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Supporting Information

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Direct Visualization of Efficient Energy Transfer in Single Oligo(*p*-phenylene vinylene) Vesicles

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General methods. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz NMR (Varian Mercury, 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) or a 300 MHz NMR (Varian Gemini, 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR). For ^{1}H-NMR and ^{13}C-NMR, chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). IR spectra were recorded on a Perkin Elmer 1600 FT-IR. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry has been performed on a PerSeptive Biosystems Voyager-DE PRO spectrometer. Elemental analysis has been carried out on a Perkin Elmer 2400. UV/vis spectra were recorded on a Perkin Elmer Lambda 900 UV/vis/NIR spectrometer, CD spectra on a Jasco J-600 spectropolarimeter and fluorescence spectra on a Perkin Elmer LS50B luminescence spectrometer. Time-correlated Single Photon Counting measurements were performed using an Edinburgh Instruments LifeSpec-PS spectrometer. The LifeSpec-PS comprises a 400 nm picosecond laser (PicoQuant PDL 800B) operated at 2.5 MHz and a Peltier-cooled Hamamatsu micro-channel plate photomultiplier (R3809U-50).

Materials. All solvents were of AR quality. Dichloromethane was freshly distilled over potassium/sodium, DMF was dried over 4Å molsieves. Other reagents used were

purchased from Acros and Aldrich and have been used without further purification. Bio-Beads SX-1 were obtained from Bio-Rad Laboratories.

Vesicle preparation for Scanning Confocal Microscopy. 100 μL of OPV stock solution (4×10^{-4} M in THF) was injected into 2.5 ml of distilled water after which the THF was evaporated by quickly heating the sample to 90 ºC. Mixtures of **OPV5** and **CN-OPV5** were prepared in two different ways. Separate vesicles were obtained by the addition of 100 μL aggregated **CN-OPV5** in water to 1 mL of aggregated **OPV5** in water (these solutions were prepared as mentioned above). To produce mixed vesicles, 2 μL **CN-OPV5** in THF was added to 100 μL of **OPV5** in THF. After sonication, the obtained mixture was injected into 2.5 mL of water and THF was removed by quickly heating the sample to 90 ºC. In order to obtain gelation, 1 mg/mL gelatine was dissolved in the desired aggregated solution at 40°C. For confocal microscopy, water/gelatine based solutions were dropcast on untreated glass and allowed to dry out in air.

Vesicle exchange experiment

Separate **OPV5** and **CN-OPV5** vesicle solutions (both 1.6×10^{-5} M in water) were prepared as described above. To both solutions 1 mg/mL gelatine was added at 40°C. Then a mixture containing 2 mol% **CN-OPV5** was prepared (by addition of the separate **OPV5** and **CN-OPV5** vesicles) which was kept at 35 °C for 48h. At different times, samples were taken from this solution which were dropcast on untreated glass and allowed to dry out in air. Fluorescence was taken from the parent solution as well as from single vesicles on the glass surface.

Scanning Confocal Microscopy. Laser light (COHERENT l = 411 nm, 25 mW) was coupled into a single-mode optical fiber, reflected by a dichroic beamsplitter (Chroma,

425dcxr) and focused on the sample by an oil immersion 100x objective (Zeiss, NA = 1.30), which was mounted on a Karl Zeiss Axiovert 200 inverted microscope. Fluorescent light coming from the focal volume was collected by the same objective, passed through the beam-splitter, filtered (Chroma, HQ435lp), guided through a 50 μm pinhole and finally focused on an avalanche photo diode (PerkinElmer SPCM-AQR-14). The sample was mounted onto a Physik Instrumente P-517.2 CL nano-positioner. Sample movement (scanning and positioning) and data collection were controlled by a LabView program. Fluorescence spectra were recorded by guiding the emitted light through a fiber optical cable into an Acton SP300I spectrograph.

Dynamic light scattering. DLS measurements were carried out using an intensity-stabilized helium-neon laser (Spectra Physics, l = 632.8 nm, 4.5 mW). The incident beam of the laser was focused in the center of a cylindrical glass cell (Helma). The light, scattered at 90° with respect to the incident beam, was transferred to a single photon detector (ALV/SO-SIPD) through a single mode fiber to meet the spatial coherence conditions. The signal was processed with a 320-channel Multiple Tau Digital Correlator (ALV-5000/E). The measurements were carried out in a temperature range of 20-80 °C (± 0.05 °C) using a laboratory-made heating stage driven by an active feedback temperature controller (LakeShore 340). Intensity fluctuations in the scattered light detected in a small volume and on a microsecond timescale are related to the Brownian motion of the particles due to density fluctuations. The normalized autocorrelation function of the intensity of the scattered light is given by:

$$g^{(2)}(t) = \frac{\langle I(t)I(t+t)\rangle}{\langle I(t)I(t)\rangle} = \frac{\langle n(t)n(t+t)\rangle}{\langle n(t)n(t)\rangle} \qquad (1)$$

where $I(t)$ is the intensity of the scattered light at time t and $n(t)$ is the photon count number detected at time t. This function can be expressed as:

$$g^{(2)}(t) = 1 + b\left|g^{(1)}(t)\right|^2 \tag{2}$$

where

$$g^{(1)}(t) = \frac{\left\langle E(t)E^*(t+t)\right\rangle}{\left\langle E(t)E^*(t)\right\rangle} \tag{3}$$

is the time autocorrelation function of the electric field of the scattered light, b is a factor that depends on the experimental set-up and $\langle\ \rangle$ indicates averaging over t.

For an ensemble of monodisperse spherical particles under translational Brownian motion, the autocorrelation function $g^{(1)}(t)$ decays exponentially on time.

$$g^{(1)}(t) = \exp(-Dq^2t) \tag{4}$$

where q is the scattering vector

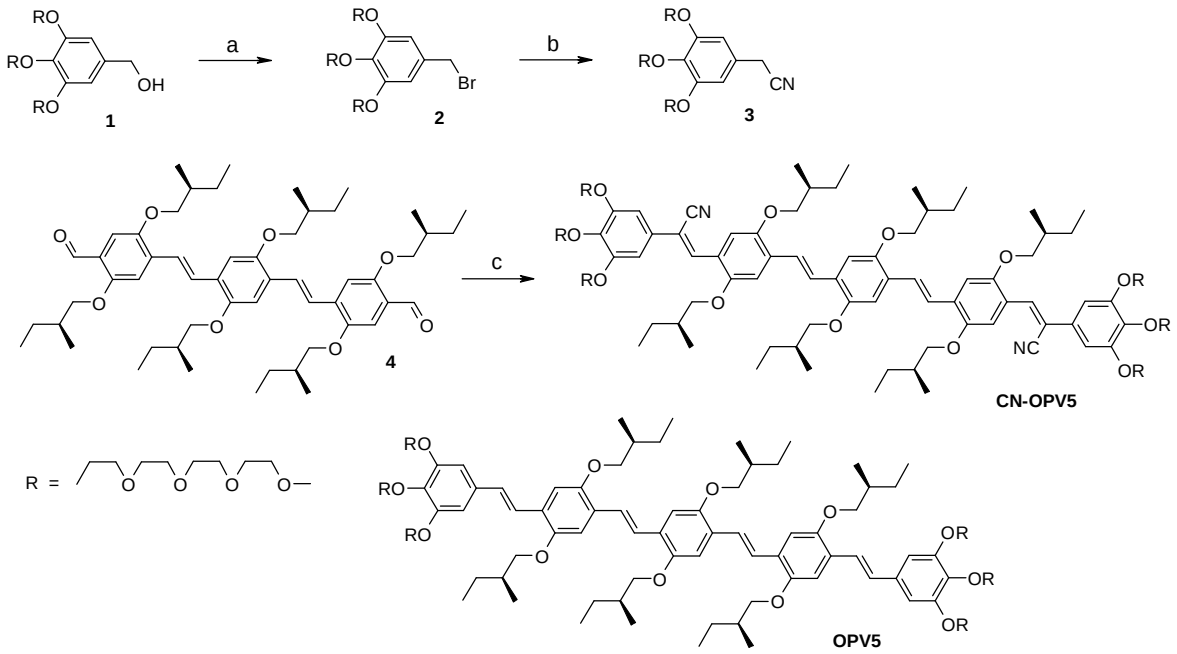
$$q = \frac{4pn}{l}\sin\left(\frac{q}{2}\right) \tag{5}$$

with n is the refractive index of the solvent, l the wavelength of the incident light, q the scattering angle and D the translational diffusion coefficient. By fitting the experimental correlation function by equation 4, D was determined, from which the hydrodynamic radius R_H of the diffusing particles was calculated from the Stokes-Einstein relationship

$$R_H = \frac{k_BT}{6phD} \tag{6}$$

where k_BT is the thermal energy and h the, temperature dependent, viscosity of the suspending medium.

Synthesis



Scheme S1. Synthesis route to **CN-OPV5**; (a) NBS, $P(Ph)_3$, dichloromethane (b) NaCN, DMF (c) **4**, KOtBu, Bu_4NOH, 10 min.

3,4,5-Tris[2-(2-{2-[2-methoxyethoxy]ethoxy}ethoxy)ethoxy]benzyl alcohol (1) and **(*E*,*E*)-1,4-Bis{4-formyl-2,5-bis[(*S*)-2-methylbutoxy]styryl}-2,5-bis[(*S*)-2-methylbutoxy]benzene (4)** were prepared according to a literature procedure.[1]

3,4,5-Tris[2-(2-{2-[2-methoxyethoxy]ethoxy}ethoxy)ethoxy]benzyl bromide

1 (1.02g, 1.40 mmol) and triphenylphosphine (0.44g, 1.68 mmol, 1.2 eq) were dissolved in 12 mL dry CH_2Cl_2 and the mixture was cooled to 0 °C. After slow addition of N-bromosuccinimide (0.38g, 1.68 mmol, 1.2 eq), the solution was stirred under Ar at room temperature for 20 hrs. After removal of the solvent *in vacuo*, the product was purified using column chromatography (SiO_2, CH_2Cl_2/MeOH 99.5:0.5 then 99:1), yielding pure **2**

as a colorless oil (0.85g, 1.08 mmol, 77%). ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.41 (s, 9H, OC*H*$_3$), 3.57-3.60 (2t, 6H, OC*H*$_2$), 3.67-3.78 (m, 30H, OC*H*$_2$), 3.82 (t, 2H, ArOCH$_2$C*H*$_2$), 3.90 (t, 4H, ArOCH$_2$C*H*$_2$), 4.17-4.22 (2t, 6H, ArOC*H*$_2$), 4.46 (s, 2H, C*H*$_2$Br), 6.68 (s, 2H, Ar*H*). ^{13}C-NMR ($CDCl_3$, ppm): δ 33.9, 58.9, 68.7, 69.5, 69.6, 70.3, 70.4, 70.5, 70.6, 71.8, 72.2, 108.6, 132.9, 138.4, 152.4. IR (UATR): ν (cm^{-1}) = 2872, 1589, 1505, 1438, 1349, 1332, 1291, 1245, 1214, 1200, 1097, 945, 849, 733, 669. MALDI-TOF MS (MW=789.72): *m/z* = 813.20 $[M+Na]^+$.

3,4,5-Tris[2-(2-{2-[2-methoxyethoxy]ethoxy}ethoxy)ethoxy]benzyl cyanide

NaCN (83 mg, 1.69 mmol) was dissolved in 7 mL dry DMF and the solution was heated to 90 °C. After removal of the oil bath, a solution of **2** (1.12g, 1.41 mmol) in 12 mL dry DMF was added dropwise and the mixture was stirred under Ar for 1.5 hrs. After cooling down to room temperature, 100 mL of water was added and the mixture was extracted with $CHCl_2$ (3×100 mL). The collected organic fractions were washed with brine (100 mL), dried with $MgSO_4$, filtered and the solvent was removed *in vacuo*. The product was purified using column chromatography (SiO_2, CH_2Cl_2/EtOH 97:3 then 95:5), yielding pure **3** as a colorless oil (0.76g, 1.03 mmol, 73%). ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.38 (s, 9H, OC*H*$_3$), 3.54-3.56 (2t, 6H, OC*H*$_2$), 3.64-3.73 (m, 32H, OC*H*$_2$, C*H*$_2$CN), 3.78 (t, 2H, ArOCH$_2$C*H*$_2$), 3.85 (t, 4H, ArOCH$_2$C*H*$_2$), 4.12-4.18 (2t, 6H, ArOC*H*$_2$), 6.56 (s, 2H, Ar*H*). ^{13}C-NMR ($CDCl_3$, ppm): δ 23.5, 58.9, 68.9, 69.5, 70.4, 70.46, 70.52, 70.7, 71.8, 72.2, 107.6, 117.7, 125.0, 138.1, 153.0. IR (ATR): ν (cm^{-1}) = 2872, 2248, 1591, 1505, 1439, 1350, 1334, 1299, 1246, 1199, 1097, 945, 850, 732, 701. MALDI-TOF MS (MW=735.86): *m/z* = 758.31 $[M+Na]^+$, 774.28 $[M+K]^+$.

(*E*,*E*,*E*,*E*)-1,4-Bis{4-[2-cyanovinyl-3,4,5-tris(2-{2-[2-(2-methoxyethoxy)-ethoxy]ethoxy}ethoxy)phenyl]-2,5-bis[(*S*)-2-ethylbutoxy]styryl}-2,5-bis[(*S*)-2-methylbutoxy]benzene (CN-OPV5)

A solution of **3** (74 mg, 0.10 mmol) and **4**[1] (43 mg, 0.050 mmol) in 3 mL dry THF/*t*-BuOH 1:3 was heated to 40 °C. Quickly, KO*t*Bu (1.1 mg, 0.010 mmol) and Bu_4NOH (100 µl of a 1M solution in MeOH) were added to the reaction mixture, after which the solution was stirred at 50 °C for 15 min. After cooling to room temperature, 1N HCL was added (50 mL) and the mixture was extracted with CH_2Cl_2 (4×50 mL). The combined organic fractions were dried with $MgSO_4$, filtered and the solvent was removed *in vacuo*. The product was purified using biobeads size exclusion chromatography, yielding pure CN-OPV5 as a red, waxy solid (90 mg, 0.039 mmol, 78%). ^{1}H-NMR ($CDCl_3$, ppm): δ = 0.96-1.02 (m, 18H, C*H*$_3$), 1.06-1.12 (m, 18H, C*H*$_3$), 1.33-1.39 (m, 6H, C*H*$_2$), 1.60-1.68 (m, 6H, C*H*$_2$), 1.94-1.96 (m, 6H, C*H*), 3.36 (s, 12H, OC*H*$_3$), 3.37 (s, 6H, OC*H*$_3$), 3.52-3.55 (2t, 12H, OC*H*$_2$), 3.62-3.74 (m, 60H, OC*H*$_2$), 3.79-3.98 (m, 24H, ArOC*H*$_2$, ArOCH$_2$C*H*$_2$O), 4.18-4.22 (2t, 12H, ArOC*H*$_2$CH$_2$O), 6.90 (s, 4H, Ar*H*), 7.19 (s, 4H, Ar*H*), 7.54 (d, 2H, ArC*H*=CH), 7.61 (d, 2H, ArCH=C*H*), 7.87 (s, 2H, Ar*H*), 7.94 (s, 2H, ArC(CN)=C*H*). ^{13}C-NMR ($CDCl_3$, ppm): δ 11.27, 11.35, 16.6, 16.68, 16.70, 26.2, 26.3, 34.8, 34.9, 58.9, 68.9, 69.6, 70.4, 70.5, 70.6, 70.7, 71.8, 72.4, 73.7, 74.0, 74.1, 77.1, 105.5, 108.8, 109.1, 110.0, 111.6, 118.6, 122.4, 122.5, 124.4, 127.3, 130.5, 130.8, 135.5, 139.1, 150.4, 151.2, 152.1, 152.8. IR (ATR): ν (cm^{-1}) = 3057, 2958, 2911, 2873, 2207, 1582, 1509, 1456, 1423, 1387, 1351, 1335, 1281, 1253, 1205, 1104, 1042, 963, 907, 854, 824, 772, 754, 718. MALDI-TOF MS (MW=2290.90): *m/z* = 2290.45 $[M]^+$, 2313.43 $[M+Na]^+$. MP = 80 °C.

Spectroscopy

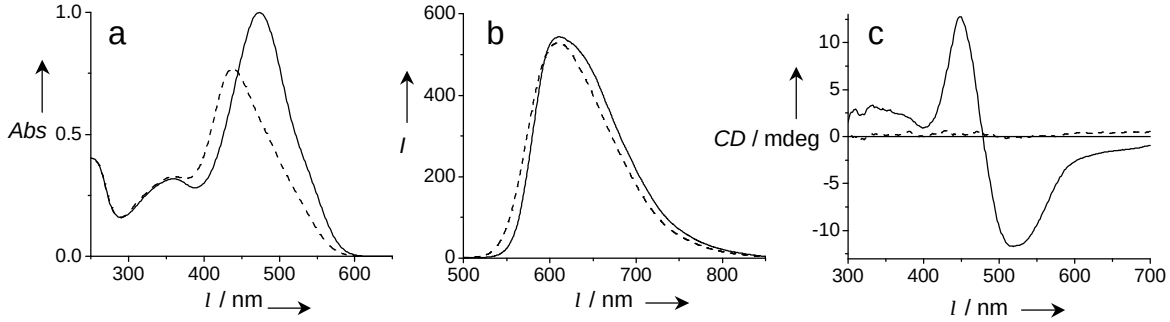


Figure S1. Temperature-dependent (a) UV/vis (b) fluorescence (λ_{exc} = respective λ_{max}) and (c) CD spectroscopy measurements on **CN-OPV5** in water (1.6×10^{-5} M, CD data at 7.3×10^{-5} M), 20 °C (solid line) and 90 °C (dashed line).

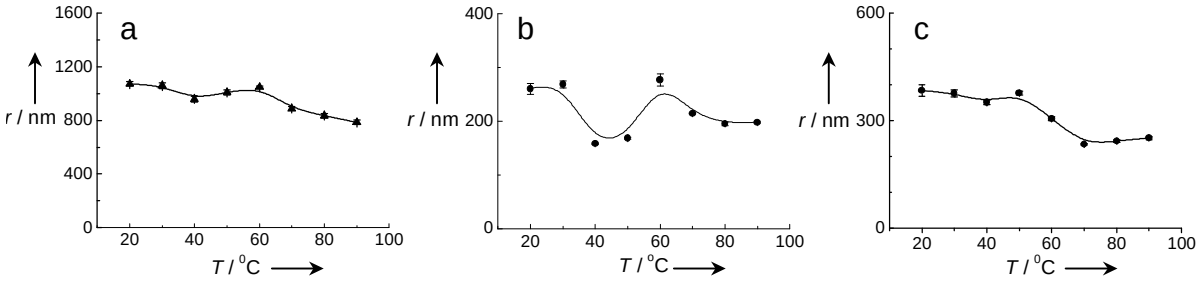


Figure S2. Dynamic light scattering (DLS) in water on (a) **OPV5**, (b) **CN-OPV5** and (c) mixed vesicles containing 9 mol% **CN-OPV5** in **OPV5** as a function of temperature.

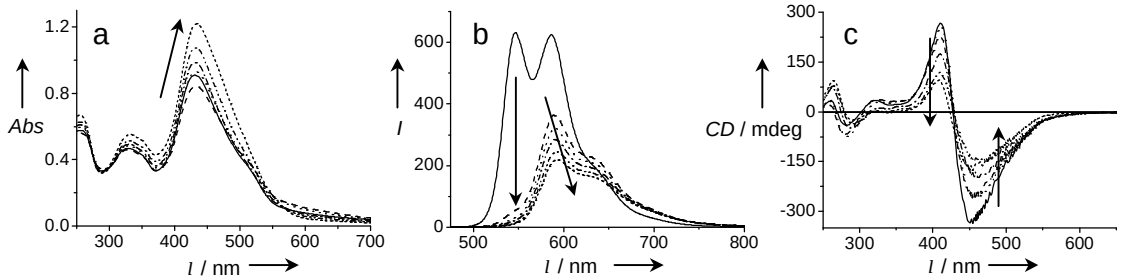


Figure S3. Mixed vesicles containing 0-31 mol% **CN-OPV5** in **OPV5** as studied with (a) UV/vis (b) fluorescence (l_{exc} = 419 nm) and (c) CD spectroscopy ([**OPV5**] = constant at 1.6×10^{-5} M in water).

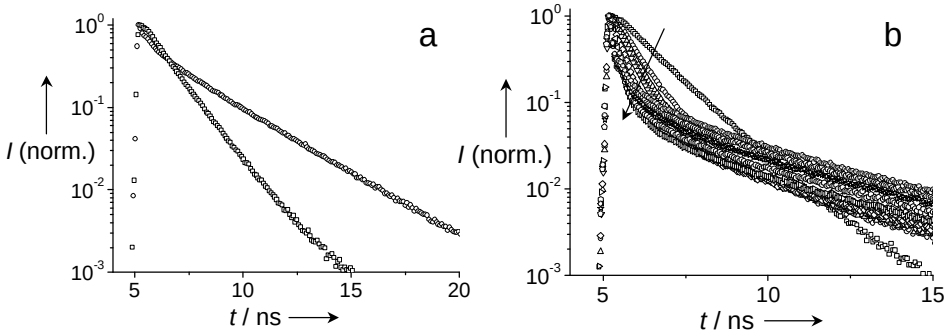


Figure S4. Time-resolved single photon counting data in water at room temperature. Shown are the decays at (a) l_{em} = 546 nm for **OPV5** (squares) and l_{em} = 634 nm for **CN-OPV5** (rounds) and (b) l_{em} = 546 nm for **CN-OPV5**/**OPV5** mixed vesicles in water, ranging from 0 (squares) – 30 mol% **CN-OPV5** ([**OPV5**] = constant at 1.6×10^{-5} M). Excitation in all cases l_{exc} = 400 nm.

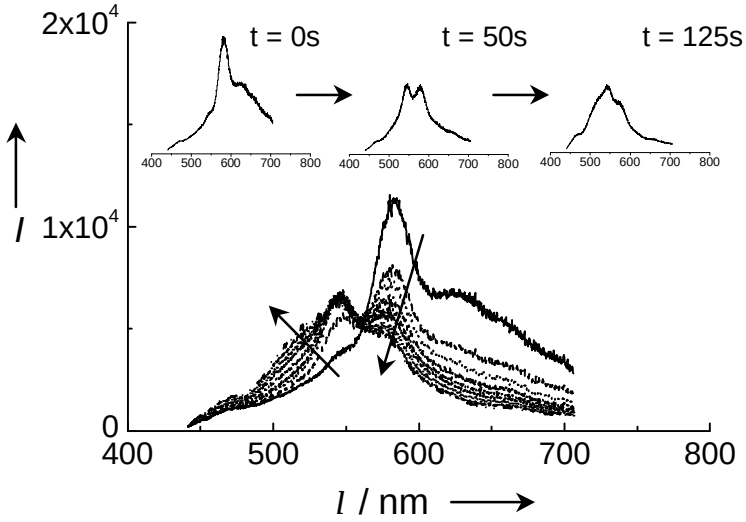


Figure S5. Extended illumination of a doped vesicle (l_{exc} = 419 nm) leads to the formation of a hot-spot at the acceptor molecule and results in the bleaching of **CN-OPV5** luminescence and restoration of **OPV5** luminescence. Further illumination also bleaches the donor **OPV5**. The time interval in the graph below is 15s.

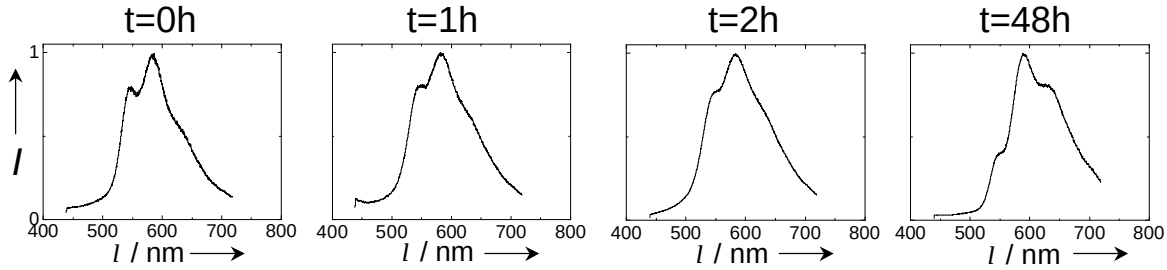


Figure S6. Normalized fluorescence plots (l_{exc} = 411 nm) for a 2 mol% **CN-OPV5** solution (starting from separate **OPV5** and **CN-OPV5** vesicles) after heating at 35 °C for the designated time. These are the parent solution data as opposed to single vesicle fluorescence as shown in figure 6.

[1] P. Jonkheijm, M. Fransen, A. P. H. J. Schenning and E. W. Meijer, *J. Chem. Soc., Perkin. Trans. 2* **2001**, 1280-1286.