Electronic Supplementary Information

Guanine anchoring: A strategy for specific targeting of a G-quadruplex using short PNA, LNA and DNA molecules

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Materials and Methods

PNA Oligomer Preparation

PNA oligomers were synthesized automatically on a Biotage Initiator+ Alstra microwave peptide synthesizer using Rink Amide ChemMatrix resin (0.47 mmol/g) at 10-40 µmol scale. The Fmoc-PNA monomers were purchased from ASM Research Chemicals. Swelling of resin was performed at 70 °C for 20 min. Fmoc deprotection was achieved at room temperature first by treating the resin with 20% piperidine in DMF for 3 min, drained off, then adding fresh 20% piperidine in DMF for another 10 min. Coupling was performed at 75 °C for 6 mins using 4 eq. of PNA monomer (or Fmoc-Lys monomer), 4 eq. Oxyma and 4 eq. DIC in DMF. Capping step was performed using DMF-lutidine-acetic anhydride (89:6:5) for 2 min at room temperature after each coupling step. The resin was washed with DMF (4 ×) after each deprotection, coupling or capping step. After the synthesis was completed, the resin was rinsed with DMF (5 ×) followed by DCM (6 ×) and dried by vacuum.

The PNA oligomers were cleaved from the resin and deprotected using a cocktail containing TFA-water-TIPS (9.5:0.25:0.25) for 90 min with constant agitation at room temperature. The resin was then filtered off and the cleavage cocktail evaporated by continuous nitrogen flow. The crude PNA oligomer was precipitated from ice cold ether and dried under vacuum. The product was then purified by HPLC-MS using a reversed-phase preparative column (19 mm x 100 mm, XBridge Peptide BEH C18 OBD Prep Column, 130 Å, 5µm) with a linear gradient of 2% to 20% ACN containing 0.1% TFA over 30 min. Solvent was evaporated from the collected product before lyophilization from water.

DNA and LNA Preparation

DNA and LNA oligonucelotides were chemically synthesized on an ABI 394 DNA/RNA synthesizer using standard phosphoramidite chemistry. Most reagents were purchased from Glen Research. LNA phosphoramidites were purchased from Sigma-Aldrich. The oligonucleotides were cleaved and deprotected in aqueous ammonia for 16 hours at 55 °C. Samples were purified using Poly-Pak II cartridge (Glen Research) and dialyzed successively against water, 25 mM KCl solution and water. The samples were then lyophilized and reconstituted in buffer containing 10 mM KCl and 10 mM potassium phosphate buffer (pH 7.0), supplemented with 1 mM EDTA and 0.1 mM NaN_3.

Nuclear Magnetic Resonance (NMR) Spectroscopy

All NMR titration experiments were performed on 600-MHz Bruker AVANCE II spectrometer at 25 °C unless otherwise stated. DNA concentrations used was 50 – 100 µM; buffer contained 10 mM KCl and 10 mM potassium phosphate (pH 7.0), supplemented with 1 mM EDTA and 0.1 mM NaN3. All spectra were processed and analyzed using TopSpin 4.0.6.

NMR Thermal Melting

All NMR thermal melting experiments were performed on 800-MHz Bruker AVANCE III HD spectrometer. A series of 1D ^{1}H NMR spectra of free *delta1* and *delta1*-PNA (*p(AG)*, *p(GA)* or *p(Lys-GA*)) complex was recorded at 5 °C interval from 25 – 75 °C in buffer containing 3 mM KCl and 3 mM potassium phosphate (pH 7.0). The DNA concentration was 100 µM and the DNA to ligand concentration ratio was 1:1. A representative peak in the imino proton region was integrated which is considered as proportional to the amount of folded species. A plot of folded species fraction against temperature was fitted using a Boltzmann distribution derived function to determine the melting temperature.

Gel electrophoresis

Gel electrophoresis experiment was performed at room temperature on a 10 cm × 7 cm native gel containing 20% acrylamide (acrylamide:bis-acrylamide = 37.5:1) with a running buffer containing 10 mM KCl in TBE (pH 8.3) at 120 V for 60 minutes. Each well contains 10 μL of DNA samples at 50 μM in solution containing 10 mM KCl, 10 mM potassium phosphate (pH 7.0), 4% sucrose. The gel was visualized by UV shadowing.

Isothermal Titration Calorimetry (ITC)

All ITC results were recorded using ITC-200 isothermal titration calorimeter at 25 °C. The initial concentration of *delta1* in the sample cell was 100 μM in buffer containing 10 mM KCl and 10 mM potassium phosphate (pH 7.0), supplemented with 1 mM EDTA and 0.1 mM NaN_3, while the ligand (*p(AG)*, *p(GA)* or *p(Lys-GA*)) stock concentrations were 1 mM in the same buffer. The titration volume was set at 2 μL for each addition. The data obtained were fitted using MicorCal-PEAQ-ITC analysis software.

Table S1. Sequences of substrates and ligands used in this study.

Substrate	Sequence[a],	
delta1	$^{5'}$TTGGTGGGTGGGTGGGT$^{3'}$	
T95-2T	$^{5'}$TTGGGTGGGTGGGTGGGT$^{3'}$	
delta1-ex	$^{5'}$TTGGTGGGTGGGTGGGCATGGACAGC$^{3'}$	
chl1-mod	$^{5'}$CGACCTGACTGGGGAAGGGGTGGGT$^{3'}$	
Ligand	Sequence[b],[c]	Target substrate
p(AG)	^{N}AG^{C}	*delta1*
p(GA)	^{N}GA^{C}	
p(Lys-GA)	^{N}Lys-GA^{C}	
DNA-G	$^{5'}$GCTGTCCATG**G**$^{3'}$	*delta1-ex*
PNA-G	^{N}GCTGTCCATG**G**^{C}	
LNA-G	$^{5'}$GcTgTcCaTg**G**$^{3'}$	
G_2-DNA	$^{5'}$**GG**AGTCAGGTCG$^{3'}$	*chl1-mod*

Flanking sequence are underlined; residues complementary to flanking sequence of target substrate are underlined; anchoring guanine residues are indicated by boldface; LNA nucleotide are indicated by lowercase.

Supplementay Figures

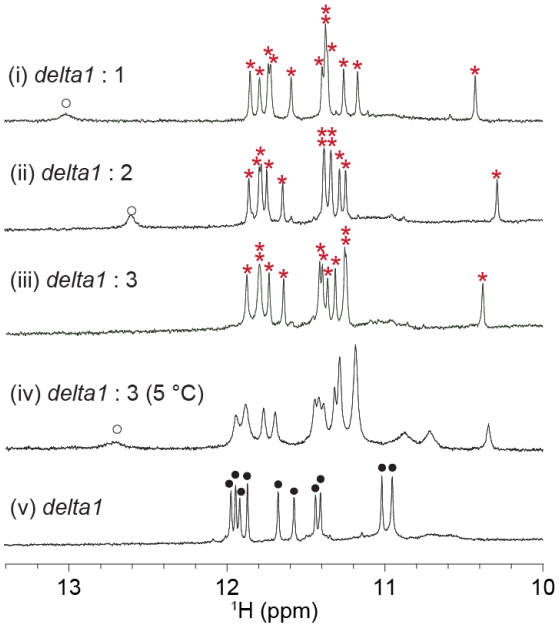

Figure S1. NMR imino proton region of *delta1* complex with (i) compound **1** at 25 °C, (ii) compound **2** at 25 °C and (iii) compound **3** at 25 °C or (iv) 5 °C and (v) free *delta1* at 25 °C. PNAs were added to 100 µM *delta1* to 1 eq. ratio. Spectra of all complexes shows the transition of 10 initial peaks (free form) to 12 final peaks (complex form). Peaks of free form are indicated by black circles and peaks from complex form are indicated by red asterisks. Peak from the A•T base pair is indicated by white circle. *delta1*: $^{5'}$TTGGTGGGTGGGTGGGT$^{3'}$

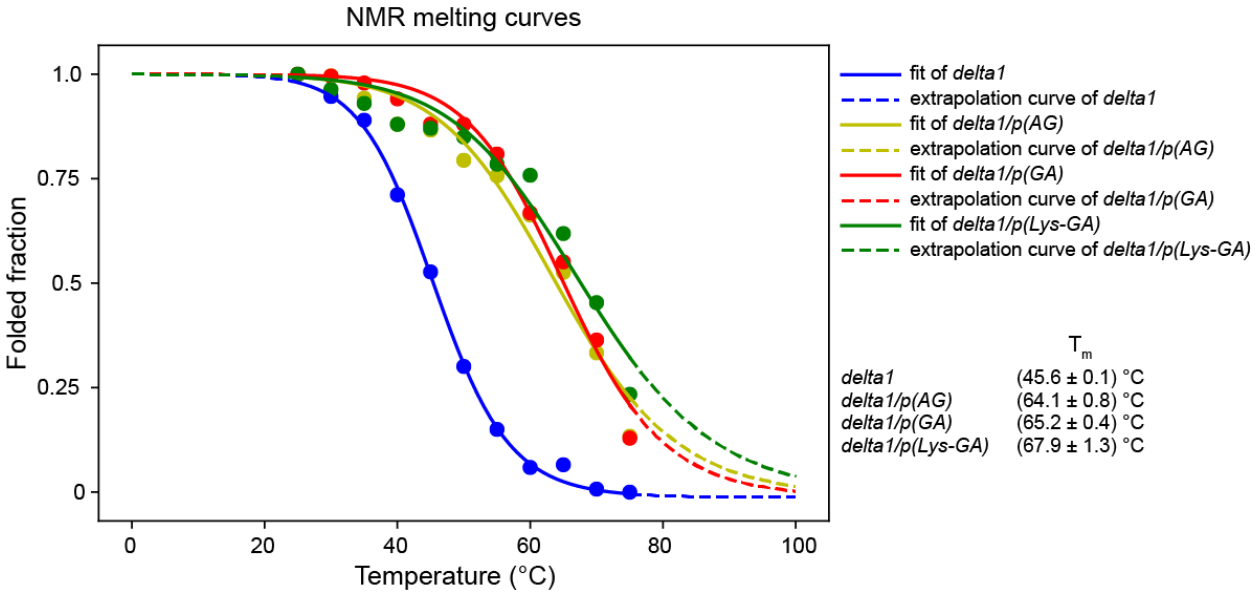

Figure S2. NMR melting experiment of free *delta1* and *delta1* complex with *p(AG)*, *p(GA)* and *p(Lys-GA)* in buffer containing 3 mM KCl and 3 mM potassium phosphate (pH 7.0). Concentration of *delta1* was 100 µM and the DNA to ligand concentration ratio was 1:1. *delta1*: $^{5'}$TTGGTGGGTGGGTGGGT$^{3'}$

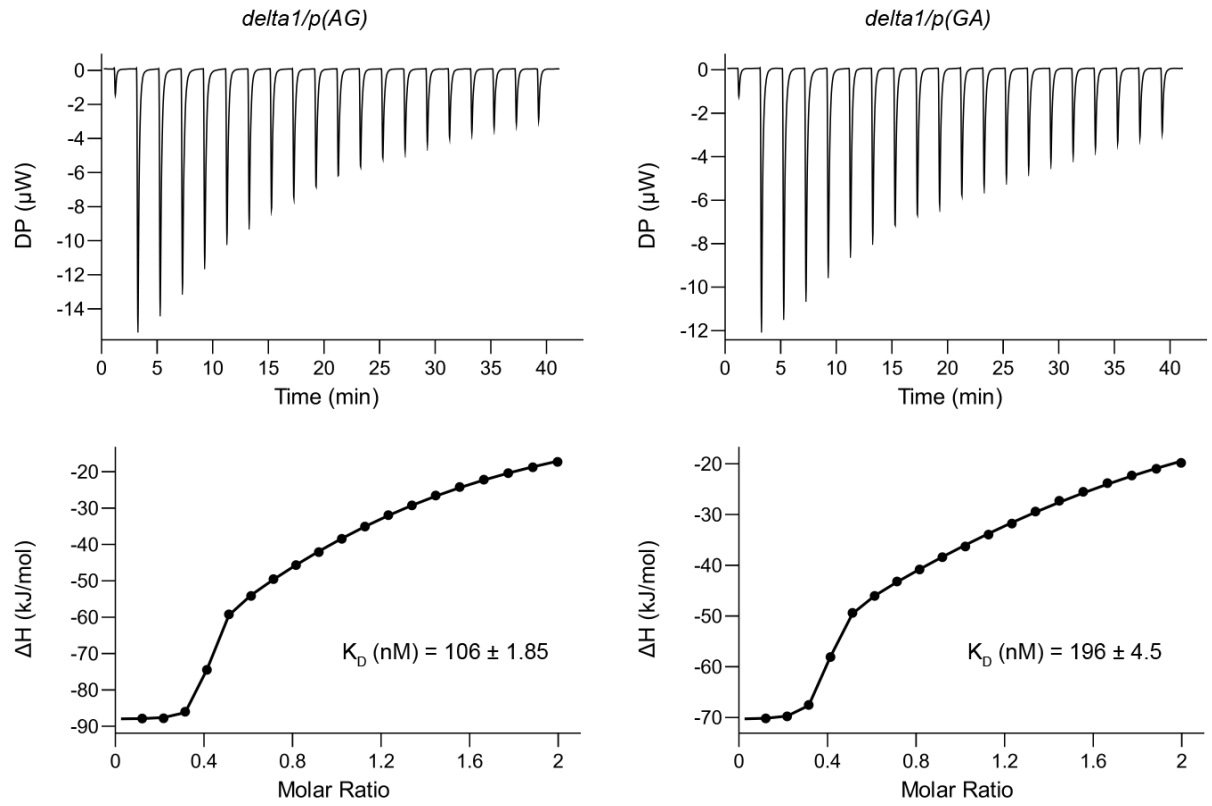

Figure S3. ITC binding isotherms of *delta1* with *p(AG)* (left) and *p(GA)* (right). *delta1*: 5′-TTGGTGGGTGGGTGGGT-3′

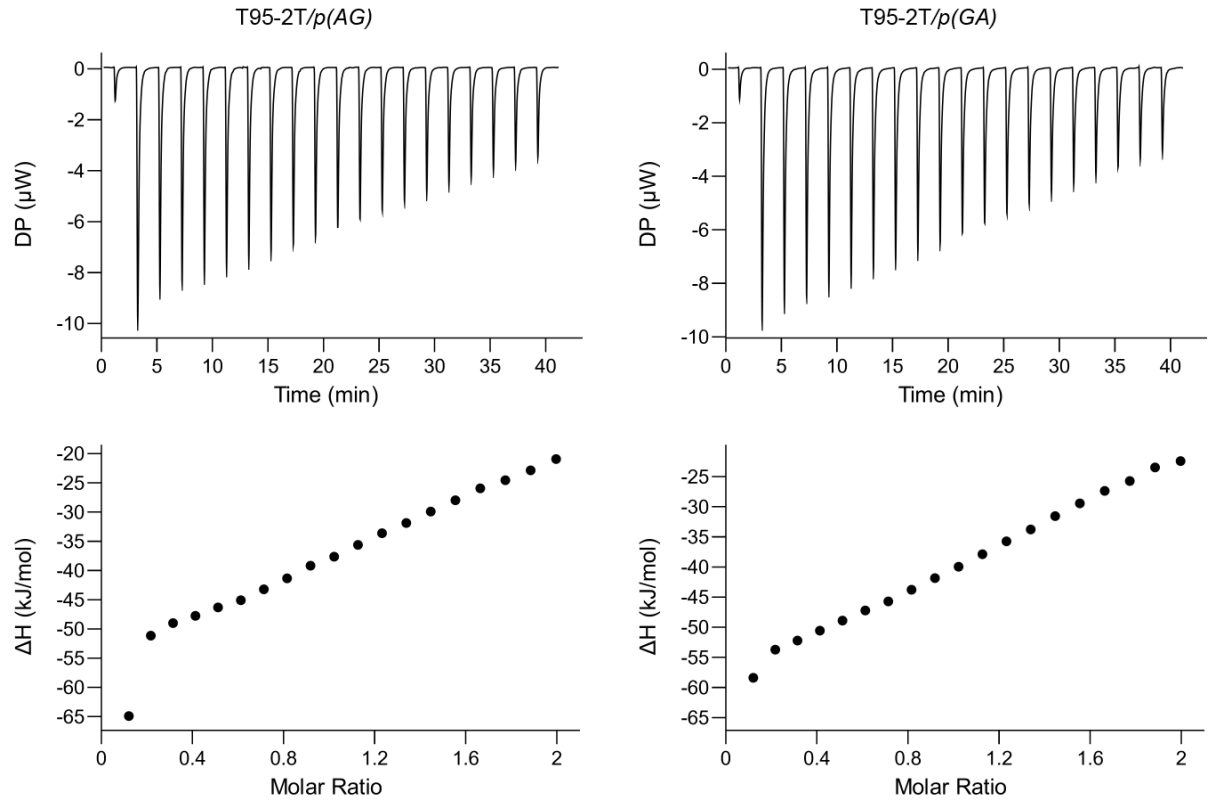

Figure S4. ITC binding isotherms of *T95-2T* with *p(AG)* (left) and *p(GA)* (right). *T95-2T*: 5′-TTGGGTGGGTGGGTGGGT-3′. Non-specific binding of the ligands toward *T95-2T* - a parallel G4 without any vacant site.

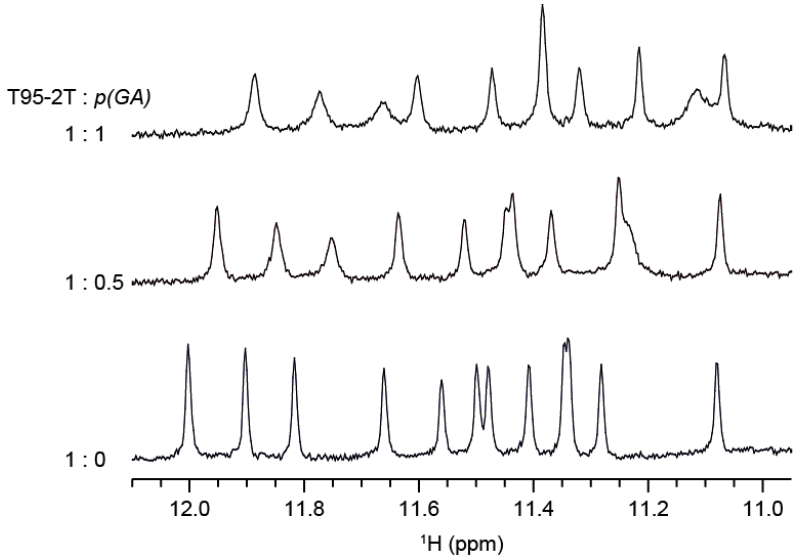

Figure S5. NMR titration experiment of *T95-2T* with *p(GA)* in buffer containing 10 mM KCl and 10 mM potassium phosphate (pH 7.0), supplemented with 1 mM EDTA and 0.1 mM NaN_3. *T95-2T*: $^{5'}$TTGGGTGGGTGGGTGGGT$^{3'}$. Concentration of *T95-2T* was 100 µM. Non-specific binding was observed in NMR titration experiment of *T95-2T* with *p(GA)*, where up-field shifts were observed for majority of the peaks.

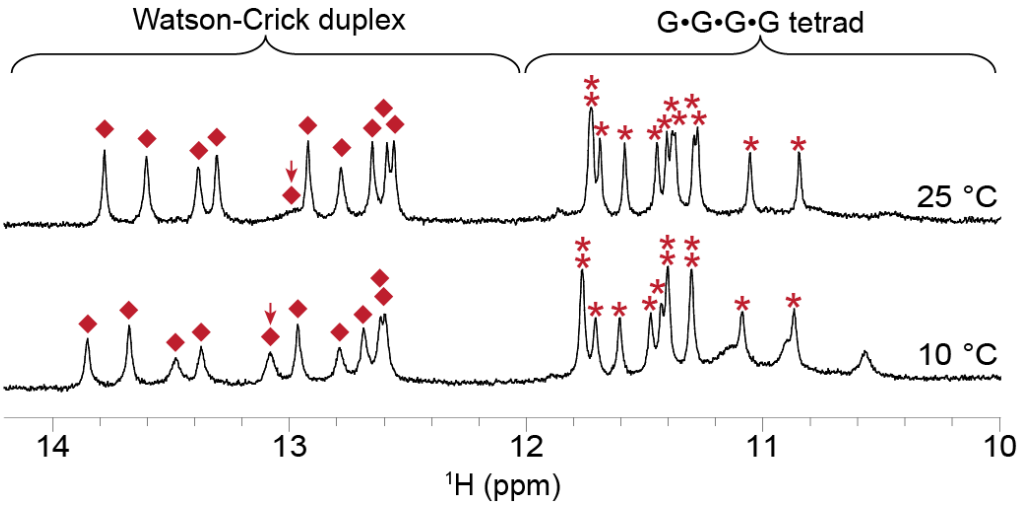

Figure S6. NMR imino proton region of *delta1-ex:DNA-G* complex showing 10 Watson-Crick duplex peaks. Imino proton of the base pair at the end of the duplex (highlighted with an arrow) was broadened at 25 °C due to fast proton exchange with solvent. *delta1-ex*: $^{5'}$TTGGTGGGTGGGTGGGCATGGACAGC$^{3'}$; *DNA-G*: $^{5'}$GCTGTCCATGG$^{3'}$; complementary segments are underlined.

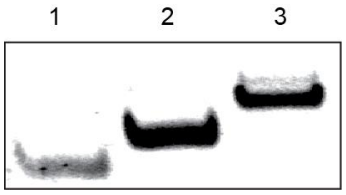

Figure S7. Native gel electrophoresis of *delta1-ex* (Lane 1), *delta1-ex*:*DNA-G* complex (Lane 2) and *delta1-ex*:*PNA-G*. Each well contains 10 µL of DNA samples at 50 µM in solution containing 10 mM KCl, 10 mM potassium phosphate (pH 7.0), 4% sucrose. *delta1-ex* to ligand concentration ratio was 1:1. The size increase after complex formation resulted in a band shift with respect to the free *delta1-ex* band. A larger shift was observed for *delta1-ex*:*PNA-G* complex as compared to its negatively-charged DNA counterpart *delta1-ex*:*DNA-G* complex as the neutral backbone of the PNA molecules lessen the band migration.

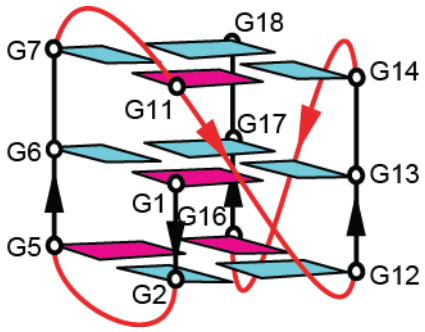

Figure S8. Schematic of *chl1* G4.

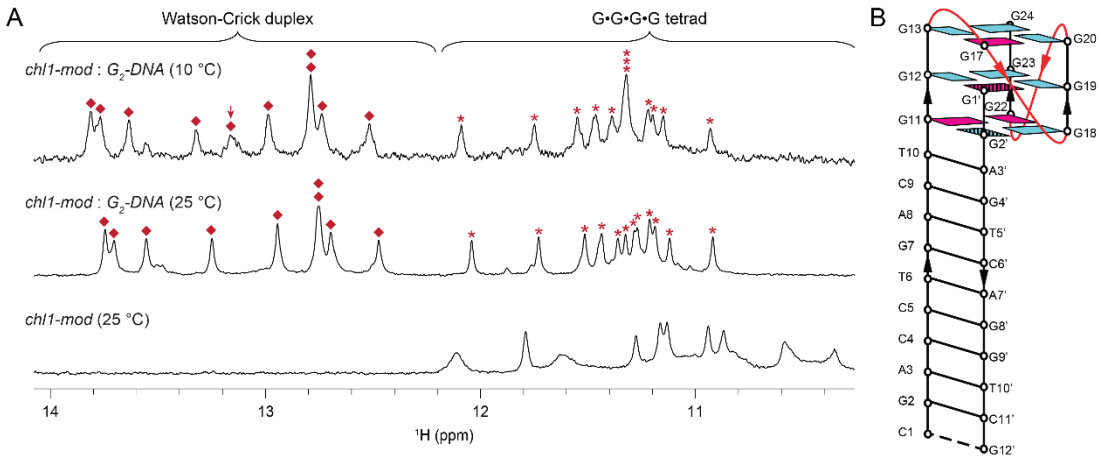

Figure S9. (A) Imino proton NMR region of free *chl1-mod* and its complex with *G_2-DNA* at 25 °C and 10 °C. Peaks from G-tetrad and peaks from Watson-Crick duplex are indicated by red asterisks and red diamond respectively. Imino proton of the base pair at the end of the duplex (highlighted with an arrow) was broadened at 25 °C due to fast proton exchange with solvent. (B) Schematic of *chl1-mod*:*G_2-DNA* quadruplex duplex hybrid complex. Target (*chl1-mod*): $^{5'}$CGACCTGACTGGGGAAGGGGTGGGT$^{3'}$; Ligand (*G_2-DNA*): $^{5'}$GGAGTCAGGTCG$^{3'}$; complementary segments are underlined.