

การศึกษาเชิงคำนวณของผลจากดีเอ็นเอต่อพลังงานอิสระ ในสถานะกระตุ้นของสารเรืองแสง Cy3

A Computational Study on the Effect of DNA to the Excited State Free Energy of Cy3 Fluorescent Dye

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บทคัดย่อ

Cy3 คือสารเรืองแสงที่มักจะถูกนำไปใช้เป็นฉลากเพื่อศึกษาโปรตีนและดีเอ็นเอ ด้วยเทคนิคฟลูออเรสเซนส์ ซึ่งสิ่งแวดล้อมรอบ Cy3 จะมีผลต่อประสิทธิภาพในการเรืองแสง อันเนื่องมาจากกระบวนการโฟโตไอโซเมอไรเซชันที่แข่งขันกับการปล่อยฟลูออเรสเซนส์เพื่อกลับลงมายังสถานะพื้น ซึ่งโดยทั่วไปแล้วการไอโซเมอไรเซชันจะลดลงหาก Cy3 ถูกจำกัดการเคลื่อนที่และจะทำให้ฟลูออเรสเซนส์มีประสิทธิภาพมากขึ้น งานวิจัยนี้ได้คำนวณหาค่ากำแพงศักย์ของพลังงานอิสระในการเกิดโฟโตไอโซเมอไรเซชันของ Cy3 ทั้งในกรณีที่เป็นอิสระ (Free-Cy3) และที่ติดกับดีเอ็นเอสายคู่ (Cy3-dsDNA) ในเมทานอล โดยใช้ระเบียบวิธี Umbrella Sampling ร่วมกับ WHAM (The weighted histogram analysis method) จากผลการคำนวณพบว่าค่ากำแพงศักย์บนสถานะกระตุ้นของการเกิด “ทราน-ซิส” ไอโซเมอไรเซชันของ Cy3-dsDNA มีค่าสูงกว่า Free-Cy3 ทั้งนี้เนื่องจาก Cy3-dsDNA มีอันตรกิริยาที่เกิดจากการซ้อนกันของโครงสร้างวงแหวนระหว่างของ Cy3 กับของ primary strand และ complementary strand ของดีเอ็นเอสายคู่ จึงทำให้การหมุนของพันธะเป็นไปได้น้อยกว่า ส่งผลให้ค่ากำแพงศักย์ของ Cy3-dsDNA มีค่าที่สูงกว่า จึงทำให้เกิดไอโซเมอไรเซชันได้ไม่ดีและเป็นการเพิ่มการเกิดฟลูออเรสเซนส์นั่นเอง

คำสำคัญ: สารเรืองแสง Cy3 โฟโตไอโซเมอไรเซชัน ค่ากำแพงศักย์

Abstract

Cy3 is a fluorescent cyanine dye, which is commonly used as a label to probe proteins and DNA using fluorescence technique. The environment surrounding Cy3 can influence its photophysical and fluorescence properties. This is due to the competition between the fluorescence and photoisomerization for Cy3 deactivation. Typically, if Cy3 is rigidified (mostly due to its surrounding), the isomerization is reduced and thus enhancing the fluorescence. In this work, the excited state barrier for photoisomerization of free Cy3 and Cy3 attached to a double-stranded DNA in methanol are presented. The free energy calculation is performed by the umbrella sampling technique with the weighted histogram analysis method (WHAM). From the calculations, the barrier of Cy3 attached to the double-stranded DNA is higher than when it is free. The stacking interaction of Cy3 and DNA is found to influence its photophysics by preventing the torsional rotation of Cy3's trimethine bridge. Therefore it is more difficult to isomerize when Cy3 is attached to the DNA.

Keywords: Cy3, photoisomerization, energy barrier

1. Introduction

Cy3 is a cyanine dye, the oldest known class of synthetic dyes. It is widely used for labeling proteins and other biomolecules, especially DNA in fluorescence probing methods. As shown in Figure 1, Cy3 is a symmetrical cationic molecule in which two indole rings are linked by a trimethine bridge (Mojzych, & Henary, 2008). The bonds on the bridge are part of the conjugated system that contains π -electrons. The stable structure of Cy3 on the singlet ground state (S_0) is an all-*trans* form.

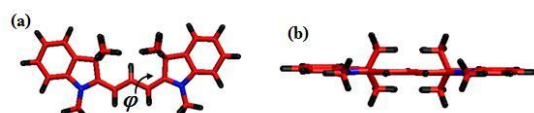


Figure 1. The optimized structure of Cy3, (a) is a top view and (b) is side view. The red, blue and black colors represent C, N and H atoms, respectively.

Upon Cy3's photoactivation to the first singlet excited state (S_1), it will relax via two main decay pathways to the ground state. These are a fluorescence process and a photoisomerization.

In such reaction, one of the bonds in the conjugated system rotates for 90° , called the intermediate twisted state (T). At this T state, Cy3 can rapidly shift to the ground state, following by evolution to either a *cis* conformation ($\phi=0^\circ$) or return back to a previous form, *trans* conformation ($\phi=180^\circ$) (Momicchioli, Baraldi, & Bertheir, 1988; Ponterini, & Momicchioli, 1991; Rulliere, 1976; Sanborn, Connolly, Gurunathan, & Levitus, 2007; Wei, XuDong et al, 2009) as shown in Figure 2.

However in most cases, Cy3 has to surpass a barrier on S_1 in order to reach the T state. From various observations, the microenvironment in which the dye is located in can influence Cy3's photoisomerization rate by altering the height of the

isomerization barrier (Cao et al., 2012; Nygren, Svanvik., & Kubista., 1998; Sanborn et al., 2007). In general, when Cy3 is attached to a biomolecule especially DNA, the photoisomerization rate decreases (implying the high barrier), concurrently increases the fluorescence quantum yield and lifetime (Lee, Henary, Strekowski, & Achilefu, 2008; Sanborn et al., 2007).

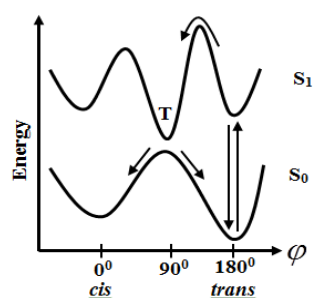


Figure 2. Schematic potential energy surfaces on the ground state (S_0) and the excited state (S_1) as functions of dihedral angle (ϕ) for photoisomerization of Cy3. T represents the intermediate twisted state.

In spite of many experimental studies of Cy3, the isomerization and fluorescence processes cannot be directly observed in a molecular level. Therefore a molecular simulation is a great approach for investigation of the dynamical phenomena of Cy3 in a microenvironment to gain an insight of the photoisomerization mechanism. So far, the computational studies of the photophysics of Cy3 and related cyanine dyes have been performed by several groups using various methods such as the density functional theory (DFT) (Spiriti, Binder, Levitus, & Vaart, 2011), a complete active space self consistent field (CASSCF) (Sanchez-Galvez et al., 2000), the

AM1 semiempirical method (Park, 2000), and the time-dependent density functional theory (TDDFT) (Cao et al., 2012).

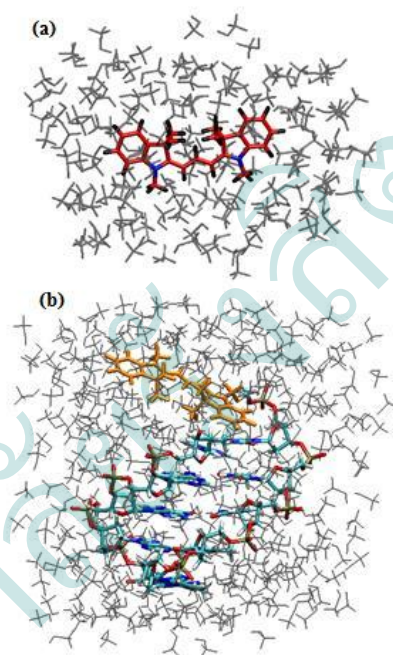


Figure 3. The optimized structures of free-Cy3 in methanol (a) and Cy3-dsDNA (b). The red, blue and black colors represent C, N and H atoms in free-Cy3. The orange color represents Cy3 in Cy3-dsDNA system. The methanol molecules are displayed in gray color.

Thus far, there are still a limited number of computational studies that consider the dynamical effects on the photoisomerization of Cy3. Therefore, in this work the excited state dynamics simulations are performed to obtain the free energy profiles of Cy3 by the umbrella sampling with the weighted histogram analysis method (WHAM). Ultimately, the isomerization barrier will be determined from the free energy profile to provide a more complete explanation for the mechanisms of Cy3 deactivation when it is free and when it is attached to DNA in solvated

environment. The QM/MM method is employed in this work. This is because the photophysics of Cy3, which involves both ground and excited state, requires the quantum mechanical (QM) description. Unfortunately, the QM method can only handle small molecules of 20-30 atoms. The MM method is then needed for treating Cy3's surrounding due to its apparent effects to the photoisomerization of Cy3. In the simulations, Cy3 is put in two different conditions. These are free Cy3 (free-Cy3); and Cy3 attached to the 5' end of the double-stranded DNA (Cy3-dsDNA), as shown in Figure 3. In all conditions, the free and DNA-attached Cy3 are solvated with methanol due to its low solubility in water.

2. Objective

To find the free energy of free-Cy3 and Cy3-dsDNA in methanol by umbrella sampling with QM/MM calculations for understanding the behavior of Cy3 on excited state in different surroundings.

3. Methodology

In QM/MM method in this work, Cy3 is calculated by QM principles while DNA and methanol is treated by MM (with classical theories). The semiempirical method is employed for the QM part due to a large size of Cy3 (56 atoms). Because of the multiple electronic state nature of Cy3's photoisomerization, the floating occupation molecular orbital configuration interaction (FOMO-CI) method (Granucci, & Toniolo, 2000) is used in the semiempirical calculations. FOMO-CI is suitable for excited state calculations since it allows for the

consideration of the multiple configurations of electrons so that the more accurate energies can be obtained (Granucci, & Toniolo, 2000). In this method, the orbitals in the active space can have fractional occupation numbers with the condition that the total number of electron remains constant. These active orbitals are first obtained via an optimization with a self-consistent field (SCF) calculation. The CAS-CI calculation is then performed in these orbitals (with optimized fractional numbers of electrons) (Granucci, & Toniolo, 2000). The FOMO-CI approach has been implemented in MOPAC2000 (Stewart, 1999) software package and is used for all geometry optimizations and energy calculations of the QM region, Cy3. The active space is composed of 4 orbitals with total 4 electrons. For better description of the excited state, the reoptimized semiempirical parameters (Punwong, Owens, & Martinez, 2014) are used. This parameter set was reparameterized for the conjugated systems and had been verified in the studies of photoisomerizable molecules (Punwong, Martinez, & Hannongbua, 2014; Punwong et al., 2014; Virshup et al., 2009). For the MM region, the double-stranded DNA is described by the AMBER force fields (Cornell et al., 1995; Kollman & Weiner, 1981; Weiner et al., 1984) and methanol molecules are described by the OPLS-AA force field (Jorgensen, Maxwell, & Tirado-Rives, 1996). The DNA sequence is TTCTT on the primary strand, same as the first five DNA bases in the experiments of Sanborn and co-workers (Sanborn et al., 2007). Consequently, the sequence in the complimentary strand is AAGAA.

The first T base on the 5' end is bonded to Cy3 via a three-carbon linker.

As mentioned before, the photophysics of Cy3 involves dynamical behaviors. The dynamics simulation is therefore necessary. However, the nanosecond time scale (Sanborn et al., 2007; Valeur, 2002) for the photoisomerization of Cy3, starting from the excited state and eventually relaxing back to the ground state is presently not practical to be directly simulated. Therefore, the umbrella sampling (Torrie & Valleau, 1977) is employed to determine the free energy and hence the energy barrier. The barrier height indicates how difficult for Cy3 to isomerize. The umbrella sampling approach is designed to facilitate the sampling of a hardly explored region in the energy landscape (which could take long computational time for direct dynamics simulations). This can be done by adding a biasing (umbrella) potential to warrant the sampling of the entire range of the interested reaction coordinate (Torrie & Valleau, 1977). In this work, the reaction coordinates (ϕ) is one of the dihedral angles on the conjugated bridge shown in Figure 1 that can undergo the rotation in the excited-state isomerization of Cy3 (Cao et al., 2012; Rodriguez, Scherlis, Estrin, Aramendia, & Negri, 1997; Sanborn et al., 2007; Wang, Zhou, Huang, & Ye, 2011).

In the umbrella sampling, the value of the dihedral angle ϕ_0 is separated into 12 windows (10° wide), each centered at 70° , 80° , ..., 180° . These are the constraints of the umbrella potential in a form of simple harmonic oscillator function, $k(\phi - \phi_0)^2$. The

force constant (k) for the umbrella potential is 100 kcal/mol. Then the constrained dynamics simulation on the excited state for each window is performed by the FMS program suite (Ben-num & Martinez, 2002; Ben-Nun, Quenneville, & Martinez, 2000), in which the umbrella sampling procedure is installed. After finishing the dynamics in all windows, the probability distributions (these are the biased one) of each window is stitched together by WHAM (Kumar, Djamal, Robert, Kollmana, & Rosenbergl, 1992). Also through WHAM, the unbiased probability distribution is obtained by removing the biasing effect from the umbrella sampling. The free energy (ΔG), which is proportional to the negative logarithmic of the probability distribution ($P(\phi)$), is then calculated (equation 1). All of these are performed by Grossfield's WHAM software package (Grossfield, 2007). The umbrella sampling of Cy3 isomerization is performed for both free-Cy3 and Cy3-dsDNA conditions.

$$\Delta G = -k_B T \ln(P(\phi)) \quad (1)$$

where k_B is Boltzmann constant and T is temperature.

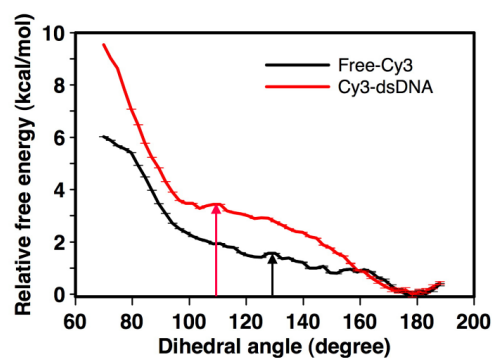


Figure 4. Relative free energy profile as a function of the dihedral angle for free-Cy3 (black) and Cy3-dsDNA (red).

4. Results and Discussion

From the umbrella sampling simulations, the energy barriers for *trans-cis* isomerization are 1.8 kcal/mol for free-Cy3, and 3.4 kcal/mol for Cy3-dsDNA as can be seen in Figure 4. These results are in good agreement with Sanborn and coworkers' report on the fluorescence quantum yield that the barrier of Cy3-dsDNA is higher than of free-Cy3 (Sanborn et al., 2007).

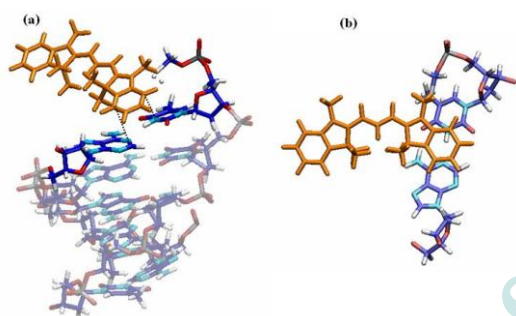


Figure 5. The configurations showing the stacking of Cy3 onto the dsDNA from side view (a) and top view (b). The black dotted lines in (a) are distances of stacking between the ring of terminal indole of Cy3 and the first base of primary and complementary strands. The Cy3 molecule is shown in orange.

The results seen in the work are possibly due to the influence of the Cy3 and DNA interactions. According to Harvey et al., the stacking interaction between Cy3 and nucleobases lowers the efficiency of photoisomerization as a result of an increased barrier (Harvey & Levitus, 2009). These can be seen from the enhanced fluorescence efficiency and lifetime (Harvey & Levitus, 2009). In order to investigate such stacking interaction in this work, the stacking distances between the atoms on the ring of terminal indole close to DNA and of T base of DNA primary strand (also to

A base on the complementary strand) are calculated. Each distance is measure from atoms on Cy3 and the base as illustrated in Figure 5. As shown in Figure 6, it was found that the distances from Cy3 to the T and A bases are approximately 4.0-4.5 Å, implying an almost persistent stacking interaction. It then can be inferred that these interactions prevent the torsional rotation, and lead to the higher energy barrier.

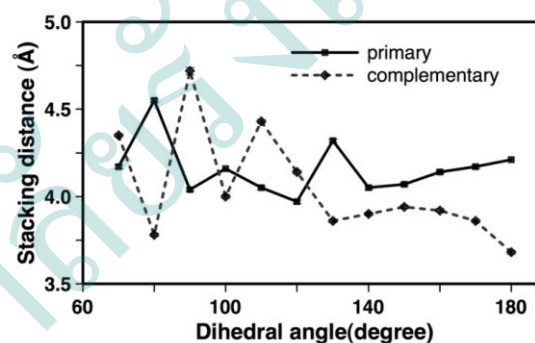


Figure 6. Stacking distances as functions of the dihedral angle (ϕ) from umbrella sampling. The distance is averaged from the 2 ps dynamics run in each window. The results from primary and complementary strands are in solid and dotted line, respectively.

5. Conclusion

The excited state free energy of Cy3 when it is free or attached to the double-stranded DNA is investigated using the QM/MM dynamics simulations with umbrella sampling technique and WHAM. The results indicate that the attachment of DNA to Cy3 is important in its fluorescence properties. Since the fluorescence emission is competed primarily by the *trans-cis* isomerization for Cy3 relaxation to the ground state, thus the isomerization barrier can be used as an indicator of fluorescence efficiency. From

this work, the barrier for *trans-cis* isomerization on the excited state is increased when Cy3 is attached to the double-stranded DNA, in good agreement with the experiments. The raised barrier is due to the stacking interaction between Cy3 and DNA bases that rigidifies the structure of the dye and prevents its rotational movement. Thus, Cy3-dsDNA is deactivated more efficiently by fluorescence emission than by *trans-cis* isomerization.

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7. References

- Ben-num, N., & Martinez, T. J. (2002). Ab initio quantum molecular dynamics. *Adv. Chem. Phys.*, 121, 439.
- Ben-Nun, M., Quenneville, J., & Martinez, T. J. (2000). Ab Initio Multiple Spawning: Photochemistry from First Principles Quantum Molecular Dynamics. *J. Phys. Chem. A*, 104A, 5161.
- Cao, J., Wu, T., Hu, C., Liu, T., Sun, W., Fan, J., & Peng, X. (2012). The nature of the different environmental sensitivity of symmetrical and unsymmetrical cyanine dyes: an experimental and theoretical study. *Phys. Chem. Chem. Phys.*, 14, 13702-13708.
- Cornell, W., Cieplak, P., Bayly, C., Gould, I., Merz, J. K., Ferguson, D., . . . Kollman, P. (1995). A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules. *J. Am. Chem. Soc.*, 117, 5179-5197.
- Granucci, G., & Toniolo, A. (2000). Molecular gradients for semiempirical CI wavefunctions with floating occupation molecular orbitals. *Chem. Phys. Lett.*, 32(1-3), 79-85.
- Grossfield, A. (2007). WHAM. version 2.0.2.
- Harvey, J. B., & Levitus, M. (2009). Nucleobase-Specific Enhancement of Cy3 Fluorescence. *J. Fluoresc.*, 19, 443-448.
- Jorgensen, W. L., Maxwell, D. S., & Tirado-Rives, J. (1996). Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J. Am. Chem. Soc.*, 118, 11225-11236.
- Kollman, P. A., & Weiner, P. K. (1981). AMBER: Assisted model building with energy refinement. A general program for modeling molecules and their interactions. *J. Comput. Chem.*, 2, 287-303.
- Kumar, S., Djamal, B., Robert, S., Kollmana, P., & Rosenbergl, J. (1992). The Weighted Histogram Analysis Method for Free-Energy

- Calculations on Biomolecules. I. The Method. *J. Comput. Chem.*, 13(8), 1011-1021.
- Lee, H., Y., B. M., Henary, M., Strekowski, L., & Achilefu, S. (2008). Fluorescence lifetime properties of near-infrared cyanine dyes in relation to their structures. *J. Photochem. Photobiol., A*, 200, 438-444.
- Mojzych, M., & Henary, M. (2008). *Synthesis of Cyanine Dyes* (Vol. 14). Berlin Heidelberg: Springer-Verlag.
- Momicchioli, F., Baraldi, I., & Bertheir, G. (1988). Theoretical-study of trans cis photoisomerism in polymethine cyanines. *Chem. Phys.*, 123, 103-112.
- Nygren, J., Svanvik, N., & Kubista, M. (1998). The Interactions Between the Fluorescent Dye Thiazole Orange and DNA. *Biopolymers.*, 46, 39-51.
- Park, J. (2000). AM1 semiempirical calculated potential energy surfaces for the isomerization of symmetrical carbocyanines. *Dyes Pigments.*, 46, 155-161.
- Ponterini, G., & Momicchioli, F. (1991). Trans cis photoisomerization mechanism of carbocyanines - experimental check of theoretical-models. *Chem. Phys.*, 151, 111-126.
- Punwong, C., Martinez, T. J., & Hannongbua, S. (2014). Direct QM/MM simulation of photoexcitation dynamics in bacteriorhodopsin and halorhodopsin. *Chem. Phys. Lett.*, 610-611(0), 213-218.
- Punwong, C., Owens, J., & Martinez, T. J. (2015). Direct QM/MM Excited-State Dynamics of Retinal Protonated Schiff Base in Isolation and Methanol Solution. *J. Phys. Chem. B.*, 119(3), 704-714.
- Rodriguez, J., Scherlis, D., Estrin, D., Aramendia, P. F., & Negri, R. M. (1997). AM1 Study of the Ground and Excited State Potential Energy Surfaces of Symmetric Carbocyanines. *J. Phys. Chem. A.*, 101, 6998-7006.
- Rulliere, C. (1976). Laser action and photoisomerization of 3,3'-diethyl oxadiazocarbocyanine iodide (dodci) -influence of temperature and concentration. *Chem. Phys. Lett.*, 43, 303-308.
- Sanborn, M. E., Connolly, B. K., Gurunathan, K., & Levitus, M. (2007). Fluorescence Properties and Photophysics of the Sulfoindocyanine Cy3 Linked Covalently to DNA. *J. Phys. Chem. B.*, 111, 11064-11074.
- Sanchez-Galvez, A., Hunt, P., Robb, M. A., Olivucci, M., Vreven, T., & Schlegel, H. B. (2000). Ultrafast Radiationless Deactivation of Organic Dyes: Evidence for a Two-State Two-Mode Pathway in Polymethine Cyanines. *J. Am. Chem. Soc.*, 122, 2911-2924.
- Spiriti, J., Binder, J. K., Levitus, M., & Vaart, A. (2011). Cy3-DNA Stacking Interactions Strongly Depend on the Identity of the Terminal Basepair. *Biophys. J.*, 100, 1049-1057.

- Stewart, J. J. P. (1999). MOPAC 2000. Tokyo, Japan: Fujitsu Limited.
- Torrie, G. M., & Valleau, J. P. (1977). Non-Physical Sampling Distributions in Monte-Carlo Free-Energy Estimation - Umbrella Sampling. *J. Comput. Phys.*, 23, 187-199.
- Valeur, B. (2002). *Molecular Fluorescence: Principles and Applications*: Wiley-VCH.
- Virshup, A. M., Punwong, C., Pogorelov, T. V., Lindquist, B. A., Ko, C., & Martinez, T. J. (2009). Photodynamics in complex environments: ab initio multiple spawning quantum mechanical/molecular mechanical dynamics. *J. Phys. Chem. B.*, 113(11), 3280-3291.
- Wang, S. F., Zhou, T., Huang, Y. C., & Ye, S. Y. (2011). DFT Investigation on the Trans-cis Photoisomerization of Pentamethine Cyanine Dye Model Molecule. *Chinese J. Struct. Chem.*, 30(3), 401-411.
- Wei, L., XuDong, C., Damir, A., & AnDong, X. (2009). Single molecule fluorescence fluctuations of the cyanine dyes linked covalently to DNA. *Sci China Ser B-Chem.*, 52, 1148-1153.
- Weiner, S. J., Kollman, P. A., Case, D. A., Singh, U. C., Ghioand, C., Alagona, G., . . . Weiner, P. (1984). A New Force Field for Molecular Mechanical Simulation of Nucleic Acids and Proteins. *J. Am. Chem. Soc.*, 106, 765-784.