Effect of Hydrophilicity-Imparting Substituents on Exciton Delocalization in Squaraine Dye Aggregates Covalently Templated to DNA Holliday Junctions

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Supplementary information

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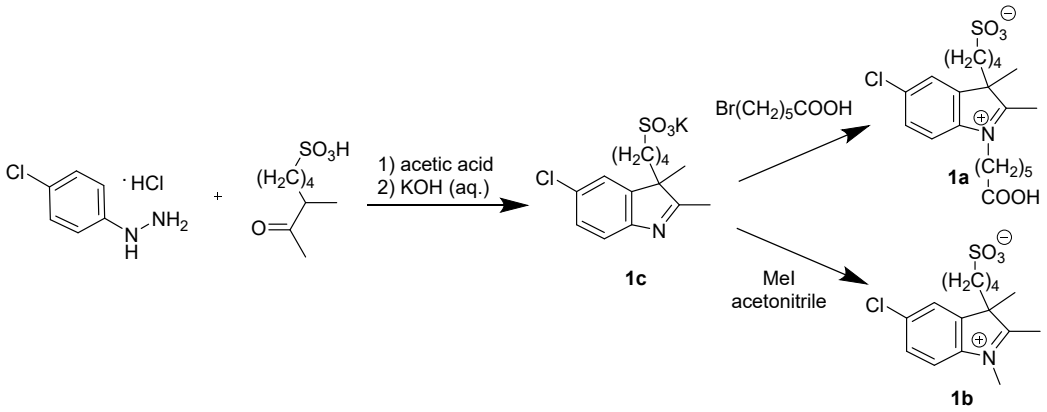
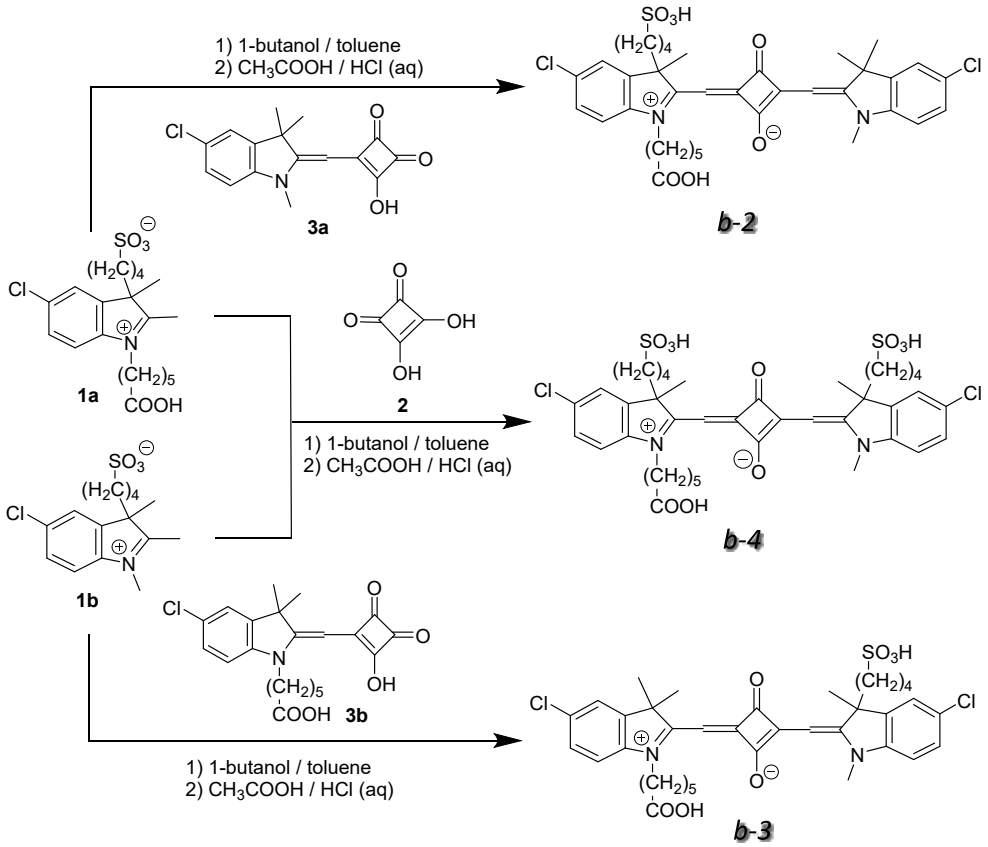
SI 1. Synthesis of hydrophilic squaraine dye

The synthetic structures for the quaternized 5-chloroindolenines **1a-1b**, the key intermediates for the synthesis of mono-reactive squaraines are presented in Scheme 1. 4-chlorophenylhydrazine was taken to the Fischer indole synthesis reaction with 5-methyl-6-oxoheptane-1-sulfonic acid[1] followed by conversion of the corresponding sulfonic acid to the potassium salt **1c** which was then quaternized with an excess of 6-bromohexanoic acid or iodomethane to give the quaternary salts **1a** and **1b,** respectively.



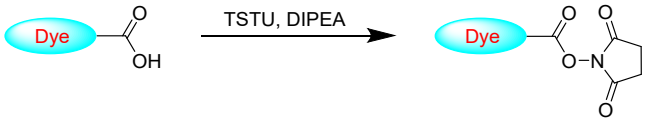
Scheme 1. Synthesis of the quaternized indolenines **1a**–**1b**.

The unsymmetrical squaraine dyes under investigation were synthesized via two ways (Scheme 2). The first approach was to react quaternized indolenines **1a** and **1b** with squaric acid (**2**) followed by hydrolysis of the formed butyl esters in a mixture of acetic and hydrochloric acids. Following this approach, a mixture of two symmetrical squaraine dyes and the unsymmetrical ***b-4*** was obtained. ***b-4*** was isolated from the mixture in low yield (7 %). A more direct and high-yield approach is based on the reaction of the mono-substituted squaric acids **3a** or **3b**[2] with the quaternized indolenines **1a** and **1b** resulting in the desired squaraine dyes ***b-2*** and ***b-3***. In this second approach, the dyes are obtained in yields of 26 % (***b-2*** and 15 % (***b-3***), respectively.



Scheme 2. Synthetic scheme for ***b-2***, ***b-3*** and ***b-4***

In order to make these dyes suitable for covalent attachment to the amino-groups of proteins, DNA and other biomolecules, the carboxylic acid groups of these dyes were converted to reactive *N*-hydroxysuccinimide (NHS) esters with *N,N,N′,N′*-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) and *N,N*-diisopropylethylamine (DIPEA) (Scheme 3).



Scheme 3. Conversion of the dyes to activated *N*-hydroxysuccinimidyl esters

Experimental

General information

The **C, H, N elemental analysis** was performed by a EuroVector Euro EA 3000 EA-IRMS elemental analyzer.

^{1}H NMR spectra were measured on a Varian 400 MR (^{1}H 400 MHz) spectrometer in DMSO-d_6 using signal of remaining non-deuterated solvent as an internal standard (2.50 ppm for DMSO [3]).

ESI mass spectra were recorded on *Waters Quattro micro API* mass spectrometer with direct injection of the sample solution to the ionization chamber. Spectra were recorded for negative and positive ions at 120 °C with energy 3 kV on capillary.

The purity of the obtained compounds were monitored by **HPLC** on Agilent Technologies 1100 (LC), column Phenomenex Luna Omega 5 μm C18 100 Å, 4.6 × 250 mm, column temperature 35 °C, eluent water-acetonitrile (ACN) + 0.05% phosphoric acid.

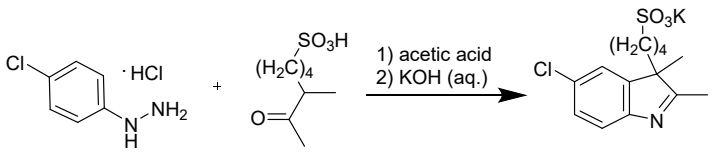
Absorption spectra were recorded in 1-cm quartz cells at 25 ºC using a PerkinElmer Lambda 35 UV/Vis spectrophotometer. Absorption maxima were determined with an accuracy of ±0.5 nm and rounded off.

Emission spectra were measured in 1-cm standard quartz cells at 25 ºC using a *Varian Cary Eclipse* spectrofluorometer. The emission spectra were corrected for wavelength-dependent instrument sensitivity. Emission maxima were determined with an accuracy of ±1.0 nm.

Synthesis

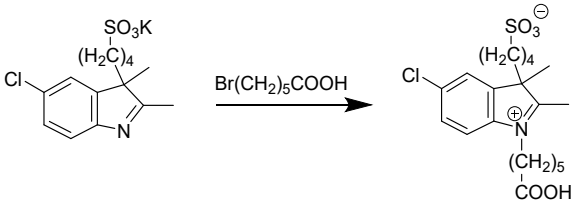
4-Chlorophenylhydrazine hydrochloride was purchased from TCI, all other reagents and Silica gel 60 for column chromatography were purchased from Aldrich and used without further purification. 3-((5-Chloro-1,3,3-trimethylindolin-2-ylidene)methyl)-4-hydroxycyclobut-3-ene-1,2-dione[2], 5-methyl-6-oxoheptane-1-sulfonic acid[1] were synthesized according to the referenced procedures.

Potassium 4-(5-chloro-2,3-dimethyl-3*H*-indol-3-yl)butane-1-sulfonate (1c)



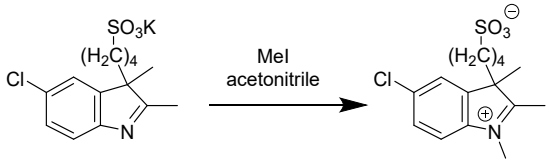
(4-Chlorophenyl)hydrazine hydrochloride (2.7 g, 15.1 mmol) and 5-methyl-6-oxoheptane-1-sulfonic acid (3.36 g, 16.1 mmol) were heated under reflux in acetic acid (20 mL) for 10 h. The acetic acid was removed under reduced pressure by rotary evaporation. The resulting viscous residue was mixed with water (5 mL) and made alkaline to pH 9 with 10 % aqueous potassium hydroxide solution. The insoluble precipitate was filtered off and the filtrate was purified by column chromatography (Silica gel 60 RP18, 0–5 % acetonitrile—water). A fraction containing the product was made alkaline to pH 9 with 10 % aqueous potassium hydroxide solution and the water was removed on a rotary evaporator. Yield: 4.1 g (69 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.47 (1H, d, arom., 2.2 Hz), 7.39 (1H, d, arom., 8.3 Hz), 7.30 (1H, dd, arom., 2.2 Hz, 8.1 Hz), 2.30–2.24 (2H, m, CH_2SO$_3$K), 2.16 (3H, s, 2-C$\underline{H_3}$), 1.95–1.69 (2H, m, C$\underline{H_2}$), 1.47–1.36 (2H, m, C$\underline{H_2}$), 1.22 (3H, s, 3-C$\underline{H_3}$), 0.74–0.59 (1H, m, C$\underline{H_2}$), 0.52–0.37 (1H, m, C$\underline{H_2}$). ESI MS, m/z calcd. for [M+H]$^+$ [$C_{14}H_{18}ClKNO_3S$]$^+$ 354.03, found: 354.09. Anal. calcd. (%) for $C_{14}H_{17}ClKNO_3S$: C, 47.51; H, 4.84; N, 3.96. Found C, 47.28; H, 4.79; N, 3.92. UV-Vis: λ_{max}(Abs) 262 nm (Methanol).

4-(1-(5-carboxypentyl)-5-chloro-2,3-dimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (1a)



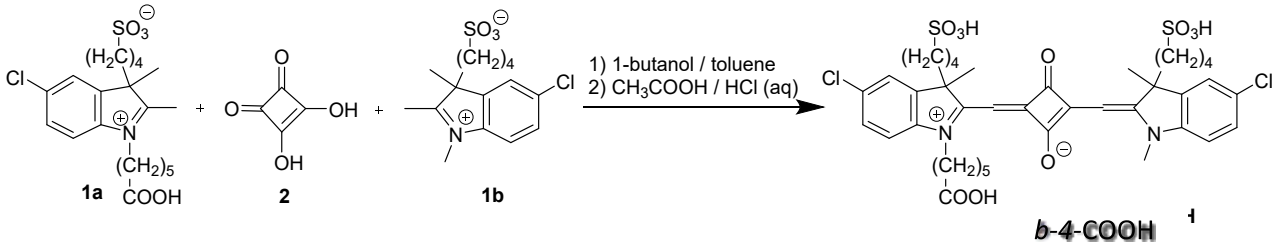
Potassium 4-(5-chloro-2,3-dimethyl-3*H*-indol-3-yl)butane-1-sulfonate (650 mg, 1.84 mmol) and 6-bromohexanoic acid (720 mg, 3.69 mmol) were thoroughly mixed in a sealed tube at heated at 110 °C for 6 h. After cooling, the resulting viscous paste was triturated with ether. A precipitate was filtered off and washed with ether to yield 910 mg of 4-(1-(5-carboxypentyl)-5-chloro-2,3-dimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (containing potassium bromide) which was used for further synthesis without additional purification. ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 8.02 (1H, d, arom., 8.8 Hz), 8.01 (1H, s, arom.), 7.72 (1H, d, arom., 8.6 Hz), 4.47 (2H, broad s, N-CH_2), 2.87 (3H, s, 2-CH_3), 2.39–2.25 (4H, m, 2×CH_2), 2.22 (2H, t, CH_2COOH, 6.9 Hz), 1.87–1.73 (2H, m, CH_2), 1.54 (3H, s, 3-CH_3), 1.60–1.46 (2H, m, CH_2), 1.46–1.33 (4H, m, CH_2), 0.88–0.67 (1H, m, CH_2), 0.64–0.45 (1H, m, CH_2). ESI MS, m/z calcd. for [M+K]$^+$ [$C_{20}H_{28}ClKNO_5S$]$^+$ 468.10, found: 468.25. UV-Vis: λ_{max}(Abs) 285 nm (Methanol).

4-(5-Chloro-1,2,3-trimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (1b)



Potassium 4-(5-chloro-2,3-dimethyl-3*H*-indol-3-yl)butane-1-sulfonate (770 mg, 2.17 mmol), iodomethane (0.5 mL, 8.03 mmol) and acetonitrile (1 mL) were mixed in a sealed tube and stirred at 40 °C for 15 h. After cooling, the solution on top was decanted and the solid product was triturated with ether to yield 1.03 g of 4-(5-chloro-1,2,3-trimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (containing potassium iodide), which was used in the further transformations without additional purification. ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.99 (1H, d, arom., 2.0 Hz), 7.94 (1H, d, arom., 8.8 Hz), 7.72 (1H, dd, arom., 2.0 Hz, 8.8 Hz), 4.00 (3H, s, N-C$\underline{H}_3$), 2.80 (3H, s, 2-C$\underline{H}_3$), 2.34–2.22 (4H, m, 2×CH_2), 1.53 (3H, s, 3-C$\underline{H}_3$), 1.47–1.35 (2H, m, C$\underline{H}_2$), 0.91–0.73 (1H, m, C$\underline{H}_2$), 0.70–0.54 (1H, m, C$\underline{H}_2$). ESI MS, m/z calcd. for $[M+K]^+$ $[C_{15}H_{20}ClKNO_3S]^+$ 368.05, found: 368.15. UV-Vis: λ_{max}(Abs) 285 nm (Methanol).

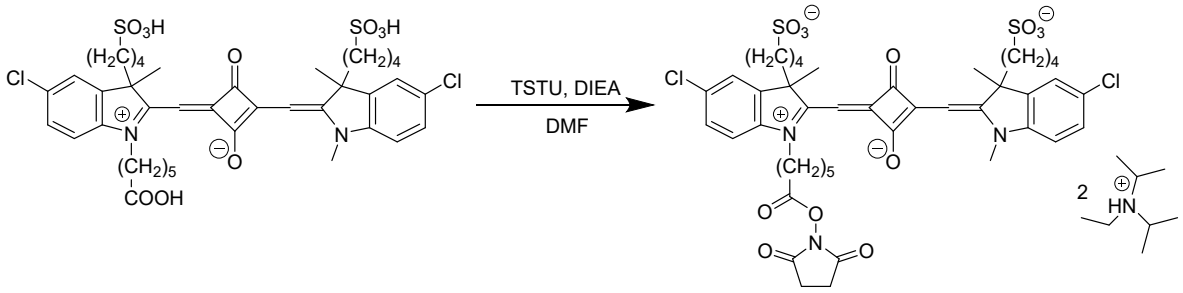
4-((1-(5-carboxypentyl)-5-chloro-3-methyl-3-(4-sulfobutyl)-3*H*-indol-1-ium-2-yl)methylene)-2-((5-chloro-1,3-dimethyl-3-(4-sulfobutyl)indolin-2-ylidene)methyl)-3-oxocyclobut-1-en-1-olate (*b-4*-COOH).



4-(1-(5-carboxypentyl)-5-chloro-2,3-dimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (**1a**) (containing potassium bromide) (260 mg, 0.45 mmol) from the previous step, 3,4-dihydroxycyclobut-3-ene-1,2-dione (**2**) (57 mg, 0.50 mmol), and 4-(5-chloro-1,2,3-trimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (**1b**) (containing potassium iodide) (250 mg) were heated under reflux in toluene (10 mL) and 1-butanol (10 mL) using a Dean-Stark apparatus for 4 h. The solvent was removed under reduced pressure by rotary evaporation. Acetic acid (6 mL) and concentrated hydrochloric acid (0.6 mL) were added to the residue and

the mixture was refluxed for 1 hour. The acids were removed by rotary evaporation and the obtained raw product was purified by column chromatography (Silica gel 60 RP18, 0—25 % acetonitrile-water) to give ***b-4*-COOH** (27 mg, 6 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.58 (2H, s, arom.), 7.40–7.34 (2H, m, arom.), 7.30 (1H, d, arom., 8.5 Hz), 7.29 (1H, d, arom., 8.7 Hz), 5.86 (1H, s, C*H*), 5.82 (1H, s, C*H*), 4.04 (2H, broad s, NCH_2), 3.56 (3H, s, NCH_3), 2.28–2.14 (4H, m, CH_2), 2.19 (2H, t, CH_2COOH, 6.9 Hz), 1.64 (6H, s, 3-CH_3), 1.72–1.60 (4H, m, CH_2), 1.59–1.49 (2H, m, CH_2), 1.45–1.29 (8H, m, CH_2), 0.82–0.62 (2H, m, CH_2), 0.59–0.36 (2H, m, CH_2). ESI MS, m/z calcd. for [M–H]$^-$ $[C_{39}H_{45}Cl_2N_2O_{10}S_2]^-$ 835.19, found: 835.27. Anal. calcd. (%) for $C_{39}H_{46}Cl_2N_2O_{10}S_2$: C, 55.91; H, 5.53; N, 3.34. Found C, 55.85; H, 5.49; N, 3.31. UV-Vis: λ_{max}(Abs) 639 nm; λ_{max}(Em) 649 nm (Methanol); λ_{max}(Abs) 637 nm, λ_{max}(Em) 647 nm (Phosphate buffer).

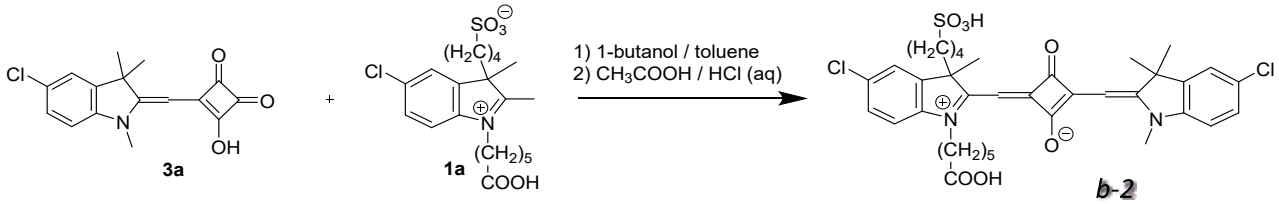
Di(*N*,*N*-diisopropylethylammonium) salt of 4-(5-chloro-2-((3-((5-chloro-1,3-dimethyl-3-(4-sulfonatobutyl)indolin-2-ylidene)methyl)-2-oxido-4-oxocyclobut-2-en-1-ylidene)methyl)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3-methyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (*N*-**Hydroxysuccinimide ester of ***b-4*-COOH)** (***b-4*-NHS**).



***b-4*-COOH** (22.0 mg, 26.2 mμmol), *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) (11 mg, 36.5 mμmol) were dissolved in DMF (1.5 mL) and *N*,*N*-diisopropylethylamine (DIEA) (20 mμL, 114.7 mμmol) was added. The solution was stirred at room temperature for 10 min. The reaction mixture was diluted with ether (20 mL) and left for precipitation. The solvent was decanted, the solid residue was washed with ether and then diluted with 5 % aqueous acetonitrile and purified by column chromatography (Silica gel 60 RP18, 5—34 % acetonitrile-water) to give ***b-4*-NHS**. Yield: 7 mg (22 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.59 (2H, s, arom.), 7.40–7.34 (2H, m, arom.), 7.34–7.27 (2H, m, arom.), 5.86 (1H, s, C*H*), 5.82 (1H, s, C*H*), 4.04 (2H, broad s, NCH_2), 3.56 (3H, s, NCH_3), 3.58–3.50 (4H, m, C*H* (DIPEA)), 3.18–3.07 (4H, m, CH_2 (DIPEA)), 2.82 (4H, s, CH_2 (succinimide)), 2.73–2.64 (4H, m, CH_2), 2.24–2.13 (2H, m, CH_2CONHS), 1.64 (6H, s, 3-CH_3), 1.72–1.60 (4H, m, CH_2), 1.60–1.48 (2H, m, CH_2), 1.45–1.29 (8H, m, CH_2), 1.33–1.19 (30H, m, DIPEA), 0.82–0.62 (2H, m, CH_2), 0.59–0.36 (2H, m, CH_2). ESI MS, m/z calcd. for [M–2DIEA+H]$^+$ $[C_{43}H_{50}Cl_2N_3O_{12}S_2]^-$ 934.22, found: 934.55. Anal. calcd.

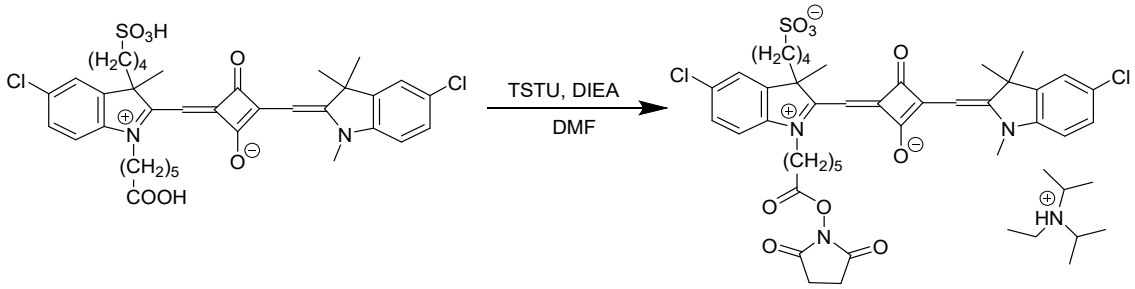
(%) for $C_{59}H_{87}Cl_2N_5O_{12}S_2$: C, 59.38; H, 7.35; N, 5.87. Found C, 59.68; H, 7.43; N, 5.96. UV-Vis: λ_{max}(Abs) 639 nm, λ_{max}(Em) 649 nm (Methanol).

4-((1-(5-carboxypentyl)-5-chloro-3-methyl-3-(4-sulfobutyl)-3*H*-indol-1-ium-2-yl)methylene)-2-((5-chloro-1,3,3-trimethylindolin-2-ylidene)methyl)-3-oxocyclobut-1-en-1-olate (***b-2***).



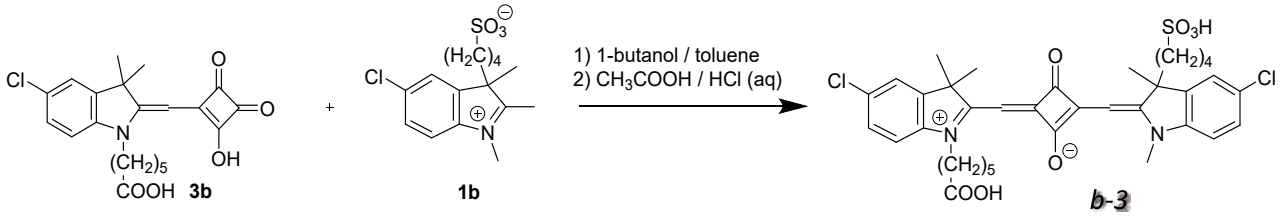
3-((5-Chloro-1,3,3-trimethylindolin-2-ylidene)methyl)-4-hydroxycyclobut-3-ene-1,2-dione (**3a**) (100 mg, 0.33 mmol) and 4-(1-(5-carboxypentyl)-5-chloro-2,3-dimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (**1a**) (containing potassium bromide) (200 mg) were heated under reflux in toluene (8 mL) and 1-butanol (8 mL) using a Dean-Stark apparatus for 6 h. The solvent was removed under reduced pressure by rotary evaporation. Acetic acid (5 mL) and concentrated hydrochloric acid (0.5 mL) were added to the residue and the mixture was refluxed for 40 min. The acids were rotary evaporated and the obtained raw product was purified by column chromatography (Silica gel 60 RP18, 20—45 % acetonitrile-water) to give ***b-2*-COOH** (60 mg, 26 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.64 (1H, s, arom.), 7.60 (1H, s, arom.), 7.45–7.35 (2H, m, arom.), 7.35–7.25 (2H, m, arom.), 5.84 (1H, s, C*H*), 5.79 (1H, s, C*H*), 4.05 (2H, broad s, NCH_2), 3.56 (3H, s, NCH_3), 2.25–2.10 (6H, m, CH_2), 1.68 (6H, s, C(CH_3)$_2$), 1.66 (3H, s, 3-CH_3), 1.72–1.60 (2H, m, CH_2), 1.59–1.43 (2H, m, CH_2), 1.43–1.24 (4H, m, CH_2), 0.82–0.62 (1H, m, CH_2), 0.60–0.36 (1H, m, CH_2). ESI MS, m/z calcd. for [M–H]$^-$ [$C_{36}H_{39}Cl_2N_2O_7S$]$^-$ 713.19, found: 713.35. Anal. calcd. (%) for $C_{36}H_{40}Cl_2N_2O_7S$: C, 60.42; H, 5.63; N, 3.91. Found C, 60.73; H, 5.68; N, 3.88. UV-Vis: λ_{max}(Abs) 636 nm (ε 173,000 M^{-1}cm^{-1}), λ_{max}(Em) 646 nm (Methanol).

N*,*N*-Diisopropylethylammonium salt of 4-(5-chloro-2-((3-((5-chloro-1,3,3-trimethylindolin-2-ylidene)methyl)-2-oxido-4-oxocyclobut-2-en-1-ylidene)methyl)-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3-methyl-3H-indol-1-ium-3-yl)butane-1-sulfonate** (*N*-Hydroxysuccinimide ester of ***b-2*-COOH)** (b-2*-NHS**).



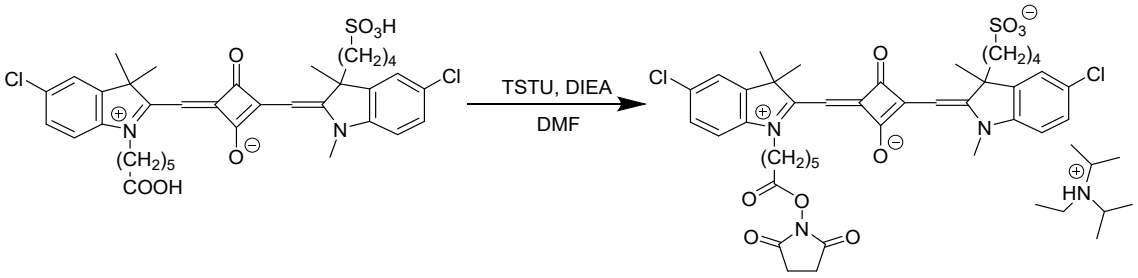
***b-2*-COOH** (55.0 mg, 76.8 mμmol), *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) (35 mg, 116.2 mμmol) were dissolved in DMF (4 mL) and *N*,*N*-diisopropylethylamine (DIEA) (37 mμL, 212.2 mμmol) was added. The solution was stirred at room temperature for 10 min. The excess of DIEA was removed under reduced pressure with a rotary evaporator and the residue was diluted with water and purified by column chromatography (Silica gel 60 RP18, 20—50 % acetonitrile-water) to give ***b-2*-NHS**. Yield: 12 mg (74 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm:7.65 (1H, s, arom.), 7.60 (1H, s, arom.), 7.43–7.35 (2H, m, arom.), 7.35–7.25 (2H, m, arom.), 5.84 (1H, s, C*H*), 5.79 (1H, s, C*H*), 4.05 (2H, broad s, NCH_2), 3.56 (3H, s, NCH_3), 3.58–3.49 (2H, m, C*H* (DIPEA)), 3.18–3.07 (2H, m, CH_2 (DIPEA)), 2.82 (4H, s, CH_2 (succinimide)), 2.72–2.64 (4H, m, CH_2), 2.21–2.10 (2H, m, CH_2CONHS), 1.68 (6H, s, C(C$\underline{H}_3$)$_2$), 1.66 (3H, s, 3-CH_3), 1.77–1.60 (2H, m, CH_2), 1.57–1.41 (2H, m, CH_2), 1.40–1.28 (4H, m, CH_2), 1.33–1.20 (15H, m, DIPEA), 0.82–0.62 (1H, m, CH_2), 0.60–0.36 (1H, m, CH_2). ESI MS, m/z calcd. for [M–DIEA-H$^+$]$^-$ [$C_{40}H_{42}Cl_2N_3O_9S$]$^-$ 810.20, found: 810.43. Anal. calcd. (%) for $C_{48}H_{62}Cl_2N_4O_9S$: C, 61.20; H, 6.63; N, 5.95. Found C, 61.33; H, 6.60; N, 6.01. UV-Vis: λ_{max}(Abs) 636 nm, λ_{max}(Em) 646 nm (Methanol).

4-((1-(5-carboxypentyl)-5-chloro-3,3-dimethyl-3*H*-indol-1-ium-2-yl)methylene)-2-((5-chloro-1,3-dimethyl-3-(4-sulfobutyl)indolin-2-ylidene)methyl)-3-oxocyclobut-1-en-1-olate (*b-3*-COOH).



6-(5-Chloro-2-((2-hydroxy-3,4-dioxocyclobut-1-en-1-yl)methylene)-3,3-dimethylindolin-1-yl)hexanoic acid (**3b**) (180 mg, 0.45 mmol) and 4-(5-chloro-1,2,3-trimethyl-3*H*-indol-1-ium-3-yl)butane-1-sulfonate (**1b**) (containing potassium iodide) (225 mg) were heated under reflux in toluene (8 mL) and 1-butanol (8 mL) using a Dean-Stark trap for 4 h. The solvent was removed under reduced pressure using a rotary evaporator. Acetic acid (6 mL) and concentrated hydrochloric acid (0.6 mL) were added to the residue and the mixture was refluxed for 1 hour. The acids were rotary evaporated and the obtained raw product was purified by column chromatography (Silica gel 60 RP18, 20—45 % acetonitrile-water) to give ***b-3*-COOH** (47 mg, 15 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.64 (1H, s, arom.), 7.60 (1H, s, arom.), 7.42–7.35 (2H, m, arom.), 7.35–7.29 (2H, m, arom.), 5.82 (1H, s, C*H*), 5.81 (1H, s, C*H*), 4.05 (2H, broad s, NCH_2), 3.55 (3H, s, NCH_3), 2.25–2.13 (6H, m, CH_2), 1.67 (6H, s, C(CH_3)$_2$), 1.66 (3H, s, 3-CH_3), 1.75–1.61 (2H, m, CH_2), 1.61–1.47 (2H, m, CH_2), 1.46–1.30 (4H, m, CH_2), 0.77–0.60 (1H, m, CH_2), 0.59–0.40 (1H, m, CH_2). ESI MS, m/z calcd. for [M–H]$^-$ [$C_{36}H_{39}Cl_2N_2O_7S$]$^-$ 713.19, found: 713.37. Anal. calcd. (%) for $C_{36}H_{40}Cl_2N_2O_7S$: C, 60.42; H, 5.63; N, 3.91. Found C, 60.38; H, 5.58; N, 3.89. UV-Vis: λ_{max}(Abs) 636 nm, λ_{max}(Em) 648 nm (Methanol).

N*,*N*-Diisopropylethylammonium salt of 4-(5-chloro-2-((3-((5-chloro-1-(6-((2,5-dioxopyrrolidin-1-yl)oxy)-6-oxohexyl)-3,3-dimethyl-3*H*-indol-1-ium-2-yl)methylene)-2-oxido-4-oxocyclobut-1-en-1-yl)methylene)-1,3-dimethylindolin-3-yl)butane-1-sulfonate** (*N*-Hydroxysuccinimide ester of ***b-3*-COOH)** (b-3*-NHS**).



***b-3*-COOH** (20.0 mg, 27.9 mμmol), *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) (10 mg, 33.2 mμmol) were dissolved in DMF (2 mL) and *N*,*N*-diisopropylethylamine (DIEA) (14 mμL, 80.3 mμmol) was added. The solution was stirred at room temperature for 10 min. The reaction mixture was diluted with ether (20 mL) and left for precipitation of the product. The formed precipitate was filtered off, washed with ether, diluted with 20 % aqueous acetonitrile and purified by column chromatography (Silica gel 60 RP18, 20—50 % acetonitrile-water) to give ***b-3*-NHS**. Yield: 12 mg (74 %). ^{1}H-NMR (400 MHz, DMSO-d_6), δ, ppm: 7.64 (1H, s, arom.), 7.60 (1H, s, arom.), 7.42–7.34 (2H, m, arom.), 7.34–7.27 (2H, m, arom.), 5.82 (1H, s, C*H*), 5.81 (1H, s, C*H*), 4.05 (2H, broad s, NCH_2), 3.55 (3H, s,

NC$\underline{H_3}$), 3.58–3.50 (2H, m, C*H* (DIPEA)), 3.18–3.06 (2H, m, CH_2 (DIPEA)), 2.83 (4H, s, CH_2 (succinimide)), 2.73–2.64 (4H, m, CH_2), 2.24–2.12 (2H, m, CH_2CONHS), 1.67 (6H, s, C(C$\underline{H_3}$)$_2$), 1.66 (3H, s, 3-C$\underline{H_3}$), 1.75–1.61 (2H, m, CH_2), 1.61–1.47 (2H, m, CH_2), 1.45–1.30 (4H, m, C$\underline{H_2}$), 1.33–1.19 (15H, m, DIPEA), 0.77–0.59 (1H, m, C$\underline{H_2}$), 0.58–0.38 (1H, m, C$\underline{H_2}$). ESI MS, m/z calcd. for [M–DIEA-H$^+$]$^-$ [$C_{40}H_{42}Cl_2N_3O_9S$]$^-$ 810.20, found: 810.47. Anal. calcd. (%) for $C_{48}H_{62}Cl_2N_4O_9S$: C, 61.20; H, 6.63; N, 5.95. Found C, 61.15; H, 6.81; N, 5.99. UV-Vis: λ_{max}(Abs) 636 nm, λ_{max}(Em) 648 nm (Methanol).

SI 2. Sequence of DNA Holliday Junction (DNA-HJ)

Table S1. DNA sequence used to synthesize the HJ-DNA

DNA strands	Base sequence
Strand A	ATATAATCGCTCG*CATATTATGACTG
Strand B	CAGTCATAATATG*TGGAATGTGAGTG
Strand C	CACTCACATTCCA*CTCAACACCACAA
Strand D	TTGTGGTGTTGAG*CGAGCGATTATAT
*Indicates the position where an extra Thymine is added to bound covalently the squaraine dye onto the DNA. Note that the extra Thymine is added only if the DNA strand has the dye as shown in Figure 1A.	

SI 3. Absorption spectra of modified squaraine monomers in DNA-HJ

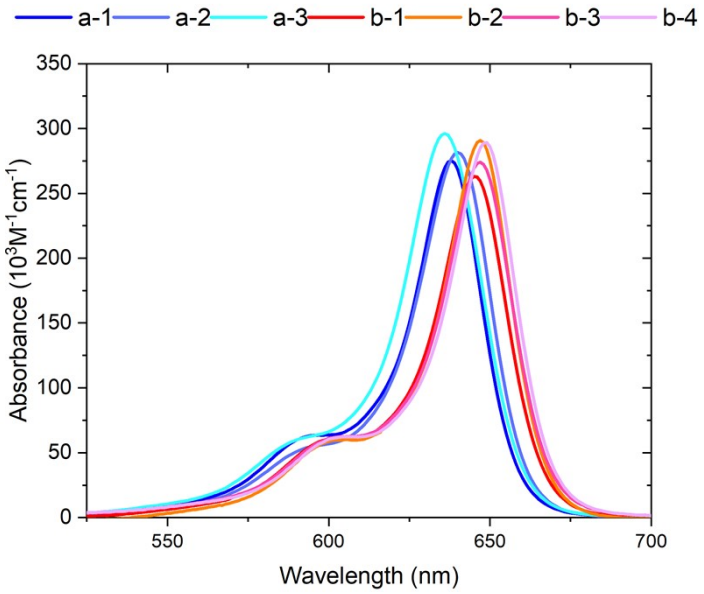


Figure S1. Absorption spectra of squaraine dyes monomers in DNA-HJ.

SI 4. Fluorescence suppression of squaraine dimers

The fluorescence suppression of dimer in reference to their respective monomers was calculated using the emission spectra scaled by the absorptance at the excitation wavelength (630 nm). The normalized emission (FL) in the wavelength range of "x" to "y" nm is defined as:

$$FL_{construct} = \frac{Emission\ spectra_{x \to y\ nm}}{Absoptane_{@excited\ wavelength}} \quad \text{(Eq. S1)}$$

Where:

$$Absoptance = 1 - 10^{-absorbance} \quad \text{(Eq. S2)}$$

The florescence suppression of the aggregates was calculated using the integrated area of monomers and dimers ($\int FL_X$) $by\ the$ following formula[4]:

$$\%\ FL\ suppression = \frac{\int FL_{Monomer} - \int FL_{Dimer}}{\int FL_{Monomer}} x\ 100\%$$

Table S2. Percentage (%) of fluorescence emission suppression of squaraine dye aggregates relative to their monomers (excitation at 630 nm)

Dye group	Dye label	Construct	Peak	Area	FL suppression (%)
Non-chlorinated dyes*	*a-1*	Monomer	646	2.47E+08	-
		Adjacent dimer	646	2.23E+07	91.0
		Transverse dimer	649	3.17E+07	87.2
	a-2	Monomer	648	2.72E+08	-
		Adjacent dimer	652	3.98E+07	85.4
		Transverse dimer	650	2.68E+07	90.2
	a-3	Monomer	648	1.52E+08	-
		Adjacent dimer	649	4.76E+07	68.7
		Transverse dimer	648	3.55E+07	76.6
Chlorinated dyes	*b-1**	Monomer	654	3.19E+08	-
		Adjacent dimer	654	2.36E+07	92.6
		Transverse dimer	654	1.85E+07	94.2
	*b-2***	Monomer	656	4.45E+07	-
		Adjacent dimer	657	1.25E+07	71.8
		Transverse dimer	658	5673110	87.2
	*b-3***	Monomer	657	3.09E+07	-
		Adjacent dimer	655	1.66E+07	46.3
		Transverse dimer	658	8480870	72.5
	*b-4***	Monomer	657	2.98E+07	-
		Adjacent dimer	660	7921470	73.4
		Transverse dimer	665	7182130	75.9

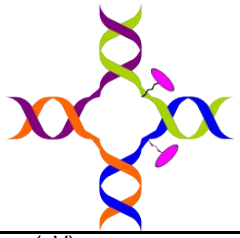
*Slit = 3 nm BadPass recorded in Mass *et al.*[4] and **Slit =2 nm of 0.5 μM SQ-DNA constructs, in 1XTBE 15 mM $MgCl_2$ recorded at room temperature. Absorbance nad fluorescence was measured under the same

experimental conditions using 5mm of SQ-DNA constructs.

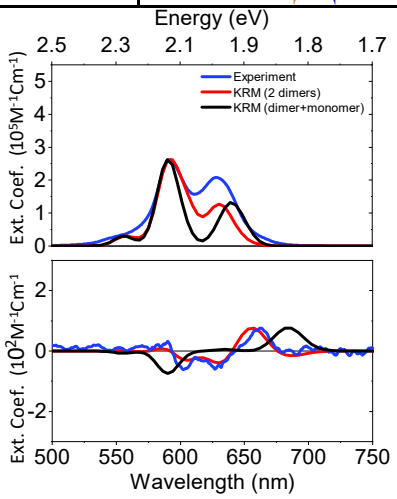
SI 5. KRM modeling

SI 5.1. Scorecards of non-chlorinated adjacent dimers

Dye a-1
Adjacent BC dimer (two dimers)
Holliday Junction DNA



Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	127	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.69	
Energy loss parameter, Γ	30	meV
Characteristic excitonic hopping parameter, J_0	53	meV*nm^3
Huang-Rhys factor (dimensionless)	0.24	
Transition dipole moment, μ	12.3	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-5	meV



Data in this scorecard represent fitting parameters of two dimers approach. Spectra in black curve suggest the presence of monomers, KRM of this run is non-shown.

Aggregate parameter	**units**	(1,2)	(3,4)				
Exciton hopping parameter, $J_{m,n}$	meV	136	53				
Center-to-center distance, $R_{m,n}$	nm	0.34	0.62				
Closest distance between dyes, dmin$_{m,n}$	nm	0.34	0.45				
Oblique angle, $\alpha_{m,n}$	degrees	1.2	4.9				
*Twist angle, $\theta_t^{m,n}$	degrees	-1.2	4.8				
^Slip angle 1, $\theta_s^{m,R}$	degrees	85.2	49.9				
^Slip angle 2, $\theta_s^{n,R}$	degrees	85.1	53.1				

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.17	85.2	0.0
2	0.00	0.00	0.17	85.1	-1.2
3	0.00	0.00	-0.31	49.9	0.0
4	0.00	0.00	0.31	53.1	4.8

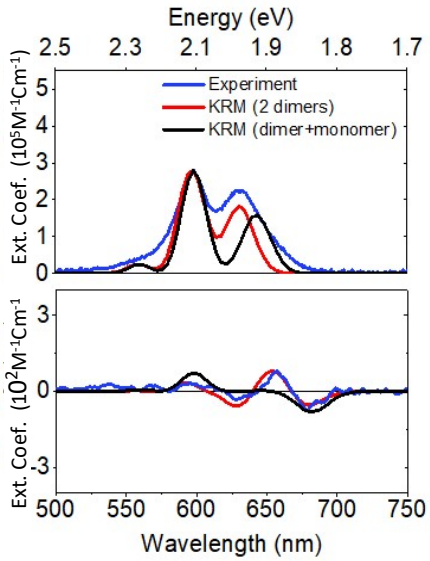
Fitting parameters	***RR***	***OI*$_{ABS}$**	***OI*$_{CD}$**	***OI*$_{total}$**	***ms*$_{ABS}$**	***ms*$_{CD}$**	***Total Fitness***
Goodness of fit results	1.12	0.96	0.82	0.89	0.93	2.38	
Fitness weight	1	0	0	n/a	1	1	3.32

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye a-2
Adjacent BC dimer (two dimers)
Holliday Junction DNA

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	140	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.62	
Energy loss parameter, Γ	30	meV
Characteristic excitonic hopping parameter, J_0	54	meV*nm^3
Huang-Rhys factor (dimensionless)	0.19	
Transition dipole moment, μ	12.4	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	0	meV



Data in this scorecard represent fitting parameters of two dimers approach. Spectra in black curve suggest the presence of monomers, KRM of this run is non-shown.

Aggregate parameter	**units**	(1,2)	(3,4)				
Exciton hopping parameter, $J_{m,n}$	meV	47	117				
Center-to-center distance, $R_{m,n}$	nm	0.70	0.39				
Closest distance between dyes, dmin$_{m,n}$	nm	0.65	0.36				
Oblique angle, $\alpha_{m,n}$	degrees	3.7	3.5				
*Twist angle, $\theta_t^{m,n}$	degrees	2.6	-2.9				
^Slip angle 1, $\theta_s^{m,R}$	degrees	78.3	85.5				
^Slip angle 2, $\theta_s^{n,R}$	degrees	75.5	83.5				

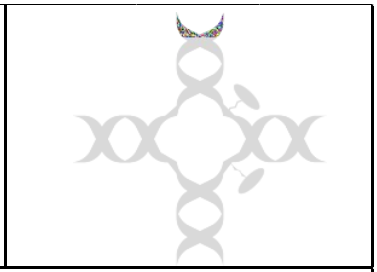
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.35	78.3	0.0
2	0.00	0.00	0.35	75.5	2.6
3	0.00	0.00	-0.19	85.5	0.0
4	0.00	0.00	0.19	83.5	-2.9

Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	0.99	0.96	0.81	0.88	1.08	2.38	
Fitness weight	1	0	0	n/a	1	1	3.47

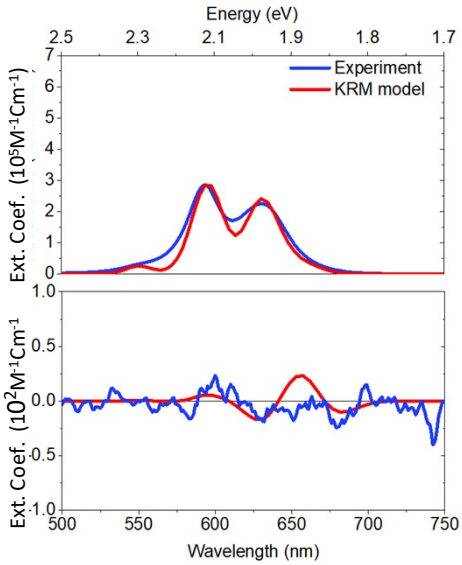
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye a-3
Adjacent BC dimer (two dimers)
Holliday Junction DNA



Number of chromophores	4	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	165	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.61	
Energy loss parameter, Γ	34	meV
Characteristic excitonic hopping parameter, J_0	61	meV*nm^3
Huang-Rhys factor (dimensionless)	0.19	
Transition dipole moment, μ	13.1	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-18	meV



Data in this scorecard represent fitting parameters of two dimers approach.

Aggregate parameter	**units**	(1,2)	(3,4)				
Exciton hopping parameter, $J_{m,n}$	meV	48	109				
Center-to-center distance, $R_{m,n}$	nm	0.87	0.44				
Closest distance between dyes, dmin$_{m,n}$	nm	0.37	0.42				
Oblique angle, $\alpha_{m,n}$	degrees	41.4	2.0				
*Twist angle, $\theta_t^{m,n}$	degrees	-1.6	-1.2				
^Slip angle 1, $\theta_s^{m,R}$	degrees	43.2	87.6				
^Slip angle 2, $\theta_s^{n,R}$	degrees	84.6	86.0				

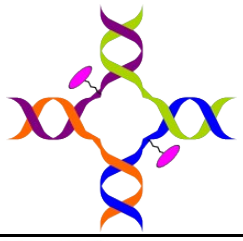
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.43	43.2	0.0
2	0.00	0.00	0.43	84.6	-1.6
3	0.00	0.00	-0.22	92.4	0.0
4	0.00	0.00	0.22	94.0	-1.2

Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.01	0.98	0.16	0.57	0.35	8.11	
Fitness weight	1	0	0	n/a	1	0	0.35

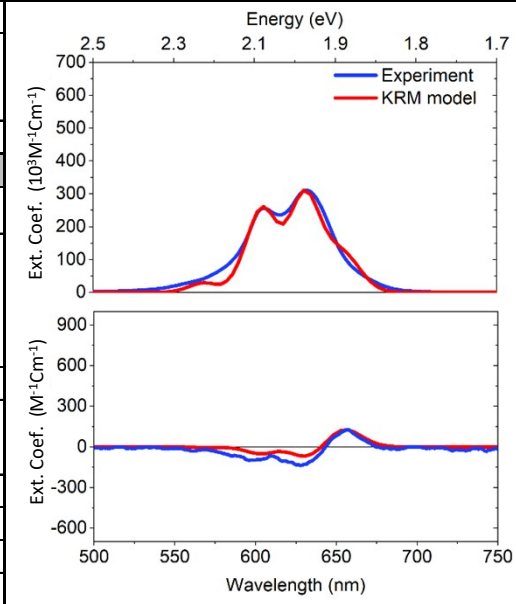
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

SI 5.2. Score cards of non-chlorinated transverse dimers

Dye a-1 Transverse AC dimer (single dimer) Holliday Junction DNA	

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	127	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.69	
Energy loss parameter, Γ	30	meV
Characteristic excitonic hopping parameter, J_0	53	meV*nm^3
Huang-Rhys factor (dimensionless)	0.24	
Transition dipole moment, μ	12.3	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-7	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	50
Center-to-center distance, $R_{m,n}$	nm	1.00
Closest distance between dyes, dmin$_{m,n}$	nm	0.36
Oblique angle, $\alpha_{m,n}$	degrees	50.4
*Twist angle, $\theta_t^{m,n}$	degrees	1.3
^Slip angle 1, $\theta_s^{m,R}$	degrees	66.2
^Slip angle 2, $\theta_s^{n,R}$	degrees	63.4

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.50	66.2	0.0
2	0.00	0.00	0.50	116.6	1.3

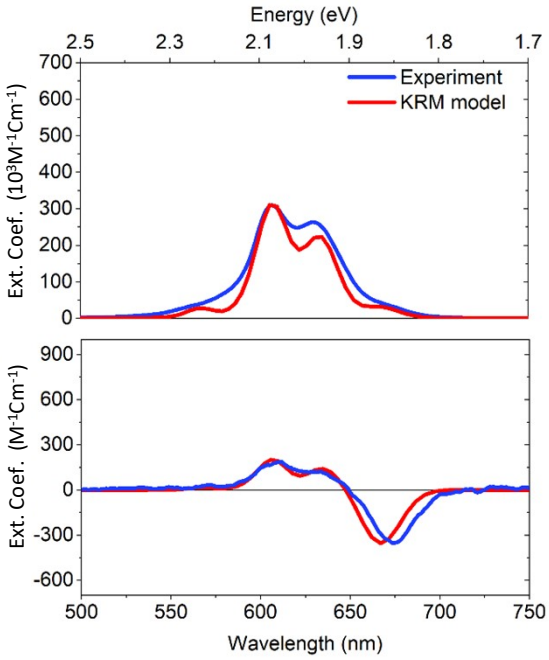
Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	0.99	0.99	0.85	0.92	0.17	2.85	
Fitness weight	1	0	0	n/a	1	1	3.03

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye a-2
Transverse AC dimer (single dimer)
Holliday Junction DNA

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	140	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.62	
Energy loss parameter, Γ	30	meV
Characteristic excitonic hopping parameter, J_0	54	meV*nm^3
Huang-Rhys factor (dimensionless)	0.19	
Transition dipole moment, μ	12.4	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-15	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	71
Center-to-center distance, $R_{m,n}$	nm	0.68
Closest distance between dyes, dmin$_{m,n}$	nm	0.34
Oblique angle, $\alpha_{m,n}$	degrees	27.7
*Twist angle, $\theta_t^{m,n}$	degrees	-4.8
^Slip angle 1, $\theta_s^{m,R}$	degrees	82.7
^Slip angle 2, $\theta_s^{n,R}$	degrees	70.1

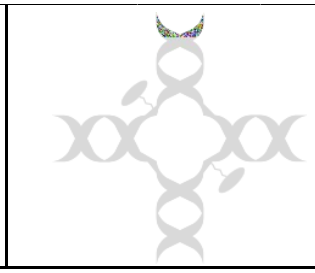
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.34	97.3	0.0
2	0.00	0.00	0.34	70.1	-4.8

Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.02	0.99	0.93	0.96	0.39	0.79	
Fitness weight	1	0	0	n/a	1	1	1.18

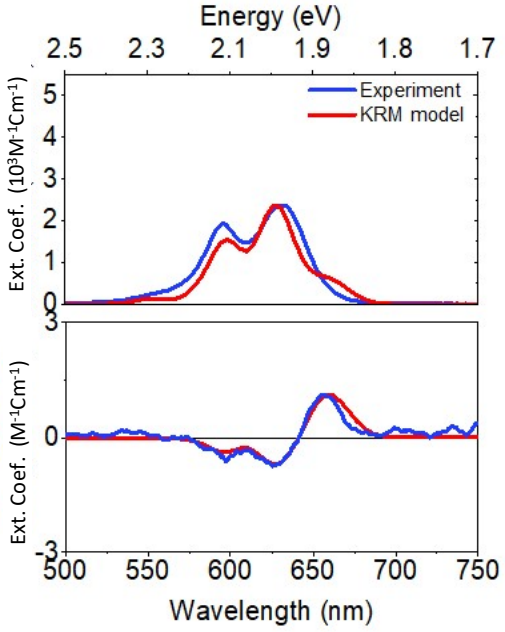
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye a-3
Transverse AC dimer (single dimer)
Holliday Junction DNA



Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	165	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.61	
Energy loss parameter, Γ	34	meV
Characteristic excitonic hopping parameter, J_0	61	meV*nm^3
Huang-Rhys factor (dimensionless)	0.19	
Transition dipole moment, μ	13.1	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-13	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	62
Center-to-center distance, $R_{m,n}$	nm	0.91
Closest distance between dyes, dmin$_{m,n}$	nm	0.35
Oblique angle, $\alpha_{m,n}$	degrees	44.0
*Twist angle, $\theta_t^{m,n}$	degrees	1.5
^Slip angle 1, $\theta_s^{m,R}$	degrees	67.2
^Slip angle 2, $\theta_s^{n,R}$	degrees	68.8

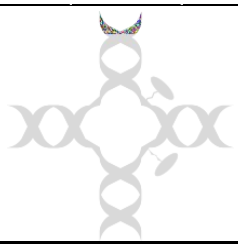
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.46	67.2	0.0
2	0.00	0.00	0.46	111.2	1.5

Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.00	0.98	0.94	0.96	0.51	0.78	
Fitness weight	1	0	0	n/a	1	1	1.29

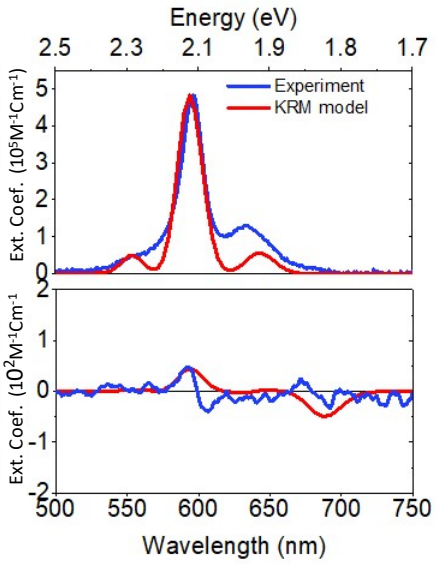
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

SI 5.3. Score cards of chlorinated adjacent dimers

Dye b-1 Adjacent BC dimer (single dimer) Holliday Junction DNA	

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.66	
Energy loss parameter, Γ	30	meV
Characteristic excitonic hopping parameter, J_0	51	meV*nm^3
Huang-Rhys factor (dimensionless)	0.22	
Transition dipole moment, μ	12.0	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-5E-05	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	132
Center-to-center distance, $R_{m,n}$	nm	0.34
Closest distance between dyes, dmin$_{m,n}$	nm	0.34
Oblique angle, $\alpha_{m,n}$	degrees	1.3
*Twist angle, $\theta_t^{m,n}$	degrees	-1.3
^Slip angle 1, $\theta_s^{m,R}$	degrees	88.9
^Slip angle 2, $\theta_s^{n,R}$	degrees	88.9

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.17	88.9	0.0
2	0.00	0.00	0.17	88.9	-1.3

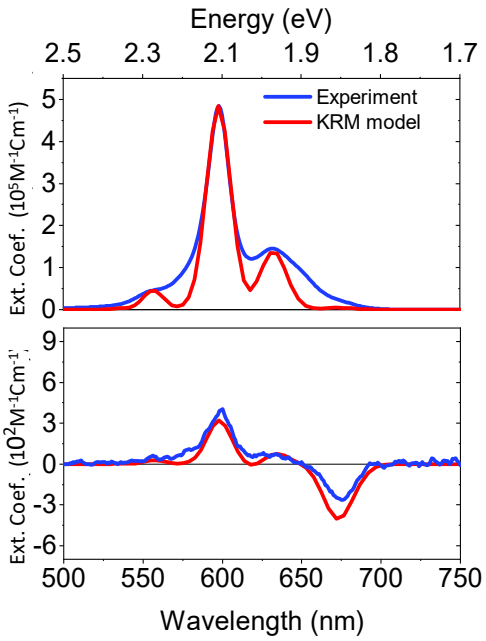
Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.03	0.95	0.35	0.65	0.41	9.27	
Fitness weight	1	0	0	n/a	1	1	9.68

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye b-2
Adjacent BC dimer (single dimer)
Holliday Junction DNA

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.64	
Energy loss parameter, Γ	26	meV
Characteristic excitonic hopping parameter, J_0	50	meV*nm^3
Huang-Rhys factor (dimensionless)	0.20	
Transition dipole moment, μ	11.9	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	17	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	100
Center-to-center distance, $R_{m,n}$	nm	0.42
Closest distance between dyes, dmin$_{m,n}$	nm	0.34
Oblique angle, $\alpha_{m,n}$	degrees	10.2
*Twist angle, $\theta_t^{m,n}$	degrees	-7.4
^Slip angle 1, $\theta_s^{m,R}$	degrees	89.8
^Slip angle 2, $\theta_s^{n,R}$	degrees	83.2

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.21	90.2	0.0
2	0.00	0.00	0.21	83.2	-7.4

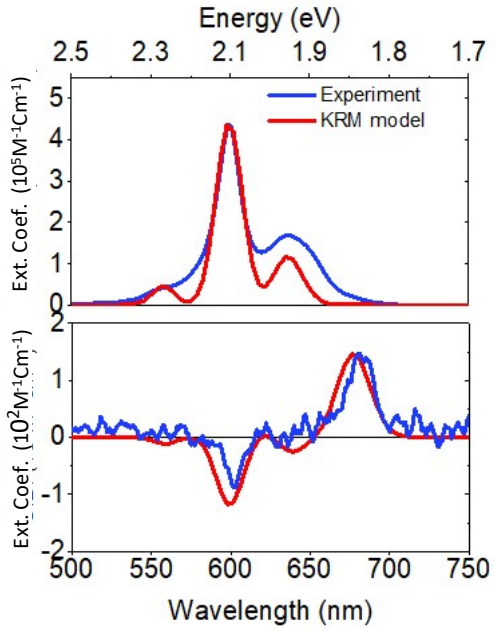
Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.00	0.97	0.91	0.94	0.29	0.99	
Fitness weight	1	0	0	n/a	1	1	1.28

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye b-3
Adjacent BC dimer (single dimer)
Holliday Junction DNA

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.67	
Energy loss parameter, Γ	28	meV
Characteristic excitonic hopping parameter, J_0	56	meV*nm^3
Huang-Rhys factor (dimensionless)	0.22	
Transition dipole moment, μ	12.6	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	8	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	103
Center-to-center distance, $R_{m,n}$	nm	0.46
Closest distance between dyes, dmin$_{m,n}$	nm	0.34
Oblique angle, $\alpha_{m,n}$	degrees	9.7
*Twist angle, $\theta_t^{m,n}$	degrees	2.8
^Slip angle 1, $\theta_s^{m,R}$	degrees	84.4
^Slip angle 2, $\theta_s^{n,R}$	degrees	86.3

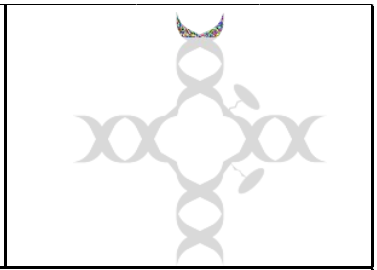
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.23	84.4	0.0
2	0.00	0.00	0.23	93.7	2.8

Fitting parameters	***RR***	***OI*$_{ABS}$**	***OI*$_{CD}$**	***OI*$_{total}$**	***ms*$_{ABS}$**	***ms*$_{CD}$**	***Total Fitness***
Goodness of fit results	1.00	0.95	0.83	0.89	0.47	1.97	
Fitness weight	1	0	0	n/a	1	0	2.44

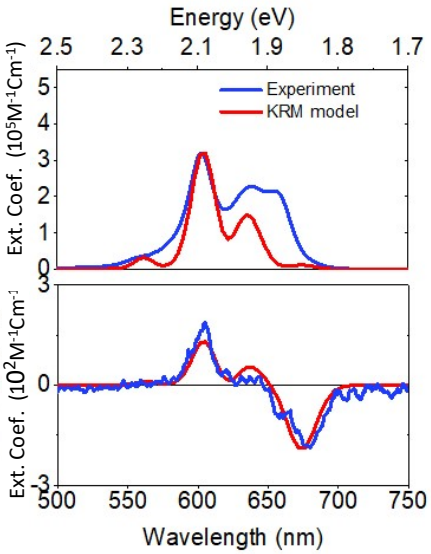
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye b-4
Adjacent BC dimer (two dimers)
Holliday Junction DNA



Number of chromophores	4	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.65	
Energy loss parameter, Γ	28	meV
Characteristic excitonic hopping parameter, J_0	57	meV*nm^3
Huang-Rhys factor (dimensionless)	0.21	
Transition dipole moment, μ		debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	13	meV



Data in this scorecard represent fitting parameters of two dimers approach.

Aggregate parameter	**units**	(1,2)	(3,4)				
Exciton hopping parameter, $J_{m,n}$	meV	96	-41				
Center-to-center distance, $R_{m,n}$	nm	0.48	1.46				
Closest distance between dyes, dmin$_{m,n}$	nm	0.34	0.35				
Oblique angle, $\alpha_{m,n}$	degrees	12.1	84.4				
*Twist angle, $\theta_t^{m,n}$	degrees	-5.9	-0.5				
^Slip angle 1, $\theta_s^{m,R}$	degrees	68.0	43.4				
^Slip angle 2, $\theta_s^{n,R}$	degrees	78.8	41.0				

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.24	112.0	0.0
2	0.00	0.00	0.24	101.2	-5.9
3	0.00	0.00	-0.73	43.4	0.0
4	0.00	0.00	0.73	139.0	-0.5

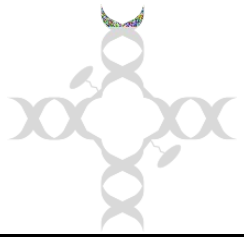
Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.01	0.98	0.94	0.96	0.39	0.76	
Fitness weight	1	0	0	n/a	1	1	1.14

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

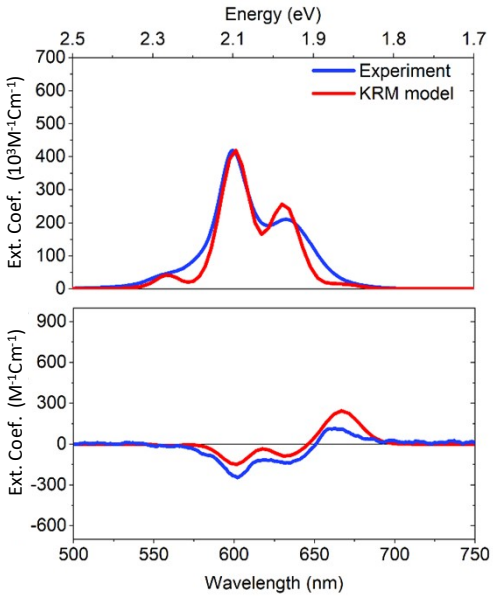
^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

SI 5.4. Score cards of chlorinated transverse dimers

Dye b-1
Transverse AC dimer (single dimer)
Holliday Junction DNA



Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.66	
Energy loss parameter, Γ	30	meV
Characteristic excitonic hopping parameter, J_0	51	meV*nm^3
Huang-Rhys factor (dimensionless)	0.22	
Transition dipole moment, μ	12.0	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	7	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	79
Center-to-center distance, $R_{m,n}$	nm	0.56
Closest distance between dyes, $dmin_{m,n}$	nm	0.34
Oblique angle, $\alpha_{m,n}$	degrees	17.1
*Twist angle, $\theta_t^{m,n}$	degrees	3.1
^Slip angle 1, $\theta_s^{m,R}$	degrees	75.4
^Slip angle 2, $\theta_s^{n,R}$	degrees	87.8

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.28	104.6	0.0
2	0.00	0.00	0.28	87.8	3.1

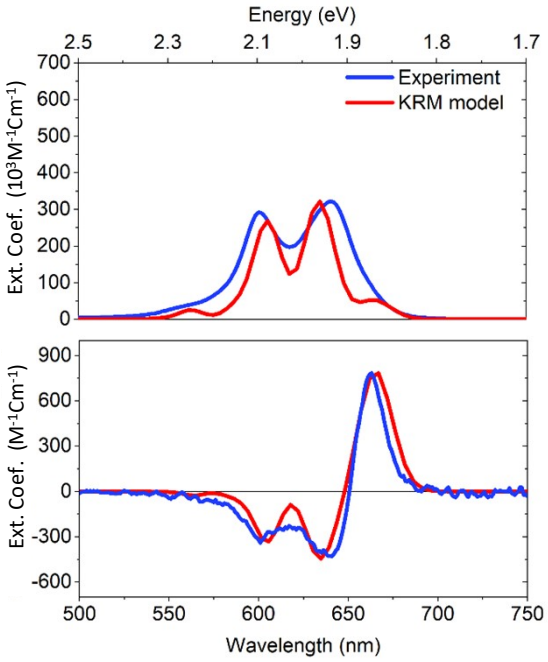
Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	0.99	0.98	0.80	0.89	0.27	2.62	
Fitness weight	1	0	0	n/a	1	1	2.89

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye b-2
Transverse AC dimer (single dimer)
Holliday Junction DNA

Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.64	
Energy loss parameter, Γ	26	meV
Characteristic excitonic hopping parameter, J_0	50	meV*nm^3
Huang-Rhys factor (dimensionless)	0.20	
Transition dipole moment, μ	11.9	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	3	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	62
Center-to-center distance, $R_{m,n}$	nm	0.73
Closest distance between dyes, dmin$_{m,n}$	nm	0.34
Oblique angle, $\alpha_{m,n}$	degrees	33.8
*Twist angle, $\theta_t^{m,n}$	degrees	8.9
^Slip angle 1, $\theta_s^{m,R}$	degrees	65.7
^Slip angle 2, $\theta_s^{n,R}$	degrees	81.7

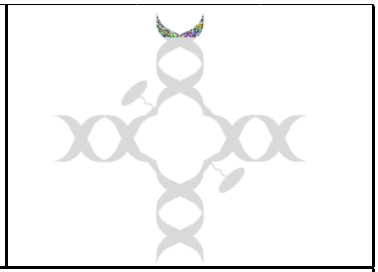
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.37	65.7	0.0
2	0.00	0.00	0.37	98.3	8.9

Fitting parameters	***RR***	***OI*$_{ABS}$**	***OI*$_{CD}$**	***OI*$_{total}$**	***ms*$_{ABS}$**	***ms*$_{CD}$**	***Total Fitness***
Goodness of fit results	1.00	0.94	0.95	0.95	1.47	0.52	
Fitness weight	1	0	0	n/a	1	1	1.99

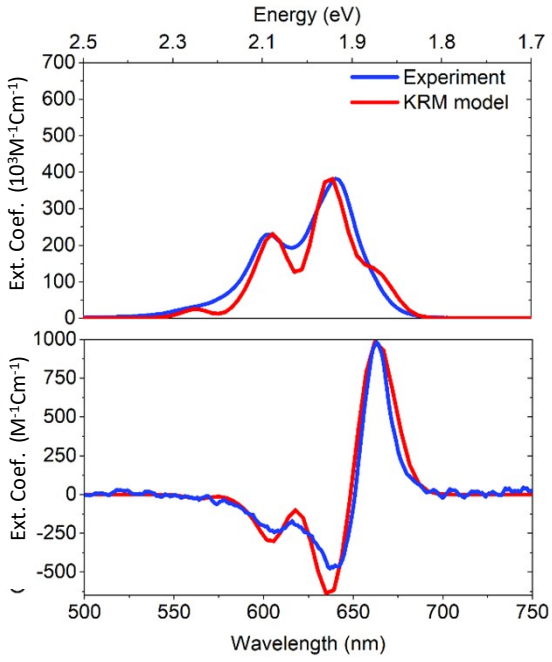
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye b-3
Transverse AC dimer (single dimer)
Holliday Junction DNA



Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.67	
Energy loss parameter, Γ	28	meV
Characteristic excitonic hopping parameter, J_0	56	meV*nm^3
Huang-Rhys factor (dimensionless)	0.22	
Transition dipole moment, μ	12.6	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-2	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	50.7
Center-to-center distance, $R_{m,n}$	nm	0.94
Closest distance between dyes, dmin$_{m,n}$	nm	0.37
Oblique angle, $\alpha_{m,n}$	degrees	50.2
*Twist angle, $\theta_t^{m,n}$	degrees	9.4
^Slip angle 1, $\theta_s^{m,R}$	degrees	81.2
^Slip angle 2, $\theta_s^{n,R}$	degrees	49.4

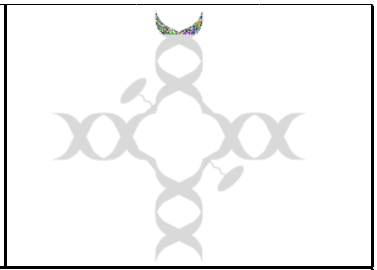
Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.47	98.8	0.0
2	0.00	0.00	0.47	49.4	9.4

Fitting parameters	***RR***	***OI$_{ABS}$***	***OI$_{CD}$***	***OI$_{total}$***	***ms$_{ABS}$***	***ms$_{CD}$***	***Total Fitness***
Goodness of fit results	1.00	0.98	0.97	0.98	0.34	0.35	
Fitness weight	1	0	0	n/a	1	1	0.68

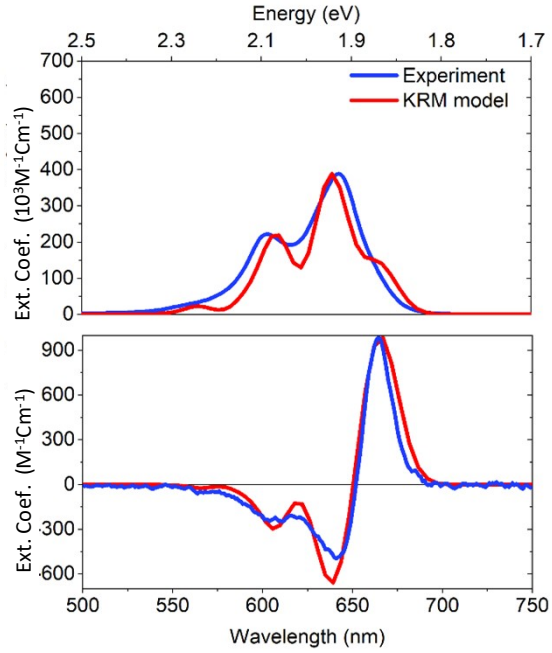
* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

Dye b-4
Transverse AC dimer (single dimer)
Holliday Junction DNA



Number of chromophores	2	
Number of vibrational levels considered, n_v	3	
Scaffold variant	4-arm HJ DNA	
Monomer property	**value**	**units**
Energy of a vibron ε_v	150	meV
Displacement of excited state potential, d, from ground state potential (dimensionless)	0.65	
Energy loss parameter, Γ	28	meV
Characteristic excitonic hopping parameter, J_0	57	meV*nm^3
Huang-Rhys factor (dimensionless)	0.21	
Transition dipole moment, μ	12.7	debye
TDM length, l	1.3	nm
Energy offset from monomer, *Eof*	-2	meV



Aggregate parameter	**units**	(1,2)
Exciton hopping parameter, $J_{m,n}$	meV	51
Center-to-center distance, $R_{m,n}$	nm	0.96
Closest distance between dyes, dmin$_{m,n}$	nm	0.37
Oblique angle, $\alpha_{m,n}$	degrees	52.5
*Twist angle, $\theta_t^{m,n}$	degrees	9.3
^Slip angle 1, $\theta_s^{m,R}$	degrees	79.9
^Slip angle 2, $\theta_s^{n,R}$	degrees	48.4

Chromophore number	**Center coordinates (nm)**			**Angles (degrees)**	
	X	**Y**	**Z**	**Zenith**	**Azimuthal**
1	0.00	0.00	-0.48	100.1	0.0
2	0.00	0.00	0.48	48.4	9.3

Fitting parameters	***RR***	***OI*** $_{ABS}$	***OI*** $_{CD}$	***OI*** $_{total}$	***ms*** $_{ABS}$	***ms*** $_{CD}$	***Total Fitness***
Goodness of fit results	1.00	0.98	0.97	0.98	0.43	0.32	
Fitness weight	1	0	0	n/a	1	1	0.75

* Twist angle is taken to be the angle betweeen TDM projections in a plane normal to the separation vector, $R_{m,n}$

^ The slip angle depends on the reference dye. Slip angle is the angle between the TDM vector and the separation vector from the reference chromophore

SI 6. Three-dimensional vector 3D plots of two dimers configuration

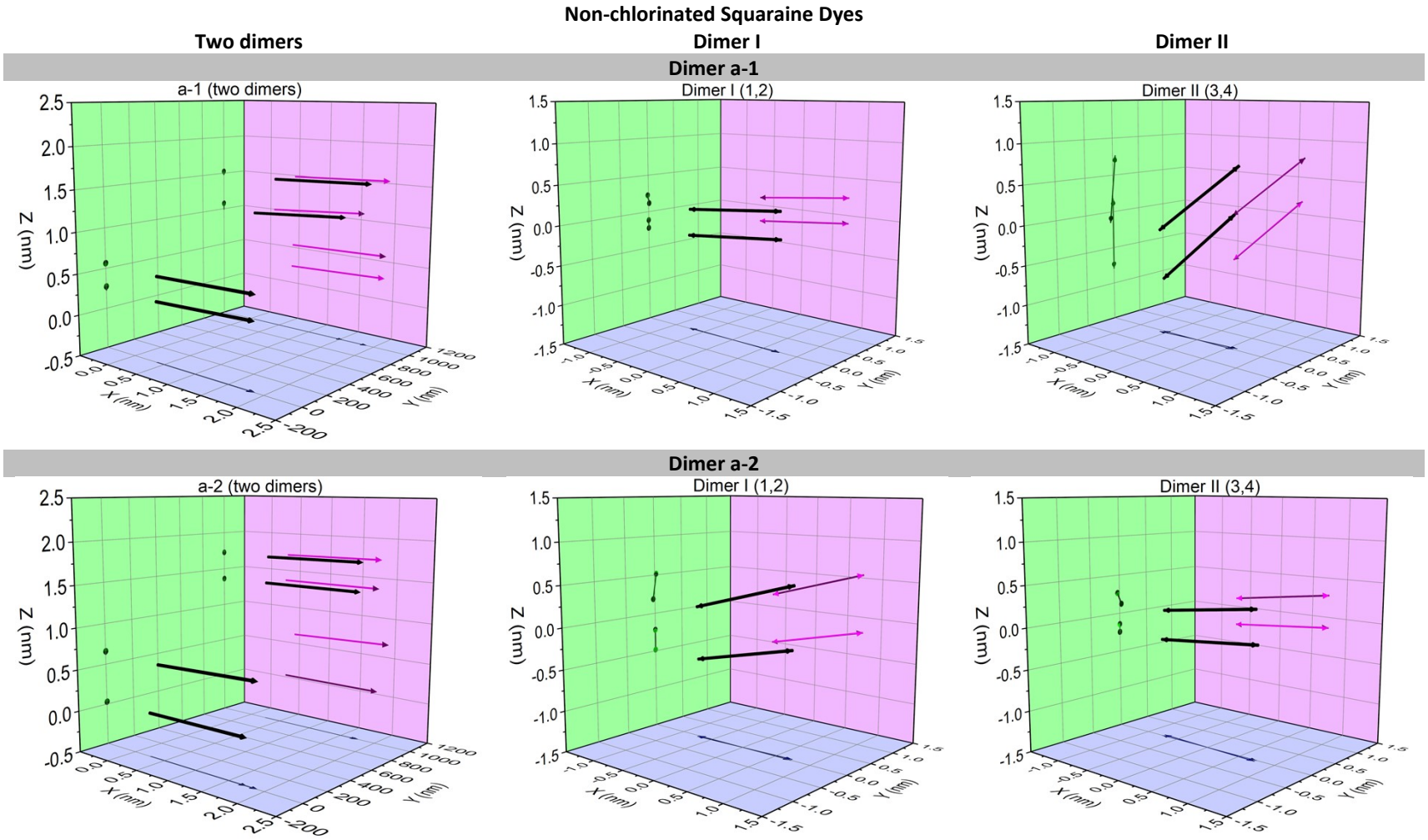
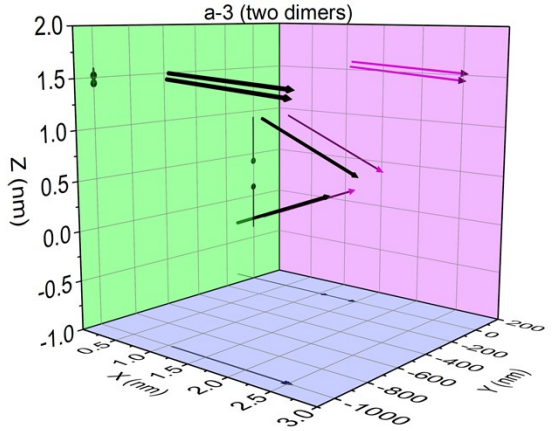
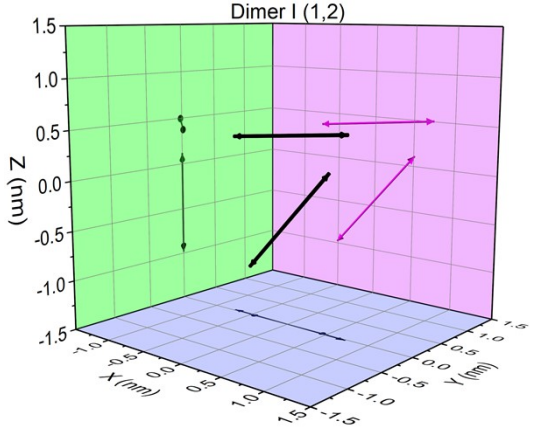
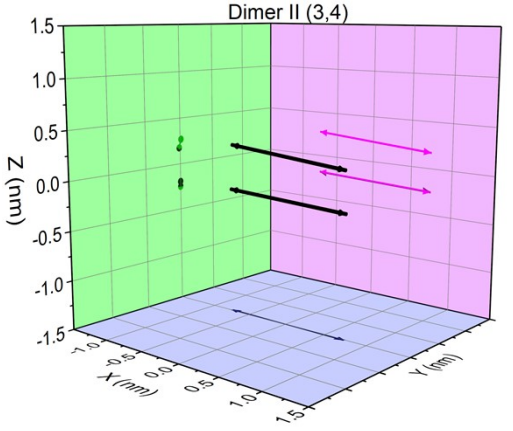
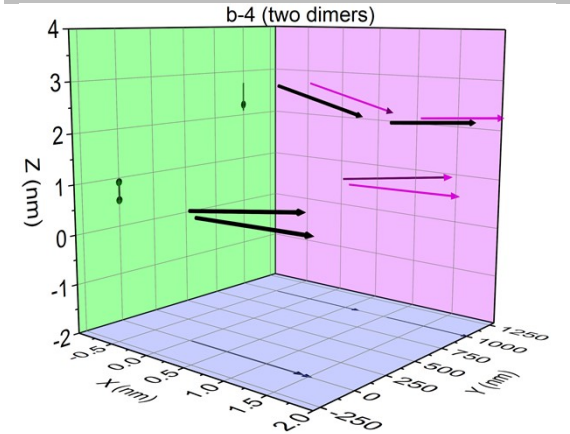
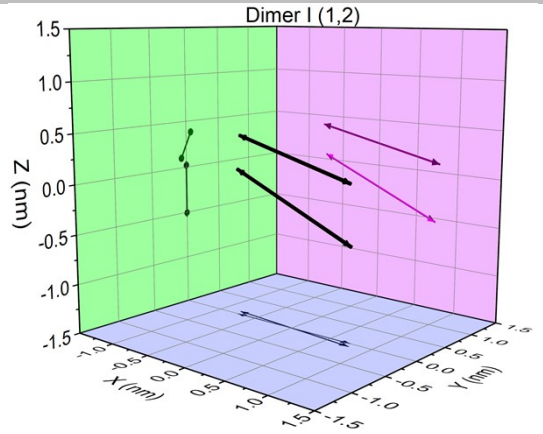
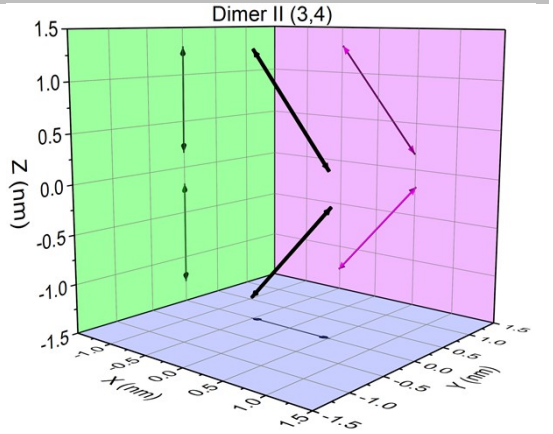


Figure S2. Vector plot with planar projections of two dimers plot and close view of each dimer, i.e., dimers I (composed of vectors 1 and 2) and II *composed of vectors 3 and 4). Vector plot represent the position and orientation of TDM of each chromophore in each aggregate (black arrow) derived from the KRM modeling.

Continuation of Figure S2

Non-chlorinated Squaraine Dyes

Two dimers	Dimer I	Dimer II
Dimer a-3		
		
Chlorinated Squaraine dyes		
Dimer b-4		
		

SI 7. Effect of physical parameters on dye aggregate

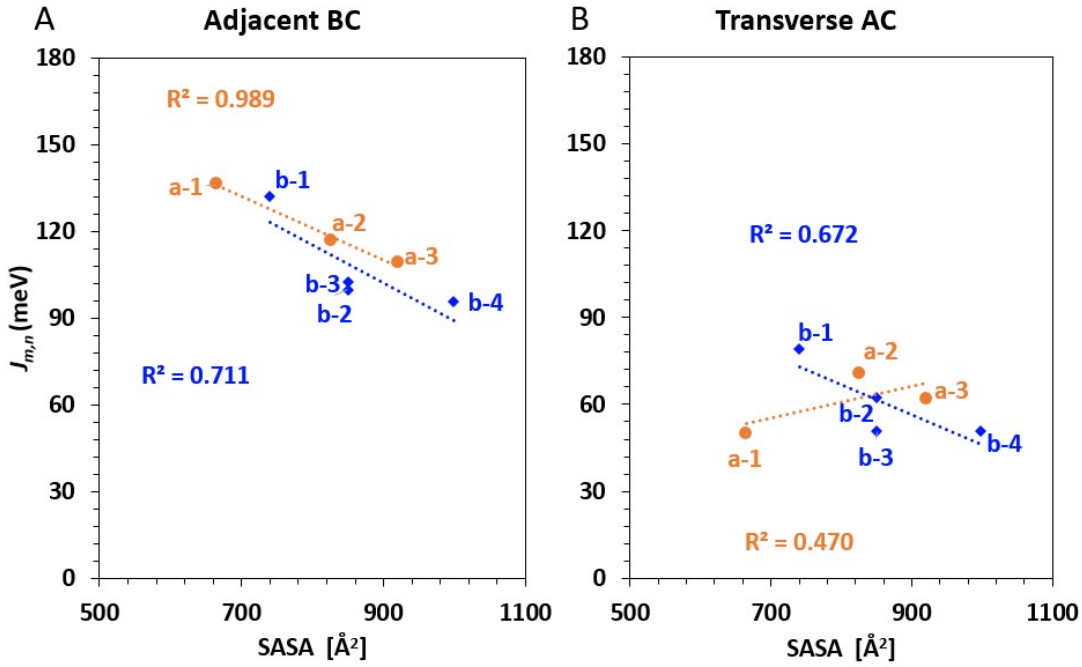


Figure S3. Effect of solvent accessible surface area (SASA) in square angstrom (Å^2) on the excitonic coupling strength ($J_{m,n}$) in meV of non-chlorinated (orange) and chlorinated (blue) squaraine dyes. (A) Adjacent dimers (A) and transverse dimers (B).

SI 8. Symmetrically substituted squaraine dyes

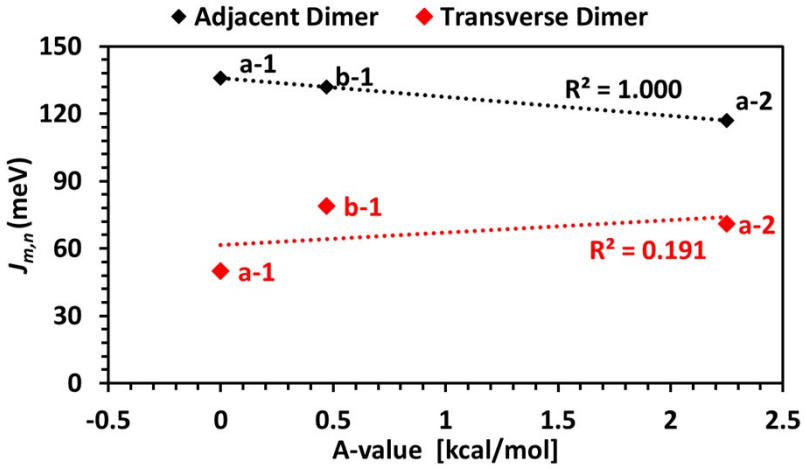


Figure S4. Relationship of A-value and exciton delocalization ($J_{m,n}$) of symmetrically substituted squaraine dyes.

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