Electronic Supplementary Material (ESI) for Faraday Discussions.

Supporting Information: Measuring local conformations and conformational disorder of $(Cy3)_2$ dimer labeled DNA fork junctions using absorbance, circular dichroism and two-dimensional fluorescence spectroscopy

Dylan Heussman,[1,†] Justin Kittell,[2,†] Loni Kringle,[1] Amr Tamimi,[1] and Andrew H. Marcus[1,*]

[1.] Department of Chemistry and Biochemistry, Center for Optical, Molecular and Quantum Science, University of Oregon, Eugene, OR 97403, USA

[2.] Department of Physics, Center for Optical, Molecular and Quantum Science, University of Oregon, Eugene, OR 97403, USA

[†]These authors contributed equally to this work.

[*]e-mail: ahmarcus@uoregon.edu

Table S1. Hamiltonian parameters of the Cy3 monomer duplex DNA construct at various temperatures, obtained from model fits of Eq. (1) to the absorbance spectra (17). The calculations used the electric transition dipole moment (EDTM) μ_{eg} = 12.8 D and the homogeneous line width Γ_H = 186 cm^{-1}. The values listed correspond to the electronic transition energy ε_{eg}, the vibrational mode frequency ω_0, the Huang-Rhys parameter λ^2, and the standard deviation of the Gaussian disorder function $\sigma_{I,mon}$.

Cy3 Monomer Duplex DNA Construct				
T (°C)	ε_{eg} (cm^{-1})	ω_0 (cm^{-1})	λ^2	$\sigma_{I,mon}$ (cm^{-1})
15	18,285 +40/−39	1,116 +99/−103	0.54 +0.07/−0.06	333 +16/−15
25	18,277 +38/−37	1,109 +88/−90	0.56 +0.06/−0.06	347 +15/−14
35	18,266 +36/−35	1,119 +82/−84	0.56 +0.06/−0.06	353 +14/−13
45	18,262 +36/−35	1,113 +82/−82	0.56 +0.06/−0.05	380 −14/ +13
55	18,280 +39/−38	1,124 +93/−96	0.55 +0.06/−0.06	380 +15/−14
65	18,301 +45/−45	1,103 +103/−107	0.54 +0.07/−0.07	367 +18/−16
75	18,323 +49/−48	1,072 +120/−120	0.54 +0.08/−0.07	376 +19/−17

Table S2. Hamiltonian parameters of the Cy3 monomer fork DNA construct at various temperatures, obtained from model fits of Eq. (1) to the absorbance spectra (17). The calculations used the same input parameters as those described in Table SI.

Cy3 Monomer Fork DNA Construct				
T (°C)	ε_{eg} (cm^{-1})	ω_0 (cm^{-1})	λ^2	$\sigma_{I,mon}$ (cm^{-1})
15	18,223 +37/−36	1,112 +83/−85	0.58 +0.06/−0.06	354 +15/−14
25	18,240 +37/−36	1,116 +81/−82	0.59 +0.06/−0.06	370 +15/−14
35	18,252 +36/−35	1,105 +80/−80	0.58 +0.06/−0.06	372 +14/−13
45	18,249 +37/−35	1,088 +79/−79	0.59 +0.06/−0.06	374 +14/ −13
55	18,257 +39/−38	1,086 +83/−83	0.58 +0.06/−0.06	387 +15/−14
65	18,263 +38/−38	1,089 +83/−85	0.58 +0.06/−0.05	398 +15/−14
75	18,285 +38/−37	1,074 +83/−83	0.58 +0.06/−0.05	405 +14/−13

Comparison between point-dipole and extended-dipole calculations for $(Cy3)_2$ dimer labeled duplex and fork DNA constructs.

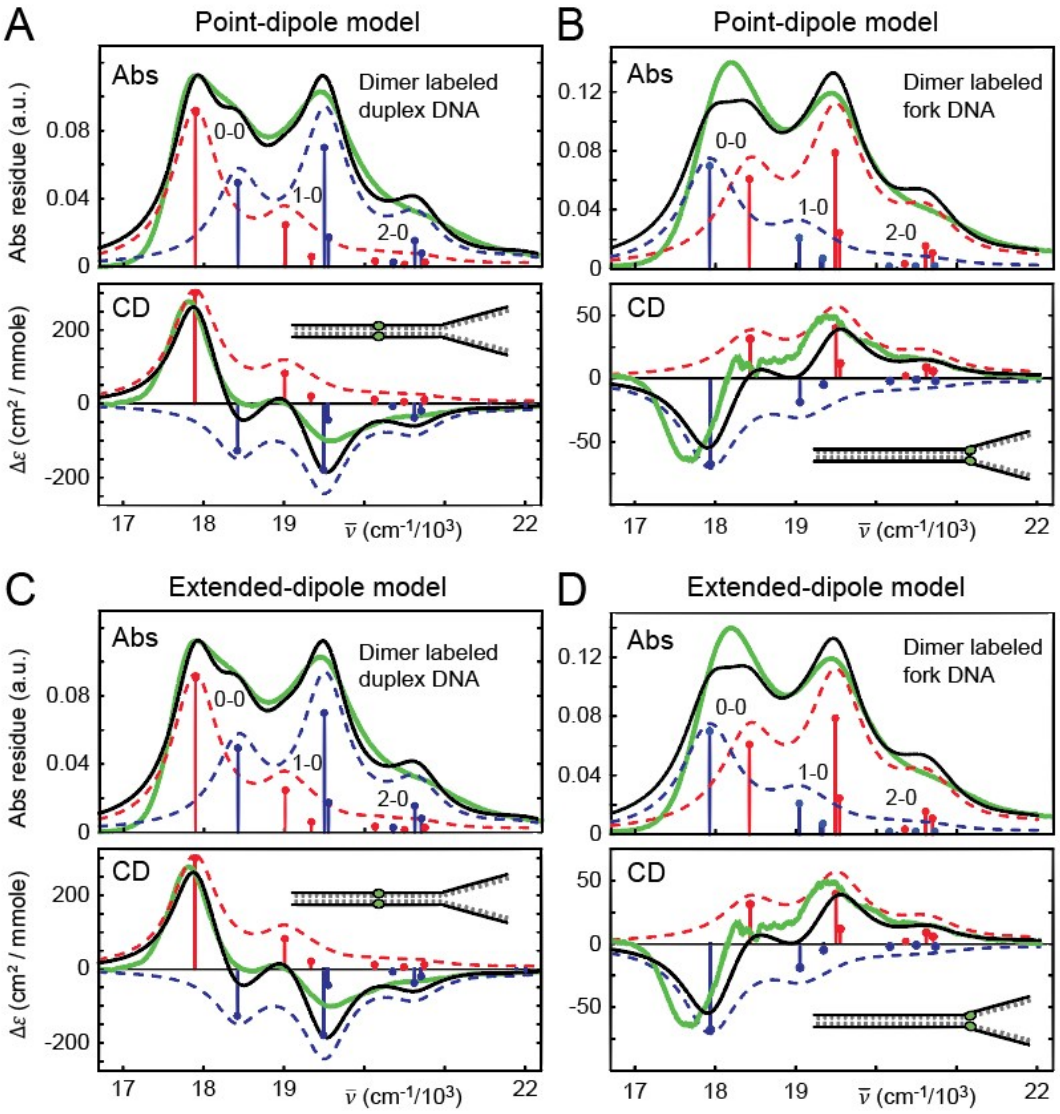

Figure S1. Comparison between 15 °C experimental and simulated absorbance and CD spectra of $(Cy3)_2$ dimer labeled duplex (***A*** & ***C***) and fork (***B*** & ***D***) DNA constructs. The simulated spectra are alternately based on the point-dipole model (***A*** & ***B***) and the extended-dipole model (***C*** & ***D***). Experimental spectra are shown in solid green, and the model total lineshapes (inhomogeneous-plus-homogeneous) in solid black. Symmetric and anti-symmetric transitions determined from the model are shown as blue and red sticks, respectively. Symmetric and anti-symmetric contributions to the inhomogeneous lineshapes are shown as

dashed blue and red curves, respectively. The corresponding optimized values are presented in Table II for the duplex DNA construct, and in Table III for the fork DNA construct.

Control 2DFS measurements of the Cy3 chromophore in methanol.

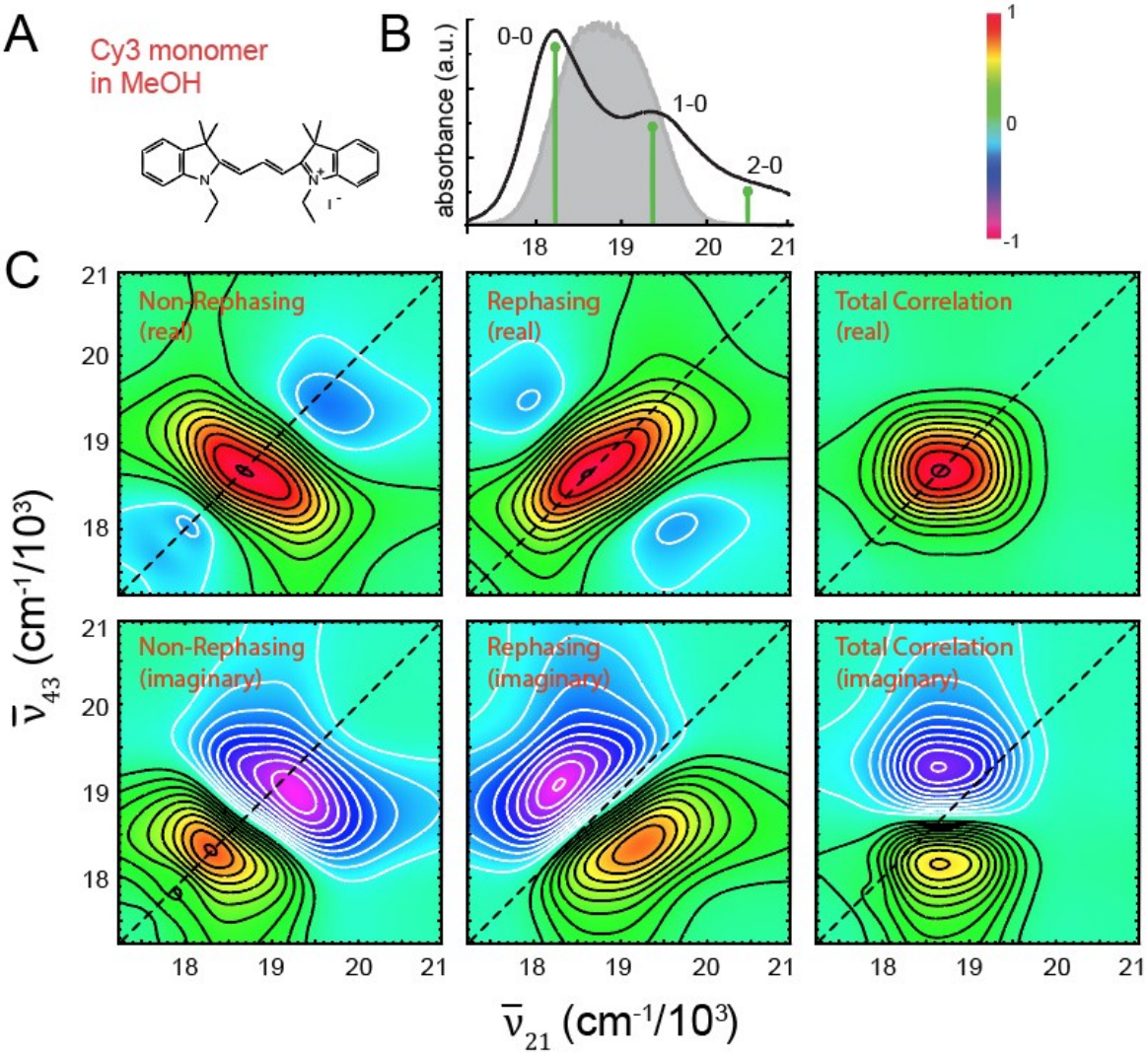

Figure S2. 2DFS measurements performed on the Cy3 chromophore in methanol at 25 °C. **(*A*)** The chemical structure of the Cy3 chromophore is shown. **(*B*)** Absorbance spectrum of the Cy3 chromophore in methanol (black curve) overlaid with the vibronic spectral features (represented as green sticks) obtained from a fit to the monomer Hamiltonian given by Eq. (1) of the text. The intra-molecular parameters are the electronic transition energy ε_{eg} = 18,500 cm^{-1}, vibrational mode frequency ω_0 = 1,120 cm^{-1}, and Huang-Rhys parameter λ^2 = 0.56. Also shown is the laser spectrum (in gray) with center frequency $\bar{\nu}_L$ = 18,796 cm^{-1} (λ_L = 532 nm) and FWHM bandwidth $\Delta\bar{\nu}_L$ = 1,300 cm^{-1} ($\Delta\lambda_L$ = 37 nm). The laser spectrum spans a

region containing both the 0–0 and 1–0 vibronic sub-bands. **(*C*)** Comparison between experimental non-rephasing (NRP, left column), rephasing (RP, middle column) and total correlation (TC, right column) spectra. Cross-sections of the 2DFS lineshapes of the RP spectra along the diagonal and anti-diagonal directions provide, respectively, values for the inhomogeneous disorder parameter σ_I = 250 cm^{-1} and the homogeneous Lorentzian FWHM Γ_H = 400 cm^{-1}.