Stereochemistry and Position-Dependent Effects of Carcinogens on TATA/TBP Binding

Supplementary Material

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Appendix S1: Remodeling of TATA DNA

The TATA DNA in the experiment has 25 basepairs (bp), but we keep the length of 1CDW PDB DNA to be 16 bp without appending since the rest 9 bp are not in contact with TBP. We then modify the sequence of the 16 bp DNA to the corresponding experimental sequence.

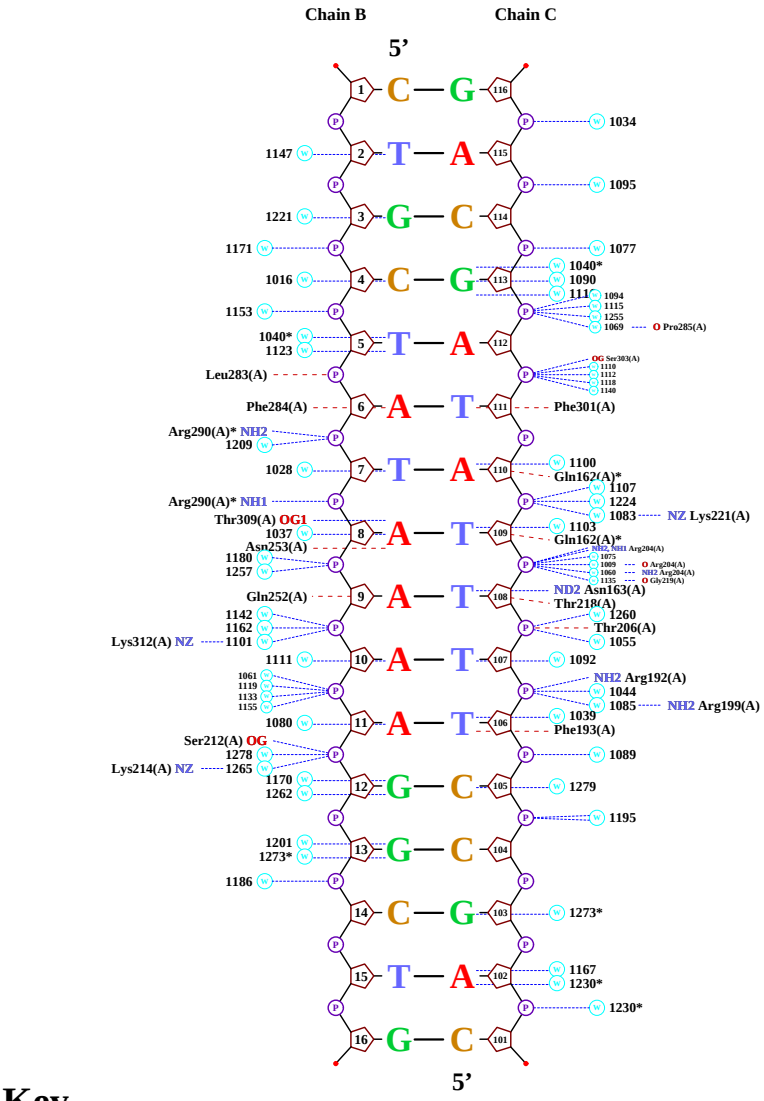
Experimental sequence: 5′–GAATTCCG TATAAATA CGTGTCGTG–3′
PDB sequence: 5′–CTGC TATAAAAG GCTG–3′
Model sequence: 5′–TCCG TATAAATA CGTG–3′

Sequence remodeling is conducted on the relevant DNA bases only, not on sugars or backbone. We then check the changes of interactions on the DNA. The interactions on DNA (from TBP and crystal water molecules) are computed and plotted in schematic diagrams using the NUCPLOT program (Luscombe N M, Laskowski R A, Thornton J M. 1997. NUCPLOT: a program to generate schematic diagams of protein-DNA interactions. *Nucleic Acids Research*, **25,** 4940-4945). Hydrogen bonds have a 3.00-Å cut-off, and nonbonded contacts have a 3.35-Å cut-off.

Since only the TATA box in the DNA contacts TBP and we only modify the bases of the last two basepairs in the TATA box (from AG to TA), we only need to know the interaction changes between TBP and the last four DNA bases in the TATA box: A11, G12, C105, T106 (see Figure 1). We find one nonbonded contact on the base of T106: T106:O2---Phe193:CZ (from phenylalanine A193) before sequence remodeling.

After sequence remodeling, all the interactions on DNA are calculated again and plotted in a new schematic diagram (Figure 2). We find there is one nonbonded contact from Phe193 on the base of A106: A106:N3---Phe193:CZ. The only difference from previous contact is that the atom T106:O2 becomes A106:N3, and both atoms lie in the minor groove, thus our remodeling process is reasonable.

Note: All the residue numbers in this appendix are from the PDB file (ID: 1CDW).



Key

3 Backbone sugar and base-number

P Phosphate group

* Residue/water on plot more than once

Hydrogen bond to DNA

Nonbonded contact to DNA (< 3.35A)

88 W Water molecule and number

1CDW

Figure 1: Interactions on 1CDW DNA before Modeling.

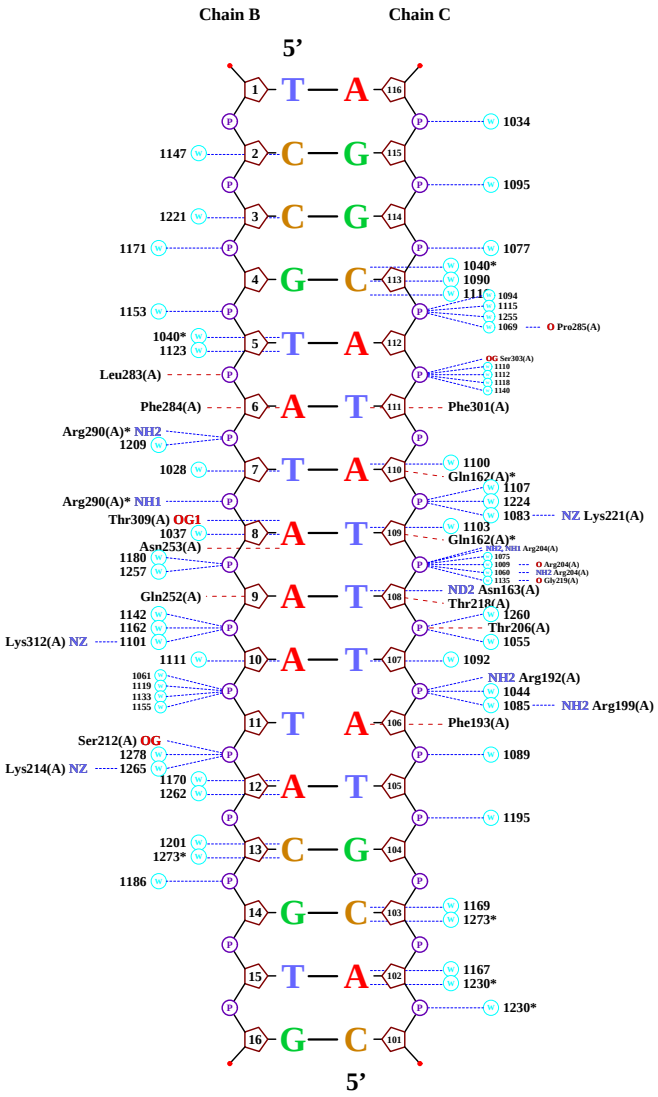


Figure 2: Interactions on 1CDW DNA after Modeling.

Appendix S2: Partial Charges and Other Added Force Field Parameters of BP-Adenine Adducts

Table 1: Partial charges, atom types, and topologies, of 10*S*(+) and 10*R*(−)-*trans*-*anti*-[BP]-N^6-dA adducts at the position A_1 of the TATA box.

atom name	atom type	topology	partial charge				
			10*S*(+)-*trans*-[A_1]		10*R*(−)-*trans*-[A_1]		
			II	III	II	III	IV
P	P	M	1.2209	1.2212	1.2212	1.2212	1.2216
O1P	O2	E	−0.7932	−0.7936	−0.7931	−0.7939	−0.7930
O2P	O2	E	−0.7932	−0.7936	−0.7931	−0.7939	−0.7930
O5′	OS	M	−0.4914	−0.4890	−0.4964	−0.4873	−0.5033
C5′	CT	M	−0.0337	−0.0152	−0.0367	−0.0023	0.0236
H5′1	H1	E	0.0795	0.0847	0.0842	0.0810	0.0655
H5′2	H1	E	0.0795	0.0847	0.0842	0.0810	0.0655
C4′	CT	M	0.2233	0.1069	0.1927	0.0831	0.0893
H4′	H1	E	0.0944	0.1329	0.1067	0.1381	0.1246
O4′	OS	S	−0.4220	−0.3721	−0.3971	−0.3492	−0.3134
C1′	CT	B	0.2145	0.1059	0.0940	0.0381	0.0161
H1′	H2	E	0.1301	0.1620	0.1610	0.1777	0.1485
N9	N*	B	−0.0899	−0.0100	−0.0344	−0.0358	−0.0697
C8	CK	S	0.1461	0.0584	0.1268	0.1150	0.1104
H8	H5	E	0.1854	0.1991	0.1938	0.2022	0.1579
C4	CB	B	0.4535	0.4741	0.4964	0.4896	0.3803
N3	NC	E	−0.7712	−0.8080	−0.7845	−0.8019	−0.4965
C5	CB	B	0.0804	−0.0081	0.0260	0.0453	0.0811
N7	NB	E	−0.5937	−0.4918	−0.5855	−0.5690	−0.5258
C6	CA	B	0.4756	0.5417	0.4930	0.4451	0.4134
N1	NC	S	−0.7193	−0.7978	−0.7339	−0.7816	−0.6829
C2	CQ	S	0.6061	0.6830	0.6118	0.6803	0.4448
H2	H5	E	0.0439	0.0373	0.0467	0.0297	0.0562
N6	N2	B	−0.4766	−0.4544	−0.4421	−0.3934	−0.3821
H6	H	E	0.3166	0.3108	0.2948	0.2860	0.2674
CC1	CT	B	0.0259	0.0116	0.0245	0.0356	0.0265
HC1	H1	E	0.1196	0.1104	0.1708	0.1665	0.1646
CC2	CT	3	−0.0224	0.0019	0.0070	0.0541	0.0192
HC2	H1	E	0.1529	0.1372	0.1807	0.1580	0.1743
O9	OH	S	−0.6752	−0.6496	−0.6666	−0.6818	−0.6812
HO9	HO	E	0.4583	0.4305	0.4641	0.4684	0.4749
CC3	CT	3	0.1060	0.0836	0.0450	0.0526	0.0371
HC3	H1	E	0.1324	0.1292	0.0878	0.0850	0.0954

O8	OH	S	−0.6475	−0.6451	−0.6541	−0.6621	−0.6592
HO8	HO	E	0.4443	0.4419	0.4407	0.4430	0.4473
CC4	CT	3	0.2329	0.2682	0.2844	0.2459	0.2314
HC4	H1	E	0.1549	0.1424	0.1203	0.1310	0.1349
O7	OH	S	−0.6733	−0.6804	−0.6847	−0.6782	−0.6780
HO7	HO	E	0.4313	0.4328	0.4214	0.4226	0.4230
CC5	CA	S	−0.1012	−0.1021	−0.0748	−0.0821	−0.0494
CC6	CA	B	−0.2048	−0.2065	−0.2274	−0.2257	−0.2443
HC6	HA	E	0.1698	0.1707	0.1631	0.1626	0.1655
CC7	CA	B	0.0282	0.0348	0.0291	0.0414	0.0491
C20	CA	E	0.0337	0.0338	0.0272	0.0181	0.0213
CC8	CA	B	−0.1631	−0.1687	−0.1540	−0.1550	−0.1626
HC8	HA	E	0.1424	0.1431	0.1413	0.1375	0.1406
CC9	CA	B	−0.2057	−0.2051	−0.2172	−0.2115	−0.2028
HC9	HA	E	0.1463	0.1476	0.1491	0.1497	0.1450
C10	CA	B	0.0532	0.0487	0.0592	0.0468	0.0534
C19	CA	E	0.0929	0.1211	0.1010	0.1225	0.0995
C11	CA	B	−0.1396	−0.1609	−0.1454	−0.1701	−0.1532
HC11	HA	E	0.1399	0.1436	0.1419	0.1476	0.1439
C12	CA	B	−0.2301	−0.2048	−0.2292	−0.1959	−0.2151
HC12	HA	E	0.1656	0.1580	0.1650	0.1578	0.1623
C13	CA	B	−0.1489	−0.1521	−0.1406	−0.1639	−0.1515
HC13	HA	E	0.1442	0.1423	0.1413	0.1443	0.1421
C14	CA	S	0.0487	0.0399	0.0411	0.0539	0.0446
C15	CA	B	−0.2041	−0.2352	−0.2104	−0.2218	−0.2029
HC15	HA	E	0.1523	0.1570	0.1556	0.1563	0.1509
C16	CA	B	−0.2045	−0.1347	−0.1823	−0.1565	−0.1454
HC16	HA	E	0.1758	0.1374	0.1561	0.1550	0.1327
C17	CA	S	0.0289	−0.0011	0.0251	0.0005	−0.0153
C18	CA	E	0.0225	0.0149	0.0301	0.0325	0.0545
C3′	CT	M	0.0337	0.0396	0.0502	0.0291	0.0375
H3′	H1	E	0.1112	0.1178	0.1126	0.1218	0.1296
C2′	CT	B	−0.0695	−0.0563	−0.0814	−0.0418	−0.0308
H2′1	HC	E	0.0502	0.0544	0.0598	0.0562	0.0526
H2′2	HC	E	0.0502	0.0544	0.0598	0.0562	0.0526
O3′	OS	M	−0.5241	−0.5151	−0.5238	−0.5111	−0.5209

Table 2: Partial charges, atom types, and topologies, of 10*S*(+) and 10*R*(−)-*trans*-*anti*-[BP]-N^6-dA adducts at the position A_2 of the TATA box.

atom name	atom type	topology	partial charge					
			10*S*(+)-*trans*-[A_2]			10*R*(−)-*trans*-[A_2]		
			III	III-2	IV	I	III	IV
P	P	M	1.2212	1.2212	1.2212	1.2211	1.2208	1.2208
O1P	O2	E	−0.7930	−0.7930	−0.7930	−0.7928	−0.7928	−0.7928
O2P	O2	E	−0.7930	−0.7930	−0.7930	−0.7928	−0.7928	−0.7928
O5′	OS	M	−0.4972	−0.4972	−0.4972	−0.4987	−0.4947	−0.4947
C5′	CT	M	−0.0085	−0.0085	−0.0085	0.0304	0.0615	0.0615
H5′1	H1	E	0.0919	0.0919	0.0919	0.0783	0.0657	0.0657
H5′2	H1	E	0.0919	0.0919	0.0919	0.0783	0.0657	0.0657
C4′	CT	M	0.0634	0.0634	0.0634	0.0704	0.0844	0.0844
H4′	H1	E	0.1397	0.1397	0.1397	0.1336	0.1239	0.1239
O4′	OS	S	−0.3451	−0.3451	−0.3451	−0.3532	−0.3647	−0.3648
C1′	CT	B	0.0575	0.0575	0.0575	0.0346	0.0506	0.0506
H1′	H2	E	0.1426	0.1426	0.1426	0.1588	0.1484	0.1484
N9	N*	B	−0.0006	−0.0006	−0.0006	−0.0455	−0.0214	−0.0214
C8	CK	S	0.0522	0.0523	0.0523	0.1695	0.1229	0.1229
H8	H5	E	0.1969	0.1969	0.1969	0.1716	0.1754	0.1754
C4	CB	B	0.4703	0.4703	0.4704	0.4591	0.4633	0.4633
N3	NC	E	−0.8104	−0.8104	−0.8104	−0.7908	−0.8108	−0.8109
C5	CB	B	−0.0120	−0.0118	−0.0119	0.0742	0.0442	0.0442
N7	NB	E	−0.4877	−0.4877	−0.4877	−0.6090	−0.5572	−0.5572
C6	CA	B	0.6011	0.6007	0.6007	0.4802	0.4633	0.4633
N1	NC	S	−0.8096	−0.8096	−0.8095	−0.7455	−0.7870	−0.7870
C2	CQ	S	0.6799	0.6798	0.6798	0.6419	0.6887	0.6887
H2	H5	E	0.0386	0.0386	0.0387	0.0399	0.0293	0.0293
N6	N2	B	−0.5362	−0.5357	−0.5357	−0.4859	−0.4078	−0.4079
H6	H	E	0.3363	0.3361	0.3361	0.3100	0.2869	0.2869
CC1	CT	B	0.0014	0.0015	0.0015	0.0258	0.0243	0.0243
HC1	H1	E	0.1119	0.1118	0.1118	0.1701	0.1692	0.1692
CC2	CT	3	0.0044	0.0044	0.0044	0.0351	0.0377	0.0377
HC2	H1	E	0.1305	0.1304	0.1304	0.1700	0.1599	0.1599
O9	OH	S	−0.6594	−0.6594	−0.6594	−0.6755	−0.6778	−0.6778
HO9	HO	E	0.4389	0.4390	0.4390	0.4631	0.4659	0.4659
CC3	CT	3	0.1252	0.1253	0.1255	0.0913	0.0628	0.0628
HC3	H1	E	0.1227	0.1226	0.1226	0.0739	0.0816	0.0816
O8	OH	S	−0.6544	−0.6544	−0.6544	−0.6629	−0.6620	−0.6620
HO8	HO	E	0.4415	0.4415	0.4416	0.4358	0.4388	0.4388
CC4	CT	3	0.2593	0.2595	0.2595	0.2940	0.3009	0.3009
HC4	H1	E	0.1434	0.1433	0.1433	0.1129	0.1138	0.1138

O7	OH	S	−0.6825	−0.6825	−0.6823	−0.6854	−0.6857	−0.6857
HO7	HO	E	0.4320	0.4319	0.4318	0.4181	0.4174	0.4174
CC5	CA	S	−0.0993	−0.0994	−0.0995	−0.0995	−0.0897	−0.0897
CC6	CA	B	−0.2022	−0.2022	−0.2023	−0.2004	−0.2305	−0.2305
HC6	HA	E	0.1702	0.1702	0.1701	0.1559	0.1597	0.1597
CC7	CA	B	0.0297	0.0297	0.0298	0.0254	0.0508	0.0509
C20	CA	E	0.0237	0.0237	0.0237	0.0180	0.0154	0.0154
CC8	CA	B	−0.1585	−0.1585	−0.1585	−0.1694	−0.1580	−0.1580
HC8	HA	E	0.1424	0.1424	0.1424	0.1457	0.1394	0.1394
CC9	CA	B	−0.2212	−0.2212	−0.2212	−0.1994	−0.2244	−0.2244
HC9	HA	E	0.1516	0.1516	0.1516	0.1440	0.1520	0.1520
C10	CA	B	0.0674	0.0674	0.0674	0.0589	0.0637	0.0637
C19	CA	E	0.1164	0.1164	0.1164	0.1024	0.1204	0.1204
C11	CA	B	−0.1758	−0.1758	−0.1758	−0.1529	−0.1691	−0.1691
HC11	HA	E	0.1482	0.1482	0.1482	0.1429	0.1480	0.1480
C12	CA	B	−0.2044	−0.2044	−0.2044	−0.2146	−0.2087	−0.2087
HC12	HA	E	0.1588	0.1588	0.1588	0.1622	0.1611	0.1611
C13	CA	B	−0.1440	−0.1440	−0.1440	−0.1549	−0.1610	−0.1610
HC13	HA	E	0.1407	0.1407	0.1407	0.1442	0.1438	0.1438
C14	CA	S	0.0364	0.0364	0.0364	0.0484	0.0598	0.0598
C15	CA	B	−0.2458	−0.2458	−0.2458	−0.2254	−0.2383	−0.2383
HC15	HA	E	0.1602	0.1602	0.1602	0.1600	0.1609	0.1609
C16	CA	B	−0.1246	−0.1246	−0.1246	−0.1701	−0.1401	−0.1401
HC16	HA	E	0.1364	0.1364	0.1364	0.1605	0.1455	0.1454
C17	CA	S	0.0101	0.0101	0.0101	0.0267	0.0017	0.0017
C18	CA	E	0.0180	0.0181	0.0182	0.0344	0.0316	0.0316
C3′	CT	M	0.1127	0.1128	0.1127	0.1311	0.1338	0.1337
H3′	H1	E	0.1131	0.1130	0.1131	0.1082	0.0988	0.0989
C2′	CT	B	−0.0954	−0.0955	−0.0955	−0.1337	−0.1032	−0.1031
H2′1	HC	E	0.0773	0.0774	0.0774	0.0880	0.0774	0.0773
H2′2	HC	E	0.0773	0.0774	0.0774	0.0880	0.0774	0.0773
O3′	OS	M	−0.5247	−0.5248	−0.5247	−0.5284	−0.5310	−0.5310

Table 3: Other added force field parameters

angle type	force constant K_θ (kcal/mol/rad^2)	angle value θ_{eq} (°)
OH−CT−CA	65.8	112.61
N2−CT−CA	50.0	108.75
H1−CT−CA	63.0	108.93

Appendix S3: Convergence of Benzo[a]pyrene Torsions

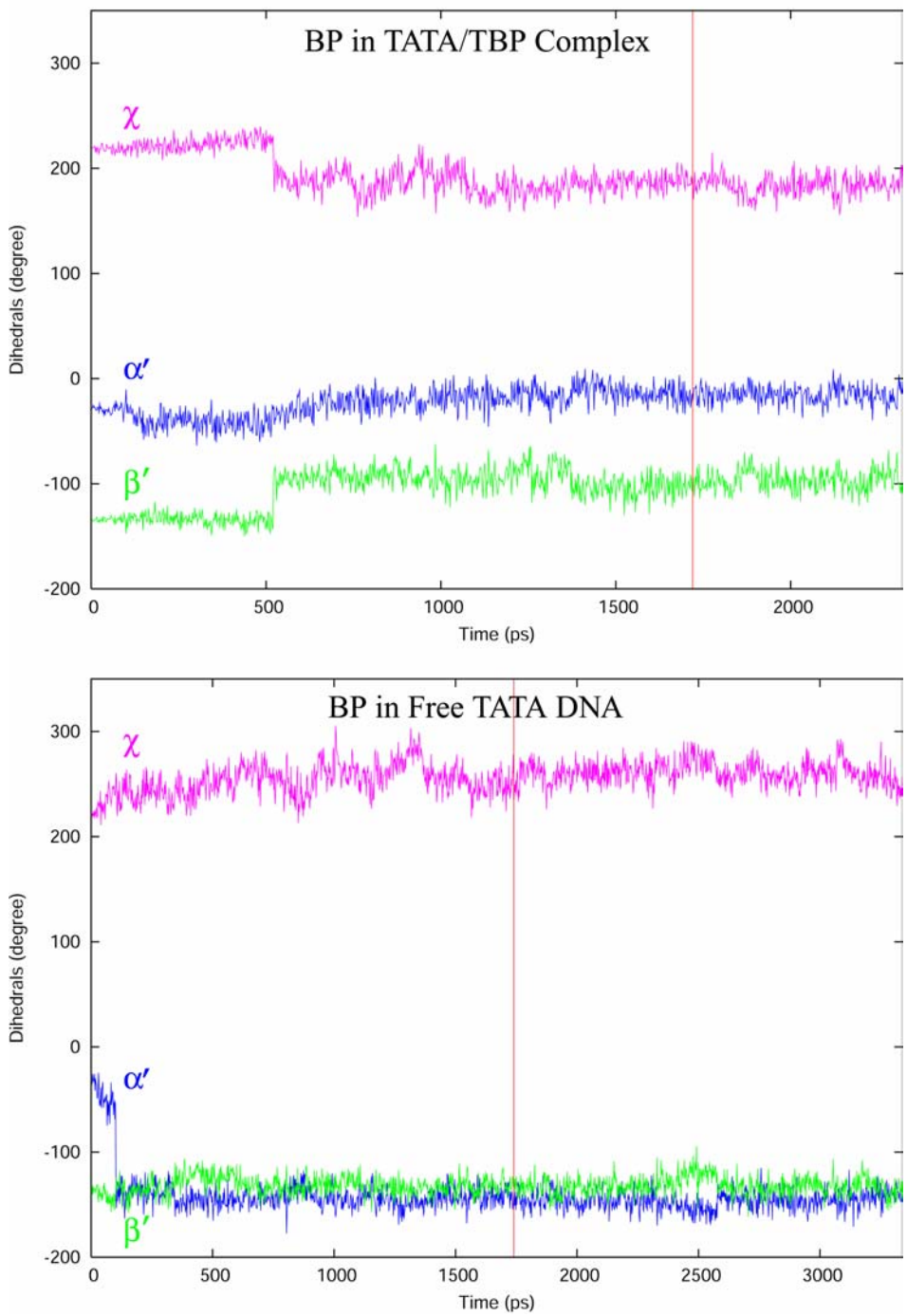


Figure 1: Torsions of benzo[a]pyrene in 10*S*(+)-*trans*-[A_1]: II as a function of simulation time. The torsions are χ (O4′−C1′−N9−C4), α' (N1−C6−N6−C10(BP)), β' (C6−N6−C10(BP)−C9(BP)) are shown in Figure 1 of the manuscript. To the right of the vertical red line is the trajectory for structural and thermodynamic analyses. Top: benzo[a]pyrene in the TATA/TBP complex. Bottom: benzo[a]pyrene in the free TATA DNA.

Appendix S4: Computing Standard Gibbs Energy Changes (Binding Free Energies) From Experimental Data (EMSA)

To compute the standard Gibbs energy (free energy) change $\Delta G°$, we have to find the equilibrium constant $K°$, which can be calculated from the activities of reactants and products at equilibrium state.

1. Defining standard Gibbs energy change and equilibrium constant

We have

$$\Delta G^o = -RT \ln K^o, \tag{1}$$

$$K^o = \prod_i (a_i)^{\upsilon_i} = \prod_i (\gamma_i x_i)^{\upsilon_i}, \tag{2}$$

where $\Delta G°$ is the standard Gibbs energy change (J); $K°$ is the equilibrium constant; R is the gas constant (8.3145J/mol·K); T is the absolute temperature (K); a_i, γ_i, x_i, and υ_i are the activity, activity coefficient, mole fraction, and stoichiometric coefficient of solute i, respectively.

In our project, we are concerned with the reaction: DNA + TBP ↔ DNA/TBP, the latter is monomeric DNA/TBP complex. Because the initial molar concentrations in reaction solution are 1 nM for DNA and 0-70 nM for TBP, this solution is dilute and the activity coefficients γ of DNA, TBP, and monomeric DNA/TBP complex are all close to 1. The stoichiometric coefficients υ of DNA, TBP, and monomeric DNA/TBP complex are 1. Thus, the equilibrium constant for this reaction is

$$K^o = \frac{x(monomericDNA/TBPcomplex)}{x(freeDNA) \cdot x(freeTBP)}. \tag{3}$$

If we use the molar concentration scale for the activities of the solutes, we will have

$$K^o = \frac{[monomericDNA/TBPcomplex]/c^o}{([freeDNA)]/c^o) \cdot ([freeTBP]/c^o)} = \frac{[monomericDNA/TBPcomplex] \times c^o}{[freeDNA] \cdot [freeTBP]}, \tag{4}$$

where $[i]$ represents the molar concentration (M) of solute i, $c°$ is the standard molar concentration (1 M).

2. Calculating standard Gibbs energy change and equilibrium constant

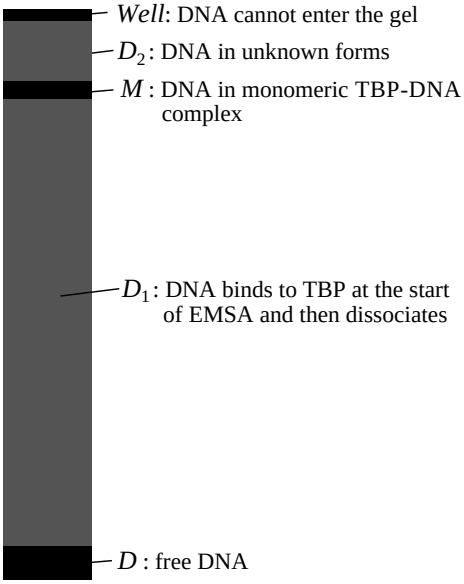


Figure 1: One lane of gel after EMSA.

To input the molar concentrations into Equation 4, we must interpret the electrophoretic mobility shift assays (EMSA) in Rechkoblit et al. 2001. Figure 1 shows one lane of a gel after EMSA.

The *Well*, D_2, M, D_1, D reflect DNA in different forms. We use *italic* font to represent these symbols as the molar concentrations. Our calculation will be based on the Table 2 in Rechkoblit et al. 2001 and the analysis of Figure 1 here. Table 2 in Rechkoblit et al. 2001 gives the apparent dissociation constants K_d (i.e., the initial molar concentration of TBP at which totally 50% DNA is bound to TBP in different forms) and the fractions of DNA in the monomeric DNA/TBP complex form corresponding to K_d.

3. **Approximations**

Since the information in the gel is complicated, we make two approximations:

1) $Well = 0$. When 50% DNA is bound to TBP, the band of *Well* is weak in each gel (see Figure 4 of Rechkoblit et al., 2001), so the amount of DNA left in the *Well* can be neglected;
2) $D_2 = 0$. D_2 part is also weak and its length is much shorter than D_1, so the initial molar concentration of DNA will be $D_{initial} = M + D + D_1 = 1$ nM.

Based on these two approximations, we can see that DNA has only two forms in the gel (excluding *Well*): free DNA and DNA in monomeric DNA/TBP complex. Since $Well = 0$ and $D_2 = 0$, we only have to consider what form of DNA is in D_1 part. The DNA in D_1 has larger mobility than monomeric DNA/TBP complex (M) and smaller mobility than free DNA (D), so D_1 has the DNA that is initially bound to TBP at the start of MESA and then dissociates during the experiment.

Based on these two approximations, it is also clear that TBP binding with DNA exists only in monomeric DNA/TBP complex. TBP dimer does not exist in the gel or in the "Well" because it dissociates during a 30 min incubation (described in "*Formation of TBP/Unmodified TATA DNA Complexes*" part of "RESULTS" section of Rechkoblit et al. 2001. So the rest of TBP exists in free form.

4. **Assumptions**

D_1 has high density in the gel, so we have to decide which part (free DNA or DNA in monomeric DNA/TBP complex) D_1 belongs to.

1) Assumption 1: D_1 belongs to free DNA, if the solution environment is the gel buffer (see "MATERIALS AND METHODS" section of Rechkoblit et al. 2001). Then $[freeDNA] = D + D_1 = D_{initial} - M = 1\text{ nM} - M$, $[monomericDNA/TBPcomplex] = M$, $[freeTBP] = TBP_{initial} - M = K_d - M$. Because 1 nM = 10^{-9} M, we have

$$K^o = K_1^o = \frac{M \times c^o}{(K_d - M)(1\times 10^{-9} - M)}; \tag{5}$$

Or

2) Assumption 2: D_1 belongs to the DNA in monomeric DNA/TBP complex, if the solution environment is the reaction buffer (see "MATERIALS AND METHODS" section of Rechkoblit et al. 2001). Then $[monomericDNA/TBPcomplex] = 50\%D_{initial} = 0.5$ nM, $[freeDNA] = 50\%D_{initial} = 0.5$ nM, $[freeTBP] = TBP_{initial} - M = K_d - 0.5$ nM. Because 1 nM = 10^{-9} M, we have

$$K^o = K_2^o = \frac{0.5\times 10^{-9} \times c^o}{(K_d - 0.5\times 10^{-9})\times 0.5\times 10^{-9}} = \frac{1}{K_d - 0.5\times 10^{-9}}. \tag{6}$$

5. **Results**

The results are shown in Table 1 following. We use K_2^o and ΔG_2^o in this work because Assumption 2 is more reasonable.

Table 1: Equilibrium Constants *K*° and Standard Gibbs Energy Changes Δ*G*°

DNA duplex	K_d	f_{DNA}	K_1^o	ΔG_1^o	K_2^o	ΔG_2^o
unmodified	9±2	14±3	1.84×10^7	−9.21	1.18×10^8	−10.23
10*S*(+)-*trans*-[A_1]	8±2	22±4	3.63×10^7	−9.59	1.33×10^8	−10.30
10*R*(−)-*trans*-[A_1]	6±1	65±13	3.47×10^8	−10.83	1.82×10^8	−10.47
10*S*(+)-*trans*-[A_2]	18±3	ND	/	/	5.71×10^7	−9.84
10*R*(−)-*trans*-[A_2]	13±3	6±1	4.93×10^6	−8.49	8.00×10^7	−10.02

K_d is the apparent dissociation constant evaluated by finding the TBP concentration at which 50% of the DNA is bound. f_{DNA} is the fraction of DNA in monomeric DNA/TBP complex when 50% of the DNA is bound ($M = f_{DNA}\cdot D_{initial}$). K_1^o and ΔG_1^o refer to the equilibrium constant and standard Gibbs energy change in Assumption 1 (D_1 in Figure 1 belongs to free DNA), while K_2^o and ΔG_2^o in Assumption 2 (D_1 belongs to DNA in monomeric DNA/TBP complex). ND means nondetected. ΔG_1^o and ΔG_2^o are calculated using Equation 1, where the absolute temperature T = 273.15 + 4.0 = 277.15 (K), the temperature in both the reaction buffer and gel buffer. K_d are in unit of nM, f_{DNA} in %, ΔG_1^o and ΔG_2^o in kcal/mol.

Reference:

- Rechkoblit O, Krzeminsky J, Amin S, Jernstrom B, Louneva N, Geacintov NE 2001 Influence of bulky polynuclear carcinogen lesions in a TATA promoter sequence on TATA binding protein-DNA complex formation. Biochemistry 40:5622-5632.

Appendix S5: Local Molecular Mechanics Interaction Analysis

Comprehensive binding free energy analyses are computationally expensive. Here we compute local molecular mechanics interaction energies ΔE_{MM} to see whether we could easily interpret the experimental binding affinities. The local region is defined by 32 residues: 3-9, 22-28, 40-41, 99, 133-135, 161-163, 166, 168, 177, 179, 183-187, where residue numbers 1-32 correspond to the DNA and 33-211 to TBP. These residues are within 5 Å from the A_1 and A_2 BP-binding sites and were selected based on the trajectories of all 12 systems. Table 1 shows the electrostatic interaction energies ΔE_{ele}, van der Waals interaction energies ΔE_{vdw}, and molecular mechanics interaction energies ΔE_{MM} between TBP and TATA DNA, as well as the molecular mechanics energies E_{MM} of the 12 TBP/DNA complexes. All energies are computed in the local region. The internal interaction energies ΔE_{int} are zero and not shown because there are no covalent bonds between TBP and TATA DNA. $\Delta E_{MM} = \Delta E_{int} + \Delta E_{vdw} + \Delta E_{ele}$.

We can see that ΔE_{MM} is dominated by ΔE_{ele} because both TBP and TATA DNA are highly charged. ΔE_{MM} differs widely, from −1068 to −1190 kcal/mol, with standard deviations around 15 to 36 kcal/mol. E_{MM} differs from −44 to −200 kcal/mol with standard deviations around 26 to 49 kcal/mol. Because the differences in both ΔE_{MM} and E_{MM} are larger than their standard deviations, we cannot interpret the experimental binding free energy differences of <1 kcal/mol. Clearly, local molecular mechanics interaction analysis is insufficient to interpret global binding affinities.

Table 1: Local molecular mechanics interaction energies

system	ΔE_{ele}	ΔE_{vdw}	ΔE_{MM}	E_{MM}
unmodified	−1063±32	−79±4	−1142±32	−151±49
10*S*(+)-*trans*-[A_1]: II	−1096±16	−77±3	−1173±15	−96±31
III	−1005±21	−77±3	−1082±22	−84±37
10*R*(−)-*trans*-[A_1]: II	−1022±20	−77±3	−1099±21	−61±39
III	−1059±27	−79±3	−1138±27	−142±49
IV	−1046±28	−75±4	−1121±29	−61±49
10*S*(+)-*trans*-[A_2]: III	−1058±21	−75±4	−1133±21	−132±35
III-2	−1062±28	−79±3	−1141±27	−200±43
IV	−1054±28	−78±3	−1133±29	−152±26
10*R*(−)-*trans*-[A_2]: I	−1116±35	−74±3	−1190±36	−136±41
III	−994±16	−74±4	−1068±17	−44±35
IV	−1058±20	−87±4	−1145±21	−115±38

ΔE_{ele}, ΔE_{vdw}, and ΔE_{MM} are the electrostatic interaction energy, van der Waals interaction energy, and molecular mechanics interaction energy between TBP and TATA DNA, respectively. E_{MM} is the molecular mechanics energy of the complexes. Internal interaction energy E_{int} (not shown) is zero for all systems because there are no covalent bonds between TBP and TATA DNA. ΔE_{MM} and E_{MM} are shown in both average and standard deviation. Underlined are the lowest energy in each of the four combinations of carcinogen location (A_1 or A_2) and stereochemistry (10*S*(+) or 10*R*(−)). All energies have the unit of kcal/mol and are shown in both average and standard deviations. The standard deviation of ΔE_{MM} is not necessarily the exact accumulation of the standard deviations of ΔE_{ele} and ΔE_{vdw}, because part of the fluctuations of these two terms is cancelled out.

Appendix S6: Stability of Free Energies of Unbound TBP and TATA DNA

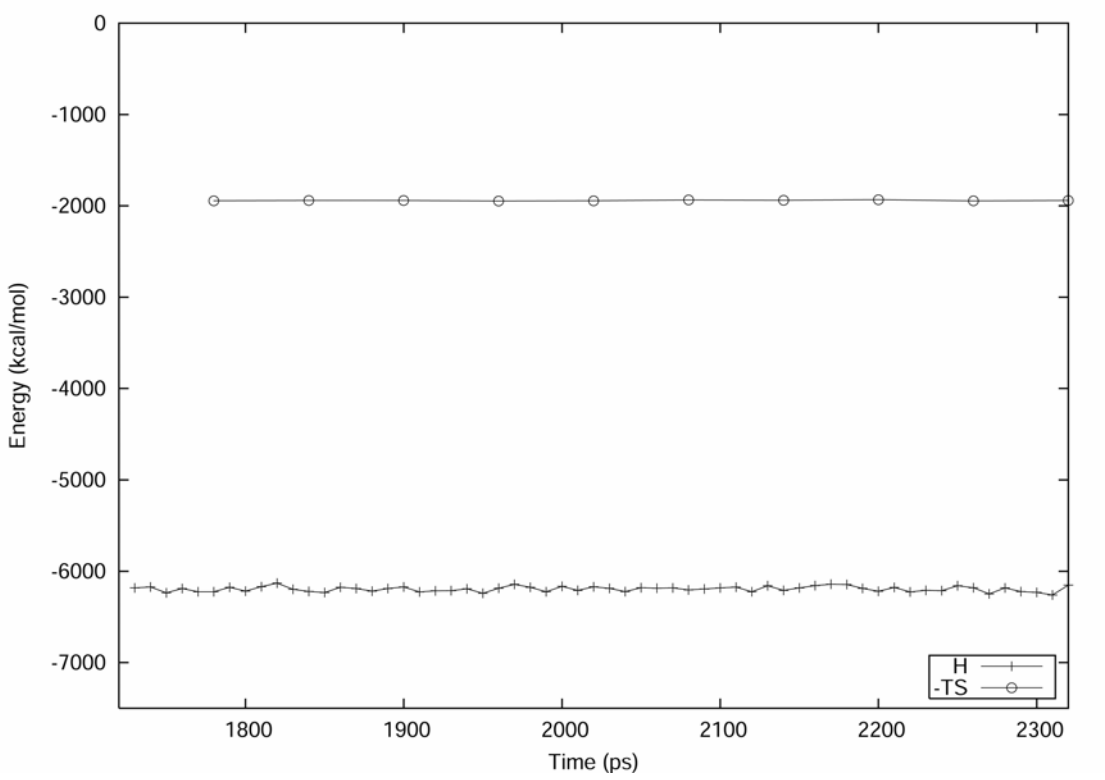


Figure 1: Free energy (enthalpy term H and entropy term –TS) of TBP a function of simulation time between 1720 and 2320 ps, in which free energy is computed.

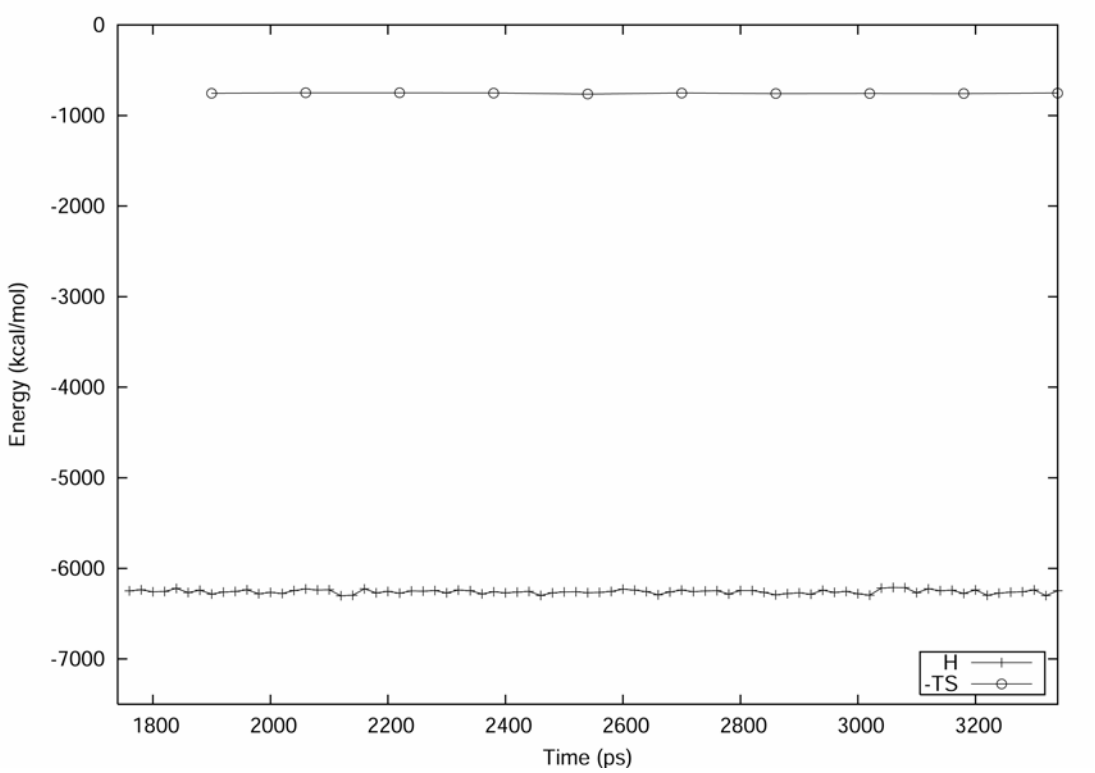


Figure 2: Free energy (enthalpy term H and entropy term –TS) of unmodified TATA DNA as a function of simulation time between 1740 and 3340 ps, in which free energy is computed.

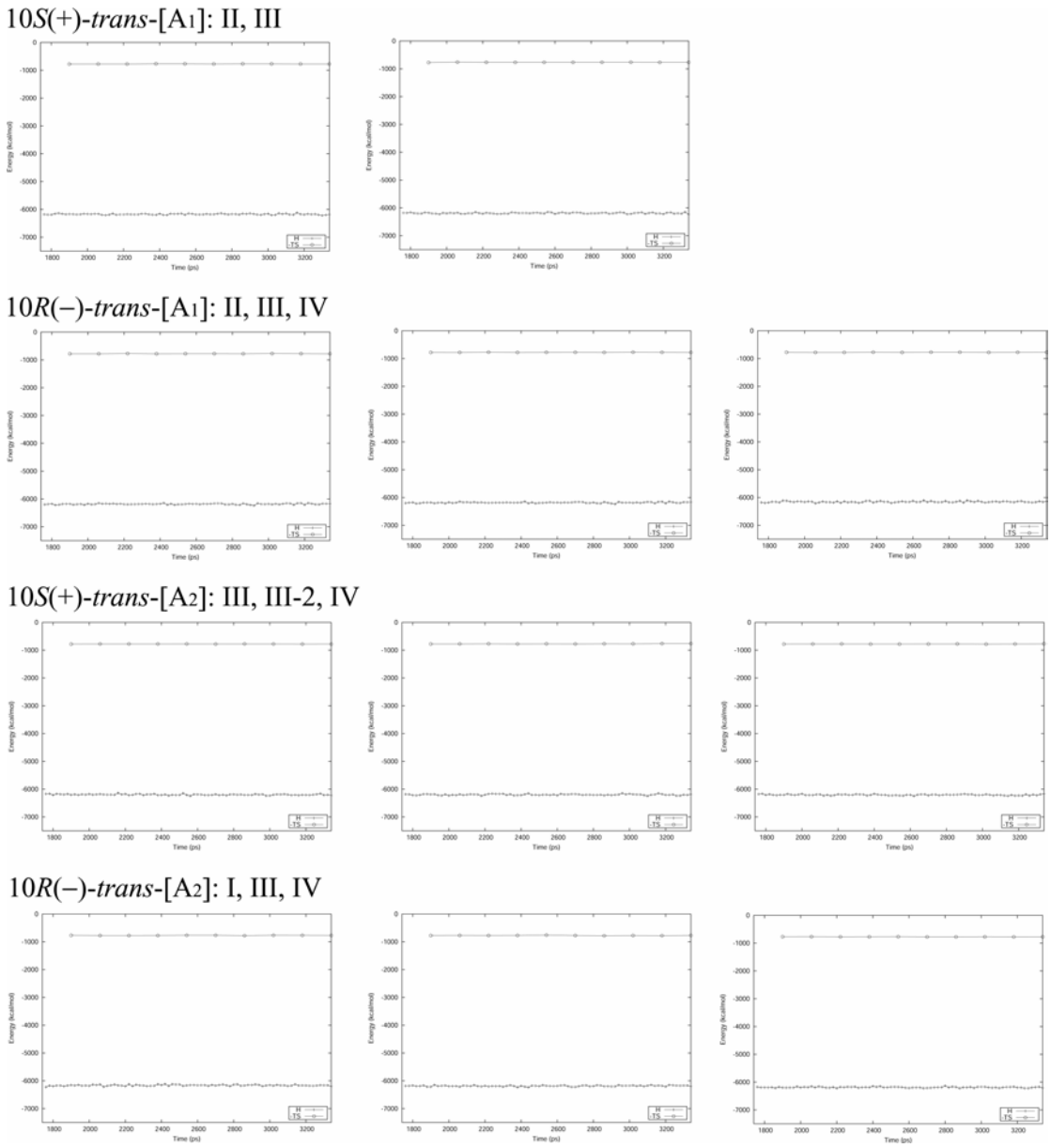


Figure 3: Free energies (enthalpy term H and entropy term –TS) of BP-modified TATA DNA as a function of simulation time between 1740 and 3340 ps, in which free energies are computed.