Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Electronic Supplementary Information

Determination of association constants and FRET in hydrazide-based molecular duplex strands

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General Information for spectroscopic measurements

Commercial $CHCl_3$ was first washed with deionized water three times and then dried over anhydrous Na_2SO_4. Fresh $CHCl_3$ was distilled from P_2O_5 before spectroscopic measurements. CH_3OH for spectroscopy measurement was distilled. UV-vis and FL spectra were recorded on an Olis DSM 172 spectrophotometer. Temperature was controlled using Northwest Quantum (Peltier controlled cell). 10 mm × 10 mm cuvettes were used. Usually data were collected at 1 nm intervals. The slitwidth was fixed at 1 mm and the integration time was set as 0.1 s. λ_{ex} = 345 nm was used to excite pyrene fluorophore and λ_{ex} = 418 nm for perylene fluorophore.

The dimerization constant was obtained by fitting the pyrene excimer intensities, using the following protocol. The excimer (λ_{max} = 474 nm) intensity was integrated from 470 nm to 600 nm to exclude as much of the monomer emission bands (λ_{max} = 378 nm and λ_{max} = 398 nm) as possible.[S1-3]

Model $\quad A + A \quad € \quad A_2$

$$K_{\dim} = \frac{[A_2]}{[A]^2} = \frac{[A_2]}{([A]_0 - 2[A_2])^2}$$

$$[A] = [A]_0 - 2[A_2]$$

$$[A_2] = \frac{4K_{\dim}[A]_0 + 1 - \sqrt{8K_{\dim}[A]_0 + 1}}{8K_{\dim}} \qquad \text{Eq. S1}$$

$$I_{excimer} = k[A_2] = k\frac{4K_{\dim}[A]_0 + 1 - \sqrt{8K_{\dim}[A]_0 + 1}}{8K_{\dim}}$$

$$\frac{I_{excimer}}{[A]_0} = k\frac{4K_{\dim}[A]_0 + 1 - \sqrt{8K_{\dim}[A]_0 + 1}}{8K_{\dim}[A]_0} \qquad \text{Eq. S2}$$

$[A]$: the concentration of free monomer

$[A]_0$: the total concentration

$[A_2]$: the concentration of molecular duplex strand

$K_{\dim}$: the dimerization constant

$I_{excimer}$: the intensity of the excimer band

k: constant

The determination of the association constant *via* titration of a non-fluorescent oligomers into a pyrene labelled oligogmer was based on the following protocol.

Model $A + A \rightleftarrows A_2$

$B + B \rightleftarrows B_2$

$A + B \rightleftarrows AB$

$$K_A = \frac{[A_2]}{[A]^2} \quad K_B = \frac{[B_2]}{[B]^2} \quad K_{AB} = \frac{[AB]}{[A][B]}$$

$$[A]_0 = [A] + 2[A_2] + [AB]$$

$$[B]_0 = [B] + 2[B_2] + [AB]$$

$$[B_2] = K_B[B]^2$$

$$2K_B[B]^2 + [B] + [AB] - [B]_0 = 0$$

$$[B] = \frac{-1 + \sqrt{1 + 8K_B([B]_0 - [AB])}}{4K_B}$$

$$\frac{[AB]}{[A]} = K_{AB}[B] = \frac{K_{AB}}{4K_B}\left(-1 + \sqrt{1 + 8K_B([B]_0 - [AB])}\right) \qquad \text{Eq. S3}$$

$$[A] = \sqrt{\frac{[A_2]}{K_A}} \quad [AB] = [A]_0 - [A] - 2[A_2] = [A]_0 - \sqrt{\frac{[A_2]}{K_A}} - 2[A_2] \quad \text{Eq. S4}$$

$[A]$: the monomer concentration of free pyrene labelled oligomer

$[A]_0$: the total concentration of pyrene labelled oligomer

$[A_2]$: the concentration of molecular duplex strand from pyrene labelled oligomer

$[B]$: the monomer concentration of non-fluorescent oligomer

$[B]_0$: the total concentration of non-fluorescent oligomer

$[B_2]$: the concentration of molecular duplex strand from non-fluorescent oligomer

K_A: the dimerization constant for pyrene labelled oligomer

K_B: the dimerization constant for non-fluorescent oligomer

K_{AB}: the association constant for heteroduplex strand

When $[B]_0 = 0$, calculate $[A_2]_0$ according to Eq. S1 and intergrate from 470 nm to 600 nm to get the corresponding $I_{excimer, 0}$

$[A_2]$ is proportional to the excimer intensity $I_{excimer}$

At a specific $[B]_0$, $[A_2] = [A_2]_0 \times \frac{I_{excimer}}{I_{excimer, 0}}$

According to Eq. S4, Calculate $[A]$, $[AB]$, $\frac{[AB]}{[A]}$, $[B]_0 - [AB]$

Fitting Eq. S3 to get K_B & K_{AB}

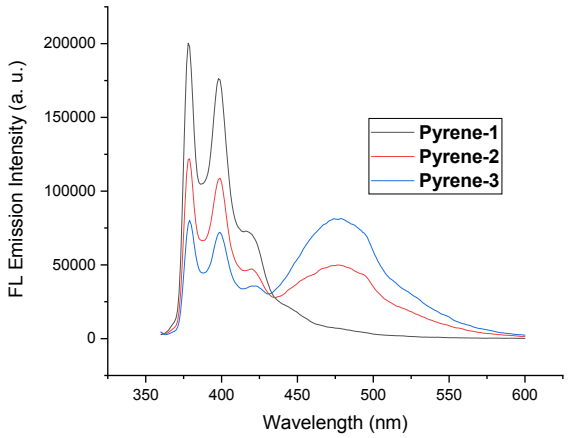


Figure S1 FL emission spectra for **Pyrene-1** ~ **Pyrene-3**, each 4×10^{-7} M in freshly distilled $CHCl_3$, 25 ℃, λ_{ex} = 345 nm.

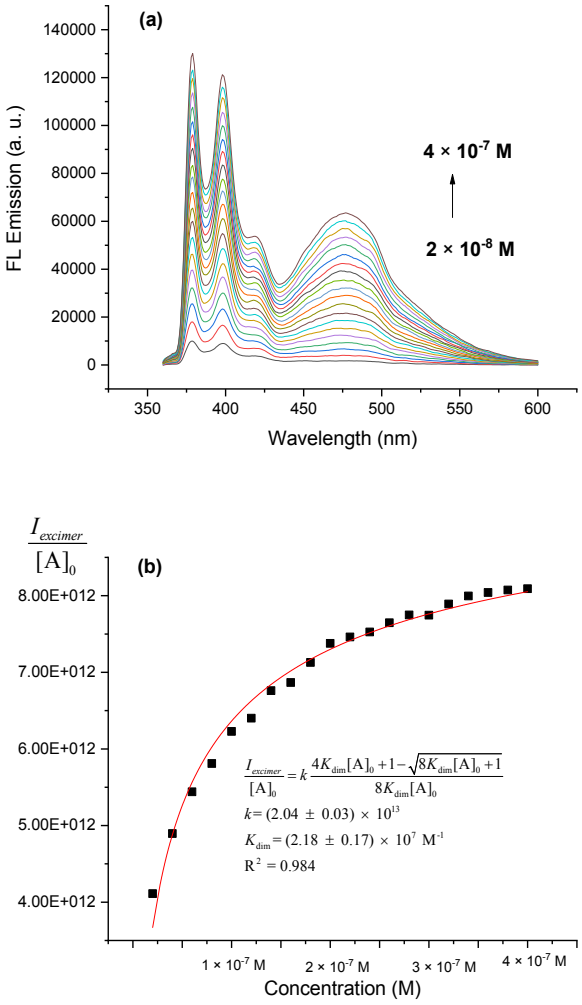


Figure S2 (a) Concentration dependent FL emission spectra for **Pyrene-2**, from 2×10^{-8} M to 4×10^{-7} M in freshly distilled CH_2Cl_2, 20 ℃, λ_{ex} = 345 nm. (b) Nonlinear fitting the excimer intensity data, yielding $K_{dim} = (2.18 \pm 0.17) \times 10^7$ M^{-1} for **Pyrene-2•Pyrene-2**.

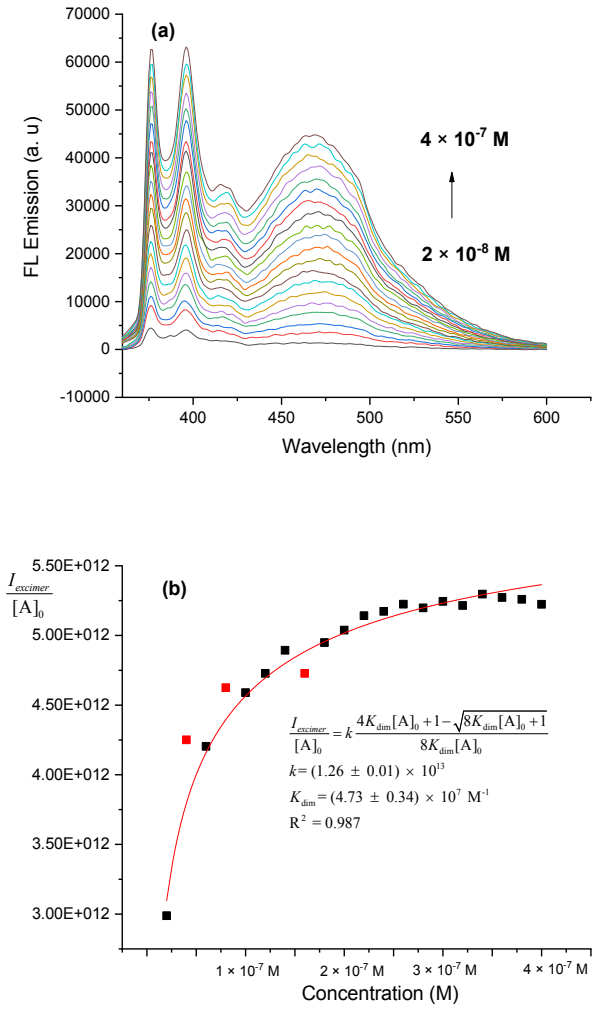


Figure S3 (a) Concentration dependent FL emission spectra for **Pyrene-2**, from 2×10^{-8} M to 4×10^{-7} M in freshly distilled toluene, 20 ℃, λ_{ex} = 345 nm. (b) Nonlinear fitting the excimer intensity data, yielding $K_{dim} = (4.73 \pm 0.34) \times 10^7$ M^{-1} for **Pyrene-2•Pyrene-2**.

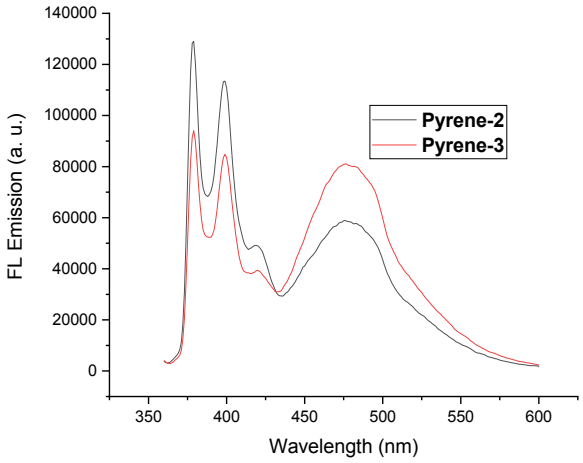


Figure S4 FL emission spectra for oligomer **Pyrene-2** and **Pyrene-3**, recorded on the same instrument conditions, each 4×10^{-7} M in freshly distilled $CHCl_3$, 20 ℃, λ_{ex} = 345 nm.

$$[A_2] = \frac{4K_{dim}[A]_0 + 1 - \sqrt{8K_{dim}[A]_0 + 1}}{8K_{dim}} \qquad \text{Eq. S1}$$

$K_{dim,\ Pyrene\text{-}2} = 9.48 \times 10^6\ M^{-1}$ $[Pyrene\text{-}2]_0 = 4 \times 10^{-7}\ M^{-1}$

$[Pyrene\text{-}2 \bullet Pyrene\text{-}2] = 1.393 \times 10^{-7}\ M$ $I_{excimer,\ Pyrene\text{-}2} = 3042220$

$[Pyrene\text{-}2] = 1.214 \times 10^{-7}\ M$

$I_{excimer,\ Pyrene\text{-}3} = 4222670$

$$[Pyrene\text{-}3 \bullet Pyrene\text{-}3] = \frac{4222670}{3042220} \times 1.393 \times 10^{-7} = 1.934 \times 10^{-7}\ M$$

$[Pyrene\text{-}3] = 1.32 \times 10^{-8}\ M$

$$K_{dim,\ Pyrene\text{-}3} = \frac{[Pyrene\text{-}3 \bullet Pyrene\text{-}3]}{[Pyrene\text{-}3]^2} = 1.11 \times 10^9\ M^{-1}$$

Figure S5 Determining the dimerization constant for **Pyrene-3•Pyrene-3** by comparing the excimer emission intensities of oligomers **Pyrene-2** and **Pyrene-3** on the same measurement conditions. The excimer emission band (λ_{max} = 474 nm) was integrated from 470 nm to 600 nm, to exclude as much of the monomer emission bands (λ_{max} = 378 nm and λ_{max} = 398 nm) as possible.

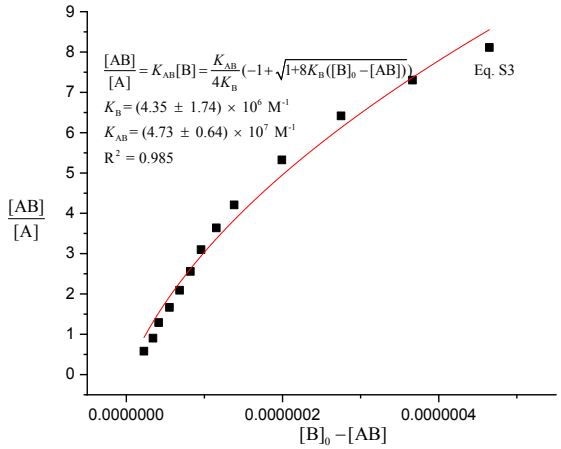
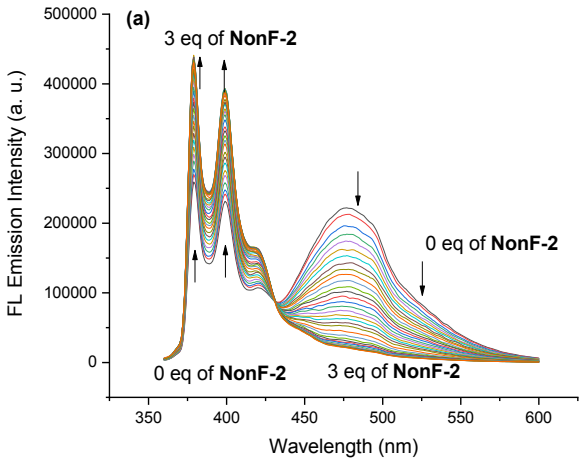
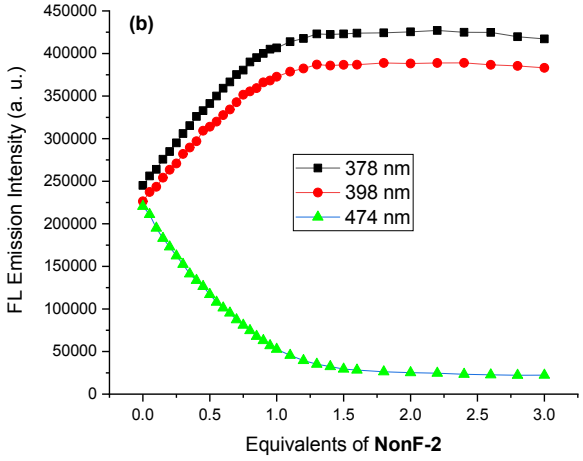


Figure S6 Fitting the excimer intensity data according to Eq. S3, yielding K_{dim} = (4.35 ± 1.74) × 10^6 M^{-1} for six-hydrogen-bonded homoduplex **NonF-1•NonF-1** and K_a = (4.73 ± 0.64) × 10^7 M^{-1} for eight-hydrogen-bonded heteroduplex **Pyrene-2•NonF-1**.





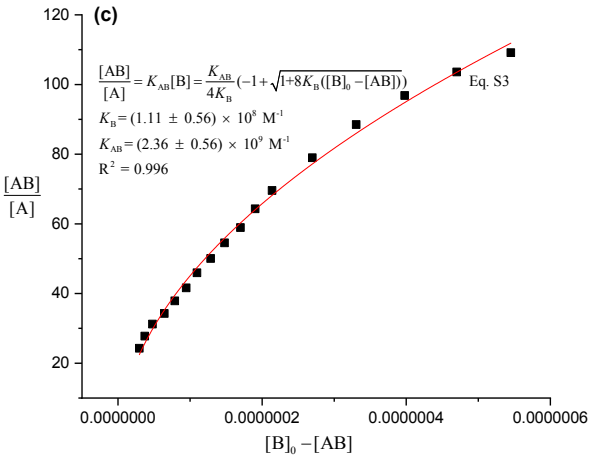


Figure S7 (a) FL emission spectra of **Pyrene-3**, 8×10^{-7} M in $CHCl_3$, 293 K, when titrated with a non-fluorescent oligomer **NonF-2** (0 ~ 3 equivalents), λ_{ex} = 345 nm, $\lambda_{isobestic}$ = 430 nm. (b) FL emission intensity changes for pyrene excimer emission (λ = 474 nm) and monomer emission (λ = 378 nm and λ = 398 nm) during titration. (c) Fitting the excimer intensity data according to Eq. S3, yielding $K_{dim} = (1.11 \pm 0.56) \times 10^8$ M^{-1} for ten-hydrogen-bonded homoduplex **NonF-2•NonF-2** and $K_a = (2.36 \pm 0.56) \times 10^9$ M^{-1} for twelve-hydrogen-bonded heteroduplex **Pyrene-3•NonF-2**.

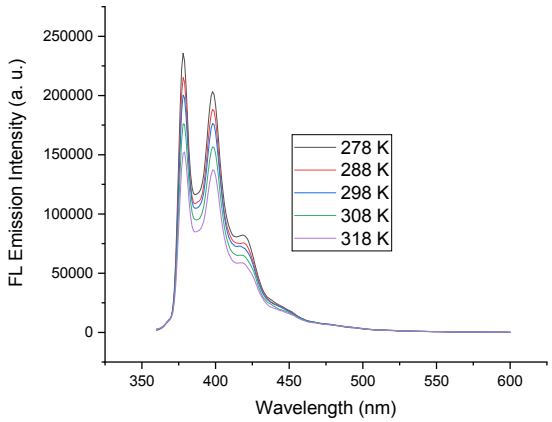


Figure S8 FL emission spectra for **Pyrene-1**, 4×10^{-7} M in freshly distilled $CHCl_3$, at different temperatures.

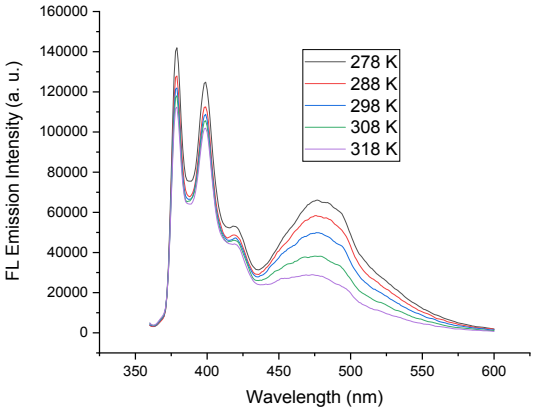


Figure S9 FL emission spectra for **Pyrene-2**, 4×10^{-7} M in freshly distilled $CHCl_3$, at different temperatures.

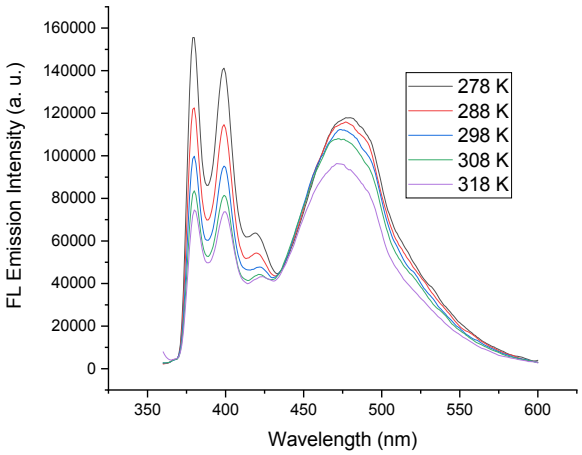


Figure S10 FL emission spectra for **Pyrene-3**, 4×10^{-7} M in freshly distilled $CHCl_3$, at different temperatures.

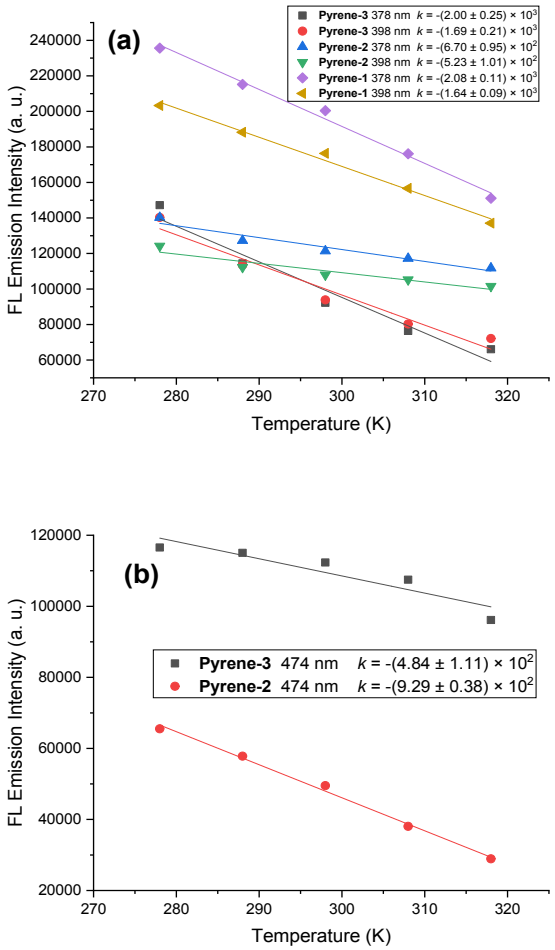


Figure S11 (a) Pyrene monomer emission (λ_{max} = 378 nm and λ_{max} = 398 nm) intensity decreased with increasing of temperature, with temperature coefficient indicated. (b) Pyrene excimer emission (λ_{max} = 474 nm) intensity changes with temperature, with temperature coefficient indicated.

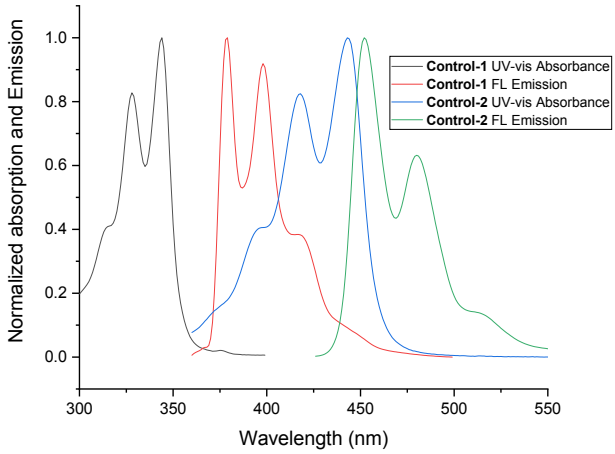


Figure S12 Normalized UV-vis absorption and FL emission spectra for pyrene (**Control-1**) and perylene (**Control-2**) labelled controls, 20 ℃. Conditions for UV-vis measurements: each 10^{-5} M in freshly distilled $CHCl_3$; Conditions for FL emission measurements: each 5×10^{-6} M in freshly distilled $CHCl_3$, λ_{ex} = 345 nm for **Control-1** and λ_{ex} = 418 nm for **Control-2**.

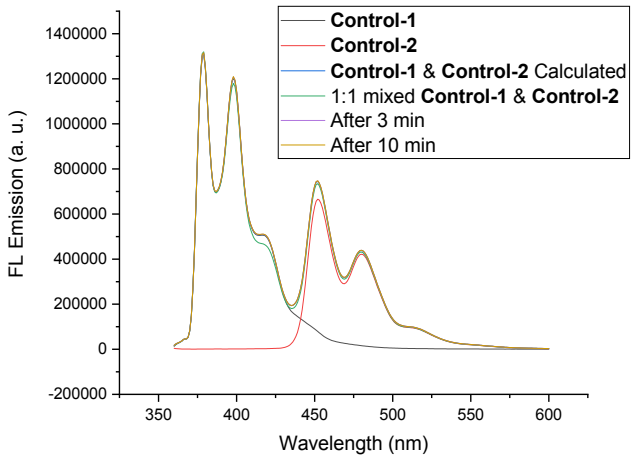


Figure S13 FL emission spectra for pyrene (**Control-1**) and perylene (**Control-2**) labelled controls, 20 ℃, each 5×10^{-6} M in freshly distilled $CHCl_3$, λ_{ex} = 345 nm.

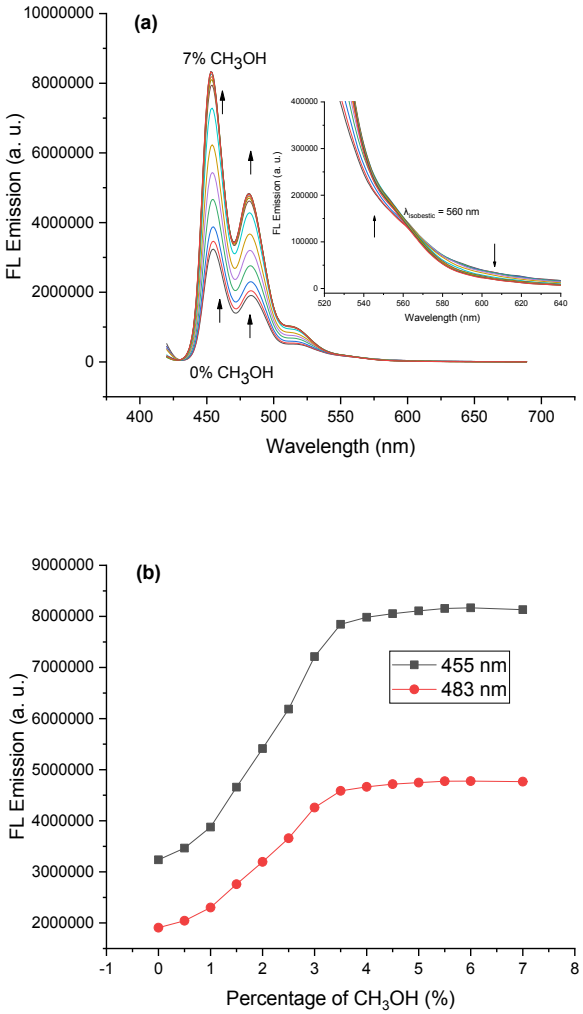


Figure S14 (a) FL emission spectra for **Perylene-1**, 2 μM in in freshly distilled $CHCl_3$, titrated with differnent percentage of CH_3OH, λ_{ex} = 418 nm, 20 ℃. The insert shows the expanded excimer emission band. (b) FL intensity changes for perylene monomer emission at λ_{max} = 455 nm and λ_{max} = 483 nm. 3.5% CH_3OH in $CHCl_3$ can recover the monomer emission completely.

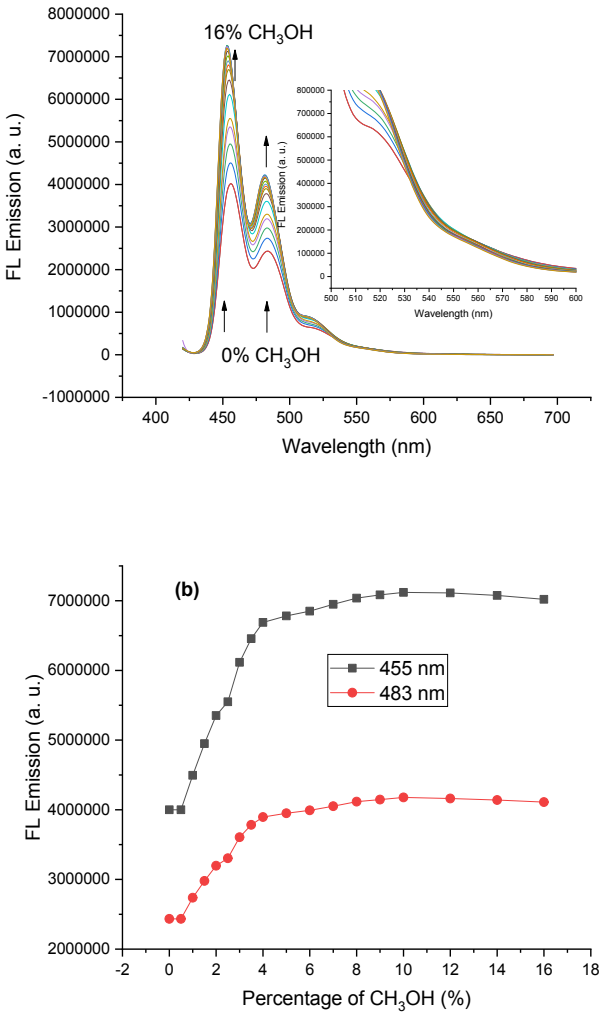


Figure S15 (a) FL emission spectra for **Perylene-2**, 2 μM in in freshly distilled $CHCl_3$, titrated with differnent percentage of CH_3OH, λ_{ex} = 418 nm, 20 ℃. The insert shows the expanded excimer emission band. (b) FL intensity changes for perylene monomer emission at λ_{max} = 455 nm and λ_{max} = 483 nm. 4% CH_3OH in $CHCl_3$ can recover the monomer emission completely.

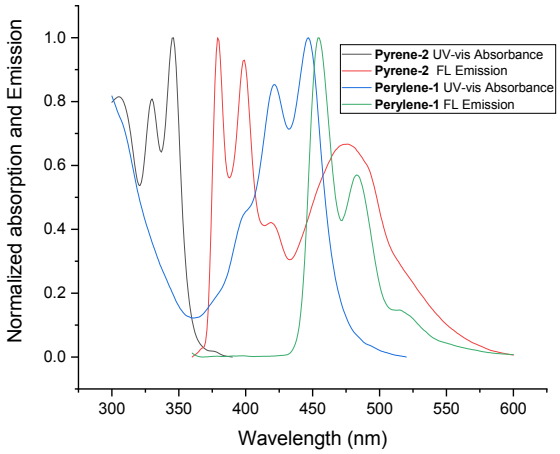


Figure S16 Normalized UV-vis absorption and FL emission spectra for **Pyrene-2** & **Perylene-1** FRET pair, 20 ℃. Conditions for UV-vis measurements: each 10^{-5} M in freshly distilled $CHCl_3$; Conditions for FL emission measurements: each 10^{-6} M in freshly distilled $CHCl_3$, λ_{ex} = 345 nm for **Pyrene-2** and λ_{ex} = 418 nm for **Perylene-1**.

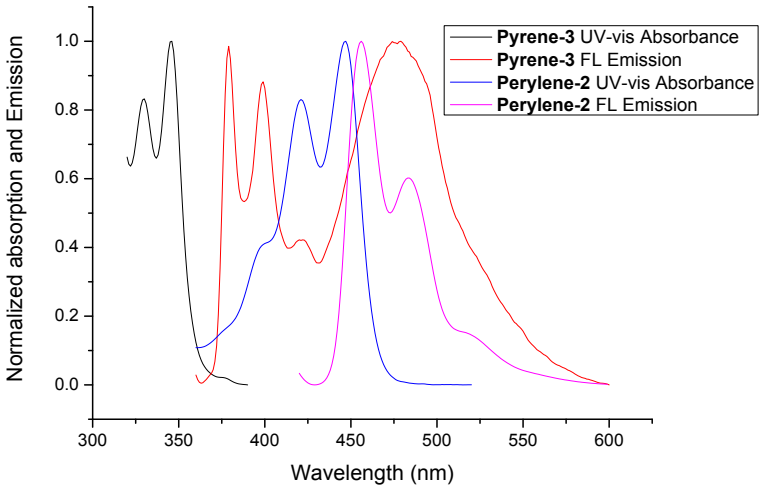


Figure S17 Normalized UV-vis absorption and FL emission spectra for **Pyrene-3** & **Perylene-2** FRET pair, 20 ℃. Conditions for UV-vis measurements: each 10^{-5} M in freshly distilled $CHCl_3$; Conditions for FL emission measurements: each 10^{-6} M in freshly distilled $CHCl_3$, λ_{ex} = 345 nm for **Pyrene-3** and λ_{ex} = 418 nm for **Perylene-2**.

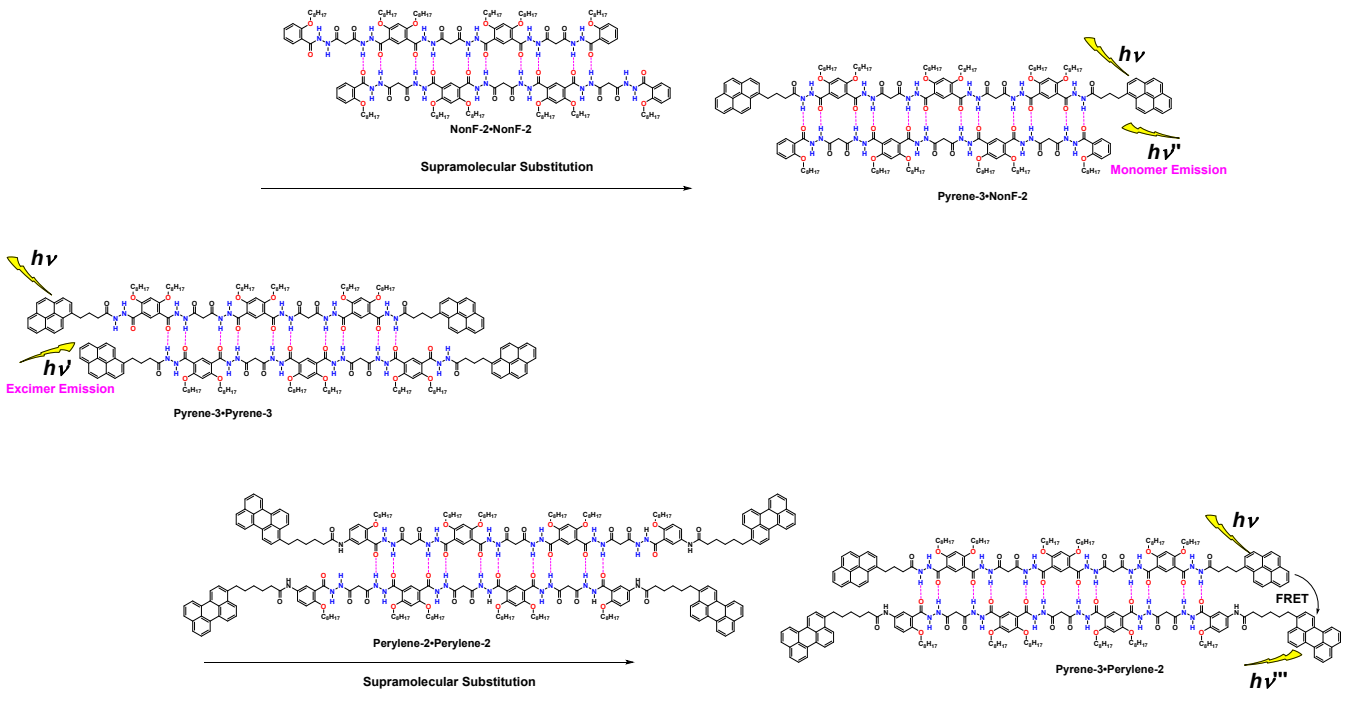
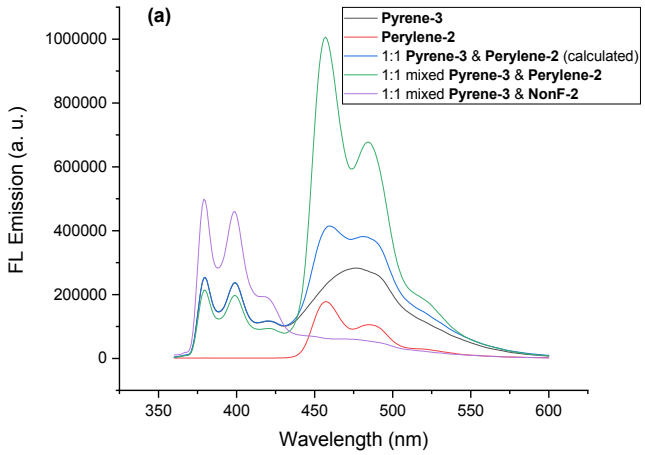
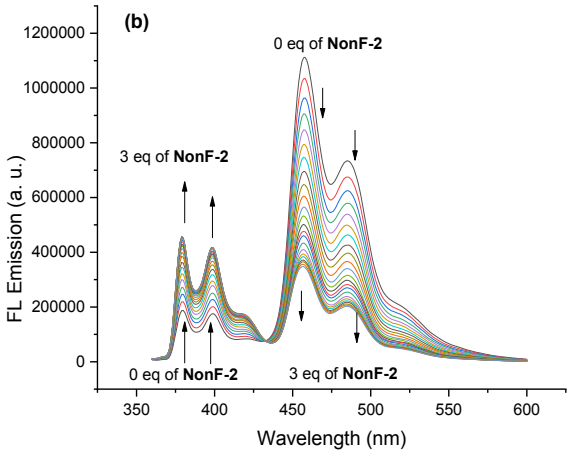


Figure S18 Representation of supramolecular substitution reactions from ten-hydrogen-bonded homoduplexes to twelve-hydrogen-bonded heteroduplexes between oligomers **Pyrene-3** & **NonF-2**, and **Pyrene-3** & **Perylene-2**, leading to excimer-monomer emission transition and FRET respectively.





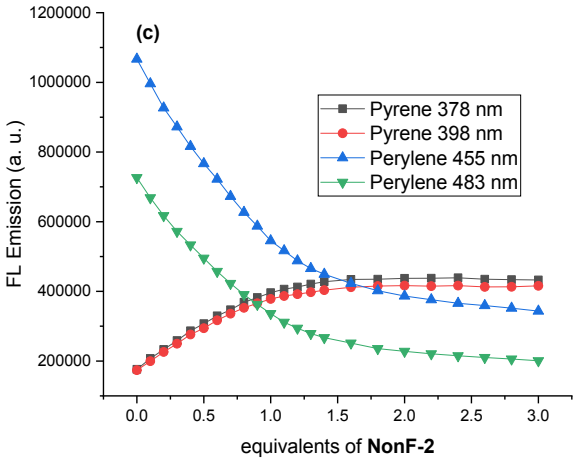
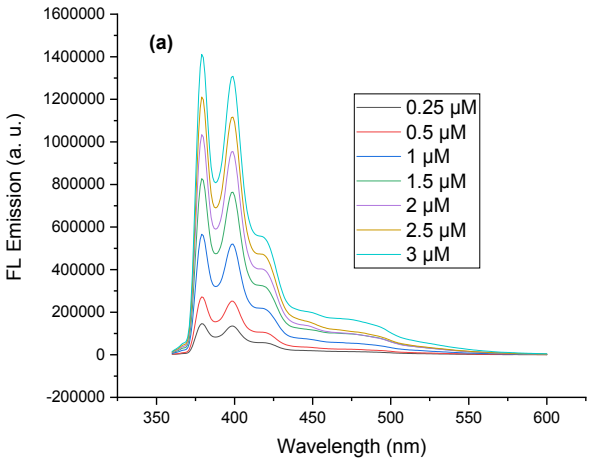
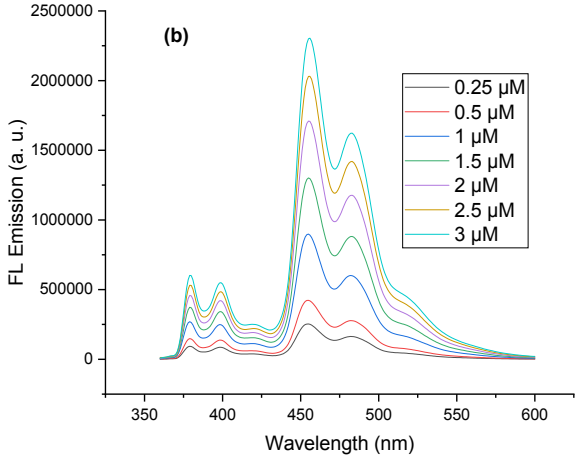


Figure S19 (a) FL emission spectra for **Pyrene-3**, **Perylene-2**, addition spectrum for **Pyrene-3** & **Perylene-2**, 1:1 mixture of **Pyrene-3** & **Perylene-2**, and 1:1 mixture of **Pyrene-3** & **NonF-2**, each 1 μM in $CHCl_3$, 293 K, λ_{ex} = 345 nm. (b) FL emission spectra for 1:1 mixture of **Pyrene-3** & **Perylene-2**, titrated with a nonfluorescent oligomer **NonF-2** (0 ~ 3 equivalents), λ_{ex} = 345 nm. (c) Increase of pyrene monomer emission (378 nm and 398 nm) and decrease of perylene monomer emission with addition of **NonF-2** (455 nm and 483 nm), $\lambda_{isobestic}$ = 432 nm.





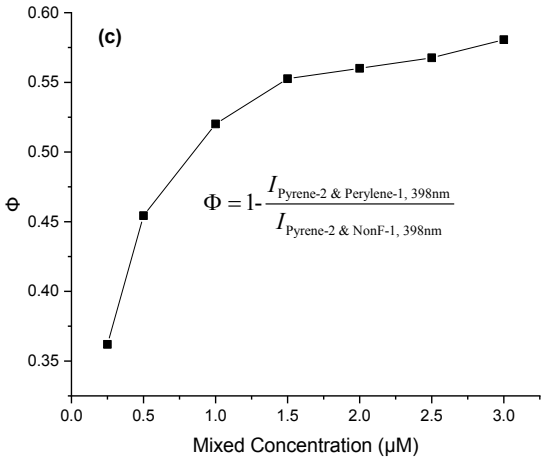
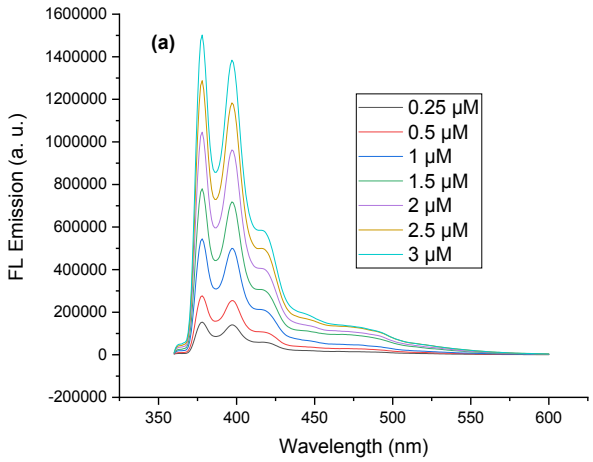
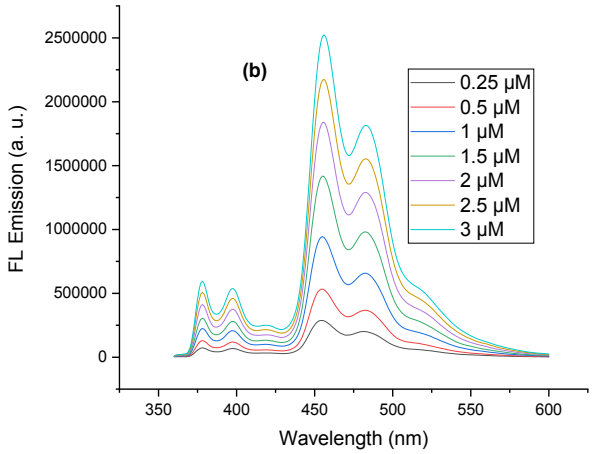


Figure S20 (a) FL emission spectra for 1:1 mixed **Pyrene-2** & **NonF-1**, from 0.25 μM to 3 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm, 20 ℃. (b) FL emission spectra for 1:1 mixed **Pyrene-2** & **Perylene-1**, from 0.25 μM to 3 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm, 20 ℃. (c) Energy transfer efficiency Φ between **Pyrene-2** & **Perylene-1** *vs* mixed concentration. Φ was measured as the fluorescence intensity change at λ = 398 nm.





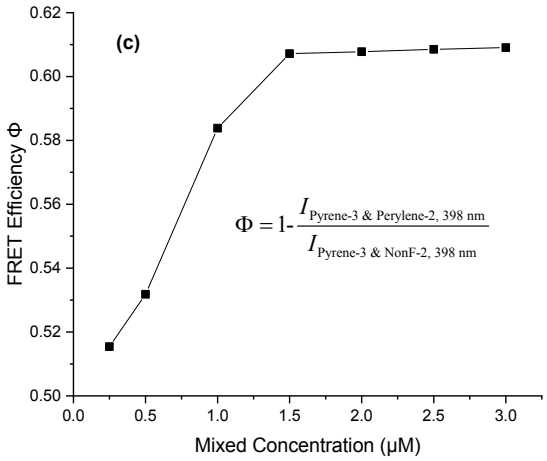


Figure S21 (a) FL emission spectra for 1:1 mixed **Pyrene-3** & **NonF-2**, from 0.25 μM to 3 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm, 20 ℃. (b) FL emission spectra for 1:1 mixed **Pyrene-3** & **Perylene-2**, from 0.25 μM to 3 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm, 20 ℃. (c) Energy transfer efficiency Φ between **Pyrene-3** & **Perylene-2** *vs* mixed concentration. Φ was measured as the fluorescence intensity change at λ = 398 nm.

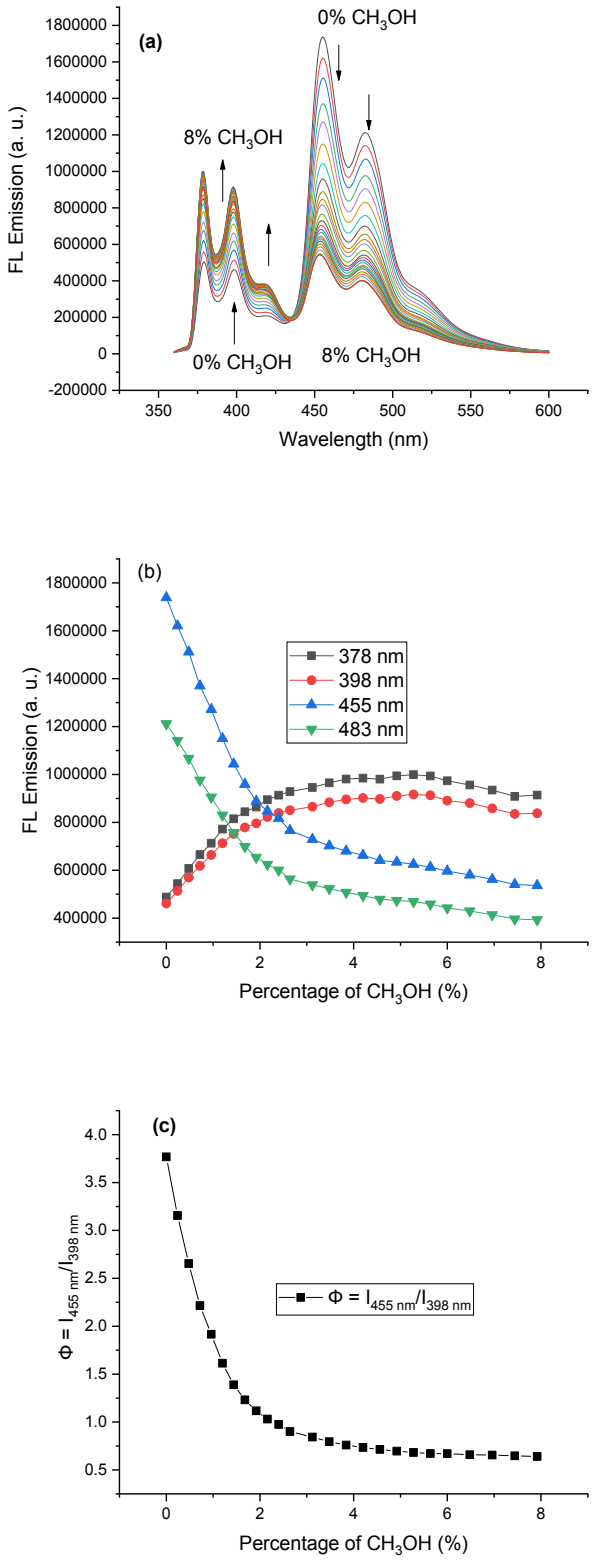


Figure S22 (a) FL emission spectra for **Pyrene-2** & **Perylene-1**, 2 μM in in freshly distilled $CHCl_3$, titrated with differnent percentage of CH_3OH, λ_{ex} = 345 nm, 20 ℃, $\lambda_{isobestic}$ = 435 nm. (b) FL intensity changes for pyrene monomer emission (λ_{max} = 378 nm and λ_{max} = 398 nm) and perylene monomer emission (λ_{max} = 455 nm and λ_{max} = 483 nm). (c) Energy transfer efficiency Φ between **Pyrene-2** and **Perylene-1**, expressed as the ratio of fluorescence intensities at λ = 455 nm (acceptor) and λ = 398 nm (donor). 3% CH_3OH in $CHCl_3$ can destroy the FRET process almost completely.

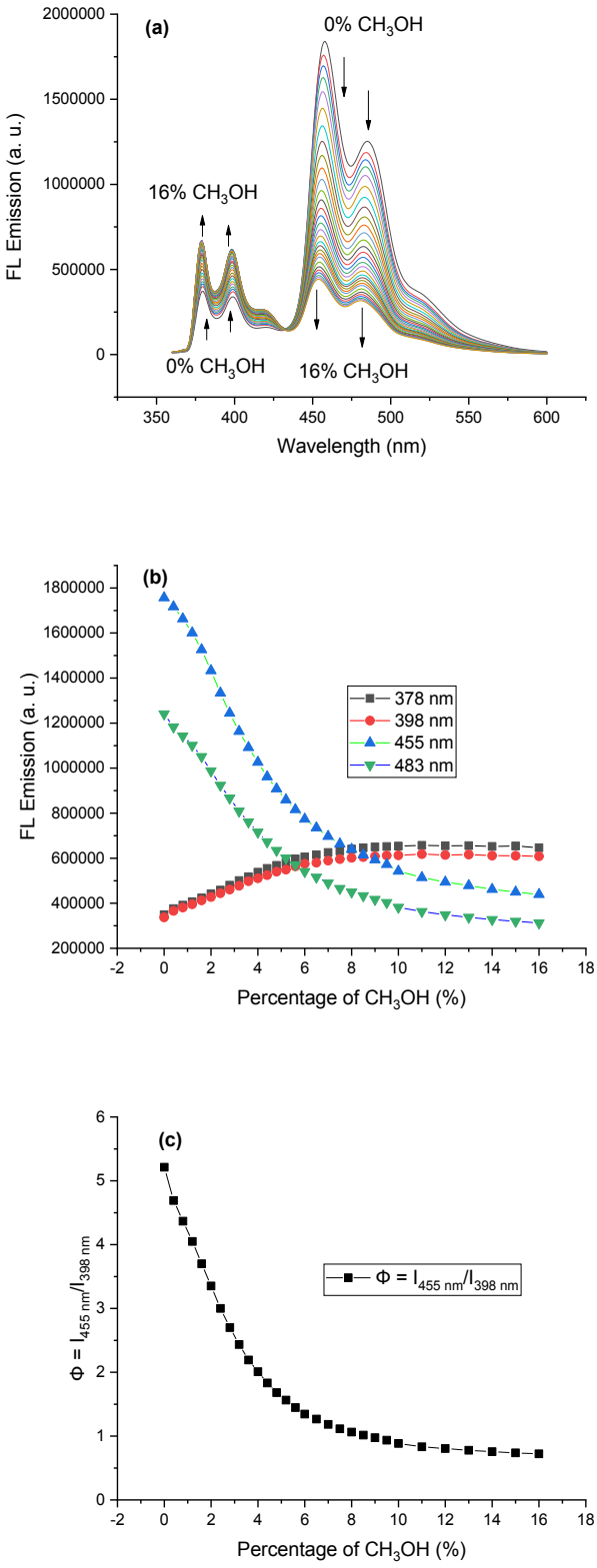
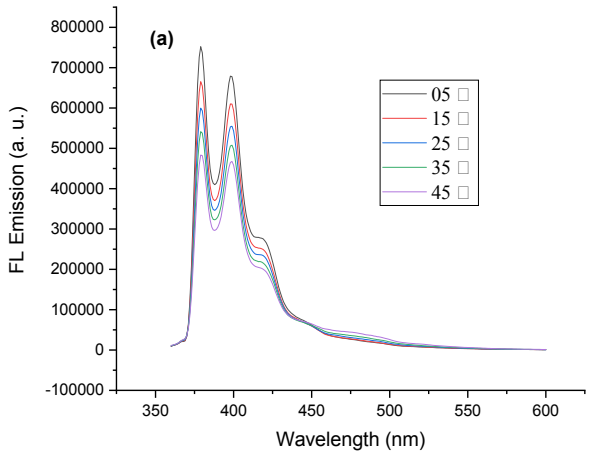
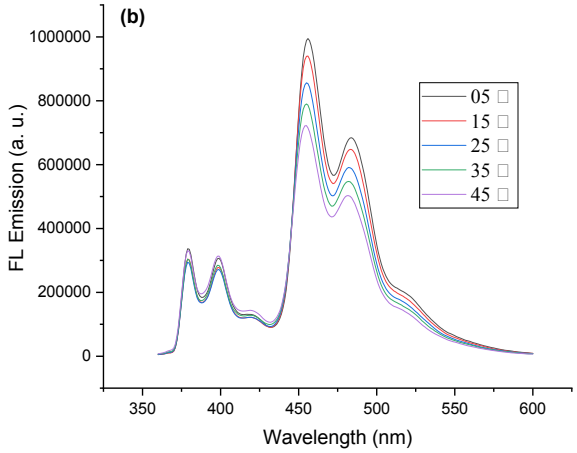


Figure S23 (a) FL emission spectra for **Pyrene-3** & **Perylene-2**, 2 μM in in freshly distilled $CHCl_3$, titrated with differnent percentage of CH_3OH, λ_{ex} = 345 nm, 20 ℃, $\lambda_{isobestic}$ = 435 nm. (b) FL intensity changes for pyrene monomer emission (λ_{max} = 378 nm and λ_{max} = 398 nm) and perylene monomer emission (λ_{max} = 455 nm and λ_{max} = 483 nm). (c) Energy transfer efficiency Φ between **Pyrene-2** and **Perylene-1**, expressed as the ratio of fluorescence intensities at λ = 455 nm (acceptor) and λ = 398 nm (donor). 6% CH_3OH in $CHCl_3$ can destroy the FRET process almost completely.





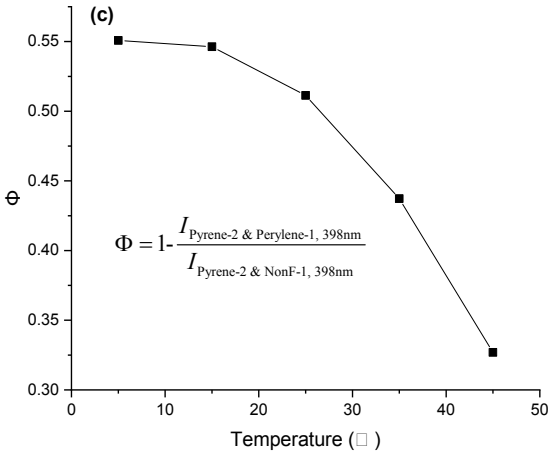


Figure S24 (a) Temperature dependent FL emission spectra for 1:1 mixed **Pyrene-2** & **NonF-1**, from 5 ℃ to 45 ℃, 1 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm. (b) Temperature dependent FL emission spectra for 1:1 mixed **Pyrene-2** & **Perylene-1**, from 5 ℃ to 45 ℃, 1 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm. (c) Energy transfer efficiency Φ between **Pyrene-2** & **Perylene-1** *vs* temperature. Φ was measured as the fluorescence intensity change at λ = 398 nm.

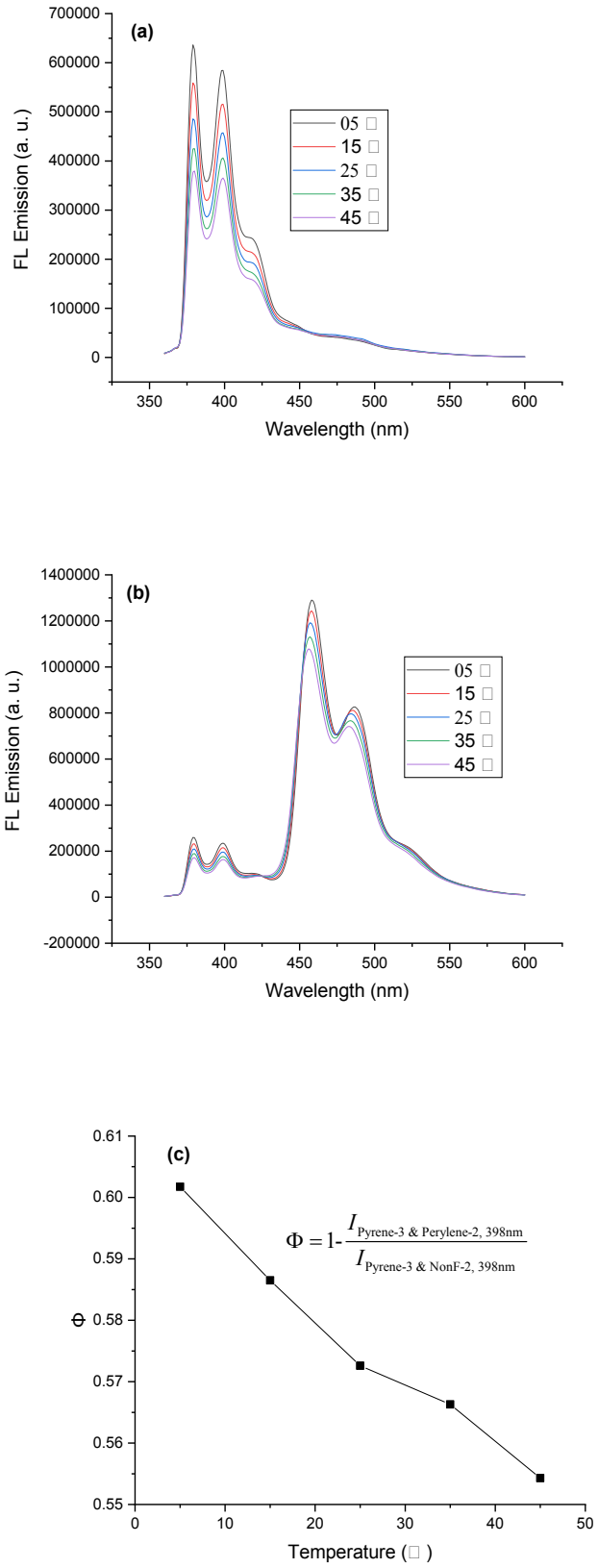


Figure S25 (a) Temperature dependent FL emission spectra for 1:1 mixed **Pyrene-3** & **NonF-2**, from 5 ℃ to 45 ℃, 1 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm. (b) Temperature dependent FL emission spectra for 1:1 mixed **Pyrene-3** & **Perylene-2**, from 5 ℃ to 45 ℃, 1 μM in freshly distilled $CHCl_3$, λ_{ex} = 345 nm. (c) Energy transfer efficiency Φ between **Pyrene-3** & **Perylene-2** *vs* temperature. Φ was measured as the fluorescence intensity change at λ = 398 nm.

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