The structural basis of actinomycin D binding induces nucleotide flipping-out, a sharp bend and a left-handed twist in CGG triplet repeats

Yu-Sheng Lo[1†], Wen-Hsuan Tseng[1†], Chien-Ying Chuang[1], and Ming-Hon Hou[1,2,3]

Supporting Information

Figure legends for supporting information

Figure S1. A refined ($2F_o - F_c$) Fourier electron density map showing the five amino acids (threonine, N-methylvaline, sarcosine, proline, and D-valine).

Figure S2. Schematic diagram of the ActD-$(ATGCGGCAT)_2$ as observed in the crystal structure.

Figure S3. Molecular interactions due to crystal packing. The flipped-out G6 **(A)** and G15 **(B)** bases of the DNA duplex form hydrogen bonds and mutual stacking interaction with the flipped out G6 and G15 bases of other duplexes with an equivalent symmetry.

Figure S4. The detailed conformation showing the stacking interactions in the ActD1-DNA (left) and ActD2-DNA (right) complexes at various base pair steps of the refined structure viewed from the minor groove direction. Hydrogen bonds are indicated as blue dotted lines.

Figure S5. Superimposition of the crystal structures of ActD1 (blue), ActD2 (green), and free ActD (red).

Figure S6. Comparison of the DNA twist (**A**), roll (**B**) and tilt (**C**) parameters in the structure of the ActD-$(ATGCGGCAT)_2$ complex. Typical values for A-DNA (solid line) and B-DNA (dashed line) are also shown.

Figure S7. (A) The crystal packing ($P6_522$) diagram of the ActD-DNA complex. **(B)** Two major different packing interactions occur between the two asymmetric units in the crystal lattice ($P6_522$): end-to-end and end-to-side, mediated by π–π stacking, and van der Waals contact.

Figure S8. Sensorgram of the ActD-DNA interaction between immobilised hairpin DNA duplexes including GG2 **(A)**, AT2 **(B)**, and GC2 **(C),** and the target ActD at various concentrations (2, 1, 0.75, 0.5, and 0.25 μM from top to bottom) in 50 mM NaCl buffered with 20 mM Tris-HCl buffer at pH 7.3. The sensorgram was obtained by subtracting the reference control. The black lines represent the fitted curves.

Figure S9. A schematic illustration of the binding of ActD to the $(CGG)_{16}$ sequence is shown.

Table SI. Crystallographic and Refinement Data of ATGCGGCA[br^{5}U]- Actinomycin D Complex.

λ for data collection (Å)	0.9062	0.9195 Inflection point	0.9189 peak	0.8563 high remote
Crystallographic data				
a=b (Å)	86.93	87.07	87.09	87.08
c (Å)	49.78	49.90	49.09	49.90
Space group			$P6_522$	
Resolution (Å)	30-2.6	50-2.8	50-2.8	50-2.8
# reflections (>0 σ(I))	48854	35451	38138	35367
<I/σ(I)>	27.0	37.1	20.5	36.1
R_{merge} (%)	9.7	7.7	6.9	7.8
Completeness (%)	99.8	99.5	99.6	99.5
Refinement data				
R-factor/R-free (5% data)	0.26/0.29			
r.m.s.d. (Å)	0.004			
r.m.s.d. (^{o})	1.40			
# of DNA atoms	366			
# of drug atoms	179			
# of waters	133			

*Including Friedel's pairs

Table SII. Base pair parameters for the ActD-$(ATGCGGCAT)_2$ complex.

Local base-pair parameters						
bp	**Shear (Å)**	**Stretch (Å)**	**Stagger (Å)**	**Buckle (˚)**	**Propeller (˚)**	**Opening (˚)**
A1-T18	-1.26	0.17	0.51	-3.39	-13.36	8.51
T2-A17	-0.34	-0.17	0.55	-11.87	-17.21	0.73
G3-C16	-0.41	-0.36	0.97	7.55	18.33	1.21
C4+G14	-0.57	2.76	0.87	4.06	28.70	-120.37
G5-C13	-0.29	-0.24	0.52	5.02	11.40	-16.18
C7-G12	-0.43	-0.07	-0.84	14.84	22.04	-3.99
A8-T11	-0.08	0.54	1.01	39.48	-21.43	11.35
T9-A10	-1.31	-0.47	0.87	29.22	-17.37	7.00

Local base-pair parameters						
step	**Shift (Å)**	**Slide (Å)**	**Rise (Å)**	**Tilt (˚)**	**Roll (˚)**	**Twist (˚)**
AT/AT	-0.95	-0.33	3.55	1.55	-13.23	32.94
TG/CA	1.01	2.00	3.38	-5.84	3.92	35.05
GC/GC	0.43	0.89	8.21	2.59	10.37	62.47
CG/CG	0.61	3.13	2.95	14.56	21.32	-43.45
GC/GC	0.80	2.94	6.68	11.52	10.20	5.84
CA/TG	0.12	1.92	2.98	-1.54	-1.17	35.43
AT/AT	0.69	-1.31	3.25	-4.43	0.89	31.70

Table SIII. Nucleic acid backbone torsion angles obtained for the ActD-$(ATGCGGCAT)_2$ complex.

Strand I								
Base	**α**	**β**	**γ**	**δ**	**ε**	**ξ**	**χ**	Puckering
A-1	----	----	51.7	146.9	-159.1	-97.6	-102.5	C2'-endo
T-2	-105.7	78.3	163.0	144.1	-121.8	-47.6	-166.2	C2'-endo
G-3	-76.6	163.5	31.1	138.1	-156.8	-128.7	-70.7	C2'-endo
C-4	-85.9	-140.4	34.4	139.4	176.1	-117.2	-58.5	C2'-endo
G-5	-53.2	-169.8	41.5	141.8	-157.5	-115.6	-58.9	C2'-endo
G-6	58.7	142.7	-50.8	162.3	-90.3	102.4	-100.6	C3'-exo
C-7	-118.0	175.1	46.5	145.2	-83.1	118.4	-83.7	C2'-endo
A-8	119.3	-113.4	149.5	145.0	-157.9	-109.2	-95.3	C2'-endo
T-9	34.2	-147.6	-85.5	142.6	----	----	-121.3	C2'-endo
Strand II								
Base	**α**	**β**	**γ**	**δ**	**ε**	**ξ**	**χ**	Puckering
T-18	-104.1	67.3	171.4	148.2	----	----	-155.6	C2'-endo
A-17	-60.7	128.2	51.4	150.4	-164.8	-75.2	-86.0	C2'-endo
C-16	101.1	-143.2	176.6	131.9	-80.6	-149.6	-162.9	C2'-endo
G-15	-47.1	174.6	46.3	148.4	-109.6	78.6	-112.3	C2'-endo
G-14	-18.2	-140.8	-50.1	153.0	-133.7	-87.8	108.0	C3'-exo
C-13	-57.1	-111.7	-58.1	149.1	-163.3	-94.7	-58.6	C3'-exo
G-12	-76.9	-168.3	51.9	144.3	-131.5	-82.5	-82.2	C2'-endo
T-11	56.3	144.4	-54.5	142.8	-177.6	-93.3	-105.1	C2'-endo
A-10	----	----	59.3	151.0	-172.7	-120.3	-143.4	C3'-exo

Figure S1

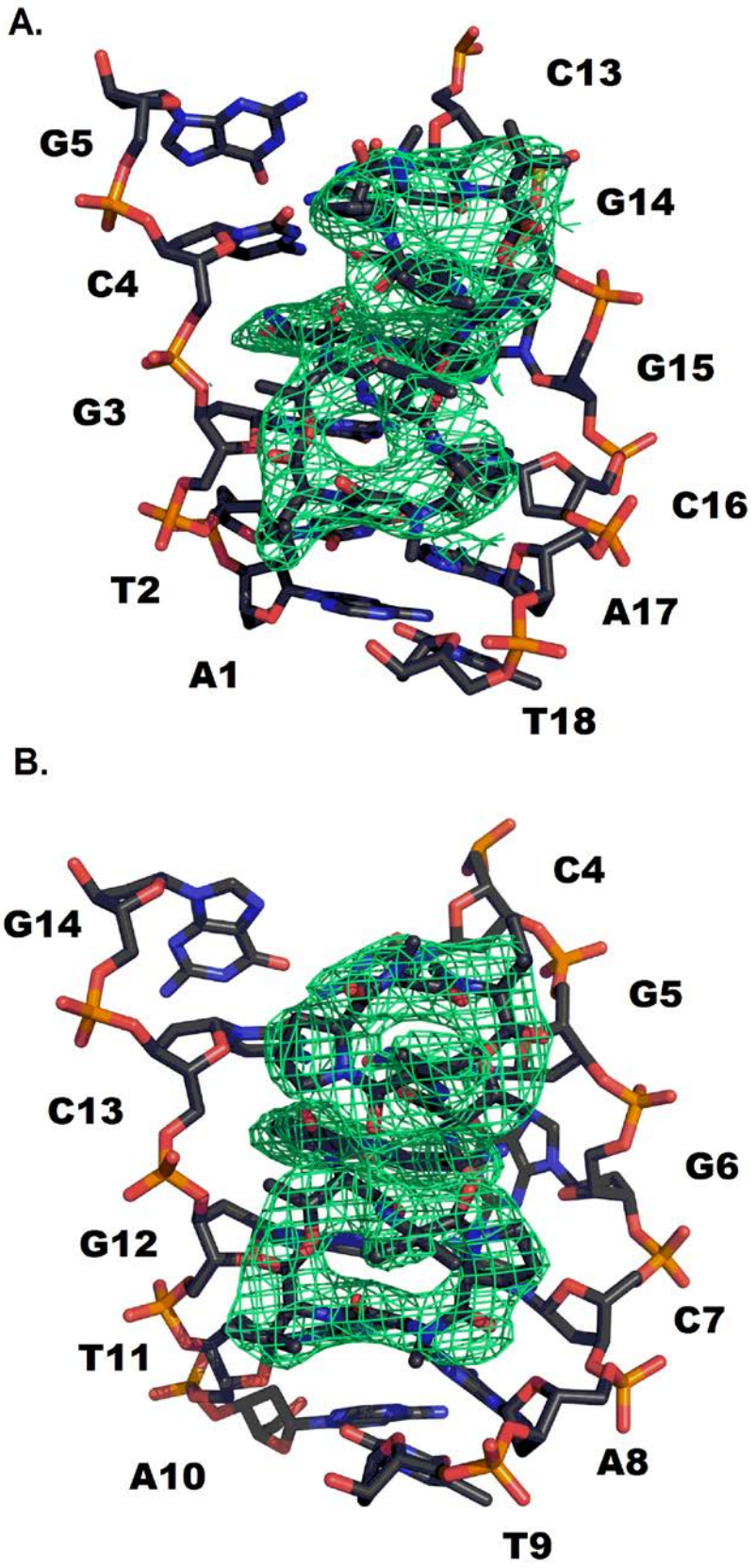

A.
C13
G5
G14
C4
G15
G3
C16
T2
A17
A1
T18
B.
C4
G14
G5
C13
G6
G12
C7
T11
A8
A10
T9

Figure S2

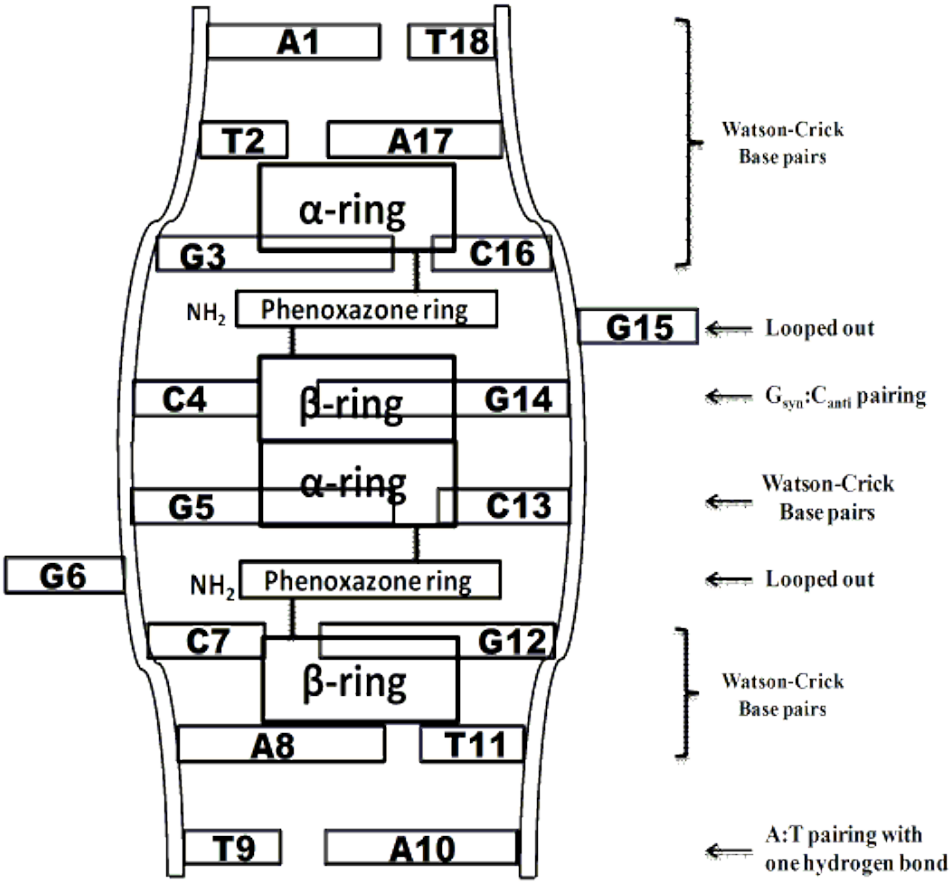
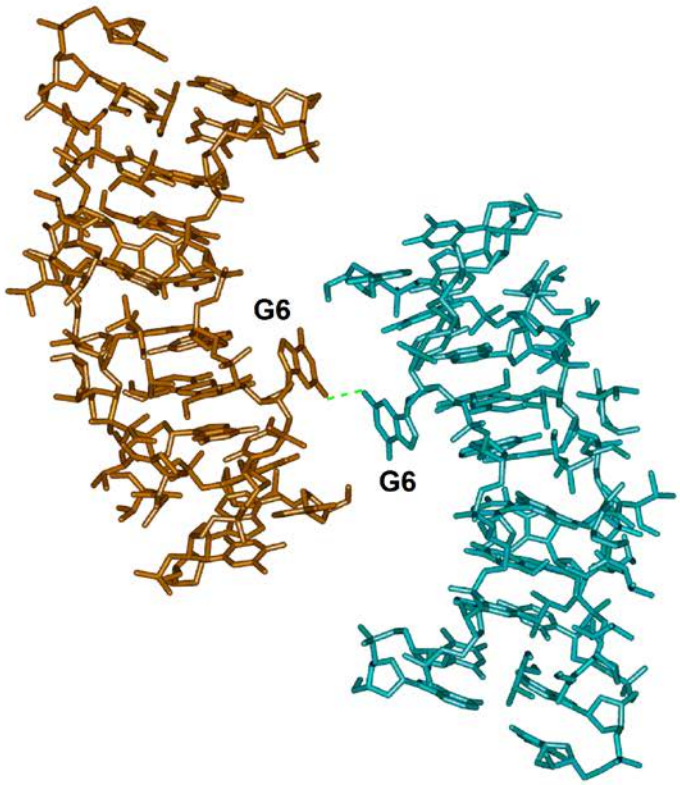

A1
T18
T2
A17
α-ring
G3
C16
NH2
Phenoxazone ring
G15
C4
β-ring
G14
α-ring
G5
C13
G6
NH2
Phenoxazone ring
C7
G12
β-ring
A8
T11
T9
A10
Watson-Crick Base pairs
Looped out
Gsyn:Canti pairing
Watson-Crick Base pairs
Looped out
Watson-Crick Base pairs
A:T pairing with one hydrogen bond

Figure S3

A.



B.

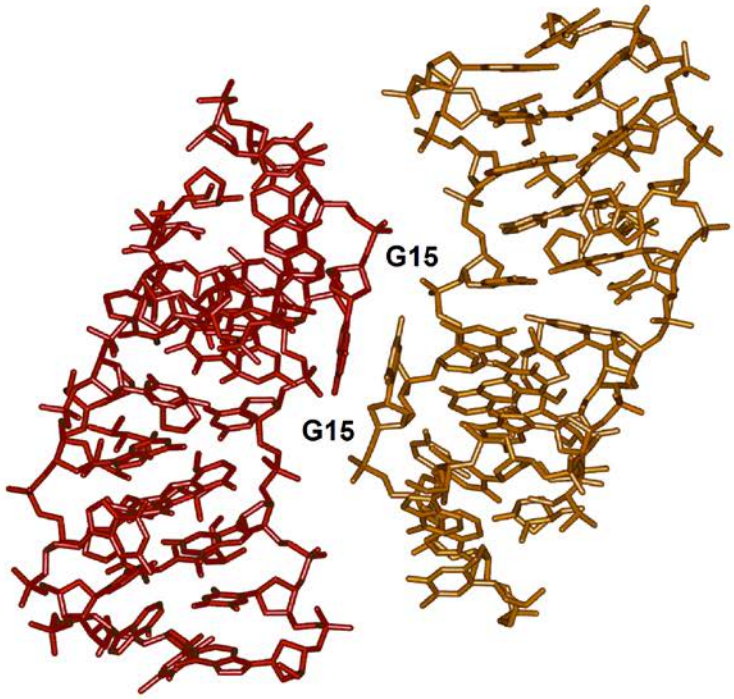


Figure S4

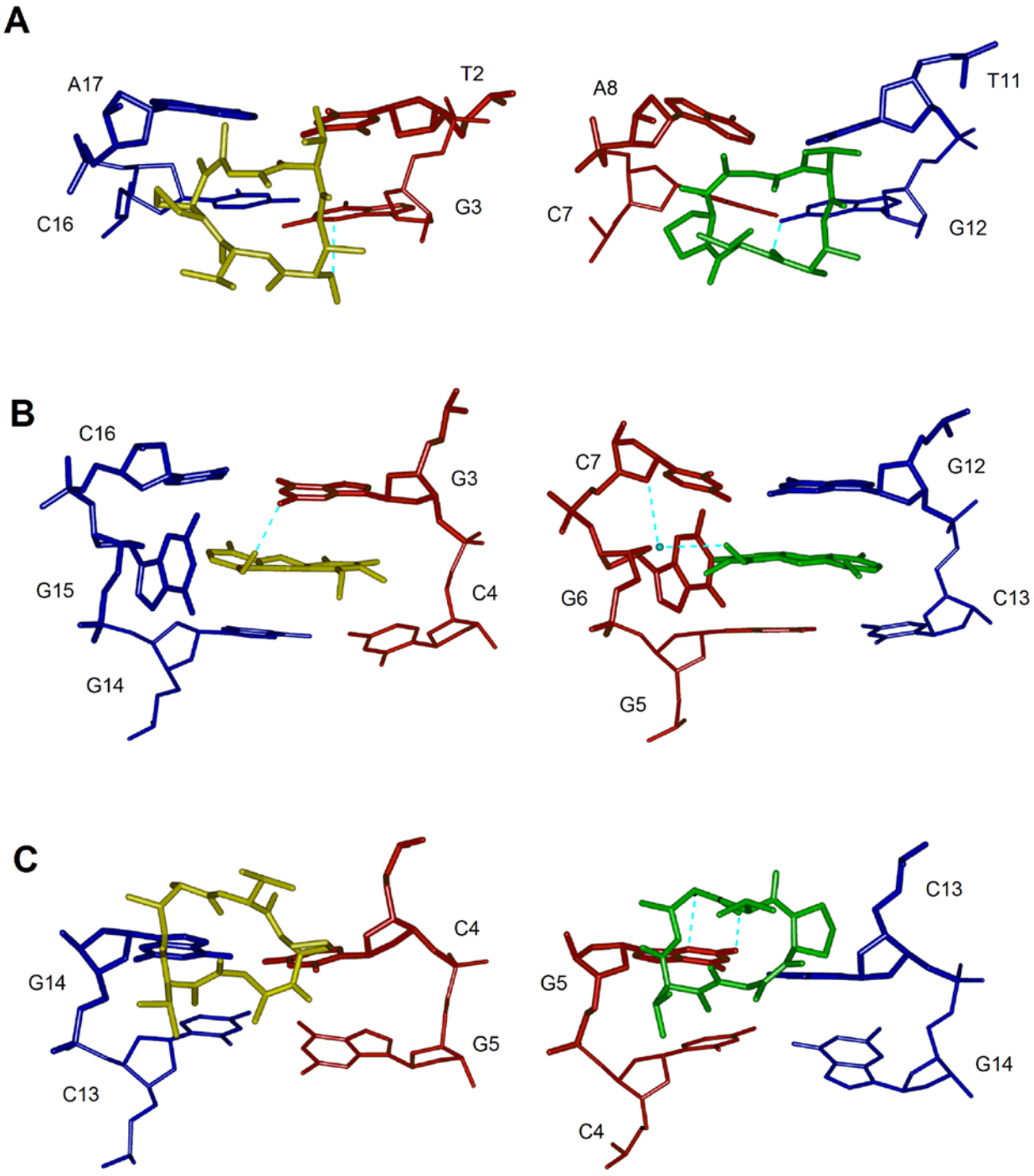
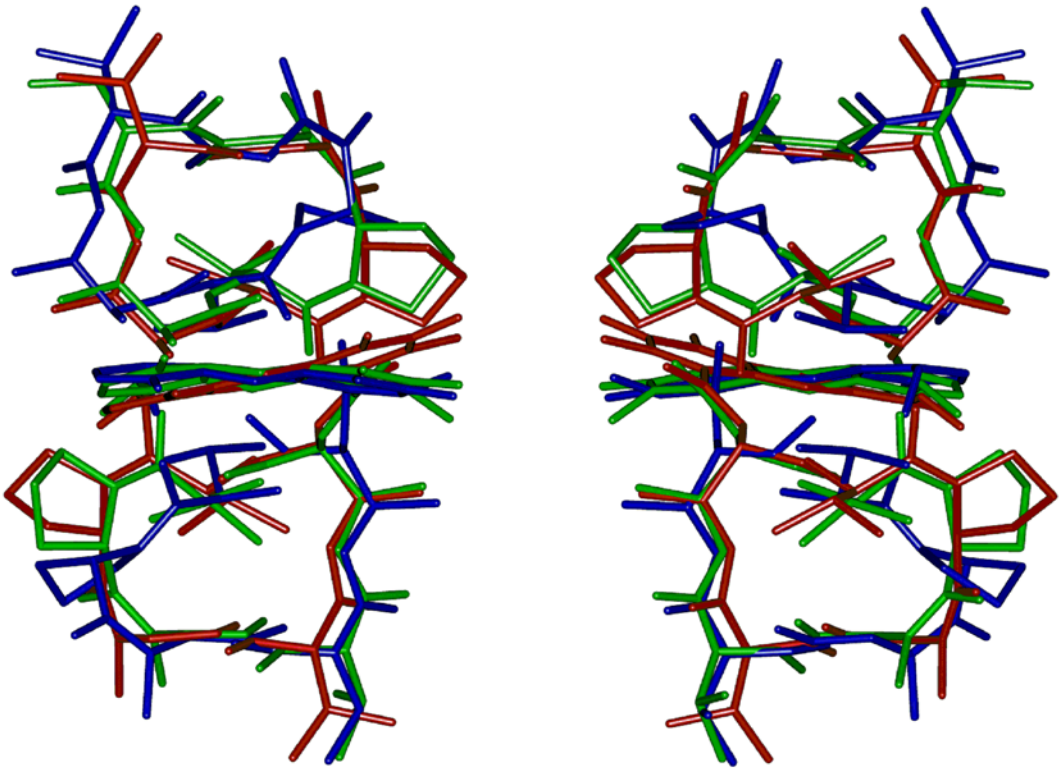

A
A17
T2
C16
G3
A8
T11
C7
G12
B
C16
G3
G15
C4
G14
C7
G12
G6
C13
G5
C
C4
G14
G5
C13
C13
G5
G14
C4

Figure S5

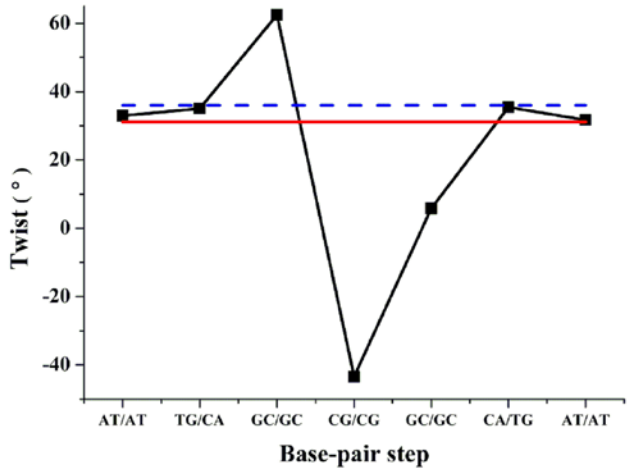


(Front view)

(Back view)

Figure S6

A.



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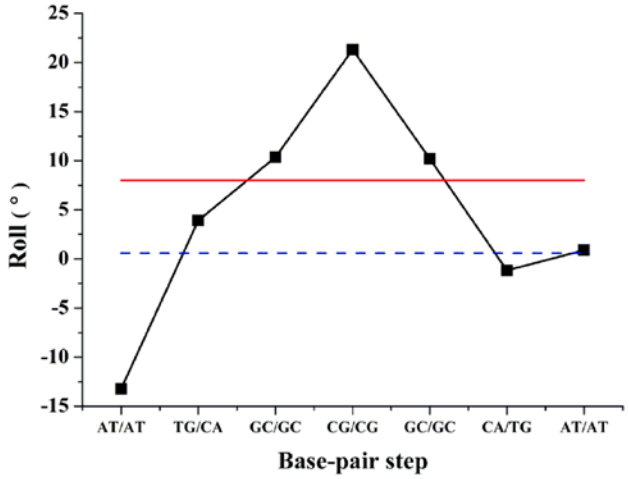

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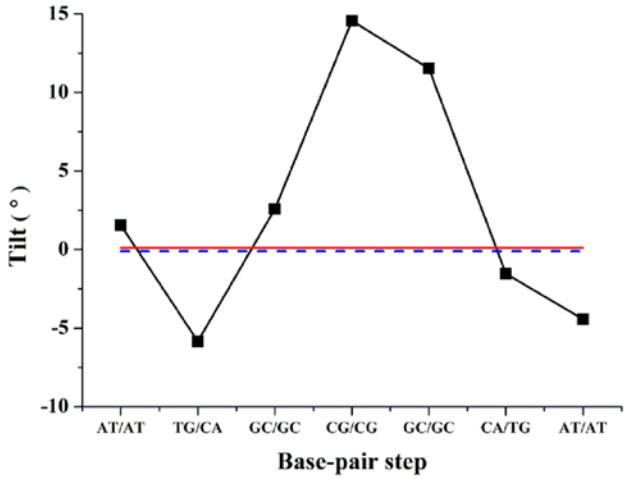
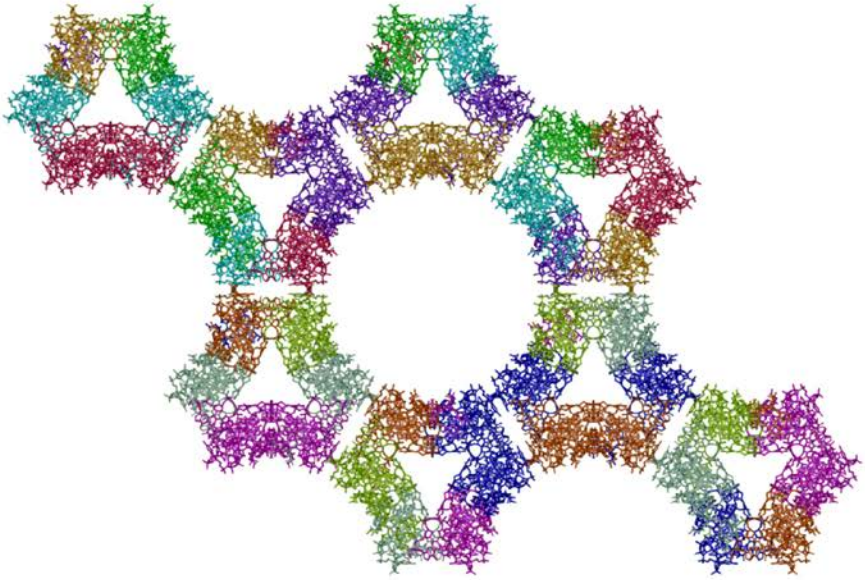


Figure S7

A.



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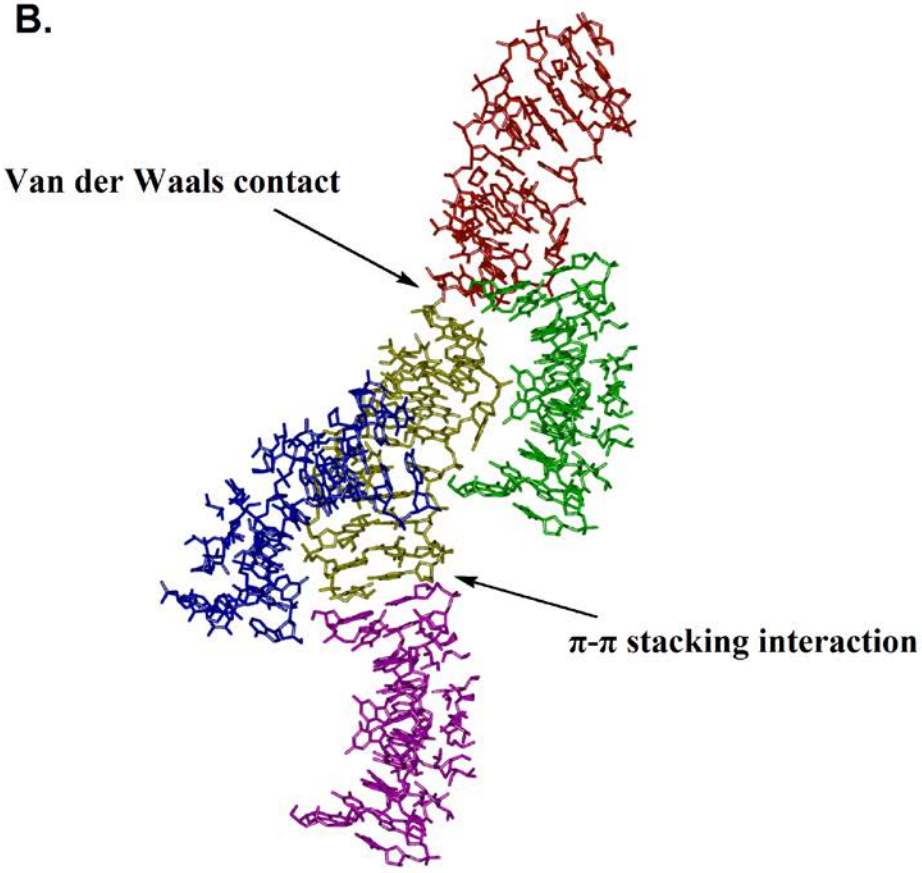
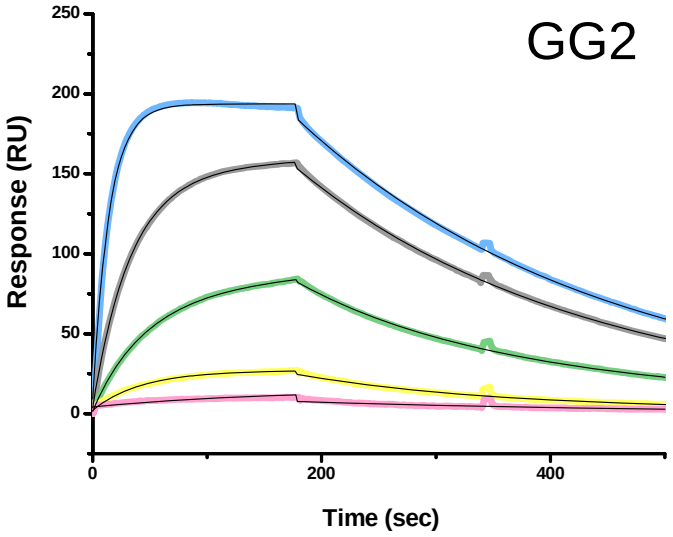


Figure S8

A.



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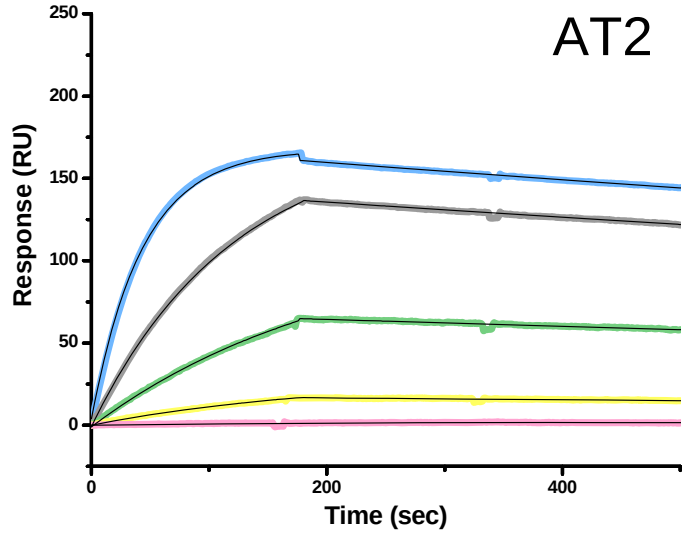

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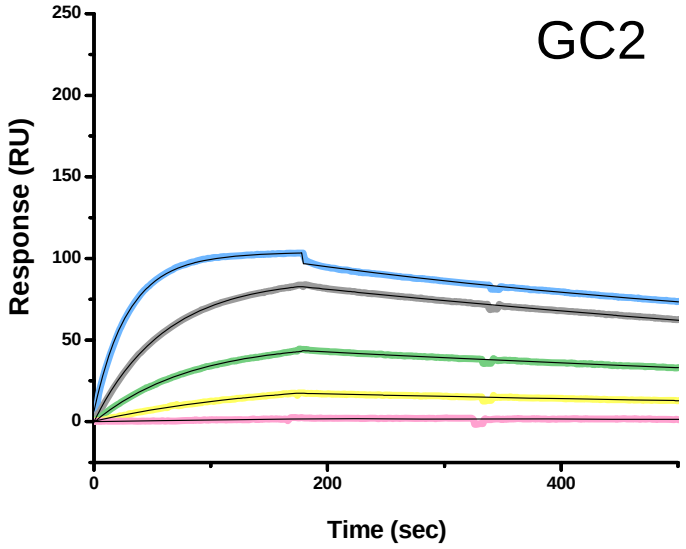

Figure S9