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Molecular Dynamics Simulations on DNA-Chromophore Complexes

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14. ABSTRACT

Molecular dynamics simulations are performed to study the effects of the hydrophobic constituents of cyanine dye, Cy5, on the binding propensity. The umbrella sampling method, one of several advanced sampling techniques, is employed to compute the potential of mean force between dyes in solution and to establish the relationship between the chemical and structural properties of dyes and the binding propensity. It is demonstrated that the binding strengths of dyes are highly correlated to the hydrophobicity values. More detailed free energy landscape of the dye-dye interactions is generated by the extensive two-dimensional umbrella sampling simulations, which showed the dyes predominantly interacting via stacked conformation. The method can easily be applied to more complex DNA-chromophore systems to study the effect of DNA scaffold on the dye-dye interactions.

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Molecular Dynamics Simulations on DNA-chromophore Complexes

Youngchan Kim (6393)

1. Objective

Develop computational framework to enhance the energy transfer in the DNA-organized chromophore systems

2. Background

The DNA-organized chromophores exhibit highly efficient excitation energy transfer, which is central to many nanophotonic applications such as OLED displays, dye-sensitized solar cells, and fluorescence-based sensing and bioimaging, as well as quantum computing. Therefore, understanding the microscopic origin of the energy transfer in the DNA-organized chromophore nanostructures is important in realizing many applications essential for the Navy's missions. Computational modeling and simulations are becoming increasingly important in not only realizing the microscopic understanding of material properties but also design of novel materials. To investigate the DNA-organized chromophore complexes requires multi-scale modeling, from quantum mechanical effects of the energy transfer to molecular arrangements. The BSU has extensive knowledge in developing first-principle-based modeling and computations of various materials properties including DNA-chromophore complexes. NRL has extensive knowledge in undertaking all-atom molecular dynamics (MD) simulations of large biomolecular systems and extracting valuable information from MD simulation data. Combining the two approach will bring closer to understanding the microscopic nature of DNA-chromophore and chromophore-chromophore interactions and benefit the Navy in developing DNA-based nanophotonic materials.

3. Model and Methods

The aggregation ability of dyes is one of key parameters that determine the performance of the dyes in the DNA-based nanophotonic applications. Dyes have been known to aggregate in natural systems, as well as in artificial systems. An ability to scan a large pool of dyes for their aggregation propensity is thus important in designing new dyes for their applications. In this respect, the MD simulations can provide a tool to measure the aggregation propensity for dyes using various advanced simulation methods.

In this report, we considered five dyes, commercially available Cy5 dye and four Cy5 modified variants with hydrophobic substituents in order to establish the relationship between the binding/aggregation propensity and the chemical/structural properties. These dyes, labeled as Cy5, Cy5-Cl, Cy5-tBu, Cy5-Hex, and Cy5-Peg are shown in Figure 1 and are hypothesized to exhibit strong dye coupling due to being hydrophobic in nature. Comparing the hydrophobicity values derived from vacuum and implicit solvent DFT calculations, Cy5-Hex is the most hydrophobic, followed by Cy5-tBu, Cy5-Cl, Cy5-Peg, and Cy5, where Cy5 is the

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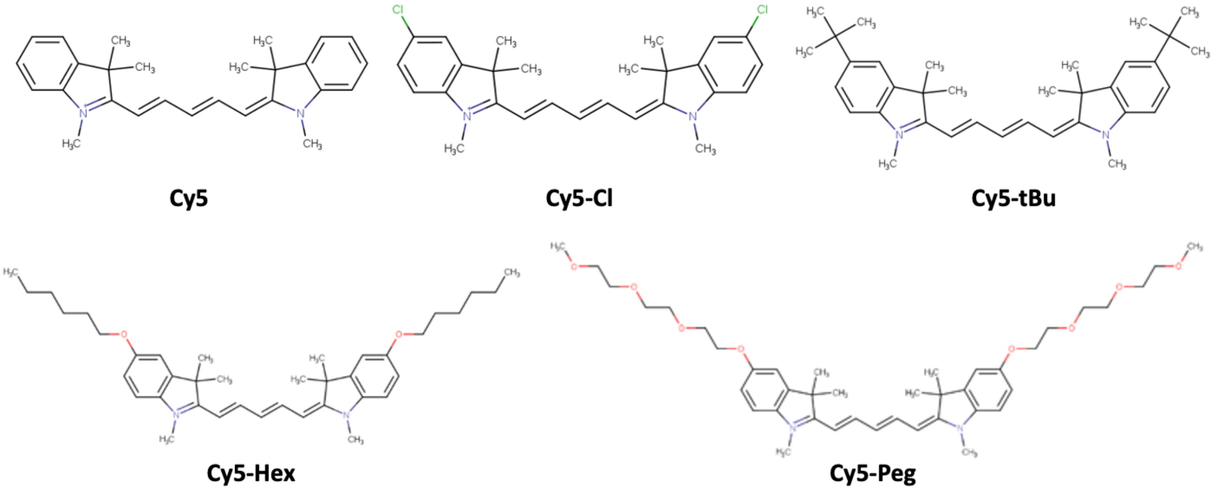
least hydrophobic [1]. It is hypothesized that more hydrophobic dyes lead to stronger aggregation ability, thus resulting in stronger dye coupling. Here we used MD simulations to calculate the binding strengths of the free (unattached) dyes in solution and relate them to the chemical/structural properties.



Cy5 **Cy5-Cl** **Cy5-tBu**

Cy5-Hex **Cy5-Peg**

Figure 1. Chemical structures of dyes (Cy5 and Cy5 variants) studied in this report.

Molecular dynamics (MD) simulations were performed with the GROMACS 2022.3 software package [2]. Dye structures were built using the UCSF ChimeraX software [3]. The generalized amber forcefield (GAFF) was used for interactions (nonbonded and bonded) between dyes [4]. Atomic charges for the dyes were calculated using the HF/6-31G* theory level [5]. The dyes were solvated in TIP3P water [6] in a truncated octahedron box. Neighbor-searching was used with a cutoff of 1.2 nm. Van der Waals interactions were limited to 1.2 nm and the particle mesh Ewald (PME) was used with a real-space coulomb cutoff of 1.2 nm. Bonds to hydrogen atoms were constrained using the LINCS algorithm [7]. The velocity-rescale thermostat was used to maintain a constant temperature of 300 K with a coupling time of 0.1 ps [8]. The Parrinello-Rahman barostat was used to keep the pressure at 1 atm with a coupling time of 1.0 ps [9]. A timestep of 2 fs was used.

To compute the aggregation propensity, we used umbrella sampling to determine the radially averaged potential of mean force (PMF) between the center of masses (COM) of two dyes. The COM of a dye is defined as the center of mass of two indolenine ends of the dye, thus excluding the effect of the substituents. To set up the system, we first generated an initial configuration to place two dyes in a cubic box, of dimension 6 nm, such that the COMs of the dyes are 5 Å apart. This initial configuration is subjected to steepest descent energy minimization to remove overlaps. Following this, we started the umbrella sampling simulations, where we applied an umbrella potential of 200 kJ mol^{-1} nm^{-2} (or 0.478 kcal/mol/Å^2) between COMs of the dyes. We used a total of ~20 replicas with centers spaced at 4 Å intervals to determine the PMF, and simulated each umbrella for 50 ns, totaling ~1 μs of simulation time, at T = 300 K and 100 mM salt concentration. We used the weighted histogram analysis (WHAM) [10] to obtain the free energy curve and corrected it with the missing Jacobian contribution to get the PMF. Figure 2 below shows histograms of distance profiles for umbrellas used in the Cy5 simulations.

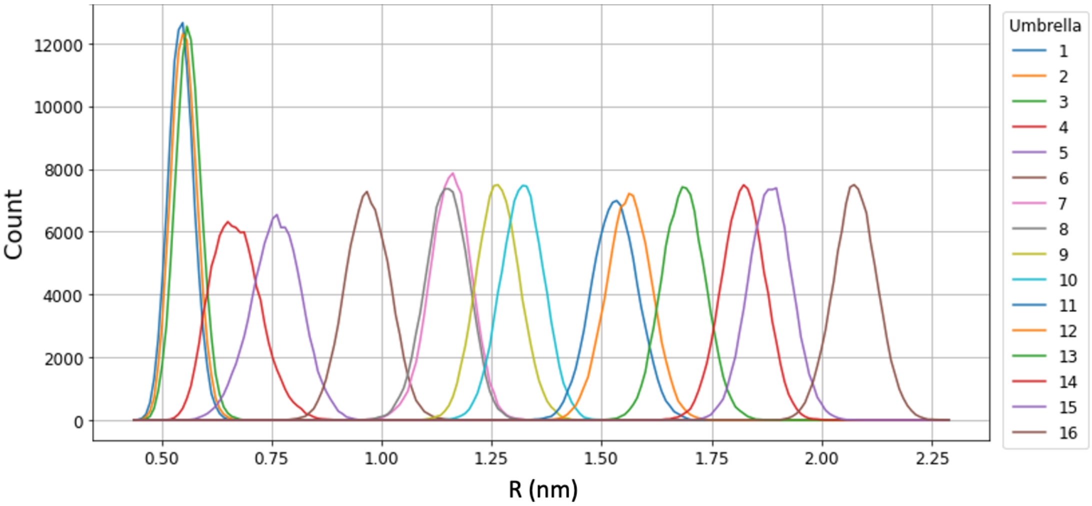


Figure 2. Histograms of the distance distributions for 16 umbrellas on Cy5 dimers.

The resulting PMF was then used to determine the molar association constant (K_a) using the following eq:

$$K_a = 4\pi N_A \int_0^b exp[-\beta F(r)] r^2 dr \quad (1)$$

where N_A is Avogadro's constant, b is the distance at which the PMF reaches its limiting value of zero, $\beta = \frac{1}{K_B T}$ (where K_B is the Boltzmann constant and T is the absolute temperature), F(r) is the PMF, and r is the intermolecular distance.

4. Results

Figure 3 shows the PMFs of Cy5 and its four derivatives calculated from the umbrella sampling simulations. First, the well depths of the PMFs closely follow the hydrophobicity values of the dyes, where the most hydrophobic Cy5-Hex has the deepest well-depth of 12 kcal/mol, closely followed by Cy5-tBu with the well-depth of 10 kcal/mol, while the least hydrophobic Cy5 has the shallowest well-depth of ~5 kcal/mol. Second, the dye-dye distance at which the PMF is minimum is about 5 Å with no apparent effects from the substituents. At this minimum distance, the dyes are stacked on each other, forming so-called H-aggregate. Interestingly, the PMFs show a shoulder (or local minimum) at a distance of ~1.2 nm, with dyes oriented head-to-tail, forming so-called J-aggregate. As the substituent becomes more hydrophobic, e.g., Cy5-Hex and Cy5-tBu, this configuration becomes more stable. We also notice that the substituents have no apparent effect on the configurations of the dye dimers. Specifically, for Cy5-Peg and Cy5-Hex, the long hydrophobic substituents did not alter the binding modes of the dyes.

To quantify the aggregation propensity, we calculated the association constants of five dyes using Eq. (1) above. The cutoff distance, b, was chosen by eyes where the PMF becomes close to zero. We note that the choice of the cutoff has little effect on the association constant estimation. Figure 4 shows the scatter plot between the association constants and the hydrophobicity values of the dyes. This confirms our hypothesis that more hydrophobic dyes lead to stronger dye-dye binding strength.

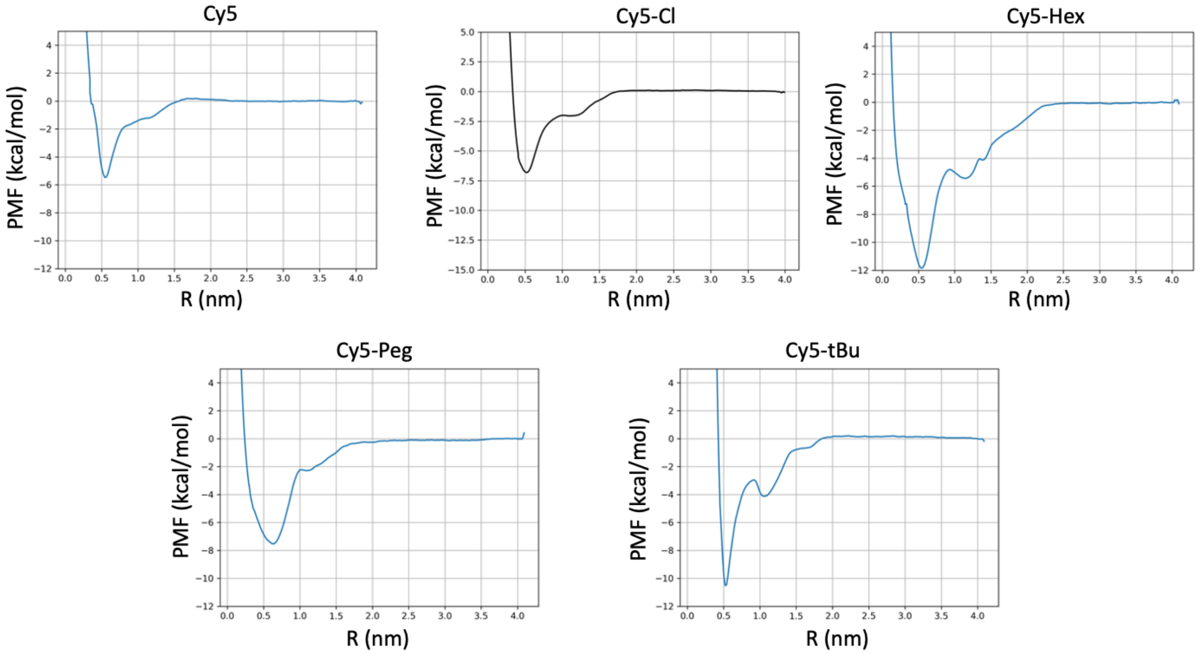


Figure 3. PMFs of Cy5 and Cy5 variants calculated from the umbrella sampling simulations.

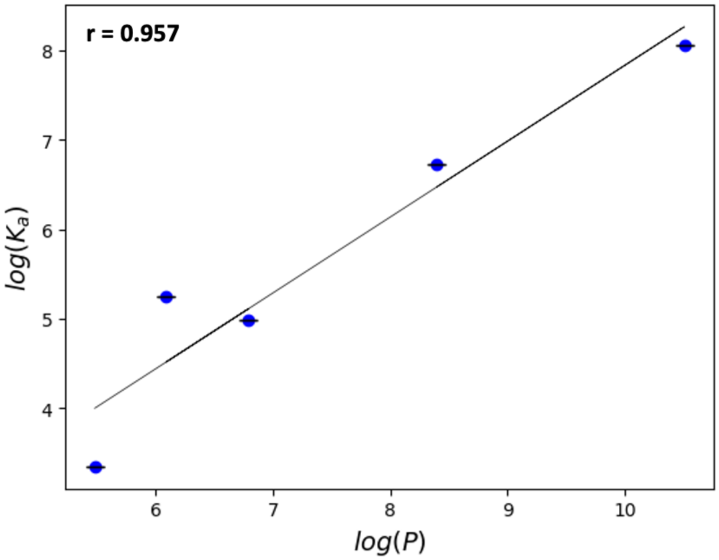


Figure 4. Scatter plot of the association constant, Ka, and the hydrophobicity, P, of the dyes. The solid line is the best linear fit to the data.

To obtain more detailed picture of the dye-dye interactions, we performed the two-dimensional umbrella sampling simulations on Cy5 with the reaction coordinates, R, the center-to-center distance, and the orientation factor, κ, defined by

$$\kappa = \hat{\boldsymbol{\mu}}_m \cdot \hat{\boldsymbol{\mu}}_n - 3\left(\hat{\mathbf{R}}_{m,n} \cdot \hat{\boldsymbol{\mu}}_m\right)\left(\hat{\mathbf{R}}_{m,n} \cdot \hat{\boldsymbol{\mu}}_n\right), \tag{2}$$

where $\hat{\boldsymbol{\mu}}_i$ is transition dipole moment unit vector of dye m or n (taken along the long axis of the dye), and $\hat{\mathbf{R}}_{m,n}$ is the unit vector between the centers of dyes m and n. When $|\kappa| = 0$ or $|\kappa| = 1.5$, the dyes are in a stacked oblique orientation or tail-to-tail oblique orientation, respectively. When $|\kappa| = 1$ or $|\kappa| = 2$, the dyes are in a stacked (H-aggregate) or tail-to-tail (J-aggregate) orientation, respectively. Figure 5 shows the resulting two-dimensional free energy landscape of the dyes in (κ,R) space. The dyes are predominantly in the stacked (H-aggregate) orientation with κ lying mostly between -1 and 1. There are, however, small populations of the head-to-tail (J-aggregate) orientation observed.

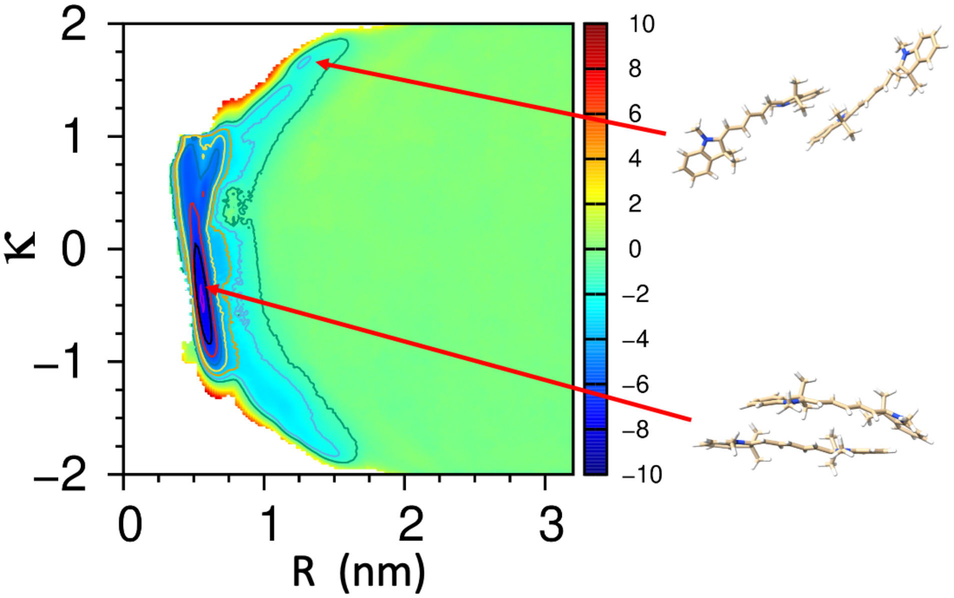


Figure 5. Two dimensional free energy landscape of Cy5 dimers calculated from the 2-dimensional umbrella sampling simulations.

As demonstrated in this report, the umbrella sampling method is a powerful tool to investigate the interactions between dyes and uncover the underlying free energy landscape. The method can easily applied to the DNA-chromophore complexes, where the DNA molecules to which dyes are attached can influence the dye-dye interactions. The simulations of the dyes attached to the DNA Holliday junction are currently underway and will be presented at later stage.

5. Conclusion

The umbrella sampling method was applied to various cyanine dyes to study the relationship between the chemical and structural properties of dyes and their binding propensity in solution. Using the empirically derived force-field parameters for the dyes, we demonstrated that the binding strengths of dyes are highly correlated to the hydrophobicity values. More detailed free energy landscape of the dye-dye interactions was generated by the two-dimensional umbrella sampling simulations, which showed the dyes predominantly interacting via stacked conformation. The method can easily be applied to more complex DNA-chromophore systems to study the effect of DNA scaffold on the dye-dye interactions.

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