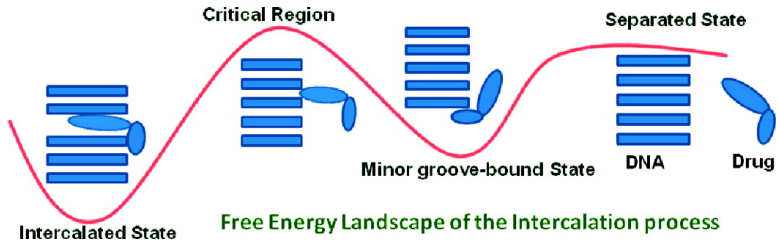
Article

On the Molecular Mechanism of Drug Intercalation into DNA: A Simulation Study of the Intercalation Pathway, Free Energy, and DNA Structural Changes

Arnab Mukherjee, Richard Lavery, Biman Bagchi, and James T. Hynes

J. Am. Chem. Soc., **2008**, 130 (30), 9747-9755 • DOI: 10.1021/ja8001666 • Publication Date (Web): 09 July 2008



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES

Published on Web 07/09/2008

On the Molecular Mechanism of Drug Intercalation into DNA: A Simulation Study of the Intercalation Pathway, Free Energy, and DNA Structural Changes

Arnab Mukherjee,*,†,‡ Richard Lavery,§ Biman Bagchi,|| and James T. Hynes†,⊥

Département de Chimie, CNRS UMR 8640 PASTEUR, Ecole Normale Supérieure, 24 Rue Lhomond, 75231 Cedex 05, France, Institut de Biologie et Chimie des Protéines, CNRS UMR 5086/Université de Lyon, 7 passage du Vercors, Lyon 69367, France, Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore 560012, India, and Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309-0215

Received January 8, 2008; E-mail: Arnab.Mukherjee@colorado.edu

Abstract: Intercalation into DNA (insertion between a pair of base pairs) is a critical step in the function of many anticancer drugs. Despite its importance, a detailed mechanistic understanding of this process at the molecular level is lacking. We have constructed, using extensive atomistic computer simulations and umbrella sampling techniques, a free energy landscape for the intercalation of the anticancer drug daunomycin into a twelve base pair B-DNA. A similar free energy landscape has been constructed for a probable intermediate DNA minor groove-bound state. These allow a molecular level understanding of aspects of the thermodynamics, DNA structural changes, and kinetic pathways of the intercalation process. Key DNA structural changes involve opening the future intercalation site base pairs toward the minor groove (positive roll), followed by an increase in the rise, accompanied by hydrogen bonding changes of the minor groove waters. The calculated intercalation free energy change is −12.3 kcal/mol, in reasonable agreement with the experimental estimate −9.4 kcal/mol. The results point to a mechanism in which the drug first binds to the minor groove and then intercalates into the DNA in an activated process, which is found to be in general agreement with experimental kinetic results.

Introduction

The anticancer anthracycline antibiotics are planar aromatic drug molecules that intercalate into DNA, i.e., insert between two successive DNA base pairs.[1–9] The drugs then inhibit topoisomerases either by stabilizing the cleaved DNA produced by topoisomerase II[10] or by preventing topoisomerase I to bind DNA;[11] some intercalators act as dual topoisomerase I/II inhibitors.[12,13] This topoisomerase inhibition stops DNA replication, leading to cell death. The relationship between intercalation and toxicity has been studied using different methods with mixed results.[14–17] Despite its importance, the mechanism that determines the kinetics of intercalation of anticancer drugs has received surprisingly little attention. The present study addresses the mechanism of intercalation into DNA of the drug daunomycin (also known as daunorubicin[18,19]), widely used in various cancer treatments,[20,21] by the calculation of a static free energy landscape for the process using an extensive set of all-atom simulations and umbrella sampling techniques.[22]

There exist a number of thermodynamic studies on several different drugs and their variants providing the free energy difference between the intercalated and free states.[3,4] Different

† Département de Chimie, CNRS.
‡ Present address: Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-0215.
§ Institut de Biologie et Chimie des Protéines, CNRS.
|| Indian Institute of Science.
⊥ University of Colorado.

(1) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18.
(2) (a) Berman, H. M.; Young, P. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87. (b) Qu, X.; Wan, C.; Becker, H.-C.; Zhong, D.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 14212.
(3) Remeta, D. P.; Mudd, C. P.; Berger, R. L.; Breslauer, K. J. *Biochemistry* **1993**, *32*, 5064.
(4) Chaires, J. B.; Satyanarayana, S.; Suh, D.; Fokt, I.; Przewloka, T.; Priebe, W. *Biochemistry* **1996**, *35*, 2047.
(5) Chaires, J. B.; Dattagupta, N.; Crothers, D. M. *Biochemistry* **1985**, *24*, 260.
(6) Macgregor, R. B.; Clegg, R. M.; Jovin, T. M. *Biochemistry* **1987**, *26*, 4008.
(7) Rizzo, V.; Sacchi, N.; Menozzi, M. *Biochemistry* **1989**, *28*, 274.
(8) Krishnamoorthy, C. R.; Yen, S. F.; Smith, J. C.; Lown, J. W.; Wilson, W. D. *Biochemistry* **1986**, *25*, 5933.
(9) Forster, W.; Stutter, E. *Int. J. Biol. Macromol.* **1984**, *6*, 114.
(10) Tewey, K. M.; Rowe, T. C.; Yang, L.; Halligan, B. D.; Liu, L. F. *Science* **1984**, *226*, 466.
(11) Crow, R. T.; Crothers, D. M. *J. Med. Chem.* **1994**, *37*, 3191.
(12) Denny, W. A.; Baguley, B. C. *Curr. Top. Med. Chem.* **2003**, *3*, 339.
(13) Denny, W. A. *Expert Opin. Investig. Drugs* **1997**, *6*, 1845.
(14) Snyder, R. D.; Ewing, D. E.; Hendry, L. B. *Environ. Mol. Mutagen.* **2004**, *44*, 163.
(15) Snyder, R. D.; Hendry, L. B. *Environ. Mol. Mutagen.* **2005**, *45*, 100.
(16) Ferguson, L. R.; Denny, W. A. *Mutat. Res.* **2007**, *623*, 14.
(17) Wilson, W. R.; Harris, N. M.; Ferguson, L. R. *Cancer Res.* **1984**, *44*, 4420.
(18) DiMarco, A.; Gaetani, M.; Orezzi, P.; Scarpinato, B.; Silvestrini, R.; Soldati, M.; Dasdia, T.; Valentini, L. *Nature* **1964**, *201*, 706.
(19) Myers, C. E.; Chabner, B. A. Anthracyclines. In *Cancer Chemotherapy: Principles and Practice*; Chabner, B. A., Collins, J. M., Eds.; Lippincott: Philadelphia, 1990; p 356.
(20) Weiss, R. B. *Semin. Oncol.* **1992**, *19*, 670.
(21) Minotti, G.; Menna, P.; Salvatorelli, E.; Cairo, G.; Gianni, L. *Pharmacol. Rev.* **2004**, *56*, 185.
(22) Torrie, G. M.; Valleau, J. P. *J. Comp. Phys.* **1977**, *23*, 187.

aspects of intercalating molecules have been studied using ultrafast methods.[2b] Kinetic studies of drug intercalation are relatively less common,[5–9] and general agreement on the mechanism has not been reached. For example, Chaires et al.[5] interpreted their kinetic data for the intercalation of the anthracycline drug daunomycin in terms of three steps: "outside" binding, intercalation and "reshuffling" of the drug in the intercalation site, while Rizzo et al.[7] proposed a five step kinetic model. Thus, while overall thermodynamic information is available, the detailed mechanism for drug intercalation remains uncertain. Accordingly, a detailed study on the molecular basis of the intercalation process is in order.

Theoretical studies of drug intercalation are few in number[23,24] and have mainly focused on the calculation of the overall free energy change using continuum solvent/environment approaches.[25–29] These rely on a simplified method of calculating the nonelectrostatic and electrostatic free energy contributions. However, this quite useful approach involves cancelation of large contributions (such as electrostatic and nonelectrostatic free energies, entropy loss etc.) to give a much smaller total free energy difference. Further, the role of molecular water is not reflected in these continuum-based studies. Finally, as noted above, the intercalation path's mechanistic aspects have not been studied. The present simulation study is a first effort to understand the intercalation process *per se* in atomistic detail and to elucidate its molecular aspects. The simulations provide an intercalation free energy in reasonable accord with experimental estimates, and point to a mechanism in which daunomycin first goes to a minor groove-bound state and then crosses a barrier to go to the intercalated state.

The outline of the remainder of this paper is as follows. We first give an overview of the simulation approach and then a recounting of the details of the simulations. The results are then presented and discussed, including a comparison with available experimental kinetic results. Finally, some concluding remarks are offered.

Materials and Methods

Drug, DNA Description; Simulation Approach. The anticancer drug daunomycin (Figure 1a) consists of an anthraquinone ring system (aglycon), the portion which intercalates into the DNA,[30] and an amino sugar group (daunosamine), which sits in the DNA minor groove.[30] We have carried out an extensive set of all-atom molecular dynamics simulations (>0.3 μs) in explicit water along with umbrella sampling techniques[22] to construct a free energy landscape and to examine other associated molecular aspects of daunomycin's intercalation into DNA.[18,19]

While we will subsequently discuss the intercalation in the direction free drug → intercalated drug, the actual calculations are performed by starting with the intercalated drug and forcing it toward the separated state along a chosen coordinate described more fully below. This calculation direction is chosen since the minimum free energy path to the intercalated state from a free state is not a priori evident and indeed is a principal object of study here. Umbrella sampling simulations were then performed starting from this initial intercalated state, and in addition from a minor groove-bound state. While the intercalated structure is constructed from the crystal structure,[30] the minor groove-bound state is hypothetical, and is selected as a plausible candidate for the intermediate "outside bound" state inferred in the experimental kinetics of daunomycin intercalation;[5] it is constructed by docking the drug into the DNA using the program HEX.[31]

Figure 1b shows the initial equilibrated intercalated state. The DNA sequence is d(GCGCACGTGCGC)$_2$. The intercalated structure's geometry is that of B-DNA except for the fifth to seventh base pairs (A5 to G7), which stay close to the crystal structure used for the starting conformation,[30] with a root-mean-square deviation of 3.2 Å for all the heavy atoms of those three basepairs. In this sequence, daunomycin is in contact with the strongest binding triplet sequence (A/T, CG).[32,33] Moreover, the inversion symmetry of the DNA oligomer studied means that inverting the intercalated drug 180° around its long axis will lead to a symmetrically identical state. The four terminal base pairs on each DNA end are chosen as CG base pairs to increase overall stability.[34] The DNA for the minor groove-bound state (Figure 1c) is constructed with a B-DNA geometry with the same sequence as for the intercalated state.

To calculate the free energy and other reaction path quantities of the drug−DNA complex as a function of separation between the two, we carry out the simulations with an extra harmonic "umbrella potential"[22] acting on the drug−DNA separation X (sampling coordinate) along a particular vectorial direction. Our coordinate choice is guided by (a) the necessity of pulling the drug out of the DNA intercalating center (IC), defined by the pair of base pairs involved in the intercalation, without affecting the drug's structure and (b) allowance of sampling of the drug on the minor groove side from where intercalation seems very likely to occur (given the experimentally observed position of the bulky daunomycin side chain in the minor groove following intercalation[30]). This sampling coordinate is measured by the projection (Figure 1d) of the vector $\vec{d}$ joining the center of mass (COM) of the IC to the COM of daunomycin onto a body-fixed unit vector $\hat{b}$, defined by the vector joining the COM of IC to the COM of two out of four ribose sugar groups (attached to the corresponding guanosine nucleotides) belonging to the intercalating set of base pairs which lie more toward the minor groove direction, i.e., $X = >\hat{b} \cdot \vec{d}$. A harmonic umbrella potential $U = 1/2k(X - X_0)^2$ is applied along the coordinate, where k is the spring constant and X_0 is the harmonic potential center.

To obtain overlapping distributions for X, we performed 35 simulations starting from the intercalated state to achieve the desired DNA-drug separation. These simulations for each umbrella window were 2.5 ns long after a previous 5 ns equilibration step. For simulations starting from the minor groove-bound state, 25 simulation windows, each of total 2.5 ns length (of which the initial 100 ps is the equilibration step), were employed. Each simulation along the sampling coordinate provides the system free energy changes as well as the DNA structural parameters and water features that help to characterize events associated with intercalation. Further simulation details are given in the next section.

(23) Baginski, M.; Fogolari, F.; Briggs, J. M. *J. Mol. Biol.* **1997**, *274*, 253.
(24) Trieb, M.; Rauch, C.; Wibowo, F. R.; Wellenzohn, B.; Liedl, K. R. *Nucleic Acids Res.* **2004**, *32*, 4696.
(25) Honig, B.; Nicholls, A. *Science* **1995**, *268*, 1144.
(26) Swanson, J. M.; Henchman, R. H.; McCammon, J. A. *Biophys. J.* **2004**, *86*, 67.
(27) Srinivasan, J.; Cheatham, T. E.; Cieplak, P.; Kollman, P. A.; Case, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 9401.
(28) Jayaram, B.; Sprous, D.; Beveridge, D. L. *J. Phys. Chem. B* **1998**, *102*, 9571.
(29) Zou, X.; Yaxiong, S.; Kuntz, I. D. *J. Am. Chem. Soc.* **1999**, *121*, 8033.
(30) Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Rich, A. *Biochemistry* **1987**, *26*, 1152.
(31) Mustard, D.; Ritchie, D. W. *Proteins* **2005**, *60*, 269.
(32) Chaires, J. B.; Herrera, J. E.; Waring, M. J. *Biochemistry* **1990**, *29*, 6145.
(33) Cieplak, P.; Rao, S. N.; Grootenhuis, P. D. J.; Kollman, P. A. *Biopolymers* **1990**, *29*, 717.
(34) Beveridge, D. L.; et al. *Biophys. J.* **2004**, *87*, 3799.
(35) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.
(36) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235.

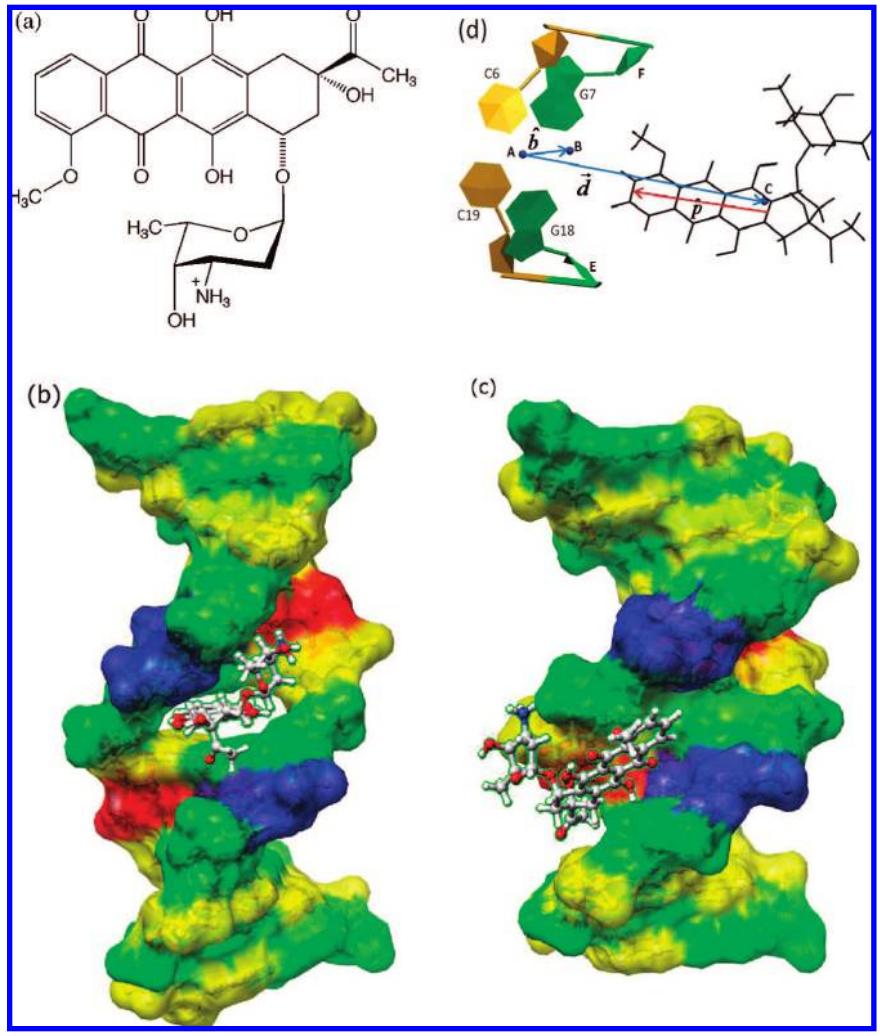


Figure 1. (a) Atomistic structure of daunomycin. Constructed and equilibrated structures of (b) the intercalated state and (c) the minor groove-bound state. The last two figures are made by Chimera.[35] In both figures, DNA is shown in a semitransparent surface representation including ball and stick atom models and residue-based color (yellow for C, green for G, red for A and blue for T), whereas daunomycin is represented via a ball and stick model with element-based CPK color. (d) Diagram of the body-fixed vector viewed along the DNA helical axis. "A" is the COM of four bases as labeled (C6, G7, C19, G18). "B" is the COM of the sugar groups of G7 and G18 marked as "F" and "E". "C" is the drug's COM. AB is the body-fixed vector $\hat{b}$ and AC is $\vec{d}$. $\hat{p}$ is the vector used to calculate an angle θ described in Figure 8.

Details of the Simulations. (a) Construction of the Intercalated and Minor Groove-Bound States. We obtained the intercalated daunomycin-DNA structure from the protein data bank (PDB),[36] with PDB id 1D11. This crystal structure with a 1.2 Å resolution, solved by Wang et al.,[30] has a repeating unit of four base pairs $d(ACGT)_2$ in which two daunomycin molecules are intercalated between two separate pairs of CG base pairs. Keeping the geometry of the three base pairs (ACG) intact, we added four base pairs above $d(CGCG)_2$ and five base pairs $d(ACGCG)_2$ below using the DNA structure building and minimization program JUMNA.[37] The complete DNA sequence is d(GCGCACGT-$GCGC)_2$. Daunomycin is intercalated at the C6-G7 site, and the sugar group of daunomycin is close to the A5 base pair.

For the minor groove-bound state, we constructed a 12 base pair DNA having the same sequence as for the intercalated DNA, but with standard B-DNA geometry using JUMNA.[37] The drug was then docked into the DNA minor groove using the docking program HEX.[31]

(b) Forcefield and Equilibration. All simulations have been carried out with the GROMACS molecular dynamics simulation package[38] with some modification to perform the umbrella sampling along our body fixed coordinate. Periodic boundary conditions are employed and Particle Mesh Ewald[39] is used for the long-range electrostatic interactions. The AMBER94 force-field[40] was selected because it well describes B-DNA[34] (a refinement to the AMBER94 force-field[41] has been published recently which rectifies the problem of α/γ transition observed in DNA trajectories longer than those studied here[42]), using AMBER ports in GROMACS.[43] Most daunomycin force-field parameters were obtained from AMBER94, with those absent in AMBER94 obtained from AMBER99[44] and

(37) Lavery, R.; Zakrzewska, K.; Sklenar, H. *Comput. Phys. Commun.* **1995**, *91*, 135.

(38) Berendsen, H. J. C.; Spoel, D. v. d.; Vandrunen, R. *Comput. Phys. Commun.* **1995**, *91*, 43.

(39) Darden, T.; York, D.; Pedersen, L. *J. Chem. Phys.* **1993**, *98*, 10089.

(40) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179.

(41) Perez, A.; Marchan, I.; Svozil, D.; Sponer, J.; Cheatham, T. E.; Laughton, C. A.; Orozco, M. *Biophys. J.* **2007**, *92*, 3817.

(42) The correction to the AMBER94 forcefield parmbsc0[41] is not used since most of the present work was performed prior to the publication. However, the problem in the α/γ transition generally occurs for longer simulations than those studied here.

(43) Sorin, E. J.; Pande, V. S. *Biophys. J.* **2005**, *88*, 2472.

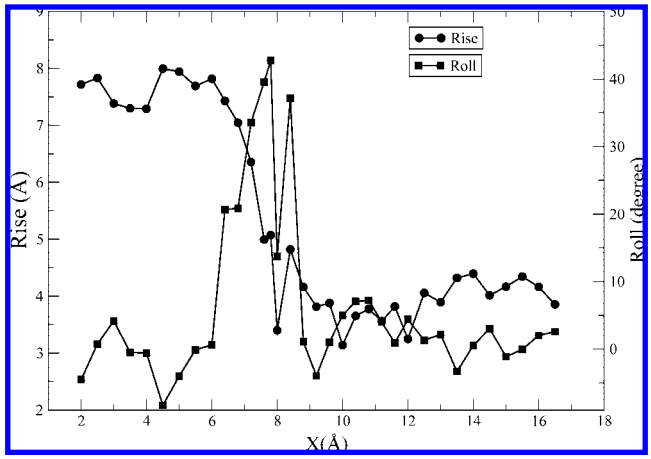


Figure 2. Calculated changes in the average rise and roll angle of the separated to the intercalated state transition along the sampling coordinate *X*. Note that the roll angle increases earlier than the rise as the intercalating drug approaches.

GAFF.[45] The RESP charges of daunomycin were calculated using the antechamber module[46] of AMBER7[47] molecular dynamics software and Gaussian03[48] using the Hartree−Fock method with the 6−31G* basis set.

The initial configurations of the intercalated and minor groove-bound states of the DNA/drug system were placed in a cubic box of side length 65 Å, and then solvated by TIP3P[49] water molecules. Twenty-two Na^+ ions and 1 Cl^- ion were placed randomly in the box to neutralize the DNA and positively charged daunomycin. In addition to the added ions, the system consists of 756 DNA atoms and 68 daunomycin atoms, solvated by 8822 water molecules, making a 27313 atom system. We also constructed a larger box of length 76.6 Å containing 14385 water molecules which allowed us to study large separation distances and to verify that the smaller box did not produce any artifacts in terms of the energetic or structural features of the intercalation process.

After initial configuration construction, we performed a standard equilibration protocol for DNA simulations.[34] The entire structure is minimized by the steepest descent method[50] to avoid close atomic contacts, followed by slow constant volume heating to 300 K over 100 ps using 2.4 kcal/mol/Å^2 harmonic restraints. These restraints were slowly reduced to zero during a series of energy minimization and 50 ps equilibration steps at constant temperature (300 K) and pressure (1 bar) with a 0.2 ps coupling constant for both parameters. The final equilibration step was a 100 ps constant volume run.

(c) Simulation Procedures. With this equilibrated system, we began a series of canonical ensemble simulations by placing the umbrella potential center close to the intercalated state and carrying out a 0.5 ns simulation. Subsequent simulations, each of 0.5 ns, were performed commencing with the previous simulations' end configuration by changing the umbrella potential center by only 0.4−0.5 Å to accelerate equilibration. For the intercalated to separated state transition, we performed 35 simulations, and for the minor groove-bound to separated state transition, 25 umbrella simulations were used. Bond lengths were constrained using the LINCS algorithm[51] and 2 fs integration time step for the simulations.

The above 0.5 ns length for each umbrella simulation gave a very large intercalation free energy (∼−30 kcal/mol). Progressively increasing that length to 5 ns reduced this to −12 kcal/mol); the PMF calculated using all the umbrella simulations for various run lengths showed that the results varied with run lengths, so that convergence with 5 ns umbrella simulations was not guaranteed. To examine this, we took the final configurations of each 5 ns umbrella simulation and performed a further 2.5 ns simulation, with random initial velocities. The PMF calculated for various time lengths using these 2.5 ns trajectories produced an intercalation free energy −13.2 kcal/mol within ± 0.5 kcal/mol, indicating satisfactory convergence (Supporting Information, Figure S1). The slow convergence of the intercalation pathway can be reasonably linked to the large structural changes which are required in the DNA oligomer. Such changes are absent for the transition from separated to minor groove-bound drug, and an umbrella simulation length of 2.5 ns was found to be sufficient for comparable convergence (Supporting Information, Figure S2).

Results and Discussion

(a) Structural Changes in DNA during Intercalation: Rise and Roll. Although DNA obviously has a complicated structure, there are nevertheless six essential degrees of freedom (dof) of the base pair steps in terms of which the DNA structure can be described reasonably well.[52] The translational dof are the rise, shift, and slide, while the rotational dof are roll, tilt, and twist. These base pair step parameters provide a standard description of the base pair geometry defined by the *Cambridge accord*.[53] We have monitored these parameters along each trajectory using the CURVES program.[54] Of these six parameters, the rise, roll and twist were found to be the important ones involved in the intercalation process. The rise denotes the distance between two base pairs along the DNA helical axis; its value in normal B-DNA is ∼3.4 Å.[55] The roll is the angle made by two base pairs toward the minor groove side, and is close to 0 in canonical B-DNA.

Figure 2 shows the average rise and roll values for each sampling window against the sampling coordinate *X* for the separated to intercalated state transition. Following the direction of the drug's approach from separated to intercalated state in this figure (i.e., from large to small *X*), the rise and roll values fluctuate around their normal values i.e., without any significant DNA structure change down to $X \approx 8.5$ Å. For smaller *X*, first the roll angle starts to increase (implying opening toward the minor groove side of the double helix), followed by the increase in the rise. The "front" part of the drug starts its insertion between the pair of base pairs around $X = 7.8$ Å, where the roll angle has its maximum. Once the drug intercalates further into the DNA, the roll angle drops again to its normal value, allowing further increase in the rise. This sequence appears to be a natural one for the intercalation.

(b) Signature of DNA Bending during Intercalation. Since the roll angle is the signature of local bending of the DNA, the change in the roll angle should have some effect also on the overall bending of the DNA. Therefore, we measured the overall bending of the DNA by calculating the ratio of the end-to-end

(44) Wang, J.; Cieplak, P.; Kollman, P. A. *J. Comput. Chem.* **2000**, *21*, 1049.
(45) Wang, J.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A. *J. Com. Chem.* **2004**, *25*, 1157.
(46) Wang, J. M.; Wang, W.; Kollman, P. A.; Case, D. A. *J. Mol. Graph Model* **2006**, *25*, 247.
(47) Case, D. A. *AMBER7*; University of California: San Francisco, 2002.
(48) Frisch, M. J. *Gaussian 03*, revision C.02; Gaussian Inc.: Wallingford, CT, 2003.
(49) Jorgensen, W. L. *J. Am. Chem. Soc.* **1981**, *103*, 335.
(50) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vetterling, W. T. *Numerical Recipes in FORTRAN: The Art of Scientific Computing*, 2nd ed.; Cambridge University Press: Cambridge, England, 1992.
(51) Hess, B.; Bekker, H.; Berendsen, H. J. C.; Fraaije, J. *J. Comput. Chem.* **1997**, *18*, 1463.
(52) Bhattacharya, D.; Bansal, M. *J. Biomol. Struct. Dyn.* **1988**, *6*, 93.
(53) Dickerson, R. E.; et al. *EMBO J.* **1989**, *8*, 1.
(54) Lavery, R.; Sklenar, H. *J. Biomol. Struct. Dyn.* **1989**, *6*, 655.
(55) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.

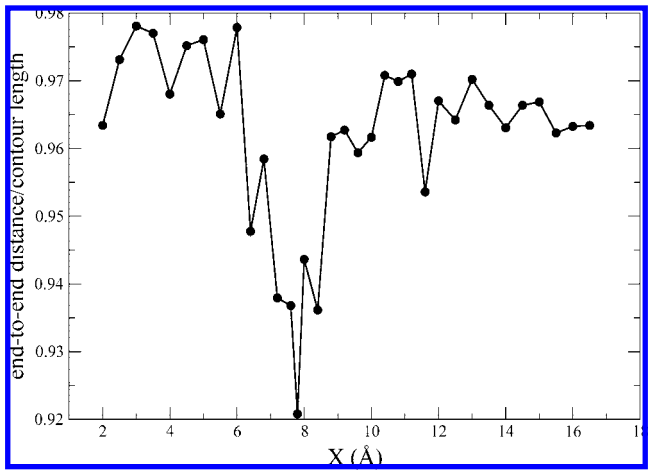


Figure 3. Bending of the DNA is observed along the intercalation process. Bending is measured by the ratio of the end-to-end distance and the contour length of the DNA.

distance to the DNA contour length using the program CURVES.[54] A decrease in the ratio signifies DNA bending. The average value (over the trajectory of each umbrella sampling simulation) of the above ratio is plotted in Figure 3. It is interesting to observe that DNA bending follows a trend similar to that of the roll angle, and a maximum DNA bending is observed around $X = 7.8$ Å, where the roll angle also has its maximum.

(c) DNA Twist during Intercalation. We also find the twist to be a significant basepair step degree of freedom for the intercalation. While a regular B-DNA has ~34° twist between two consecutive basepairs,[55] intercalation results in untwisting of the DNA,[30,56,57] to a degree depending on the drug.[30,56,57] Crystal structures of daunomycin show a slight 6° untwisting (twist angle 28°) in the pair of basepairs adjacent to the basepair involved in intercalation.[30,58,59] Solution sedimentation coefficient studies indicate a 11° untwisting for daunomycin intercalation[60] (and 15.4° for circular DNA[56]), although the precise location of the twist cannot be determined from these studies. By contrast, a recent all-atom simulation study on the structure of DNA-intercalated daunomycin has found a large untwisting (twist angle 6.6°) at the intercalation site when only one daunomycin is intercalated[61] and a smaller untwisting for each pair of basepairs (twist angle 19−21°) when two daunomycin molecules are intercalated in the DNA.[61]

Unfortunately, we have not been able to discern from our simulations any clear pattern of the average twist values along the sampling coordinate X, due to the presence of large fluctuations (Supporting Information, Figure S3). Accordingly, we limit the discussion to the simulated values of twist found for the pair of basepairs involved in the intercalation for the separated state (which corresponds to B-DNA) and the most stable intercalated state ($X = 3$ Å). The separated state twist value is 28° (±4°), which is lower than the experimental B-DNA twist value[55] ~34°. This underestimation is evidently due to the AMBER94 force-field[40] which is known to produce a slightly unwound DNA helical structure with a sequence-average twist of 30°.[34,62,63] In the intercalated state, we find a twist angle 17° (±5°) of the pair of basepairs involved in the intercalation, i.e, an untwisting of 11° for this pair. Note that the experimental 6° untwisting in the crystal structure occurs in the pair of basepairs *adjacent* to the pair involved in intercalation. The discrepancy concerning the position of the untwisting may arise from the difference in the intercalation sites for the crystal and simulated structures. In the former, two daunomycin molecules are intercalated in terminal pairs of basepairs[30] while in the present simulation, one daunomycin is intercalated in the middle of a twelve basepair long DNA. In addition, for free B-DNA, the experimental value of 34° quoted above is sequence-averaged and can vary considerably as a function of the actual base-sequence studied.[62,64]

(d) Hydration Changes during Intercalation. Osmotic stress experiments on intercalation of different drugs indicate differing water uptake by the DNA,[65,66] with an uptake of 18 water molecules observed for the daunomycin case.[65] The present all-atom explicit water simulations allow a direct measurement of the water involvement in the intercalation process. Thus, to address this phenomenon, we have calculated the number of water hydrogen (H)-bonds to the DNA intercalation site (three basepairs, A5-G7) and the drug in both the separated ($X = 16.5$ Å) and intercalated ($X = 3$ Å) states. We have also calculated the total number of water molecules in the first hydration shell (within distance of the first minimum in the proximal radial distribution function,[67–69] found to be 3.4 Å) of the same. We used two different H-bond criteria (i) HB1: the donor−acceptor distance is within 3.5 Å and the donor−acceptor-hydrogen angle is within 30°[70] and (ii) HB2: the maximum H-bond distance (H-acceptor) is 2.5 Å and the H-bond angle (donor-H-acceptor) is greater than 135°, the criterion employed by Feig and Petitt to calculate the number of H-bonds to C/G and A/T basepairs in the free DNA.[71]

Table 1 displays the calculated values of the H-bonds and total number of first hydration shell water molecules around the DNA intercalation site and the drug. The number of H-bonds and of the first hydration shell water molecules for the intercalation site (A5-G7) in the separated state agree well with the values calculated by Feig and Pettitt.[71] Further, the H-bond numbers calculated via the two criteria are very similar. To investigate via the Table 1 results the water uptake by the intercalated system compared to the free DNA, we first calculated the average number of water H-bonds and the average number of first hydration shell water molecules around the

(56) Fritzsche, H.; Triebel, H.; Chaires, J. B.; Dattagupta, N.; Crothers, D. M. *Biochemistry* **1982**, *21*, 3940.
(57) Tsai, C.-C.; Jain, S. C.; Sobell, H. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 628.
(58) Quigley, G. J.; Wang, A. H. J.; Ughetto, G.; Marel, G. V. D.; Boom, J. H. V.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204.
(59) Frederick, C. A.; Williams, L. D.; Ughetto, G.; Marel, G. A. V. D.; Boom, J. H. V.; Rich, A.; Wang, A. H.-J. *Biochemistry* **1990**, *29*, 2538.
(60) Waring, M. J. *J. Mol. Biol.* **1970**, *54*, 247.
(61) Trieb, M.; Rauch, C.; Wellenzohn, B.; Wibowo, F.; Loerting, T.; Mayer, E.; Liedl, K. R. *J. Biomol. Struct. Dyn.* **2004**, *21*, 713.
(62) Dixit, S. B.; Andrews, D. Q.; Beveridge, D. L. *Biophys. J.* **2005**, *88*, 3147.
(63) We did not use the more recent AMBER99[44] force field, which attempted to correct for the low twist, since this was not in fact successful and also introduced new problems.
(64) Cooper, V. R.; Thonhauser, T.; Puzder, A.; Schroder, E.; Lundqvist, B. I.; Langreth, D. C. *J. Am. Chem. Soc.* **2008**, *130*, 1304.
(65) Qu, X.; Chaires, J. B. *J. Am. Chem. Soc.* **1999**, *121*, 2649.
(66) Qu, X.; Chaires, J. B. *J. Am. Chem. Soc.* **2001**, *123*, 1.
(67) Mehrotra, P. K.; Beveridge, D. L. *J. Am. Chem. Soc.* **1980**, *102*, 4287.
(68) Mezei, M.; Beveridge, D. L. *Methods Enzymol.* **1986**, *127*, 21.
(69) Lounnas, V.; Pettitt, B. M. *Proteins-Structure Function Genetics* **1994**, *18*, 133.
(70) vanderSpoel, D.; vanMaaren, P. J.; Larsson, P.; Timneanu, N. *J. Phys. Chem. B* **2006**, *110*, 4393.
(71) Feig, M.; Pettitt, B. M. *J. Mol. Biol.* **1999**, *286*, 1075.

Table 1. Water Uptake upon Intercalation

sites[e]	HB1[a]	HB2[b]	total[c]
A5-G7 (free)	56.2	55.9	89.2
A5-G7(intercalated)	55.0	54.7	86.9
Drug (free)	11.0	11.0	45.0
Drug(intercalated)	9.2	9.2	32.2
A5-G7+drug (intercalated)	64.1	63.9	103.5
Uptake[d]	7.9	8.0	14.3

[a] H-bonds are defined when the donor−acceptor distance is within 3.5 Å and the acceptor−donor−hydrogen angle is within 30°. [b] H-bonds are defined such that the maximum hydrogen−acceptor distance is 2.5 Å and the H-bond angle (donor−hydrogen−acceptor) is more than 135°. [c] Number of water oxygen atoms for which the minimum distance from the DNA intercalation site is less than 3.4 Å. [d] The water uptake is calculated from the difference of A5-G7 in the free state from A5-G7+drug in the intercalated state. [e] "Free" and "intercalated" correspond to the configurations at $X = 16.5$ Å and $X = 3$ Å along the sampling coordinate.

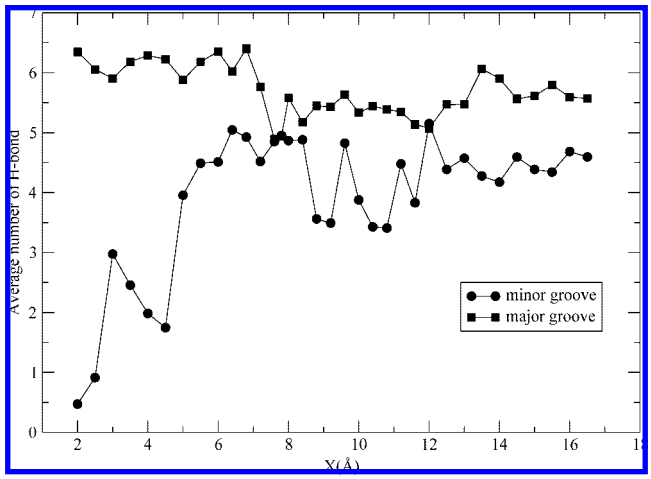


Figure 4. Average number of waters H-bonded to the minor and major groove atoms of the set of base pairs (C6-G7) involved in the daunomycin intercalation is plotted against the sampling coordinate *X*. Note first a decrease and then an increase in the number of H-bond to the minor groove atoms.

combined system of the DNA intercalation site and the drug in the intercalated state and then subtracted from that the values for the DNA intercalation site in the separated state. Thus the net changes in the H-bonds and first hydration shell water molecules due to intercalation are obtained. We find an increase of ∼8 H-bonds and ∼14 water molecules due to the daunomycin intercalation, in reasonable agreement with the experimental findings of Chaires et al.,[65] particularly in view of the fact that assessment of the water uptake calculation based on geometrical criteria involving only the first hydration layer could underestimate the results obtained by thermodynamic measurement. The components of this uptake can be readily addressed via Table 1. Upon intercalation, both the drug and the DNA intercalation site lose water. However, the loss of water by the DNA intercalation site is overcompensated by the water around the drug in the intercalated state.

We have also examined some local aspects of water's participation in the process of daunomycin intercalation. In particular, we monitored the number of water H-bonds (using HB1; HB2 gives similar results) formed specifically to the major and minor groove atoms of the pair of basepairs (C6-G7, without the sugar phosphate backbone) along the sampling coordinate *X*, plotted in Figure 4, for the separated to intercalated state transition.

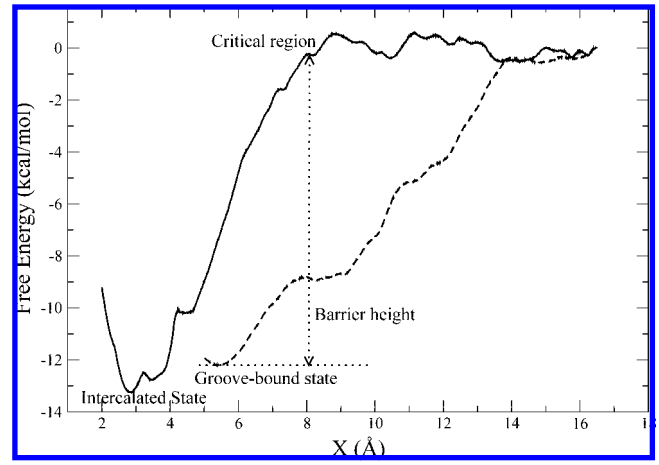


Figure 5. Potential of mean force (PMF) along the sampling coordinate *X* leading to intercalation (solid line, *X* decreases as the drug approaches DNA) and leading to the minor groove-bound state transition (dashed line). The dotted line shows the barrier height for intercalation from the minor groove-bound state.

As the drug approaches the DNA, there is a slight decrease in the average number of waters H-bonded to the minor groove atoms of the set of base pairs because of the obstruction created by the drug which hovers in fluctuating orientation just outside the entrance to the intercalation site. As the front part of the drug begins to intercalate (at $X \approx 8$ Å in Figure 4), water molecules in the minor groove arrange above and below the plane of the drug and an increase in the average number of H-bonds is observed. On further progress of intercalation of the drug, the daunosamine group, the acetyl and hydroxyl group (see Figure 1a) come close to the minor groove atoms of the set of base pairs (at $X < 5$ Å in Figure 4) and block the approach of water, so that the average number of H-bonds decreases significantly. For the major groove, the average number of water molecules remains constant (since the drug approaches from the minor groove side) until the drug intercalates and causes significant base pair separation (increased rise at $X \approx 7$ Å, see Figure 2) resulting (see Figure 4) in an increase in the average number of water H-bonds due to the increased space created, thus increasing the water accessibility to the pair of base pairs on the major groove side. The water H-bonding remains approximately constant for further progress of intercalation, since the DNA rise has been largely accomplished (see Figures 2 and 4).

(e) Potential of Mean Force of Intercalation. The potential of mean force (PMF) gives the system free energy along a chosen reaction coordinate, and has for example recently been calculated in atomistic detail for protein - small molecule association.[72,73] The umbrella sampling technique[22] allows obtaining our sampling coordinate (*X*) probability distributions, which then are combined by the weighted histogram analysis method (WHAM)[74,75] to obtain the PMF or free energy along *X*. This is useful not only to understand the driving force for intercalation, but also to give some information on its kinetic aspects. Figure 5 (solid line) shows the PMF along *X*. The separated state ($X = 16.5$) is taken to have a reference zero value for the free energy.

(72) Woo, H.-J.; Roux, B. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 6825.
(73) Lee, M. S.; Olson, M. A. *Biophys. J.* **2006**, *90*, 864.
(74) Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M. *J. Comput. Chem.* **1992**, *13*, 1011.
(75) Roux, B. *Comput. Phys. Commun.* **1995**, *91*, 275.

Table 2. Contributions to the ΔG Values[a] for the Transitions from the Separated to Intercalated and Minor Groove-Bound States

ΔG	PMF	$(-\Delta G_{conc})$	(ΔG_{ion})	total	expt
Intercalated	−13.2	+2.3	−1.4	−12.3 (±0.5)	−9.4[b] (±0.1)
Minor Groove-bound	−12.2	+2.3	−1.4	−11.3 (±0.1)	−7.5[c]

[a] Units are kcal/mol. [b] Value obtained from ref.[4] [c] Value estimated from the rate constants of the experimental kinetics results of the Chaires et al.[5] after correcting for ionic concentration. For the concentration and ionic contribution free energy corrections, see Supporting Information. The error estimates for the calculated and experimental values are shown in parentheses.

Several features of the daunomycin intercalation free energy profile in Figure 5 can be noted. First, the free energy minimum coincides with the crystal structure. Second, the PMF gives the difference in the free energy of the intercalated and separated states as ∼−13.2 kcal/mol. To compare this with the experimental estimate of this intercalation free energy, we need to include two corrections, both connected to concentrations (see Supporting Information). With these two corrections, the resulting intercalation free energy is −12.3 kcal/ mol (Table 2), which is in reasonable agreement with the experimental value (∼−9.4 kcal/mol).[4]

We note parenthetically that the calculated intercalation enthalpy −7.1 kcal/mol is also in reasonable agreement with the experimental estimate −8.2 kcal/mol,[76] although as is well-known,[77,78] such energetic calculations are subject to large uncertainties.

(f) Minor Groove-Bound State Analysis. We now investigate possible pathways of intercalation. To this end, we have created a minor groove-bound state which we consider a probable intermediate state in the intercalation process. Figure 5 shows the PMF of the separated state to the minor groove-bound state along the sampling coordinate X (dashed line), together with the corresponding PMF ending in the intercalated state for comparison.

Figure 5 indicates that the drug will first arrive at the minor groove-bound state following the minimum free energy path, i.e., there is a downhill free energy path to the minor groove when the daunomycin approaches the DNA from a completely separated state. Moreover, the drug's planar aglycon portion is farther out from the intercalating center than when the drug is in the minor groove. To provide a perspective of the real distance of the drug's intercalating part from the intercalating set of base pairs, Figure 6 displays the free energy of each umbrella window against the average radial distance of the aglycon center of mass from the intercalating center for the corresponding umbrella window.

(g) Minor Groove-Bound to Intercalated State Transition. We now consider the transition from the groove-bound state to the intercalated state. To intercalate starting from the former, the drug needs to be oriented in such a way that the tip of the drug's planar aglycon portion (Figure 1a) could insert between a pair of base pairs. This criterion compels the drug to be in a correct orientation at a correct distance ($X \approx 8$ Å, see below) from the intercalating base pairs. (Once the drug is intercalated, the orientation becomes automatically restricted due to the geometrical constraints imposed by the DNA.) The combination

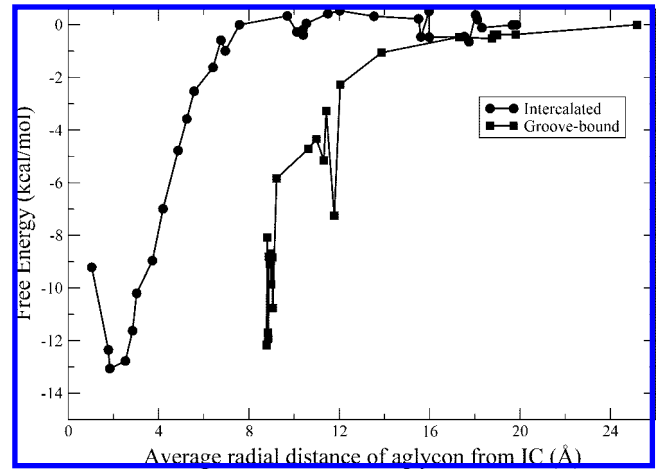


Figure 6. Free energy versus the average of the radial distance of the aglycon part of daunomycin from the intercalating center (IC; defined by the pair of base pairs (C6-G7) involved in the intercalation) for each umbrella window. The real distance of the aglycon part of the drug from the IC when it is in the minor groove-bound state is much larger than it appears to be in Figure 5.

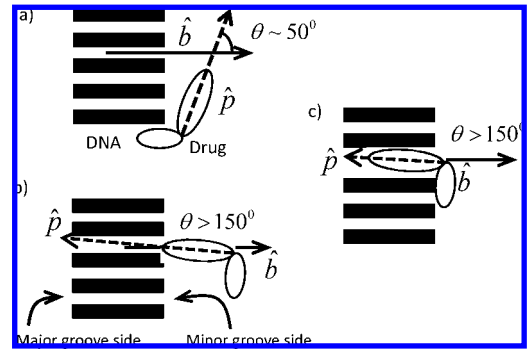


Figure 7. Schematic picture for the angle θ for (a) the minor groove-bound state, (b) the critical region and (c) the intercalated state. The DNA is shown as a stack of bars and the drug is shown as a combination of two ellipses. θ is the angle for the dot product of unit vectors $\hat{b}$ and $\hat{p}$, see Figure 1d.

of distance and orientation could be termed a "critical" or "gate" region (defined below) through which the drug has to pass to intercalate. An estimate of the barrier height can be derived from Fig. 5 by noting that the free energy is downhill from the critical region to the intercalated state. Therefore, the barrier height for the minor groove-bound to intercalated state transition would be obtained from the difference of free energy at the critical region at $X \approx 8$ Å (≈−0.3 kcal/mol) to the free energy of the minor groove-bound state (−12.2 kcal/mol) giving rise to a barrier of 11.9 kcal/mol. This value is fairly close to the experimental estimate of 14.9 kcal/mol calculated (see below) using the rate constant of k_{23} of the kinetic model of Chaires et al.[5]

(h) Two Dimensional (2D) Free Energy Landscape of Daunomycin Intercalation. Rigorous calculation of a 2D free energy surface for the present system is extremely difficult and time-consuming. However, an approximate calculation can give insight into the reaction path not readily obtained in a 1D approach. We have calculated two 2D free energy surfaces from two independent sets of simulations, one leading to the intercalated state from the separated state and another leading to the minor groove-bound state from the separated state, using the probability distributions of X and an angle θ and combining

(76) Remeta, D. P.; Mudd, C. P.; Berger, R. L.; Breslauer, K. J. *Biochemistry* **1991**, *30*, 9799.
(77) Kollman, P. *Chem. Rev.* **1993**, *93*, 2395.
(78) Stephen, H. F.; Charles, L. B., III *J. Chem. Phys.* **1987**, *87*, 3029.

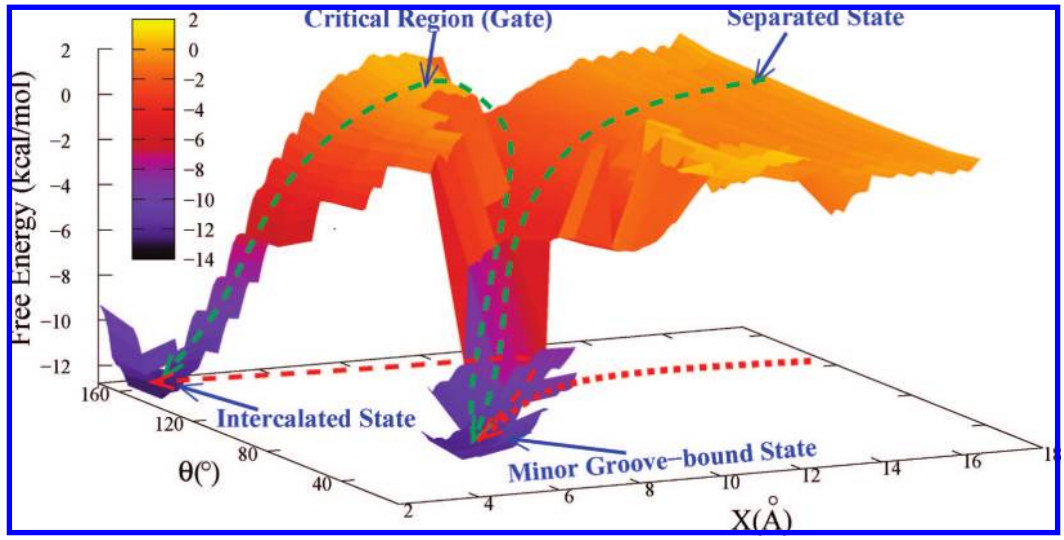


Figure 8. Free energy landscape in the umbrella sampling coordinate X and the angle θ (see Figures 1d and 7). The intercalated, minor groove-bound, and separated states are indicated, as is the critical region. The green arrowed dashed lines (and their red projections onto the X-θ plane) are schematic guides to show the most probable path from separated → minor groove-bound → intercalated state through the critical region (see Figure 7b). Due to the approximate nature of the calculations, it is not possible at this stage to say whether or not the small corrugations in the surface are physically meaningful.

the distributions using WHAM.[74,75] The second dimension θ is the angle made by the body-fixed vector ($\hat{b}$) and the vector ($\hat{p}$) parallel to the aglycon plane pointing toward the tip of the drug (see Figure 1d), and is a useful parameter to distinguish the minor groove-bound state from the intercalated state (Figure 7): θ is small (≈50°) for the former and large (>150°) for the latter. The essential approximation is that no umbrella potential was used for θ. The angle is instead free to fluctuate, which naturally does not ensure sufficient sampling in all regions of the individual free energy surfaces. To approximately overcome this, we have combined the two 2D free energy surfaces into a single 2D free energy surface (Figure 8), in which we average the values for each grid point wherever the two individual surfaces overlap.

The free energy contour plot Figure 8 indicates that to intercalate, the drug first follows the minimum free energy path to the minor groove-bound state. It then climbs up a free energy barrier by changing both distance and orientation to reach the critical region (loosely defined by $X \approx 8$ Å and $\theta > 150°$), which serves a gate for the subsequent intercalated state, and finally descends to the intercalated state. From Figure 8, the estimated free energy barrier for the minor groove-bound to intercalated state transition is ∼12 kcal/mol, very close to our previous estimate.

(i) Comparison with Experimental Kinetics Results. The calculations presented above for daunomycin intercalation into DNA have given an overall intercalation free energy in reasonable agreement with experimental estimates. They have indicated a sequence of DNA roll and rise distortions as well as changes in water H-bonds associated with the process. In terms of the free energy calculations presented, the overall mechanistic picture for the reaction pathway is, first, a downhill transition in free energy from the completely separated drug to a DNA minor groove-bound state, and second, an activated transition of the drug from this groove-bound state to the intercalated state, with a free energy barrier ∼11.9 kcal/mol (Figure 5). While these free energy calculations do not directly address the intercalation dynamics, we nonetheless discuss this mechanistic picture in the context of the two most extensive experimental kinetic studies for daunomycin intercalation (which as noted in the Introduction are not in complete accord with each other).

Chaires et al.[5] proposed a three sequential step mechanism from three different time scales obtained from stopped-flow absorption studies. The first step (with second order rate constant 3×10^6 $M^{-1}sec^{-1}$) was argued to be production of an "outside" bound state, while the unimolecular second step (with an estimated free energy barrier 14.9 kcal/mol) was interpreted as intercalation *per se*. The third and final step was interpreted as a further adjustment of either the intercalated drug or the DNA (All estimated 'experimental' free energy barrier heights in this section are calculated by assuming that the experimental unimolecular rate constants satisfy the Transition State Theory relation,[79] $k = k_B T/h\ e^{-\Delta G^{\#}/RT}$).

Rizzo et al.[7] also obtained three relaxation times from a stopped-flow study, but proposed a five-step kinetic model. A main difference from Chaires et al.[5] is that two different bimolecular association steps were proposed, one leading to a very weakly bound state, not leading directly to intercalation, while the second is on the pathway to intercalation and has a second-order rate constant (6×10^6 $M^{-1}sec^{-1}$) similar to that of Chaires et al.[5] Subsequent kinetic scheme steps were loosely associated with drug and/or DNA relaxation.[7]

The candidate in the present mechanism for comparison with the first bimolecular step on the pathway to intercalation of Chaires et al.[5] and Rizzo et al.[7] is the downhill first step to the minor groove-bound site. The experimental bimolecular rate is similar to that of a diffusion-limited association with orientation dependence,[80,81] which could be consistent with the character of the minor groove-bound state as an associated state with the drug in a certain restricted orientation. Thus, the minor groove-bound state would be identified as the "outside" bound state of Chaires et al.[5,8] However, one should note that the energetically favorable minor groove-bound state found here need not be unique, and could be one representative of several possible "outside" bound states in an experimental situation.

The candidate in the present mechanism for comparison with the second, unimolecular step on the pathway to intercalation

of Chaires et al.[5] and Rizzo et al.[7] is the activated transition from the minor groove-bound state to the intercalated state. This would be consistent with this step's identification in Chaires et al.[5] as an activated intercalation step. Indeed, the theoretical (∼11.9 kcal/mol) and experimental (14.9 kcal/mol)[5] barrier estimates are very similar. The corresponding step in the Rizzo et al.[7] kinetic scheme was not interpreted but has a similar rate constant.

The final unimolecular step in the mechanism proposed by Chaires et al.[5] was postulated to be an activated readjustment of either the intercalated drug or the DNA in the presence of the intercalated drug. (The Rizzo et al.[7] scheme replaces this single step with two branches, with a similar interpretation, with rate constants similar to the Chaires et al.[5] third step rate constant.) It is thus possible that the second and third steps observed by Chaires et al.[5] represent a first intercalation to less stable site, followed by intercalation to the most stable site, whereas our calculations involve intercalation to the most stable site. Another possibility is the following. It is important to recall that in the present PMF calculations the entire drug/DNA system is equilibrated at each sampling coordinate(s) value, as in Figures 5 and 8. In such calculations, any nonequilibrium dynamical character of adjustments in the intercalated drug/DNA system would be absent. It is thus possible that second and third step in Chaires et al.[5] are folded together, in an equilibrium path fashion, in the second, groove-bound state → intercalated state transition of the present work. This possibility can be investigated in the future via appropriate nonequilibrium simulations, for which the present study provides an important starting point.

Concluding Remarks

The extensive simulations presented here for daunomycin intercalation into DNA have given an overall intercalation free energy in reasonable agreement with experimental estimates. They indicate a sequence of DNA roll and rise distortions as well as changes in water H-bonds associated with the process. The free energy calculations presented suggest that the overall mechanistic picture for the reaction pathway is, first, a downhill transition in free energy from the completely separated drug to a DNA minor groove-bound state, and second, an activated transition of the drug from this groove-bound state to the intercalated state, with a free energy barrier ∼11.9 kcal/mol. Comparison with available kinetic results indicates reasonable quantitative agreement with this mechanistic picture, although the experiments suggest that the final intercalation step may be more complex than the one depicted in the present equilibrium calculations. This issue is currently under investigation, using the present results as a starting point.

Acknowledgment. We thank P. Kiefer, D. Laage, S. Re, and J. Nandakumar for discussions. This work was supported by CEFIPRA-IFCPAR (Project No. 3105-1).

Supporting Information Available: Figures showing the convergence of the PMF for intercalated and minor groove-bound state transitions; figure of the average twist value of the basepairs involved in intercalation along the sampling coordinate; free energy corrections due to drug and ionic concentration. Complete refs 34, 47, 48, and 53. This material is available free of charge via the Internet at http://pubs.acs.org.

JA8001666

(79) Fersht, A. *Enzyme Structure and Mechanism*; Freeman W. H. and Company: San Francisco, 1985.
(80) Janin, J. *Proteins* **1997**, *28*, 153.
(81) Northrup, S. H.; Erickson, H. P. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3338.