Supporting Information

Tailoring the Luminescence of FRET Systems Built by Supramolecular Polymeric Nanotubes

Qiao Song[*a,b], Jingyu Zhang,[a] Xinxin Yu,[a] Zihe Cheng,[b] Jie Yang,[b,c] Stephen C. L. Hall,[b] Sébastien Perrier[*b, d, e]

a. Shenzhen Grubbs Institute, Southern University of Science and Technology, Shenzhen 518055, China. E-mail: songq@sustech.edu.cn
b. Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK. E-mail: s.perrier@warwick.ac.uk
c. College of Science, Nanjing Forestry University, Nanjing 210037, China.
d. Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK.
e. Faculty of Pharmacy and Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia

S1. Materials and Characterization

Materials

Fmoc-protected amino acids and coupling agents were purchased from Iris Biotech GmbH. α-Methoxy-ω-amino PEG (CH_3O-PEG-NH_2, M_n=5000 g mol^{-1}) and α-methoxy-ω-NHS ester PEG (CH_3O-PEG-NHS, M_n=5000 g mol^{-1}) were purchased from Rapp Polymere GmbH. Cyanine5 NHS ester (Cy5-NHS) was purchased from Lumiprobe GmbH. (1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl *N*-succinimidyl carbonate (BCN-NHS) and other chemicals were purchased from Sigma-Aldrich. Solvents were purchased from several departmental suppliers, Honeywell, Fisher and Sigma-Aldrich.

Characterization

Nuclear Magnetic Resonance Spectroscopy (NMR): ^{1}H NMR spectra were measured using a Bruker Avance III HD 400 MHz NMR spectrometer. The residual solvent peaks were used as internal references.

Gel Permeation Chromatography (GPC): GPCs were measured using Agilent Infinity II MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and variable wavelength UV detectors. The system was equipped with two PLgel Mixed D columns (300 × 7.5 mm) and a PLgel 5 μm guard column. The eluent used was DMF with 5 mmol NH_4BF_4 additive. Samples were run at 1mL/min at 50 °C. The samples were prepared by filtering them through 0.45 μm pore size PTFE membranes before injection. Agilent EasyVial poly(methyl methacrylate) standards were used to calibrate the instrument and output data were analysed using Agilent GPC/SEC software..

High-Performance Liquid Chromatography (HPLC): High-performance liquid chromatograms were measured using a Shimadzu Prominence HPLC, equipped with an Agilent Eclipse Plus C18 column (100 mm × 4.6 mm) with 3.5 μm micron packing. Water and acetonitrile were used as mobile phase A and B, respectively. All solvents contained 0.04 vol% TFA. The gradient used for HPLC analysis was increased from 5% to 95% B in 30 minutes. Detection was achieved *via* monitoring UV absorption at different wavelengths. Samples were dissolved in mobile phase A with concentration of 0.5 mg mL^{-1} and the injection volume was 20 μL.

Mass Spectrometry (ESI-TOF): ESI-TOF mass spectra were measured using an Agilent 6130B single Quad to characterize the peptides in both positive and negative ionisation modes. Samples were dissolved in methanol.

Ultraviolet–Visible (UV–Vis) Absorption Spectroscopy: UV–vis absorption spectra were measured using either an Agilent Technologies Cary 60 UV–vis spectrometer or a Shimadzu UV-2600i UV–vis spectrometer. The path length of the cuvette is 10 mm.

Fluorescence Emission Spectroscopy: Fluorescence emission spectra were measured using either an Agilent Technologies Cary Eclipse Fluorescence spectrometer or an Edinburgh FLS1000 Photoluminescence Spectrometer.

Time-resolved Fluorescence Spectroscopy: Fluorescence lifetime measurements were performed using the Fluorolog®-3 Spectrofluorometer (Horiba, Japan). Measurements were performed using NanoLEDs as pulsed light sources for fluorescence lifetime as fast as 20 ns. An excitation wavelength of 345 nm (318 nm NanoLED) was used, and the emitted fluorescence was monitored at 460 nm.

Small Angle Neutron Scattering (SANS): SANS was carried out on Larmor at the ISIS Pulsed Neutron Source (STFC Rutherford Appleton Laboratory, Didcot, UK). Prior to measurement, each sample was dissolved in deuterated solvent and placed in a 2 mm quartz cuvette. The scattering cross-section was measured over a Q-range of 0.004 - 0.5 $Å^{-1}$ where Q is defined as:

$$Q = \frac{4\pi sin\frac{\theta}{2}}{\lambda}$$

Here, θ is the scattered angle, and λ is the incident neutron wavelength.

A Q-range of 0.004 - 0.5 $Å^{-1}$ was achieved utilizing an incident wavelength range of 0.9 - 13.3 Å. The detector is located 4.1 m from the sample and is 664 mm wide × 664 mm high with the beam in the centre of the detector. The beam size is 6 mm wide and 8 mm high. Each raw scattering data set was corrected for the detector efficiencies, sample transmission and background scattering and converted to scattering cross-

section data ($\partial\Sigma/\partial\Omega$ vs. Q) using the instrument-specific software. These data were placed on an absolute scale (cm^{-1}) using the scattering from a standard sample (a solid blend of hydrogenous and perdeuterated polystyrene) in accordance with established procedures.

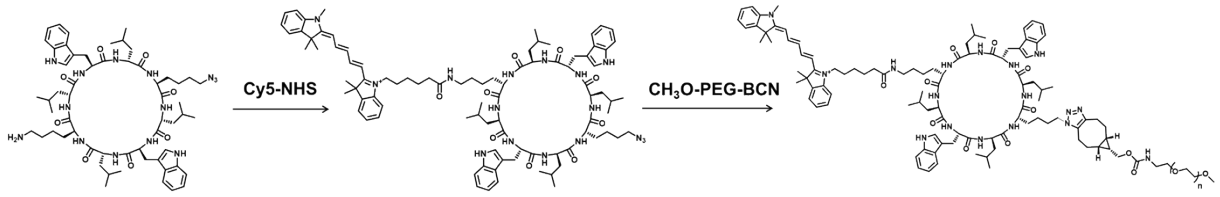
Transmission Electron Microscopy (TEM): 10 μL of the conjugate aqueous solution was drop-casted on the carbon-coated grid. After 3 min, the solution on the grid was absorbed with filter paper. After 15 min, 10 μL of a 0.2% uranyl acetate solution was dropped onto the grid and absorbed after 30 s. Bright field TEM micrographs were obtained with a JEOL 2100Plus microscope operating at 200 kV, equipped with a Gatan OneView IS camera.

S2. Synthesis

1. H_2N-CP-N_3, CH_3O-PEG-BCN, NTI-CP-PEG and Cy3-CP-PEG

H_2N-CP-N_3, CH_3O-PEG-BCN, NTI-CP-PEG, and Cy3-CP-PEG were synthesized according to a previously reported procedure.[1]

2. Cy5-CP-PEG



a. Cy5-CP-N_3

H_2N-CP-N_3 (10.0 mg, 0.009 mmol) and Cy5-NHS (8.7 mg, 0.0135 mmol) were dissolved in 0.7 mL DMF, with the addition of NMM (2.7 mg, 0.027 mmol). The reaction was left for 48 h. The DMF solution was then precipitated in THF and washed twice to obtain **Cy5-CP-N_3** (yield: 12.5 mg).

MS (ESI-ToF) (m/z): $[M]^+$ 1546.8 (calculated: 1545.9).

b. Cy5-CP-PEG

Cy5-CP-N_3 (12.0 mg, 0.0073 mmol) and CH_3O-PEG-BCN (57 mg, 0.0110 mmol) were dissolved in 1 mL DMF. The reaction was left for 3 days. Then the DMF solution was precipitated in cold diethyl ether. The precipitate was collected using centrifugation and dried under N_2. The resulting solid was then redissolved in 2 mL DCM and 10 mL diethyl ether was added dropwise to obtain precipitate. This process was repeated twice. Finally, **Cy5-CP-PEG** was obtained by drying under vacuum as a blue solid (yield: 44 mg).

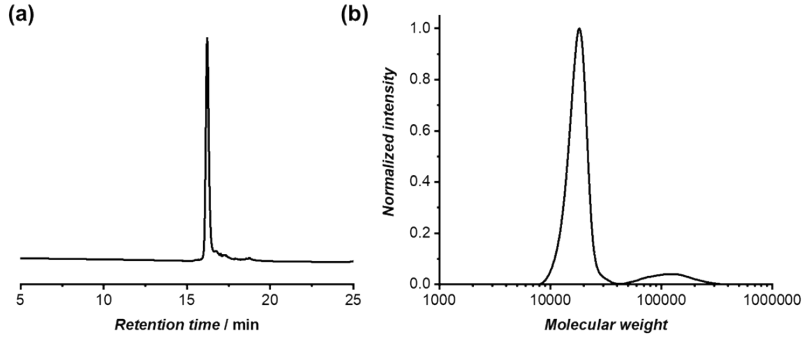
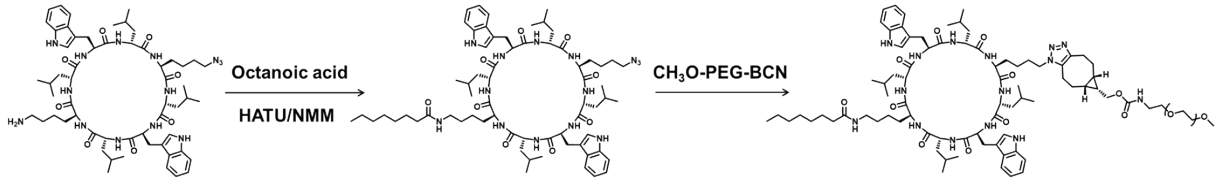


Figure S1 (a) HPLC spectrum of Cy5-CP-PEG monitored by a UV detector at 641 nm; (b) GPC trace of Cy5-CP-PEG.

3. Oct-CP-PEG



a. Oct-CP-N_3

H_2N-CP-N_3 3 (20.0 mg, 0.018 mmol) and octanoic acid (5.2 mg, 0.036 mmol) were dissolved in 1 mL DMF, with the addition of HATU (13.7 mg, 0.036 mmol) and NMM (5.5 mg, 0.054 mmol). The reaction was left for 72 h. The DMF solution was then precipitated in cold diethyl ether and washed twice to obtain **Oct-CP-N_3** (yield: 12.5 mg).

MS (ESI-ToF) (m/z): $[M+Na]^+$ 1256.8 (calculated: 1555.8).

b. Oct-CP-PEG

Oct-CP-N_3 (10.2 mg, 0.0082 mmol) and CH_3O-PEG-BCN (64 mg, 0.0124 mmol) were dissolved in 1 mL DMF. The reaction was left for 48 h. Then the DMF solution was precipitated in cold diethyl ether. The precipitate was collected using centrifugation and dried under N_2. The resulting solid was then redissolved in 1.5 mL DCM and 8.25 mL diethyl ether was added dropwise to obtain precipitate. This process was repeated twice. Finally, **Oct-CP-PEG** was obtained by drying under vacuum as a white solid (yield: 38 mg).

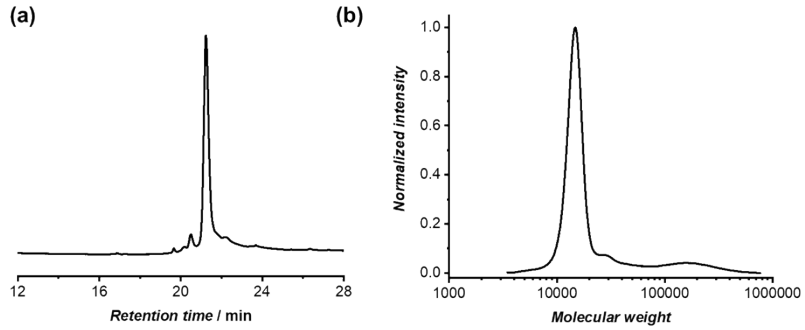


Figure S2 (a) HPLC spectrum of Oct-CP-PEG monitored by a UV detector at 280 nm; (b) GPC trace of Oct-CP-PEG.

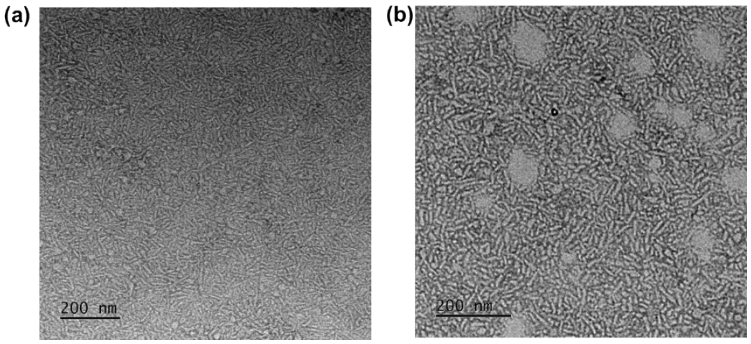
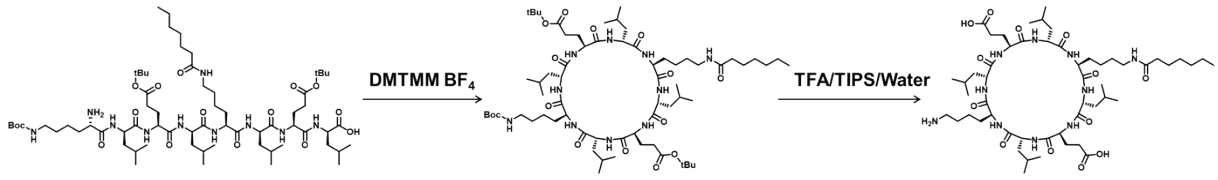


Figure S3 TEM images of Cy5-CP-PEG (a) and Oct-CP-PEG (b).

4. Hep-CP-NH_2



Linear peptide

H_2N-L-Lys(Boc)-D-Leu-L-Glu(OtBu)-D-Leu-L-Lys(Heptanoyl)-D-Leu-L-Glu(OtBu)-D-Leu-COOH

Fully protected linear octapeptide was prepared *via* solid phase peptide synthesis (SPPS) on a Prelude Automated Peptide SynthesizerTM (Protein Technologies Inc.) using 2-chlorotrityl chloride resin as the solid support. The first Fmoc protected amino acid was coupled to the resin using DIPEA (4 eq.) in DMF, followed by capping of unreacted resin sites using a solution of MeOH:DIPEA:DCM (7:1:2, *v*/*v*/*v*). Deprotection of the Fmoc group of the amino acids was done using 20% piperidine in DMF. Subsequent amino acids were coupled using Fmoc-amino acids (5 eq.), HCTU (5 eq.) and NMM (10 eq.) in DMF. In the last step, the linear octapeptide was cleaved from the resin (while keeping protecting groups on) by a

solution of 20 vol % 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in DCM. The linear peptide was obtained by evaporating solvent under reduced pressure.

MS (ESI-ToF) (m/z): $[M+H]^+$ 1309.8 (calculated: 1309.9), $[M+Na]^+$ 1331.8 (calculated: 1331.9).

Protected cyclic peptide

Linear peptide (200 mg, 0.153 mmol) was cyclized by stirring at room temperature for 5 days in the presence of 1.2 equivalents of DMTMM·BF_4 (60 mg, 0.183 mmol) in 50 mL DMF under the protection of N_2. The solution was then concentrated to 5 mL under reduced pressure and then precipitated with cold methanol/water=1/1 to obtain a white powder as protected cyclic peptide (yield: 120 mg).

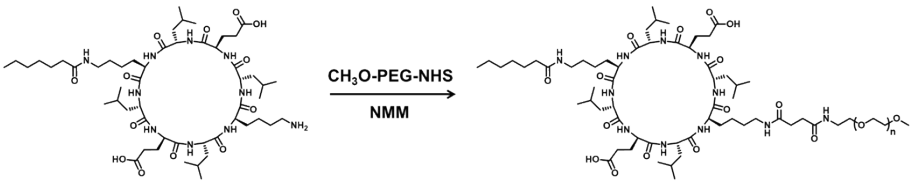
MS (ESI-ToF) (m/z): $[M+Na]^+$ 1313.8 (calculated: 1313.9).

Deprotected cyclic peptide Hep-CP-NH_2

Removal of the -Boc and -OtBu protecting groups was achieved by adding a mixture of trifluoroacetic acid (TFA, 1.8 mL), triisopropylsilane (TIPS, 0.1 mL) and water (0.1 mL) to the protected cyclic peptide (100 mg) and stirring for 3 hours. The resulting solution was then precipitated in ice cold diethyl ether and washed twice with ice cold diethyl ether to give an off-white powder as the deprotected cyclic peptide **Hep-CP-NH_2** (yield: 85 mg).

MS (ESI-ToF) (m/z): $[M+H]^+$ 1079.7 (calculated: 1079.7).

5. Hep-CP-PEG



Hep-CP-NH_2 (12 mg, 0.0111 mmol) and CH_3O-PEG-NHS (84 mg, 0.0168 mmol) were dissolved in 1 mL DMF, followed by the addition of NMM (3.4 mg, 0.0336 mmol). The reaction was left for 3 days. Then the DMF solution was precipitated in cold diethyl ether. The precipitate was collected using centrifugation and dried under N_2. The resulting solid was then redissolved in 2 mL DCM and 10 mL diethyl ether was added dropwise to obtain precipitate. This process was repeated twice. Finally, **Hep-CP-PEG** was obtained by drying under vacuum as a white solid (yield: 44 mg).

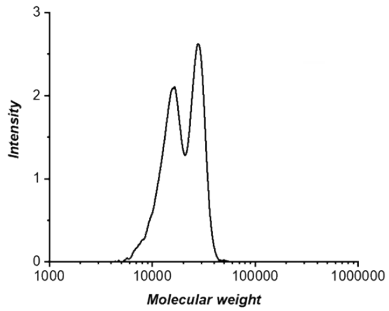


Figure S4 GPC trace of Hep-CP-PEG (the peak at higher molecular weight is most likely caused by the aggregation of conjugate).

S3. FRET between PYR-CP-PEG and NTI-CP-PEG, Cy3-CP-PEG, or Cy5-CP-PEG

1. Sample Preparation and Measurement

The co-assembly of the conjugates was realized by firstly premixing PYR-CP-PEG with NTI-CP-PEG, Cy3-CP-PEG, or Cy5-CP-PEG with certain molar ratios in a small amount of DMSO, followed by the addition of DI water, resulting clear solutions with DMSO/H_2O ratio of 5/95. PYR-CP-PEG concentration was fixed at 10 μM (in case of time-resolved fluorescence spectroscopy, 25 μM was used for better signals). Emission spectra were measured using excitation wavelength of 345 nm.

2. Time-resolved Fluorescence Spectroscopy

Table S1 Fluorescence lifetimes of PYR-CP-PEG, PYR-CP-PEG/NTI-CP-PEG, PYR-CP-PEG/Cy3-CP-PEG, and PYR-CP-PEG/Cy5-CP-PEG assemblies in aqueous solutions.

	τ_1/ns	τ_2/ns	τ_3/ns	α_1/%	α_2/%	α_3/%	τ_{ave}	χ^2	Φ_{ET}/%
PYR-CP-PEG	4.6	32.7	80.7	60.6	20.5	19.0	24.8	1.38	-
PYR-CP-PEG/6%NTI-CP-PEG	2.6	16.3	60.7	65.7	28.0	6.3	10.1	1.34	85.7
PYR-CP-PEG/10%Cy3-CP-PEG	1.3	8.6	36.8	78.6	18.5	2.9	3.7	1.25	95.8
PYR-CP-PEG/8%Cy5-CP-PEG	2.4	13.7	51.3	73.2	21.7	5.1	7.3	1.24	85.4

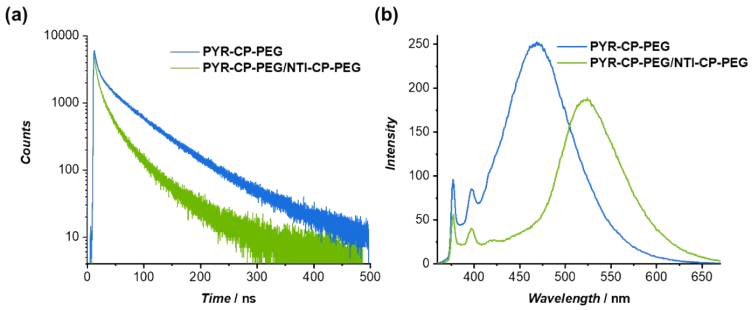


Figure S5 Fluorescence decay profiles of PYR-CP-PEG and PYR-CP-PEG/NTI-CP-PEG.

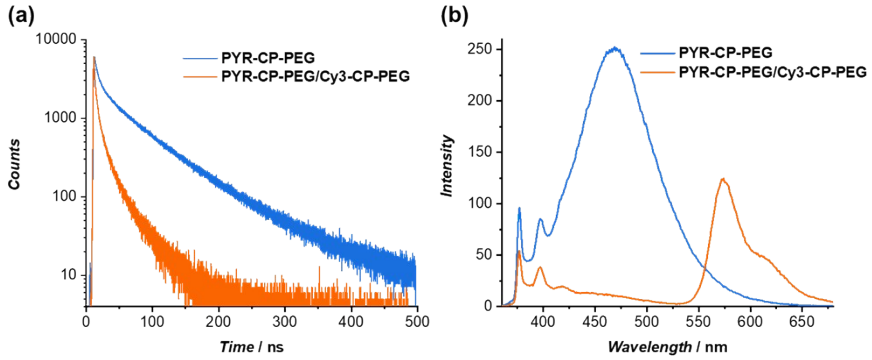


Figure S6 Fluorescence decay profiles of PYR-CP-PEG and PYR-CP-PEG/Cy3-CP-PEG.

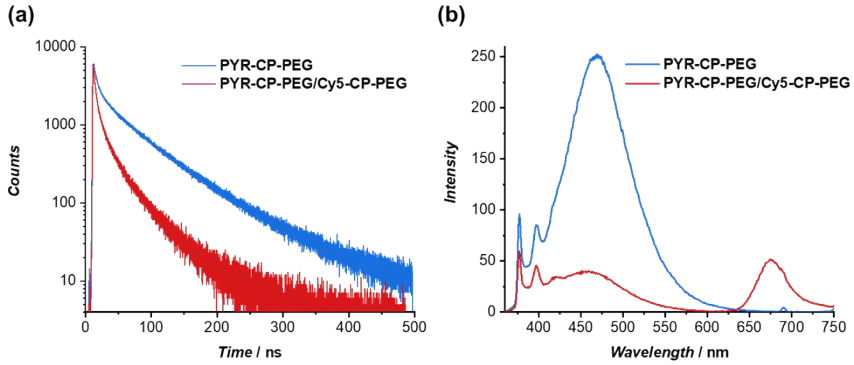


Figure S7 Fluorescence decay profiles of PYR-CP-PEG and PYR-CP-PEG/Cy5-CP-PEG.

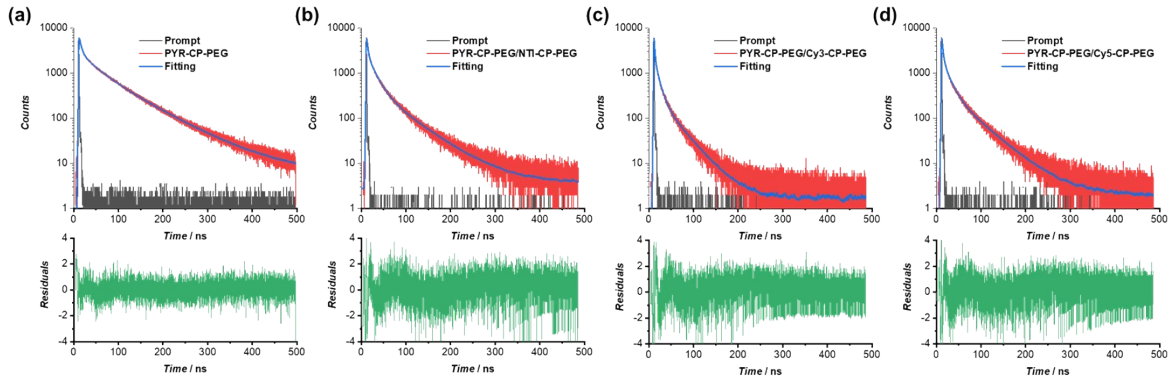


Figure S8 Fluorescence decay profiles of (a) PYR-CP-PEG, (b) PYR-CP-PEG/NTI-CP-PEG, (c) PYR-CP-PEG/Cy3-CP-PEG=100/8, and (d) PYR-CP-PEG/Cy5-CP-PEG and the corresponding fitting curves using a three-exponential decay model.

3. Energy Transfer Efficiency (Φ_{ET}) Calculation

Each emission spectrum was convoluted into 3 peaks including PYR monomer emission, PYR excimer emission, and acceptor (NTI, Cy3, or Cy5) emission. Mathematically, the emission spectrum of a D/A system could be expressed as:

$$F_{D/A}(\lambda) = a * F_{PYRMon}(\lambda) + b * F_{PYRExc}(\lambda) + c * F_{A}(\lambda)$$

Where $F_{D/A}(\lambda)$ is the emission spectrum of the D/A system, $F_{PYRMon}(\lambda)$, $F_{PYRExc}(\lambda)$, and $F_A(\lambda)$ represent the normalized emission spectrum of PYR monomer, PYR excimer, and acceptor, respectively.

Φ_{ET} from PYR monomer to acceptor:

$$\Phi_{ET,Mon-A} = 1 - \frac{a_{D/A}}{a_D}$$

Φ_{ET} from PYR excimer to acceptor:

$$\Phi_{ET,Exc-A} = 1 - \frac{b_{D/A}}{b_D}$$

Overall Φ_{ET} from PYR to acceptor:

$$\Phi_{ET,total} = \frac{a_D}{a_D + b_D} * \Phi_{ET,Mon-A} + \frac{b_D}{a_D + b_D} * \Phi_{ET,Exc-A}$$

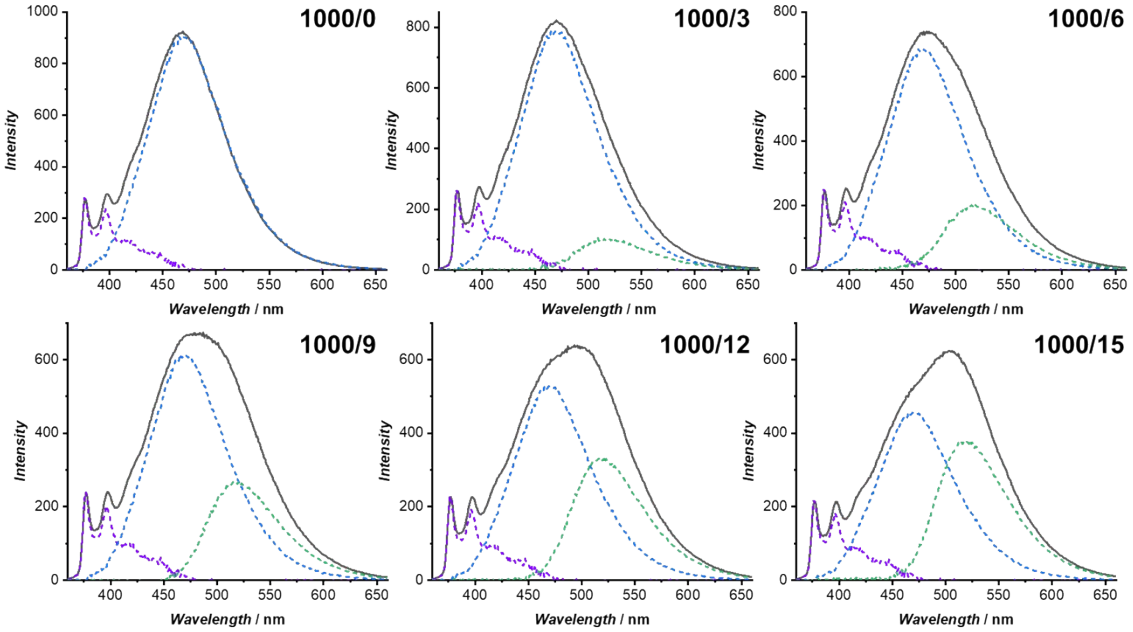


Figure S9 Convolution of emission spectra of PYR-CP-PEG/NTI-CP-PEG (grey) into emission bands of PYR monomer (purple), PYR excimer (blue), and NTI (green).

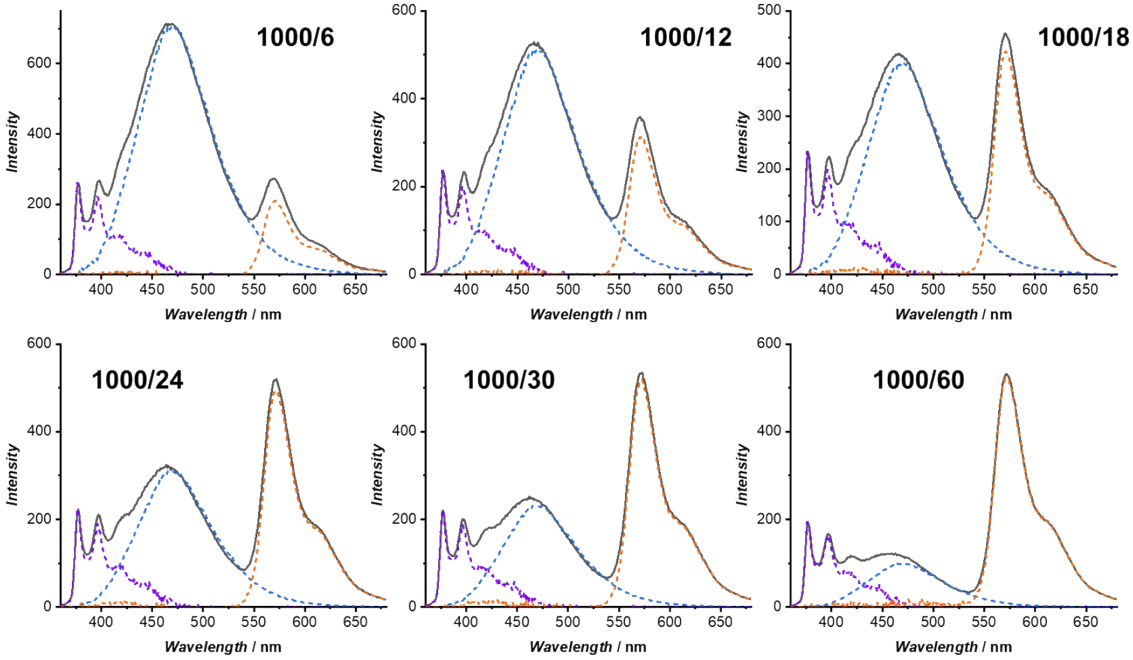


Figure S10 Convolution of emission spectra of PYR-CP-PEG/Cy3-CP-PEG (grey) into emission bands of PYR monomer (purple), PYR excimer (blue), and Cy3 (orange).

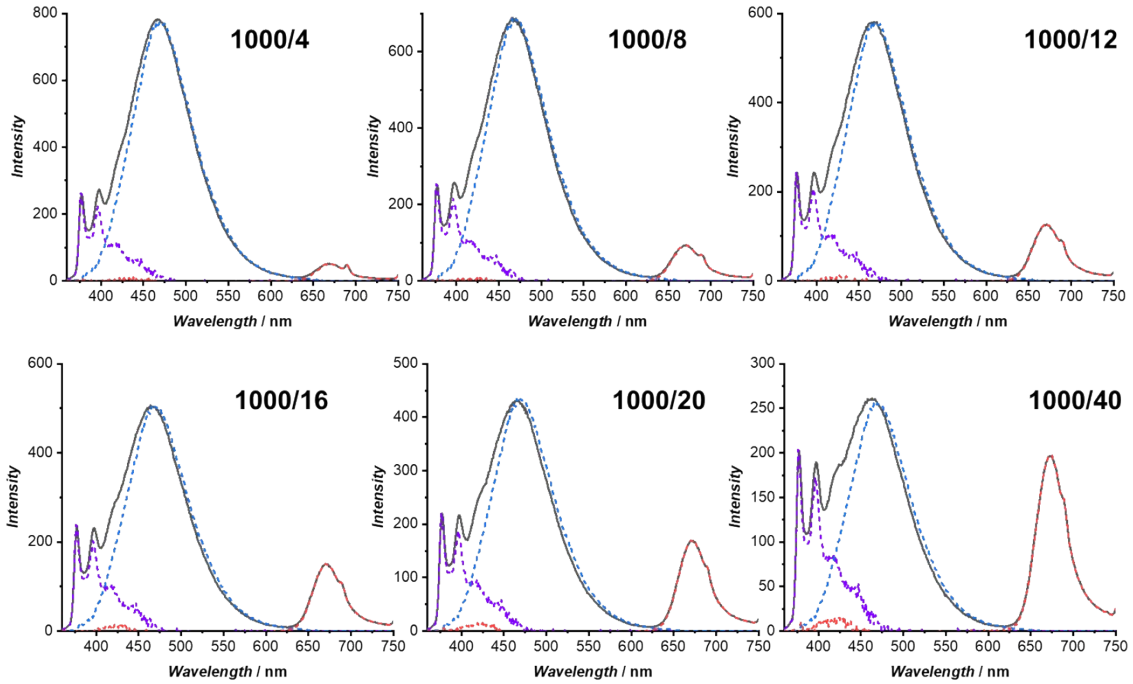


Figure S11 Convolution of emission spectra of PYR-CP-PEG/Cy5-CP-PEG (grey) into emission bands of PYR monomer (purple), PYR excimer (blue), and Cy5 (red).

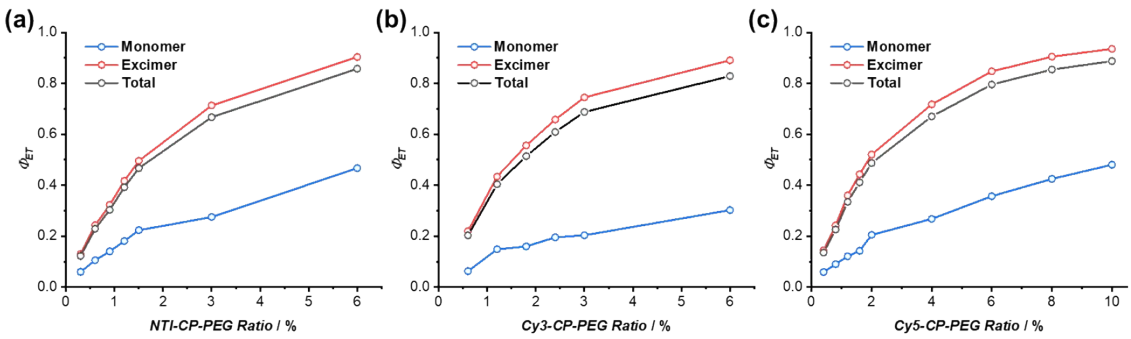


Figure S12 Φ_{ET} at different PYR-CP-PEG/NTI-CP-PEG (a), PYR-CP-PEG/Cy3-CP-PEG (b), and PYR-CP-PEG/Cy5-CP-PEG (c) ratios.

S5. Tuning PYR Monomer-to-Excimer Emission Ratios Using Oct-CP-PEG as Spacer

The co-assembly of PYR-CP-PEG and Oct-CP-PEG was realized by firstly premixing the two conjugates with certain molar ratios in a small amount of DMSO, followed by the addition of DI water, resulting clear solutions with DMSO/H_2O ratio of 5/95. PYR-CP-PEG concentration was fixed at 10 μM. Emission spectra were measured using excitation wavelength of 345 nm.

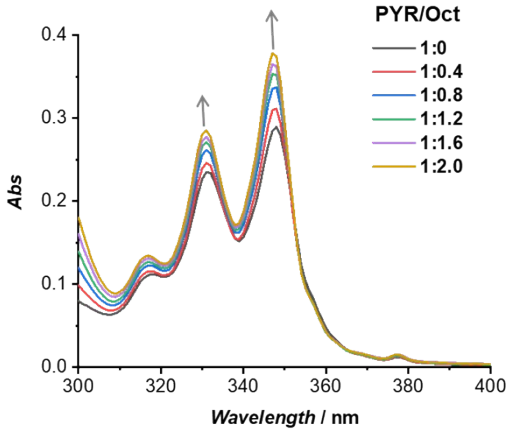


Figure S13 UV/vis spectra of PYR-CP-PEG/Oct-CP-PEG at different molar ratios.

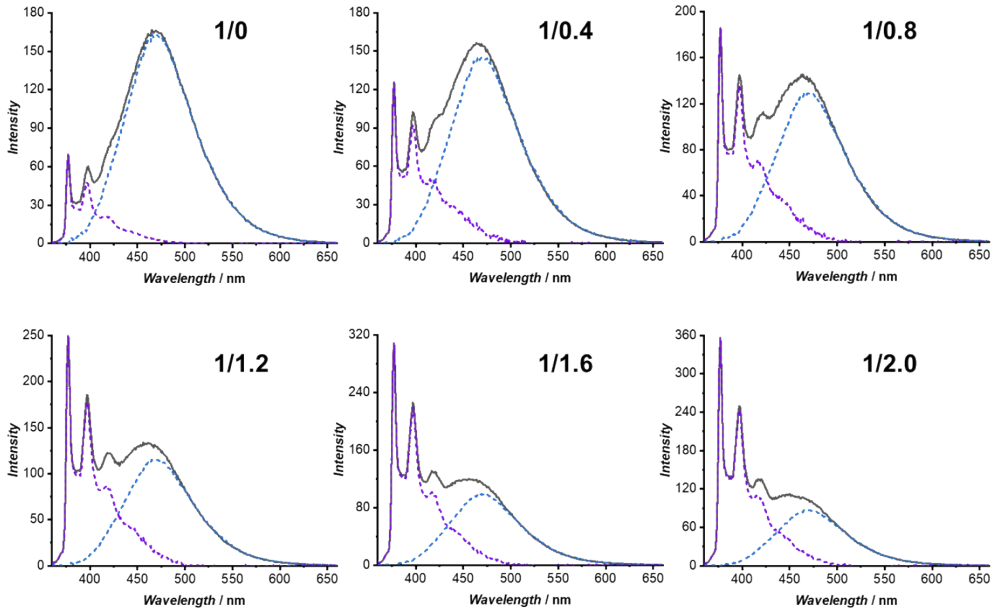


Figure S14 Convolution of emission spectra of PYR-CP-PEG/Oct-CP-PEG (grey) into emission bands of PYR monomer (purple) and PYR excimer (blue).

S6. Tuning Emission Colour of FRET Systems Using Oct-CP-PEG as Spacer

The co-assembly of PYR-CP-PEG, Oct-CP-PEG, and NTI-CP-PEG, Cy3-CP-PEG, or Cy5-CP-PEG was realized by firstly premixing the three conjugates with certain molar ratios in a small amount of DMSO, followed by the addition of DI water, resulting clear solutions with DMSO/H_2O ratio of 5/95. PYR-CP-PEG concentration was fixed at 10 μM. NTI-CP-PEG, Cy3-CP-PEG, and Cy5-CP-PEG concentrations were fixed at 0.3, 0.6, and 1.0 μM, respectively. Emission spectra were measured using excitation wavelength of 345 nm.

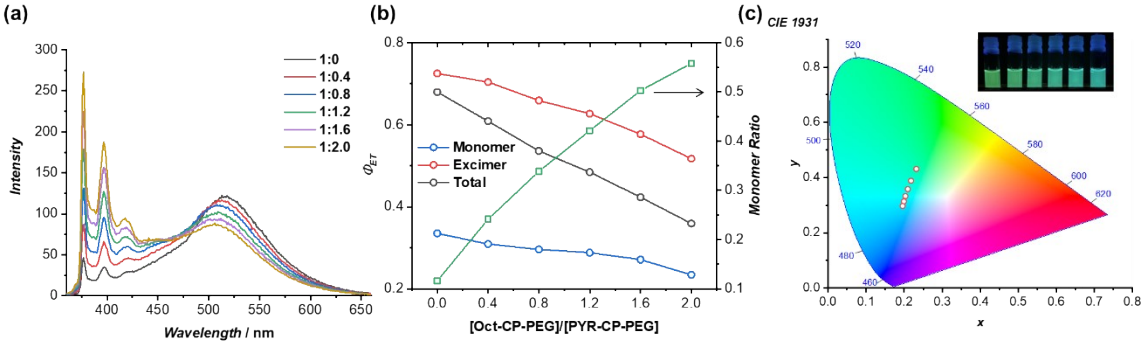


Figure S15 Fluorescence spectra of PYR-CP-PEG/3%NTI-CP-PEG with the addition of different molar ratios of Oct-CP-PEG; (b) Φ_{ET} and PYR monomer ratio at different Oct-CP-PEG/PYR-CP-PEG ratios; (c) CIE 1931 diagram showing CIE coordinates of (a) (insert: photograph showing fluorescent emissions of (a) under a 365 nm UV lamp).

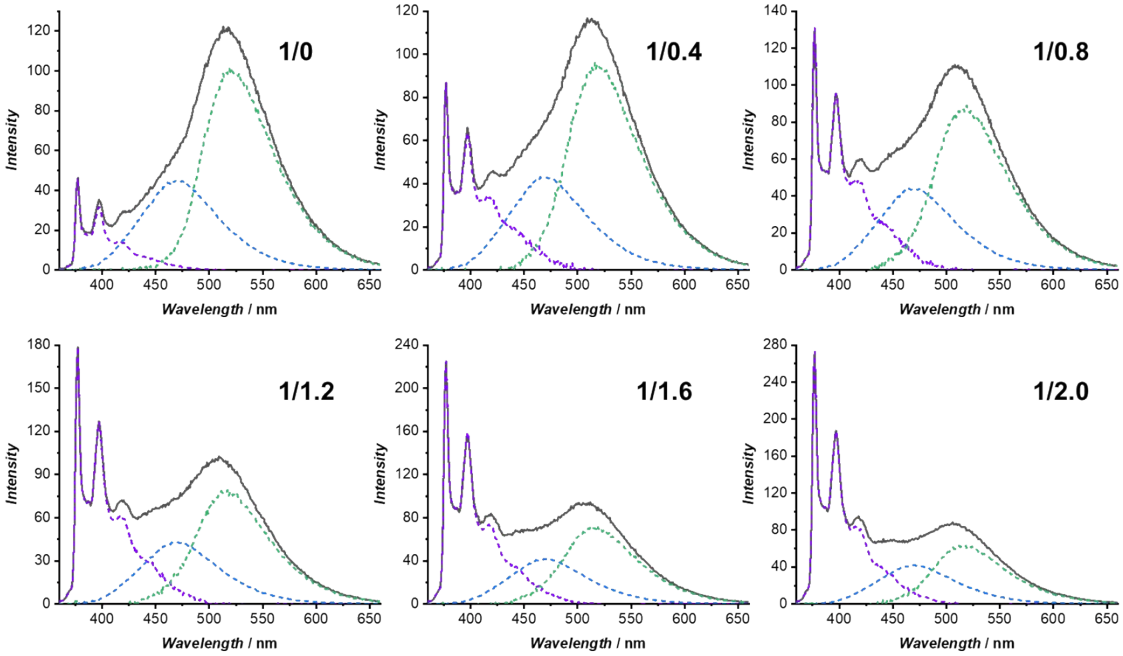


Figure S16 Convolution of emission spectra of PYR-CP-PEG/3%NTI-CP-PEG/Oct-CP-PEG (grey) into emission bands of PYR monomer (purple), PYR excimer (blue), and NTI (green).

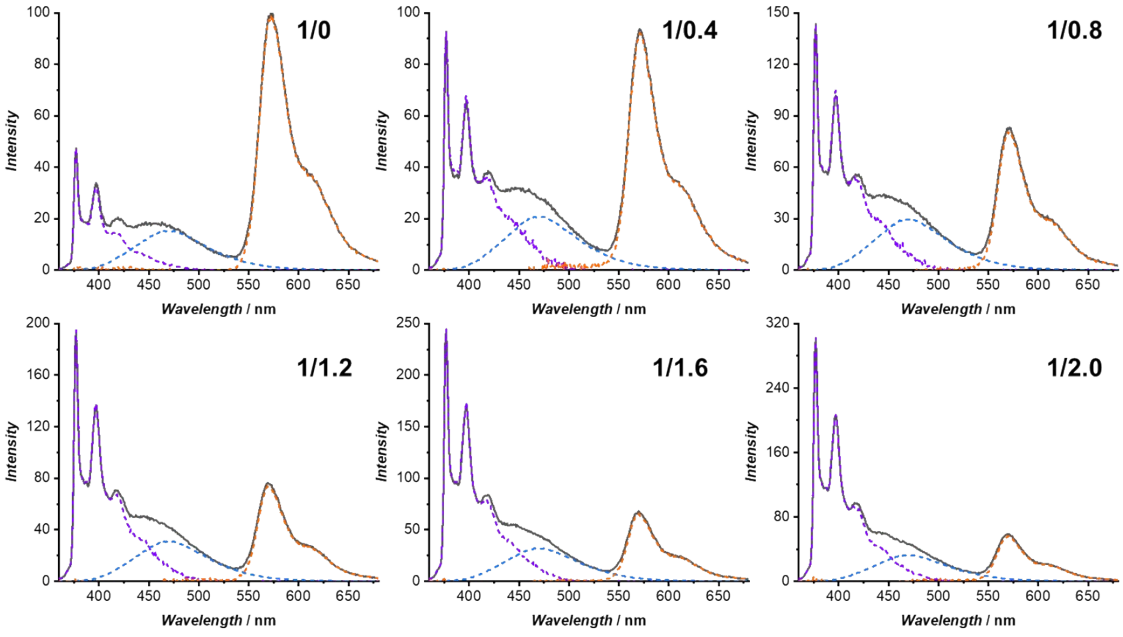


Figure S17 Convolution of emission spectra of PYR-CP-PEG/6%Cy3-CP-PEG/Oct-CP-PEG (grey) into emission bands of PYR monomer (purple), PYR excimer (blue), and Cy3 (orange).

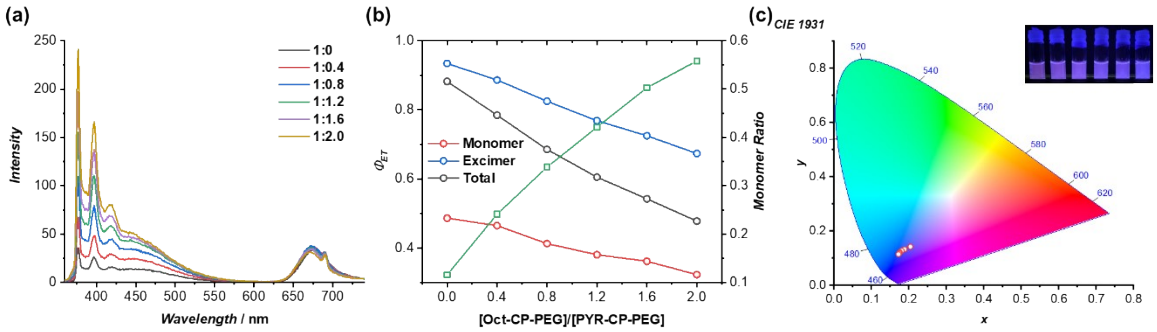


Figure S18 Fluorescence spectra of PYR-CP-PEG/10%Cy5-CP-PEG with the addition of different molar ratios of Oct-CP-PEG; (b) Evolution of Φ_{ET} values and PYR monomer ratio with different Oct-CP-PEG/PYR-CP-PEG ratios; (c) CIE 1931 diagram showing CIE coordinates of (a) (insert: photograph showing fluorescent emissions of (a) under a 365 nm UV lamp).

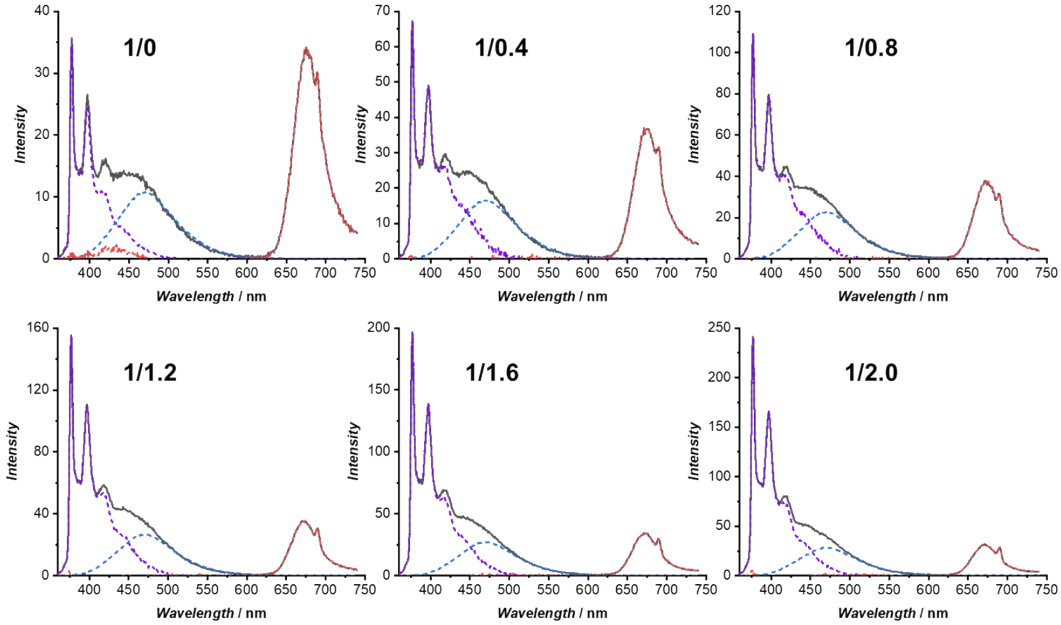


Figure S19 Convolution of emission spectra of PYR-CP-PEG/10%Cy5-CP-PEG/Oct-CP-PEG (grey) into emission bands of PYR monomer (purple), PYR excimer (blue), and Cy5 (red).

S7. pH-responsiveness of Hep-CP-PEG

SASfit software was used to fit the SANS data of Hep-CP-PEG at pH=2 in D_2O, using a cylindrical polymer micelle model (CYL+Chains(RW)). SLD and brush volume values were calculated based on the molecular structures of the conjugates and solvents.

Table S2 Fitting parameters using a cylindrical polymer micelle model.

Parameter	Hep-CP-PEG (pH=2)
Scale	0.01484 ± 0.00066
Background* / cm^{-1}	0.0013
Core Radius / Å	33.07 ± 0.38
Grafting Density / $Å^{-2}$	0.00329 ± 0.00003
Brush Volume* / $Å^3$	7413
SLD_{core}*/ × 10^{-6} $Å^{-2}$	1.27
SLD_{shell}*/ × 10^{-6} $Å^{-2}$	0.63
$SLD_{solvent}$*/ × 10^{-6} $Å^{-2}$	6.33
Core Solvation*	0
R_g / Å	33.40 ± 0.17
Core Penetration*	1.0
Length / Å	544.9 ± 22.6
Reduced Chi^2	2.63

* The parameters were held constant during the fitting procedure.

SasView software was used to fit the SANS data of Hep-CP-PEG at pH=10 in D_2O, using a monodisperse Gaussian Coil model + Power Law (Sum of two separate models).

Table S3 Fitting parameters using a Monodisperse Gaussian Coil model + Power Law.

Parameter	Hep-CP-PEG (pH=10)
Background* / cm^{-1}	0.001
Scale* (Gaussian Coil)	1
I_0 / cm^{-1}	0.04381 ± 0.00306
R_g / Å	38.03 ± 1.68
Scale (Power Law)	$1.59*10^{-7}$ ± $7.25*10^{-8}$
Exponent	3.0661 ± 0.0805
Reduced Chi^2	1.01

* The parameters were held constant during the fitting procedure.

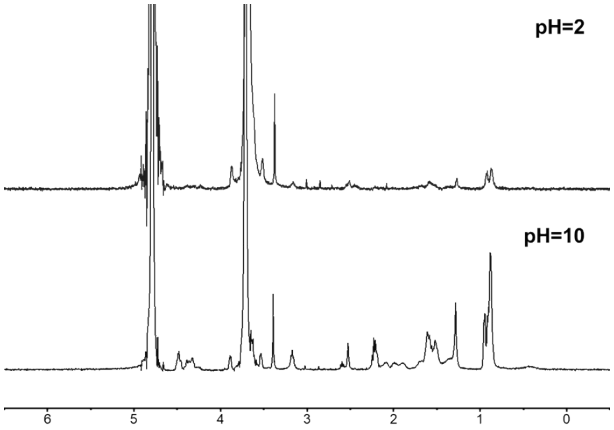


Figure S20 ^{1}H NMR spectra of Hep-CP-PEG in D_2O at pH=2 and pH=10 (400 MHz).

S8. Tuning PYR Monomer-to-Excimer Emission Ratios Using Hep-CP-PEG as Spacer and its pH-Responsiveness

The co-assembly of PYR-CP-PEG and Hep-CP-PEG was realized by firstly premixing the two conjugates with certain molar ratios in a small amount of DMSO, followed by the addition of buffer at specific pH (10 mM NaOH for pH=12, and 10 mM HCl for pH=2), resulting clear solutions with DMSO/buffer ratio of 5/95. PYR-CP-PEG concentration was fixed at 10 μM. Emission spectra were measured using excitation wavelength of 345 nm. (It should be noted that Hep-CP-PEG is more hydrophilic than Oct-CP-PEG as the two hydrophobic Trp moieties of Oct-CP-PEG are replaced by two Glu moieties. Therefore, Hep-CP-PEG is believed to exhibit a weaker assembling capability, thus resulting a less effective ability when tuning the monomer-to-excimer ratio of PYR-CP-PEG.)

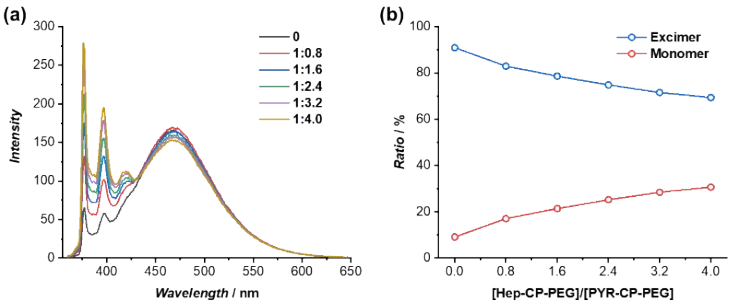


Figure S21 (a) Fluorescence spectra of PYR-CP-PEG/Hep-CP-PEG at different molar ratios at pH=2; (b) Evolution of the ratio of PYR monomer and excimer emission upon Hep-CP-PEG/PYR-CP-PEG molar ratio.

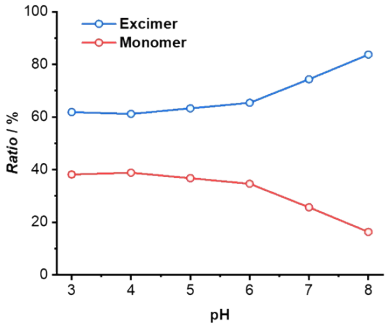


Figure S22 Evolution of the ratio of PYR monomer and excimer emission upon pH.

S9. Tuning Emission Colour of FRET Systems Using Hep-CP-PEG as Spacer

The co-assembly of PYR-CP-PEG, Hep-CP-PEG, and NTI-CP-PEG, Cy3-CP-PEG, or Cy5-CP-PEG was realized by firstly premixing the three conjugates with certain molar ratios in a small amount of DMSO, followed by the addition of buffer at specific pH (10 mM NaOH for pH=12, and 10 mM HCl for pH=2), resulting clear solutions with DMSO/buffer ratio of 5/95. PYR-CP-PEG concentration was fixed at 10 μM. Emission spectra were measured using excitation wavelength of 345 nm.

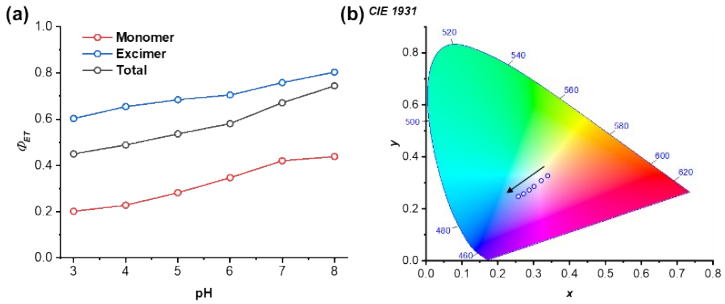


Figure S23 (a) Evolution of Φ_{ET} values with pH; (b) CIE 1931 diagram showing CIE coordinates of fluorescent emission colours of PYR-CP-PEG/Cy3-CP-PEG/Hep-CP-PEG=1/0.06/4 at different pHs.

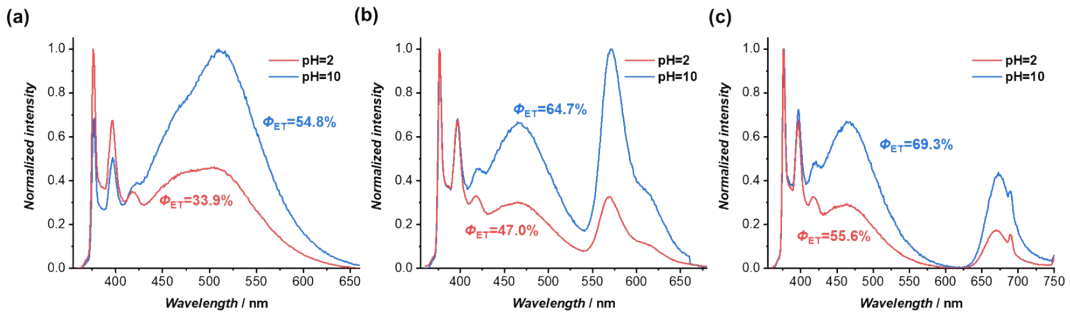


Figure S24 Fluorescence spectra of PYR-CP-PEG/NTI-CP-PEG/Hep-CP-PEG (a), PYR-CP-PEG/Cy3-CP-PEG/Hep-CP-PEG (b), and PYR-CP-PEG/Cy5-CP-PEG/Hep-CP-PEG (c) at pH=2 and pH=10.

[1] Q. Song, S. Goia, J. Yang, S. C. L. Hall, M. Staniforth, V. G. Stavros and S. Perrier, *J. Am. Chem. Soc.*, 2021, **143**, 382-389.