Electronic Supplementary Material (ESI) for Molecular BioSystems

SUPPORTING INFORMATION

Bi and tri-substituted Phenyl Ring Containing Bisbenzimidazoles Bind Differentially with DNA Duplexes: A Biophysical and Molecular Simulation study

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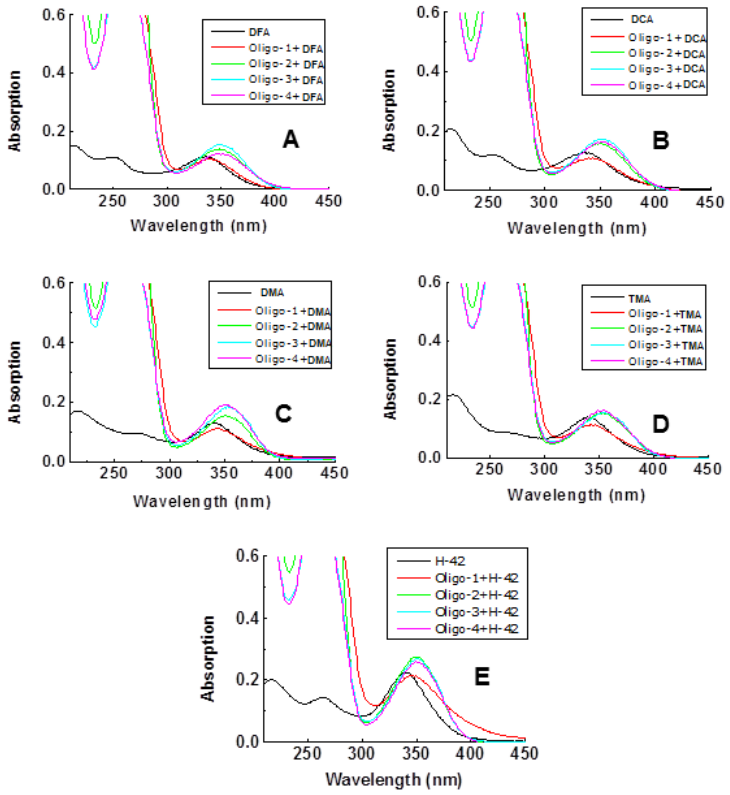

Fig. S1

Fig. S1 UV absorption spectra of **DFA** (A), **DCA** (B), **DMA** (C), **TMA** (D) & **Hoechst 33342** (E) alone and in the presence of synthetic duplex at drug/oligomer ratio r=1. The ligand and DNA concentration was 5µM. Oligo-1: $d(GCGCGCGCGCGCGCGC)_2$; Oligo-2: $d(ATATATATATATATAT)_2$; Oligo-3: $d(GAAAATTTTC)_2$; Oligo-4: $d(GTTTTAAAAC)_2$

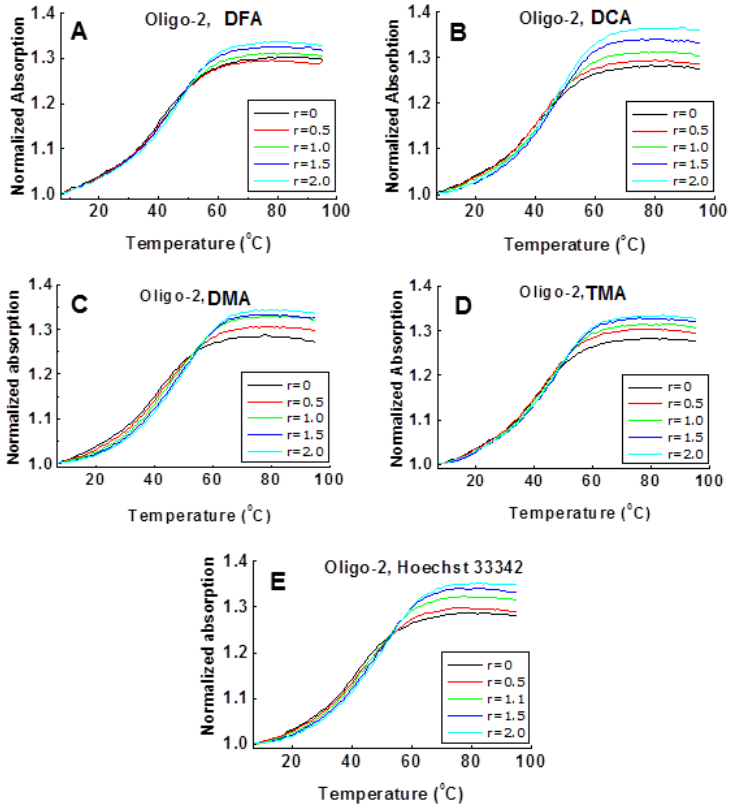

Fig. S2

Fig. S2 UV melting profiles of oligomer-2 i.e.; d(ATATATATATATATAT)$_2$ alone and in the presence of ligands (**DFA, DCA, DMA, TMA** & **Hoechst 33342**) at drug/oligomer ratio, r = 0-2. Samples of DNA (2.5 μM) were mixed with ligand (1.25-5 μM) in buffer [20mM sodium cacodylate, 100mM NaCl (pH 7.2)] before being heated at 95ºC for 5 min and slowly annealed to 4ºC before UV analysis at 260 nm from 7 to 95ºC at a heating rate of 0.2 ºC/min. T_m values were determined by first-derivative analysis.

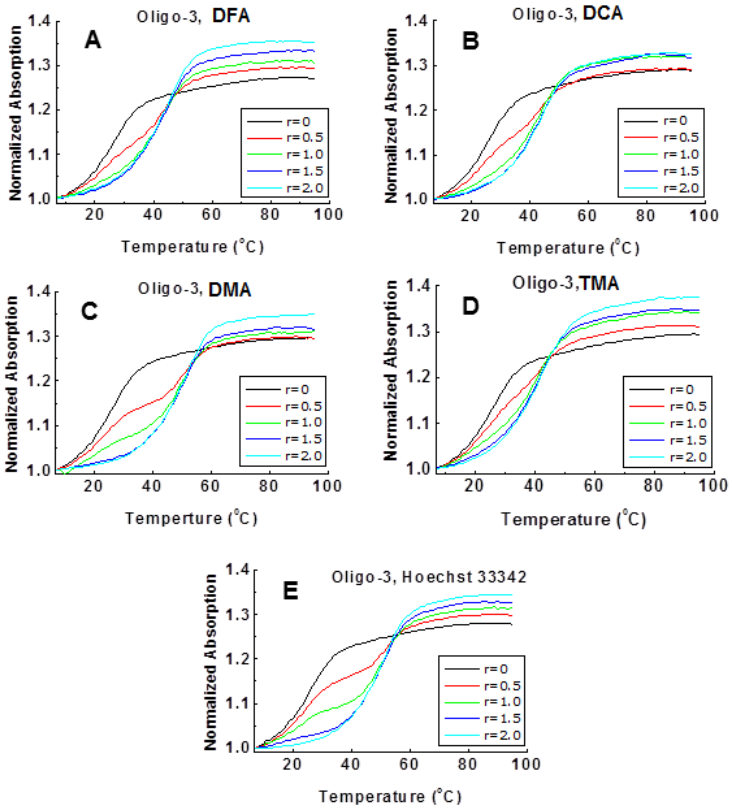

Fig. S3

Fig. S3 UV melting profiles of oligomer-3 i.e.; d(GAAAATTTTC)$_2$ alone and in the presence of ligands **(DFA, DCA, DMA, TMA** & **Hoechst 33342**) at drug/oligomer ratio, r = 0-2. Samples of DNA (2.5 µM) were mixed with ligand (1.25-5 µM) in buffer [20mM sodium cacodylate, 100mM NaCl (pH 7.2)] before being heated at 95ºC for 5 min and slowly annealed to 4ºC before UV analysis at 260 nm from 7 to 95ºC at a heating rate of 0.2ºC/min. T_m values were determined by first-derivative analysis.

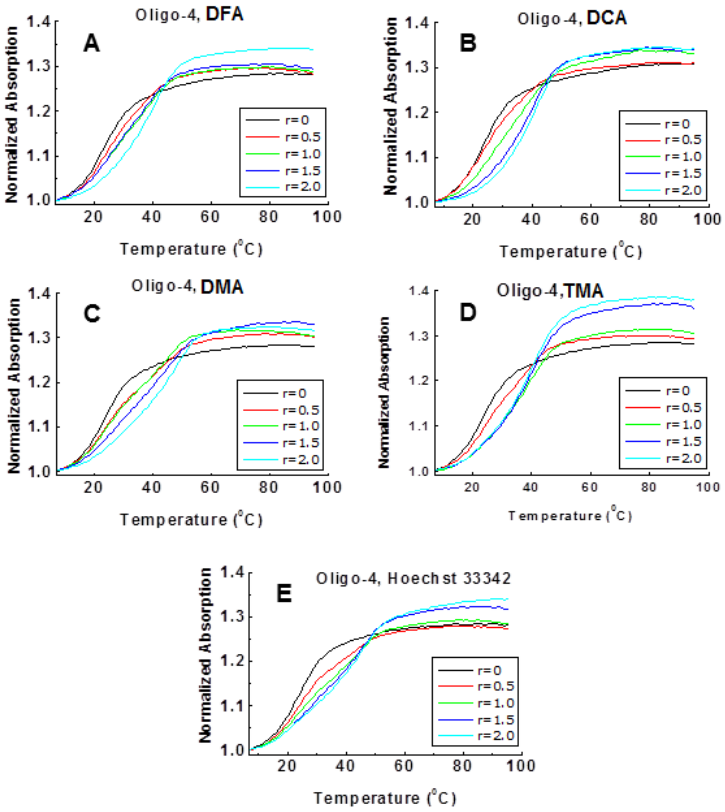

Fig. S4

Fig. S4 UV melting profiles of oligomer-4 i.e.; d(GTTTTAAAAC)$_2$ alone and in the presence of ligands (**DFA, DCA, DMA, TMA** & **Hoechst 33342**) at drug/oligomer ratio, r = 0-2. Samples of DNA (2.5 µM) were mixed with ligand (1.25-5 µM) in buffer [20mM sodium cacodylate, 100mM NaCl (pH 7.2)] before being heated at 95ºC for 5 min and slowly annealed to 4ºC before UV analysis at 260 nm from 7 to 95ºC at a heating rate of 0.2ºC/min. T_m values were determined by first-derivative analysis.

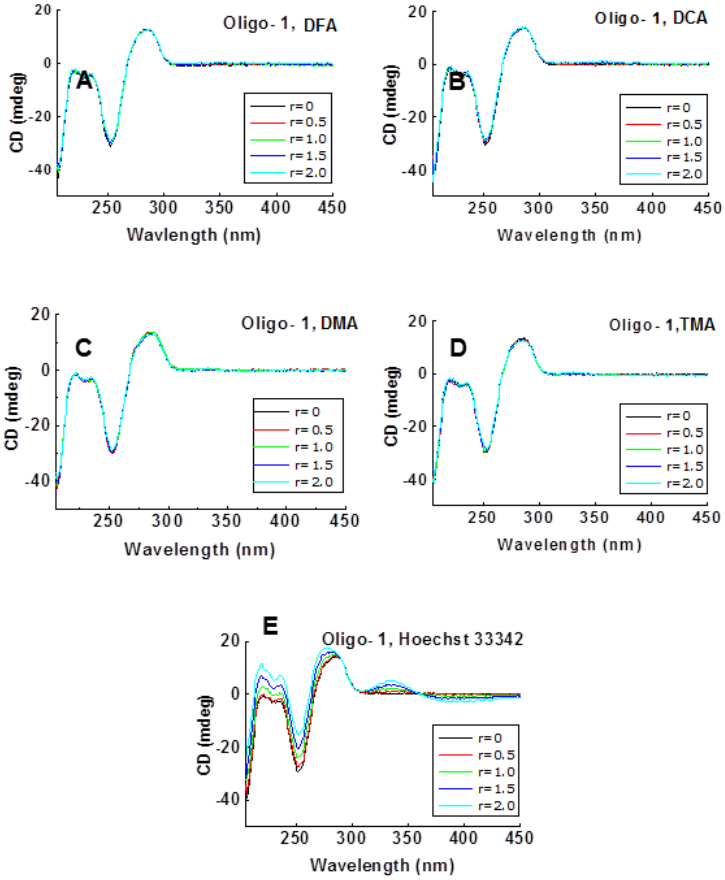

Fig. S5

Fig. S5 CD scans of oligomer-1 i.e.; d(GCGCGCGCGCGCGCGC)$_2$ with increasing concentrations of ligands (**DFA, DCA, DMA, TMA** & **Hoechst 33342**) at 10^{o}C. Samples of oligomer (5 µM) were scanned from 460 to 210 nm after serial additions of concentrated ligand with stirring. Peaks around 350 nm correspond to ligand-oligomer complex. Buffer: 20mM sodium cacodylate, 100mM NaCl (pH 7.2). A) **DFA**-oligomer DNA; B) **DCA**-oligomer; C) **DMA**-oligomer; D) **TMA**-oligomer; E) **Hoechst 33342**-oligomer; r stands for drug/oligomer ratio.

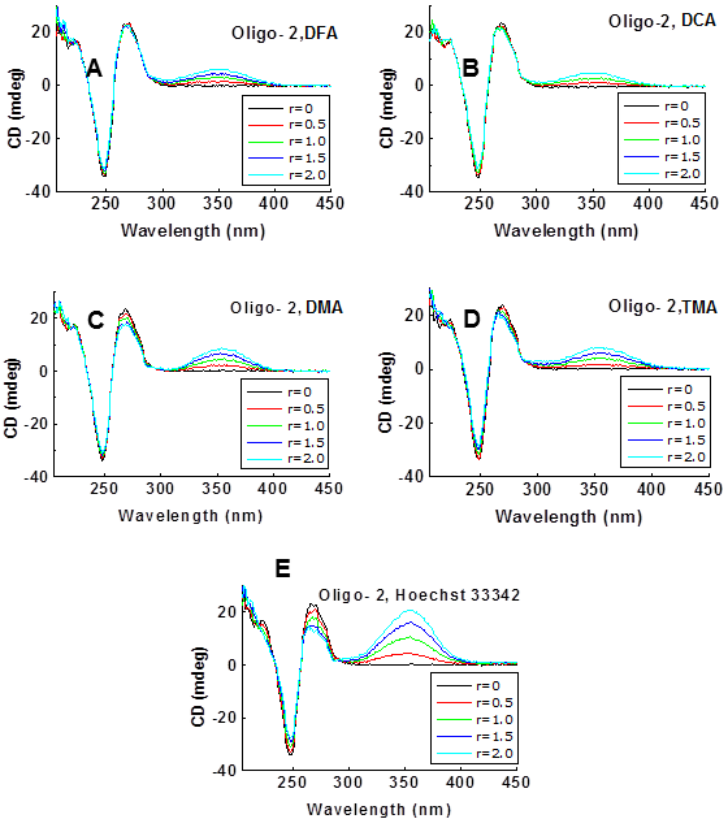

Fig. S6

Fig. S6 CD scans of oligomer-2 i.e.; d(ATATATATATATATAT)$_2$ with increasing concentrations of ligands (**DFA, DCA, DMA, TMA** & **Hoechst 33342**) at 10^{o}C. Samples of oligomer (5 µM) were scanned from 460 to 210 nm after serial additions of concentrated ligand with stirring. Peaks around 350 nm correspond to ligand-oligomer complex. Buffer: 20mM sodium cacodylate, 100mM NaCl (pH 7.2). A) **DFA**-oligomer DNA; B) **DCA**-oligomer; C) **DMA**-oligomer; D) **TMA**-oligomer; E) **Hoechst 33342**-oligomer; r stands for drug/oligomer ratio.

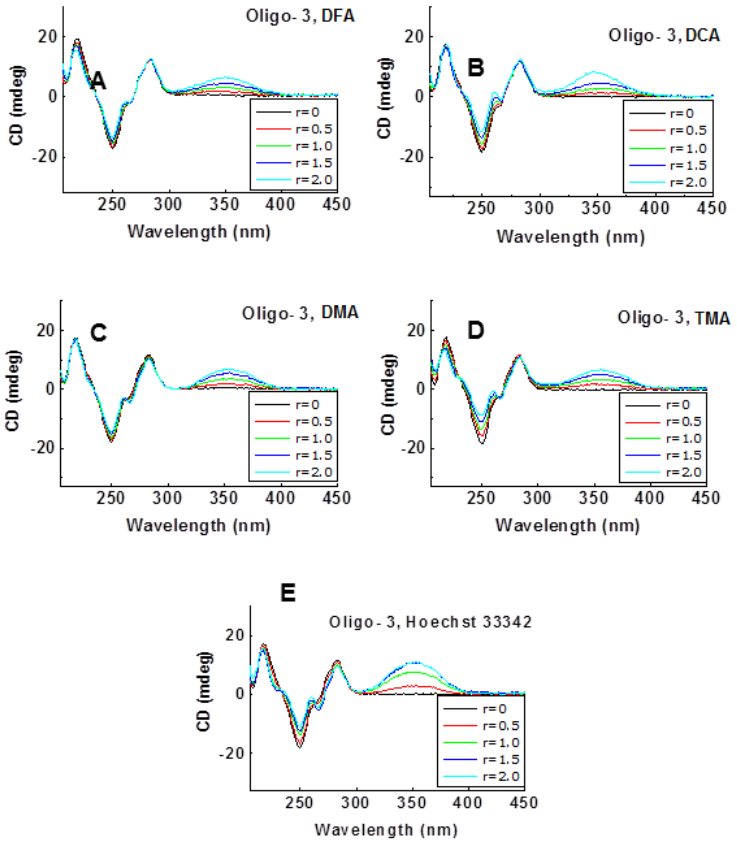

Fig. S 7

Fig. S7 CD scans of oligomer-3 i.e.; d(GAAAATTTTC)$_2$ with increasing concentrations of ligands (**DFA, DCA, DMA, TMA** & **Hoechst 33342**) at 10^{o}C. Samples of oligomer (5 µM) were scanned from 460 to 210 nm after serial additions of concentrated ligand with stirring. Peaks around 350 nm correspond to ligand-oligomer complex. Buffer: 20mM sodium cacodylate, 100mM NaCl (pH 7.2). A) **DFA**-oligomer DNA; B) **DCA**-oligomer; C) **DMA**-oligomer; D) **TMA**-oligomer; E) **Hoechst 33342**-oligomer; r stands for drug/oligomer ratio.

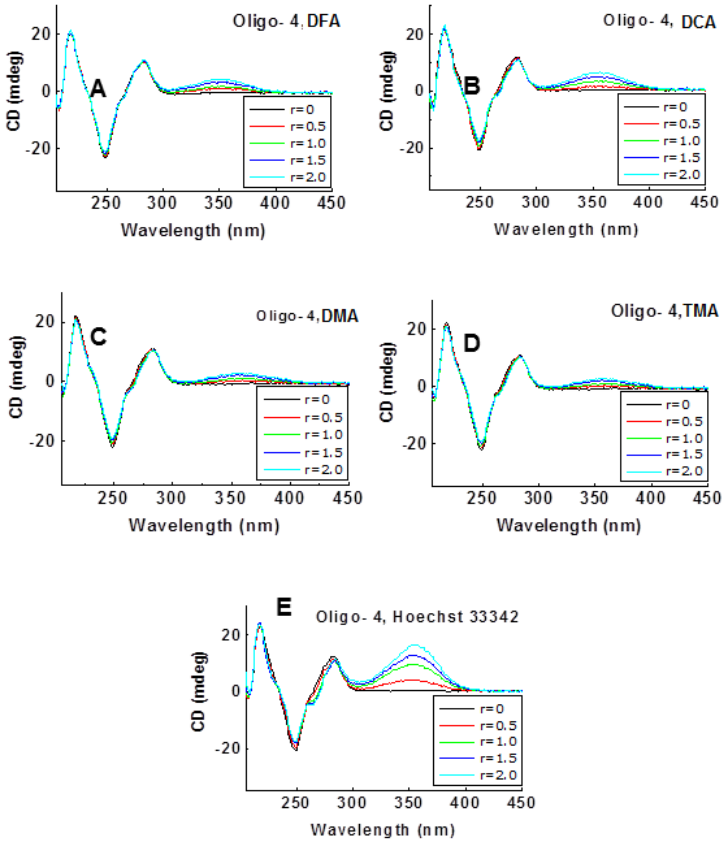

Fig. S8

Fig. S8 CD scans of oligomer-4 i.e.; d(GTTTTAAAAC)$_2$ with increasing concentrations of ligands (**DFA, DCA, DMA, TMA** & **Hoechst 33342**) at 10^{o}C. Samples of oligomer (5 µM) were scanned from 460 to 210 nm after serial additions of concentrated ligand with stirring. Peaks around 350 nm correspond to ligand-oligomer complex. Buffer: 20mM sodium cacodylate, 100mM NaCl (pH 7.2). A) **DFA**-oligomer DNA; B) **DCA**-oligomer; C) **DMA**-oligomer; D) **TMA**-oligomer; E) **Hoechst 33342**-oligomer; r stands for drug/oligomer ratio.

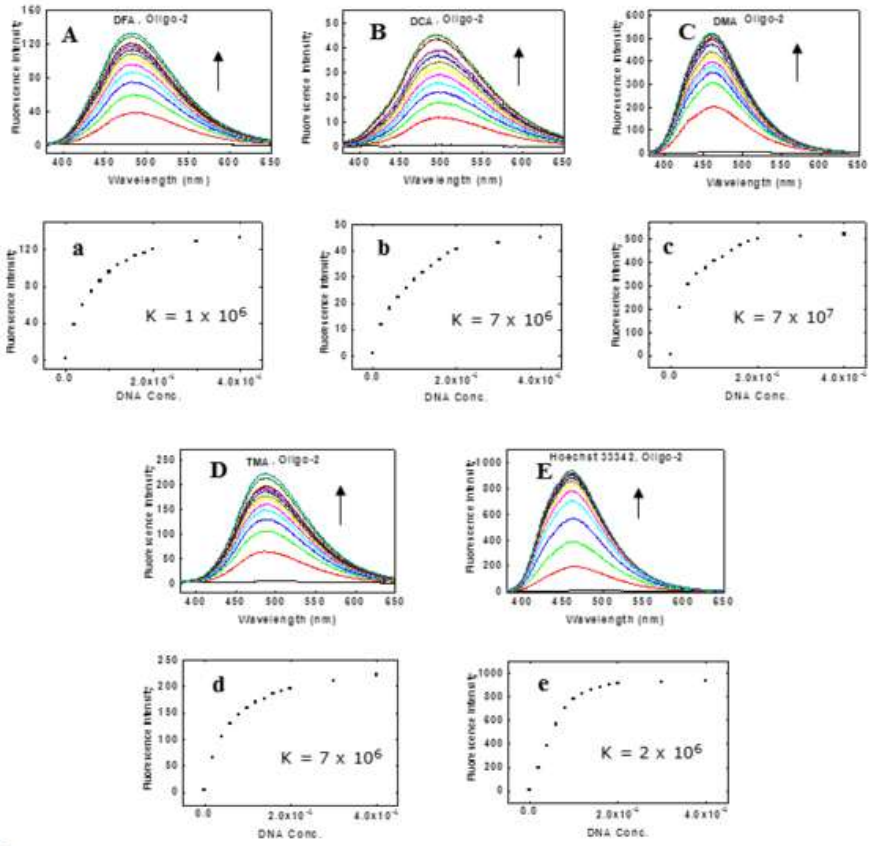

Fig. S9

Fig. S9 Fluorescence-detected binding of oligomer-3 i.e.; d(ATATATATATATATAT)$_2$ with ligands. Small aliquots (1-30 µL) of DNA (1 µM) were added to a solution of ligand (1ml of a 1µM solution) before fluorescence analysis (emission scanning from 400 to 650 at 2 nm/s; 5 nm slit width; T = 10ºC). Buffer: 20 mM sodium cacodylate, 100 mM NaCl (pH 7.2). A) **DFA**-oligomer, excitation 348 nm; B) **DCA**-oligomer, excitation 352 nm; C) **DMA**-oligomer, excitation 352 nm; D) **TMA**-oligomer, excitation 355 nm; E) **Hoechst 33342**-oligomer, excitation 350 nm.

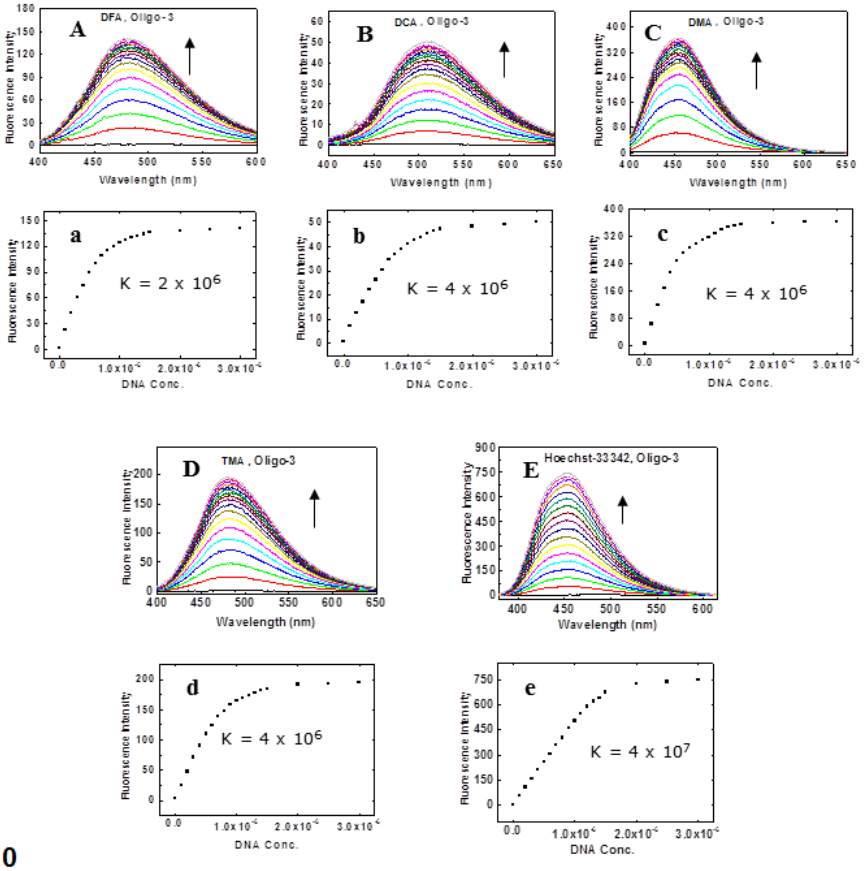

Fig. S10

Fig. S10 Fluorescence-detected binding of oligomer-3 i.e.; d(GAAAATTTTC)$_2$ with ligands. Small aliquots (1-30 µL) of DNA (1 µM) were added to a solution of ligand (1ml of a 1µM solution) before fluorescence analysis (emission scanning from 400 to 650 at 2 nm/s; 5 nm slit width; T = 10ºC). Buffer: 20 mM sodium cacodylate, 100 mM NaCl (pH 7.2). A) **DFA**-oligomer, excitation 348 nm; B) **DCA**-oligomer, excitation 352 nm; C) **DMA**-oligomer, excitation 352 nm; D) **TMA**-oligomer, excitation 355 nm; E) **Hoechst 33342**-oligomer, excitation 350 nm.

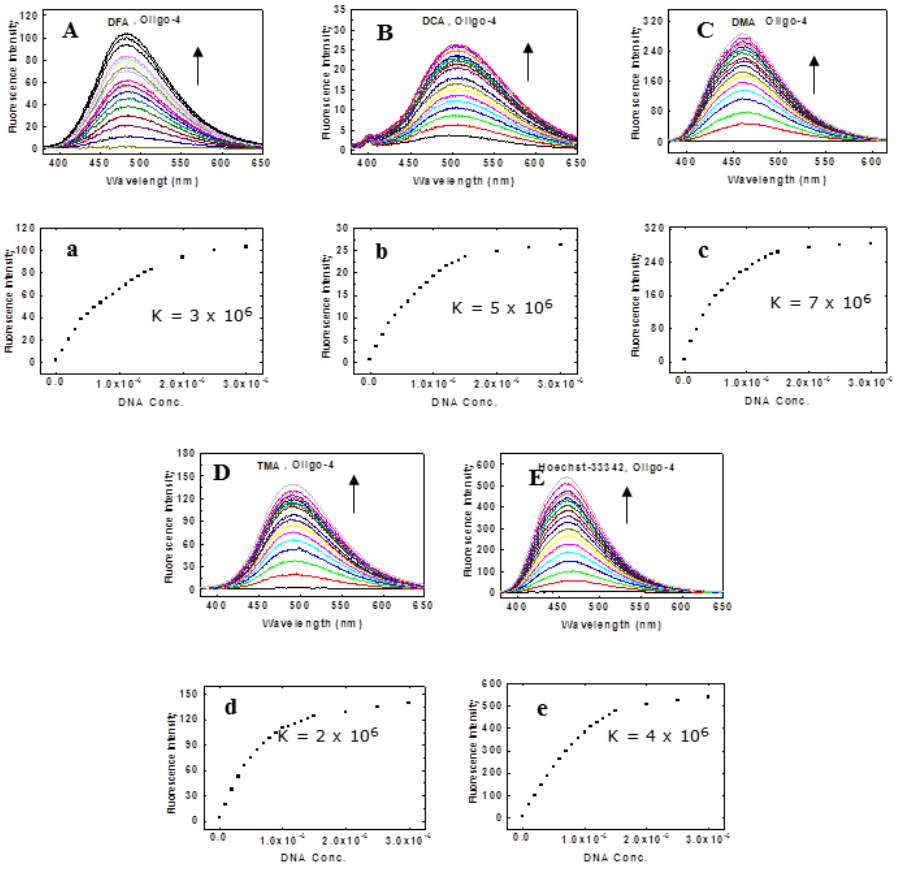

Fig. S11

Fig. S11 Fluorescence-detected binding of oligomer-4 i.e.; d(GTTTTAAAAAC)$_2$ with ligands. Small aliquots (1-30 µL) of DNA (0.1 mM) were added to a solution of ligand (1ml of a 1µM solution) before fluorescence analysis (emission scanning from 380 to 650 at 2 nm/s; 5 nm slit width; T = 10ºC). Buffer: 20 mM sodium cacodylate, 100 mM NaCl (pH 7.2). A)**DFA**-oligomer, excitation 349 nm; B) **DCA**-oligomer, excitation 352nm. C) **DMA**-oligomer, excitation 350; D) **TMA**-oligomer, excitation 353 nm; E) **Hoechst 33342**-oligomer, excitation 350 nm.

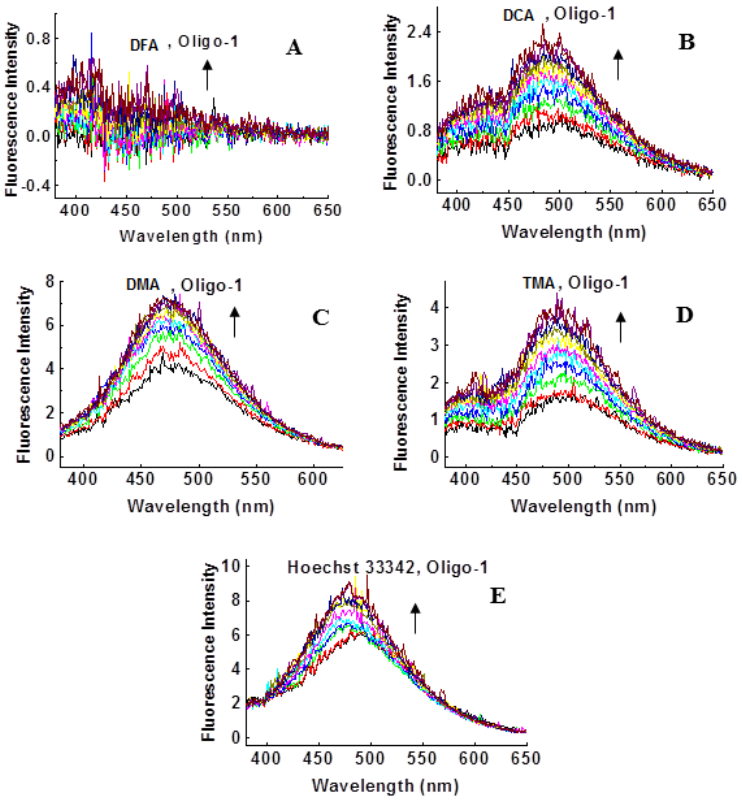

Fig. S12

Fig. S12 Fluorescence-detected binding of oligomer-1 i.e.; d(GCGCGCGCGCGCGCGC)$_2$ with ligands. Small aliquots (1-20 µL) of DNA (0.1 mM) were added to a solution of ligand (1ml of a 1µM solution) before fluorescence analysis (emission scanning from 380 to 650 at 2 nm/s; 5 nm slit width; T = 10ºC). Buffer: 20 mM sodium cacodylate, 100 mM NaCl (pH 7.2). A) **DFA**-oligomer, excitation 349 nm; B) **DCA**-oligomer, excitation 341; C) **DMA**-oligomer, excitation 341nm; D) **TMA**-oligomer, excitation 341nm; E) **Hoechst 33342**-oligomer, excitation 350nm.

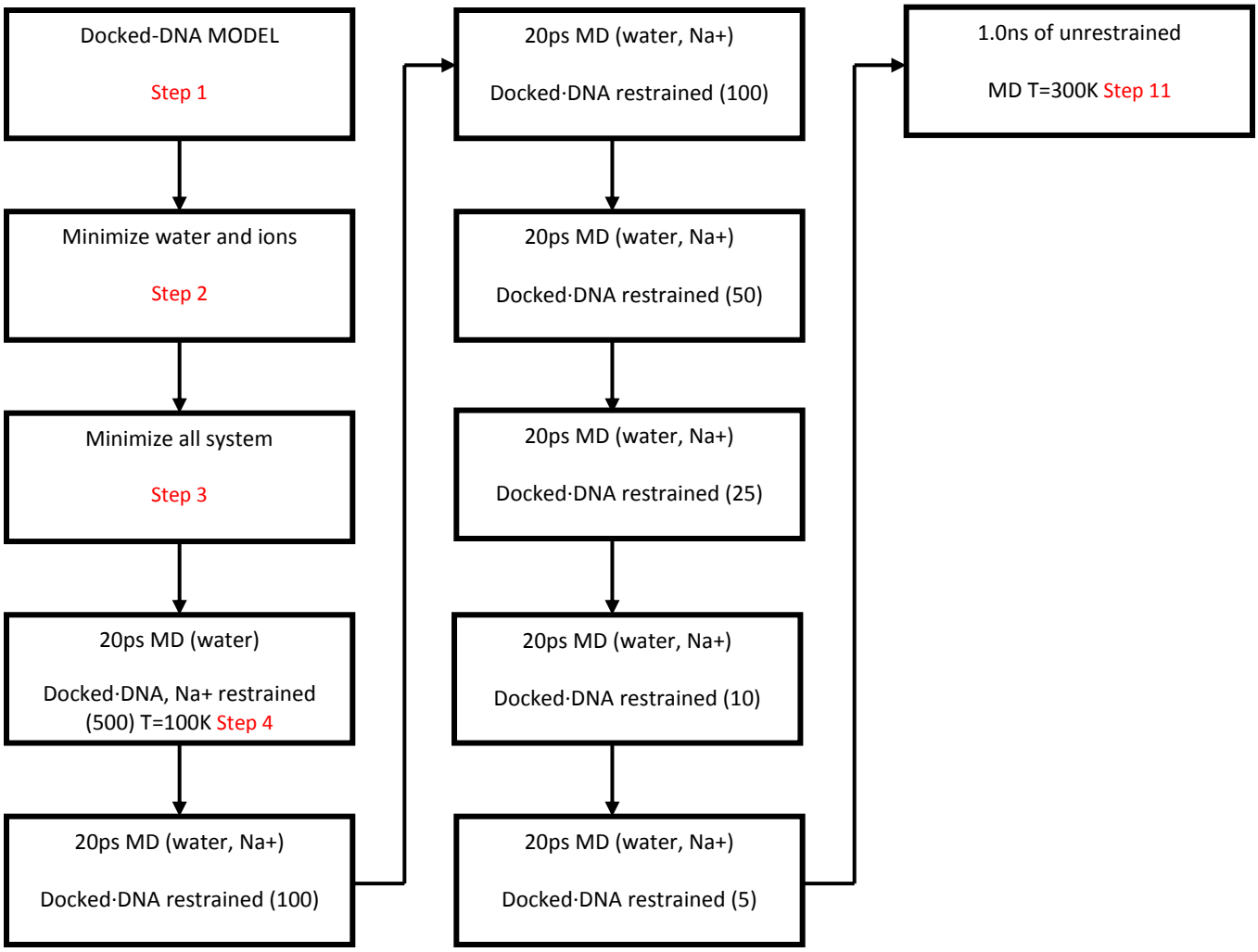

Fig. S13: MD-Simulation Protocol

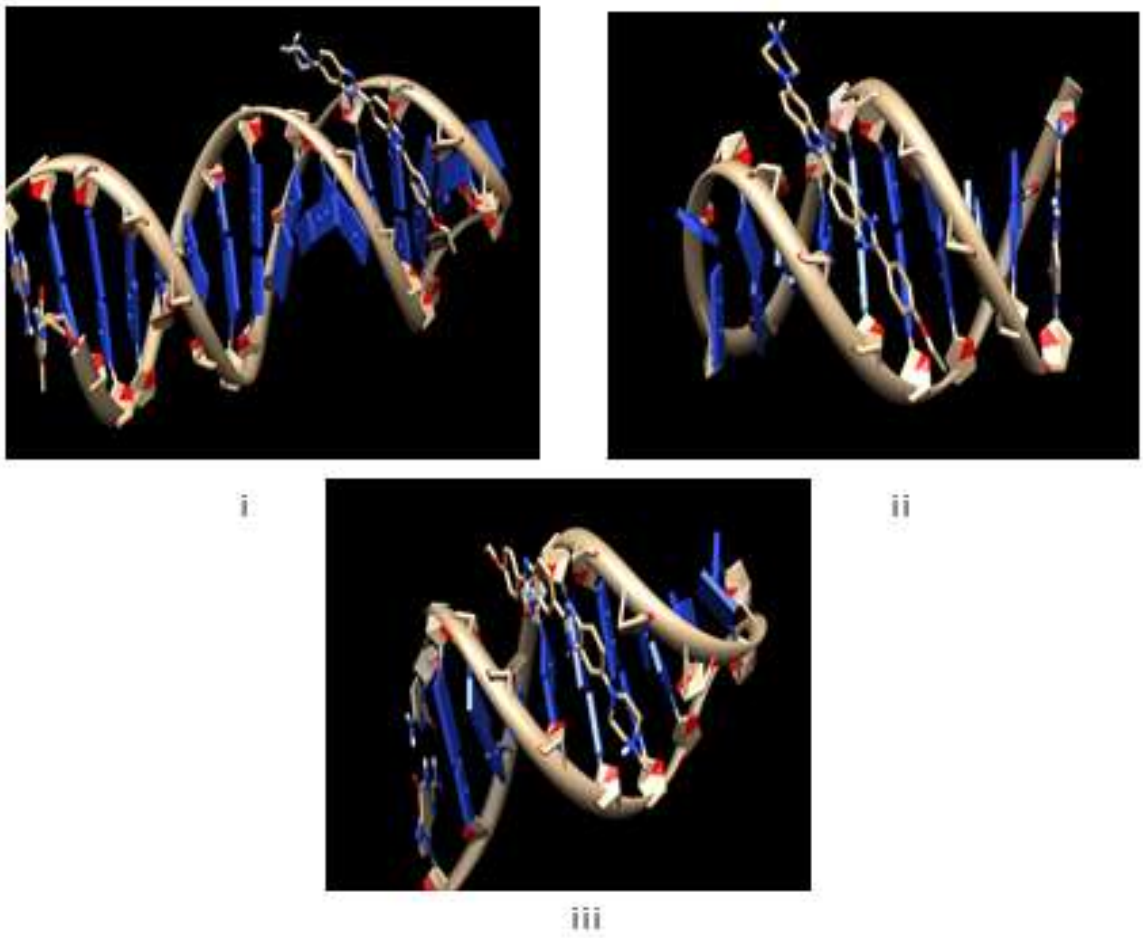

Fig. S14: Ligand-Docked structure of Hoechst-33342 with (i) d(ATATATATATATATAT)$_2$,(ii) d(GAAAATTTTC)$_2$ and (iii) d(GTTTTAAAAC)$_2$

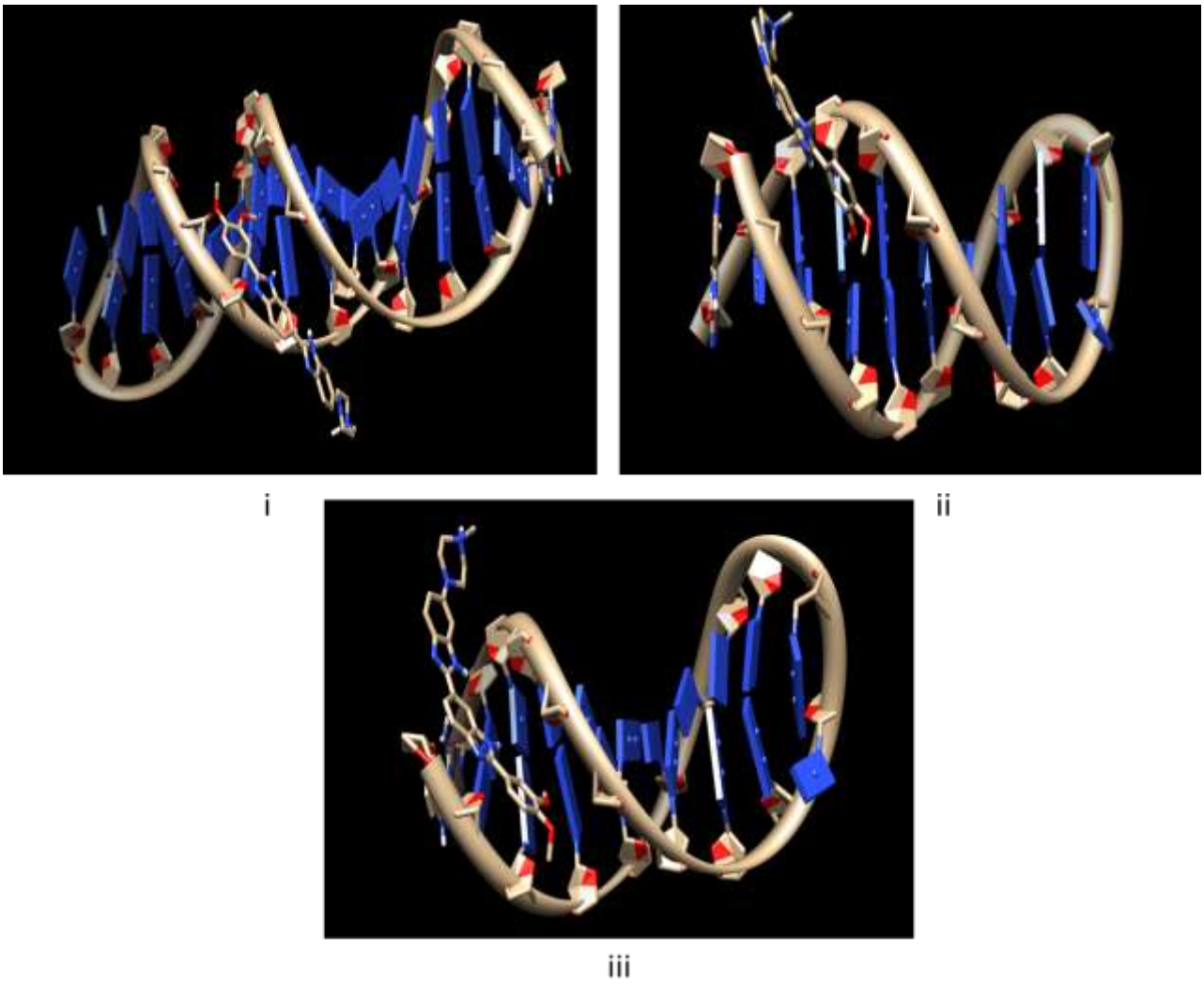

Fig. S15: Ligand-Docked structure of DMA with (i) d(ATATATATATATATAT)$_2$,(ii) d(GAAAATTTTC)$_2$ and (iii) d(GTTTTAAAAC)$_2$

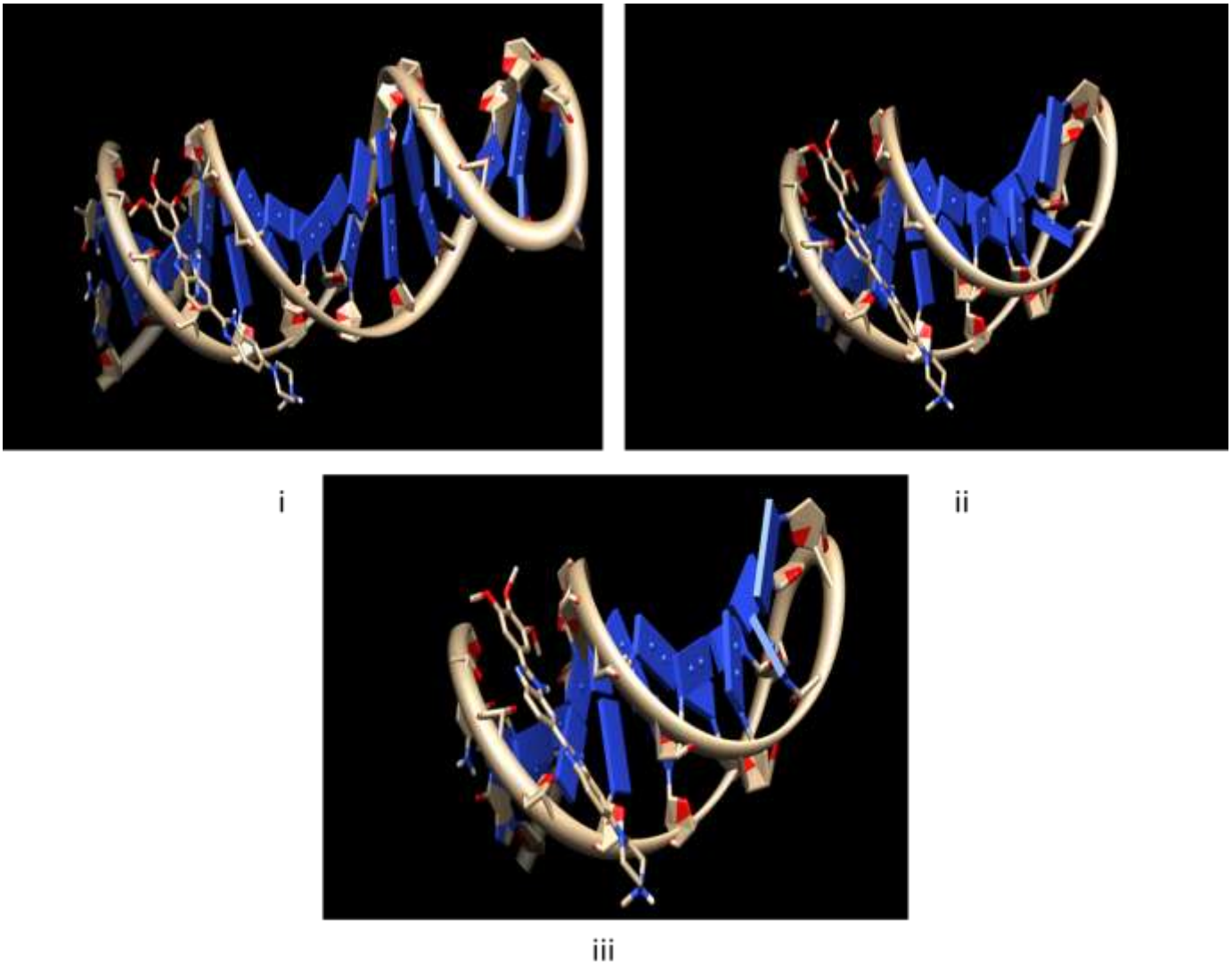

Fig. S16: Ligand-Docked structure of TMA with (i) d(ATATATATATATATAT)$_2$,(ii) d(GAAAATTTTC)$_2$ and (iii) d(GTTTTAAAAC)$_2$

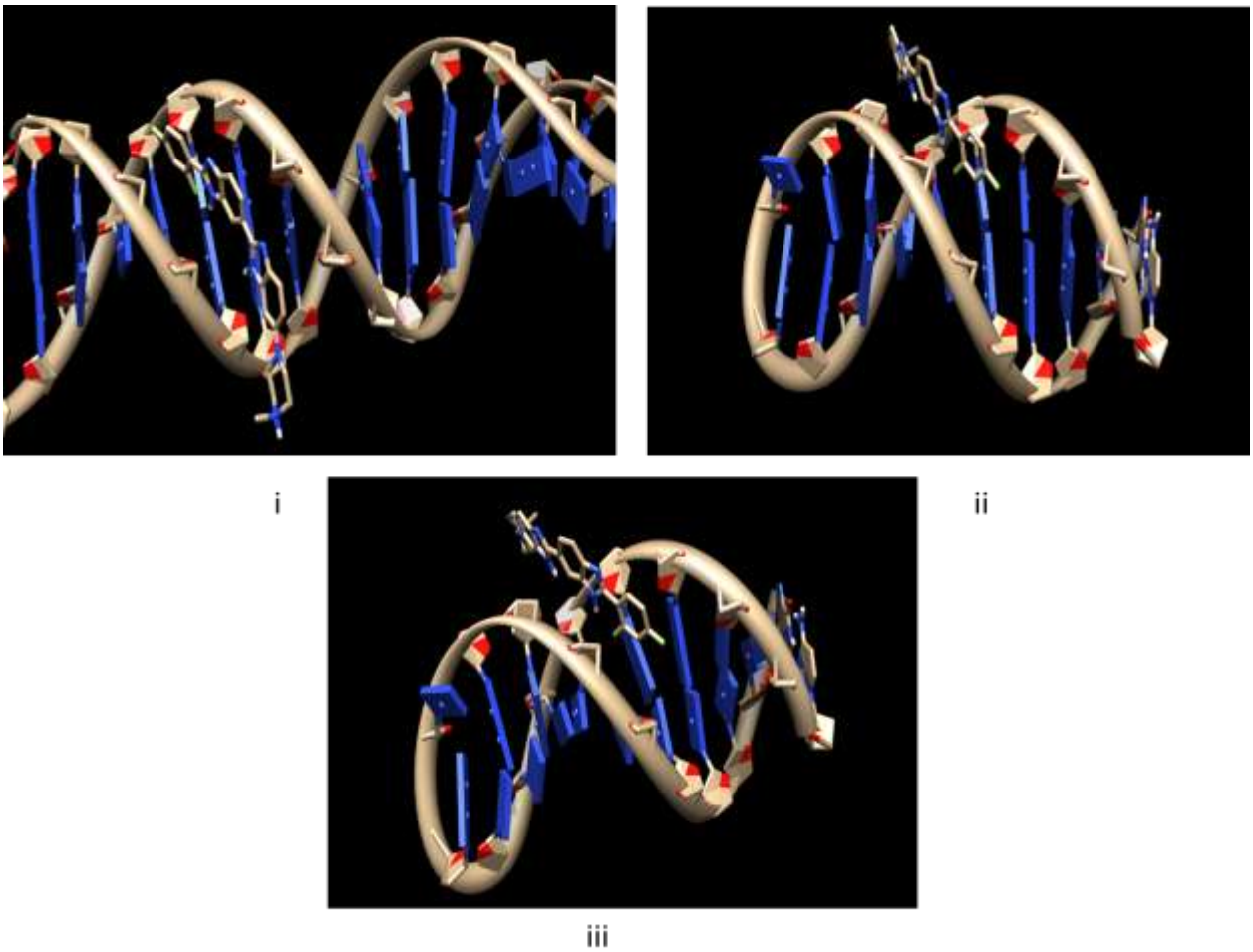

Fig. S17: Ligand-Docked structure of DFA with (i) d(ATATATATATATATAT)$_2$,(ii) d(GAAAATTTTC)$_2$ and (iii) d(GTTTTAAAAC)$_2$

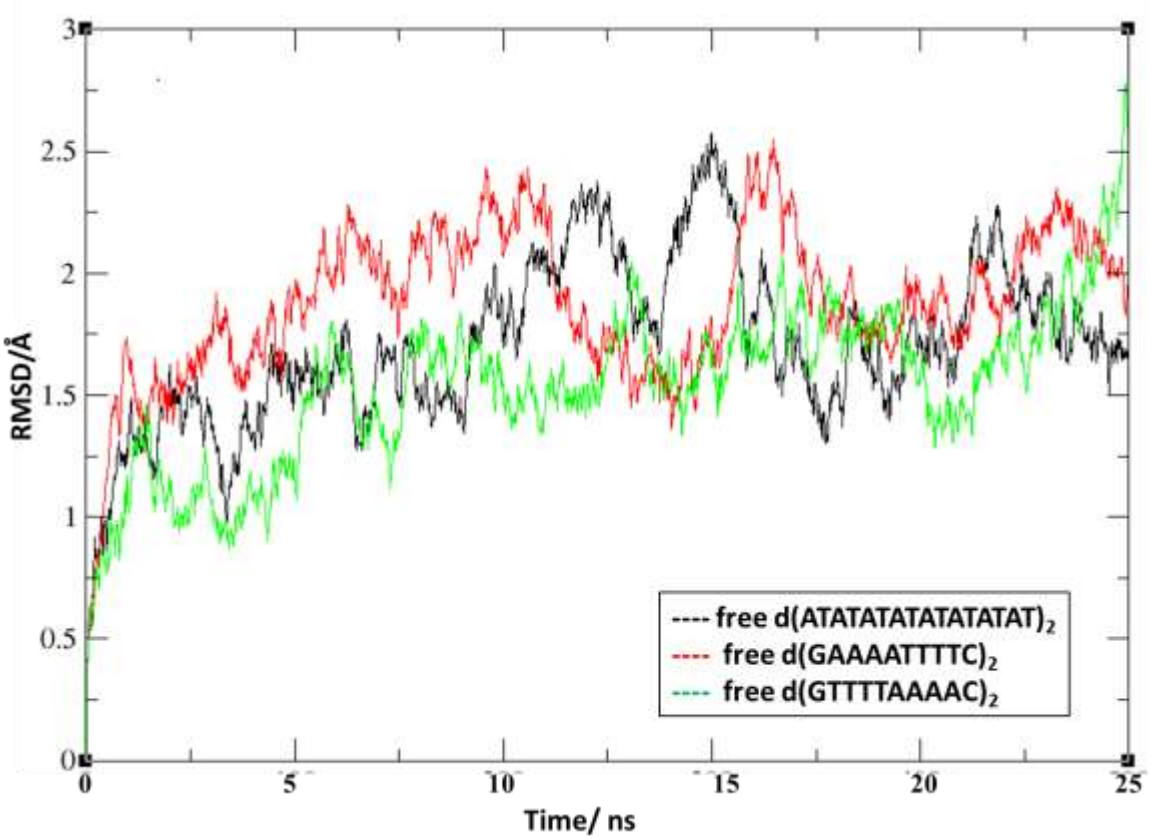

Fig.-S18: RMSD plots of Free-DNA duplexes up to 25ns simulation. :d(GAAAATTTTC)$_2$ (red), d(GTTTTAAAAC)$_2$ (green) and d(ATATATATATATATAT)$_2$ (black).

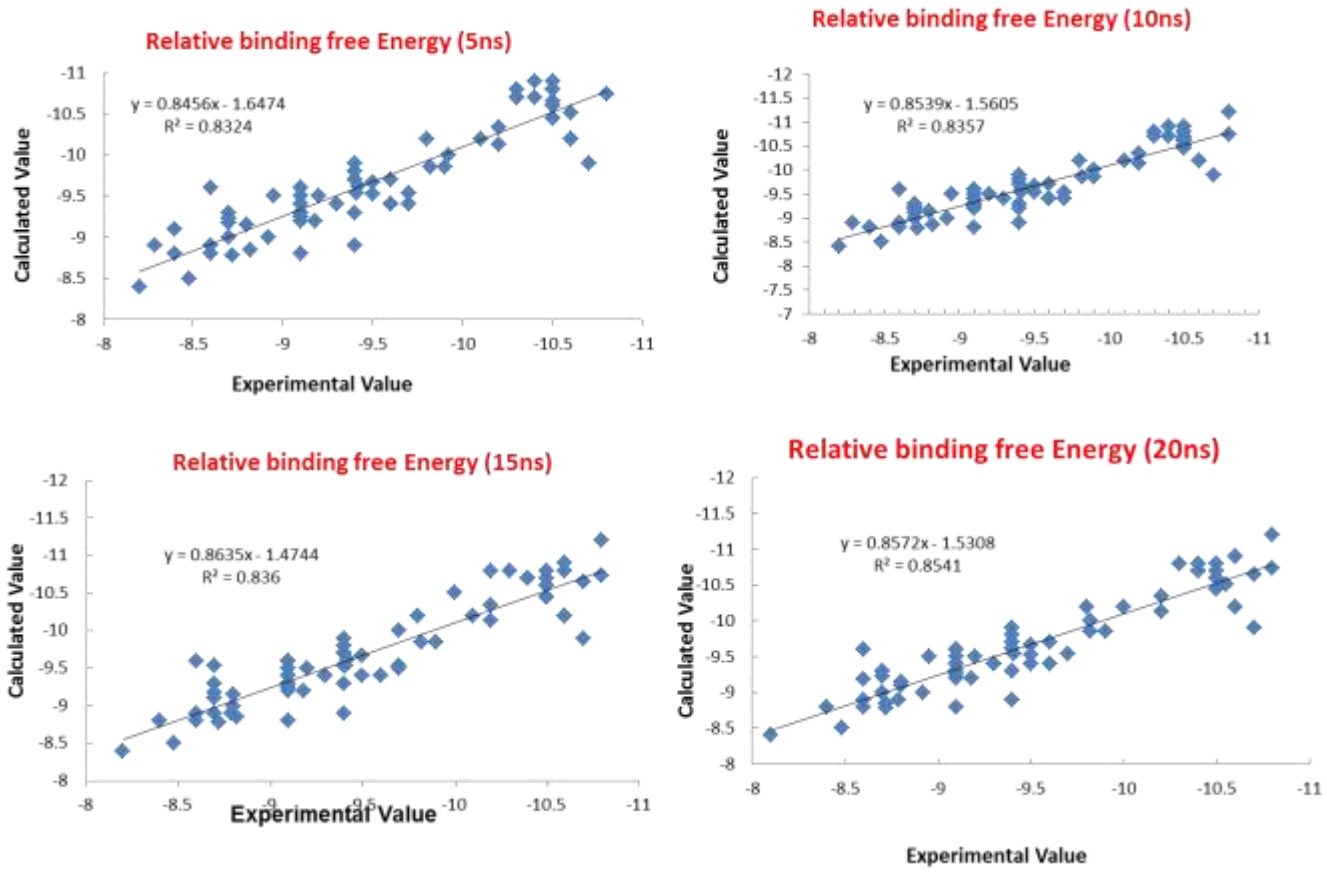

Fig. S19: Correlation diagram of Experimental and Calculated Binding Free Energy profiles after 5, 10, 15 and 20ns simulation.

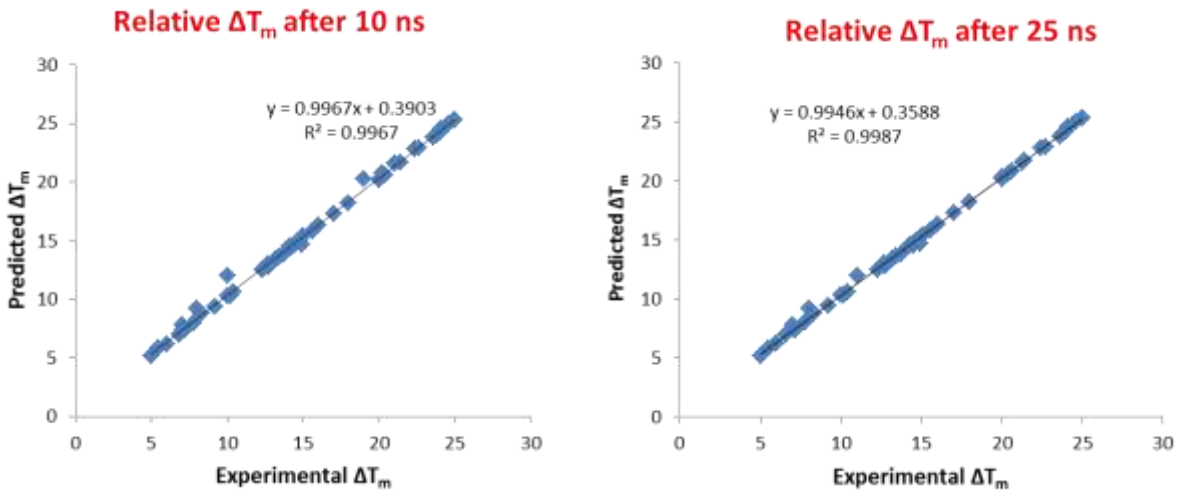

Fig. S20: Correlation diagram of Experimental and Calculated ΔT_m profiles after 5, 10, 15 and 20ns simulation.