Electronic Supplementary Material (ESI) for Nanoscale.

Supporting information: Towards Control of Excitonic Coupling in DNA-Templated Cy5 Aggregates: The Principal Role of Chemical Substituent Hydrophobicity and Steric Interactions.

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Table S1: Oligonucleotide sequences

Name	Sequence (5'-3')	Name	Sequence (5'-3')

Unlabeled A	ATATAATCGCTCGCATATTATGACTG	Labeled A	ATATAATCGCTCG-X-CATATTATGACTG
Unlabeled B	CAGTCATAATATGTGGAATGTGAGTG	Labeled B	CAGTCATAATATG-X-TGGAATGTGAGTG
Unlabeled C	CACTCACATTCCACTCAACACCACAA	Labeled C	CACTCACATTCCA-X-CTCAACACCACAA
Unlabeled d	TTGTGGTGTTGAGCGAGCGATTATAT	Labeled D	TTGTGGTGTTGAG-X-CGAGCGATTATAT

X indicates the position of Cy5-R

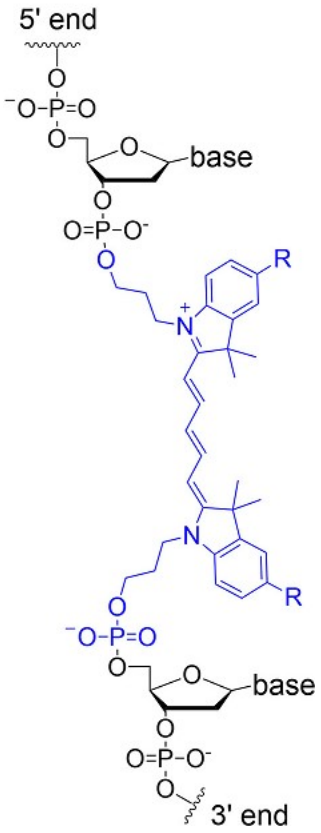

Figure S1. General structure of the Cy5 dye derivatives inserted internally into DNA oligonucleotides during phosphoramidite synthesis and attached to the DNA at both the 3′ and 5′ ends.

Additional Spectra and Characterization

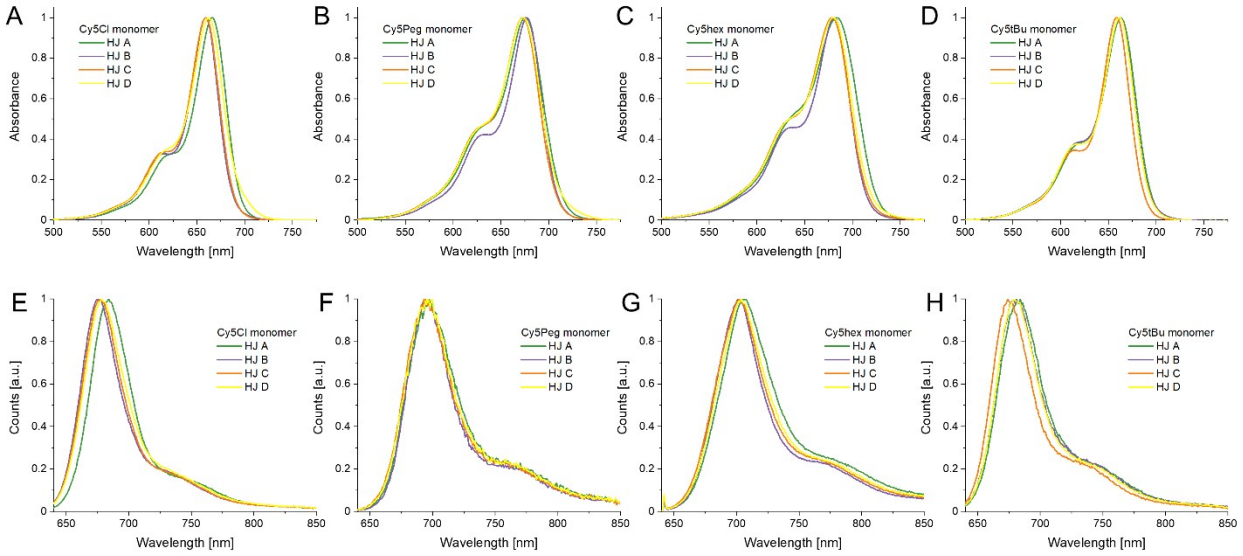

Figure S2: (A-D) Absorbance of Cy5-R monomers. (E-H) Fluorescence emission of Cy5-R monomers. 615 nm excitation wavelength.

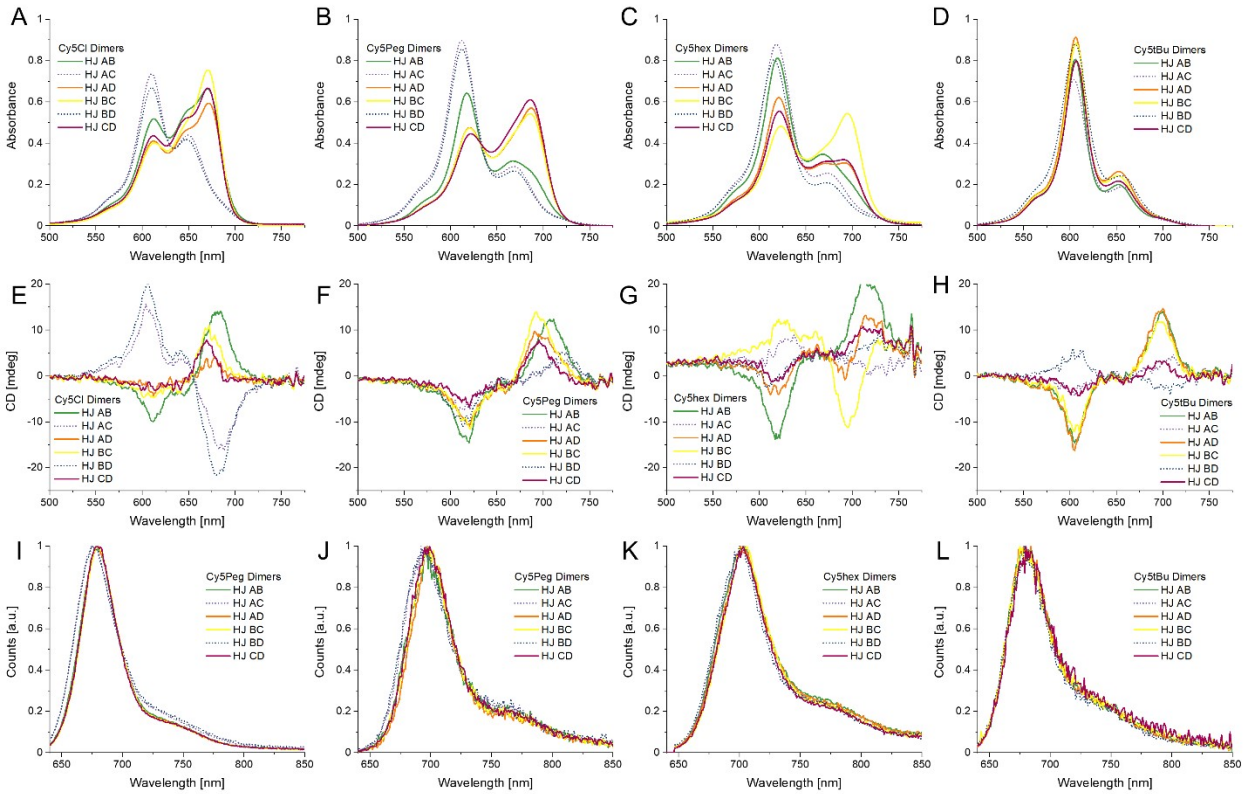

Figure S3: Adjacent dimers are full lines while transverse dimers are dotted lines. (A-D) Absorbance of Cy5-R dimers. (E-H) CD spectra of Cy5-R dimers. (I-L)Fluorescence emission of Cy5-R. 615 nm excitation wavelength.

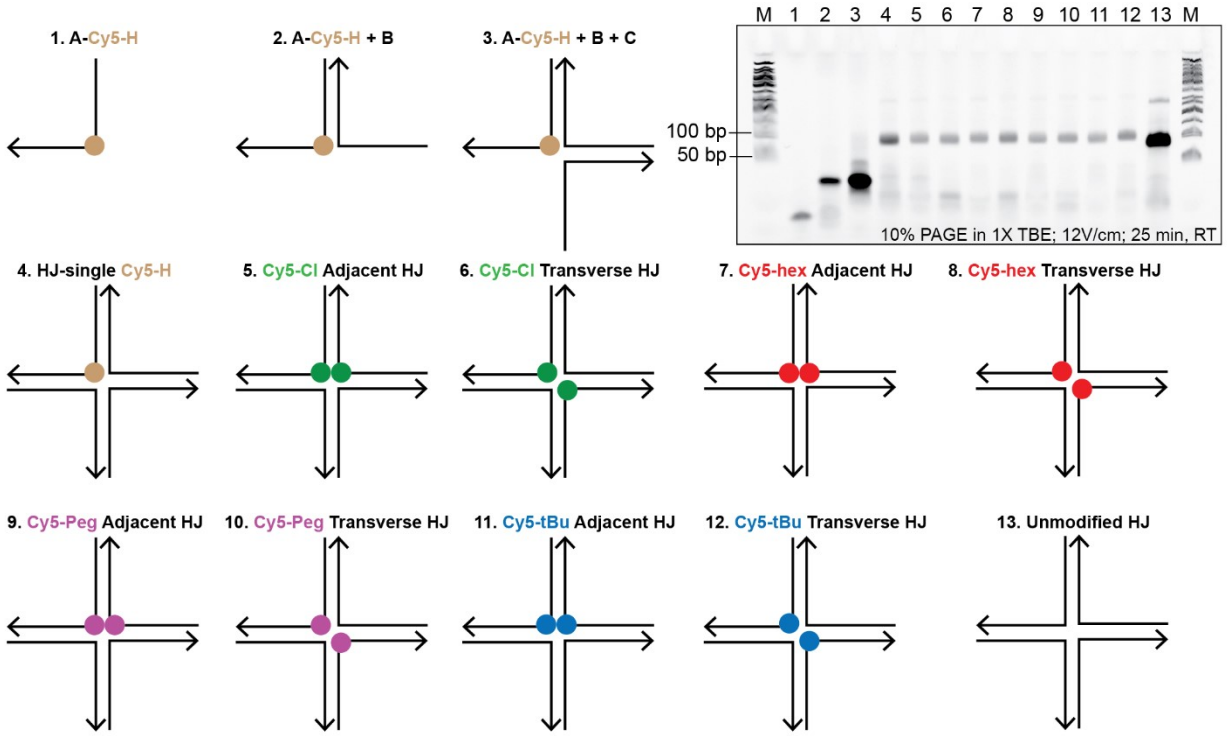

Figure S4: 10% Polyacrylamide gel electrophoresis of variations of the HJ Cy5-R homodimers in 1× TAE and 15 mM Mg^{+2} as the sample buffer as well as control structures that include partial structures and unlabeled HJ. It can be seen that all structures form with relatively high efficiency. Quantification on the gels was >85% HJ efficiency.

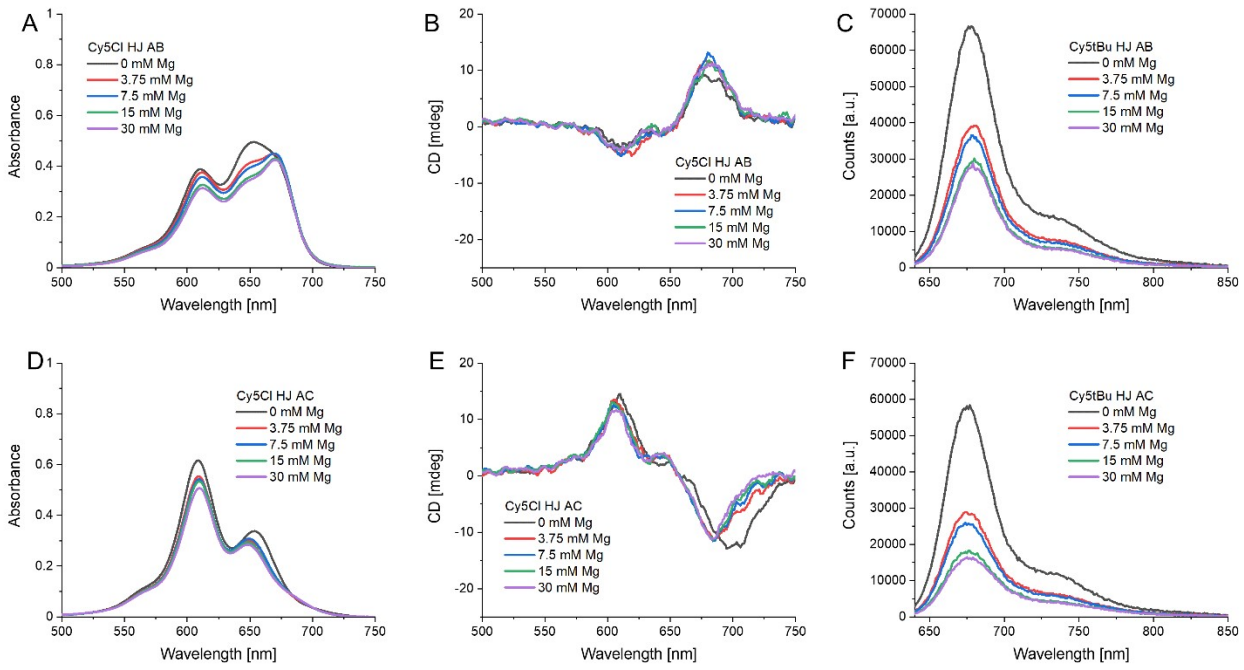

Figure S5: Spectra of Cy5Cl in 1× TAE with increasing concentration of Mg^{+2}. (A-C) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AB dimer. (D-F) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AC dimer.

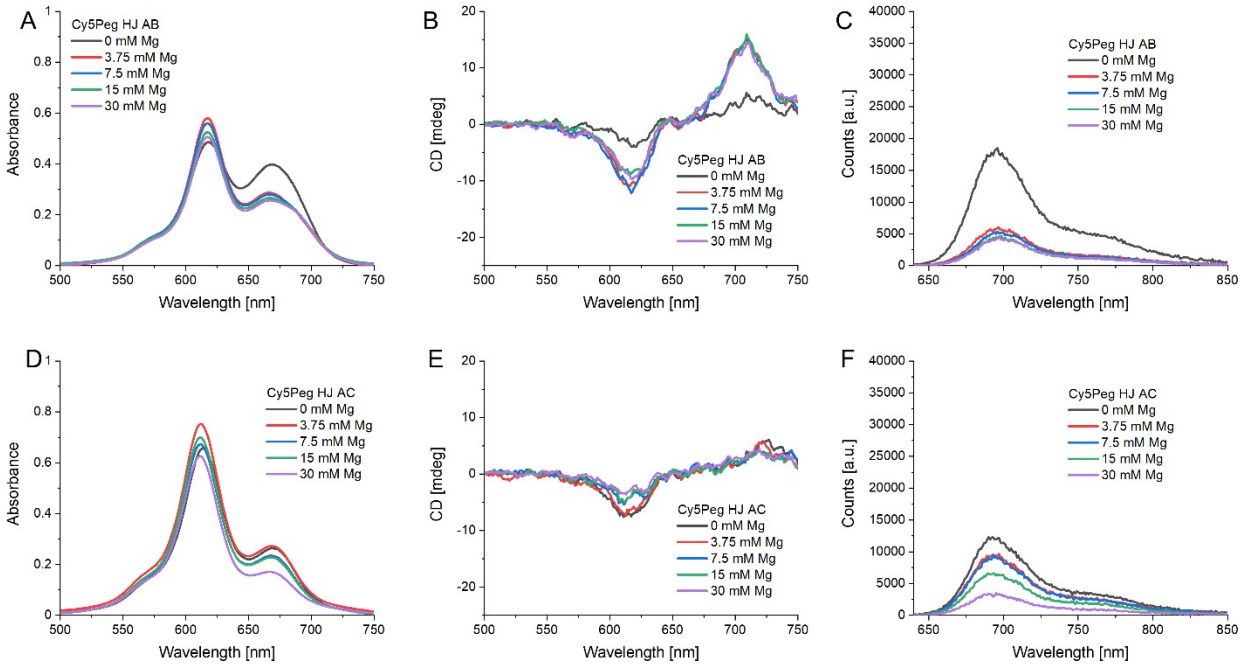

Figure S6: Spectra of Cy5Peg in 1× TAE with increasing concentration of Mg^{+2}. (A-C) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AB dimer. (D-F) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AC dimer.

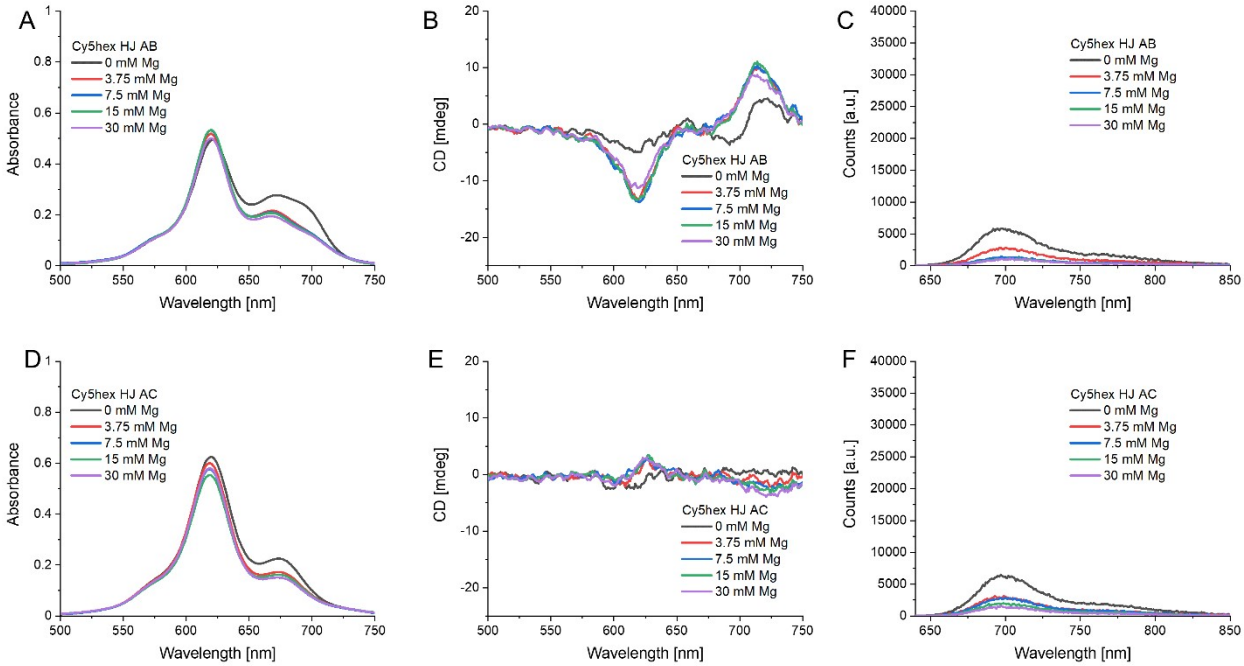

Figure S7: Spectra of Cy5hex in 1× TAE with increasing concentration of Mg^{+2}. (A-C) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AB dimer. (D-F) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AC dimer.

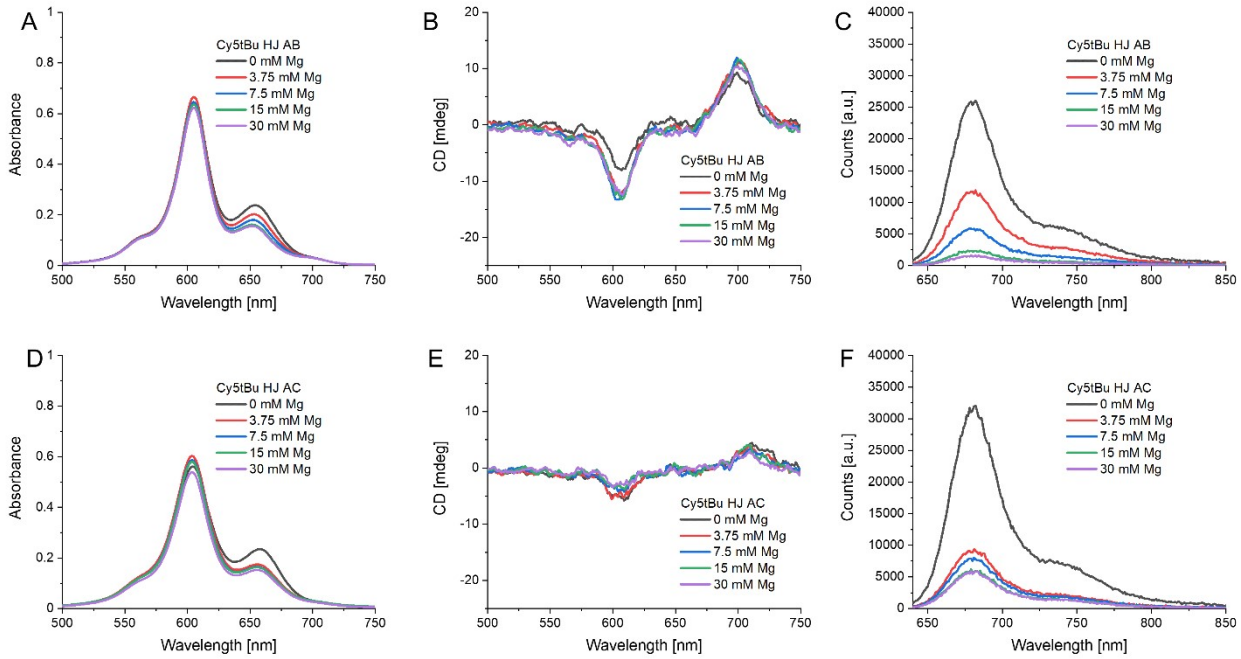

Figure S8: Spectra of Cy5tBu in 1× TAE with increasing concentration of Mg^{+2}. (A-C) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AB dimer. (D-F) Absorbance, CD, and fluorescence emission (615 nm excitation) of HJ AC dimer.

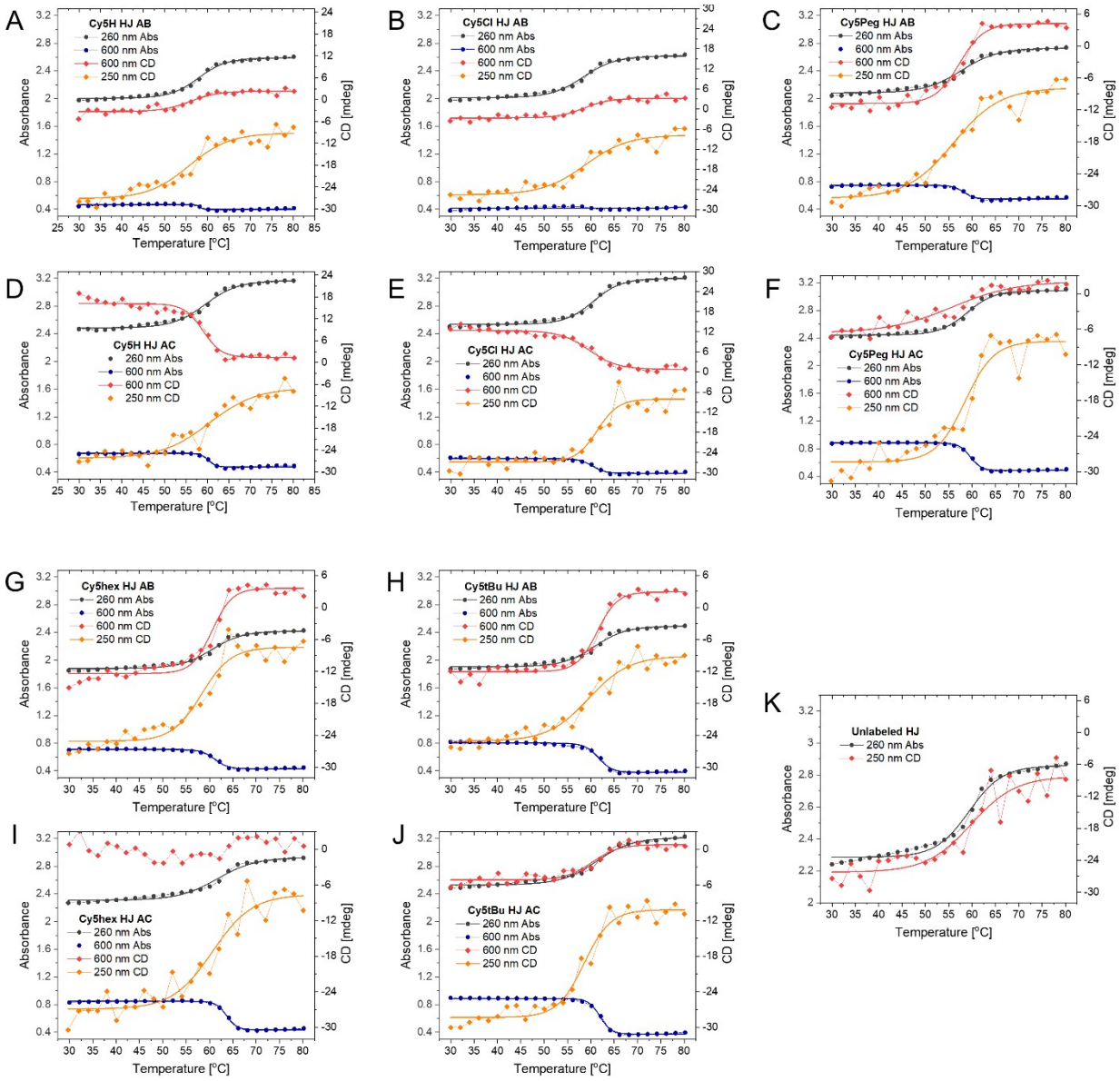

Figure S9: Melting curves of Cy5-R HJ in 1× TAE with 15 mM Mg^{+2} following both absorbance (260 nm, black ; 600 nm, blue) and CD (250 nm, orange ; 600 nm, red) signals. (A, D) Cy5H HJ AB and AC, respectively. (B, E) Cy5Cl HJ AB and AC, respectively. (C, F) Cy5Peg HJ AB and AC, respectively. (G, I) Cy5hex HJ AB and AC, respectively. The 600 nm CD signal for Cy5hex HJ AC was not fit as the signal change was not clear. (H, J) Cy5tBu HJ AB and AC, respectively. (K) Unlabeled HJ.

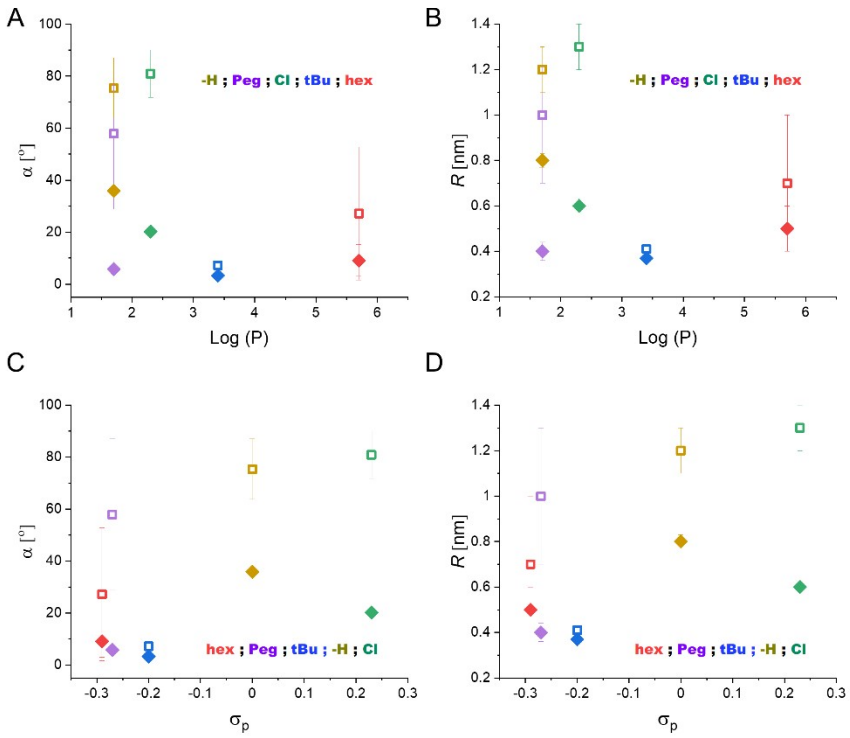

Figure S10: A) α as a function of hydrophobicity. B) R as a function of hydrophobicity. C) α as a function of the electron donating capability of the substituent. D) R as a function of electron donating capability of the substituent.

$$y = A_2 + \frac{(A_1 - A_2)}{1 + \left(\frac{x}{x_0}\right)^p}$$

Table S2: Fitting parameters of logistic fits from Figure 6C-E main text. Equation:

Data	A_1	A_2	X_0	p	R^2
$J_{m,n}$ - Adjacent	51 ± 20	115 ± 20	0.91 ± 0.04	25 ± 6	0.90
$J_{m,n}$ - Transverse	69.7 ± 1.2	131 ± 2	0.59 ± 0.03	6.4 ± 1.2	0.99
α - Adjacent	79 ± 10	7.2 ± 1.6	0.90 ± 0.04	31 ± 8	0.97
α - Transverse	35.9 ± 0.3	3.1 ± 0.3	0.49 ± 0.01	4.0 ± 0.2	0.99
R - Adjacent	1.26 ± 0.06	0.41 ± 0.02	0.87 ± 0.04	9 ± 2	0.98
R - Transverse	0.80 ± 0.03	0.37 ± 0.01	0.51 ± 0.03	3.5 ± 1.5	0.99

Supporting Analysis of KRM Results

Parameter List (See Figure 5 in the main text for schematics)

Ev - The energy of vibron (*eV*)

d - Displacement of excited state vibronic potential (dimensionless units)

Γ - Energy loss damping constant (*eV*)

J_0 - Characteristic exchange energy ($meV\text{-}nm^3$)

M - Dipole Moment (*Debye*)

nv - Vibrational state Hilbert space

E_{of} - Energy offset from monomer (*eV*)

l - Length of the transition dipole moment (*nm*)

cdis - Closest distance between the long axes of any pair of dyes (*nm*)

r - The ratio of theoretical to experimental values of the ratio of the max abs CD peak height to max absorbance peak height

OI_{AB} - Normalized overlap integral for the experimental and theoretical absorbance curves

OI_{CD} - Normalized overlap integral for the experimental and theoretical CD spectra

OI_{Tot} - Mean of OI_{AB} and OI_{CD}

MSD_{abs} - Absorbance spectrum mean-square deviation

MSD_{cd} - CD spectrum mean-square deviation

$w_{abscd}rms$ - Weighted mean-squared deviation between the experimental and theoretical ABS and CD spectra

$J_{m,n}$ – The excitonic hopping parameter (*meV*)

R - Center-to-Center distance (Å)

dmin - The shortest distance between the transition dipole moment vectors (*nm*)

α - Oblique angle (°)

θ_{1s} - Slip angle, the angle between dye 1 and the line connecting dye centers (°)

θ_{2s} - Slip angle, the angle between dye 2 and the line connecting dye centers (°)

θ_t - Twist angle, the angle between dye 1 and dye 2 in the XY plane (rotation about Z-axis or R) (°)

As detailed in the main text there were a few cases where the KRM fitting was improved by assuming that the spectra were composed of a mixed population of Cy5-R dimers. We note that the fitting program necessarily assumed 50% population for each dimer, though of course there is no experimental evidence to support this specific distribution. The particular case of Cy5hex HJ BC is shown in Figure S9, while a compilation of the specific cases is provided in Table S2. All other systems not found in Table S2 were the best fit by a single dimer.

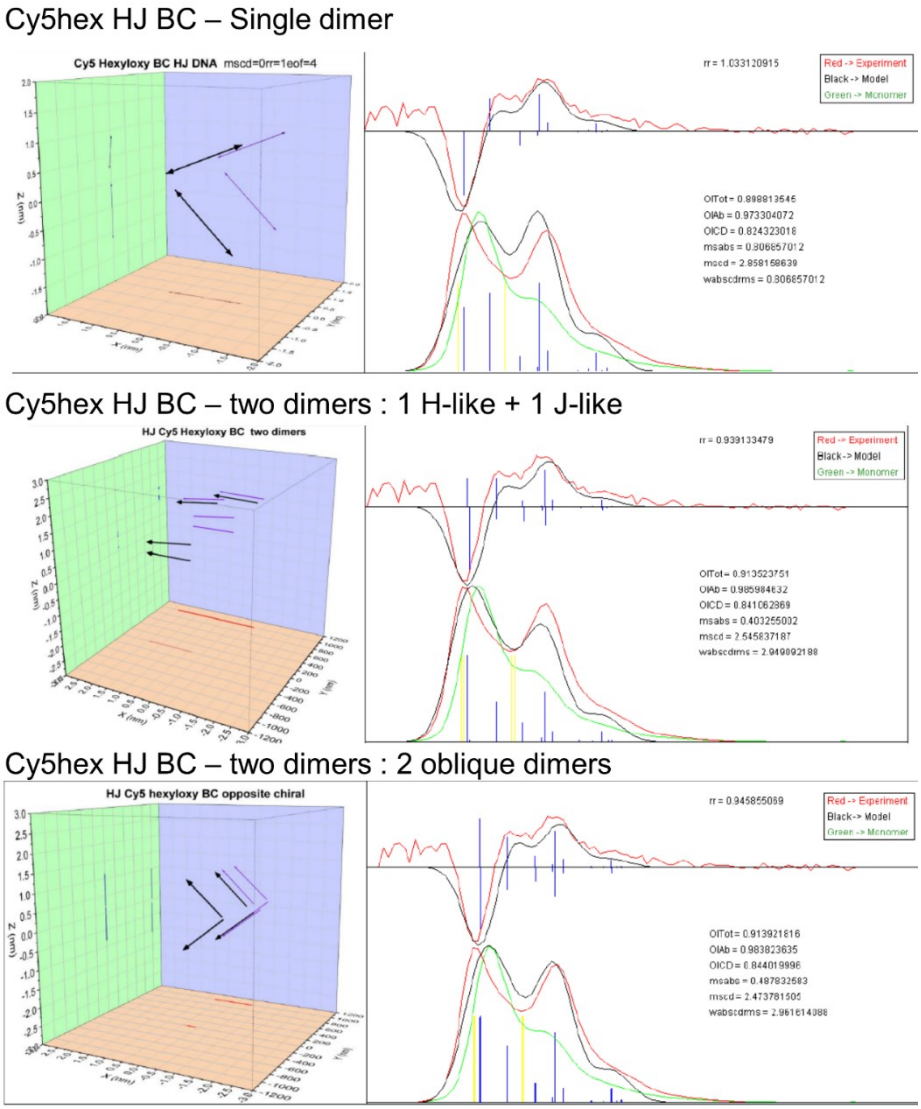

Figure S11: Three KRM fittings of Cy5hex HJ BC denoting the single dimer fitting (top), a result where the fit was improved by using two dimers which resulted in an H-like and J-like dimer pair (middle), a third fit which was also improved where the resulting packing was two dimers of oblique nature with opposite chirality.

Table S3: Comparison of coupling strength for dimers presenting heterogeneous configuration, suggested by the KRM modeling.

Structure	Single Dimer		Two dimers			
			Dimer I		Dimer II	
	Type	$J_{m,n}$ [meV]	Type	$J_{m,n}$ [meV]	Type	$J_{m,n}$ [meV]
Cy5Cl AB	oblique	52.8	H-like	62.8	oblique	47.8
Cy5Cl AD	oblique	48.9	oblique	60.2	oblique	60.0
Cy5Cl CD	oblique	48.0	oblique	59.4	oblique	57.2
Cy5Peg AD	oblique	57.0	H-like	82.2	J-like	-53.2
Cy5Peg BC	oblique	55.1	J-like	-44.2	H-like	85.4
Cy5hex AD	H	109.4	oblique	67.8	oblique	96.6
Cy5hex CD	H	95.5	oblique	77.8	oblique	67.4
Cy5hex BC	oblique	57.7	oblique	59.5	oblique	62.1

The oblique type configuration of dimer I and dimer II indicate that they are chiral opposites of the same packing, thereby they have similar coupling strength. When two $J_{m,n}$ are reported that is because the coupling strength is unique to each dimer. The coupling strength is presented in the same order as the dimer type in the column to the left.

Of the 30 structures we looked at the majority (73%) were fit optimally by a single dimer KRM approach. In the remaining systems, five of the eight, had improved fits by assuming two dimers of the same type (still oblique) with opposite chiral orientations. These cases do not modify the interpretation of the parameters of the Cy5-R that determine dimer structure in DNA HJ templates. The more interesting cases are where the 'Double Dimer' fitting shift the prediction from one configuration in the single dimer to either a different configuration (e.g. Cy5hex AD) or a heterogeneous mixture (e.g. Cy5Peg AD). In all cases, the dimers in the 'Double Dimers' do not couple between each other as seen below. The cases where two dimer configurations are proposed open up the possibility of greater heterogeneity within the structures, though again we highlight that our modeling inherently fixes the distribution at 50% of each dimer. With our current spectroscopy approaches we cannot parse the validity of the hypothesis. Work is underway utilizing more advanced approaches to test for heterogeneity within structures, particularly looking for the J-like dimers in Cy5Peg AD and BC, which will be the focus of future manuscripts.

Single Cy5Cl Dimer

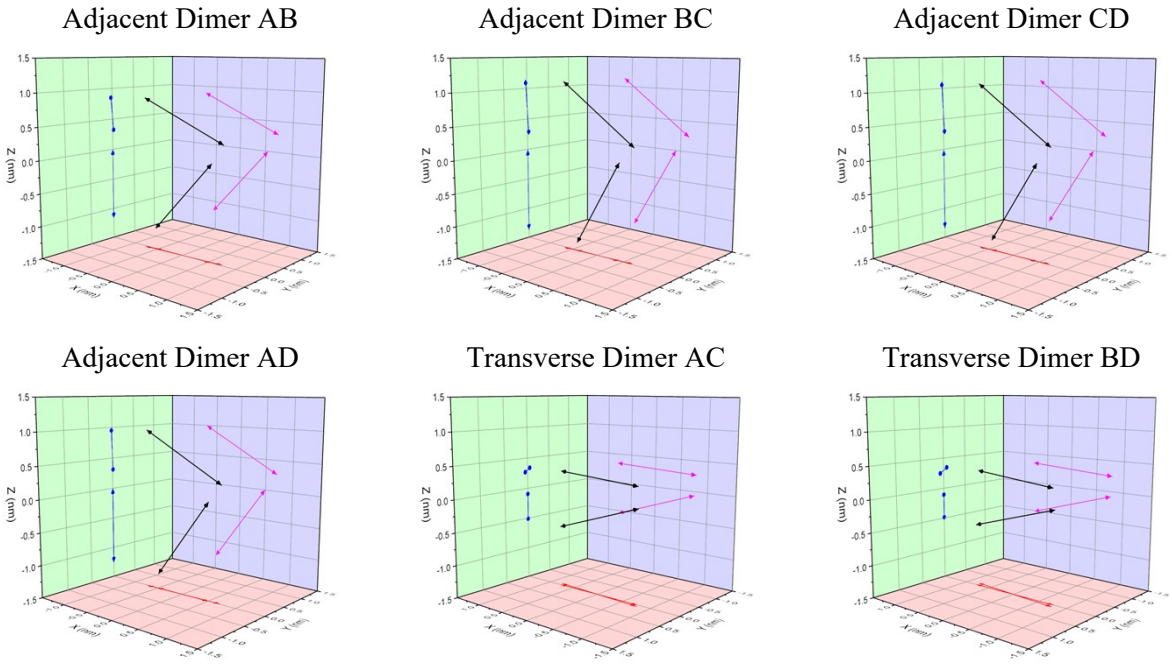

Figure S12. Three-dimensional vector plots of the KRM single dimer positions for Cy5Cl showing the different plane projections XY, XZ, and YZ.

Two Cy5Cl Dimers

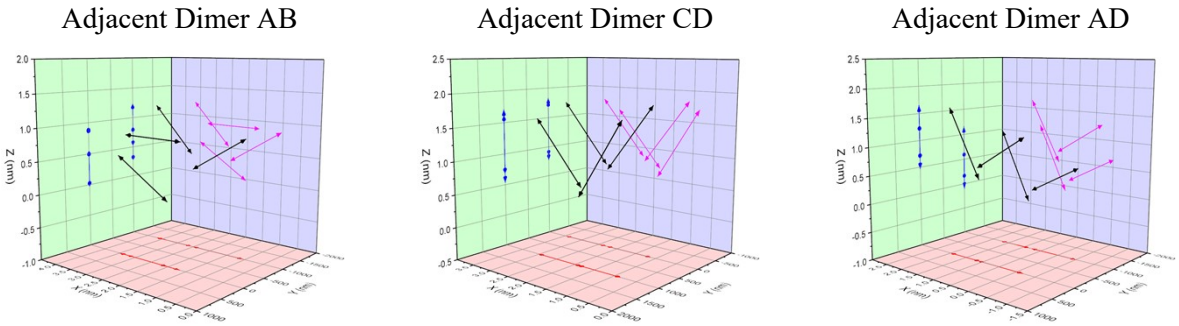

Figure S13. Three-dimensional vector plots of the KRM two dimer positions for Cy5Cl showing the different plane projections XY, XZ, and YZ.

Single Cy5Cl Dimer: Absorbance and Circular Dichroism

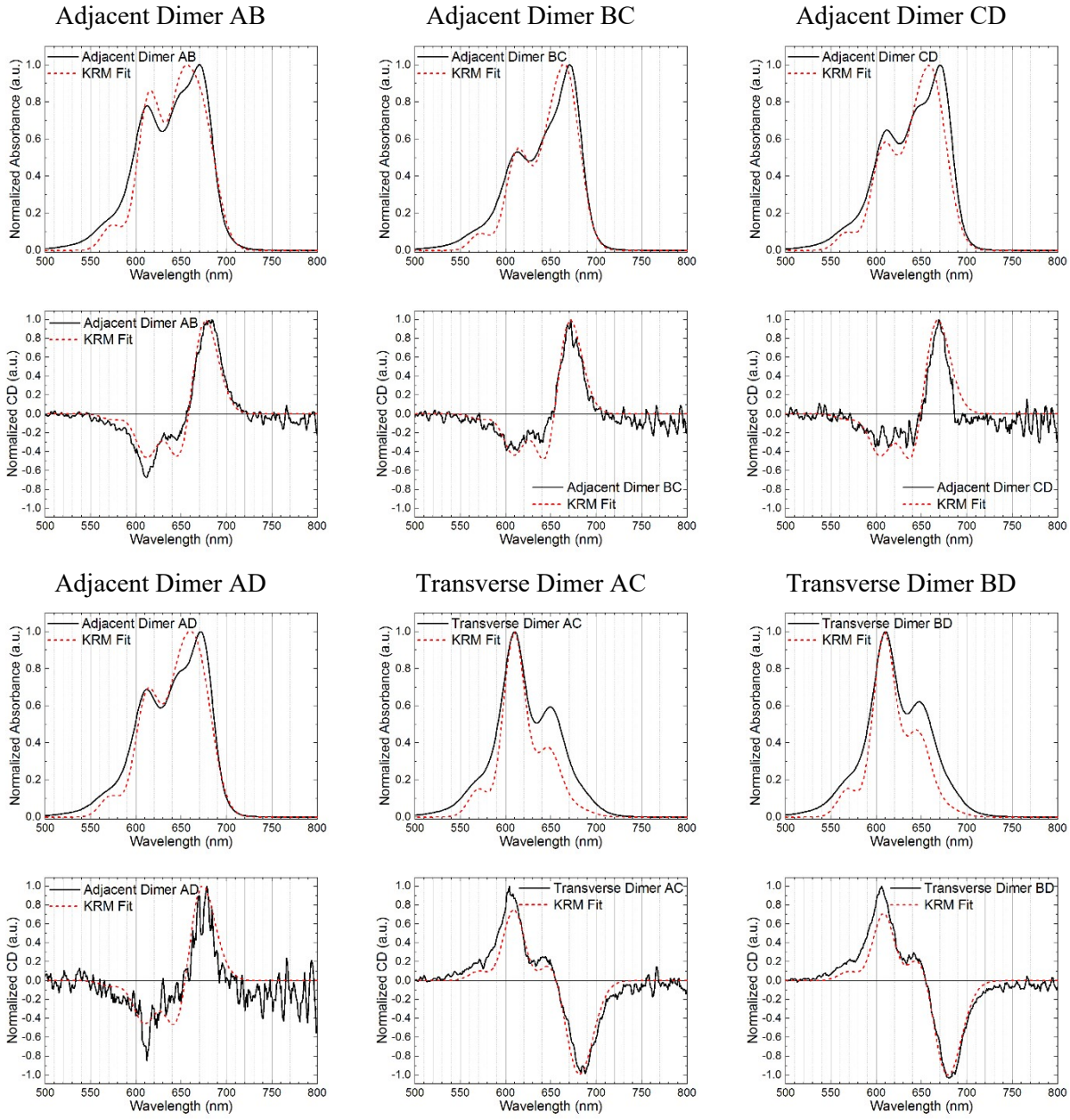

Figure S14. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5Cl covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

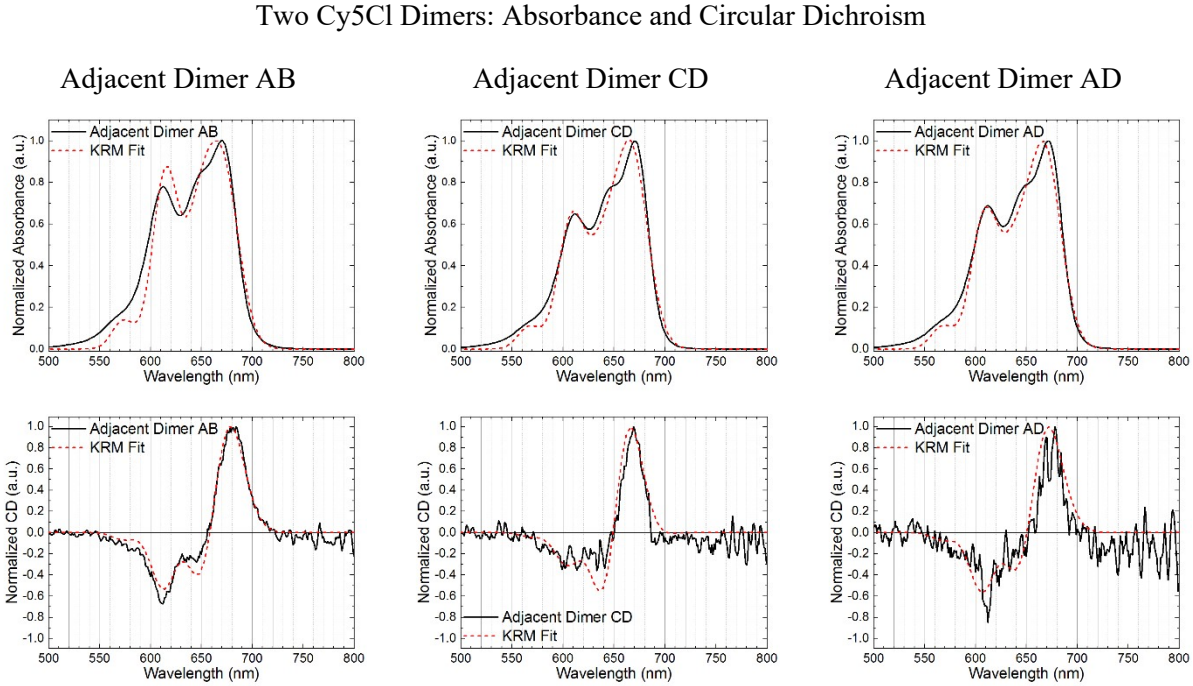

Figure S15. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5Cl covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot **line**). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

Table S4. Various monomer parameters for Cy5Cl.

Parameter	Monomer A	Monomer B	Monomer C	Monomer D
E_v, (eV)	0.135	0.136	0.138	0.143
d (dimensionless units)	0.807	0.805	0.815	0.820
Γ (eV)	0.039	0.037	0.040	0.043
J_0^* $(meV\text{-}nm^3)$	65.3	60.9	66.5	60.3
M^* $(Debye)$	13.627	13.162	13.761	13.096

Table S5. Input fitting parameters used in calculations of single Cy5Cl dimers.

Parameter	Adjacent AB	Adjacent BC	Adjacent CD	Adjacent AD	Transverse AC	Transverse BD
n_v	3	3	3	3	3	3
J_0^* (m$eV{\cdot}nm^3$)	65.3	63.7	63.4	62.8	65.9	60.6
E_{of} (eV)	0.020	0.012	0.024	0.028	0.032	0.014
E_v (eV)	0.140	0.137	0.140	0.139	0.136	0.139
d (dimensionless units)	0.806	0.810	0.818	0.814	0.811	0.813
Γ (eV)	0.039	0.038	0.041	0.041	0.039	0.040
l (nm)	1.4	1.4	1.4	1.4	1.4	1.4
$cdis$ (nm)	0.34	0.34	0.34	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S6. Input fitting parameters used in calculations of two Cy5Cl dimers.

Parameter	Adjacent, AB	Adjacent, CD	Adjacent, AD
n_v	3	3	3
J_0^* (m$eV{\cdot}nm^3$)	65.3	63.4	62.8
E_{of} (eV)	0.020	0.022	0.041
E_v (eV)	0.14	0.14	0.139
d (dimensionless units)	0.806	0.818	0.814
Γ (eV)	0.039	0.041	0.041
l (nm)	1.4	1.4	1.4
$cdis$ (nm)	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S7. The goodness of the fit parameters for absorbance and CD spectra for Cy5Cl dimers (both for one and two dimers).

Construct	r	OI_{AB}	OI_{CD}	OI_{Tot}	MSD_{abs}	MSD_{cd}	$w_{abscd}rms$
Adjacent Dimer, AB	0.962	0.987	0.960	0.973	0.359	0.547	0.907
Adjacent Dimer, BC	0.982	0.988	0.947	0.968	0.218	0.647	0.866
Adjacent Dimer, CD	1.013	0.981	0.910	0.946	0.432	1.212	1.644
Adjacent Dimer, AD	1.010	0.987	0.808	0.898	0.303	2.322	0.303
Transverse Dimer, AC	1.008	0.973	0.963	0.968	0.694	0.759	1.454
Transverse Dimer, BD	0.998	0.980	0.974	0.977	0.544	0.626	1.170
Two Adjacent Dimers, AB	0.999	0.989	0.975	0.982	0.300	0.340	1.498
Two Adjacent Dimers, CD	1.000	0.995	0.914	0.955	0.115	0.981	1.096
Two Adjacent Dimers, AD	1.026	0.995	0.825	0.910	0.118	2.231	2.349

Table S8. Kühn-Renger-May model fitting outputs describing each dye orientation and position in Cy5Cl dimers for one dimer.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Adjacent Dimer, AB					
Dye 1	43.3	0.0	0.000	0.000	-0.586
Dye 2	112.1	2.2	0.000	0.000	0.586
Adjacent Dimer, BC					
Dye 1	30.9	0.0	0.000	0.000	-0.689
Dye 2	124.4	2.5	0.000	0.000	0.689
Adjacent Dimer, CD					
Dye 1	33.5	0.0	0.000	0.000	-0.676
Dye 2	122.5	2.0	0.000	0.000	0.676
Adjacent Dimer, AD					
Dye 1	37.8	0.0	0.000	0.000	-0.631
Dye 2	116.9	1.1	0.000	0.000	0.631
Transverse Dimer, AC					
Dye 1	107.3	0.0	0.000	0.000	-0.304
Dye 2	87.4	-4.2	0.000	0.000	0.304
Transverse Dimer, BD					
Dye 1	105.6	0.0	0.000	0.000	-0.297
Dye 2	86.5	-6.0	0.000	0.000	0.297

Table S9. Kühn-Renger-May model fitting outputs describing each dye in standard orientation and position in Cy5Cl dimers for two dimers.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Two Adjacent Dimers, AB					
Dye 1	64.2	0.0	0.000	0.000	-0.361
Dye 2	87.6	4.0	0.000	0.000	0.361
Dye 3	43.9	0.0	0.000	0.000	-0.765
Dye 4	160.5	2.1	0.000	0.000	0.765
Two Adjacent Dimers, CD					
Dye 1	42.7	0.0	0.000	0.000	-0.602
Dye 2	130.8	-33.2	0.000	0.000	0.602
Dye 3	48.7	0.0	0.000	0.000	-0.534
Dye 4	130.4	38.3	0.000	0.000	0.534
Two Adjacent Dimers, AD					
Dye 1	47.1	0.0	0.000	0.000	-0.547
Dye 2	131.1	36.8	0.000	0.000	0.547
Dye 3	50.0	0.0	0.000	0.000	-0.589
Dye 4	135.3	-32.1	0.000	0.000	0.589

* Standard orientation and position of Dimer I (dyes 1 & 2) and Dimer II (dyes 3 & 4) separated by ~1,000 nm.

Table S10. Calculated $J_{m,n,}$ and geometric parameters of Cy5Cl dimers (both for one and two dimers).

Dimer Aggregate	$J_{m,n}$ (meV)	R, ($Å$)	d_{min} (nm)	α (°)	θ_{1s} (°)	θ_{2s} (°)	θ_t (°)
Adjacent, AB	52.8	1.17	0.34	68.9	43.3	67.9	2.2
Adjacent, BC	48.1	1.38	0.34	86.5	30.9	55.6	2.5
Adjacent, CD	48.0	1.35	0.34	89.1	33.5	57.5	2.0
Adjacent, AD	48.9	1.26	0.34	79.2	37.8	63.1	1.1
Transverse, AC	85.0	0.61	0.34	20.4	72.7	87.4	-4.2
Transverse, BD	79.5	0.59	0.34	20.0	74.4	86.5	-6.0
Two Adjacent dimers, AB	62.8	0.72	0.41	23.7	64.2	87.6	4.0
	47.8	1.53	0.35	63.4	43.9	19.5	2.1
Two Adjacent dimers, CD	59.4	1.20	0.34	87.0	42.7	49.2	-33.2
	57.2	1.07	0.38	88.8	48.7	49.6	38.3
Two Adjacent dimers, AD	60.2	1.09	0.36	89.6	47.1	48.9	36.8
	60.0	1.18	0.34	90.0	50.0	44.7	-32.1

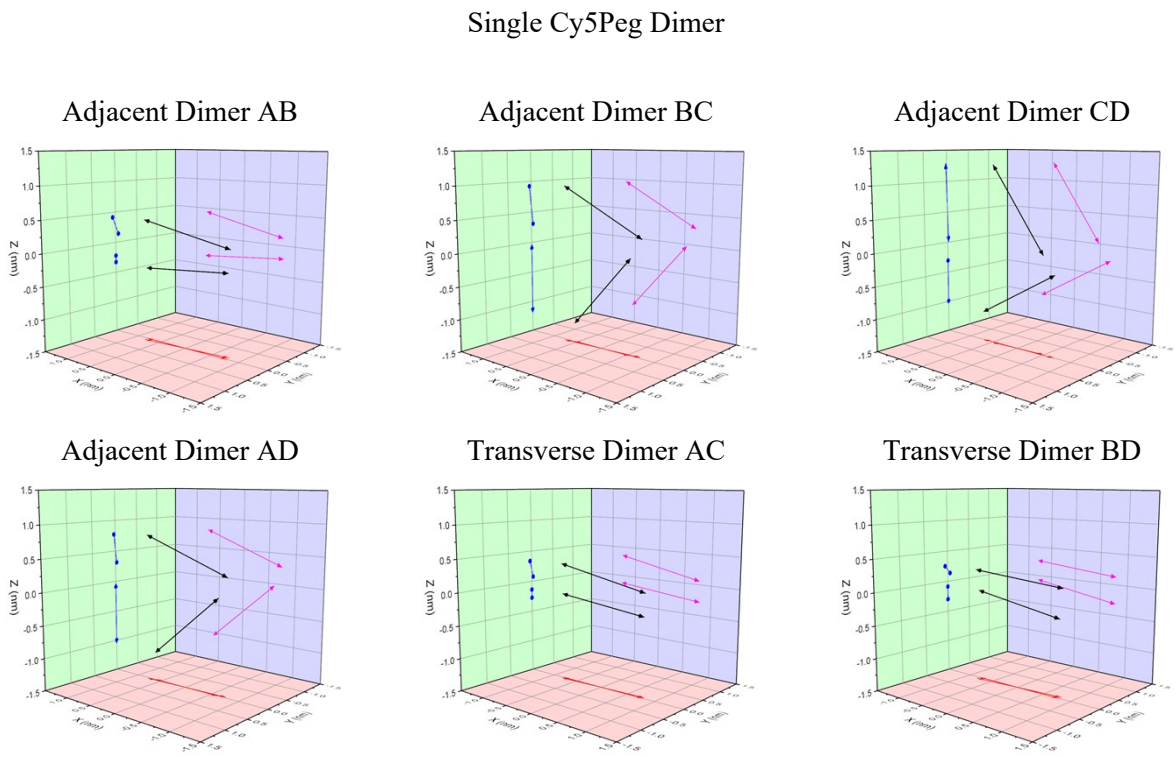

Figure S16. Three-dimensional vector plots of the KRM single dimer positions for Cy5Peg showing the different plane projections XY, XZ, and YZ.

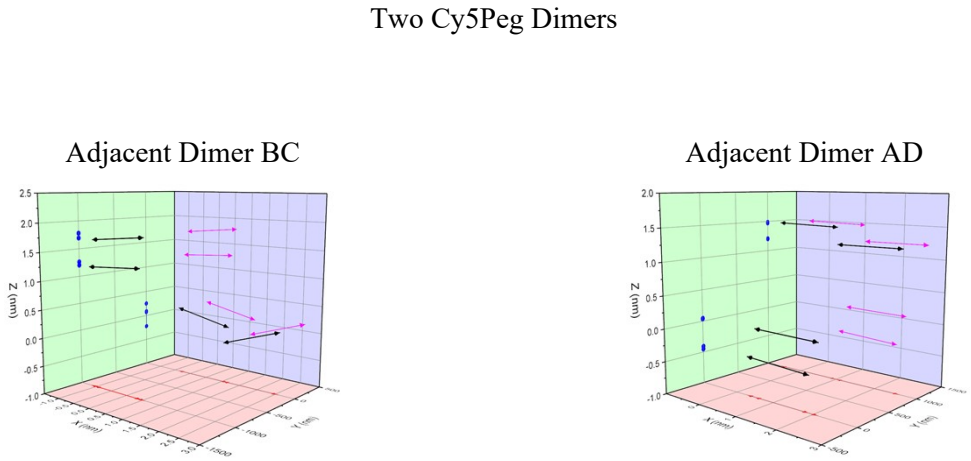

Figure S17. Three-dimensional vector plots of the KRM two dimer positions for Cy5Peg showing the different plane projections XY, XZ, and YZ.

Single Cy5Peg Dimer: Absorbance and Circular Dichroism

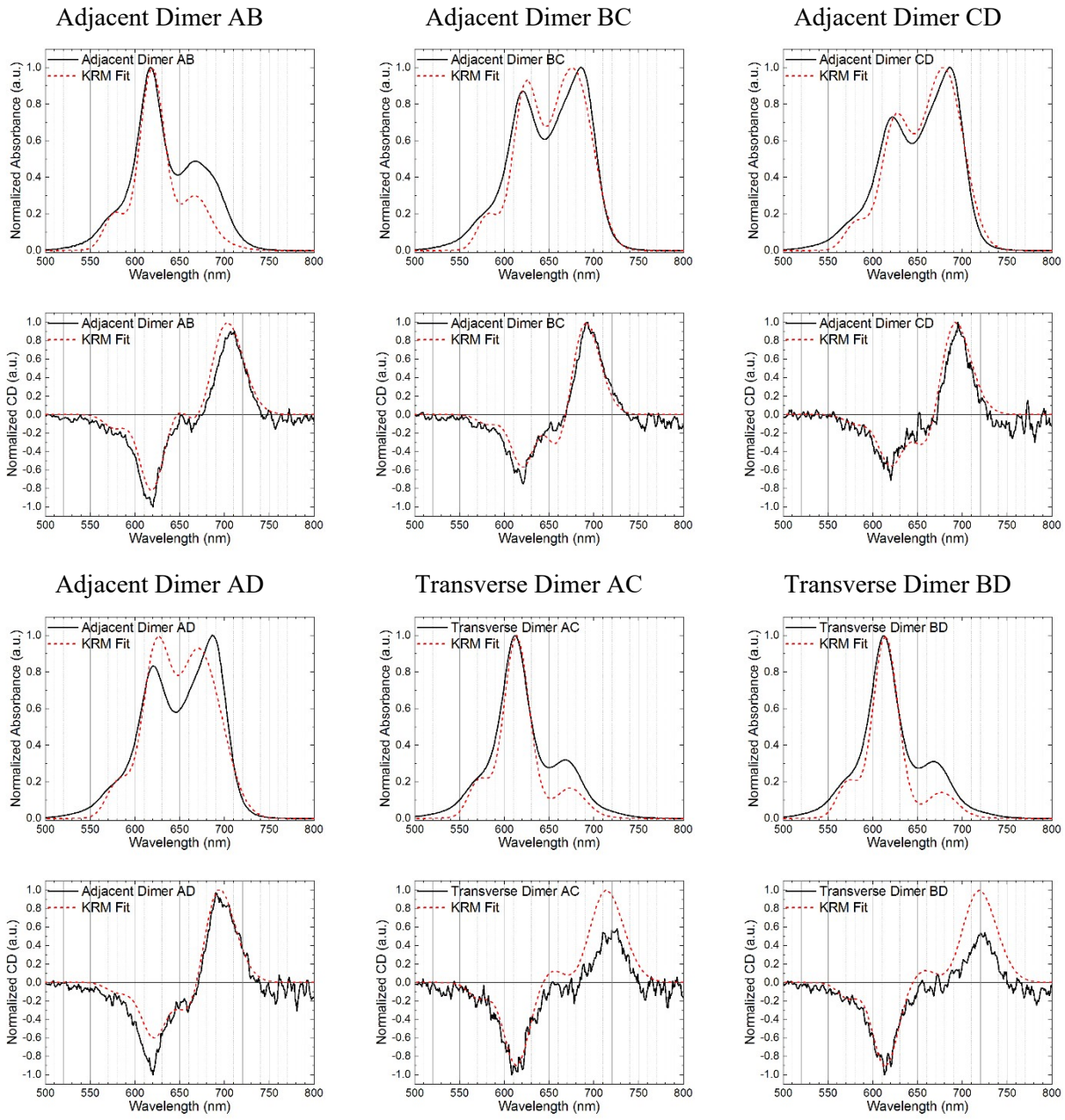

Figure S18. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5Peg covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

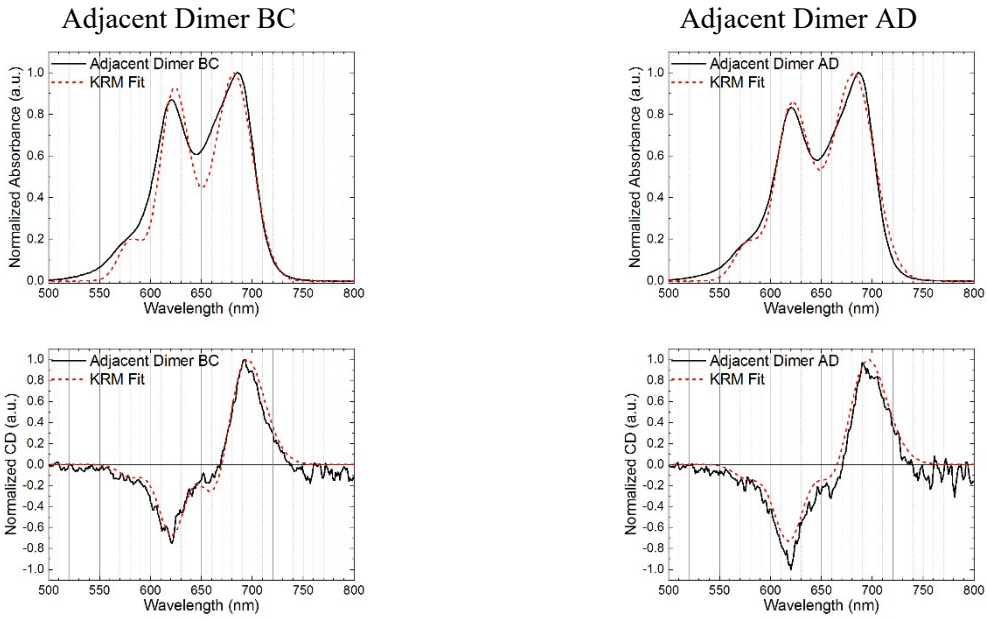

Figure S19. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5Peg covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

Table S11. Various monomer parameters for Cy5Peg.

Parameter	Monomer A	Monomer B	Monomer C	Monomer D
E_v, (eV)	0.140	0.138	0.140	0.143
d (dimensionless units)	0.944	0.920	0.944	0.926
Γ (eV)	0.047	0.041	0.045	0.049
J_0 (meV-nm^3)	68.0	70.0	68.5	65.4
M (*Debye*)	13.911	14.112	13.957	13.641

Table S12. Input fitting parameters used in calculations of single Cy5Peg dimers.

Parameter	Adjacent AB	Adjacent BC	Adjacent CD	Adjacent AD	Transverse AC	Transverse BD
n_v	3	3	3	3	3	3
J_0^* (m$eV{\cdot}nm^3$)	69.0	69.2	66.9	66.7	68.2	67.7
E_{of} (eV)	0.010	0.014	0.000	0.006	0.008	0.006
Energy of vibron, E_v (eV)	0.139	0.139	0.141	0.141	0.140	0.140
d (dimensionless units)	0.930	0.932	0.935	0.933	0.942	0.923
Γ (eV)	0.0439	0.0430	0.0470	0.0479	0.0459	0.0450
l (nm)	1.4	1.4	1.4	1.4	1.4	1.4
$cdis$ (nm)	0.34	0.34	0.34	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S13. Input fitting parameters used in calculations of two Cy5Peg dimers.

Parameter	Adjacent, BC	Adjacent, AD
n_v	3	3
J_0^* (m$eV{\cdot}nm^3$)	69.2	66.7
E_{of} (eV)	0.010	0.015
E_v (eV)	0.139	0.141
d (dimensionless units)	0.932	0.933
Γ (eV)	0.0430	0.0479
l (nm)	1.4	1.4
$cdis$ (nm)	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S14. The goodness of the fit parameters for absorbance and CD spectra for Cy5Peg dimers (both for one and two dimers).

Construct	r	OI_{AB}	OI_{CD}	OI_{Tot}	MSD_{abs}	MSD_{cd}	$w_{abscd}rms$
Adjacent Dimer, AB	1.015	0.970	0.963	0.966	0.625	0.686	1.310
Adjacent Dimer, BC	1.016	0.987	0.968	0.977	0.391	0.469	0.859
Adjacent Dimer, CD	1.006	0.991	0.949	0.970	0.231	0.763	0.994
Adjacent Dimer, AD	0.996	0.976	0.952	0.964	0.763	1.023	1.786
Transverse Dimer, AC	1.004	0.980	0.878	0.929	0.371	2.581	2.953
Transverse Dimer, BD	1.003	0.975	0.893	0.934	0.458	2.377	2.835
Two Adjacent Dimers, BC	1.001	0.990	0.977	0.983	0.332	0.368	0.700
Two Adjacent Dimers, AD	1.010	0.997	0.965	0.981	0.090	0.717	0.808

Table S15. Kühn-Renger-May model fitting outputs describing each dye orientation and position in Cy5Peg single dimers.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Adjacent Dimer, AB					
Dye 1	94.5	0.0	0.000	0.000	-0.277
Dye 2	78.5	5.2	0.000	0.000	0.277
Adjacent Dimer, BC					
Dye 1	137.0	0.0	0.000	0.000	-0.614
Dye 2	64.0	3.2	0.000	0.000	0.614
Adjacent Dimer, CD					
Dye 1	119.1	0.0	0.000	0.000	-0.648
Dye 2	36.2	2.4	0.000	0.000	0.648
Adjacent Dimer, AD					
Dye 1	129.6	0.0	0.000	0.000	-0.542
Dye 2	70.7	2.3	0.000	0.000	0.542
Transverse Dimer, AC					
Dye 1	95.6	0.0	0.000	0.000	-0.207
Dye 2	100.8	2.7	0.000	0.000	0.207
Transverse Dimer, BD					
Dye 1	98.7	0.0	0.000	0.000	-0.194
Dye 2	95.0	4.5	0.000	0.000	0.194

Table S16. Kühn-Renger-May model fitting outputs describing each dye in standard orientation and position in Cy5Peg two dimers.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Two Adjacent Dimers, BC					
Dye 1	150.6	0.0	0.000	0.000	-0.754
Dye 2	173.4	-42.8	0.000	0.000	0.754
Dye 3	74.7	0.0	0.000	0.000	-0.264
Dye 4	72.6	4.6	0.000	0.000	0.264
Two Adjacent Dimers, AD					
Dye 1	66.7	0.0	0.000	0.000	-0.261
Dye 2	66.0	6.3	0.000	0.000	0.261
Dye 3	12.5	0.0	0.000	0.000	-0.818
Dye 4	12.8	-9.1	0.000	0.000	0.818

*Standard orientation and position of Dimer I (dyes 1 & 2) and Dimer II (dyes 3 & 4) separated by ~1,000 nm.

Table S17. Calculated $J_{m,n,}$ and geometric parameters of Cy5Peg dimers (both for one and two dimers).

Dimer Aggregate	$J_{m,n}$ (meV)	R, ($Å$)	d_{min} (nm)	α (°)	θ_{1s} (°)	θ_{2s} (°)	θ_t (°)
Adjacent, AB	95.8	0.55	0.34	16.8	85.5	78.5	5.2
Adjacent, BC	55.1	1.23	0.34	73.1	43.0	64.0	3.2
Adjacent, CD	51.6	1.30	0.34	82.9	60.9	36.2	2.4
Adjacent, AD	57.0	1.08	0.34	58.9	50.4	70.7	2.3
Transverse, AC	124.0	0.41	0.34	5.8	84.4	79.2	2.7
Transverse, BD	131.0	0.39	0.34	5.8	81.3	85.0	4.5
Two Adjacent dimers, BC	-44.2	1.51	0.41	24.9	29.4	6.6	-42.8
	85.4	0.53	0.49	4.9	74.7	72.6	4.6
Two Adjacent dimers, AD	82.2	0.52	0.48	5.8	66.7	66.0	6.3
	-53.2	1.64	0.36	2.0	12.5	12.8	-9.1

Single Cy5hex Dimer

Adjacent Dimer AB Adjacent Dimer BC Adjacent Dimer CD

Single Cy5hex Dimer: Absorbance and Circular Dichroism

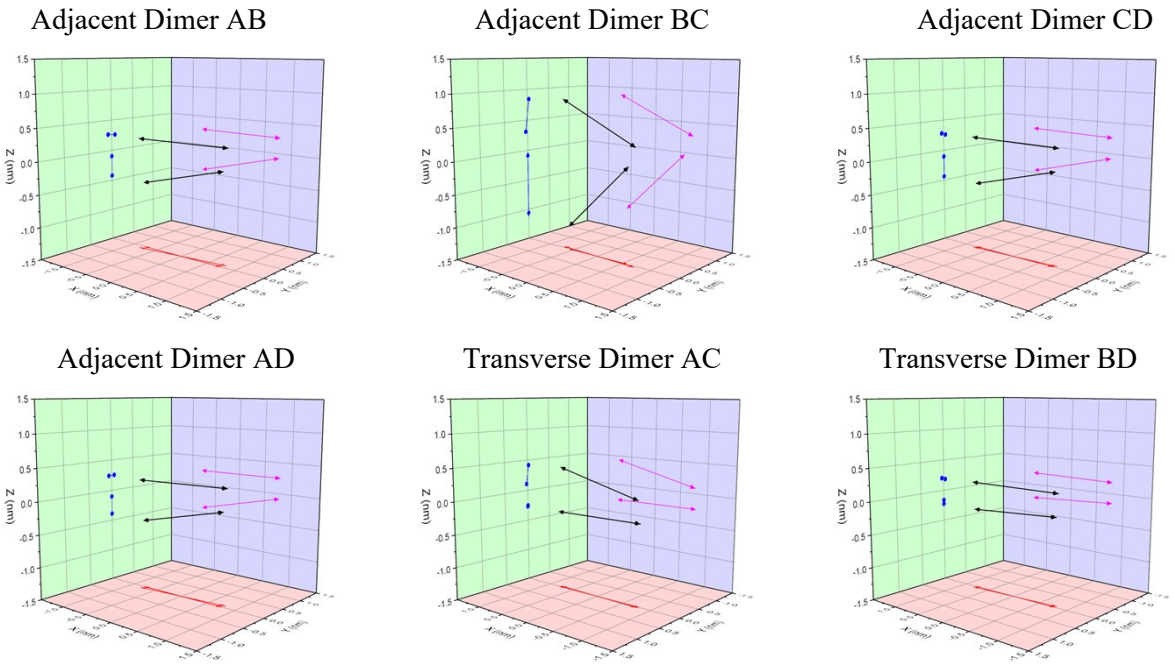

Figure S19. Three-dimensional vector plots of the KRM single dimer positions for Cy5hex showing the different plane projections XY, XZ, and YZ.

Two Cy5hex dimers

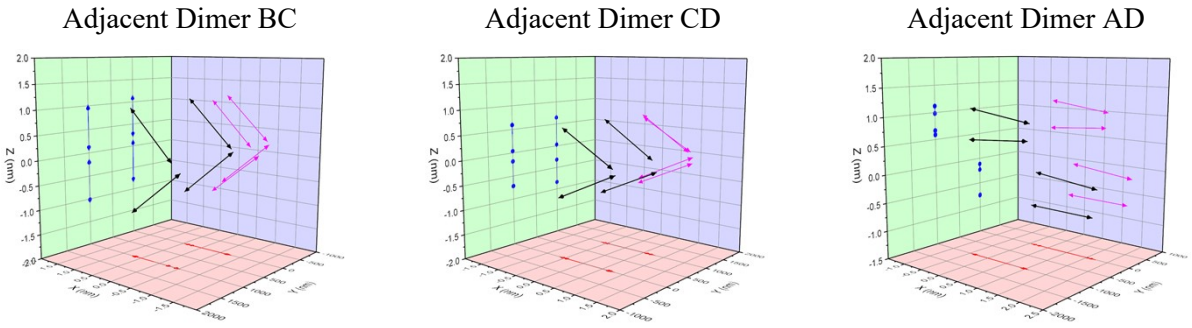

Figure S20. Three-dimensional vector plots of the KRM two dimer positions for Cy5hex showing the different plane projections XY, XZ, and YZ.

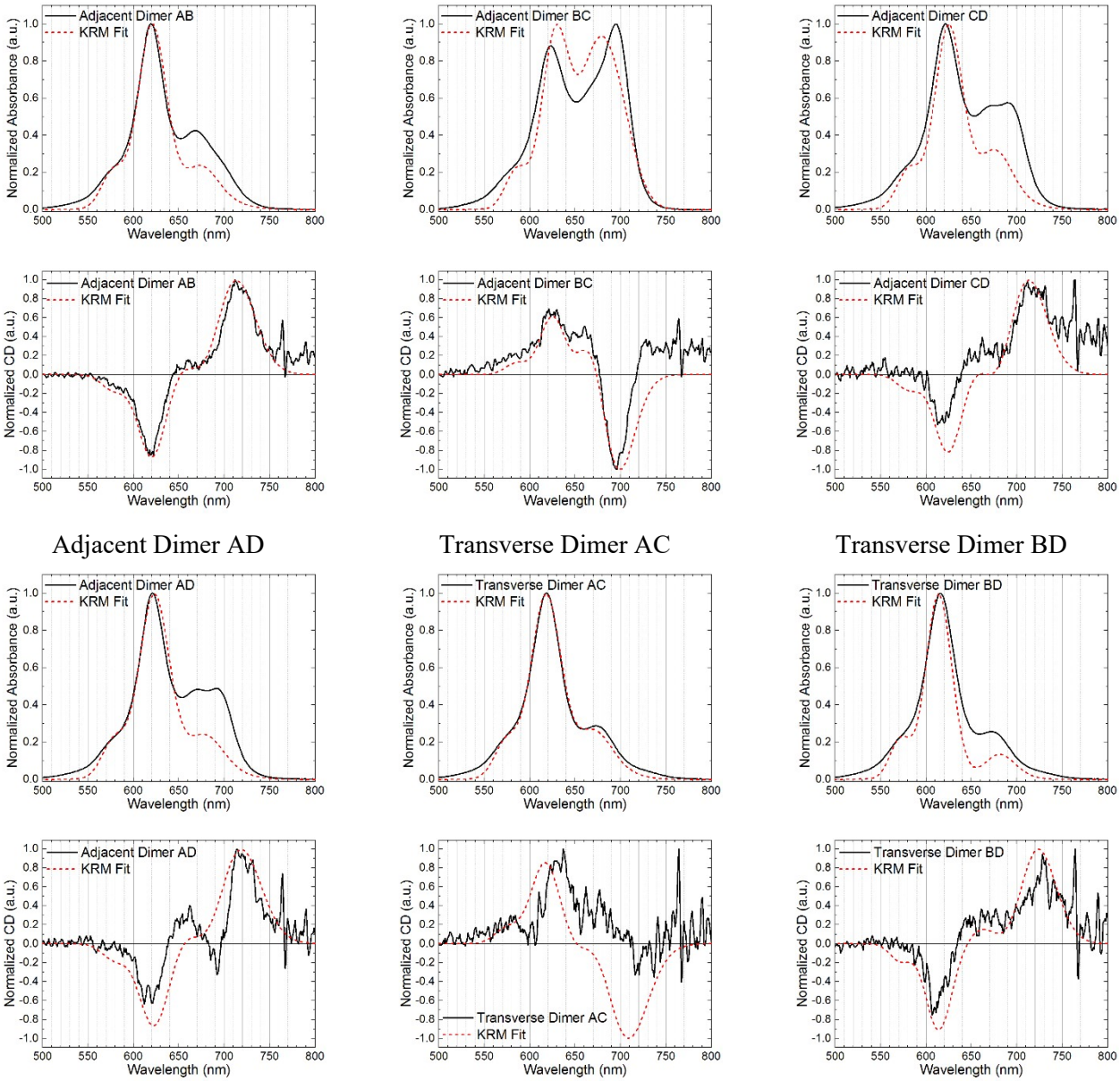

Figure S21. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5hex covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

Two Cy5hex Dimers: Absorbance and Circular Dichroism

Adjacent Dimer BC Adjacent Dimer CD Adjacent Dimer AD

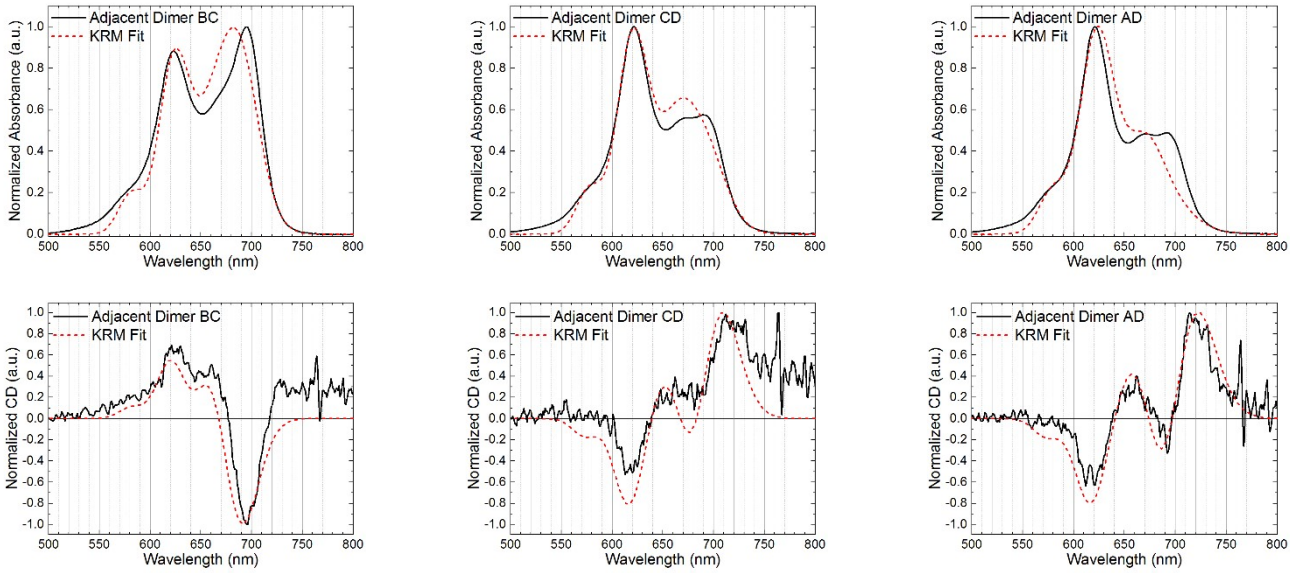

Figure S22. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5hex covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

Table S18. Monomer parameters for Cy5hex.

Parameter	Monomer A	Monomer B	Monomer C	Monomer D
E_v, (eV)	0.141	0.137	0.141	0.144
d (dimensionless units)	0.958	0.946	0.969	0.971
Γ (eV)	0.057	0.043	0.049	0.048
J_0 (meV-nm^3)	75.4	72.8	67.2	67.7
M $(Debye)$	14.645	14.389	13.823	13.885

Table S19. Input fitting parameters used in calculations of single Cy5hex dimers.

Parameter	Adjacent AB	Adjacent BC	Adjacent CD	Adjacent AD	Transverse AC	Transverse BD
n_v	3	3	3	3	3	3
J_0^* ($meV{\cdot}nm^3$)	74.1	70.0	67.4	71.6	71.3	70.3
E_{of} (eV)	0.014	0.004	-0.010	0.002	0.022	0.006
E_v (eV)	0.139	0.139	0.142	0.142	0.141	0.140
d (dimensionless units)	0.9520	0.9572	0.9699	0.9647	0.9635	0.9583
Γ (eV)	0.0498	0.0460	0.0485	0.0523	0.0528	0.0455
l (nm)	1.4	1.4	1.4	1.4	1.4	1.4
$cdis$ (nm)	0.34	0.34	0.34	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S20. Input fitting parameters used in calculations of two Cy5hex dimers.

Parameter	Adjacent, BC	Adjacent, CD	Adjacent, AD
n_v	3	3	3
J_0^* ($meV{\cdot}nm^3$)	70.0	67.4	71.6
E_{of} (eV)	0.020	0.015	0.014
E_v (eV)	0.139	0.142	0.142
d (dimensionless units)	0.957	0.970	0.965
Γ (eV)	0.046	0.049	0.052
l (nm)	1.4	1.4	1.4
$cdis$ (nm)	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S21. The goodness of the fit parameters for absorbance and CD spectra for Cy5hex dimers (both for one and two dimers).

Construct	r	OI_{AB}	OI_{CD}	OI_{Tot}	MSD_{abs}	MSD_{cd}	$w_{abscd}rms$
Adjacent Dimer, AB	1.017	0.981	0.973	0.977	0.344	0.679	1.023
Adjacent Dimer, BC	1.033	0.973	0.824	0.899	0.807	2.858	0.807
Adjacent Dimer, CD	0.984	0.956	0.801	0.878	1.124	3.833	4.957
Adjacent Dimer, AD	1.021	0.960	0.841	0.901	0.802	3.635	4.437
Transverse Dimer, AC	1.005	0.997	0.392	0.694	0.047	11.767	0.047
Transverse Dimer, BD	0.987	0.977	0.887	0.932	0.430	2.683	3.113
Two Adjacent Dimers, BC	0.946	0.984	0.844	0.914	0.488	2.474	2.962
Two Adjacent Dimers, CD	0.981	0.995	0.780	0.888	0.138	3.972	4.110
Two Adjacent Dimers, AD	0.956	0.984	0.941	0.962	0.339	1.355	1.694

Table S22. Kühn-Renger-May model fitting outputs describing each dye in standard orientation and position in Cy5hex dimers for one dimer.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Adjacent Dimer, AB					
Dye 1	76.6	0.0	0.000	0.000	-0.261
Dye 2	90.6	6.3	0.000	0.000	0.261
Adjacent Dimer, BC					
Dye 1	46.7	0.0	0.000	0.000	-0.576
Dye 2	112.2	-3.4	0.000	0.000	0.576
Adjacent Dimer, CD					
Dye 1	76.2	0.0	0.000	0.000	-0.271
Dye 2	91.1	3.5	0.000	0.000	0.271
Adjacent Dimer, AD					
Dye 1	78.1	0.0	0.000	0.000	-0.248
Dye 2	89.8	4.9	0.000	0.000	0.248
Transverse Dimer, AC					
Dye 1	90.6	0.0	0.000	0.000	-0.261
Dye 2	77.3	-2.0	0.000	0.000	0.261
Transverse Dimer, BD					
Dye 1	92.7	0.0	0.000	0.000	-0.194
Dye 2	89.1	3.2	0.000	0.000	0.194

Table S23. Kühn-Renger-May model fitting outputs describing each dye in standard orientation and position in Cy5hex dimers for two dimers.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Two Adjacent Dimers, BC*					
Dye 1	130.5	0.0	0.000	0.000	-0.516
Dye 2	56.3	-36.6	0.000	0.000	0.516
Dye 3	121.2	0.0	0.000	0.000	-0.577
Dye 4	44.2	26.2	0.000	0.000	0.577
Two Adjacent Dimers, CD*					
Dye 1	64.0	0.0	0.000	0.000	-0.384
Dye 2	114.4	27.0	0.000	0.000	0.384
Dye 3	67.3	0.0	0.000	0.000	-0.460
Dye 4	119.8	-21.2	0.000	0.000	0.460
Two Adjacent Dimers, AD*					
Dye 1	78.1	0.0	0.000	0.000	-0.301
Dye 2	86.4	-18.0	0.000	0.000	0.301
Dye 3	73.1	0.0	0.000	0.000	-0.247
Dye 4	97.9	24.5	0.000	0.000	0.247

* Standard orientation and position of Dimer I (dyes 1 & 2) and Dimer II (dyes 3 & 4) separated by ~1,000 nm.

Table S24. Calculated $J_{n,m}$ and geometric parameters of Cy5hex dimers (both for one and two dimers).

Dimer Aggregate	$J_{m,n}$ (meV)	R, ($Å$)	d_{min} (nm)	α (°)	θ_{1s} (°)	θ_{2s} (°)	θ_t (°)
Adjacent, AB	107.8	0.52	0.34	15.3	76.6	89.4	6.3
Adjacent, BC	57.7	1.15	0.34	65.6	46.7	67.8	-3.4
Adjacent, CD	95.5	0.54	0.34	15.3	76.2	88.9	3.5
Adjacent, AD	109.4	0.50	0.34	12.7	78.1	89.8	4.9
Transverse, AC	104.3	0.52	0.34	13.4	89.4	77.3	-2.0
Transverse, BD	136.6	0.39	0.34	4.8	87.3	89.1	3.2
Two Adjacent Dimers, BC	59.5	1.03	0.39	81.5	49.5	56.3	-36.6
	62.1	1.15	0.34	80.6	58.8	44.2	26.2
Two Adjacent, Dimers CD	77.8	0.77	0.34	56.8	64.0	65.6	27.0
	67.4	0.92	0.34	56.3	67.3	60.2	-21.2
Two Adjacent, Dimers AD	67.8	0.60	0.54	19.6	78.1	86.4	-18.0
	96.6	0.49	0.34	34.6	73.1	82.1	24.5

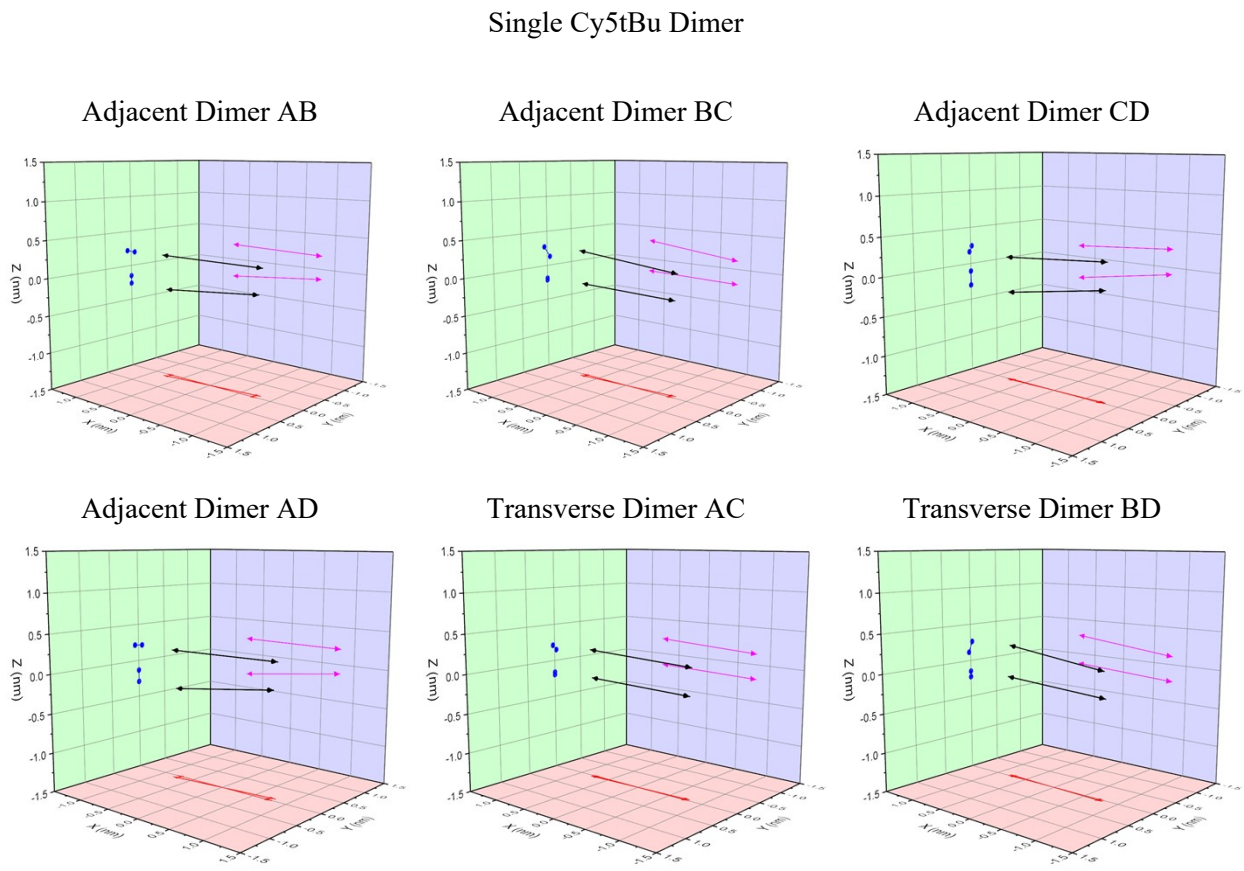

Figure S23. Three-dimensional vector plots of the KRM single dimer positions for Cy5tBu showing the different plane projections XY, XZ, and YZ.

Single Cy5tBu Dimer: Absorbance and Circular Dichroism

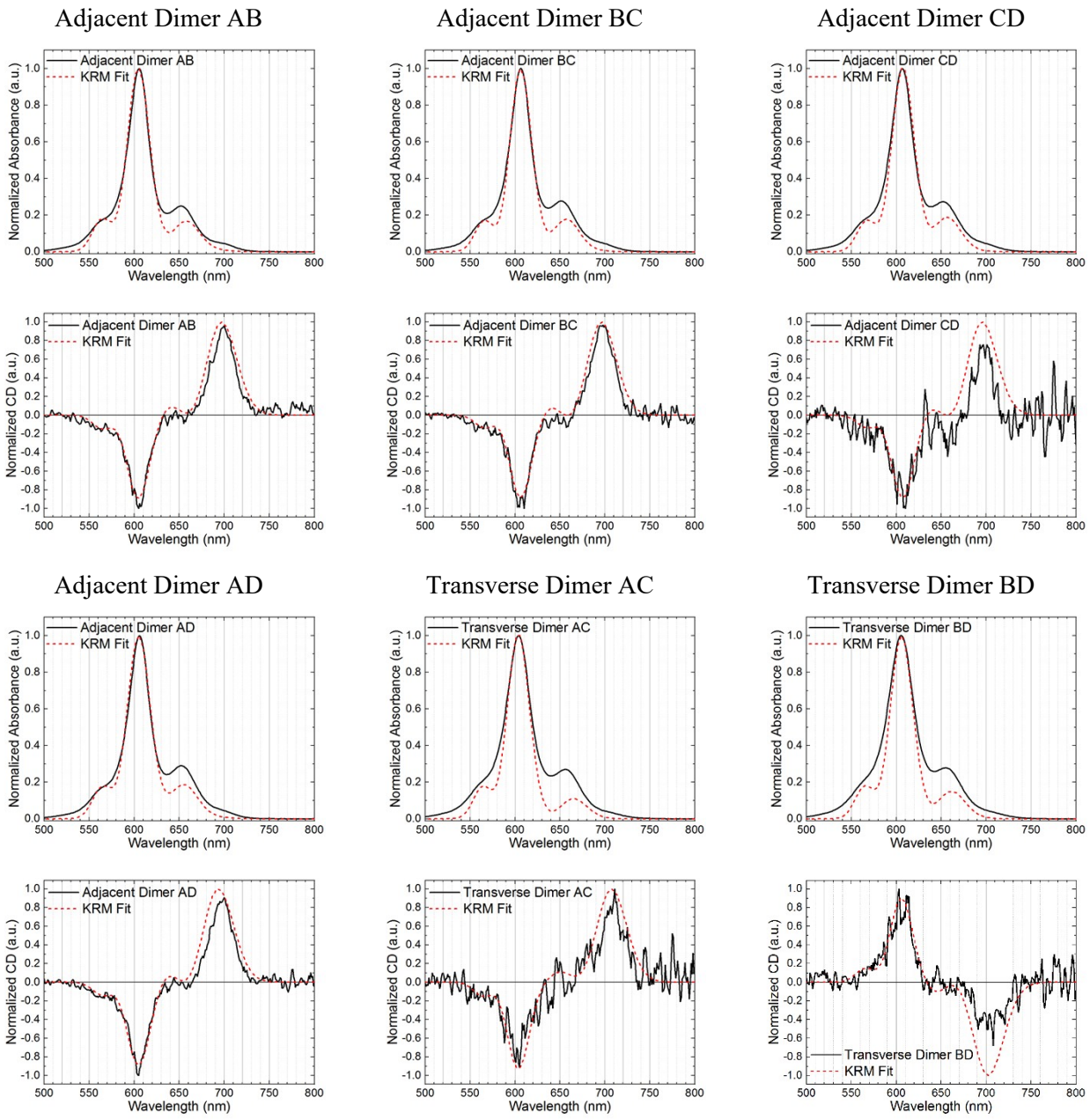

Figure S24. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5tBu covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

Table S25. Various monomer parameters for Cy5tBu.

Parameter	Monomer A	Monomer B	Monomer C	Monomer D
E_v, (eV)	0.137	0.141	0.137	0.139
d (dimensionless units)	0.867	0.873	0.833	0.862
Γ (eV)	0.043	0.041	0.040	0.043
J_0 (meV-nm^3)	69.2	63.4	61.0	60.4
M (*Debye*)	14.031	13.431	13.174	13.114

Table S26. Input fitting parameters used in calculations of Cy5tBu dimers.

Parameter	Adjacent AB	Adjacent BC	Adjacent CD	Adjacent AD	Transverse AC	Transverse BD
n_v	3	3	3	3	3	3
J_0^* (meV·nm^3)	66.3	62.2	60.7	64.8	65.1	61.9
E_{of} (eV)	0.012	0.008	-0.004	0.016	-0.010	0.001
E_v (eV)	0.139	0.139	0.138	0.138	0.137	0.140
d (dimensionless units)	0.870	0.853	0.848	0.865	0.850	0.868
Γ (eV)	0.042	0.040	0.041	0.043	0.041	0.042
l (nm)	1.4	1.4	1.4	1.4	1.4	1.4
cdis (nm)	0.34	0.34	0.34	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S27. The goodness of the fit parameters for absorbance and CD spectra for Cy5tBu dimers.

Construct	r	OI_{AB}	OI_{CD}	OI_{Tot}	MSD_{abs}	MSD_{cd}	$w_{abscd}rms$
Adjacent Dimer, AB	1.013	0.991	0.979	0.985	0.106	0.446	0.552
Adjacent Dimer, BC	0.997	0.988	0.980	0.984	0.163	0.377	0.539
Adjacent Dimer, CD	1.010	0.988	0.865	0.926	0.167	2.439	2.605
Adjacent Dimer, AD	0.996	0.991	0.974	0.982	0.121	0.620	0.741
Transverse Dimer, AC	1.002	0.971	0.946	0.959	0.451	1.245	1.696
Transverse Dimer, BD	0.998	0.977	0.900	0.938	0.437	2.172	2.609

Table S28. Kühn-Renger-May model fitting outputs describing each dye orientation and position in Cy5tBu dimers.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Adjacent Dimer, AB					
Dye 1	94.5	0.0	0.000	0.000	-0.207
Dye 2	88.7	5.9	0.000	0.000	0.207
Adjacent Dimer, BC					
Dye 1	88.8	0.0	0.000	0.000	-0.202
Dye 2	83.9	4.5	0.000	0.000	0.202
Adjacent Dimer, CD					
Dye 1	98.0	0.0	0.000	0.000	-0.204
Dye 2	93.2	1.8	0.000	0.000	0.204
Adjacent Dimer, AD					
Dye 1	83.5	0.0	0.000	0.000	-0.214
Dye 2	90.4	5.4	0.000	0.000	0.214
Transverse Dimer, AC					
Dye 1	88.5	0.0	0.000	0.000	-0.181
Dye 2	87.3	2.5	0.000	0.000	0.181
Transverse Dimer, BD					
Dye 1	86.8	0.0	0.000	0.000	-0.188
Dye 2	84.2	-2.5	0.000	0.000	0.188

Table S29. Calculated $J_{m,n}$ and geometric parameters of Cy5tBu dimers.

Dimer Aggregate	$J_{m,n}$ (meV)	R, ($Å$)	d_{min} (nm)	α (°)	θ_{1s} (°)	θ_{2s} (°)	θ_t (°)
Adjacent, AB	119.1	0.41	0.34	8.3	85.5	88.7	5.9
Adjacent, BC	115.4	0.40	0.34	6.7	88.8	83.9	4.5
Adjacent, CD	112.4	0.41	0.34	5.1	82.0	86.8	1.8
Adjacent, AD	113.2	0.43	0.34	8.8	83.5	89.6	5.4
Transverse, AC	137.5	0.36	0.35	2.8	88.5	87.3	2.5
Transverse, BD	124.7	0.38	0.34	3.6	86.8	84.2	-2.5

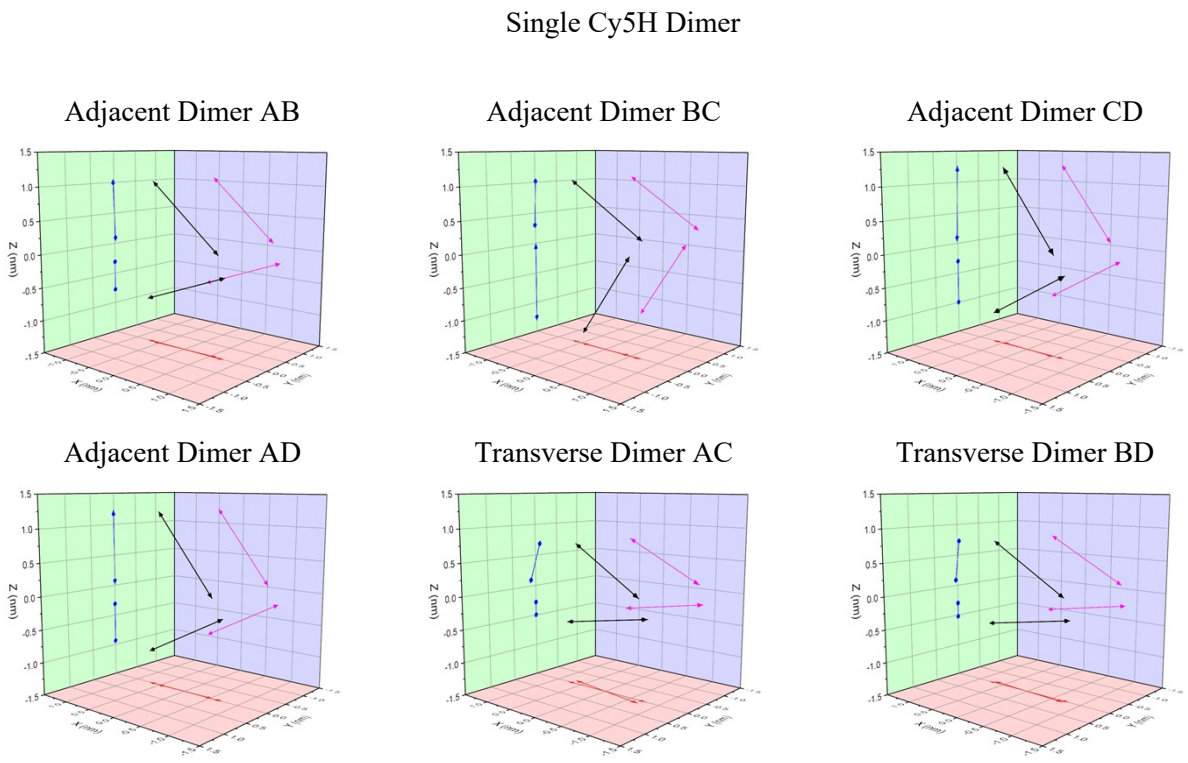

Figure S25. Three-dimensional vector plots of the KRM single dimer positions for Cy5H showing the different plane projections XY, XZ, and YZ.

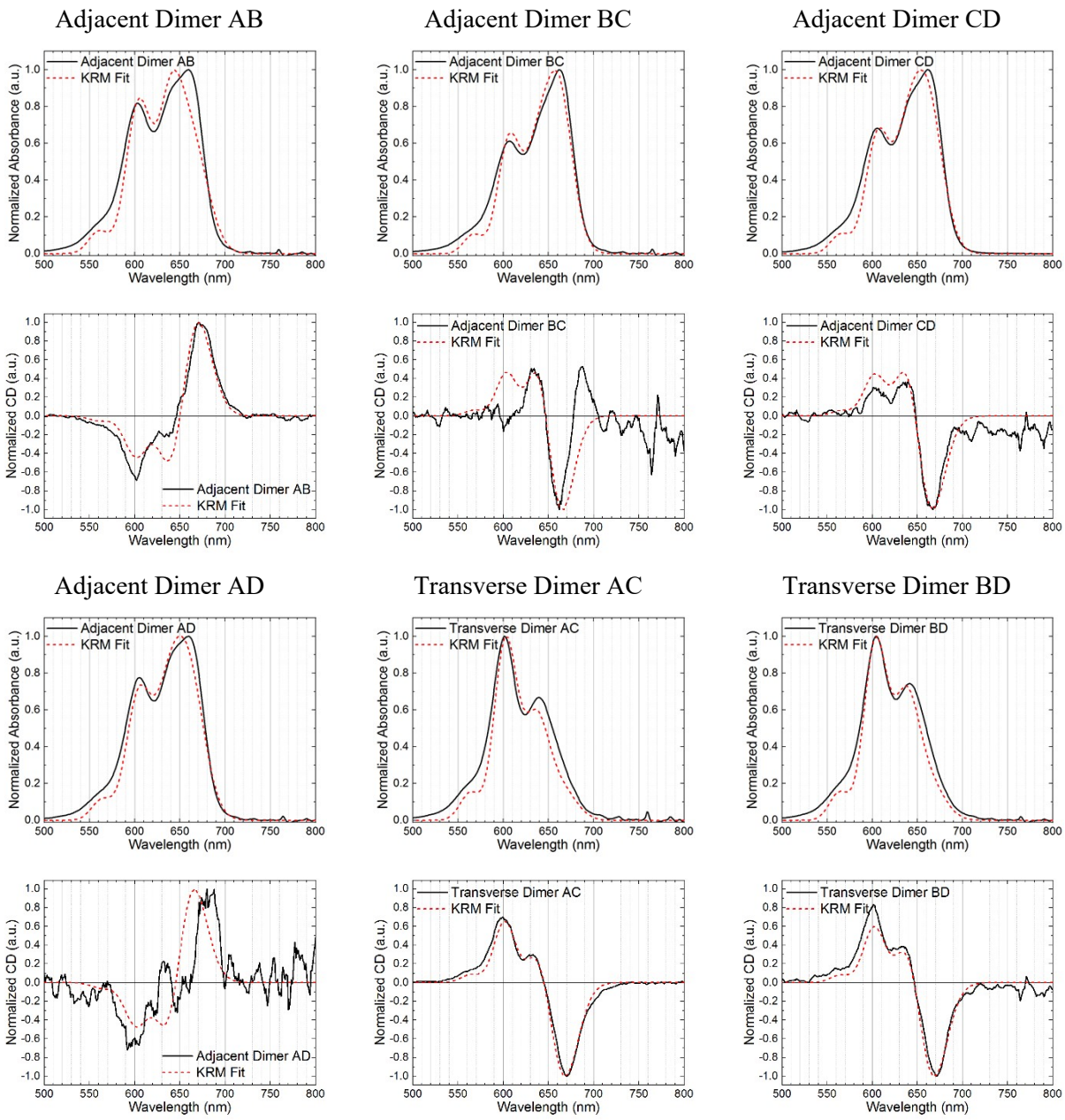

Figure S26. Normalized obtained steady-state absorption and circular dichroism spectra (solid line) of Cy5H covalently templated by DNA HJ with matching one dimer KRM Fit (dash-dot line). These spectra were recorded at 20 °C with the dye-DNA construct concentration at 2 μM in 1× TBE, 15 mM $MgCl_2$.

Table S30. Various monomer parameters for Cy5H.

Parameter	Monomer A	Monomer B	Monomer C	Monomer D
E_v, (eV)	0.138	0.136	0.139	0.145
d (dimensionless units)	0.810	0.810	0.800	0.810
Γ (eV)	0.044	0.039	0.039	0.044
J_0 (meV-nm^3)	13.831	13.736	13.854	13.388
M (*Debye*)	67.2	66.3	67.5	63.0

Table S31. Input fitting parameters used in calculations of Cy5H dimers.

Parameter	Adjacent, AB	Adjacent, BC	Adjacent, CD	Adjacent, AD	Transverse, AC	Transverse, BD
n_v	3	3	3	3	3	3
J_0^* (meV·nm^3)	67.2	66.9	65.2	65.1	67.3	64.6
E_{of} (eV)	0.005	0.017	0.008	0.015	0.021	0.019
E_v (eV)	0.155	0.137	0.142	0.1416	0.1383	0.141
d (dimensionless units)	0.780	0.805	0.805	0.810	0.805	0.810
Γ (eV)	0.043	0.039	0.042	0.044	0.0415	0.042
l (nm)	1.4	1.4	1.4	1.4	1.4	1.4
cdis (nm)	0.34	0.34	0.34	0.34	0.34	0.34

*J_0 was calculated from the fitting monomer absorption spectrum and used as an input parameter in the theoretical fitting of dimers. For dimers, each monomer J_0 was averaged between the two participating dyes.

Table S32. The goodness of the fit parameters for absorbance and CD spectra for Cy5H dimers.

Construct	r	OI_{AB}	OI_{CD}	OI_{Tot}	MSD_{abs}	MSD_{cd}	$w_{abscd}rms$
Adjacent Dimer, AB	0.951	0.986	0.949	0.968	0.415	0.735	1.149
Adjacent Dimer, BC	0.973	0.992	0.626	0.809	0.179	4.288	0.179
Adjacent Dimer, CD	0.986	0.992	0.926	0.959	0.203	0.951	1.154
Adjacent Dimer, AD	1.048	0.994	0.627	0.810	0.197	5.393	0.197
Transverse Dimer, AC	1.022	0.986	0.988	0.987	0.333	0.192	0.525
Transverse Dimer, BD	0.994	0.992	0.974	0.983	0.214	0.502	0.716

Table S33. Kühn-Renger-May model fitting outputs describing each dye orientation and position in Cy5H dimers.

Dye	θ_i (°)	φ_i (°)	x_i (nm)	y_i (nm)	z_i (nm)
Adjacent Dimer, AB					
Dye 1	70.5	0.0	0.000	0.000	-0.544
Dye 2	129.6	1.8	0.000	0.000	0.544
Adjacent Dimer, BC					
Dye 1	34.4	0.0	0.000	0.000	-0.659
Dye 2	120.4	-1.2	0.000	0.000	0.659
Adjacent Dimer, CD					
Dye 1	119.5	0.0	0.000	0.000	-0.648
Dye 2	39.2	-1.7	0.000	0.000	0.648
Adjacent Dimer, AD					
Dye 1	116.3	0.0	0.000	0.000	-0.623
Dye 2	40.1	0.6	0.000	0.000	0.623
Transverse Dimer, AC					
Dye 1	99.1	0.0	0.000	0.000	-0.388
Dye 2	64.9	-9.6	0.000	0.000	0.388
Transverse Dimer, BD					
Dye 1	99.2	0.0	0.000	0.000	-0.409
Dye 2	63.1	-3.9	0.000	0.000	0.409

Table S34. Calculated $J_{m,n}$ and geometric parameters of Cy5H dimers.

Dimer Aggregate	$J_{m,n}$ (meV)	R, ($Å$)	d_{min} (nm)	α (°)	θ_{1s} (°)	θ_{2s} (°)	θ_t (°)
Adjacent, AB	57.5	1.09	0.34	59.1	70.5	50.4	1.8
Adjacent, BC	51.1	1.32	0.34	86.0	34.4	59.6	-1.2
Adjacent, CD	50.3	1.30	0.34	80.3	60.5	39.2	-1.7
Adjacent, AD	51.2	1.25	0.34	76.3	63.7	40.1	0.6
Transverse, AC	72.5	0.78	0.34	35.5	80.9	64.9	-9.6
Transverse, BD	66.9	0.82	0.34	36.3	80.8	63.1	-3.9