

Supporting Information

On the Molecular Mechanism of Drug Intercalation into DNA: A Computer
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; [§]
Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309-
0215, USA.

*Arnab.Mukherjee@colorado.edu

Convergence of the Potential of Mean Force (PMF) for Separated to Intercalated State Transition.

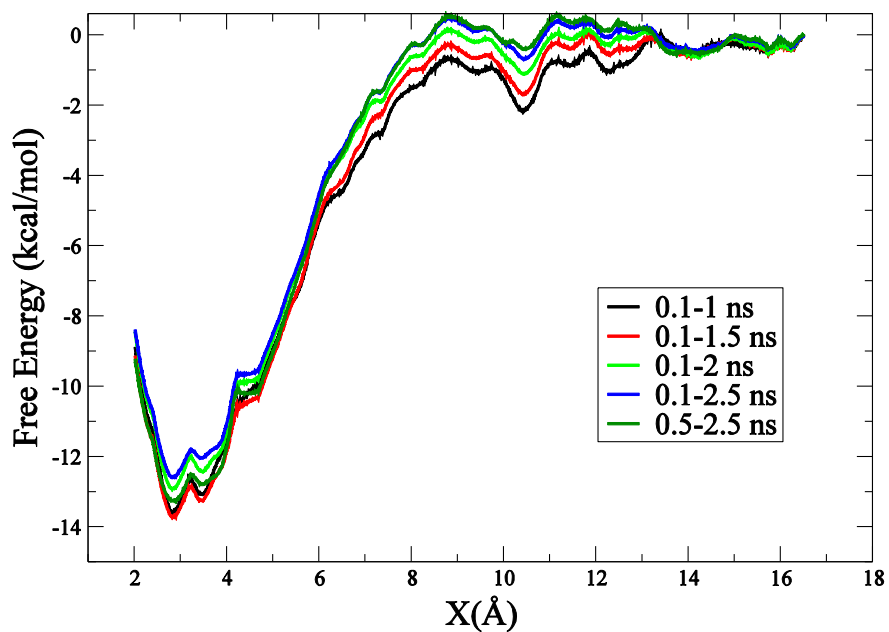


Figure S1. Convergence of the PMF for the separated state to intercalated state transition. Legends indicate the different time windows selected from each of the 35 simulations to construct the PMF. An initial 5 ns equilibration step was performed for each simulation.

Convergence of the Potential of Mean Force (PMF) for Separated to Minor Groove-bound State Transition.

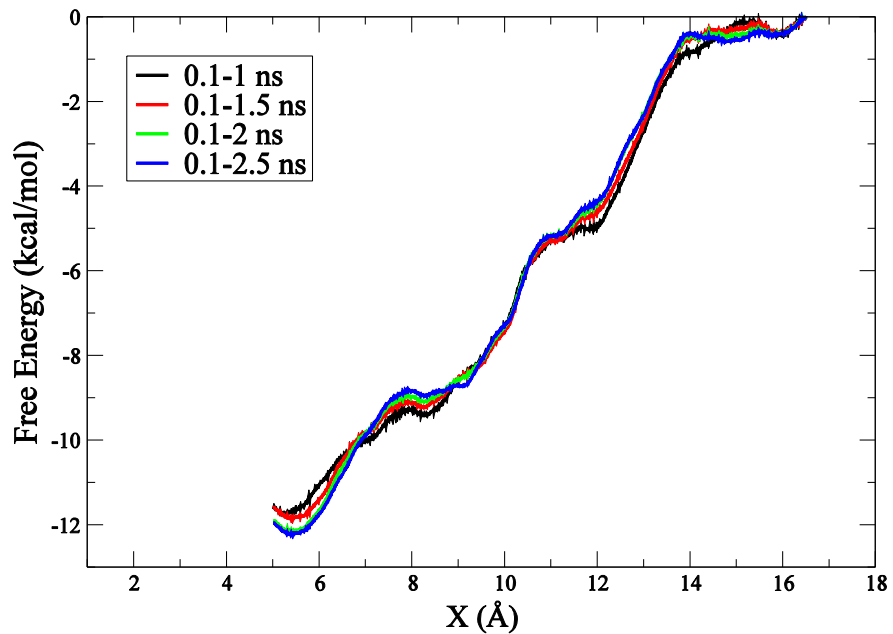


Figure S2. Convergence of the PMF for the separated state to minor groove-bound state transition. Legends indicate the different time windows selected from each of the 25 simulations to construct the PMF.

DNA Twist during Intercalation.

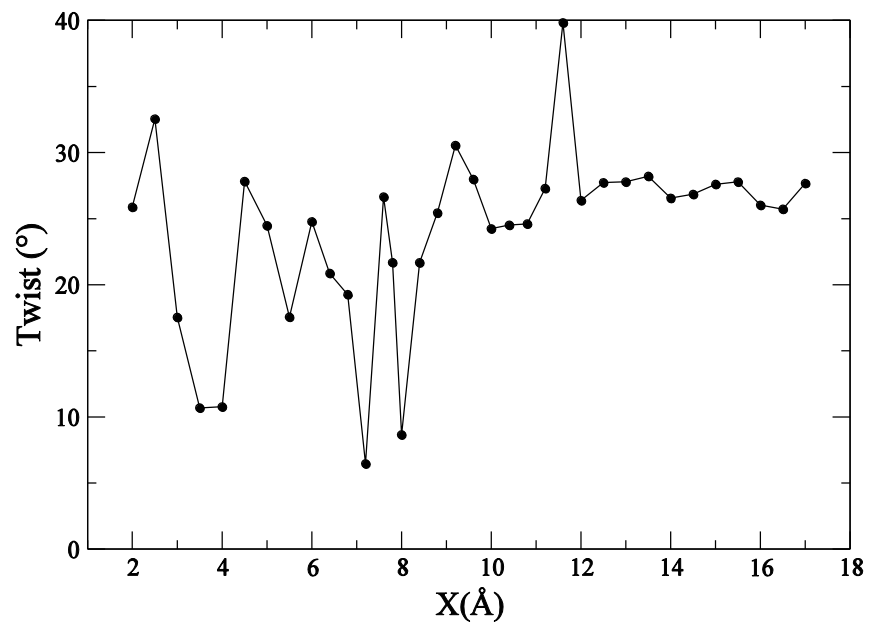


Figure S3. Average value of the twist for the pair of basepairs involved in the intercalation versus the sampling coordinate X . Note that there is untwisting of 11° for the intercalated state ($X=3\text{\AA}$) with respect to the separated state ($X=16.5\text{\AA}$).

Free Energy Corrections

Here we describe two corrections for the intercalation free energy necessary to compare the present results to experiment. The first of these is the correction to a standard drug concentration. The standard free energy of binding of B and A particles to form an AB particle is, in the dilute concentration limit¹, $\Delta G_{AB}^0 = -RT \ln \left(\frac{C^0 C_{AB}}{C_A C_B} \right) = -RT \ln K_{AB}$, where C^0 is the standard concentration 1M and the C_i 's are the respective species' concentrations. Here A denotes the drug and B denotes the binding site of the DNA, which is equal to the number of base pairs divided by the binding site size or the exclusion parameter, which is approximately 3 base pairs for daunomycin². Therefore C_A and C_{AB} cancel each other and a net difference in free energy appears due to the concentrations C_B and C^0 . Since $C_A = 0.006$ M (one molecule in a cubic box of 65 Å length) and $C_B = (12/3) C_A = 0.02$ M, this gives a free energy difference $\Delta G_{\text{conc}} = -RT \ln (C^0/C_B) = -2.3$ kcal/mol. This correction should be subtracted from our intercalation free energy (which is then increased) to compare with the experimental standard free energy (Table 2). The same correction has been applied for the transition to the minor groove, assuming the same exclusive parameter.

The second correction involves the free energy dependence on the solution ionic concentration. Experimentally, the binding constant K (and consequently the free energy) depends on the ionic concentration, which can be written as $m = \frac{\partial \log K}{\partial \log [Na^+]}$, where $[Na^+]$ is the positive ion concentration. For daunomycin, $m = -1.08^3$. The above equation indicates that magnitude of the (negative) intercalation free energy is smaller at higher ionic

concentration ($\Delta G^0 = -RT \ln K$). Due to the difference in ionic concentrations in the reported standard experimental daunomycin intercalation free energy estimate³ and in the present simulation, a correction ΔG_{ion} should be incorporated as,
$$\Delta G_{ion} = \Delta G_{exp} - \Delta G_{sim} = -mRT \ln \left(\frac{[Na_{exp}^+]}{[Na_{sim}^+]} \right)$$
. ΔG_{exp} is the experimental free energy at $[Na_{exp}^+] = 0.016$ M and ΔG_{sim} is the simulated free energy with the higher theoretical ionic concentration $[Na_{sim}^+]$ of 0.14 M (22 Na⁺ in 8822 water molecules). Therefore, $\Delta G_{ion} = -1.4$ kcal/mol. Since the theoretical ionic concentration is higher than the experimental one, the theoretical intercalation free energy magnitude is less negative than the experimental value by $|\Delta G_{ion}|$. The theoretical value should then be corrected by adding ΔG_{ion} as done in Table 2.

References

1. Gilson, M. K.; Given, J. A.; Bush, B. L.; McCammon, J. A. *Biophys. J.* **1997**, *72*, 1047.
2. Wang, A. H.-J.; Ughetto, G.; Quigley, G. J.; Rich, A. *Biochemistry* **1987**, *26*, 1152.
3. Chaires, J. B.; Satyanarayana, S.; Suh, D.; Fokt, I.; Przewlaka, T.; Priebe, W. *Biochemistry* **1996**, *35*, 2047.

Complete Author-list of the References of the Manuscript

34. Beveridge, D. L.; Barreiro, G.; Byun, K. S.; Case, D. A.; Cheatham, T. E.; Dixit, S. B.; Giudice, E.; Lankas, F.; Lavery, R.; Maddocks, J. H.; Osman, R.; Seibert, E.; Sklenar, H.; Stoll, G.; Thayer, K. M.; Varnai, P.; Young, M. A. *Biophys. J.* **2004**, *87*, 3799

47. Case, D. A.; Pearlman, D. A.; Caldwell, J. W.; III, T. E. C.; Wang, J.; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Merz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Tsui, V.; Gohlke, H.; Radmer, R. J.; Duan, Y.; Pitera, J.; Massova, I.; Seibel, G. L.; Singh, U. C.; Weiner, P.; Kollman, P. A., *AMBER 7*, University of California: CA San Francisco, 2002.

48. Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A., *Gaussian 03, Revision C.02*. 2003.

53. Dickerson, R. E.; Bansal, M.; Calladine, C. R.; Diekmann, S.; Hunter, W. N.; Kennard, O.; Kitzing, E. v.; Lavery, R.; Nelson, H. C. M.; Olson, W. K.; Saenger, W.;

Shakke, Z.; Sklenar, H.; Soumpasis, D. M.; Tung, C.-S.; Wang, A. H.-J.; Zhurkin, V. B.
EMBO J. **1989**, *8*, 1.