Supporting Information for

Inter-helical Excitonic Coupling Dye Assemblies Templated by Anti-parallel and Parallel DNA Motifs

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Contents

[1] The design and sequences of the multi-motif construct

The design of the multi-motif construct and sequence details are shown in Figure S1 and Table S1. The cyanine dye was incorporated into the DNA backbone as a nucleotide surrogate using phosphoramidite chemistry, as shown in Figure S1D.

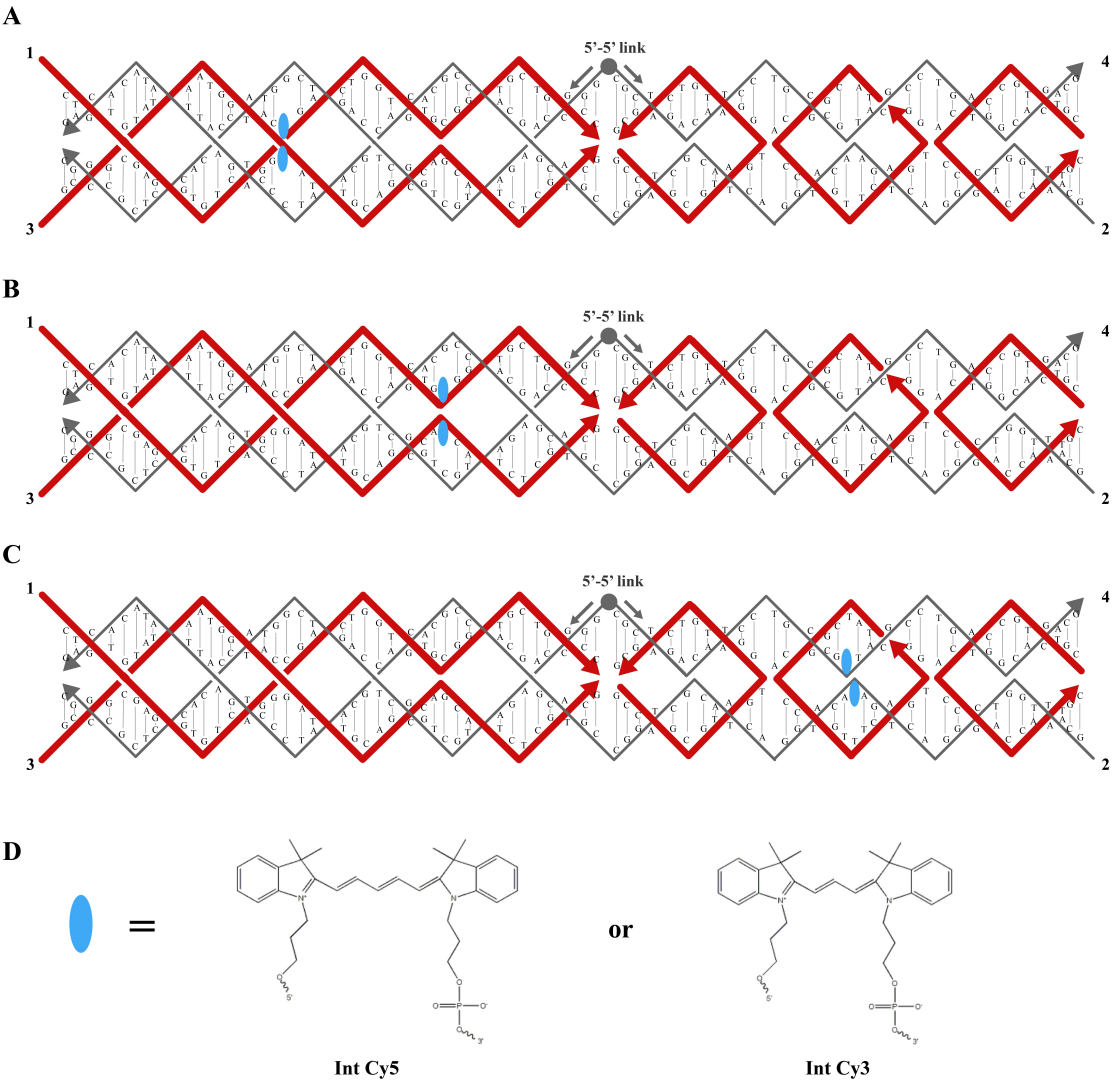

Figure S1. The multi-motif construct modified with cyanine dyes. (A) Dimeric Cy5 (or Cy3) in PX. **(B)** Dimeric Cy5 (or Cy3) in DPE. **(C)** Dimeric Cy5 (or Cy3) in DAE. **(D)** The blue oval label stands for a cyanine dye (Cy5 or Cy3) molecule incorporated into the DNA backbone.

Table S1. Sequences used for DNA multi-motif constructs

Name	Sequence	Length	Used in motif	
PX-DPE 1	5’-CTGTGGAGGTGTCTGGCGACTGGTGTGCGGTGCTGCCC-3’	38	DAE	
PX-DPE 1 Cy3	5’-CTGTGGAGGTGTCTGG-**Cy3**-GACTGGTGTGCGGTGCTGCCC-3’	38		PX
PX-DPE 1' Cy3	5’-CTGTGGAGGTGTCTGGCGACTGGTGTG-**Cy3**-GGTGCTGCCC-3’	38	DPE	
PX-DPE 1 Cy5	5’-CTGTGGAGGTGTCTGG-**Cy5**-GACTGGTGTG CGGTGCTGCCC-3’	38		PX
PX-DPE 1’ Cy5	5’-CTGTGGAGGTGTCTGGCGACTGGTGTG-**Cy5**-GGTGCTGCCC-3’	38	DPE	
PX-DPE 3	5’-GCGGCTATAATGGTACGATTGCAGGCAGCATCTCGACG-3’	38	DAE	
PX-DPE 3 Cy3	5’-GCGGCTATAATGGTAC-**Cy3**-ATTGCAGGCA GCATCTCGACG-3’	38		PX
PX-DPE 3’ Cy3	5’-GCGGCTATAATGGTACGATTGCAGGCA-**Cy3**-CATCTCGACG-3’	38	DPE	
PX-DPE 3 Cy5	5’-GCGGCTATAATGGTAC-**Cy5**-ATTGCAGGCAGCATCTCGACG-3’	38		PX
PX-DPE 3’ Cy5	5’-GCGGCTATAATGGTACGATTGCAGGCA-**Cy5**-CATCTCGACG-3’	38	DPE	
PX-DPE-DAE 2	5’-GCATTGGTCGGACTGAACAGGACTACGCTGGCCGTCGGCACCGCACACGCAATCCCAGAATTATACACAG-3’	71	DPE	PX
PX-DPE-DAE 2 Cy3	5’-GCATTGGTCGGACTGA-**Cy3**-ACAGGACTACGCTGGCCGTCGGCACCGCACACGCAATCCCAGAATTATACACAG-3’	72	DAE	
PX-DPE-DAE 2 (PX) Cy3	5’-GCATTGGTCGGACTGAACAGGACTACGCTGGCCGTCGGCACCGCACACGCAAT-**Cy3**-CCAGAATTATACACAG-3’	71		PX
PX-DPE-D	5’-GCATTGGTCGGACTGA-**Cy5**-ACAG	72	DAE	

AE 2 Cy5	GACTACGCTGGCCGTCGGCACCGCA CACGCAATCCCAGAATTATACACAG-3’				
PX-DPE-DAE 4	3’-GCACACGGAGTCCGATGCCGTCCT ACAGACGC **5’- 5’** GGGCAAGATGCTGCCTCAGTCGGTA CCCACCTCGCCGC-3’	71		DPE	PX
PX-DPE-DAE 4 Cy3	3’-GCACACGGAGTCCGAT-**Cy3**-GCCG TCCTACAGACGC **5’- 5’** GGGCAAGATGCTGCCTCAGTCGGTA CCCACCTCGCCGC-3’	72	DAE		
PX-DPE-DAE 4 (PX) Cy3	3’-GCACACGGAGTCCGATGCCGTCCT ACAGACGC **5’- 5’** GGGCAAGATGCTGCCTCAGTC-**Cy3**-G TACCCACCTCGCCGC-3’	71			PX
PX-DPE-DAE 4 Cy5	3’-GCACACGGAGTCCGAT-**Cy5**-GCCG TCCTACAGACGC **5’- 5’** GGGCAAGATGCTGCCTCAGTCGGTA CCCACCTCGCCGC-3’	72	DAE		
DAE 1	5’-GCCAGCGTAGTGGATGTCTGCG-3’	22	DAE	DPE	PX
DAE 3	5’-CGTGTGCCTCACCGACCAATGC-3’	22	DAE	DPE	PX
DAE 5	5’-TATCGGCACCTGTTTCAGTGGC-3’	22	DAE		
DAE 5’	5’-TACGGCACCTGTTCAGTGGC-3’	20		DPE	PX
DAE-1-dlc	5’-GCATCTCGACGGCCAGCGTAGTG GATGTCTGCG-3’	33			PX-dlc
PX-DPE-3-dlc	5’-TAATGGTACGATTGCAGGCA-3’	20			PX-dlc

[2] The synthesis of DNA strands with 5’-5’ link

The DNA strands with 5’-5’ linkage were prepared through the reverse DNA phosphoramidite synthesis. In addition to the standard phosphoramidite synthesis of the DNA fragment in the 3’-5’ direction, reverse nucleotides phosphoramidite with a DMT protecting group at the 3’ (Figure S2) was used to create a 5’-5’ linkage and perform 5’-3’ synthesis afterward (Figure S2).

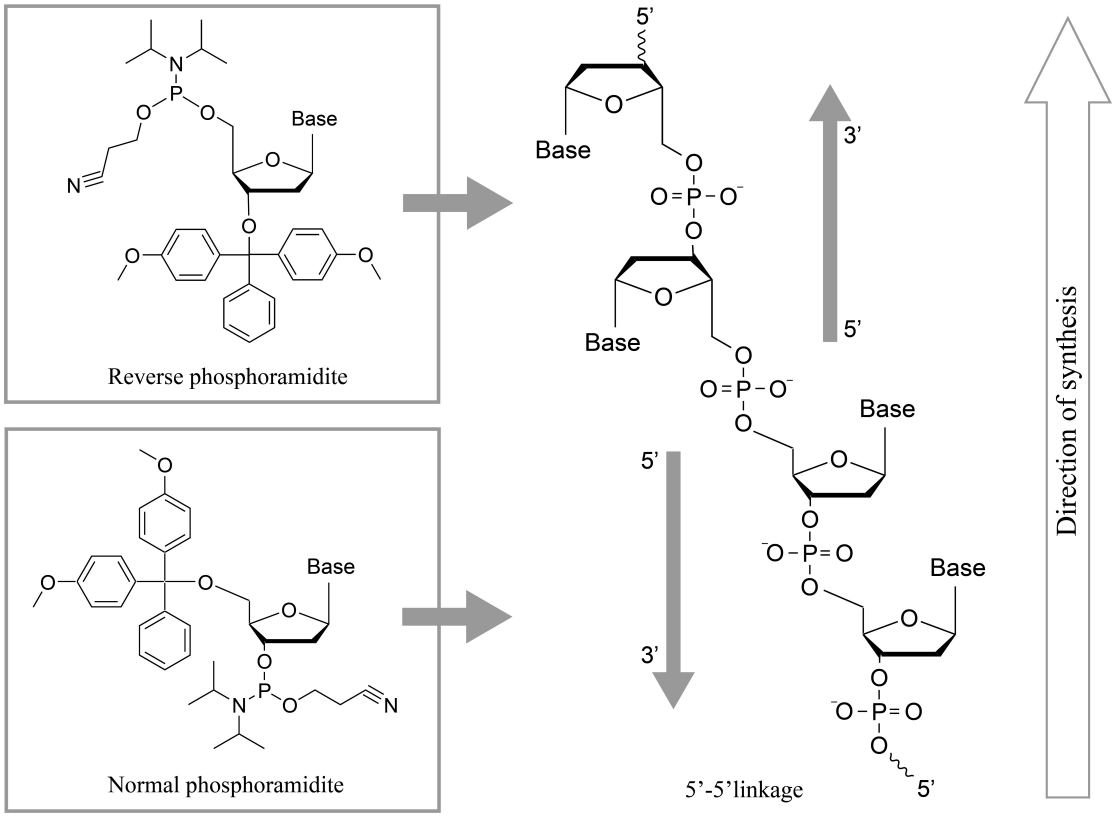

Figure S2. Reversed DNA synthesis. Reverse nucleotides phosphoramidites were used as special monomers in the DNA solid-phase synthesis to carry out the 5’-3’ synthesis. The right part shows the polarity reversing region, which contains a 5’-5’ linkage.

[3] Non-denaturing gel electrophoresis characterization of dye-DNA assemblies

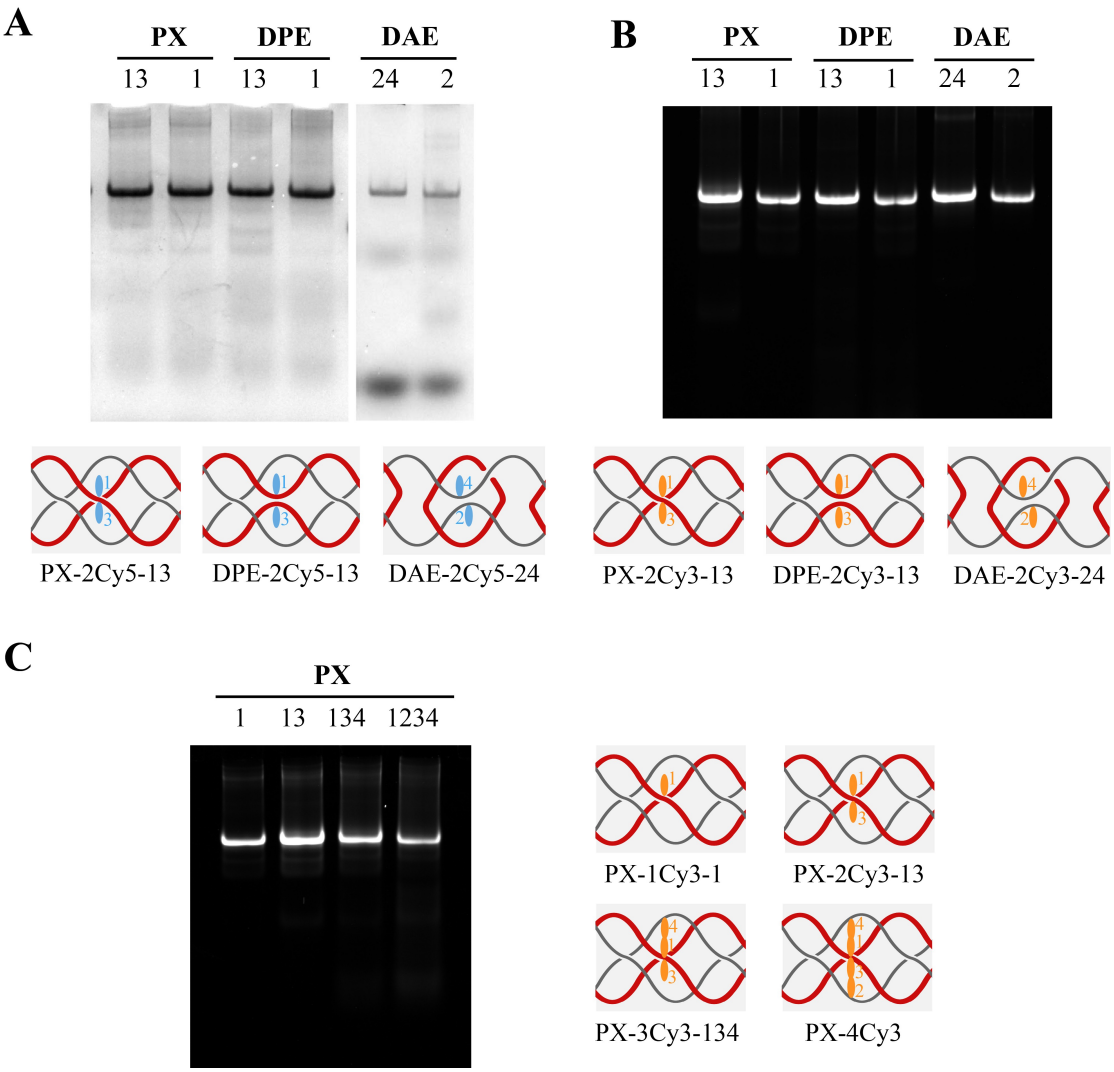

Figure S3. Native gel electrophoresis characterization of the multi-motif construct with cyanine dyes modifications. (A) Gel image of the multi-motif construct with mono- and dimeric Cy5 templated by PX, DPE, and DAE motifs (stained with stains-all). **(B)** Gel image of the multi-motif construct with mono- and dimeric Cy3 modified PX, DPE, and DAE motifs under the blue light (470 nm). **(C)** Gel image of the multi-motif construct with PX motif region modified with increasing number of Cy3 dyes under the blue light (470 nm). Note: A clear band in 10% native gel electrophoresis can be observed for each dye modification version, which suggests the successful self-assembly of all constructs (average yield of ~80%).

[4] Overlap of fluorescence excitation and absorption spectra

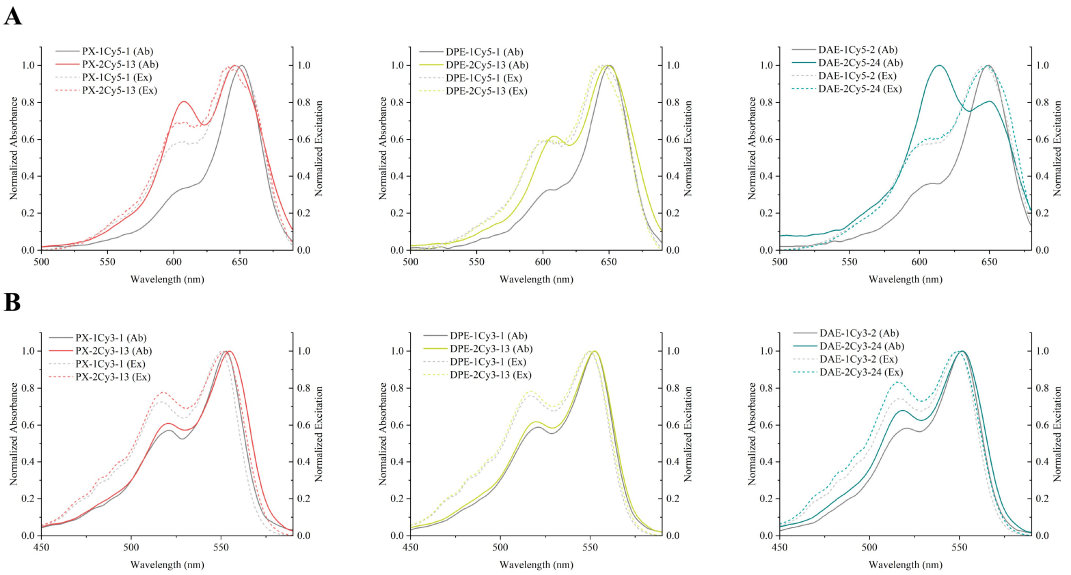

Figure S4. Overlap of fluorescence excitation and absorption spectra. (A) Normalized Ab/Ex overlap spectra of dimeric Cy5 in PX, DPE, and DAE, compared to their monomer versions. **(B)** Normalized Ab/Ex overlap spectra of dimeric Cy3 in PX, DPE, and DAE, compared to their monomer versions.

[5] Influence of Cy3 arrangement pattern in PX motif

The excitonic coupling was affected by the position of dye molecules in PX motif. In the dimeric constructs, intrahelical coupling (PX-2Cy3-23) leads to the most visible H-type spectral shift (the increase of the 0-1 peak and hypsochromic shift of both peaks), which is descended when the distance between dyes increases (PX-2Cy3-12, PX-2Cy3-24). Though PX-2Cy3-13 is also supposed to have a close distance between dyes, similar to PX-2Cy3-23, the J-type coupling (bathochromic shift of 0 – 0 peak) suggests a different configuration of Cy3 dimer at the PX crossover points. The results of trimeric constructs are consistent with that in dimers. A 134-construct exhibits a J-type spectrum feature, while the 124-construct seems to prefer the H-type configuration as the result of the intrahelical dimer.

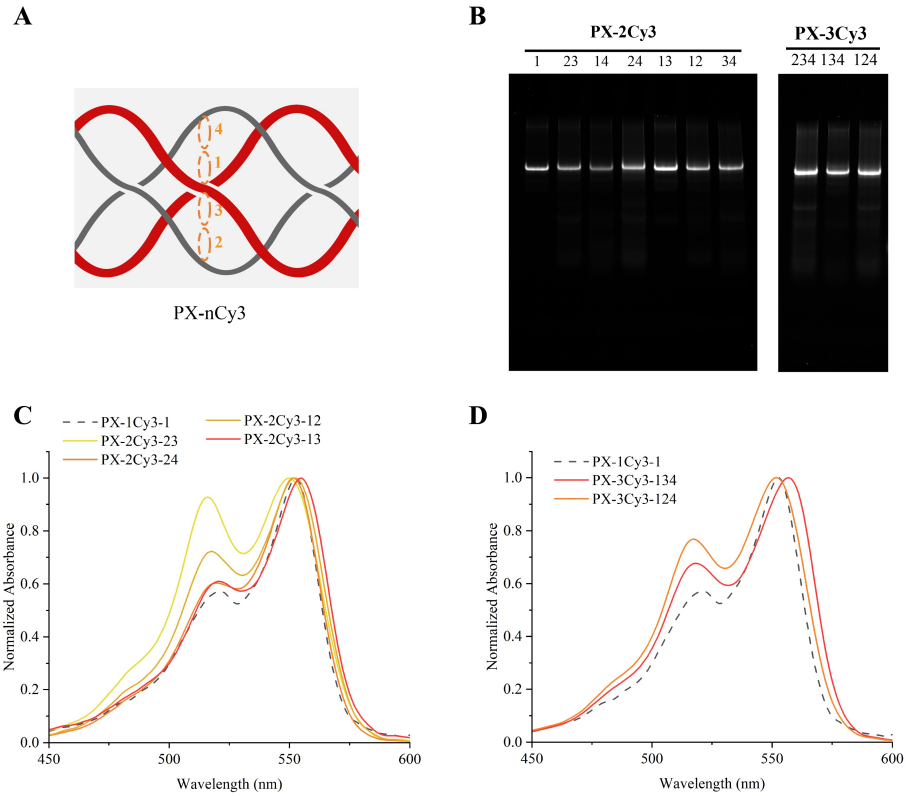

Figure S5. Excitonic coupling of multiple Cy3 molecules in different positions templated by PX motifs. (A) The numeric labels of Cy3 modification sites in the PX motif. **(B)** 10% native PAGE characterization of the dimeric and trimeric Cy3 in the PX motifs, respectively (PX-2Cy3 and PX-3Cy3). **(C)** Normalized linear absorption spectra of dimeric constructs with Cy3 molecules at different positions of the PX motif. **(D)** Normalized linear absorption spectra of trimeric constructs with Cy3 molecules at different positions of the PX motif.

[6] Fluorescence emission spectra of dye-DNA assemblies

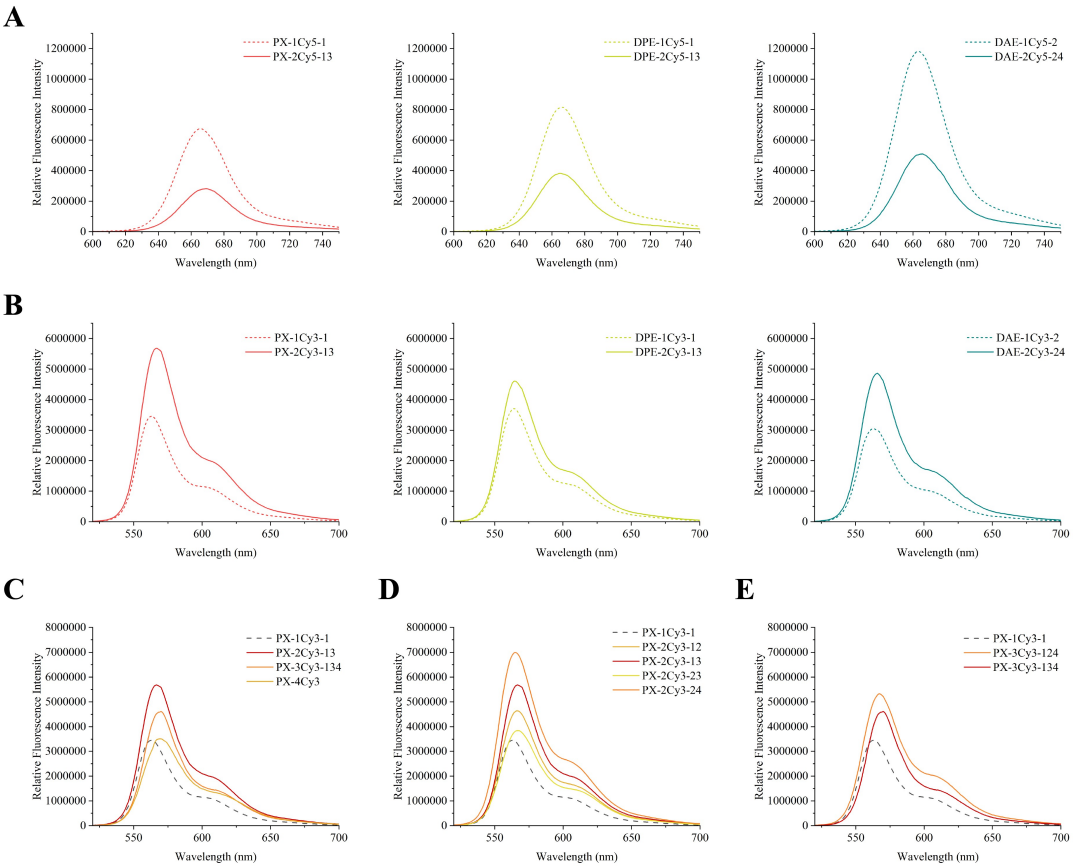

Figure S6. Fluorescence emission spectra of all constructs. (A) Emission spectra of dimeric Cy5 in PX, DPE, and DAE, compared to their monomer versions. **(B)** Emission spectra of dimeric Cy3 in PX, DPE, and DAE, compared to their monomer versions. **(C)** Emission spectra of different number of Cy3 molecules in PX, compared to the monomer version. **(D)** Emission spectra of dimeric Cy3 in different positions in PX, compared to the monomer version. **(E)** Emission spectra of trimeric Cy3 in different positions in PX, compared to the monomer version. All measurements were done at a sample concentration of 40 nM.

[7] Quantum yields of different constructs

Table S2. Quantum yields of the constructs used in this study

Cy3-DNA assemblies		Cy5-DNA assemblies	
sample	**quantum yield**	**sample**	**quantum yield**
DAE-1Cy3-2	27.00%	DAE-1Cy5-2	33.00%
DAE-2Cy3-24	27.71%	DAE-2Cy5-24	6.43%
DPE-1Cy3-1	23.69%	DPE-1Cy5-1	29.80%
DPE-2Cy3-13	25.08%	DPE-2Cy5-13	13.63%
PX-1Cy3-1	41.36%	PX-1Cy5-1	31.69%
PX-2Cy3-13	28.98%	PX-2Cy5-13	4.31%
PX-2Cy3-24	24.63%		
PX-2Cy3-12	18.72%		
PX-2Cy3-23	12.81%		
PX-3Cy3-134	15.27%		
PX-2Cy3-124	13.74%		
PX-4Cy3	9.27%		

[8] Time-correlated single photon counting of Cy3 assemblies in PX motif

The fluorescence lifetime data of Cy3 assemblies of different number is shown in Table S2-S5. Each result contains 9 parallel measurements, reporting the multi-exponential tailfit lifetimes (τ_1,τ_2), the average lifetime (τ_{AV}) and the proportion of τ_1 / τ_2, along with the chi-square indicating the fit quality. The final average lifetime and standard deviation are also provided. The increased proportion of the short time component (τ_2) and the decreased average lifetime can demonstrate a J-type coupling, while the opposite demonstrates an H-type coupling.

Table S3. The fluorescence lifetime results for PX-1Cy3-1 construct (Cy3 monomer assembly)

Replicate	**Two-components lifetime（τ_1/τ_1） (ns)**	**Average lifetime-τAv (ns)**	**Proportion of τ_1/τ_1**	**Chi-square**
1	2.45/1.6	2.290	0.735//0.265	1.059
2	2.8/1.91	2.300	0.366/0.634	1.038
3	2.88/1.99	2.300	0.273/0.727	0.943
4	2.83/1.9	2.290	0.316/0.684	1.057
5	2.89/1.94	2.300	0.293/0.707	0.954
6	2.68/1.82	2.300	0.471/0.529	0.914
7	2.7/1.85	2.310	0.436/0.564	1.070
8	2.8/1.91	2.310	0.332/0.668	1.063
9	2.53/1.7	2.290	0.63/0.37	1.062
Average		2.299		
Standard deviation		0.008		

Table S4. The fluorescence lifetime results for PX-2Cy3-13 construct (dimeric Cy3 assembly)

Replicate	**Two-components lifetime (τ_1/τ_1) (ns)**	**Average lifetime-τAv (ns)**	**Proportion of τ_1/τ_1**	**Chi-square**
1	2.68/1.35	2.060	0.37/0.63	1.025
2	2.66/1.34	2.070	0.382/0.618	0.978
3	2.59/1.26	2.070	0.436/0.564	1.001
4	2.52/1.23	2.060	0.463/0.537	0.971
5	1.38/2.67	2.060	0.633/0.367	1.091
6	2.598/1.28	2.060	0.431/0.569	1.117
7	2.6/1.28	2.050	0.405/0.595	1.039
8	1.17/2.51	1.970	0.587/0.413	0.995
9	2.55/1.25	2.060	0.445/0.555	0.954
Average		2.051		
Standard deviation		0.031		

Table S5. The fluorescence lifetime results for PX-3Cy3-134 construct (Trimeric Cy3 assembly)

Replicate	**Two-components lifetime (τ_1/τ_1) (ns)**	**Average lifetime-τAv (ns)**	**Proportion of τ_1/τ_1**	**Chi-square**
1	2.4/0.781	1.533	0.225/0.775	1.062
2	2.27/0.739	1.473	0.23/0.77	1.122
3	2.3/0.745	1.448	0.21/0.79	1.064
4	2.3/0.75	1.428	0.2/0.8	0.930
5	2.2/0.72	1.400	0.22/0.78	0.944
6	2.2/0.717	1.384	0.2/0.79	0.929
7	2.35/0.76	1.518	0.22/0.78	1.017
8	2.3/0.75	1.463	0.21/0.79	1.079
9	2.33/0.75	1.467	0.21/0.79	1.085
Average		1.457		
Standard deviation		0.049		

Table S6. The fluorescence lifetime results for PX-4Cy3 construct (Tetrameric Cy3 assembly)

Replicate	**Two-components lifetime (τ_1/τ_1) (ns)**	**Average lifetime-τAv (ns)**	**Proportion of τ_1/τ_1**	**Chi-square**
1	2.5/0.885	1.900	0.371/0.629	1.013
2	2.4/0.913	1.902	0.412/0.588	0.982
3	2.55/0.954	1.932	0.4/0.6	1.041
4	2.35/0.824	1.825	0.4/0.6	0.998
5	2.4/0.872	1.887	0.42/0.58	1.001
6	2.38/0.85	1.890	0.43/0.57	1.004
7	2.53/0.869	1.810	0.307/0.693	1.004
8	2.45/0.874	1.863	0.375/0.625	1.054
9	2.47/0.89	1.892	0.38/0.62	1.054
Average		1.878		
Standard deviation		0.039		

[9] Strand displacement reaction on PX motif

For the strand displacement design, the invasion strand is one of the Cy3 modified strands in the PX-tetrameric Cy3 construct, which displaces the short orange strand (strand 3-dlc) through a 7 nt toehold to generate the tetrameric Cy3 assembly.

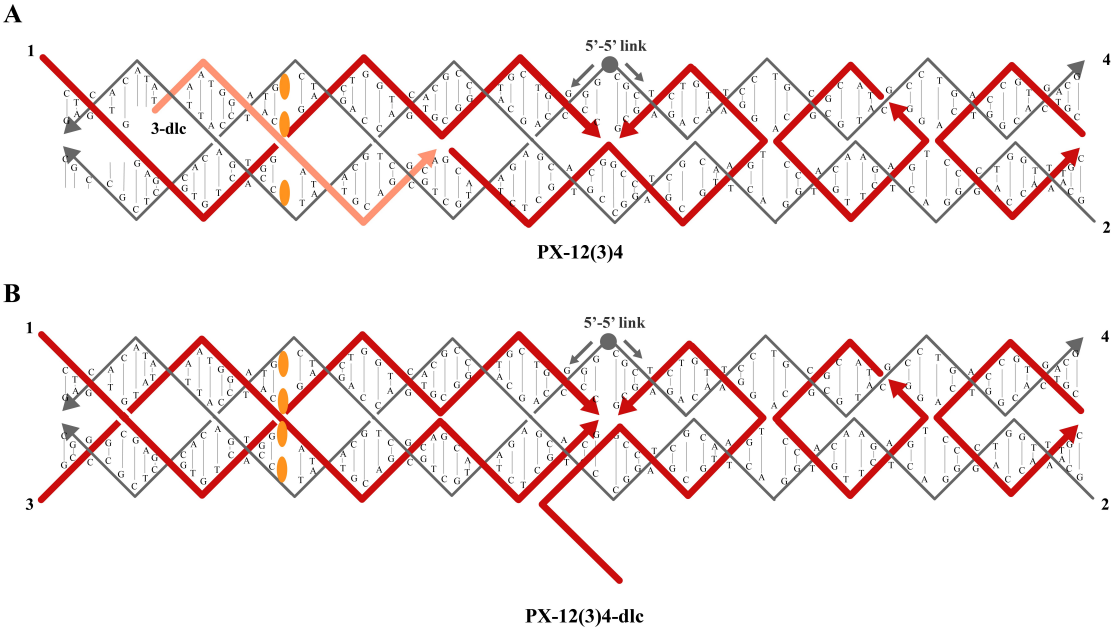

Figure S7. Tetrameric Cy3 assembly generated from trimeric Cy3 assembly by strand displacement reaction on PX motif. (A) The design and sequences of PX-12(3)4 before the strand displacement reaction. **(B)** The design and sequences of PX-12(3)4-dlc after the strand displacement reaction. The short orange strand (strand 3-dlc) was displaced by the dark red Cy3 modified strand (strand 3-Cy3) to obtain a tetrameric Cy3 assembly.

[10] Quantitative spectra analysis

Table S6 shows the main peak wavelengths of absorption and emission spectra for different constructs and the full width at half maximum (FWHM) of absorption spectra in PX-nCy3 constructs. The gradually increased FWHM of 0-0 transition from PX-1Cy3-1 to PX-4Cy3 indicates a composited J- and H- type coupling in the multi-dye constructs.

In Figure S7, "0-0 Peak Wavelength" and "0-1 Peak Increase" are chosen as parameters to better visualize the spectral differences. The position of the points on the x-axis represents the blue or red shift of the 0-0 peak, and the position on the y-axis represents the increased proportion of the 0-1 peak relative to the control sample (the monomer construct).

Table S7. Quantitative data analysis of the linear absorption and emission spectra

	Absorption				**Emission**
	0-1 transition		**0-0 transition**		**0-0 transition**
	Wavelength (nm)	FWHM (nm)	Wavelength (nm)	FWHM (nm)	Wavelength (nm)
DAE-1Cy5-2	605		649		663
DAE-2Cy5-24	614		648		666
DPE-1Cy5-1	606		651		666
DPE-2Cy5-13	608		649		665
PX-1Cy5-1	606		651		666
PX-2Cy5-13	608		646		669
DAE-1Cy3-2	521		551		563
DAE-2Cy3-24	519		552		566
DPE-1Cy3-1	521		553		563
DPE-1Cy3-13	520		553		565
PX-1Cy3-1	521	42.1989	553	21.1101	563
PX-2Cy3-13	521	42.7441	555	23.596	567
PX-3Cy3-134	518	41.4523	557	24.7495	570
PX-4Cy3	517	29.1394	552	25.3349	569
PX-12(3)4	518		552		567
PX-12(3)4-dlc	517		552		569

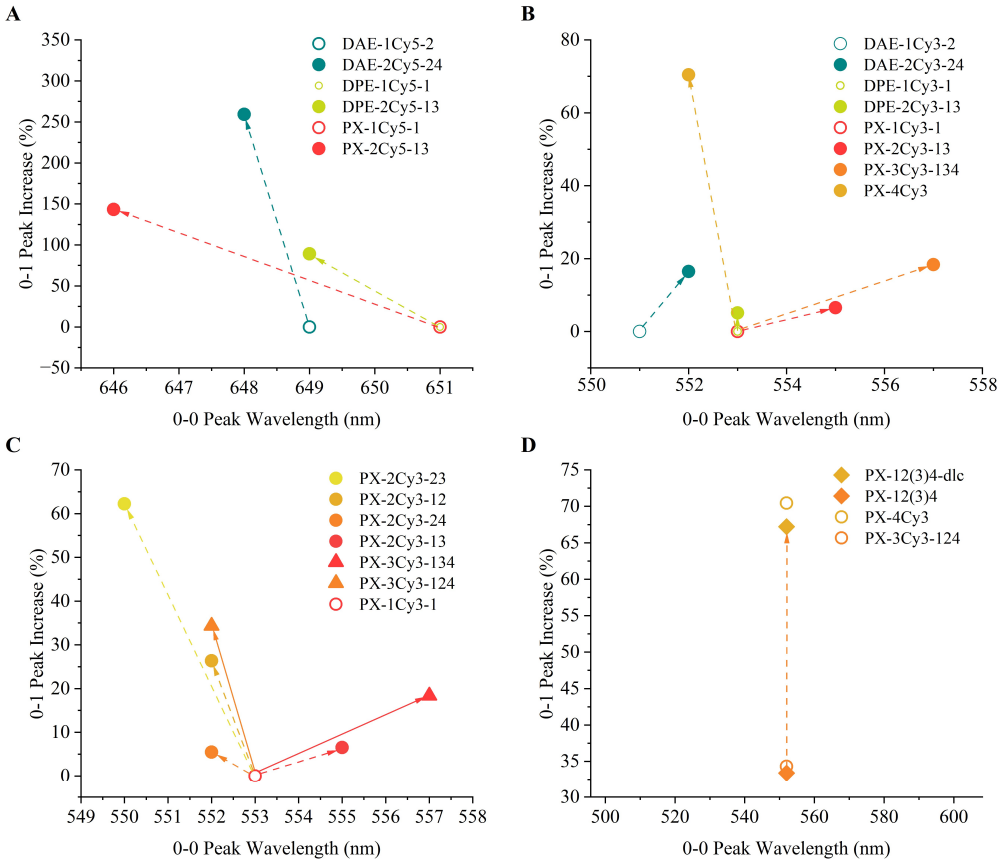

Figure S8. The visualization of spectral differences indicating J- and H- type coupling. The arrows point from the hollow circles to the solid circles (or triangles and diamonds), representing the spectral change from the mono-dye controls to the multi-dye experiment groups. **(A)** The dimeric Cy5 constructs in DAE, DPE and PX motifs. **(B)** The dimeric Cy3 constructs in DAE, DPE and multi-Cy3 coupling in the PX motif. **(C)** Different arrangement patterns of dimeric and trimeric Cy3 assemblies in the PX motif. **(D)** Tetrameric Cy3 assembly generated from trimeric Cy3 assembly by strand displacement on the PX motif.