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Supporting information

Light Upconverting soft particles: Triplet-triplet annihilation in the phospholipid bilayer of self-assembled vesicles

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Content

General methods and materials

Diphenylantracen **DPA** was purchased from TCI chemicals and **PtOEP** from Sigma Aldrich, both compounds were used as delivered. 9-Bromo-10-phenylanthracene[1] and amphiphilic sensitizer Tris(bipyridine)ruthenium(II)[2] **1** were prepared according to published procedure. All used lipids were purchased from Avanti Polar Lipids Inc. All aqueous solutions were prepared in MiliQ deionised water.

NMR-Spectroscopy: NMR-spectra were recorded on a Bruker Avance 300 (^{1}H: 300 MHz, ^{13}C: 75 MHz, T = 295 K) using the solvent residual peak as internal reference ($CDCl_3$: δ H 7.26). The chemical shifts are reported in δ [ppm] relative to internal standards (solvent residual peak). The spectra were analyzed by first order, the coupling constants *J* are given in Hertz [Hz]. Integration is determined as the relative number of atoms. Error of reported values: chemical shift: 0.01 ppm for ^{1}H-NMR, 0.1 ppm for ^{13}C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Thin Layer Chromatography: Aluminium plates coated with silica gel (ALUGRAM Xtra SIL G/UV_{254} from Macherey-Nagel) were used. Detection was done by UV light (254 nm, 366 nm) or oxidation, using a $KMnO_4$-solution.

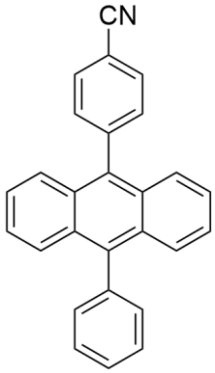
Spectroscopy: UV/Vis Spectra were recorded on a Cary 50 UV/Vis spectrophotometer, fluorescence of dyes on Horiba Fluoromax.

Dynamic Light Scattering: DLS measurements were performed on a Malvern Zetasizer Nano at 25 °C using 1 cm disposable polystyrene cuvettes (VWR).

Time resolved measurements: The time resolved fluorescence spectra were recorded using an optical parametric oscillator pumped by a Nd:YAG Laser (Surelite II and Sureleite OPO Plus, Continuum) as excitation source. The fluorescence light was recorded by the combination of a spectrograph (Burker 200is, grating 100 lines / 100mm), a streak camera (C7700, Hamamatsu Photonics) and a CCD camera (ORCA-CR, Hamamatsu Photonics). The software provided by Hamamatsu (HPD-TA, Hamamatsu Photonics) was used to record the fluorescence in photon counting mode.

Synthesis

4-(10-Phenylanthracen-9-yl)benzonitrile (DPA-CN)[3]



9-Bromo-10-phenylanthracene (473 mg, 1.42 mmol), 4-cyanophenylboronic acid (314 mg, 2.14 mmol) and K_2CO_3 (1969 mg, 14.25 mmol) were suspended in ethanol (5 mL) and toluene (20 mL). The reaction mixture was flushed with nitrogen for 20 min. Afterwards $Pd(PPh_3)_4$ (83 mg, 0.07 mmol) was added and the reaction was refluxed under nitrogen for 24 h. After cooling to room temperature, brine (10 mL) was added and the mixture was extracted with toluene (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, solids were filter off and the solvent removed under reduced pressure. The residue was purified by flash chromatography (gradient: 100 % petroleum ether to 50 % petroleum ether and 50 % dichloromethane) to obtain 471 mg (93 %) of product **DPA-CN** in form of white crystals.

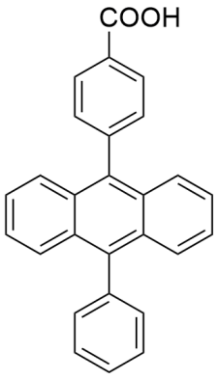
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 – 7.89 (m, 2H), 7.79 – 7.68 (m, 2H), 7.68 – 7.51 (m, 7H), 7.51 – 7.43 (m, 2H), 7.43 – 7.31 (m, 4H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 144.6, 138.7, 138.3, 134.5, 132.4, 132.3, 131.2, 129.8, 129.4, 128.5, 127.7, 127.3, 126.0, 125.8, 125.2, 119.0, 111.6.

MS (EI(+)): m/z = 355.13 [M^+]

MP: 278 – 279 °C

4-(10-Phenylanthracen-9-yl)benzoic acid (DPA-COOH)[4]



A pellet of KOH (662 mg, 11.82 mmol) was added to a suspension of derivative **DPA-CN** (300 mg, 0.84 mmol) in water (15 mL) and 2-ethoxyethanol (30 mL). The reaction mixture was refluxed for 4 h. After cooling aq. HCl was added dropwise to reach acidic pH followed by additional water (50 mL). The suspension was extracted with diethylether (3 × 50 mL), combined organic layers were washed with water (10 mL), brine (10 mL) and dried over $MgSO_4$. Solids were filter off and the residue was purified via column chromatography (gradient: petroleum ether with 50 % ethyl acetate to 100 % ethyl acetate) to obtain 242 mg (77 %) product **DPA-COOH** in form of white solid.

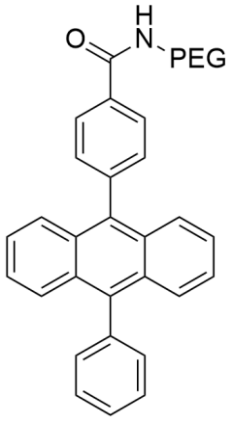
^{1}H NMR (300 MHz, DMSO) δ 8.27 – 8.13 (m, 3H), 7.72 – 7.50 (m, 10H), 7.49 – 7.40 (m, 5H).

^{13}C NMR (75 MHz, DMSO) δ 167.7, 141.2, 138.0, 136.8, 135.7, 133.6, 130.7, 130.8, 129.1, 128.9, 128.6, 127.7, 126.4, 126.1, 125.6, 125.5.

MS (ESI(+)): m/z = 374.15 [MH^+]

MP: 304 – 308 °C

Polyethyleneglycol 4-(10-phenylanthracen-9-yl)benzamid (DPA-PEG)[5]



In nitrogen atmosphere the acid **DPA-COOH** (100 mg, 0.26 mmol) was suspended in dry DCM (15 mL) and additional dry DMF was added under stirring until the acid dissolved. DCC (28 mg, 0.13 mmol) was added and stirring continued for 15 min. Consecutively methoxypolyethylene glycol amine 5000 (668 mg, 0.134 mmol) was added as a solution in dry DCM (5 mL) followed by HOBT (20 mg, 0.134 mmol) in dry DMF (5 mL). The reaction mixture was stirred at RT for 24 h. DCM was removed in *vacuo*. The residue was treated with diethylether, a precipitate was filtered off and washed with cold diethylether. The obtained solid was recrystallized three times from ethanol and dried in *vacuo* to obtain 300 mg (41 %) of polymer **DPA-PEG** in form of a white solid. Loading of the dye in the polymer was calculated to be: 74 % (via ^{1}H NMR).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.7 Hz, 2H), 7.74 – 7.28 (m, 15H), 3.85 – 3.31 (m, 614H).

MP: 50 – 57 °C

Preparation and characterization of the vesicles

Vesicle preparation

Vesicles were prepared according to known procedures.[6] From stock solutions of lipids and dyes the required ratio of compounds was mixed, solvent was evaporated in a stream of nitrogen and in *vacuo*. The residue was dissolved in MiliQ water to obtain the solution of all amphiphiles with a concentration of 1mM. The emulsion was sonicated and extruded through polycarbonate membrane with 100 nm pores to obtain vesicles with the desired size. Samples were bubbled with nitrogen for 15 min during sonication step and 10 min prior and during the measurements.

Different systems

Absorption spectra for sensitizer **1** and annihilator **DPA** were measured individually and compared with the mixture of the components under three different conditions (Fig. S1).

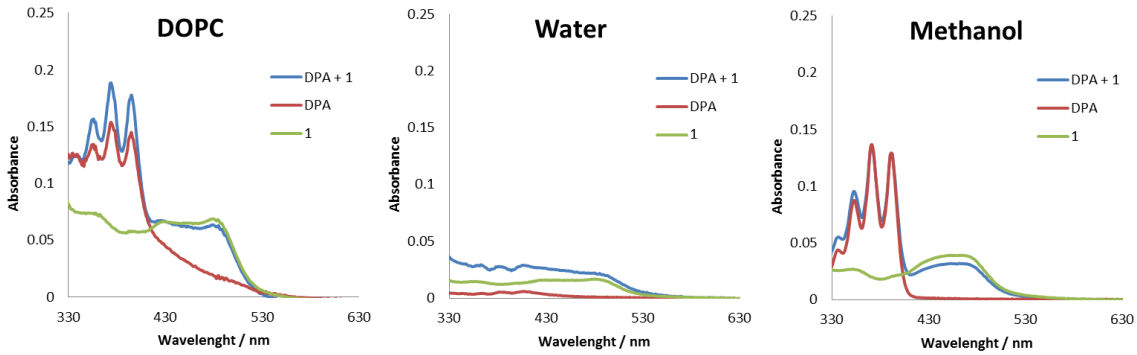


Figure S1: UV-Vis spectra of different systems containing **DPA** (0.04 mM) or **1** (0.01 mM) or their mixture in aq. DOPC vesicles (0.95 mM), MiliQ water or Methanol.

All concentrations are identical. **DPA** is nearly insoluble in water.

Loading of DPA in Vesicles

Different loadings of **DPA** in DOPC vesicles were examined. Highest fluorescence intensity and absorption for **DPA** is observed for system with 4 mol % of **DPA** in DOPC membrane (with 1 mol % of **1**) (Fig. S2).

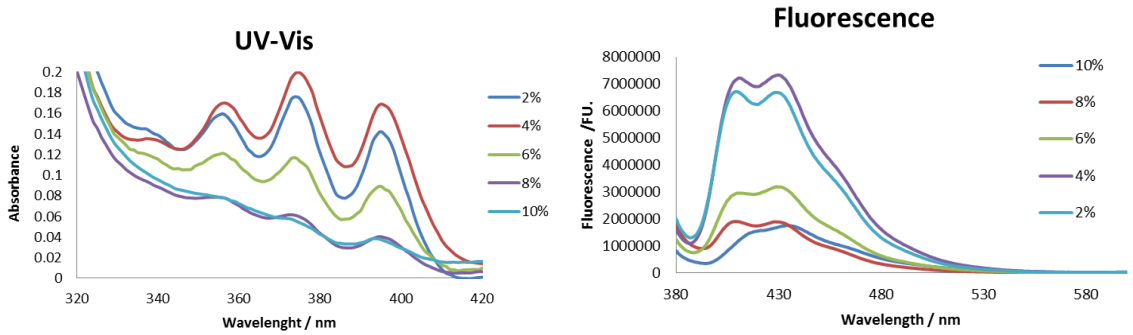


Figure S2: Absorption and emission spectra of DOPC vesicles with different molar loading of **DPA** in presence of 1 mol % of **RuBiPy** (0.01 mM). Concentration of DOPC is always calculated to give 1 mM of all amphiphiles (0.89–0.97 mM).

Vesicles are stable after extrusion for 24 h at room temperature (Fig. S3) and only vesicles with 10 mol. % of **DPA** exhibited a higher polydispersity index showing inferior stability with increased loading.

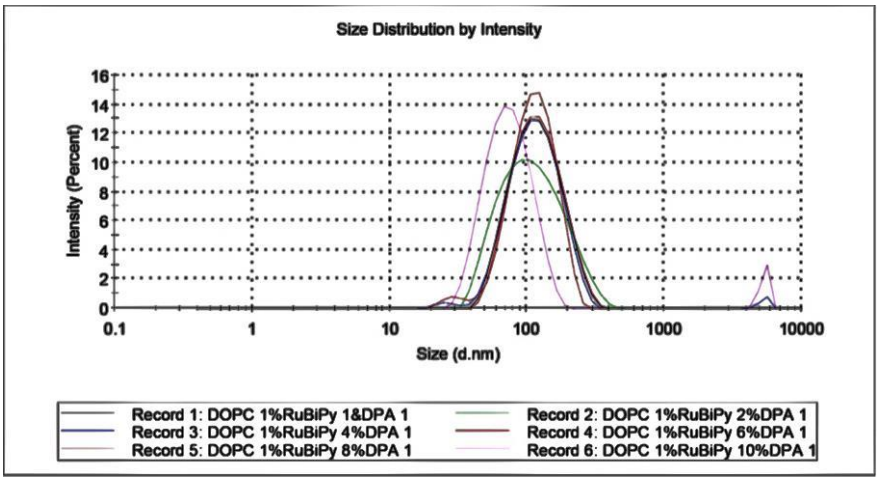


Figure S3: DLS measurement for DOPC (0.89–0.97 mM) vesicles with 1 mol. % **1** (0.01 mM) and different loadings of **DPA** (1–10 mol. %) after 24 h.

Disassembling of vesicles by methanol addition

Extruded vesicles can be dissolved by addition of 3 vol. eq. of methanol. The recovered fluorescence and absorbance indicates an aggregation and self-quenching of **DPA** in the bilayer (Fig. S4). With 2 vol. eq. of methanol vesicles do not disintegrate immediately.

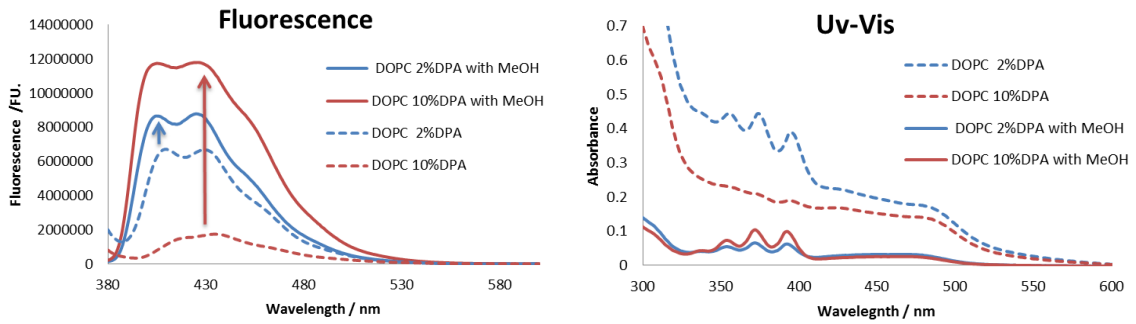


Figure S4: Absorption and emission spectra of aq. vesicular solution of DOPC (0.89, 0.97 mM) vesicles functionalised with 1 mol % of **1** (0.01 mM) and 2 or 10 mol % of **DPA** (0.02, 0.1 mM) before and after addition of 3 vol. eq of MeOH.

Amphiphilic DPA derivatives

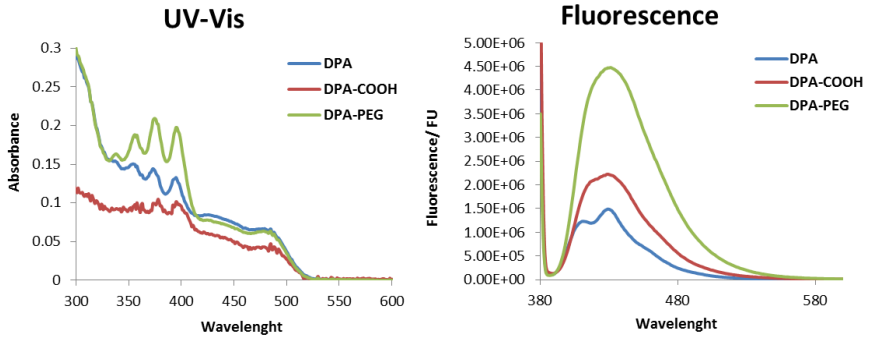


Figure S5: Absorption and emission spectra of aq. vesicular solution of DOPC (0.95 mM) vesicles functionalised with 1 mol % of **1** (0.01 mM) and 4 mol % of **DPA**, **DPA-COOH** or **DPA-PEG** (0.04 mM).

Vesicles with amphiphilic derivatives of **DPA** (**DPA-COOH**, **DPA-PEG**) were prepared in the same manner as for **DPA** and yielded stable 100 nm vesicles just by simple sonication (Fig. S6)

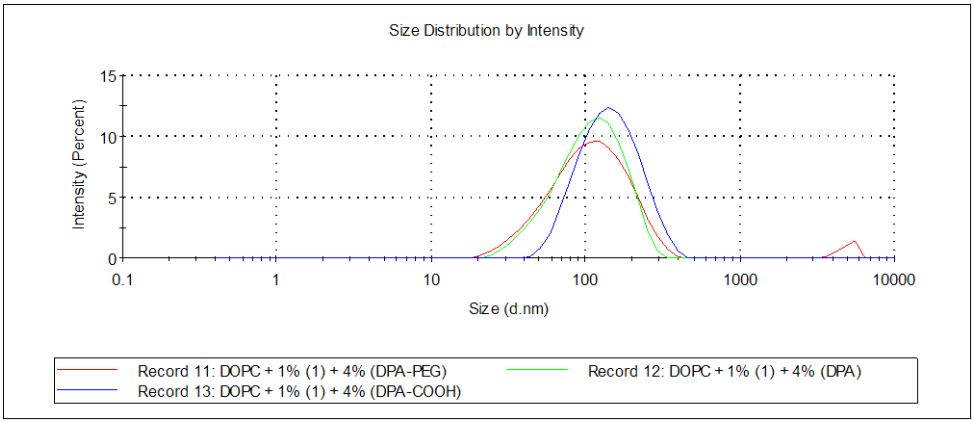


Figure S6: Size distribution of DOPC vesicles: 1 mol % of **1** (0.01 mM) and 4 mol % of **DPA** derivatives (0.04 mM) and 95 mol % DOPC (0.95 mM).

Lipids

Vesicular systems with different lipids were prepared in the same manner as for DOPC with exception for DSPC where the solution during sonication and extrusion was heated to 70 °C to be above the transition temperature of the lipid (for DSPC 55 °C). Optical spectra of the obtained systems were measured (Fig. S7).

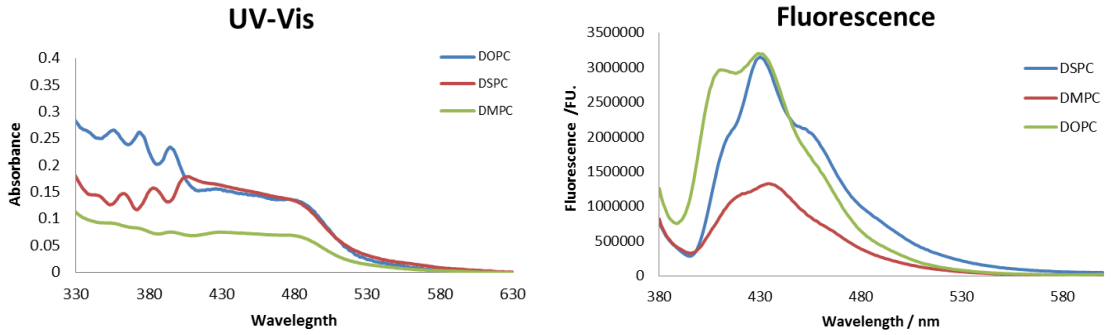


Figure S7: Absorption and emission spectra of vesicular systems with different lipids (0.97 mM) functionalised with 1 mol % **1** (0.01 mM) and 6 mol % of **DPA** (0.06 mM) in MiliQ water.

TTA measurements

TTA delayed fluorescence was recorded by photon counting. Measurement data is saved as a 2D Matrix for 512 lines and 512 columns, so that every line is a spectrum at a given time and every column is a time trace at a given wavelength. The intensity values for the delayed fluorescence were calculated by summation over all cells in the 2D Matrix that fall in a specified time and wavelength range. This range was from 370 nm to 470 nm and from 2µs (to avoid the scattered excitation light) after the excitation event to the end of the measurement. Fluorescence spectra and time traces were always displayed as a sum over a range several µs or nm.

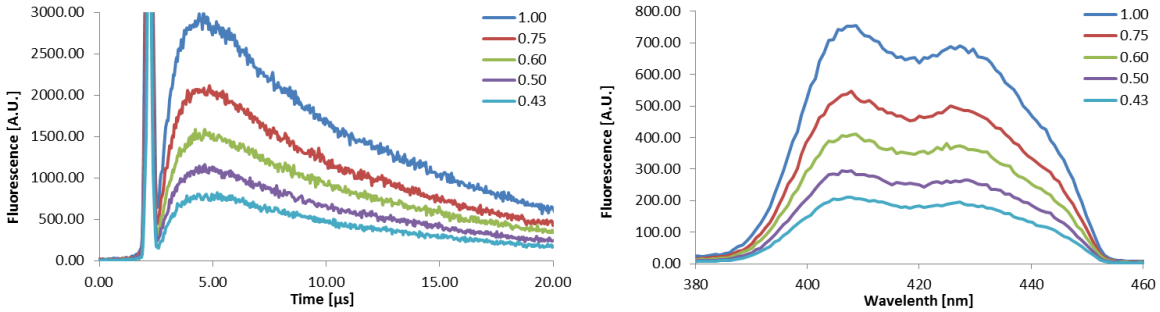


Figure S8: Example of measured delayed fluorescence in dilution experiments for DOPC vesicles (0.93 mM) with **1** (0.01 mM) and **DPA** (0.06 mM). Spectra correspond with relative dilution c/c_0. The time traces are averaged over 405 nm to 430 nm and the spectra are the average from 2µs after the excitation to the end of the measurement.

The time dependence of the delayed fluorescence could be modeled very well by a simple model. The sensitizer triplet can decay spontaneously (k_s), through energy transfer to the annihilator ($k_{ET}[A]$), through quenching by other compounds in the sample, and by triplet-triplet annihilation. If we neglect the last process, the time dependence will be monoexponential.

$$c_s(t) = c_s(0)exp(-k_1 t)\ ;\ k_1 = k_s + k_{ET}[A]_0$$

Where we have subsumed all pseudo first order quenching processes into k_s. The annihilator triplet is produced from the sensitizer and decays both spontaneously and by triplet triplet annihilation (TTA)

$$\frac{dc_A}{dt} = k_{ET}[A]_0 c_S - k_A c_A - k_{TTA} c_A^2$$

If we assume that TTA makes a negligible contribution to the decay, the time dependence of c_A is given by

$$c_A(t) = \frac{k_{ET}[A]_0}{k_1 - k_A}\left(exp(-k_A t) - exp(-k_1 t)\right)$$

And the time dependence of the delayed fluorescence is given by

$$F(t) = \Phi_F k_{TTA} c_A^2$$

Where Φ_F is the fluorescence quantum yield of the excited singlet state of the acceptor. A fit of this model to the data yields the two rate constants and the amplitude. We observe values from 5.0 to 5.2 × 10^4 s^{-1} for k_A and values from 1.44 to 1.61 × 10^6 s^{-1} for k_1. The amplitude increase linearly with the acceptor concentration (see figure S9).

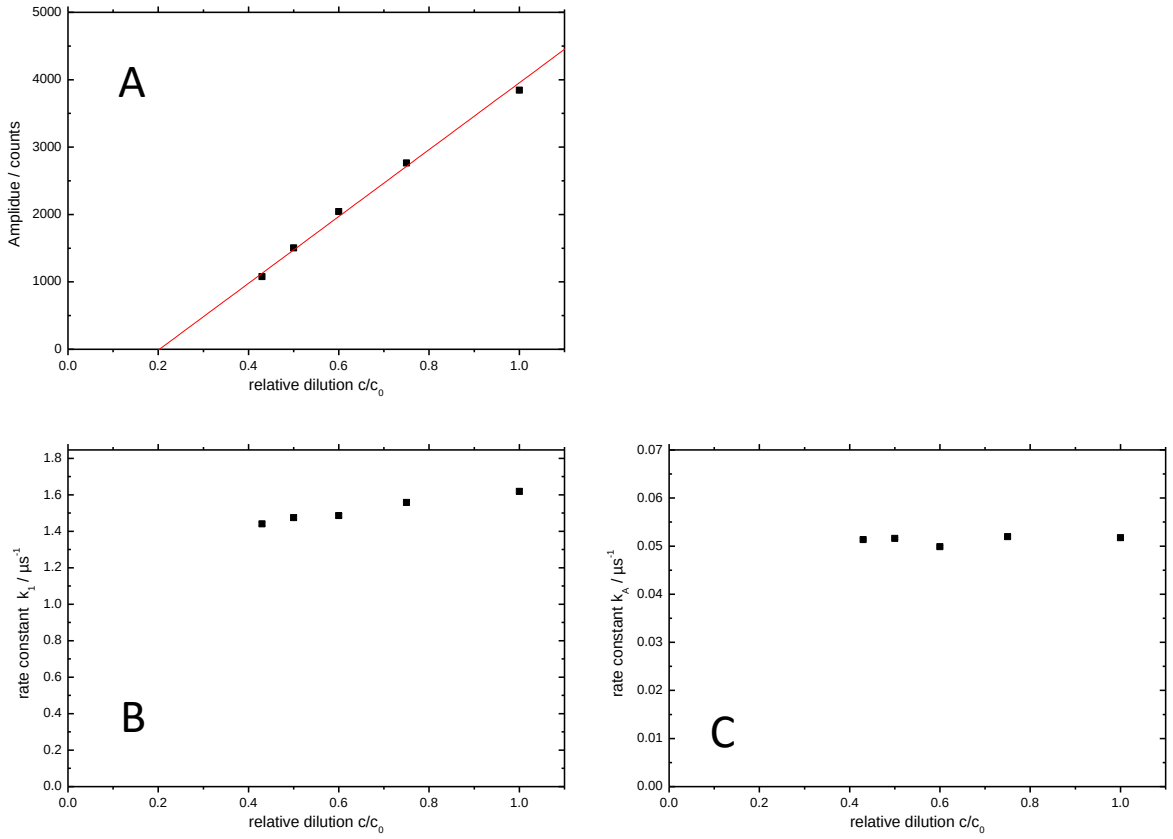
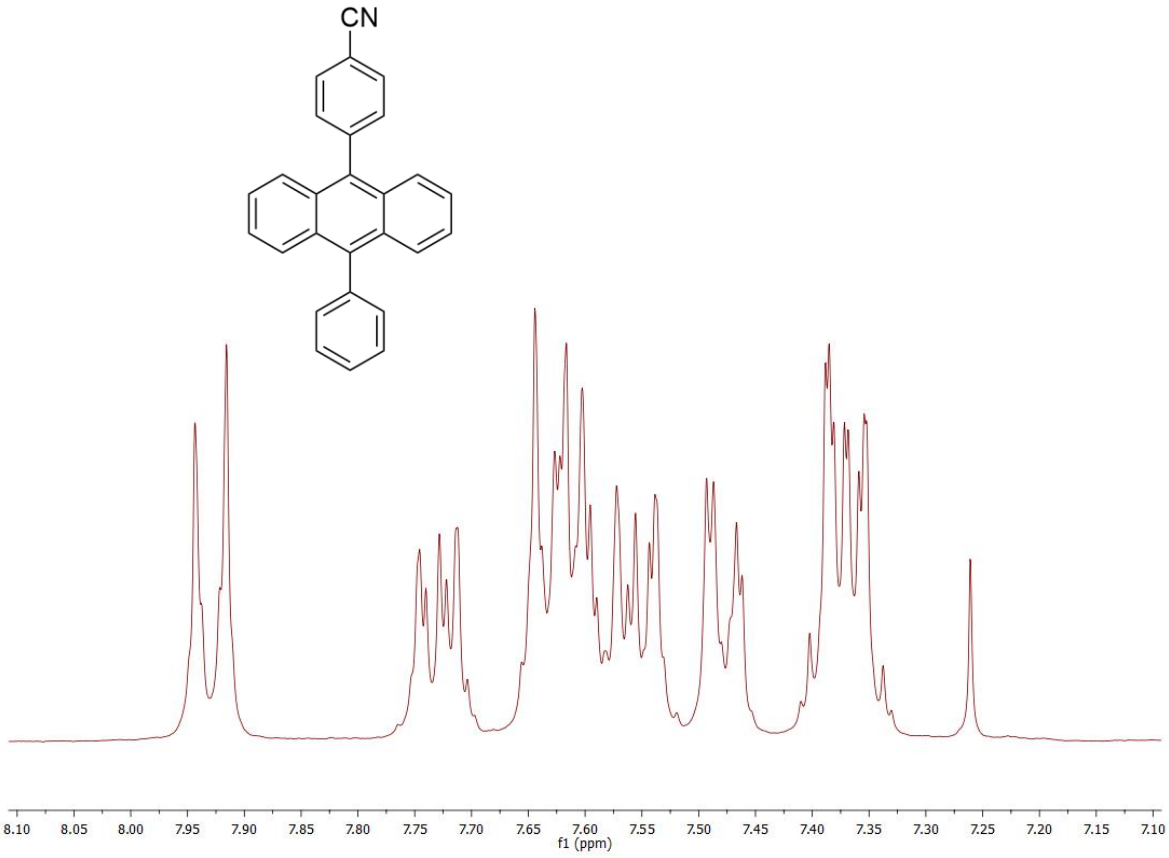
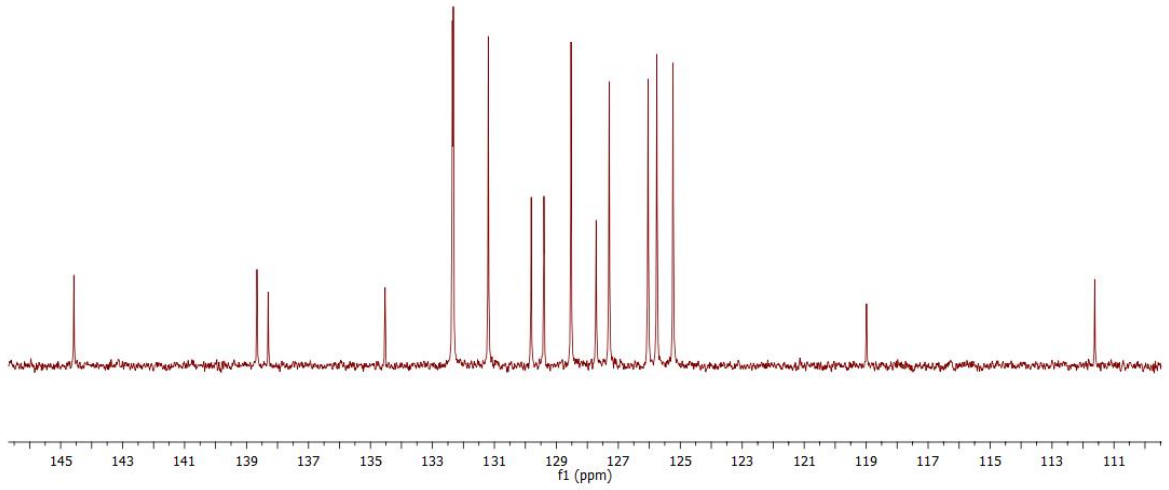
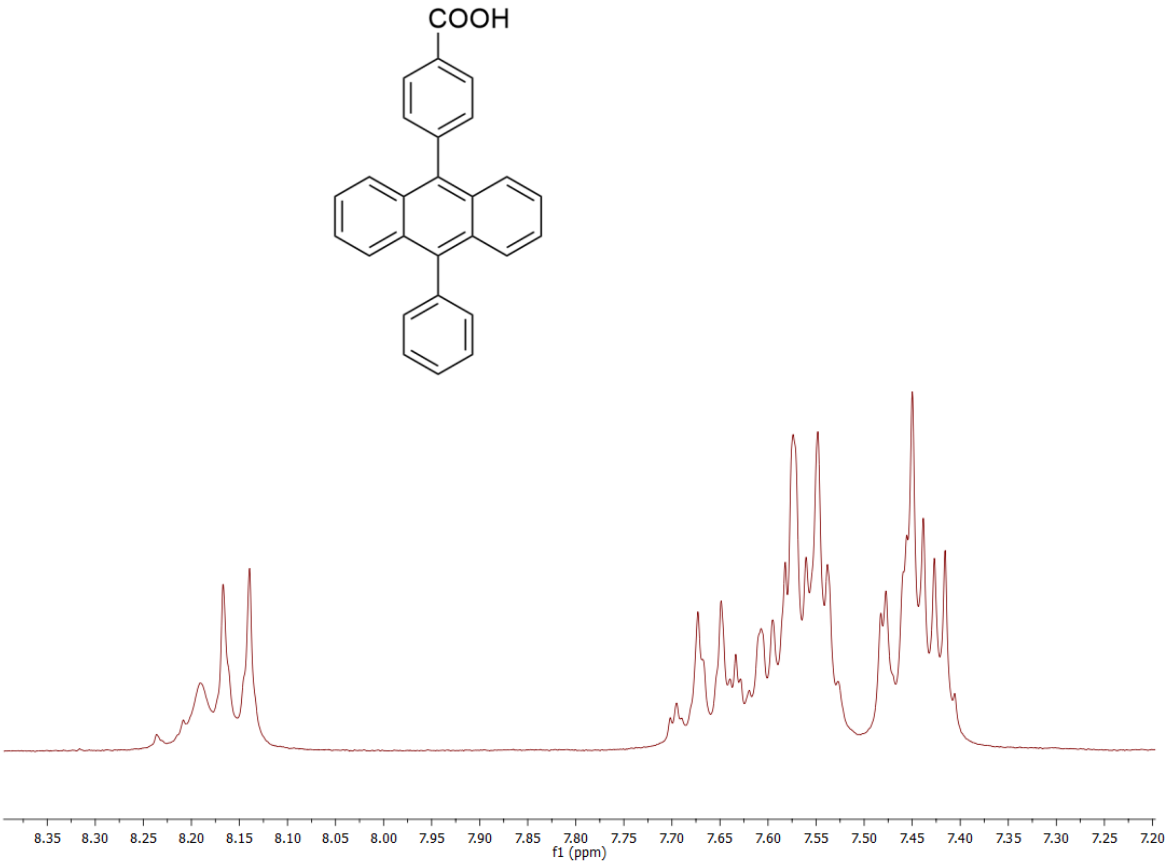


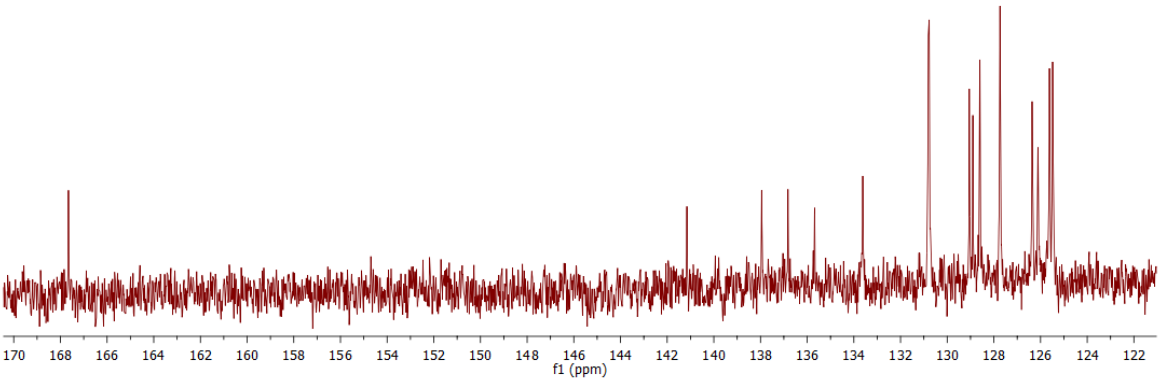
Figure S9: Plots of the Amplitude (**A**) rate constants k_A (**B**) and k_1 (**C**) vs the relative concentration of vesicles corresponding to the measurements displayed in S8.

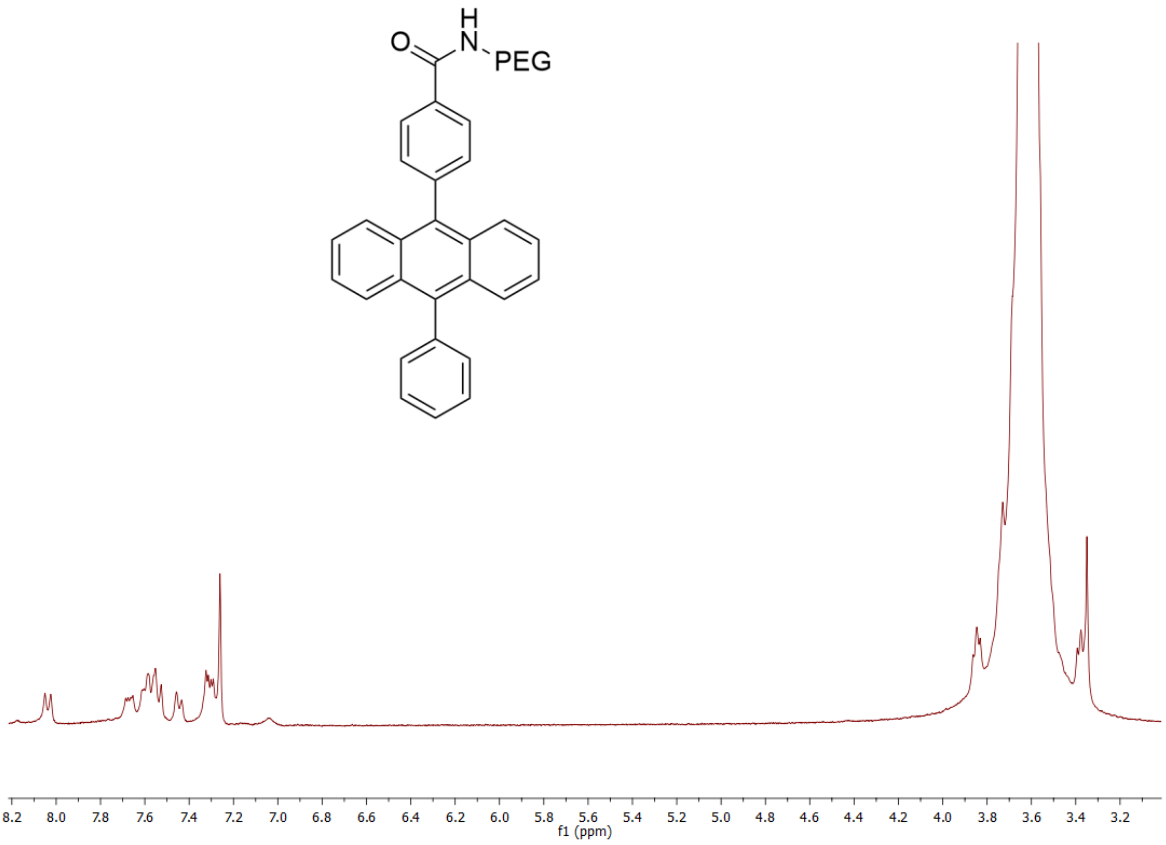
NMR spectra


CN
8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20 7.15 7.10
f1 (ppm)


145 143 141 139 137 135 133 131 129 127 125 123 121 119 117 115 113 111
f1 (ppm)


COOH
8.35 8.30 8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20
f1 (ppm)


170 168 166 164 162 160 158 156 154 152 150 148 146 144 142 140 138 136 134 132 130 128 126 124 122
f1 (ppm)



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