Supporting Material

Cy3 – DNA stacking interactions strongly depend on the identity of the terminal base pair

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Construction of initial Cy3-DNA configurations

B-form DNA structures were created with the fiber program of the 3DNA package (1). Upon alignment of the phosphorus atoms of the Cy3-labeled chain to the NMR structure of a Cy3+Cy5 labeled DNA strand (2), the coordinates of Cy3 and its linker were transferred to the model DNA. The systems were minimized using the GBMV implicit solvent model (3, 4) with harmonic restraints on the base atoms. After minimization, each system was placed in a rhombic dodecahedral box and solvated in explicit water (5), surrounding Cy3-DNA by at least 12 Å. The total charge was neutralized by 22 potassium ions, which were placed using the SOLVATE program (6). After a 450 ps heating of the water and ions while keeping Cy3-DNA fixed, the entire system was gradually heated to 300 K over 300 ps with harmonic restraints of 1 kcal/(mol Å^2) on all non-hydrogen atoms. These restraints were gradually removed over 500 ps, followed by short unrestraint equilibration of 100 ps, and the production run.

Quantum Chemical Calculations for Indocyanine Ring System

A normal mode calculation on dimethylindocarbocyanine in a C_{2v}-symmetric conformation was performed at the B3LYP/6-31+G(d,p) (7, 8) level using Gaussian 03 (9). Dimethylindocarbocyanine differs from Cy3 only in having the –$NCH_2CH_2CH_2OH$ linkers replaced by –NCH_3. Numerical integrations for the DFT were performed using an ultrafine grid. The resulting frequencies were scaled by a factor of 1.0117 as recommended for low frequencies for this level of theory in a previous study (10). The lowest 10 frequencies are shown in Table S1.

In addition, a relaxed torsional scan on the C9A-N1A-CL3A-CL2A dihedral was performed at the B3LYP/6-31+g(d,p) level on a model compound that included one of the rings in the indocyanine ring system, an ethyl substituent on the nitrogen atom of the ring, and a –$CH=CH_2$ substitutent to emulate part of the conjugated linker between the rings (Fig. S3).

Parameterization of the Cy3 Fluorescent Dye

Parameters for the Cy3 dye were obtained by combining parameters for 3*H*-indole, 1,3,5-hexatriene, and n-propanol with additional parameters for guanine, methylcyclopentane, benzimidazole, neopentane, and retinol and its Schiff base modification where chemically appropriate and necessary to provide a complete parameter set. Atom names were assigned as shown in Fig. S1. All original parameters were taken from the CHARMM General Force Field (11). The atom types and partial charges are shown in Table S2. Several of the bond, angle, and dihedral parameters were adjusted as described (12) to match as many as possible of the lowest vibrational modes (those shown in Table S1) between the quantum calculation and the force field. The resulting parameters are shown in Tables S3, S4, and S5. The vibrational density of states for Cy3 from the force field and from the quantum calculation are shown in Fig. S2.

In addition, the CG321-CG324-NG2R52-CG2R52 and CG321-CG324-NG2R52-CG2RC0 dihedral parameters (for the C9-N1-CL3-CL2 and C2-N1-CL3-CL2 dihedrals) were adjusted so that a relaxed torsional scan on these dihedrals gave energy barriers similar to the quantum calculations. Energy profiles for this dihedral are shown in Fig. S3. Test simulations of a free Cy3 in explicit water were also performed, and the parameters were adjusted to prevent excessive twisting of the two rings about the conjugated linker.

Time Trace of Center of Mass Distance Between Dye and First Base

A time trace of the center of mass distance between the Cy3 ring system and the first base on the strand to which Cy3 is attached is shown in Fig. S4.

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Table S1. Comparison of first 10 vibrational modes of Cy3 from the force field to those from the quantum chemistry calculation.

Mode number	Irreducible representation of C_{2v}	Scaled QM vibrational frequency (cm^{-1})	Force field mode number	Force field vibrational frequency (cm^{-1})
1	A_2	19.8	7	22.3
2	B_1	26.3	8	30.7
3	A_1	36.5	9	41.7
4	A_2	46.7	10	64.0
5	B_1	66.4	n/a*	n/a
6	A_2	91.1	12	98.4
7	B_1	93.6	11	89.9
8	A_2	111.0	n/a*	n/a
9	B_2	118.0	13	124.1
10	B_1	131.5	14	137.4

*No unique force field mode could be identified corresponding to these quantum modes.

Table S2. Atom types and partial charges for Cy3 dye.

Atom names	Atom type	Partial charge (electron)
N1A, N1B	NG2R52	-0.11
C2A, C2B	CG2R52	0.29
C3A, C3B	CG3C50	0.09
C4A, C4B	CG2RC0	0.25
C5A, C5B	CG2R61	-0.22
H5A, H5B	HGR61	0.20
C6A, C6B	CG2R61	-0.22
H6A, H6B	HGR61	0.21
C7A, C7B	CG2R61	-0.21
H7A, H7B	HGR61	0.21
C8A, C8B	CG2R61	-0.34
H8A, H8B	HGR61	0.26
C9A, C9B	CG2RC0	0.23
CM1A, CM1B, CM2A, CM2B	CG331	-0.27
HM1A, HM2A, HM3A, HM4A, HM5A, HM6A, HM1B, HM2B, HM3B, HM4B, HM5B, HM6B	HGA3	0.09
OL1A, OL1B	OG311	-0.65
HO1A, HO1B	HGP1	0.42
CL1A, CL1B	CG321	0.05
CL2A, CL2B	CG321	-0.18
CL3A, CL3B	CG324	-0.18
H11A, H12A, H21A, H22A, H31A, H32A, H11B, H12B, H21B, H22B, H31B, H32B	HGA2	0.09
CX1, CX2, CX3	CG2DC2	-0.15
HX1, HX2, HX3	HGA4	0.15

Table S3. Bond parameters.

Atom types	Force constant (kcal/(mol Å^2))	Equilibrium length (Å)
CG2RC0-NG2R52	310.00	1.3650
CG2R52-CG2DC2	450.00	1.3000
CG324-NG2R52	300.00	1.4330
CG331-CG3C50	222.50	1.5280
CG2RC0-CG3C50	305.00	1.5200
CG2R52-CG3C50	350.00	1.5050

Table S4. Angle and Urey-Bradley parameters

Atom types	Force constant (kcal/mol/rad^2)	Equilibrium angle (°)	Urey-Bradley force constant (kcal/mol-Å^2)	Equilibrium Urey-Bradley distance (Å)
CG2R52-NG2R52-CG2RC0	60.00	107.20		
CG2R52-CG3C50-CG2RC0	105.00	105.00		
CG2RC0-CG2RC0-CG3C50	143.00	110.00		
CG2R61-CG2RC0-CG3C50	60.00	130.00		
CG3C50-CG2R52-NG2R52	170.00	112.00		
CG2RC0-NG2R52-CG324	62.30	112.30		
CG2R52-NG2R52-CG324	62.30	140.50		
CG2R61-CG2RC0-NG2R52	130.00	130.00		
CG3C50-CG331-HGA3	33.43	110.10	22.53	2.17900
CG2DC2-CG2DC2-CG2DC2	17.30	123.00		
CG2DC2-CG2DC2-CG2R52	40.00	123.00		
CG2DC2-CG2R52-NG2R52	10.00	125.60		
CG2R52-CG3C50-CG331	32.00	112.20		
CG2RC0-CG3C50-CG331	32.00	112.20		
CG331-CG3C50-CG331	58.35	113.50	11.16	2.561
CG2DC2-CG2R52-CG3C50	10.00	123.50		
CG2RC0-CG2RC0-NG2R52	100.00	110.00		
NG2R52-CG331-HGA3	33.43	110.10		
NG2R52-CG321-HGA2	33.43	110.10		
NG2R52-CG324-HGA2	33.43	110.10		
CG321-CG324-NG2R52	70.00	113.70		
CG2R52-CG2DC2-HGA4	25.50	119.00		

Table S5. Dihedral parameters.

Atom types	Force constant (kcal/(mol rad^2))	Multiplicity	Phase (°)
CG2DC2-CG2R52-NG2R52-CG324	1.0000	2	180.00
CG2DC2-CG2R52-NG2R52-CG2RC0	1.5000	2	180.00
HGA4-CG2DC2-CG2R52-NG2R52	0.5000	2	180.00
CG3C50-CG2R52-NG2R52-CG324	1.0000	2	180.00
HGA2-CG324-NG2R52-CG2R52	0.1500	3	180.00
HGA2-CG324-NG2R52-CG2RC0	0.1500	3	180.00
CG3C50-CG2R52-CG2DC2-CG2DC2	0.9000	1	0.00
CG3C50-CG2R52-CG2DC2-CG2DC2	2.1000	2	180.00
CG3C50-CG2R52-CG2DC2-CG2DC2	0.2200	3	0.00
CG3C50-CG2R52-CG2DC2-CG2DC2	0.2500	5	180.00
CG3C50-CG2R52-CG2DC2-CG2DC2	0.1000	6	0.00
CG3C50-CG2R52-CG2DC2-HGA4	0.5000	2	180.00
NG2R52-CG2R52-CG3C50-CG331	0.5000	2	0.00
NG2R52-CG2R52-CG3C50-CG331	0.4000	3	0.00
CG2DC2-CG2R52-CG3C50-CG331	0.3000	3	0.00
CG2RC0-CG2RC0-CG3C50-CG331	0.5000	2	0.00
CG2RC0-CG2RC0-CG3C50-CG331	0.4000	3	0.00
CG2R61-CG2RC0-CG3C50-CG331	0.5000	2	0.00
CG2R61-CG2RC0-CG3C50-CG331	0.4000	3	0.00
CG2R52-CG3C50-CG331-HGA3	0.1600	3	0.00
CG2RC0-CG3C50-CG331-HGA3	0.1600	3	0.00
CG331-CG3C50-CG331-HGA3	0.1600	3	0.00
CG2DC2-CG2R52-CG3C50-CG2RC0	0.0000	3	0.00
NG2R52-CG2R52-CG3C50-CG2RC0	1.3000	3	180.00
CG3C50-CG2R52-NG2R52-CG2RC0	6.0000	2	180.00
CG2R61-CG2R61-CG2RC0-CG3C50	0.0000	2	180.00
CG2R61-CG2R61-CG2RC0-NG2R52	1.5000	2	180.00
CG2R61-CG2RC0-CG2RC0-CG3C50	2.5000	2	180.00
CG2RC0-CG2RC0-CG3C50-CG2R52	0.5000	3	180.00
CG2R61-CG2RC0-NG2R52-CG2R52	4.0000	2	180.00
CG2R61-CG2RC0-CG3C50-CG2R52	3.5000	3	0.00
CG2RC0-CG2RC0-NG2R52-CG2R52	4.0000	2	180.00
CG3C50-CG2RC0-CG2RC0-NG2R52	6.5000	2	180.00
CG2R61-CG2RC0-CG2RC0-NG2R52	1.5000	2	180.00
HGR61-CG2R61-CG2RC0-NG2R52	0.8000	2	180.00
HGR61-CG2R61-CG2RC0-CG3C50	0.0000	2	180.00
CG2RC0-CG2RC0-NG2R52-CG324	9.0000	2	180.00
CG2R61-CG2RC0-NG2R52-CG324	9.0000	2	180.00
CG2R52-CG2DC2-CG2DC2-CG2DC2	0.5600	1	180.00
CG2R52-CG2DC2-CG2DC2-CG2DC2	4.0000	2	180.00
NG2R52-CG2R52-CG2DC2-CG2DC2	0.5600	1	180.00
NG2R52-CG2R52-CG2DC2-CG2DC2	9.0000	2	180.00
CG2DC2-CG2DC2-CG2DC2-HGA4	5.2000	2	180.00
CG2R52-CG2DC2-CG2DC2-HGA4	5.2000	2	180.00
HGA2-CG321-CG324-NG2R52	0.1950	3	0.00
CG321-CG321-CG324-NG2R52	0.1950	3	0.00
CG321-CG324-NG2R52-CG2R52	2.2500	1	0.00
CG321-CG324-NG2R52-CG2R52	0.9000	2	180.00
CG321-CG324-NG2R52-CG2RC0	2.2500	1	180.00
CG321-CG324-NG2R52-CG2RC0	0.9000	2	180.00
CG324-CG321-CG321-OG303	0.1950	3	0.00
CG324-CG321-CG321-OG311	0.1950	3	0.00
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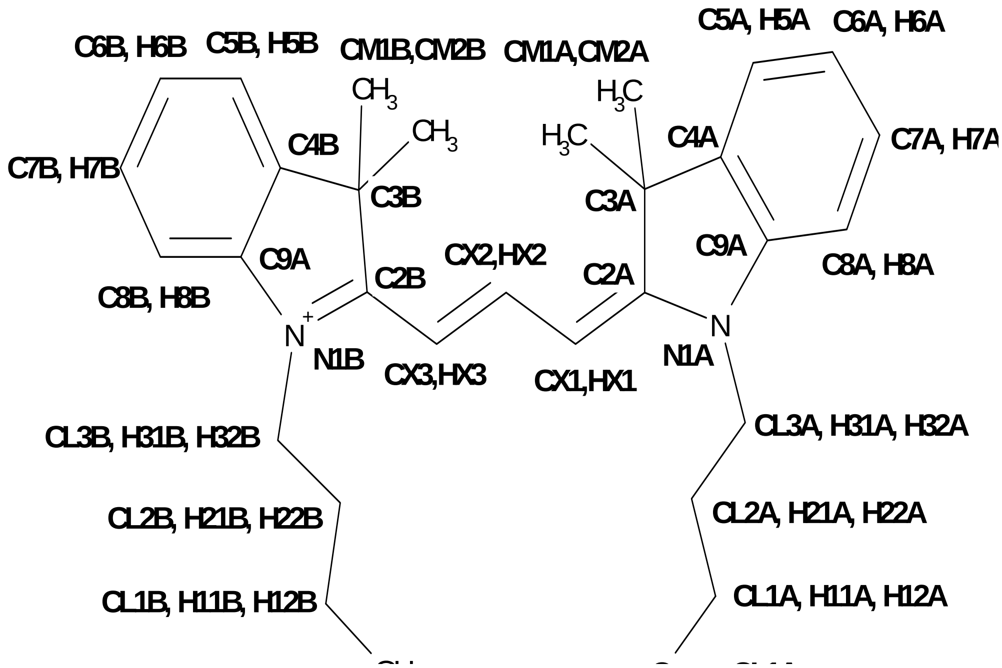

Figure S1. Structure of Cy3 with CHARMM atom names in bold.

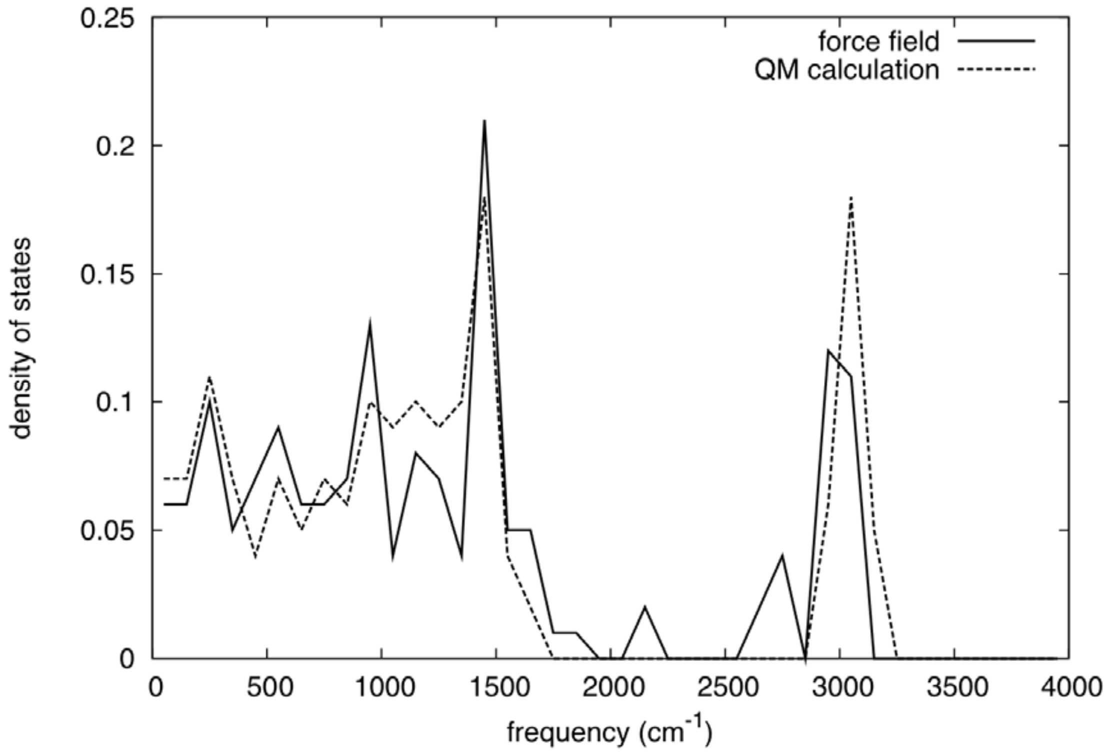

Figure S2. Vibrational density of states for Cy3 from force field and from QM calculation.

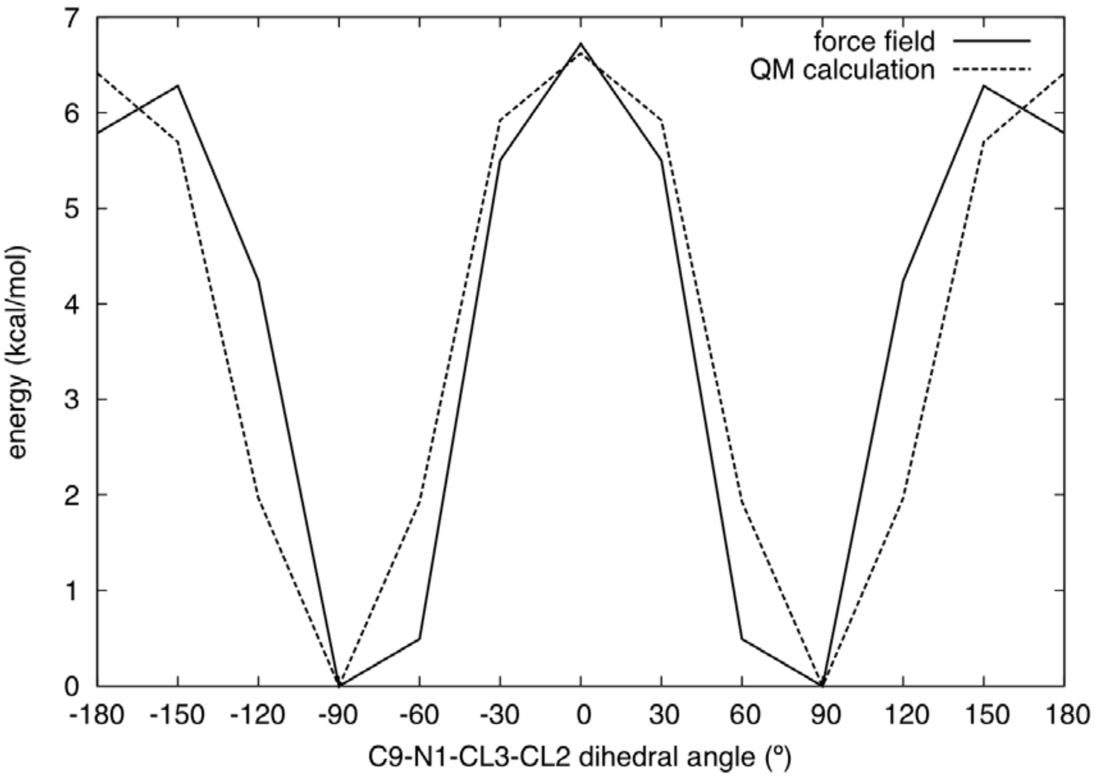

Figure S3. Comparison of energy profiles around C9-N1-CL3-CL2 dihedral from force field and quantum calculation.

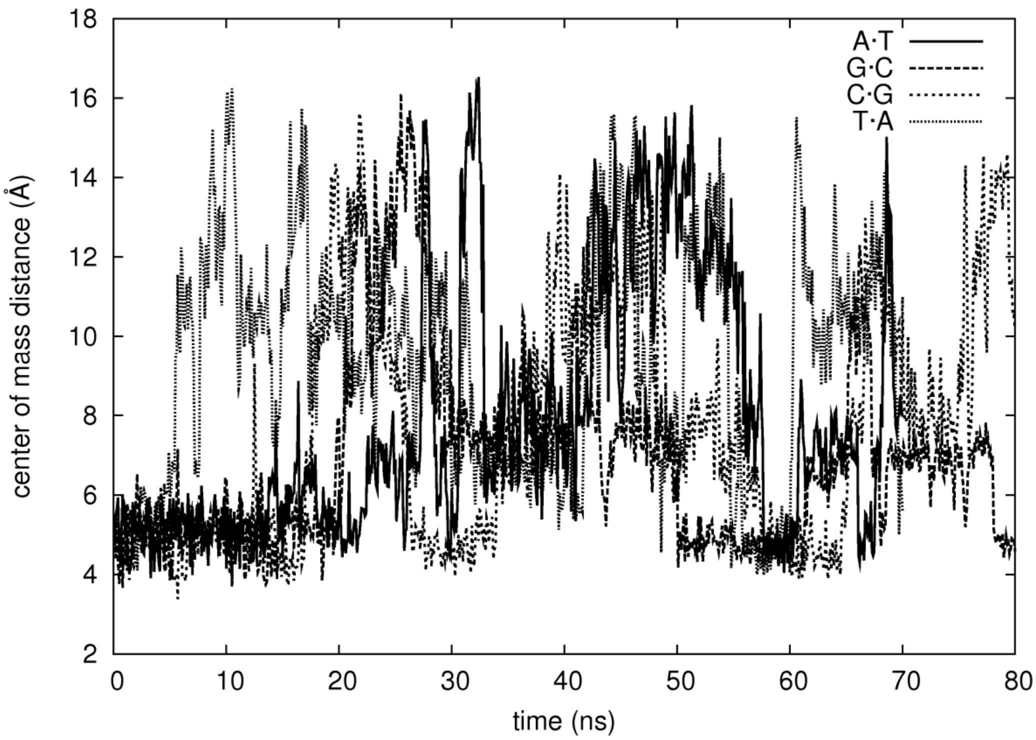

Figure S4. Center of mass distance between Cy3 and first base as a function of time.