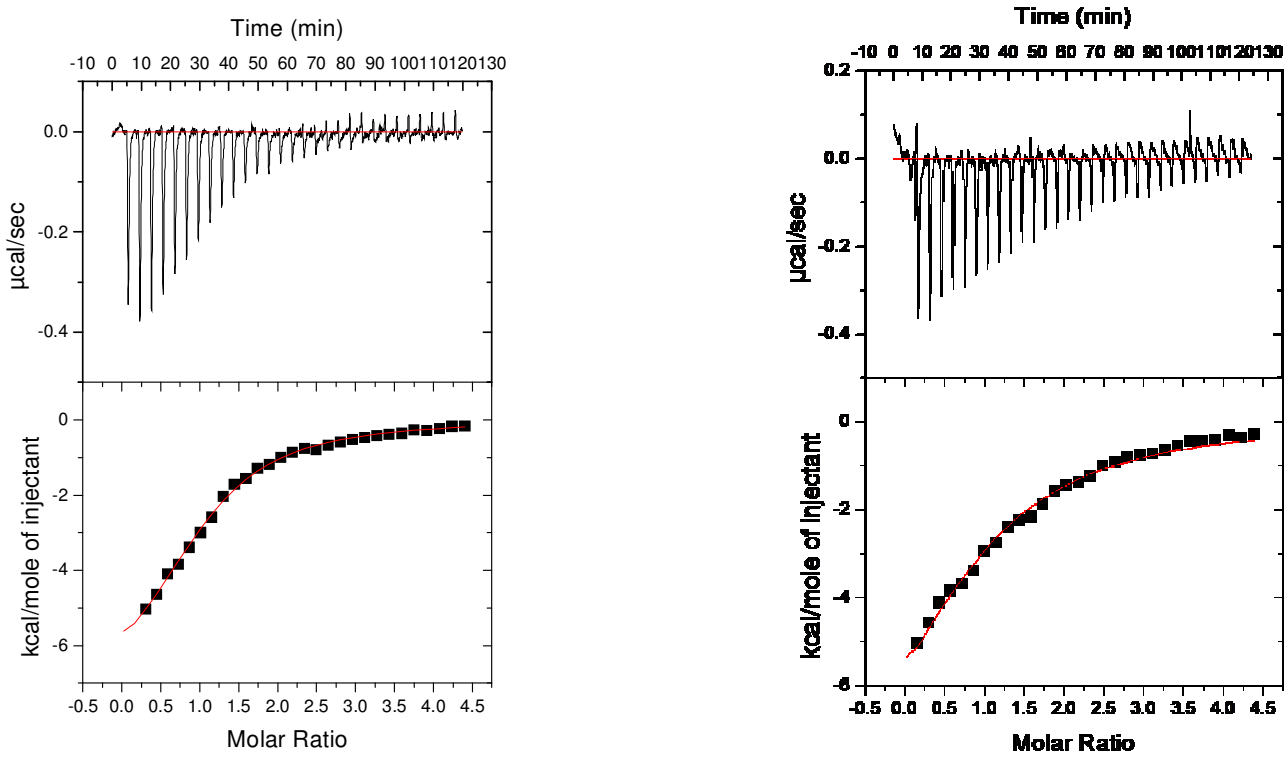

Figure S4: ITC titration of Oxytricha Nova quadruplex $(GGGGTTTTGGGG)_2$ in 30mM KCl , 0.5mM EDTA, 10mM Sodium cacodylate at pH 7.0 (Left 30^0C and right 40^0C) .

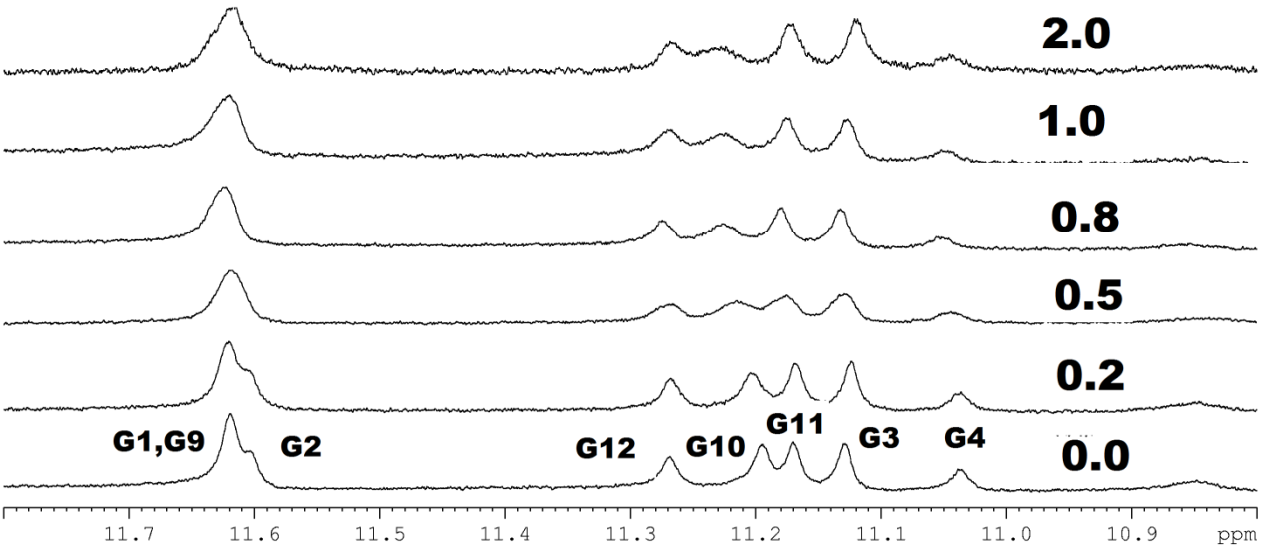

Figure S5: A plot showing ^{1}HNMR titration of neomycin into DNA. At 1:1 ligand:quadruplex ratio, complex was in precipitated state).

[Salt] **30mM KCl**	**n** **(drug/tetraplex)**	**K(M^{-1})**
20^0C	1.34±0.02	(2.76±0.21) ×10^5
30^0C	1.02±0.02	(1.78±0.11) ×10^5
40^0C	1.01±0.01	(0.73±0.03) ×10^5

Table S1: ITC titration of Oxytricha Nova telomeric quadruplex with Neomycin at various temperatures in buffer 100 mM NaCl, 10 mM SC, 0.5 mM EDTA, pH 7.0.

Association constant of neomycin binding to human telomeric DNA quadruplex obtained using ITC			
Salt	Temperaure	N	K_a
100mM NaCl	20^0C	1.05±0.03	(2.93±0.11)10^4

Table S2: Association constant and binding stoichiometry obtained from the ITC titration of neomycin into human telomeric quadruplex DNA in buffer 100mM NaCl ,0.5mM EDTA, 10mM Sodium cacodylate at pH 7.0 (T= 20^0C).

PDB Accession Codes of different nucleic acids studied for docking studies

Nucleic Acid	**PDB Accession Code**
A-Site RNA	2ET4
Triplex DNA	1D3R
Oxytricha DNA	156D
Human Telomeric DNA	143D
B-DNA (Major groove)	1BNA

Table S3: PDB ID of nucleic acid used in the docking studies.

Procedure for determination of thermodynamic parameters using Van't Hoffs analysis:

The raw data from the thermal melting curves were first plotted in Kaleidagraph (version 4.0) and the upper and lower baselines were linearly fit. The resulting equations were then used to convert the raw data to fraction folded using the equation

$$\theta = \frac{m(U).T+A(U)-A(T)}{\{m(U)-m(L)\}T+A(U)-A(L)\}} \quad \text{..... (1)}$$

where,

θ= Fraction Folded, m(U)= Slope of upper baseline, A(U)= Intercept of the upper baseline, m(L)= slope of the lower baseline, A(L)= intercept of the lower baseline, A(T)= Absorbance of at the corresponding temperature (T).

This represents the data between 1(fully folded state) to 0(fully unfolded state). The data was then converted to yield association constants using equation-

$$K_a = \frac{\theta}{2C_0(1-\theta)^2} \quad \text{..... (2)}$$

where,

K_a= Association constant, θ= Fraction Folded, C_0= Initial strand concentration of the quadruplex.

Following this, ln(K_a) was plotted versus 1/T to afford a linear relationship which gave –ΔH/R as slope and ΔS/R as intercept where,

ΔH= Molar enthalpy (van't Hoff), ΔS= Molar entropy (van't Hoff), R= Gas conatant

The data in the range of θ= 0.87 to θ= 0.13 was analyzed for van't Hoff analysis.