Supplementary Tables

Table S1. Representation of heavy atoms in both WC and Hoogsteen hydrogen bonding interactions in the triplex structures

WC base-pair						
	1^{st} - 2^{nd}	1^{st} - 2^{nd}	1^{st} - 2^{nd}			
A ------ T	N1 – N3	N6 – O4				
C ------ G	O2 – N2	N3 – N1	N4 – O6			
Hoogsteen base-pair						
	3^{rd} - 1^{st}	3^{rd} - 1^{st}	3^{rd} – 2^{nd}	3^{rd} – 2^{nd}	3^{rd} - 1^{st}	3^{rd} - 1^{st}
T ------ A	N3-N7	O4-N6				
T ------ C	O4-N4	N3-C5				
T ------ G	N3-N7	O4-O6				
C ------ G	N3-O6	O2-N7				
5a--A	N3-N7	N4-N6	N44-O2	N43-O2	N45-N6	O2-N6
5a--C	N3-N4	O2-N4	N44-N7	N43-O6	O2'-H5	
5a--G	O2-N7	N3-O6	N44-O2	N45-N3	N44-O6	N43-N7

Base pairing is shown for the following A:T, G:C, C:G duplexes and A:T•T, A:T•5a, G:C•T, G:C•5a, C:G•T, C:G•5a triplexes

Table S2. Average WC hydrogen bond distances (Å) in triplexes calculated over 5-3-ns portion of the MD simulations.

	1st -- 2nd		A:T·T		A:T•5a		G:C·T		G:C•5a		C:G·T		C:G•5a	
16	A-----T	N1-N3	2.92	(0.10)	2.93	(0.11)	2.95	(0.12)	2.94	(0.11)	2.94	(0.12)	2.92	(0.10)
17	A-----T	N1-N3	2.93	(0.11)	2.92	(0.11)	2.92	(0.11)	2.91	(0.10)	2.92	(0.11)	2.92	(0.10)
18	A-T/G-C/C-G	O2-N2	2.95	(0.12)	8.72	(0.41)	2.86	(0.13)	9.45	(2.67)	2.86	(0.14)	2.87	(0.13)
19	A-----T	N1-N3	2.92	(0.10)	2.97	(0.13)	2.96	(0.12)	2.92	(0.10)	2.91	(0.10)	2.93	(0.11)
20	G-----C	N2-O2	2.91	(0.16)	2.92	(0.16)	3.19	(0.26)	2.91	(0.15)	2.93	(0.18)	2.89	(0.16)
16	A-----T	N6-O4	3.05	(0.22)	3.00	(0.20)	3.06	(0.22)	3.09	(0.23)	3.09	(0.25)	3.08	(0.21)
17	A-----T	N6-O4	3.09	(0.23)	3.03	(0.20)	3.14	(0.25)	3.10	(0.23)	2.98	(0.20)	3.18	(0.27)
18	A-T/G-C/C-G	N3-N1	3.06	(0.22)	11.32	(0.36)	2.99	(0.14)	9.84	(3.36)	3.00	(0.17)	2.98	(0.10)
19	A-----T	N6-O4	2.98	(0.20)	3.03	(0.23)	2.97	(0.18)	3.09	(0.26)	3.05	(0.23)	2.97	(0.19)
20	G-----C	N1-N3	3.00	(0.12)	3.00	(0.12)	4.17	(0.52)	3.01	(0.12)	3.01	(0.17)	3.01	(0.14)

Table S3. Average Hoogsteen hydrogen bond distances (Å) calculated over the 5-30ns portion of the MD simulations

	3rd -- 1st		A:T·T		A:T•5a		G:C·T		G:C•5a		C:G·T		C:G•5a	
8	T ----- A	N3-N7	2.95	(0.12)	2.95	(0.12)	2.91	(0.11)	2.93	(0.12)	2.93	(0.11)	2.95	(0.12)
9	T ----- A	N3-N7	2.93	(0.11)	2.94	(0.11)	2.90	(0.10)	2.92	(0.11)	2.90	(0.11)	2.92	(0.11)
10	5a-A/G/C		2.92	(0.11)	5.78	(0.31)	3.50	(0.33)	5.58	(2.79)	4.60	(0.90)	3.74	(1.17)
11	T ----- A	N3-N7	2.89	(0.10)	6.07	(0.24)	2.99	(0.16)	3.07	(0.39)	3.79	(1.14)	2.94	(0.11)
12	C ----- G	N3-O6	3.87	(0.35)	3.95	(0.39)	5.14	(0.35)	3.83	(0.43)	4.07	(0.86)	4.82	(1.06)
8	T ----- A	O4-N6	2.93	(0.16)	2.91	(0.15)	2.96	(0.17)	2.97	(0.18)	2.95	(0.18)	2.93	(0.17)
9	T ----- A	O4-N6	2.93	(0.15)	2.85	(0.13)	3.00	(0.19)	3.08	(0.23)	3.14	(0.28)	2.96	(0.17)
10	5a-A/G/C		2.95	(0.17)	5.78	(0.28)	2.95	(0.12)	5.34	(4.28)	5.13	(0.58)	3.71	(1.16)
11	T ----- A	O4-N6	3.12	(0.25)	6.87	(0.33)	2.89	(0.18)	3.83	(0.86)	4.03	(1.21)	2.91	(0.17)
12	C ----- G	N3-O6	4.06	(0.29)	3.95	(0.31)	5.35	(0.33)	3.86	(0.37)	4.30	(0.85)	4.84	(0.84)

Supplementary Figure Legends

S1. Thermal stability analyses of triplexes formed by the TFO containing 2'OMe ribose at all positions and thymine or cystosine bases. This TFO is designed to bind the wild type target sequence, with A:T at the X:Y position in the schematic in Fig 2. The nature of the X:Y base pair is noted in each panel. The triplexes were formed on hairpin duplexes.

S2. Thermal stability analyses of triplexes formed by the TFO containing 2'OMe ribose at all positions and the modified base with a butylamine side chain, corresponding to 3c in Fig 3a, at the Z position (Fig 2). Triplexes were formed on hairpin duplexes.

S3. Thermal stability analyses of triplexes formed by the TFO containing 2'OMe ribose at all positions and the modified base (Z) with the ethyl guanidine side chain corresponding to 5a in Fig 4a. Triplexes were formed on open duplexes.

S4. Thermal stability analyses of triplexes formed by the TFO containing 2'OMe ribose, a cluster of four 2'aminoethoxy residues, and the modified base 5a at the Z position. Triplexes were formed on open duplexes.

S5. RMS Deviation (Å) of heavy atoms as a function of time (ps) for six triplex systems: A:T•T, A:T•5MeC-5a, G:C•T, G:C•5MeC-5a, C:G•T and C:G•5MeC-5a.

S6. Time series of hydrogen bond distances (Å) for atoms in the TFO strand of ATT triplex involved in Hoogsteen base pairing with 1st strand of the WC duplex.

S7. Time series of hydrogen bond distances (Å) for atoms in the TFO strand of A:T•5MeC-5a triplex involved in Hoogsteen base pairing with 1st strand of the WC duplex. C10 of the TFO is defined separately.

S8. Time series of hydrogen bond distances (Å) for atoms in the TFO strand of G:C•T triplex involved in Hoogsteen base pairing with 1st strand of the WC duplex.

S9. Time series of hydrogen bond distances (Å) for atoms in the TFO strand of G:C•5MeC-5a triplex involved in Hoogsteen base pairing with 1st strand of the WC duplex.

S10. Time series of hydrogen bond distances (Å) for atoms in the TFO strand of C:G•T triplex involved in Hoogsteen base pairing with 1st strand of the WC duplex.

S11. Time series of hydrogen bond distances (Å) for atoms in the TFO strand of C:G•5MeC-5a triplex involved in Hoogsteen base pairing with 1st strand of WC duplex.

S12. Snapshots of G:C•5MeC-5a triplet taken at 22 ns, 22.5 ns and 25 ns time step showing the flipping of the G15 out of the duplex due to the interaction of the guanidinium tail with WC duplex.

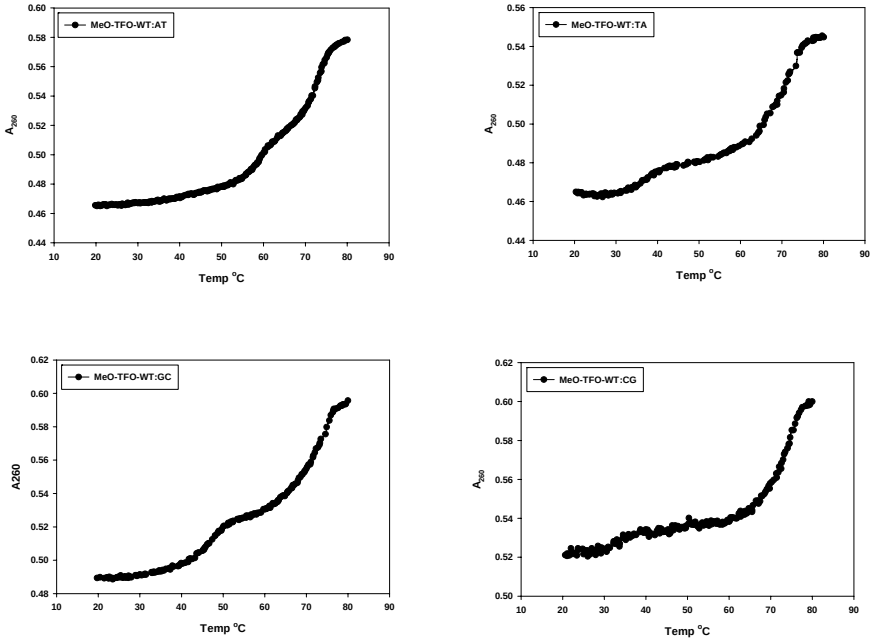
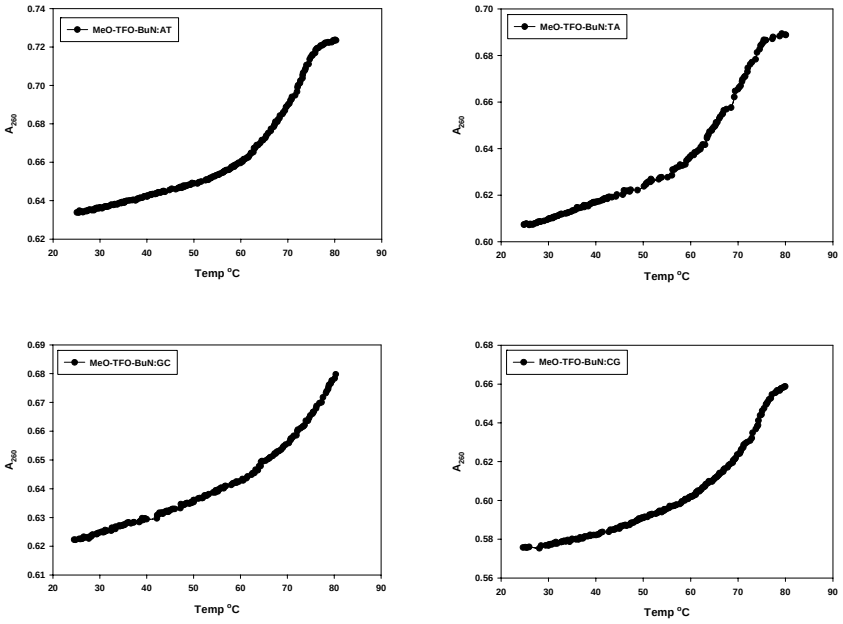

MeO-TFO-WT:AT
MeO-TFO-WT:TA
MeO-TFO-WT:GC
MeO-TFO-WT:CG
A260
Temp °C

Fig S1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MeO-TFO-BuN:TA
MeO-TFO-BuN:GC
MeO-TFO-BuN:CG
Temp °C

Fig S2

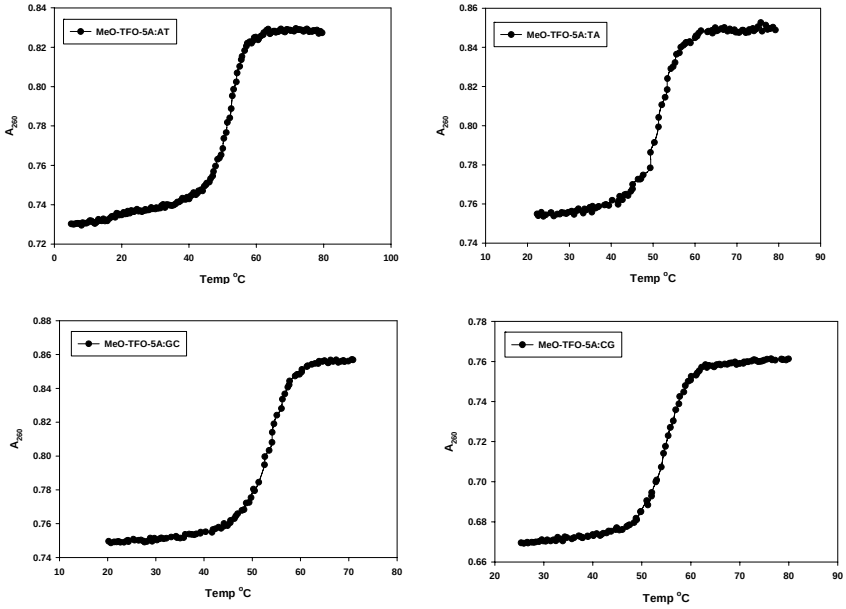
MeO-TFO-5A:AT
MeO-TFO-5A:TA
MeO-TFO-5A:GC
MeO-TFO-5A:CG
A260
Temp °C

Fig S3

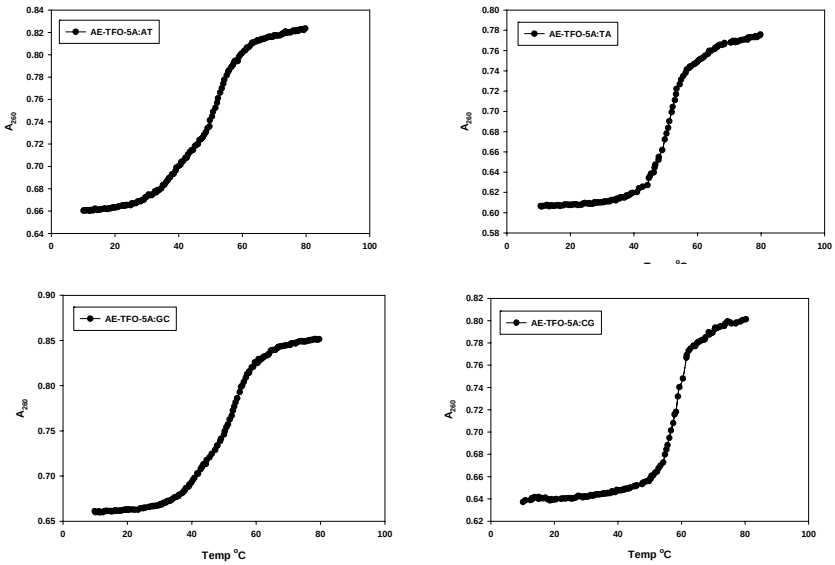
AE-TFO-5A:AT
AE-TFO-5A:TA
AE-TFO-5A:GC
AE-TFO-5A:CG
A260
Temp °C

Fig S4

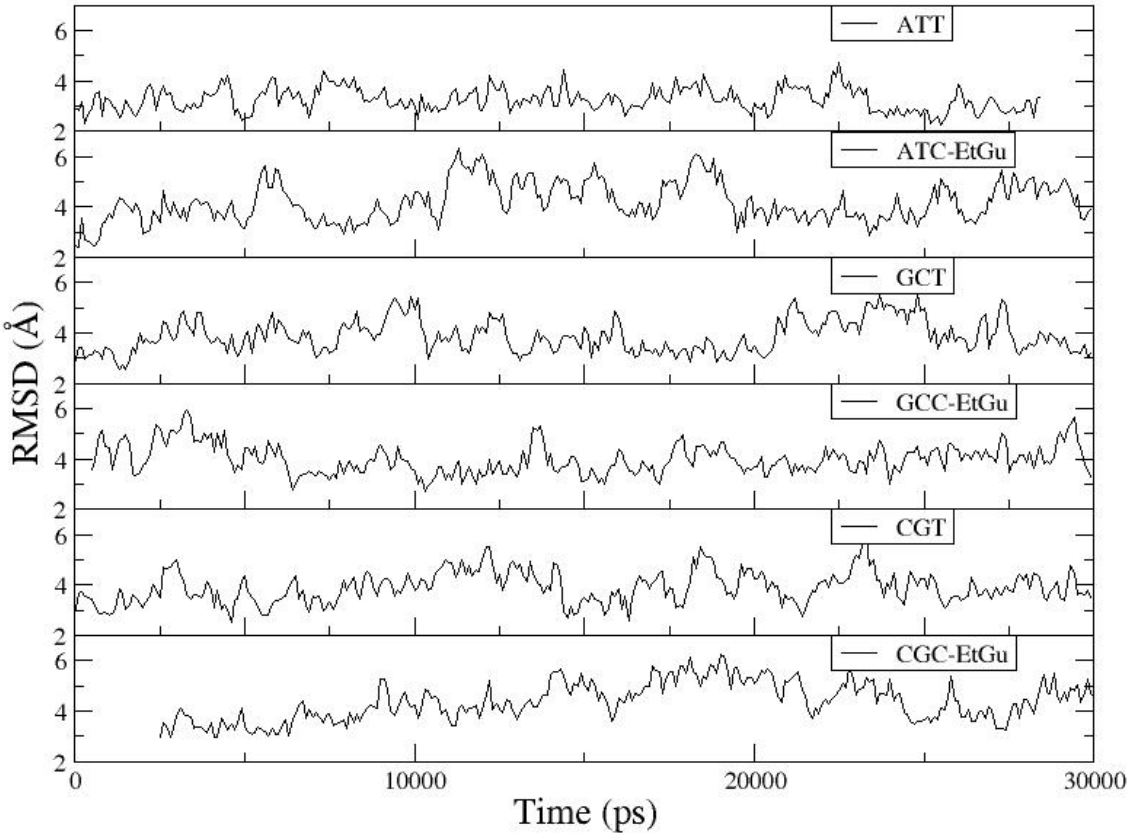
ATT
ATC-EtGu
GCT
GCC-EtGu
CGT
CGC-EtGu
RMSD (Å)
Time (ps)
0
10000
20000
30000
2
4
6

Fig S5.

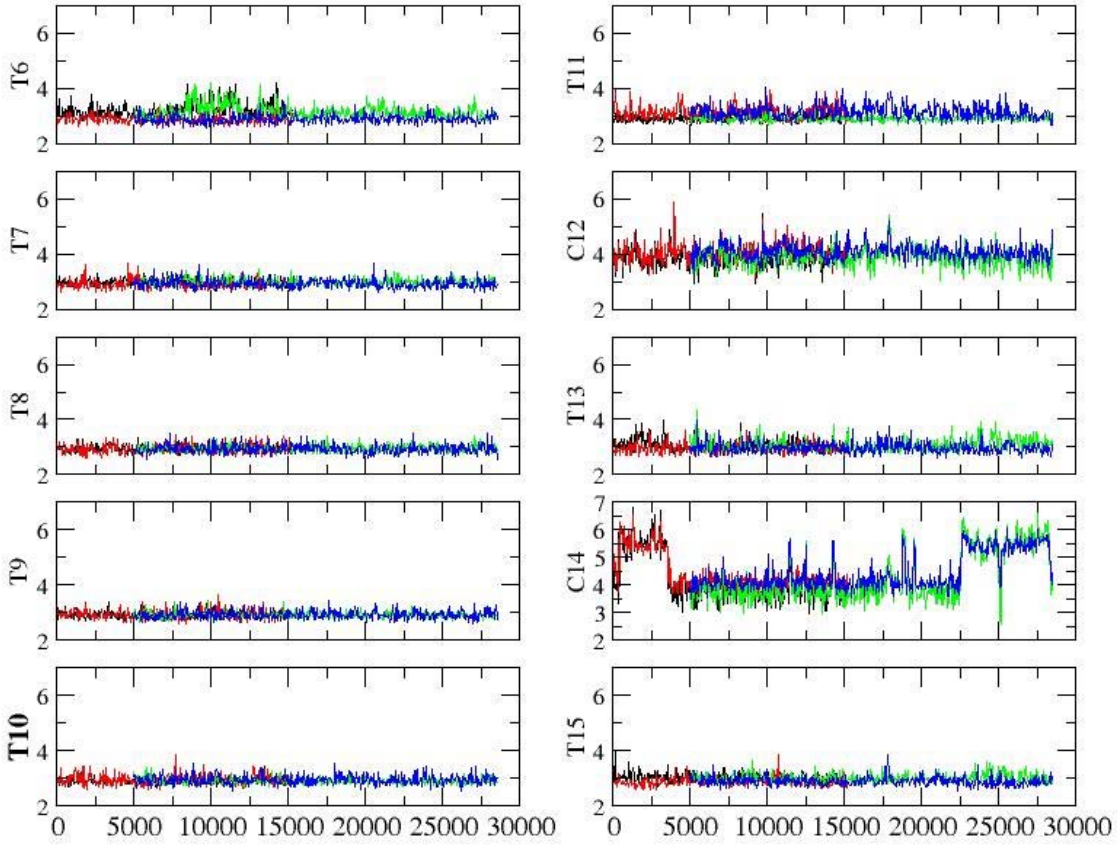
T6
T11
T7
C12
T8
T13
T9
C14
T10
T15
0 5000 10000 15000 20000 25000 30000

Fig S6

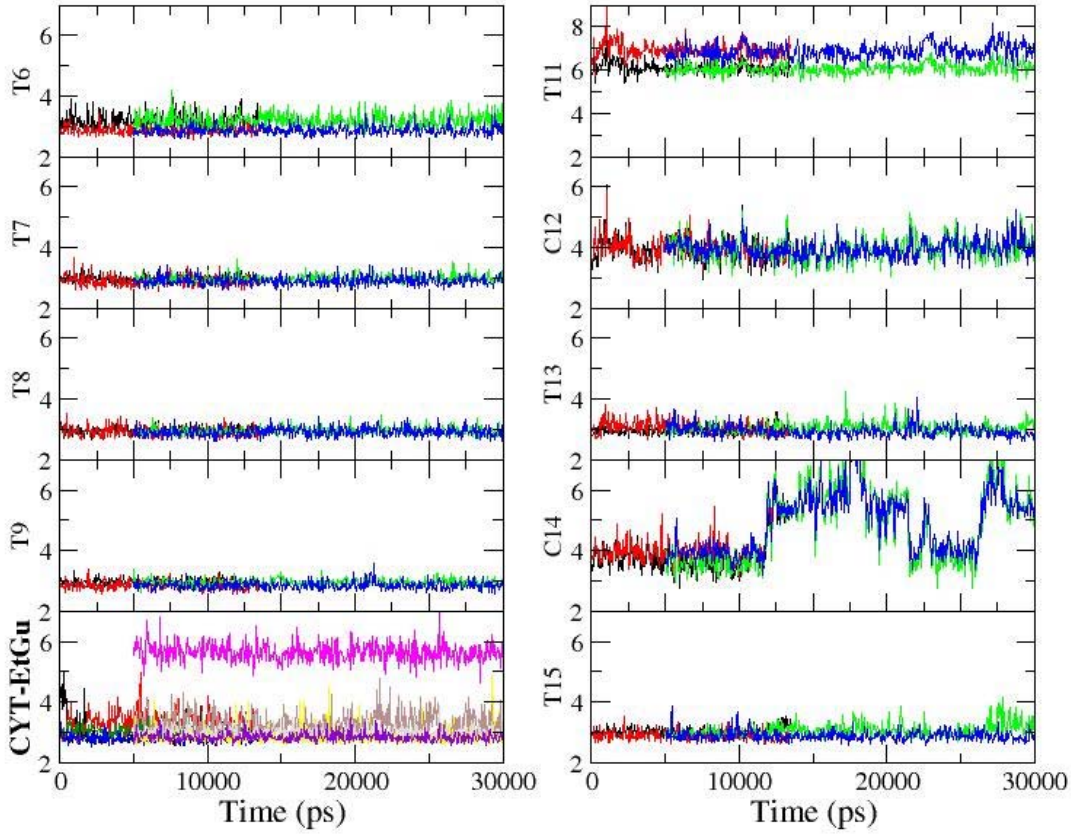

Fig S7.

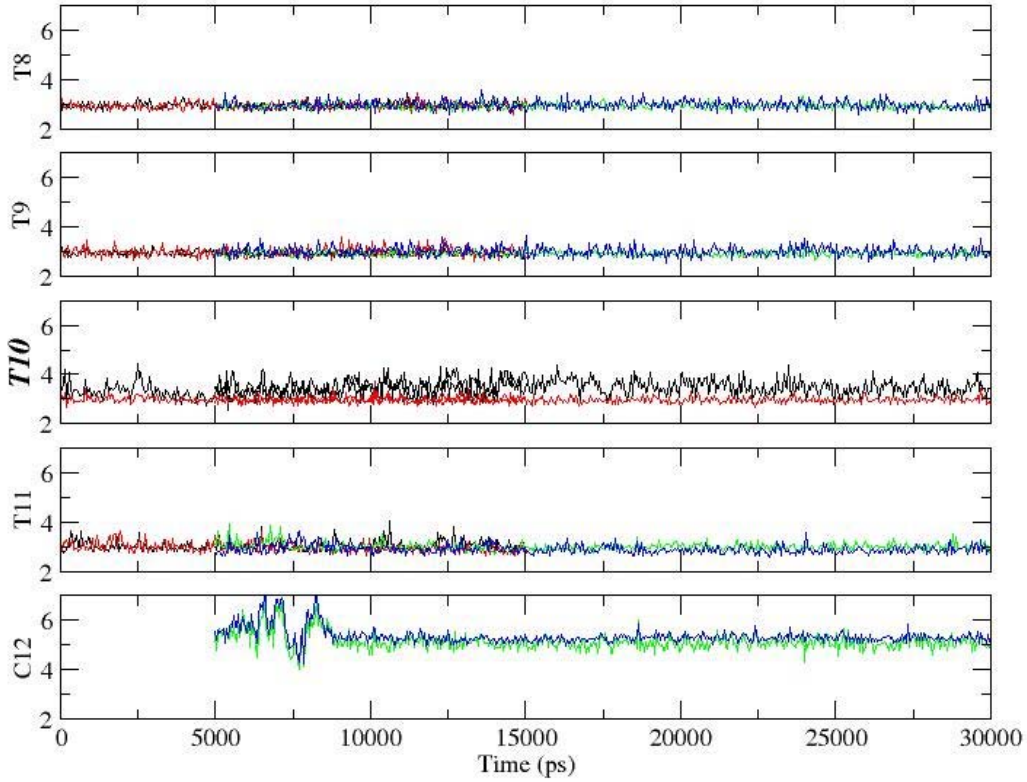
T8
T9
T10
T11
C12
6
4
2
0
5000
10000
15000
20000
25000
30000
Time (ps)

Fig S8.

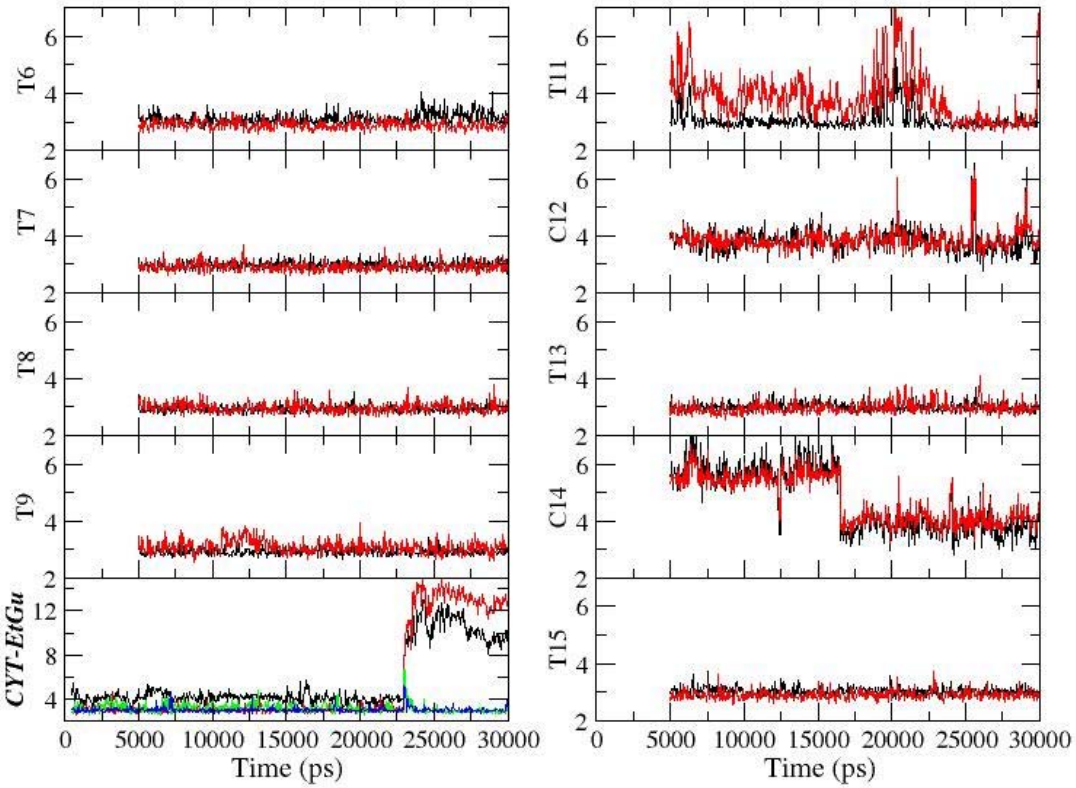

Fig S9.

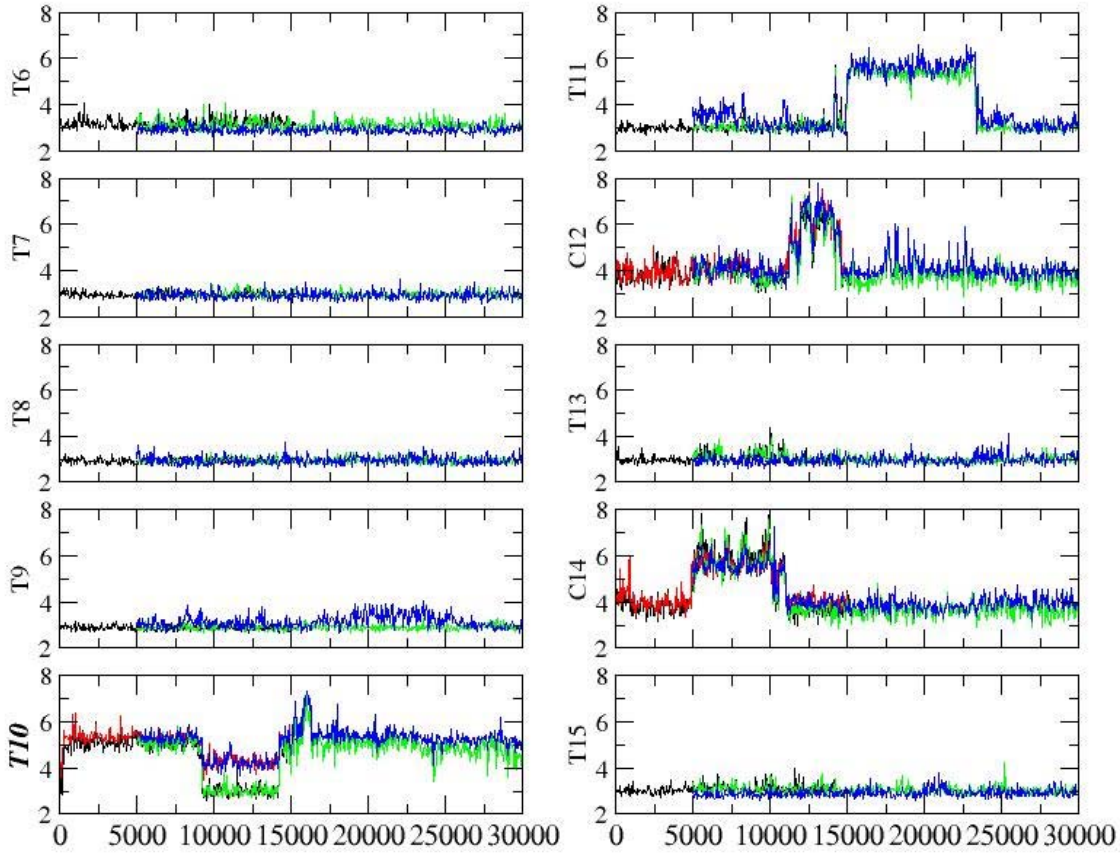
T6
T7
T8
T9
T10
T11
C12
T13
C14
T15
2
4
6
8
0
5000
10000
15000
20000
25000
30000

Fig S10.

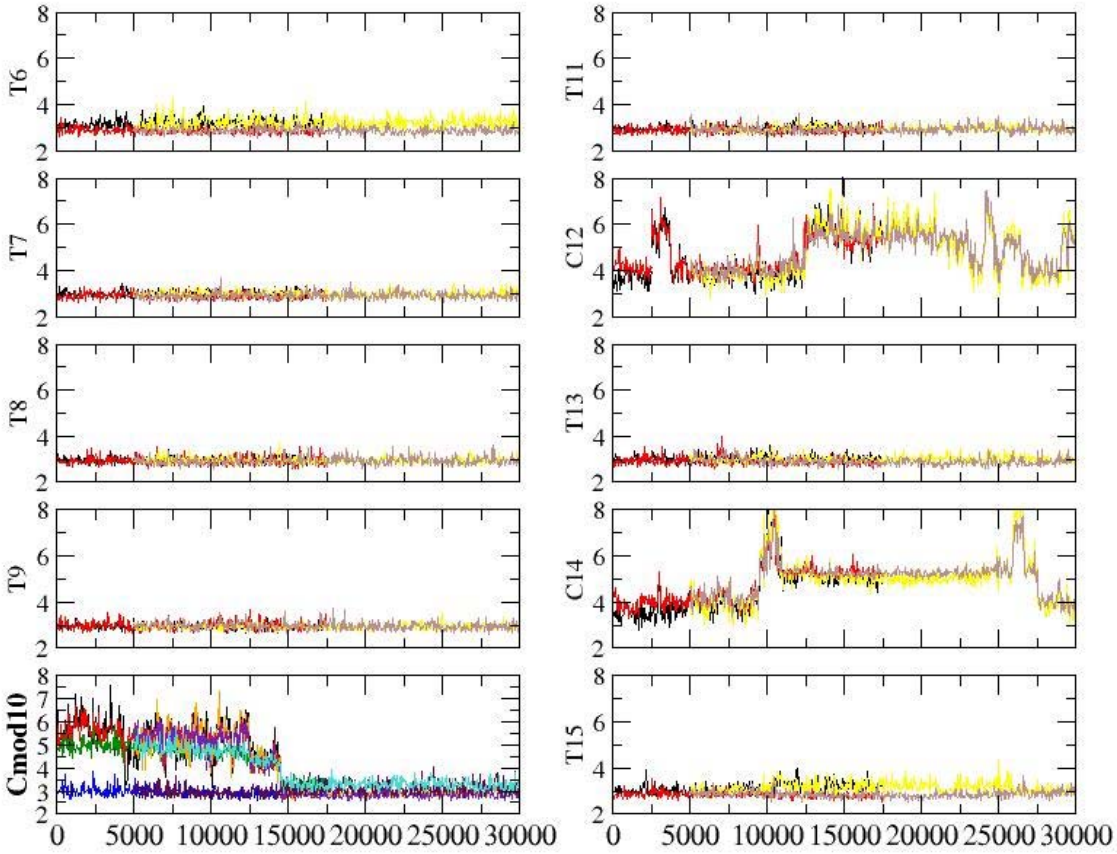
T6
T7
T8
T9
Cmod10
T11
C12
T13
C14
T15
0
5000
10000
15000
20000
25000
30000

Fig S11.

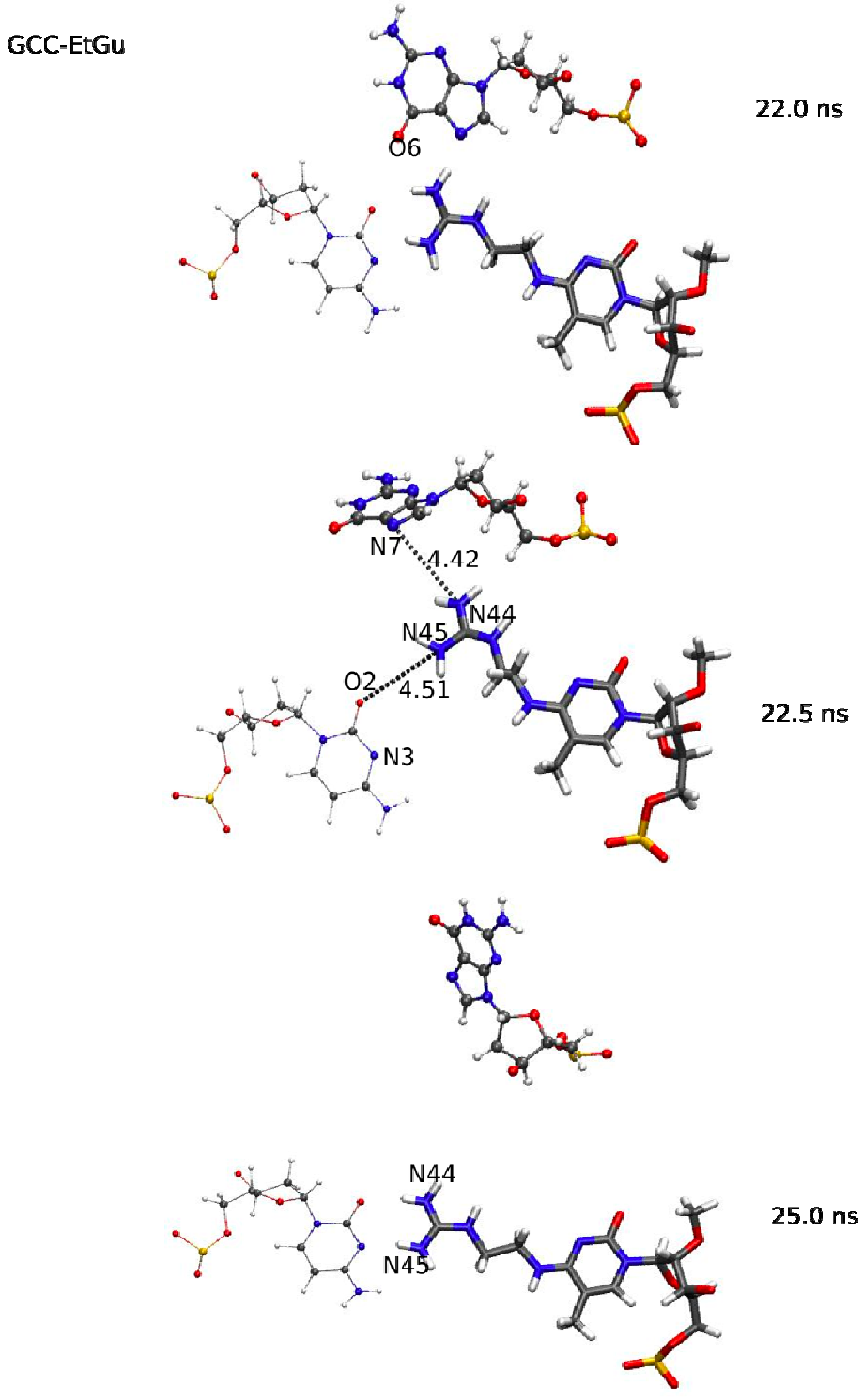

Fig S12.