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Supporting Information

Molecular Mechanism and Binding Free Energy of Doxorubicin intercalation in DNA

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I. Binding Free Energy Computational Methods

We have applied *Molecular Mechanics Poisson–Boltzmann Surface Area* (MM-PBSA) and *Molecular Mechanics Generalized-Born surface area* (MM-GBSA) continuum solvation methods to calculate the binding free energy (BFE) of DOX-DNA intercalation complexes. The free energy can be extracted by three main methods, depending on the system: (1) if the stability of the two conformations of a molecule is required, simulations and free energy calculations should be conducted independently. We used this type of calculations to determine the DNA deformation penalty when DOX intercalates into DNA; (2) The BFE is evaluated as the difference between the free energies of the bound state (complex) and unbound state (separate receptor and ligand) from a single trajectory of the complex ("single-trajectory protocol", STP), or (3) Use "multiple-trajectory protocol" (MTP). Here the simulation involves multiple separate trajectories of the complex, receptor, and/or ligand.[1] In the MM–PBSA or MM-GBSA methods, ΔG_{bind} is obtained according to **Eq. (1)** and their associated equation are presented in the main text (see section computational theory of manuscript).

The polar contribution G_p is calculated with the implicit solvation model, either by solving the Poisson–Boltzmann (PB) equation[2, 3] or alternatively by the Generalized Born[3] (MM-GBSA) approach. The value of the interior dielectric constant is set to 1 while the exterior dielectric constant is set to either 78.3 or 80.[4]

The G_{np} is calculated from the *solvent-accessible surface area* (SASA) using the hard-sphere atomic model. The probe radius of 1.4 Å is used for the solvents.

$$G_{np} = \gamma\Delta_{SASA} + \beta \quad \text{(S1)}$$

Here γ is the surface tension and β is the offset value used to correct for the nonpolar free energy contribution.[4]

If we assume that the biological systems obey a rigid rotor model, the entropic contributions are the sum of translational, rotational and vibrational entropies. The translational and rotational entropies can be calculated using the standard statistical mechanical formulas.[5, 6] For the vibrational entropy, it can be approximated using one of two methods. First, the vibrational frequencies of normal modes can be calculated at various local minima of the potential energy surface. This method is referred as *nmode*.[6, 7] The normal modes are calculated by the diagonalization of the Hessian matrix for highly optimized geometries. Alternatively, the eigenvalues of the mass-weighted covariance matrix constructed from every member of the ensemble can be approximated as frequencies of global, orthogonal motions. This technique is referred to the *quasi-harmonic approximation*.[6, 7] In our study, normal mode analyses were used.
Here, two types of calculations that used by MM-PB(GB)SA are briefly discussed.

a) Single Trajectory Protocol (STP) of MM-PB(GB)SA Calculation

In this approach, all ensembles can be extracted from a single molecular dynamics (MD) trajectory of the bound DOX-DNA complexes that only simulates the complex and creates the average ensemble of the free receptor (DNA) and ligand (DOX). STP is less computationally expensive than MTP, because only a single trajectory is used to generate all three ensembles (DOX-DNA complex, DNA and DOX). In addition, the internal potential terms (e.g., bonds, angles, and dihedrals) cancel out exactly because the conformations in the bound and unbound ensembles are the same, leading to lower fluctuations and easier convergence for BFE.[1, 6] This protocol is mentioned in **Fig. 2 (a)** in the main text.

b) Multiple Trajectory Protocol (MTP) of MM-PB(GB)SA Calculation

In the MTP approach, each ensemble (DOX-DNA complex, free ligand DOX and unbound receptor DNA) can be simulated separately in different MD simulations. This is called the *three trajectories protocol* (3TP).[8] It can also be estimated from only two separated simulations, one from simulations of the complex, and the other from the free ligand or the unbound receptor. This is called the *two trajectories protocol* (2TP). The conformations populating the unbound ensembles typically adopt strained configurations when extracted from the bound state ensemble in STP, thereby overstabilizing the binding, compared to the MTP.[6] Performance of 3TP and STP depended on the test system and solvation model.[9] However, the 3TP approach is also more expensive in terms of computational cost and the standard error is larger.[10] The 3TP is sketched in **Fig. 2 (b).**

In general, STP of MM-PB(GB)SA methods gives more accurate results than the 3TP approach.[11, 12] In our study, we adopted the STP of MM-PB(GB)SA methods to calculate free energy of intercalating DOX-DNA complexes. Further, the 2TP of MM-PB(GB)SA methods can be used to improve the accuracy of the results that calculated by these methods,[13, 14] so in the case of calculating the DNA deformation energy the 2TP approach is adopted.

II. Modeling Procedure

Since our goal is to understand the intercalation process of DOX-DNA complex at molecular level, we constructed different models of the DOX-DNA complex with two 6-base pair dsDNA. The first sequence of dsDNA is d(CG**AT**CG) or DNA1 is selected from the structure resolved by X-ray diffraction (PDB ID:1D12),[15] while the second sequence of dsDNA is d(CG**TA**CG) or DNA2 from another structure resolved by X-ray diffraction (PDB ID:1D11).[16] It should be mentioned that the (1D11) source contains two daunorubicin (DNR) drugs with the DNA2 sequence, so we adopted this structure by replacing DNR with doxorubicin (DOX) to construct the DOX-DNA complex for DNA2. To accentuate the important role played by analyzing energy components of total BFE in the intercalation process of DOX into DNA base pairs, we explicitly targeted three different models for each dsDNA sequence. They are DNA only, DOX-DNA with one DOX (1:1 complex) and DOX-DNA with two DOX (2:1 complex). The first two models are only DNA models from unbounded DNAs with different sequences of dsDNA obtained separately from two crystal structures (1D12) and (1D11), respectively. These two models were used as a reference model for DNA1 and DNA2. The second two models are 1:1 complexes constructed from only one DOX molecule that intercalated into DNA of each sequence. The final two models are 2:1 complexes created from two DOX molecules within dsDNA sequences in DNA1 or DNA2. For all models, nucleotides on strand 1 are labelled Cl to G6 in the 5' to 3' direction and C7 to G12 in the 5' to 3'direction on symmetry-related strand 2. The DOX molecule is numbered DOX1 in 1:1 complex models, and DOX1 and DOX2 in 2:1 complex models as shown in **Fig. S1**. Each of these six structural models (M1, M2, M3, M4, M5, and M6) are appropriately solvated with water molecules and described below.

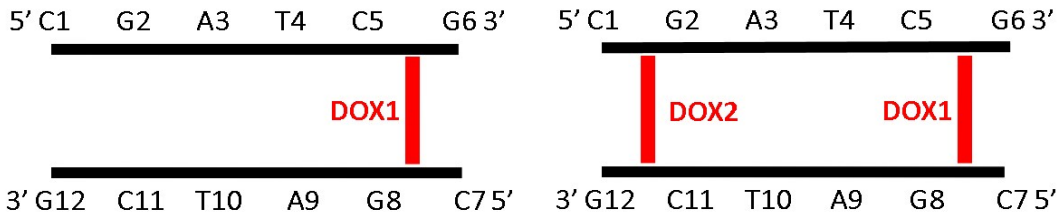


Fig. S1. The nucleotide complementary bases of DNA d(CG**AT**CG) or DNA1 and the intercalated sites of DOX drug.

Models:

Firstly, we take the isolated double stranded DNA1 and DNA2 structure by removing the DOX or DNR molecule and all the other bathing solution molecules from the crystal structure of PDB ID: 1D12 and 1D11, respectively. In order to solvate the DNAs, we have inserted the isolated dsDNAs into the water box of 10 Å in each direction with periodic boundary conditions by using the TIP3P model[17] in AMBER package.[18] Water box is maintained to be cubic which having dimensions of 52× 52×52 $Å^3$ by specifying a list of numbers to the solvateBox command through the LEaP[18] module from AMBER 11. The dsDNAs fragment has six base pairs with total charge of −10e. 10 Na^+ ions are added as counter ions to neutralize the system and called model M1 and M2 for DNA1 and DNA2 respectively.

Secondly, the intercalated structure of DOX-DNA1 complex is taken from PDB ID 1D12. In this case, we considered only dsDNA molecules with one protonated DOX molecule (1:1 complex), that has a molecular structure of (C27H30NO11, 69 atoms), by again removing all the other molecules to form this DOX-DNA1 complex. For solvation of DOX-DNA complex, we have used the same approach as in DNA only models.

For charge compensation, we have added 9 Na^+ ions in and this model is called M3. Similarly, the intercalated DOX-DNA2 complex, we have started from original source data 1D11. We should mention here that the crystal structure in initial data contains DNR instead of DOX. In this case, we replaced DNR molecule with just one protonated DOX molecule that was in 1D12. After this, all the other bathing solution molecules were removed then we have solvated according to same procedure as in previous models. The same number of Na^+ counter ions (9 Na^+ ions) as in M3 was used to neutralize the system and called model M4.

Thirdly, the DOX-DNAs complex with two DOX molecules (2:1 complexes) is constructed according to exactly same procedure applying in 1:1 complex just differ is we inserted two DOX molecules instead of one DOX molecules in DNAs. There are two protonated DOX molecules so only 8 Na^+ ions are needed as counter ions to neutralize the systems and are called model M5 and M6 for DNA1 and DNA2 respectively. The illustration of these six models are shown in **Fig. S2**. The summary of all six models is tabulated in **Table 1**. The specific purposes of using these six models are: first, to analyze the contributions to BFE of various DOX-DNA intercalating complexes though MM-PB(GB)SA methods. Second, to ascertain the changes rooted from two different sequences of dsDNA in M3 to M6 on how they can affect the BFE of DOX-DNA. Third, by comparing between M1 with M3 and M5 or M2 with M4 and M6 models to see the DNA conformational changes when DOX intercalate with DNA. We can also calculate the penalty of deforming DNA. Finally, to see how the BFE can be affected if we have two DOX molecules with different DNA sequences, or the 2:1 complex.

III. Computational Methods

The value of computational approaches using MD for investigating studies of biomolecules such as DNA and drugs-DNA complexes for a deeper understanding on the structural, dynamical and energetic properties of biomolecular systems have already been amply demonstrated.[19-21] In the following, we provide the detailed descriptions of the calculations methodology and the results obtained as applied to the six models described above.

1. Doxorubicin Preparation

As mentioned earlier, a free DOX molecule was taken from crystal structure (PDB ID:1D12) as in models M3 and M4, as well as for the two DOX molecules in M5 and M6. The molecular mechanics (MM) parameters for free DOX drug are obtained following the standard AMBER 11 protocol.[22] In particular, the electrostatic potential of DOX was obtained after geometry optimization using Gaussian 09 at the HF/6-31G* level.[23] The partial charges are obtained by fitting the electrostatic potential using the RESP (Restrained Electrostatic Potential) method[24] in the R.E.D. server.[25, 26] They are listed in **Table S1**. Other parameters of DOX drug are taken from the AMBER GAFF[27] parameter set.

2. Complexes of DOX-DNA Preparation

DNA sequence plays an important role in the binding of a ligand to DNA strands. As already described earlier, the DOX-DNA intercalating complexes are constructed from the different dsDNA sequences with one DOX molecule, 1:1 complexes (M3 and M4), and with two DOX molecules, 2:1 complexes (M5 and M6). The parameters for DNA were generated using parmbsc0 force field[28] with periodic boundary conditions. As mentioned earlier, all six models were solvated in a rectangular box of TIP3P water molecules of 10 Å in each direction and by reducing the water box to be cubic which having dimensions of 52× 52×52 $Å^3$ by specifying a list of numbers for the solvateBox command through the LEaP module

from AMBER 11. To neutralize the solvated systems, counter ions of Na^+ were randomly placed on grid points that had the largest positive Columbic potential around the molecule.

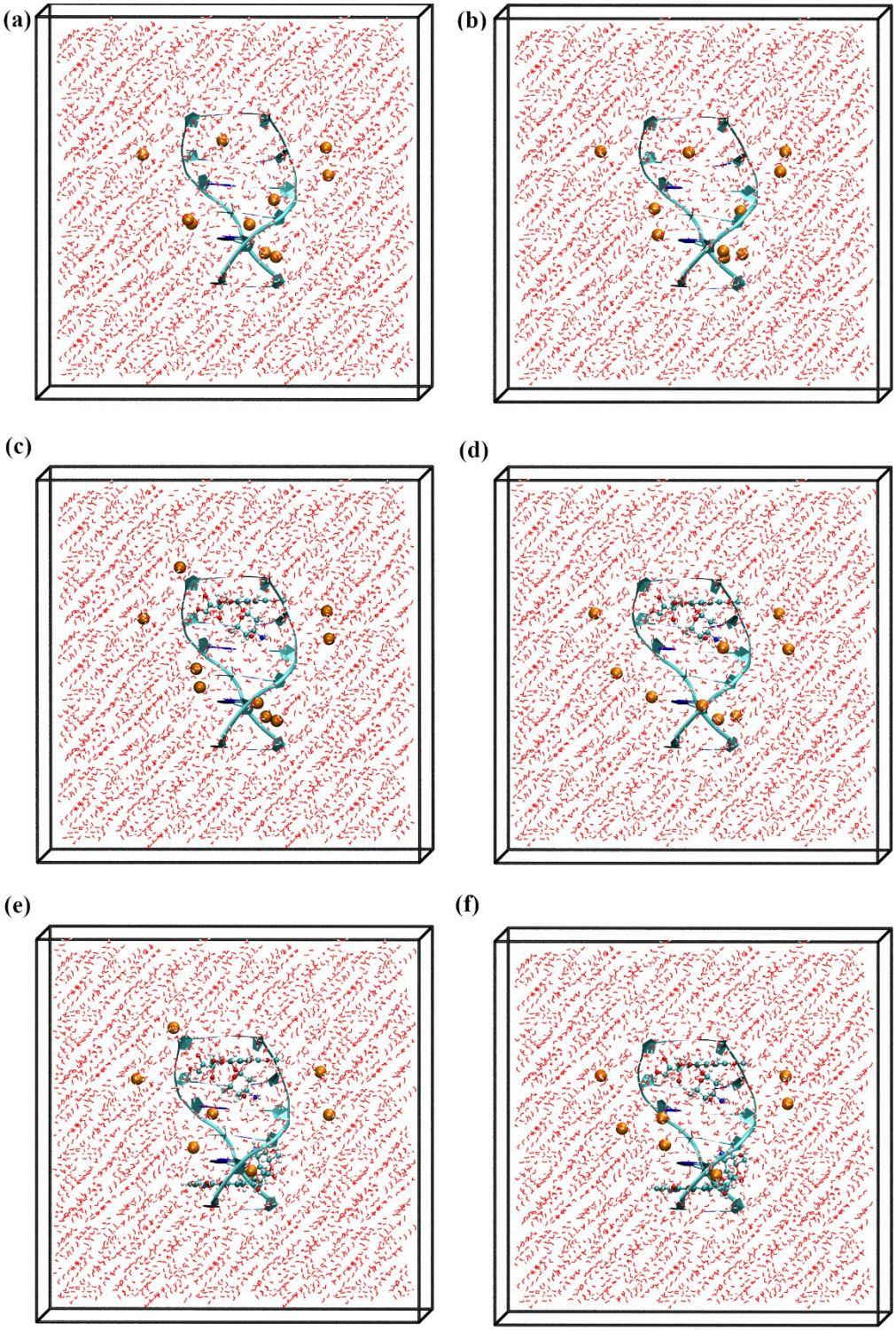


Fig. S2: The structure of simulations (a): Model 1; (b): Model 2; (c): Model 3; (d) Model 4 (e) Model 5; and (f) Model 6.

Table S1: Atom types used for DOX and RESP atomic charges that calculated by R.E.D server.

Atom Number	Atom Type	RESP Charge	Atom Number	Atom Type	RESP Charge	Atom Number	Atom Type	RESP Charge
1	C1	-0.0976	24	C17	0.3361	47	H12	0.499
2	C2	-0.1718	25	O17	-0.5944	48	H142	0.07
3	C3	-0.1318	26	C18	-0.3139	49	H143	0.07
4	C4	0.2316	27	C19	0.6228	50	HO14	0.4496
5	O4	-0.245	28	O19	-0.5217	51	H152	0.0811
6	C5	-0.102	29	C20	-0.125	52	H153	0.0811
7	C6	0.5709	30	C21	-0.0755	53	H17	0.4733
8	O6	-0.5788	31	C1'	0.0867	54	H211	0.0938
9	C7	-0.1253	32	C2'	-0.0591	55	H212	0.0938
10	C8	0.0807	33	C3'	0.0644	56	H213	0.0938
11	O8	-0.444	34	N3'	-0.4467	57	H1'	0.161
12	C9	-0.018	35	C4'	0.0355	58	H2'	0.0776
13	C10	0.0403	36	O4'	-0.6283	59	H2''	0.0776
14	O10	-0.3547	37	C5'	0.1345	60	H3'	0.1255
15	C11	-0.1272	38	O5'	-0.3166	61	HN31	0.3485
16	C12	0.1239	39	C6'	-0.1304	62	HN32	0.3485
17	O12	-0.7507	40	H1	0.1515	63	HN33	0.3485
18	C13	0.627	41	H2	0.1854	64	H4'	0.1058
19	O13	-0.5056	42	H3	0.136	65	HO4'	0.4671
20	C14	0.0789	43	H8	0.4155	66	H5'	0.0798
21	O14	-0.6771	44	H10	0.1321	67	H6'1	0.0634
22	C15	-0.014	45	H112	0.0789	68	H6'2	0.0634
23	C16	0.008	46	H113	0.0789	69	H6'3	0.0634

3. Molecular Dynamics (MD) Simulations

All MD simulations for the 6 models are performed using the AMBER 11 simulation package in explicit solvent with periodic boundaries. Prior to MD simulations, we use two-stage approaches for minimization our models using the *Particle Mesh Ewald* (PME)[29] potential function. These minimization steps are necessary to remove possible steric clashes and adapt the systems to the chosen force field. The first stage kept the solute (DOX-DNA complex or DNA) fixed with a force constant of 500 kcal/mol-Å^2 and just optimize the positions of the water and ions, necessary to get the water and ions properly randomized. In this step, each one of the six models were first minimized for 5000 steps with steepest descent, followed by 5000 steps of conjugate gradient while keep the positions of solute fixed. In the second stage of minimization, the entire system is minimized without the restraints for additional 10000 cycles (steepest descent 5000 steps, followed by 5000 steps for conjugate gradient). After minimization, the next stage is to gradually heat our system from 0 K to 310 K for 310 picoseconds (ps) using the NVT ensemble with a

10 kcal/mol-Å^2 weak restraint on the solute (complex or DNA). Then, 0.5 ns without restraint of constant pressure of 1 bar and temperature 310 K (NPT) to allow the system to reach the proper density. The systems were then equilibrated over 3 ns using NPT ensemble through six multiple simulations, the length of each one is 0.5 ns. The following settings were activated in all of the equilibration MD simulations: Langevin dynamics for temperature scaling, 2 ps as the pressure relaxation time, long-range electrostatic interactions were calculated with the *Particle-Mesh Ewald method* (PME), both the direct space PME and Lennard-Jones cutoffs were set at 10 Å, the SHAKE algorithm[30] was used to constraint bond length of hydrogen atoms to avoid high-frequency motions involving hydrogen atoms, and 1 fs time step. All the minimizations and equilibration steps were conducted using the SANDER module of the AMBER 11. Finally, 30 ns NPT production run with 30 multiple MD runs was performed at constant pressure (1 bar) and temperature (310 K). The length of each independent MD production run is 1 ns. During the production run, the atomic coordinates from trajectories were saved every 2 ps for subsequent MM-PB(GB)SA analyses. All settings that were activated in the equilibration MD simulations were kept during the production run except 2 fs time step is used instead of 1 fs time step. The PMEMD program in AMBER11 was used for production MD simulations.

4. Principal Component Analysis (PCA) of our MD Trajectory

PCA is a standard mathematical tool that can be used to extract large-scale motions occurring in the MD trajectory, providing a brief picture of the underlying structure of atomic fluctuations by applying the dimensionality reduction method. This technique is based on the determination of a new set of collective coordinates called the principal components (PCs) or “modes” through a linear transformation of the atomic coordinates. The PCs are described as the eigenvectors of the atomic displacement covariance matrix. They represent a correlated motion of a number of atoms in a 3-dimensional space, and the corresponding eigenvalues indicate the extent of the total motion occurring in each direction. Usually, the first few principal components (PCs) are sufficient to describe the most important slow modes of the total motion observed during the dynamic, which are related to the functional motions of a biomolecular system.[31] PCA has been widely used to study the intrinsic motions of various biomolecular systems such as nucleic acids[32] and proteins.[33]

In the present study, PCA was performed with R software using the bio3D package.[34] We applied PCA to the heavy atoms of the DNA and DOX in order to investigate and compare the functional motions of DNA-free and DNA-bound complex. Our PC analysis based on Cartesian coordinates. The trajectory snapshots were extracted from MD production runs for whole 30 ns, so we have 15000 snapshots for each model. The total number of atoms that included in PCA analysis is various in three different situations of each DNA sequence (free DNA, 1:1 complex and 2:1 complex). A total of 240 atoms were included in the analysis of free DNA in both sequences, resulting in 720 PCs. In 1:1 complex case for both sequences, a total of 279 atoms were adopted in the analysis, resulting in 837 PCs. While in 2:1 complex case, 318 atoms were included in the analysis, resulting in 954 PCs.

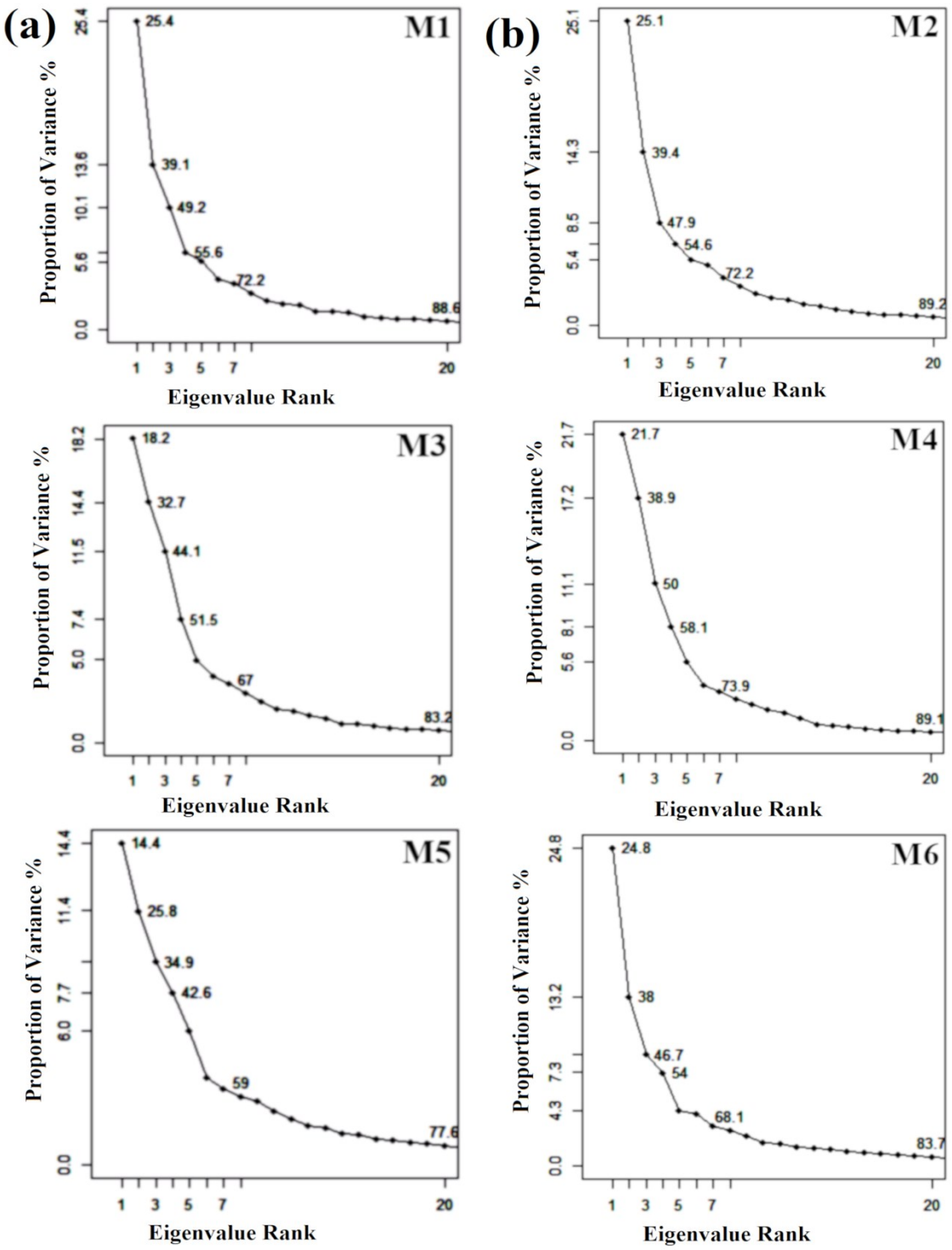


Fig. S3: Scree plot for principal component analysis on the MD coordinate data of three different environments (free DNA, 1:1 complex, 2:1 complex) of (a) three situations of DNA1 sequence and (b) three situations of DNA2 sequence. The magnitude of each eigenvalue is expressed as the proportion of the total variance (mean-square fluctuation) captured by the corresponding eigenvector. Labels on each point indicate the cumulative sum of variance accounted for by a particular eigenvector and its preceding eigenvectors.

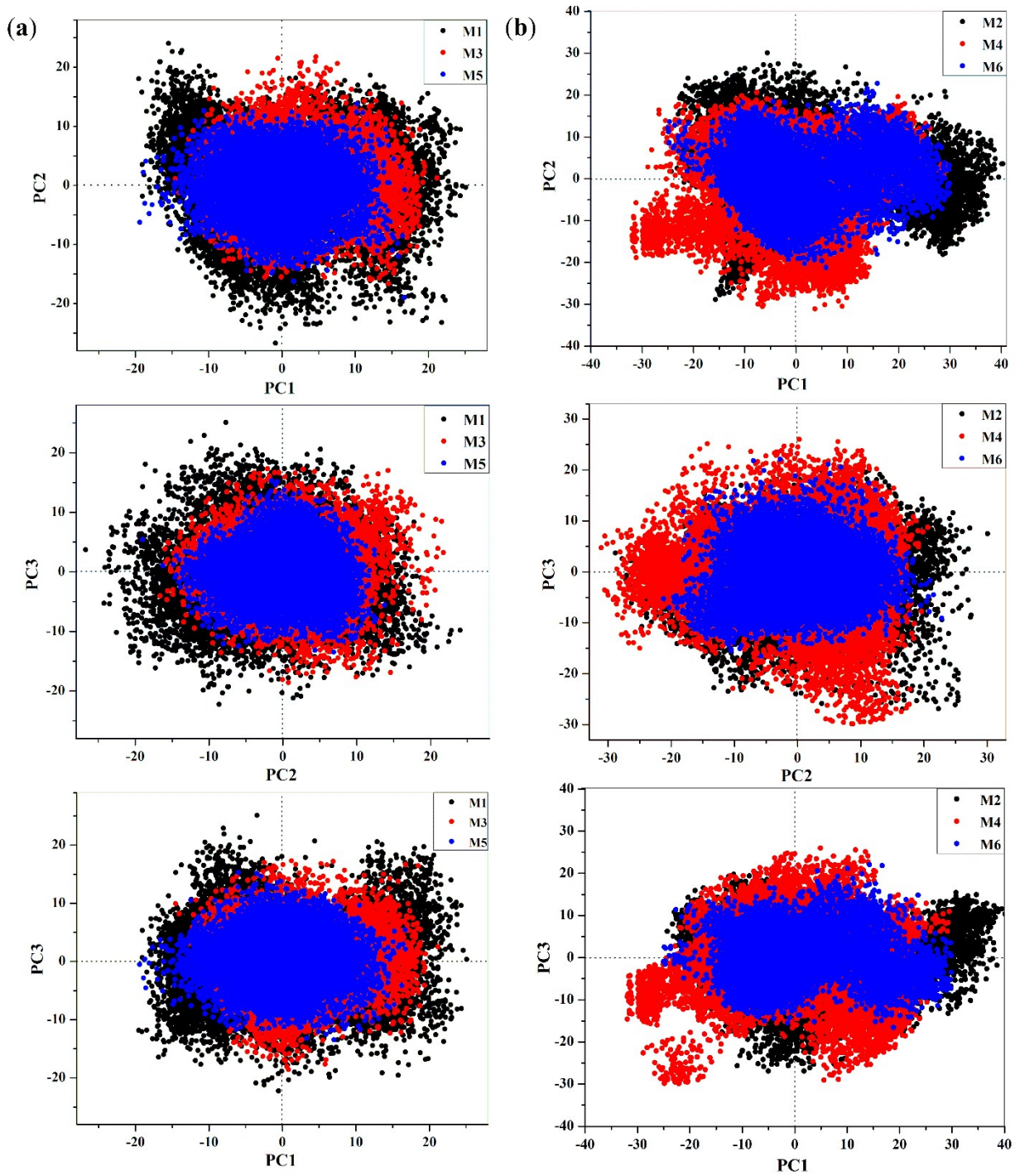


Fig. S4: Projection of MD simulations with three different environments (free DNA which represents by black circle , 1:1 complex which represents by red circle, and 2:1 complex which represents by blue circle) onto the corresponding first, second and third PC modes from the principal component analysis (PCA) of (a) the three situations of DNA1 sequence (M1 is free DNA, M3 is 1:1 complex and M5 is 2:1 complex), (b) the three situations of DNA2 sequence (M2 is free DNA, M4 is 1:1 complex and M6 is 2:1 complex).

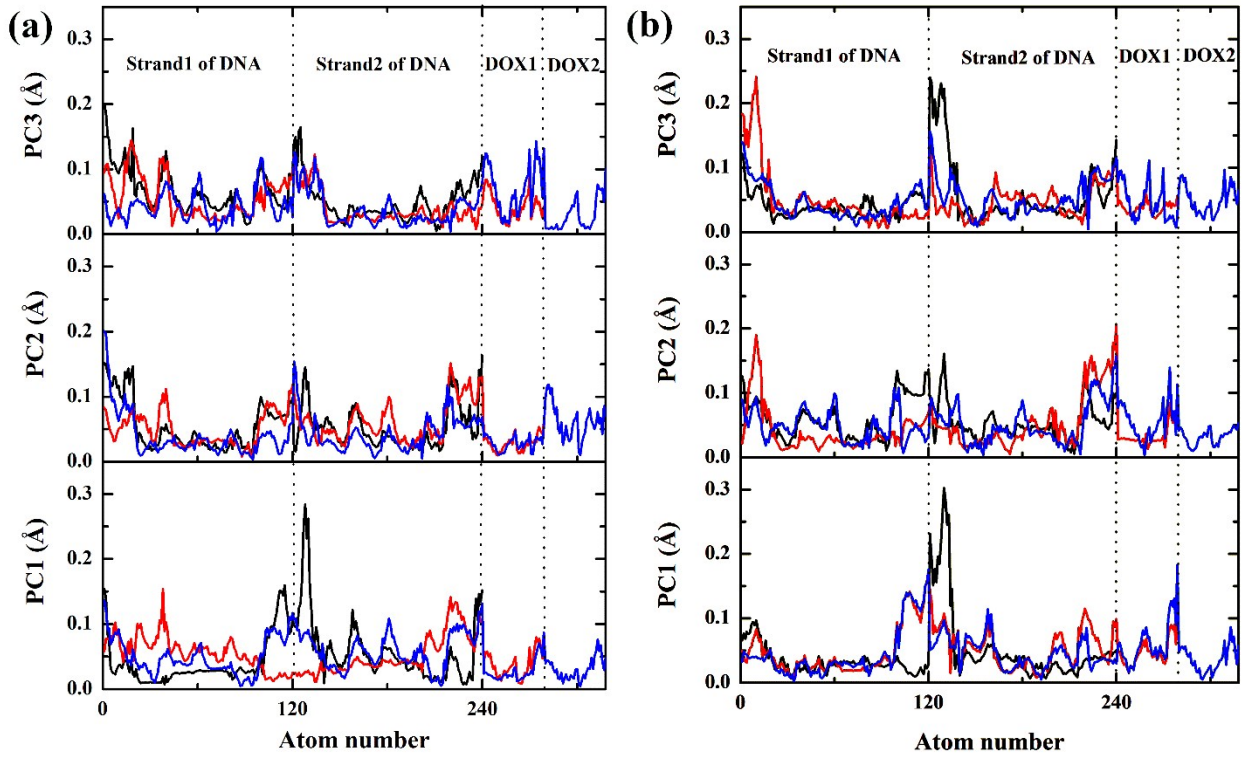


Fig. S5: Residue-wise loadings for the first three principal components of three different environments (free DNA which represents by black line, 1:1 complex which represents by red line, and 2:1 complex which represents by blue line) for (a) the three situations of DNA1 sequence (M1 is free DNA, M3 is 1:1 complex and M5 is 2:1 complex), (b) the three situations of DNA2 sequence (M2 is free DNA, M4 is 1:1 complex and M6 is 2:1 complex). The dashed lines are used to separate DNA's and DOX's atom numbers.

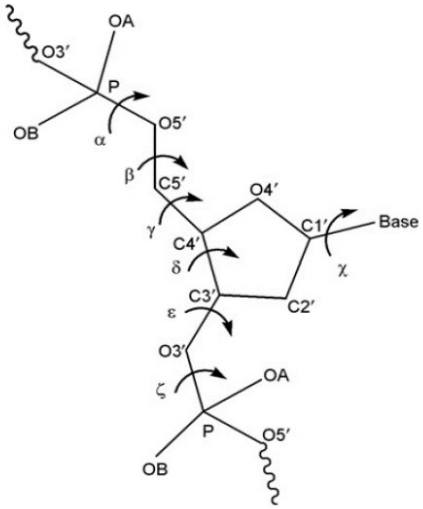
5. Geometrical Parameters for Distinguishing DNA Conformation

There are various parameters to describe helical geometry of the dsDNA including six local base-pair parameters, six local base pair-step parameters and four local base pair-axis parameters in a Cartesian coordinate system (X: short axis of paired base plane, Y: the long axis of paired base plane and Z: the DNA helix direction). The six base pair parameters are defined as the deviation of one base with respect to its paired one. Three of them are shear, stretch and stagger which describe the translational deviations from ideal base pair geometry. The other three are buckle, propeller and opening which defined as rotational deviations from ideal base pair geometry with respect to X, Y and Z axis respectively. The deviation of two successive base pairs from their ideal geometries with respect to each other is called the base pair-step parameters that include translational deviations (Shift, Slide and Rise) and rotational deviations (Tilt, Roll and Twist). The four local base pair-axis parameters (X-displacement, Y-displacement, inclination, and tip) depict the position and orientation of a base-pair relative to the helical axis, defined here by the repetition of a two-base-pair unit. Because we expect that there are specific changes in DNA conformation accompany the intercalation, our special interest lies at intercalation sites of DNA. Based on the previous observations from X-ray structures[15, 16, 35, 36] which indicated that there was an increase in the rise parameter of DNA of about 4.1 Å per each intercalation site of drug, and also significant increase in buckle parameter for base pair above and below the intercalator. These two important geometrical parameters rise and twist of the dsDNA were thoroughly analyzed using 3DNA program.[37] Sugar-phosphate backbone

and glycosyl torsion angles as shown in **Fig. S6** and sugar conformations were also tested using 3DNA program.

Fig. S6: The backbone and glyosidic torsion angles in a unit nucleotide.[38] The torsion angles along the backbone of the oligonucleotide in 3DNA program are defined as [37]:

Alpha (α): O3'(i-1)-P-O5'-C5'
Beta (β): P-O5'-C5'-C4'
Gamma (γ): O5'-C5'-C4'-C3'
Delta (δ): C5'-C4'-C3'-O3'
Epsilon (ε): C4'-C3'-O3'-P(i+1)
Zeta (ζ): C3'-O3'-P(i+1)-O5'(i+1)
The glycosyl torsion angle is defined as:
Chi (χ) for pyrimidines (Y): O4'-C1'-N1-C2
Chi (χ) for purines (R): O4'-C1'-N9-C4.
P is the phase angle of pseudorotation of the sugar ring.



For identifying the global structural features, we focus only on the hydrogen bonds (HBs) between the drug and DNA which are identified by using the HBonds plugin in VMD.[39] In general, there would be a HB between an electronegative atom (the donor, D) and another electronegative atom (the acceptor, A). In this study, the HBs are counted as those within the 3.5 Å distance cut-off and 25° angle cut-off. For HBs within DNA base pairings, 3DNA program can be used for the purpose of achieving the Watson-Crick (WC) base pairs which are three HBs for each C-G base pairing and two for each A-T base pairing. Thus, the total number of HBs for this dsDNA hexamer is 16 in the standard B-type DNA. In this study, the average coordinate structure with respect to each modeled system is extracted from the whole simulation range (30 ns). Here, our goals are firstly investigated the B-form of our models for DNA-only as in M1 and M2 by comparing their parameters (e.g., torsion angles, rise and twist parameters) with parameters of B-DNA form.[40] Then, the structural changes of DNA when mono or double intercalated by DOX are compared with reference models (M1 and M2). Furthermore, our results of DNA conformations from models 5 and 6 can be used to compare with the available parameter values from both sources (1D12 and 1D11) as well as the validation of the AMBER force field of DNA and drug from these comparisons.

6. Free Energy Calculations

After MD simulations, snapshots were taken for every 10 ps from 0 to 30 ns, so 1500 snapshots were extracted for post-process binding free energies using MM-PB(GB)SA methods. BFE calculations by MM-PBSA or MM-GBSA methods were performed using the MMPBSA.py module of AMBER 11.[6] They are described succinctly in the following four subsections: a) MM-PBSA method, b) MM-GBSA method, c) Entropy calculations, d) Binding free energy corrections.

a) MM-PBSA Method

In MM-PBSA approach, the following setting are used to calculate the BFE of drug-DNA intercalating complexes: the value of an exterior dielectric constant was set to 80 while for solute dielectric constant was set to 1, the MM-PBSA surface tension (γ) and the non-polar free energy correction term (β) were set to 0.00542 kcal/mol-Å^2 and 0.92 kcal/mol, respectively according to the PARSE and mbondi2 sets.[4]

b) MM-GBSA Method

The model developed by Onufriev et al. (GB^{OBC} which GB=2)[41] was used as GB model in this study. By the antechamber program in AMBER11, the mbondi2 radii set was prepared. The default setting of MM-GBSA surface tension (γ = 0.005 kcal / mol Å^2) and the non-polar free energy correction term (β = 0) were applied. The value of an exterior dielectric constant was set to 78.3 and for solute dielectric constants was used 1 as in MM-PBSA method.

c) The Entropy Calculations

The normal mode analysis was used to evaluate the solute entropic contribution of DOX-DNA complexes. In the normal-mode analysis, the conformational entropy change (-TΔS) upon binding DOX to DNA was estimated using the nmode program through MMPBSA.py module of AMBER 11.[6, 18] Due to the limitation of computationally expensive normal-mode analysis, we only considered the residues within a 12 Å sphere centered at the ligand, and these residues were retrieved from an MD snapshot for each DOX-DNA complex. Then, each structure was fully minimized for 10000 steps using a distance-dependent dielectric of $4r_{ij}$ (r_{ij} is the distance between two atoms) to mimic the solvent dielectric change from the solute to solvent. To reduce the computational demand, 150 snapshots were taken from 0 to 30 ns to estimate the contribution of the entropy to binding. The final conformational entropy was obtained from the average over the snapshots.

d) Binding Free energy Corrections

For comparing our present results of the intercalation BFE with experimental result from Chaires and co-workers[42] for DOX-DNA intercalating complexes, three necessary corrections of the BFE are described here.

The DNA structure is usually stable B-form conformation before binding with DOX. After the binding, this B-form conformation can deform due to the generation of the intercalation site, which is the first step in the binding process of each intercalator. Unlike the undisturbed DNA (B-form of DNA), the creation of intercalation site causes a doubling of the base–base distance. Therefore, the DNA deformation penalty is a significant term for corrected binding free energy, and this part is the first correction that should be incorporated to estimate correct binding free energy. The BFE without any corrections is denoted as $\Delta G_{uncorrected}$. The DNA deformation energy is not related to MM-PB(GB)SA method, but it is arising from the fact that DNA deformation energy is indispensable in the calculating of the intercalation binding process. In our present study, this DNA deformation penalty can be computed by subtracting the energy of bounded DNA (only DNA energy from complex without ligand) and energy of unbounded B-form DNA using MD simulations. Then, the corrected binding energy of a bound DOX-DNA complex was obtained by adding this DNA deformation energy to the uncorrected BFE from MM-PB(GB)SA methods.

The second of these corrections is to a standard one to account for drug concentration. In the dilute concentration limit, the standard free energy to form an AB particle from binding of a particle A to particles B is[43]:

$$\Delta G^{0}_{AB} = -RT \ln\left(\frac{C^{0}\, C_{AB}}{C_A\, C_B}\right) = -RT \ln\left(K_{AB}\right) \qquad \text{(S2)}$$

Where R is the gas constant, T is the temperature in Kelvin (310 K in our case), K_{AB} is the binding constant, C^0 is the standard concentration (1M), and the C_i's are the concentration of respective species. Here A denotes the drug (DOX) and B denotes the DNA-binding site which is equal to the number of base pairs

divided by the binding site size (base pairs per bound drug), which is approximately 3 base pairs for DOX.[16, 36] Thus, C_A and C_{AB} cancel each other and a net difference in binding free energy seems from C_B and C^0 concentrations.[44] This correction should be subtracted from our results that calculated by MM-PB(GB)SA approaches.

The third correction comes from the dependence of the intercalation BFE on the ionic concentration of the solution. From experimental study by Chaires et al.[42], the binding constant (K_{AB}) in the last term of **Eq. (S2)** was used to calculate the binding free energy. The salt dependence of the binding constant (K) can be obtained from the slope[45]:

$$S = -\left(\frac{\delta \ln K}{\delta \ln\left[Na^{+}\right]}\right) \tag{S3}$$

where $[Na^+]$ is the positive ion concentration. The salt dependence of the binding constant S can be used to evaluate the ionic contribution ΔG_{ion} to the standard free energy ΔG^0 at a given salt concentration by the relation[42, 45]:

$$\Delta G_{ion} = \left[(S)RT \ln(Na^{+})\right]_{\exp} \tag{S4}$$

The difference between the standard free energy and ΔG_{ion} defines the binding free energy, ΔG_t, that is defined the nonelectrostatic free energy contribution, referring to a standard state of about 1 M monovalent salt.[46] This means ΔG_{ion} equals zero and not dependent on salt concentration and is given by the relation [38,41]:

$$\Delta G_t = (\Delta G^0 - \Delta G_{ion})_{\exp} \tag{S5}$$

Form this experimental study at $[Na^+]$ = 0.016 M and T = 293.15 K, they found that S of DOX is equal -0.97, ΔG_{ion} = -2.3 kcal/mol, ΔG^0 = -9.98 kcal/mol and ΔG_t = -7.7 ± 0.3 kcal/mol.[42] Also, they found ΔG_{ion} = -1 kcal/mol at 0.2 M of NaCl and T = 293.15 K.[46]

In our present study for the analysis of ionic concentrations dependence, the calculations of intercalation BFE were firstly accomplished at 0.1, 0.15, 0.2, 0.25, 0.5 and 1.0 M theoretical ionic concentrations by MM-PB(GB)SA methods. Then, the dependence of ionic concentration of BFE, ΔG_{ion}, is calculated as the difference between the free energy at specific ionic concentration and the free energy at 1 M ionic concentration, when $\Delta G_{ion} = 0$ according to **Eq. (S4)**. Further, it can be compared the calculated value of S in our study with experimentally values[42] and other theoretical predicted values,[45, 47] salt dependence of the binding constant (K), by using following procedure: first finding the standard BFE by considering the first two corrections into the calculated BFE by MM-PB(GB)SA methods which these two corrections are arising from the DNA deformation energies and a standard drug concentration effect, then by straightforward application of thermodynamic **Eq. (S2)** to find the corresponding binding constant, K, and then find S by applying **Eq. (S3)**. These procedure's steps are necessary to be reasonably compared our present results with corresponding values from experimental study by Chaires et al.[42] because of the difference in the calculating of the DOX intercalation free energy with respect to change ionic concentrations experimentally, and in the present MD simulations.

Finally, the true binding free energy, $\Delta G_{t\text{-}sim}$, in our present study is obtained when the above three corrections using MM-PB(GB)SA methods are considered, and can be compared with the experimental free energy $\Delta G_{t\text{-}exp}$.

TABLE S2: Backbone and Glycosyl Torsion Angles (Degrees) and Sugar Conformations [#] (deg.)

a) Model 1 of dsDNA d(CG**AT**CG) or DNA1 sequence

Residue	α	β	γ	δ	ε	ζ	χ	P	Puckering
C1	---	---	63	121	-156	-92	-119	133	C1'-exo
G2	-80	166	51	134	-142	-141	-103	146	C2'-endo
A3	-86	161	47	137	-175	-91	-112	165	C2'-endo
T4	-68	167	58	110	-175	-86	-131	122	C1'-exo
C5	-67	170	61	120	-170	-94	-118	129	C1'-exo
G6	-73	172	55	121	---	---	-114	129	C1'-exo
C7	---	---	63	115	-156	-92	-126	123	C1'-exo
G8	-85	170	54	134	-158	-131	-107	146	C2'-endo
A9	-75	166	56	126	-178	-91	-124	128	C1'-exo
T10	-65	170	59	107	-173	-89	-128	110	C1'-exo
C11	-69	171	58	122	-167	-93	-121	130	C1'-exo
G12	-72	170	54	130	---	---	-107	142	C1'-exo
Aver.	-74	168	57	123	-165	-100	-118	134	
B-DNA*	-63	171	54	123	-169	-108	-117		

b) Model 2 of dsDNA d(CG**TA**CG) or DNA2 sequence

Residue	α	β	γ	δ	ε	ζ	χ	P	Puckering
C1	---	---	62	127	-155	-81	-133	137	C1'-exo
G2	-85	163	53	133	-139	-131	-101	138	C1'-exo
T3	-94	161	59	122	-169	-100	-119	133	C1'-exo
A4	-69	174	58	139	-177	-99	-102	168	C2'-endo
C5	-68	166	56	106	-159	-106	-120	103	O4'-endo
G6	-77	169	55	131	---	---	-107	146	C2'-endo
C7	---	---	58	126	-162	-81	-128	137	C1'-exo
G8	-84	173	50	138	-136	-143	-100	146	C2'-endo
T9	-84	160	54	132	-163	-96	-119	147	C2'-endo
A10	-78	169	49	139	-165	-111	-98	144	C1'-exo
C11	-78	161	51	126	-141	-97	-122	136	C1'-exo
G12	-88	162	52	126	---	---	-103	138	C1'-exo
Aver.	-81	166	55	129	-157	-105	-113	140	
B-DNA*	-63	171	54	123	-169	-108	-117		

c) Model 3 of 1:1 complex with DNA1 dsDNA sequence

Residue	α	β	γ	δ	ε	ζ	χ	P	Puckering
C1	---	---	63	133	-167	-78	**-45**	153	C2'-endo
G2	-72	169	56	137	-145	-169	-101	153	C2'-endo
A3	-80	163	50	135	-177	-95	-113	160	C2'-endo
T4	-66	170	58	114	-162	-82	-124	118	C1'-exo
C5	-76	169	53	139	**-97**	**142**	**-84**	149	C2'-endo
G6	-59	179	32	141	---	---	**-93**	170	C2'-endo
C7	---	---	35	125	**-105**	-70	**-148**	134	C1'-exo
G8	-76	162	49	127	-139	**103**	**-86**	132	C1'-exo
A9	-94	**153**	53	136	-175	-94	-115	167	C2'-endo
T10	-69	168	61	108	-168	-87	-128	109	C1'-exo
C11	-70	173	58	137	-175	-85	-118	153	C2'-endo
G12	-67	169	56	130	---	---	-106	132	C1'-exo
Aver.	-73	168	52	130	-151	-51	-105	144	
B-DNA*	-63	171	54	123	-169	-108	-117		

d) Model 4 of 1:1 complex with DNA2 dsDNA sequence

Residue	α	β	γ	δ	ε	ζ	χ	P	Puckering
C1	---	---	61	119	-151	-65	**-41**	143	C1'-exo
G2	-113	167	60	135	-176	-103	-100	170	C2'-endo
T3	-71	167	58	117	-172	-94	-121	104	O4'-endo
A4	-70	174	57	127	-170	-88	-108	148	C2'-endo
C5	-72	166	57	116	**-101**	**163**	**-86**	121	C1'-exo
G6	-65	166	41	132	---	---	**-94**	135	C1'-exo
C7	---	---	43	127	**-109**	-156	**-143**	131	C1'-exo
G8	-91	163	57	134	-140	**120**	**-91**	149	C2'-endo
T9	-81	**151**	67	129	-163	-92	-125	147	C2'-endo
A10	-78	170	57	128	-147	-133	-105	141	C1'-exo
C11	-68	162	56	124	-165	-87	-118	134	C1'-exo
G12	-75	169	56	124	---	---	-107	132	C1'-exo
Aver.	-78	166	56	126	-149	-51	-103	138	
B-DNA*	-63	171	54	123	-169	-108	-117		

e) Model 5 of 2:1 complex with DNA1 dsDNA sequence

Residue	α	β	γ	δ	ε	ζ	χ	P	Puckering
C1	---	---	40	128	**-101**	-70	**-149**	138	C1'-exo
G2	-75	169	48	130	-144	**121**	**-86**	135	C1'-exo
A3	-96	**156**	52	139	-176	-94	-116	171	C2'-endo
T4	-69	172	59	120	-163	-83	-123	128	C1'-exo
C5	-77	169	53	138	**-96**	**164**	**-81**	148	C2'-endo
G6	-71	178	42	139	---	---	**-96**	186	C3'-exo
C7	---	---	50	124	**-109**	-72	**-146**	134	C1'-exo
G8	-76	164	50	129	-140	**135**	**-88**	134	C1'-exo
A9	-94	**155**	53	138	-175	-94	-116	168	C2'-endo
T10	-69	172	60	119	-163	-84	-121	127	C1'-exo
C11	-76	168	54	138	**-103**	**148**	**-83**	148	C2'-endo
G12	-72	179	43	138	---	---	**-97**	180	C3'-exo
Aver.	-77	168	50	132	-137	7	-109	150	
B-DNA*	-63	171	54	123	-169	-108	-117		

f) Model 6 of 2:1 complex with DNA2 dsDNA sequence

Residue	α	β	γ	δ	ε	ζ	χ	P	Puckering
C1	---	---	37	130	**-96**	-69	**-149**	139	C1'-exo
G2	-74	164	46	133	-139	**127**	**-86**	138	C1'-exo
T3	-92	**151**	56	130	-172	-96	-119	147	C2'-endo
A4	-73	176	58	132	-166	-90	-105	153	C2'-endo
C5	-74	166	52	129	**-107**	**180**	**-85**	137	C1'-exo
G6	-61	177	32	139	---	---	**-96**	180	C3'-exo
C7	---	---	46	130	**-100**	-76	**-146**	139	C1'-exo
G8	-76	161	48	131	-143	**141**	**-88**	138	C1'-exo
T9	-87	**150**	59	126	-168	-101	-116	139	C1'-exo
A10	-71	173	57	133	-167	-88	-120	152	C2'-endo
C11	-74	167	53	131	**-107**	**151**	**-83**	138	C1'-exo
G12	-67	179	39	135	---	---	**-97**	178	C2'-endo
Aver.	-75	166	49	131	-137	8	-107	148	
B-DNA*	-63	171	54	123	-169	-108	-117		

The torsion angles are defined according to 3DNA Program as in the caption of **Fig. S4**.

* The torsion angle values of B-DNA are taken from Drew et al. study.[48]

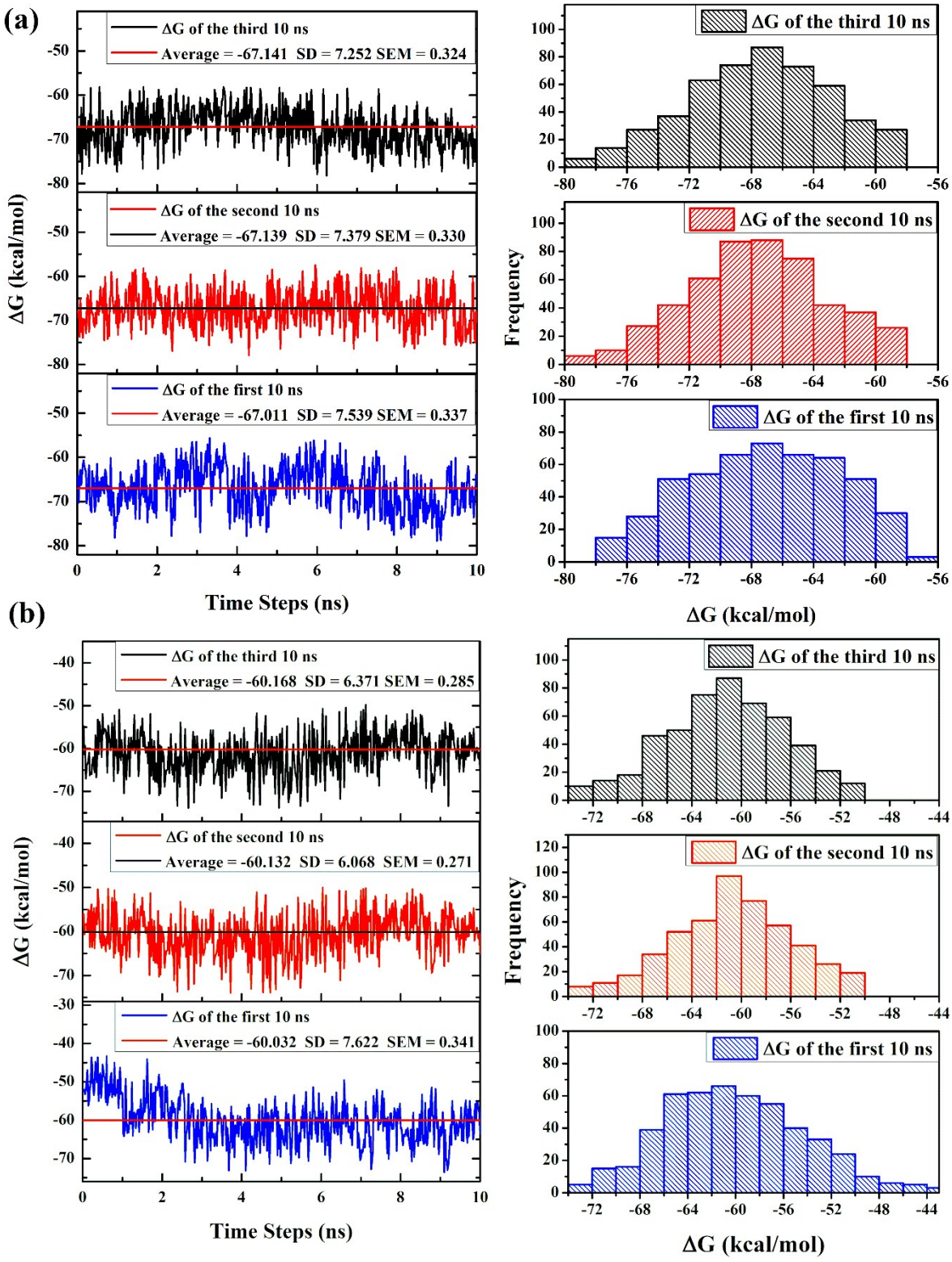


Fig. S7: The binding free energy against the first, second and third 10 ns segments of whole 30 ns time simulation and histogram distribution of BFE in 2:1 complex at 0.2 M salt concentration using MM-GBSA method without any corrections for: (a) DOX-DNA1 or M5 and (b) DOX-DNA2 or M6.

TABLE S3. The distribution of the binding free energy of the intercalated complexes for the first, second and third 10 ns segments of MD that calculated from single trajectory protocol (STP) at a theoretical salt concentration 0.2 M. The value of BFE without any corrections.

a) For Model 3 (M3) of 1:1 complex

ΔG (kcal/mol)	Frequency of ΔG at 1st 10 ns	Percentage of frequency of ΔG at 1st 10 ns (%)	Frequency of ΔG at 2nd 10 ns	Percentage frequency of ΔG at 2nd 10 ns (%)	Frequency of ΔG at 3rd 10 ns	Percentage of frequency of ΔG at 3rd 10 ns (%)
-39	1	0.2	2	0.4	2	0.4
-37	5	1	7	1.4	15	3
-35	16	3.2	23	4.6	54	10.8
-33	64	12.8	78	15.6	105	21
-31	128	25.6	133	26.6	139	27.8
-29	135	27	119	23.8	120	24
-27	86	17.2	75	15	45	9
-25	47	9.4	43	8.6	17	3.4
-23	14	2.8	18	3.6	2	0.4
-21	3	0.6	2	0.4	1	0.2
-19	0	0	0	0	0	0
-17	1	0.2	0	0	0	0

b) For Model 4 (M4) of 1:1 complex

ΔG (kcal/mol)	Frequency of ΔG at 1st 10 ns	Percentage of frequency of ΔG at 1st 10 ns (%)	Frequency of ΔG at 2nd 10 ns	Percentage frequency of ΔG at 2nd 10 ns (%)	Frequency of ΔG at 3rd 10 ns	Percentage of frequency of ΔG at 3rd 10 ns (%)
-37	1	0.2	3	0.6	2	0.4
-35	19	3.8	9	1.8	11	2.2
-33	48	9.6	26	5.2	29	5.8
-31	54	10.8	66	13.2	52	10.4
-29	94	18.8	93	18.6	99	19.8
-27	98	19.6	113	22.6	124	24.8
-25	65	13	100	20	107	21.4
-23	44	8.8	58	11.6	58	11.6
-21	35	7	29	5.8	15	3
-19	22	4.4	3	0.6	3	0.6
-17	11	2.2	0	0	0	0
-15	9	1.8	0	0	0	0

c) For Model 5 (M5) of 2:1 complex

ΔG (kcal/mol)	Frequency of ΔG at 1st 10 ns	Percentage of frequency of ΔG at 1st 10 ns (%)	Frequency of ΔG at 2nd 10 ns	Percentage frequency of ΔG at 2nd 10 ns (%)	Frequency of ΔG at 3rd 10 ns	Percentage of frequency of ΔG at 3rd 10 ns (%)
-79	0	0	6	1.2	6	1.2
-77	15	3	10	2	14	2.8
-75	28	5.6	27	5.4	27	5.4
-73	51	10.2	42	8.4	37	7.4
-71	54	10.8	61	12.2	63	12.6
-69	66	13.2	87	17.4	74	14.8
-67	72	14.4	87	17.4	86	17.2
-65	66	13.2	75	15	73	14.6
-63	64	12.8	42	8.4	59	11.8
-61	51	10.2	37	7.4	34	6.8
-59	30	6	26	5.2	27	5.4
-57	3	0.6	0	0	0	0
-79	0	0	6	1.2	6	1.2
-77	15	3	10	2	14	2.8

d) For Model 6 (M6) of 2:1 complex

ΔG (kcal/mol)	Frequency of ΔG at 1st 10 ns	Percentage of frequency of ΔG at 1st 10 ns (%)	Frequency of ΔG at 2nd 10 ns	Percentage frequency of ΔG at 2nd 10 ns (%)	Frequency of ΔG at 3rd 10 ns	Percentage of frequency of ΔG at 3rd 10 ns (%)
-73	5	1	8	1.6	10	2
-71	15	3	11	2.2	14	2.8
-69	16	3.2	17	3.4	18	3.6
-67	39	7.8	34	6.8	46	9.2
-65	61	12.2	52	10.4	50	10
-63	62	12.4	61	12.2	75	15
-61	66	13.2	97	19.4	87	17.4
-59	60	12	77	15.4	69	13.8
-57	55	11	57	11.4	59	11.8
-55	40	8	41	8.2	39	7.8
-53	33	6.6	26	5.2	21	4.2
-51	24	4.8	19	3.8	12	2.4
-49	10	2	0	0	0	0
-47	6	1.2	0	0	0	0
-45	5	1	0	0	0	0
-43	3	0.6	0	0	0	0

TABLE S4. Energy contributions of DOX-DNA complex for model 3 (M3) that calculated from single trajectory protocol (STP) by subtracting DOX and DNA contributions from the values of complex at a theoretical salt concentration 0.2 M. std is Standard Deviation.

	Complex		DNA		DOX		**Delta G** = COM-(DNA+DOX)	
Energy (kcal/mol)	Average	std	Average	std	Average	std	Average	std
$E_{internal}$	641.72	10.40	564.44	10.20	77.05	4.10	0.23	0.10
$E_{electrosatic}$	332.90	20.10	719.58	20.24	-41.52	7.34	-345.16	10.68
E_{VDW}	-234.44	6.94	-169.91	5.85	-0.38	2.20	-64.15	2.90
E_{gas}	740.18	26.42	1114.11	25.41	35.15	9.47	-409.08	10.61
G_{np} by GBSA	12.28	0.15	12.19	0.12	2.95	0.03	-2.86	0.12
G_{p} by GBSA	-1632.48	13.67	-1903.26	15.02	-91.52	4.27	362.30	9.07
G_{solv} by GBSA	-1620.20	13.66	-1891.07	15.00	-88.57	4.26	359.44	9.05
G_{np} by PBSA	15.22	0.15	15.82	0.15	5.10	0.04	-5.70	0.08
G_{p} by PBSA	-1677.86	13.11	-1944.74	14.74	-94.58	4.36	361.46	8.81
G_{solv} by PBSA	-1662.64	13.12	-1928.92	14.76	-89.48	4.35	355.76	8.78
$T*S_{tran}$	15.74	0.00	15.61	0.00	13.87	0.00	-13.74	0.00
$T*S_{rot}$	14.84	0.01	14.61	0.03	11.83	0.01	-11.60	0.10
$T*S_{vib}$	346.91	1.56	296.94	1.93	44.28	0.25	5.69	2.45
$T*S_{total}$	377.49	1.57	327.16	1.95	69.98	0.25	-19.65	2.46
$G_{uncorrected}$ by GBSA	-1257.51	20.79	-1104.12	19.00	-123.40	7.41	**-29.99**	4.39
$G_{uncorrected}$ by PBSA	-1299.95	21.44	-1141.97	19.23	-124.31	7.56	**-33.67**	5.34

TABLE S5. Energy contributions of DOX-DNA complex for M4 that calculated from single trajectory protocol (STP) by subtracting DOX and DNA contributions from the values of complex at a theoretical salt concentration 0.2 M.

	Complex		DNA		DOX		**Delta G**= COM-(DNA+DOX)	
Energy (kcal/mol)	Ave.	std	Ave.	std	Ave.	std	Ave.	std
$E_{internal}$	643.61	10.80	568.11	9.90	75.29	4.10	0.21	0.10
$E_{electrosatic}$	353.65	24.03	724.32	19.95	-43.35	7.06	-327.32	22.57
E_{VDW}	-228.99	8.56	-168.60	6.52	0.70	2.57	-61.09	5.00
E_{gas}	768.26	27.33	1123.82	23.45	32.65	9.05	-388.21	26.26
G_{np} by GBSA	12.90	0.32	12.35	0.19	3.29	0.04	-2.75	0.26
G_{p} by GBSA	-1657.49	20.83	-1911.01	14.70	-91.26	3.74	344.78	20.61
G_{solv} by GBSA	-1644.59	20.64	-1898.66	14.63	-87.97	3.73	342.04	20.41
G_{np} by PBSA	15.63	0.38	15.94	0.30	5.12	0.06	-5.43	0.27
G_{p} by PBSA	-1705.33	21.91	-1956.60	14.91	-92.85	3.81	344.12	21.45
G_{solv} by PBSA	-1689.70	21.69	-1940.66	14.84	-87.73	3.79	338.68	21.21
$T*S_{tran}$	15.74	0.00	15.61	0.00	13.87	0.00	-13.74	0.00
$T*S_{rot}$	14.86	0.04	14.64	0.05	11.84	0.02	-11.62	0.04
$T*S_{vib}$	347.21	2.45	296.28	2.59	44.64	0.32	6.29	2.60
$T*S_{total}$	377.81	2.48	326.52	2.63	70.35	0.33	-19.06	3.14
$G_{uncorrected}$ by GBSA	-1254.13	10.76	-1101.35	10.89	-125.66	6.56	**-27.11**	4.55
$G_{uncorrected}$ by PBSA	-1299.24	10.94	-1143.36	10.92	-125.42	6.46	**-30.47**	5.30

TABLE S6. Energy contributions of 2DOX-DNA complex for model 5 (M5) that calculated from single trajectory protocol (STP) by subtracting 2DOX and DNA contributions from the values of complex at a theoretical salt concentration 0.2 M.

	Complex		DNA		2DOX		**Delta G**= COM-(DNA+2DOX)	
Energy (kcal/mol)	Ave.	std	Ave.	std	Ave.	std	Ave.	std
$E_{internal}$	722.15	10.62	570.00	9.43	151.68	5.24	0.47	0.11
$E_{electrosatic}$	-29.52	20.00	703.30	18.10	-41.06	11.39	-691.76	16.54
E_{VDW}	-282.20	7.51	-152.54	5.52	-0.97	2.95	-128.69	3.97
E_{gas}	410.43	26.66	1120.76	24.11	109.66	14.60	-819.99	16.78
G_{np} by GBSA	13.08	0.18	12.83	0.13	5.89	0.04	-5.64	0.17
G_{p} by GBSA	-1387.04	13.19	-1901.49	13.54	-219.26	7.30	733.71	14.19
G_{solv} by GBSA	-1373.96	13.18	-1888.66	13.52	-213.37	7.30	728.07	14.16
G_{np} by PBSA	15.77	0.18	16.95	0.17	9.21	0.06	-10.39	0.10
G_{p} by PBSA	-1437.14	12.65	-1943.43	13.46	-224.33	7.51	730.62	13.80
G_{solv} by PBSA	-1421.37	12.61	-1926.48	13.46	-215.12	7.51	720.23	13.77
$T*S_{tran}$	15.85	0.00	15.61	0.00	14.51	0.00	-14.27	0.00
$T*S_{rot}$	15.07	0.01	14.62	0.03	12.97	0.25	-12.52	0.15
$T*S_{vib}$	397.75	1.11	296.45	2.22	99.34	4.87	1.95	4.65
$T*S_{total}$	428.67	1.11	326.67	2.24	126.82	5.11	-24.82	5.33
$G_{uncorrected}$ by GBSA	-1392.20	22.34	-1094.57	19.36	-230.53	10.82	**-67.09**	7.43
$G_{uncorrected}$ by PBSA	-1439.60	22.83	-1132.39	19.29	-232.28	11.01	**-74.93**	8.67

TABLE S7. Energy contributions of 2DOX-DNA complex for M6 that calculated from STP by subtracting 2DOX and DNA contributions from the values of complex at a theoretical salt concentration 0.2 M.

	Complex		DNA		2DOX		**Delta G**= COM-(DNA+2DOX)	
Energy (kcal/mol)	Ave.	std	Ave.	std	Ave.	std	Ave.	std
$E_{internal}$	725.41	10.56	573.65	9.39	151.38	5.02	0.38	0.11
$E_{electrosatic}$	-22.09	20.32	705.15	17.90	-43.57	11.84	-683.66	22.80
E_{VDW}	-274.06	9.11	-150.69	5.47	-0.48	3.28	-122.89	5.29
E_{gas}	429.26	27.97	1128.10	24.04	107.33	14.76	-806.17	25.77
G_{np} by GBSA	13.17	0.31	12.86	0.15	5.91	0.05	-5.59	0.25
G_{p} by GBSA	-1395.97	21.06	-1906.14	14.43	-216.82	7.43	727.00	20.14
G_{solv} by GBSA	-1382.80	20.87	-1893.29	14.40	-210.91	7.43	721.40	19.99
G_{np} by PBSA	15.87	0.34	16.91	0.21	9.22	0.07	-10.26	0.21
G_{p} by PBSA	-1449.60	20.86	-1952.26	14.54	-221.82	7.53	724.48	20.46
G_{solv} by PBSA	-1433.73	20.63	-1935.35	14.54	-212.60	7.53	714.22	20.31
$T*S_{tran}$	15.85	0.00	15.61	0.00	14.51	0.00	-14.26	0.00
$T*S_{rot}$	15.06	0.01	14.63	0.03	12.94	0.27	-12.52	0.23
$T*S_{vib}$	398.33	0.97	295.92	2.15	100.28	3.32	2.13	3.21
$T*S_{total}$	429.24	0.97	326.16	2.18	127.73	3.54	-24.65	3.34
$G_{uncorrected}$ by GBSA	-1382.77	19.88	-1091.35	19.48	-231.31	11.15	**-60.11**	6.86
$G_{uncorrected}$ by PBSA	-1433.71	20.36	-1133.41	19.55	-233.00	11.34	**-67.30**	7.77

TABLE S8: The details of energy contribution of uncomplexed and complexed DNA1 (without ligands) at different theoretical salt concentrations.

a) Energy contribution of unbounded DNA from M1 that calculate from two trajectory protocol (2TP) with different theoretical salt concentration.

Energy (kcal/mol)	DNA at a theoretical salt concentration (in Molarity) from 2TP					
	0.1	0.15	0.2	0.25	0.5	1
$E_{internal}$	561.75	561.75	561.75	561.75	561.75	561.75
$E_{electrosatic}$	735.01	735.01	735.01	735.01	735.01	735.01
E_{VDW}	-186.90	-186.90	-186.90	-186.90	-186.90	-186.90
E_{gas}	1109.86	1109.86	1109.86	1109.86	1109.86	1109.86
G_{np} by GBSA	11.59	11.59	11.59	11.59	11.59	11.59
G_{p} by GBSA	-1910.27	-1911.53	-1912.42	-1913.11	-1915.15	-1916.96
G_{solv} by GBSA	-1898.68	-1899.94	-1900.83	-1901.52	-1903.56	-1905.38
G_{np} by PBSA	15.03	15.03	15.03	15.03	15.03	15.03
G_{p} by PBSA	-1953.23	-1953.49	-1953.76	-1953.89	-1954.84	-1955.20
G_{solv} by PBSA	-1938.20	-1938.46	-1938.74	-1938.86	-1939.81	-1940.17
$T*S_{tran}$	15.61	15.61	15.61	15.61	15.61	15.61
$T*S_{rot}$	14.70	14.70	14.70	14.70	14.70	14.70
$T*S_{vib}$	300.30	300.30	300.30	300.30	300.30	300.30
$T*S_{tot}$	330.60	330.60	330.60	330.60	330.60	330.60
G_{total} by GBSA	-1119.43	-1120.69	-1121.58	-1122.27	-1124.31	-1126.12
G_{total} by PBSA	-1158.95	-1159.21	-1159.48	-1159.61	-1160.56	-1160.92

b) Energy contribution of bounded DNA from M3 that calculated from single trajectory protocol (STP) with different theoretical salt concentration.

Energy (kcal/mol)	DNA at a theoretical salt concentration (in Molarity) from STP					
	0.1	0.15	0.2	0.25	0.5	1
$E_{internal}$	564.44	564.44	564.44	564.44	564.44	564.44
$E_{electrosatic}$	719.58	719.58	719.58	719.58	719.58	719.58
E_{VDW}	-169.91	-169.91	-169.91	-169.91	-169.91	-169.91
E_{gas}	1114.11	1114.11	1114.11	1114.11	1114.11	1114.11
G_{np} by GBSA	12.19	12.19	12.19	12.19	12.19	12.19
G_{p} by GBSA	-1901.14	-1902.38	-1903.26	-1903.93	-1905.89	-1907.64
G_{solv} by GBSA	-1888.96	-1890.19	-1891.07	-1891.74	-1893.70	-1895.45
G_{np} by PBSA	15.82	15.82	15.82	15.82	15.82	15.82
G_{p} by PBSA	-1944.25	-1944.49	-1944.74	-1944.84	-1945.68	-1945.97
G_{solv} by PBSA	-1928.43	-1928.68	-1928.92	-1929.02	-1929.87	-1930.15
$T*S_{tran}$	15.61	15.61	15.61	15.61	15.61	15.61
$T*S_{rot}$	14.61	14.61	14.61	14.61	14.61	14.61
$T*S_{vib}$	296.94	296.94	296.94	296.94	296.94	296.94
$T*S_{tot}$	327.16	327.16	327.16	327.16	327.16	327.16
G_{total} by GBSA	-1102.01	-1103.24	-1104.12	-1104.79	-1106.75	-1108.50
G_{total} by PBSA	-1141.48	-1141.73	-1141.97	-1142.07	-1142.92	-1143.20

c) Energy contribution of bounded DNA alone from M5 that calculated from single trajectory protocol (STP) with different theoretical salt concentration.

Energy (kcal/mol)	DNA at a theoretical salt concentration (in Molarity) from STP					
	0.1	0.15	0.2	0.25	0.5	1
$E_{internal}$	570.00	570.00	570.00	570.00	570.00	570.00
$E_{electrosatic}$	703.30	703.30	703.30	703.30	703.30	703.30
E_{VDW}	-152.54	-152.54	-152.54	-152.54	-152.54	-152.54
E_{gas}	1120.76	1120.76	1120.76	1120.76	1120.76	1120.76
G_{np} by GBSA	12.83	12.83	12.83	12.83	12.83	12.83
G_{p} by GBSA	-1899.41	-1900.63	-1901.49	-1902.13	-1904.09	-1905.78
G_{solv} by GBSA	-1886.58	-1887.80	-1888.66	-1889.30	-1891.26	-1892.95
G_{np} by PBSA	16.95	16.95	16.95	16.95	16.95	16.95
G_{p} by PBSA	-1942.94	-1943.18	-1943.43	-1943.52	-1944.40	-1944.66
G_{solv} by PBSA	-1925.99	-1926.23	-1926.48	-1926.57	-1927.45	-1927.71
$T*S_{tran}$	15.61	15.61	15.61	15.61	15.61	15.61
$T*S_{rot}$	14.62	14.62	14.62	14.62	14.62	14.62
$T*S_{vib}$	296.45	296.45	296.45	296.45	296.45	296.45
$T*S_{tot}$	326.67	326.67	326.67	326.67	326.67	326.67
G_{total} by GBSA	-1092.49	-1093.71	-1094.57	-1095.21	-1097.17	-1098.86
G_{total} by PBSA	-1131.90	-1132.14	-1132.39	-1132.48	-1133.36	-1133.62

TABLE S9: The details of energy contribution of uncomplexed and complexed DNA (without ligands) of d(CG**TA**CG), DNA2, sequence at different theoretical salt concentrations.

a) Energy contribution of free DNA alone of M2 that calculated from multiple trajectory protocol (2TP) with different theoretical salt concentration.

Energy (kcal/mol)	DNA at a theoretical salt concentration (Molarity) from 2TP					
	0.1	0.15	0.2	0.25	0.5	1
$E_{internal}$	565.38	565.38	565.38	565.38	565.38	565.38
$E_{electrosatic}$	748.48	748.48	748.48	748.48	748.48	748.48
E_{VDW}	-186.97	-186.97	-186.97	-186.97	-186.97	-186.97
E_{gas}	1126.89	1126.89	1126.89	1126.89	1126.89	1126.89
G_{np} by GBSA	11.61	11.61	11.61	11.61	11.61	11.61
G_{p} by GBSA	-1928.09	-1929.37	-1930.32	-1931.01	-1933.10	-1934.94
G_{solv} by GBSA	-1916.48	-1917.76	-1918.71	-1919.40	-1921.49	-1923.33
G_{np} by PBSA	14.93	14.93	14.93	14.93	14.93	14.93
G_{p} by PBSA	-1975.12	-1975.45	-1975.78	-1975.93	-1976.74	-1977.18
G_{solv} by PBSA	-1960.19	-1960.52	-1960.85	-1961.00	-1961.81	-1962.25
$T*S_{tran}$	15.61	15.61	15.61	15.61	15.61	15.61
$T*S_{rot}$	14.80	14.80	14.80	14.80	14.80	14.80
$T*S_{vib}$	297.72	297.72	297.72	297.72	297.72	297.72
$T*S_{tot}$	328.13	328.13	328.13	328.13	328.13	328.13
G_{total} by GBSA	-1117.71	-1119.00	-1119.94	-1120.63	-1122.72	-1124.56
G_{total} by PBSA	-1161.43	-1161.76	-1162.09	-1162.24	-1163.05	-1163.49

b) Energy contribution of bounded DNA alone from M4 that calculated from single trajectory protocol (STP) with different theoretical salt concentration.

Energy (kcal/mol)	DNA at a theoretical salt concentration (Molarity) from STP					
	0.1	0.15	0.2	0.25	0.5	1
$E_{internal}$	568.11	568.11	568.11	568.11	568.11	568.11
$E_{electrosatic}$	724.32	724.32	724.32	724.32	724.32	724.32
E_{VDW}	-168.60	-168.60	-168.60	-168.60	-168.60	-168.60
E_{gas}	1123.82	1123.82	1123.82	1123.82	1123.82	1123.82
G_{np} by GBSA	12.35	12.35	12.35	12.35	12.35	12.35
G_{p} by GBSA	-1908.84	-1910.09	-1911.01	-1911.68	-1913.70	-1915.46
G_{solv} by GBSA	-1896.49	-1897.74	-1898.66	-1899.33	-1901.35	-1903.11
G_{np} by PBSA	15.94	15.94	15.94	15.94	15.94	15.94
G_{p} by PBSA	-1956.02	-1956.32	-1956.60	-1956.73	-1957.49	-1957.86
G_{solv} by PBSA	-1940.08	-1940.38	-1940.66	-1940.79	-1941.55	-1941.92
$T*S_{tran}$	15.61	15.61	15.61	15.61	15.61	15.61
$T*S_{rot}$	14.64	14.64	14.64	14.64	14.64	14.64
$T*S_{vib}$	296.28	296.28	296.28	296.28	296.28	296.28
$T*S_{tot}$	326.52	326.52	326.52	326.52	326.52	326.52
G_{total} by GBSA	-1099.18	-1100.43	-1101.35	-1102.02	-1104.04	-1105.80
G_{total} by PBSA	-1142.78	-1143.08	-1143.36	-1143.49	-1144.25	-1144.62

c) Energy contribution of bounded DNA alone from M6 that calculated from single trajectory protocol (STP) with different theoretical salt concentration.

Energy (kcal/mol)	DNA at a theoretical salt concentration (Molarity) from STP					
	0.1	0.15	0.2	0.25	0.5	1
$E_{internal}$	573.65	573.65	573.65	573.65	573.65	573.65
$E_{electrosatic}$	705.15	705.15	705.15	705.15	705.15	705.15
E_{VDW}	-150.69	-150.69	-150.69	-150.69	-150.69	-150.69
E_{gas}	1128.10	1128.10	1128.10	1128.10	1128.10	1128.10
G_{np} by GBSA	12.86	12.86	12.86	12.86	12.86	12.86
G_{p} by GBSA	-1904.00	-1905.24	-1906.14	-1906.78	-1908.79	-1910.57
G_{solv} by GBSA	-1891.14	-1892.39	-1893.29	-1893.92	-1895.93	-1897.71
G_{np} by PBSA	16.91	16.91	16.91	16.91	16.91	16.91
G_{p} by PBSA	-1951.67	-1951.96	-1952.26	-1952.38	-1953.13	-1953.50
G_{solv} by PBSA	-1934.76	-1935.05	-1935.35	-1935.47	-1936.22	-1936.59
$T*S_{tran}$	15.61	15.61	15.61	15.61	15.61	15.61
$T*S_{rot}$	14.63	14.63	14.63	14.63	14.63	14.63
$T*S_{vib}$	295.92	295.92	295.92	295.92	295.92	295.92
$T*S_{tot}$	326.16	326.16	326.16	326.16	326.16	326.16
G_{total} by GBSA	-1089.20	-1090.45	-1091.35	-1091.98	-1093.99	-1095.77
G_{total} by PBSA	-1132.82	-1133.11	-1133.41	-1133.53	-1134.28	-1134.65

TABLE S10. Energy contributions of DOX-DNA complex in M3 that calculated from STP of MM-PB(GB)SA methods at different theoretical salt concentrations, $\Delta G_{uncorrected}$, and adding to the corresponding DNA deformation energy and then subtracting from ΔG_{con} to find corresponding standard free energy ΔG^0_{sim}.

Salt Con. (M)	0.1	0.15	0.2	0.25	0.5	1.0
Energy (kcal/mol)	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.
ΔE_{gas}	-409.08	-409.08	-409.08	-409.08	-409.08	-409.08
ΔG_{solv} by GBSA	358.98	359.23	359.44	359.57	359.96	360.40
ΔG_{solv} by PBSA	355.31	355.55	355.76	355.92	356.25	356.66
$\Delta T*S_{total}$	-19.65	-19.65	-19.65	-19.65	-19.65	-19.65
$\Delta G_{uncorrected}$ by GBSA	-30.45	-30.20	-29.99	-29.86	-29.47	-29.03
$\Delta G_{uncorrected}$ by PBSA	-34.12	-33.87	-33.67	-33.51	-33.18	-32.77
ΔG_{deform} by GBSA	17.42	17.44	17.46	17.47	17.56	17.62
ΔG_{deform} by PBSA	17.46	17.49	17.51	17.54	17.64	17.72
ΔG_{con}	-2.31	-2.31	-2.31	-2.31	-2.31	-2.31
ΔG^0_{sim} by GBSA	**-10.72**	**-10.45**	**-10.22**	**-10.08**	**-9.60**	**-9.10**
ΔG^0_{sim} by PBSA	**-14.35**	**-14.08**	**-13.85**	**-13.66**	**-13.23**	**-12.74**

TABLE S11. Energy contributions of DOX-DNA complex for M4 that calculated from STP of MM-PB(GB)SA methods at different theoretical salt concentrations, $\Delta G_{uncorrected}$, and adding to the corresponding DNA deformation energy and then subtracting from ΔG_{con} to find corresponding standard free energy ΔG^0_{sim}.

Salt Con. (M)	0.1	0.15	0.2	0.25	0.5	1.0
Energy (kcal/mol)	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.
ΔE_{gas}	-388.21	-388.21	-388.21	-388.21	-388.21	-388.21
ΔG_{solv} by GBSA	341.62	341.86	342.05	342.18	342.58	342.98
ΔG_{solv} by PBSA	338.25	338.50	338.68	338.80	339.21	339.62
$\Delta T*S_{total}$	-19.06	-19.06	-19.06	-19.06	-19.06	-19.06
$\Delta G_{uncorrected}$ by GBSA	-27.53	-27.29	-27.11	-26.97	-26.57	-26.17
$\Delta G_{uncorrected}$ by PBSA	-30.90	-30.65	-30.47	-30.35	-29.94	-29.53
ΔG_{deform} by GBSA	18.53	18.56	18.59	18.61	18.68	18.76
ΔG_{deform} by PBSA	18.65	18.68	18.73	18.75	18.80	18.87
ΔG_{con}	-2.31	-2.31	-2.31	-2.31	-2.31	-2.31
ΔG^0_{sim} by GBSA	**-6.69**	**-6.42**	**-6.21**	**-6.05**	**-5.58**	**-5.10**
ΔG^0_{sim} by PBSA	**-9.94**	**-9.66**	**-9.43**	**-9.29**	**-8.83**	**-8.35**

TABLE S12. Energy contributions of 2DOX-DNA complex for M5 that calculated from STP of MM-PB(GB)SA methods at different theoretical salt concentrations, $\Delta G_{uncorrected}$, and adding to the corresponding DNA deformation energy and then subtracting from ΔG_{con} to find corresponding standard free energy ΔG^0_{sim}.

Salt Con. (M)	0.1	0.15	0.2	0.25	0.5	1.0
Energy (kcal/mol)	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.
ΔE_{gas}	-819.99	-819.99	-819.99	-819.99	-819.99	-819.99
ΔG_{solv} by GBSA	727.17	727.67	728.07	728.29	729.16	729.99
ΔG_{solv} by PBSA	719.29	719.83	720.23	720.47	721.32	722.16
$\Delta T*S_{total}$	-24.82	-24.82	-24.82	-24.82	-24.82	-24.82
$\Delta G_{uncorrected}$ by GBSA	-68.00	-67.50	-67.10	-66.88	-66.01	-65.18
$\Delta G_{uncorrected}$ by PBSA	-75.88	-75.34	-74.94	-74.70	-73.85	-73.01
ΔG_{deform} by GBSA	26.94	26.98	27.01	27.06	27.14	27.26
ΔG_{deform} by PBSA	27.05	27.07	27.09	27.13	27.20	27.30
ΔG_{con}	-2.31	-2.31	-2.31	-2.31	-2.31	-2.31
ΔG^0_{sim} by GBSA	**-38.75**	**-38.21**	**-37.78**	**-37.51**	**-36.56**	**-35.61**
ΔG^0_{sim} by PBSA	**-46.52**	**-45.96**	**-45.54**	**-45.26**	**-44.34**	**-43.40**

TABLE S13. Energy contributions of 2DOX-DNA complex for M6 that calculated from STP of MM-PB(GB)SA methods at different theoretical salt concentrations, $\Delta G_{uncorrected}$, and adding to the corresponding DNA deformation energy and then subtracting from ΔG_{con} to find corresponding standard free energy ΔG^0_{sim}.

Salt Con. (M)	0.1	0.15	0.2	0.25	0.5	1.0
Energy (kcal/mol)	Ave.	Ave.	Ave.	Ave.	Ave.	Ave.
ΔE_{gas}	-806.17	-806.17	-806.17	-806.17	-806.17	-806.17
ΔG_{solv} by GBSA	720.54	721.04	721.40	721.69	722.53	723.39
ΔG_{solv} by PBSA	713.32	713.85	714.22	714.50	715.40	716.23
$\Delta T*S_{total}$	-24.65	-24.65	-24.65	-24.65	-24.65	-24.65
$\Delta G_{uncorrected}$ by GBSA	-60.98	-60.48	-60.12	-59.83	-58.99	-58.13
$\Delta G_{uncorrected}$ by PBSA	-68.2	-67.67	-67.3	-67.02	-66.12	-65.29
ΔG_{deform} by GBSA	28.51	28.55	28.6	28.65	28.73	28.79
ΔG_{deform} by PBSA	28.6	28.64	28.67	28.7	28.76	28.83
ΔG_{con}	-2.31	-2.31	-2.31	-2.31	-2.31	-2.31
ΔG^0_{sim} by GBSA	**-30.16**	**-29.62**	**-29.21**	**-28.87**	**-27.95**	**-27.03**
ΔG^0_{sim} by PBSA	**-37.29**	**-36.72**	**-36.32**	**-36.01**	**-35.05**	**-34.15**

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