Encapsulation and covalent binding of molecular payload in enzymatically activated micellar nanocarriers

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Supporting Information

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Instrumentation:

HPLC: All measurements were recorded on a Waters Alliance e2695 separations module equipped with a Waters 2998 photodiode array detector. All solvents were purchased from Bio-Lab Chemicals and were used as received. All solvents are HPLC grade. **^{1}H and ^{13}C NMR:** spectra were recorded on Bruker Avance III 400MHz spectrometer. Chemical shifts are reported in ppm and referenced to the solvent. The molecular weights of the PEG-dendron hybrids were determined by comparison of the areas of the peaks corresponding to the PEG block (3.63 ppm) and the protons peaks of the dendrons. **GPC:** All measurements were recorded on Viscotek GPCmax by Malvern using refractive index detector and PEG standards (purchased from Sigma-Aldrich) were used for calibration. **Infrared spectra:** All measurements were recorded on a Bruker Tensor 27 equipped with a platinum ATR diamond. **Fluorescence spectra:** All measurements were recorded on an Agilent Technologies Cary Eclipse Fluorescence Spectrometer using quartz cuvettes or TECAN Infinite M200Pro device. **MALDI-TOF MS:** Analysis was conducted on a Bruker AutoFlex MALDI-TOF MS (Germany) and also on a Waters MALDI synapt (USA). DHB matrix was used. **TEM:** Images were taken by a Philips Tecnai F20 TEM at 200kV. **DLS:** All measurements were recorded on a Malvern Zetasizer NanoZS. **UV lamp**: UVP® Model XX-15L UV Bench Lamp, 15-watt, 365nm UV.

Materials:

2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%), Esterase from porcine liver (PLE), N,N'-dicyclohexylcarbodiimide (DCC, 99%), Sephadex® LH20 and dry DMF were purchased from Sigma-Aldrich. 2-Mercaptoethanol (99%) was purchased from Alfa Aesar and Phenyl acetic acid was purchased from Fluka. All solvents were purchased from Bio-Lab and were used as received. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories, Inc.

Synthesis

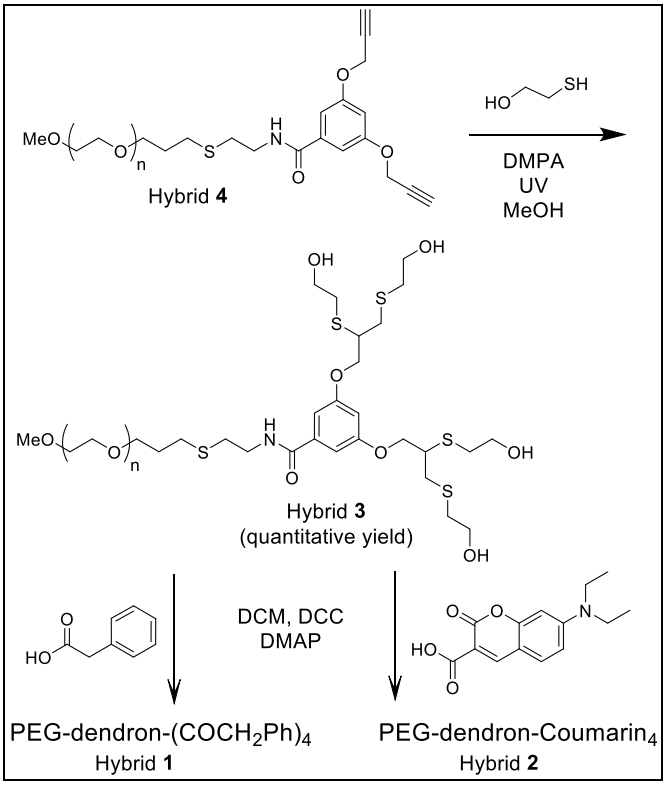

Figure S1: Preparation of hybrids **1** and **2.**

Hybrid 3:

185 mg (34.7µmol) of hybrid **6**[1] were dissolved in MeOH (1mL). 2-Mercaptoethanol (194 µL, 2.77 mmol, 40 eq. per yne) and DMPA (7.10 mg, 27.7 µmol, 0.4 eq. per yne) were added. The solution was purged with nitrogen for 15 minutes and then placed under UV light at 365nm for 2 hours. The crude mixture was loaded on a MeOH based LH20 SEC column. The fractions that contained the product were unified and the MeOH was evaporated in vacuum to yield an oily residue. In order to facilitate the removal of residual MeOH and solidification of the product, the oily residue was re-dissolved in DCM (5mL per 1g) followed by addition of Hexane (20mL per 1g). DCM and Hexane were evaporated to dryness and the obtained solid was dried under high vacuum. The product was obtained as an off-white solid (195mg, quantitative yield).

^{1}H-NMR ($CDCl_3$): δ 7.1 (t, *J* = 5.6 Hz, 1H, -N***H***-CO-), 7.00 (d, *J* = 2.2 Hz, 2H, Ar-H), 6.62 (t, *J* = 2.2 Hz 1H, Ar-H), 4.17-4.28 (m, 4H, Ar-O-C***H***$_2$-), 3.43-3.89 (m, PEG backbone + C***H***$_2$-OH), 3.36 (s, 3H, C***H***$_3$-O-PEG), 3.26-3.29 (m, 2H, -C***H***-S-), 2.73-3.01 (m, 14H, -C***H***$_2$-S-) 2.64 (t, *J* = 7.1 Hz, 2H, -O-CH_2-CH_2-C***H***$_2$-S-),1.85 (qui, *J* = 7.0 Hz, 2H, -O-CH_2-C***H***$_2$-CH_2-S-); ^{13}C-NMR ($CDCl_3$) δ 167.2, 159.6, 136.9, 106.3, 105.4, 72.0, 70.7, 70.3, 70.2, 69.5, 61.8, 61.2, 60.5, 59.1, 45.4, 41.4, 39.3, 36.3, 35.3, 35.0, 31.7, 29.8, 29.7, 28.5; FT-IR, ν(cm^{-1}):

2889, 1592, 1541, 1468, 1360, 1341, 1279, 1240, 1105, 945, 842; GPC (DMF+LiBr): Mn = 7.1kDa, PDI = 1.03. MALDI-TOF MS: molecular ion centered at 5.7kDa. Expected Mn = 5.6KDa.

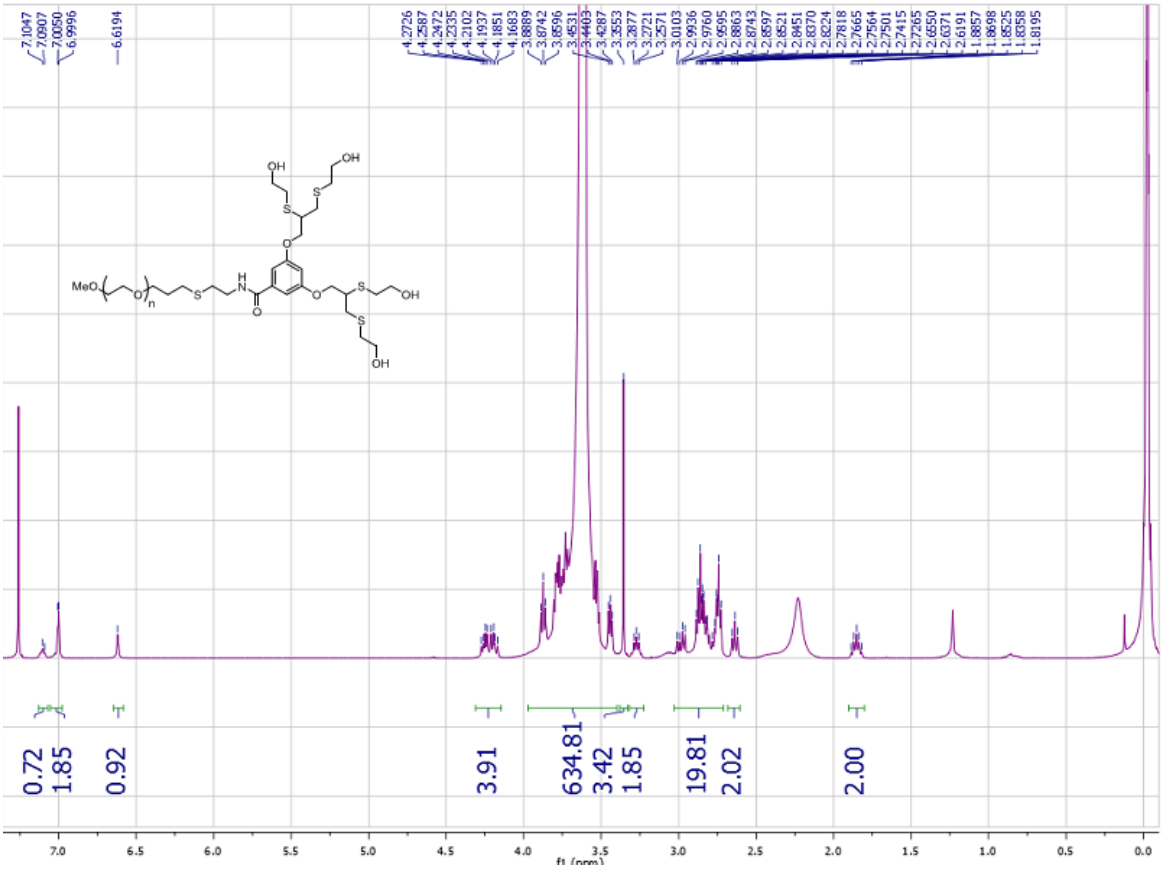

Figure S2: ^{1}H-NMR spectrum of hybrid **3** in $CDCl_3$.

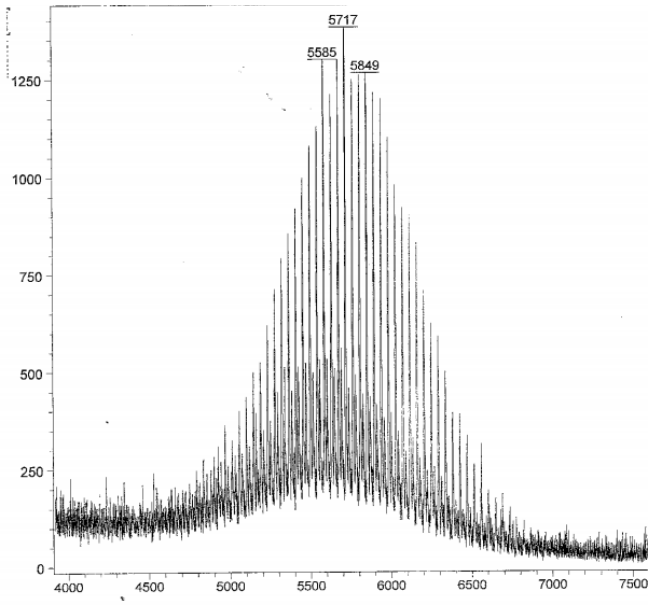

Figure S3: MALDI spectrum of hybrid **3**

Hybrid 1:

40 mg (7.3 µmol) of hybrid **3** were dissolved in dry DCM (3mL), Phenyl acetic acid (12mg, 87.5µmol, 3eq. per OH) was added. The solution was cooled to 0° followed by the addition of DCC (18mg, 88 µmol, 3eq. per OH) and DMAP (catalytic) dissolved in dry DCM (1mL cooled to 0°).

The reaction was heated to 30° and allowed to stir overnight. The crude mixture was filtered and evaporated to dryness. The crude mixture was dissolved in MeOH and loaded on a MeOH based LH20 SEC column. The fractions that contained the product were unified and the MeOH was evaporated in vacuum to yield an oily residue. In order to facilitate the removal of residual MeOH and solidification of the product, the oily residue was re-dissolved in DCM (5mL per 1g) followed by addition of Hexane (20mL per 1g). DCM and Hexane were evaporated to dryness and the obtained solid was dried under high vacuum. The product was obtained as an off-white solid (38mg, 87% yield).

^{1}H-NMR ($CDCl_3$): δ 7.22-7.30 (m, 20H, Ar-H), 6.95 (d, *J* = 2.2 Hz, 2H, Ar-H), 6.86 (t, *J* = 5.6 Hz, 1H, -N***H***-CO-), 6.56 (t, *J* = 2.2 Hz, 1H, Ar-H), 4.21-4.27 (m, 8H, C***H₂*** -O-CO-CH_2-Ph), 4.06-4.16 (m, 4H, Ar -O-C***H₂***-), 3.43-3.81 (m, PEG backbone), 3.36 (s, 3H, C***H₃***-O-PEG), 3.1-3.13 (m, 2H, -C***H***-S-), 2.65-2.94 (m, 14H, -CH-C***H₂***-S- + -S-C***H₂***-), 2.61 (t, *J* = 7.1 Hz, 2H, -O-CH_2-CH_2-C***H₂***-S-) 1.84 (qui, *J* = 6.9 Hz, 2H, -O-CH_2-C***H₂***-CH_2-S-);

^{13}C-NMR ($CDCl_3$) δ 171.6, 171.5, 166.9, 159.6, 136.9, 133.82*, 133.79*, 129.4, 128.7, 127.3, 127.28*, 106.3, 104.6, 72.0, 70.7, 70.3, 70.2, 69.8, 69.6, 64.2, 63.9, 59.1, 45.46, 41.32, 41.31, 39.3, 36.3, 34.9, 31.5, 30.4, 29.8, 29.7, 28.4; FT-IR, ν(cm^{-1}): 2890, 1735, 1592, 1537, 1468, 1360, 1341, 1279, 1239, 1107, 945, 842; GPC (DMF+LiBr): M_n = 7.1kDa, PDI = 1.03. MALDI-TOF MS: molecular ion centered at 6.2kDa. Expected Mn = 6.1KDa.

*These peaks have very close chemical shift and hence another digit was added in order to distinguish between them.

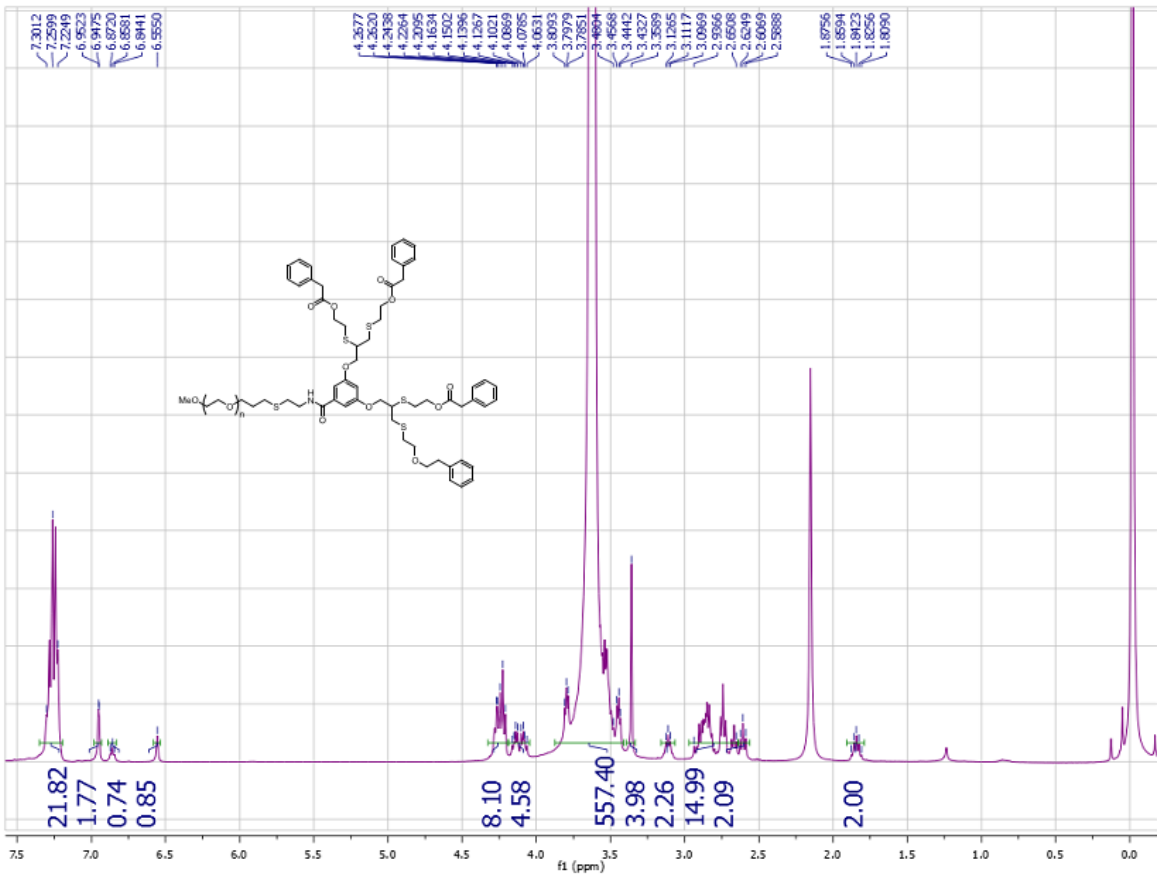

Figure S4: ^{1}H-NMR spectrum of hybrid **1** in $CDCl_3$.

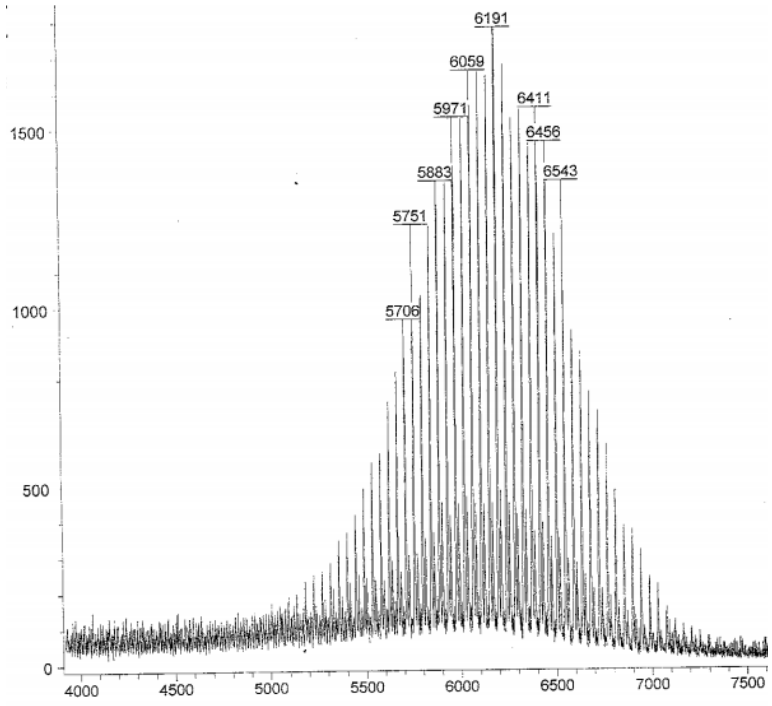

Figure S5: MALDI spectrum of hybrid **1.**

Hybrid 2:

38 mg (7.1 μmol) of hybrid **3** were dissolved in dry DCM (3mL), 7-diethylamino-3-carboxycoumarin[2] (coumarin acid) (74 mg, 0.28 mmol, 10eq. per OH) was added. The solution was cooled to 0° followed by the addition of DCC (59 mg, 0.28 mmol, 10eq. per OH) and DMAP (catalytic) dissolved in dry DCM (1mL cooled to 0°).

The reaction was heated to 30° and allowed to stir overnight. The crude mixture was filtered and evaporated to dryness. The crude mixture was dissolved in DCM and loaded on a MeOH:DCM (1:1) based LH20 SEC column. The fractions that contained the product were unified and the solvent was evaporated in vacuum to yield an oily residue. In order to facilitate the removal of residual MeOH and solidification of the product, the oily residue was re-dissolved in DCM (5mL per 1g) followed by addition of Hexane (20mL per 1g). DCM and Hexane were evaporated to dryness and the obtained solid was dried under high vacuum. The product was obtained as a yellow solid (33mg, 74% yield).

^{1}H-NMR ($CDCl_3$): δ 8.39 (s, 2H, Ar-H), 8.37 (s, 2H, Ar-H), 7.4 (t, *J* = 5.8 Hz, 1H, N***H***-CO-), 7.32 (d, *J* = 2 Hz, 2H, Ar-H), 7.30 (d, *J* = 2 Hz, 2H, Ar-H), 7.01 (d, *J* = 1.9 Hz, 2H, Ar-H), 6.62 (t, *J* = 2.1 Hz, 1H, Ar-H), 6.57 (d, *J* = 1.1 Hz, 2H, Ar-H), 6.55 (d *J* = 1.1 Hz, 2H, Ar-H), 6.38 (s, 2H, Ar-H), 6.37 (s, 2H, Ar-H), 4.41-4.48 (m, 8H, C***H₂*** -O-CO-CH_2-Ph), 4.17-4.28 (m, 4H, Ar -O-C***H₂***-), 3.49-3.80 (m, PEG backbone), 3.38-3.45 (m, 16H, N-*C**H₂***-CH_3-), 3.35 (s, 3H, C***H₃***-O-PEG), 2.75-3.1 (m, 16H, -C***H***-S- + -CH-C***H₂***-S- + -S-C***H₂***-), 2.63 (t, *J* = 7.2Hz, 2H, -O-CH_2-CH_2-C***H***$_2$-S-) 1.84 (qui, *J* = 7.1Hz, 2H, -O-CH_2-C***H₂***-CH_2-S-), 1.2 (t, *J* = 7 Hz, 24H, N-CH_2-***CH₃***-) ;

^{13}C-NMR ($CDCl_3$) δ 167.1, 163.9, 163.8, 159.6, 158.57*, 158.56*, 158.28*, 158.25*, 153.14*, 153.12*, 149.67*, 149.56*, 136.8, 131.4, 131.38*, 109.75*, 109.74*, 108.13*, 108.06*, 107.77*, 107.75*,106.5, 104.4, 99.5, 96.7, 72.0, 70.6, 70.3, 70.0, 69.7, 64.5, 64.1, 59.1, 45.55, 45.18, 39.8, 35.0, 31.5, 31.3, 30.4, 29.8, 29.7, 28.4, 12.5; FT-IR, ν(cm-1): 2890, 1756, 1583, 1512, 1468, 1359, 1341, 1279, 1241, 1101, 945, 842; GPC (DMF+LiBr): Mn = 7.3kDa, PDI = 1.03. MALDI-TOF MS: molecular ion centered at 6.7kDa. Expected Mn = 6.6KDa.

*These peaks have very close chemical shifts and hence another digit was added in order to distinguish between them.

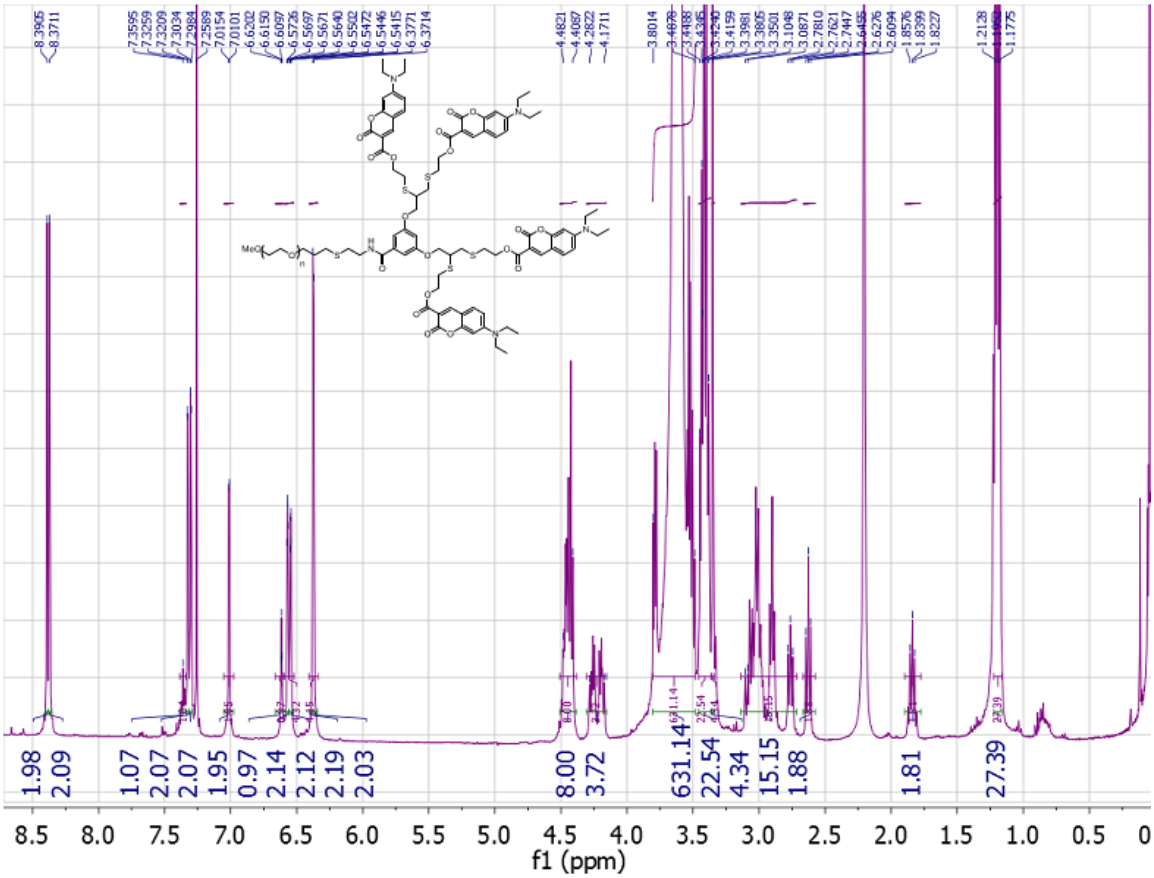

Figure S6: ^{1}H-NMR spectrum of hybrid **2** in $CDCl_3$.

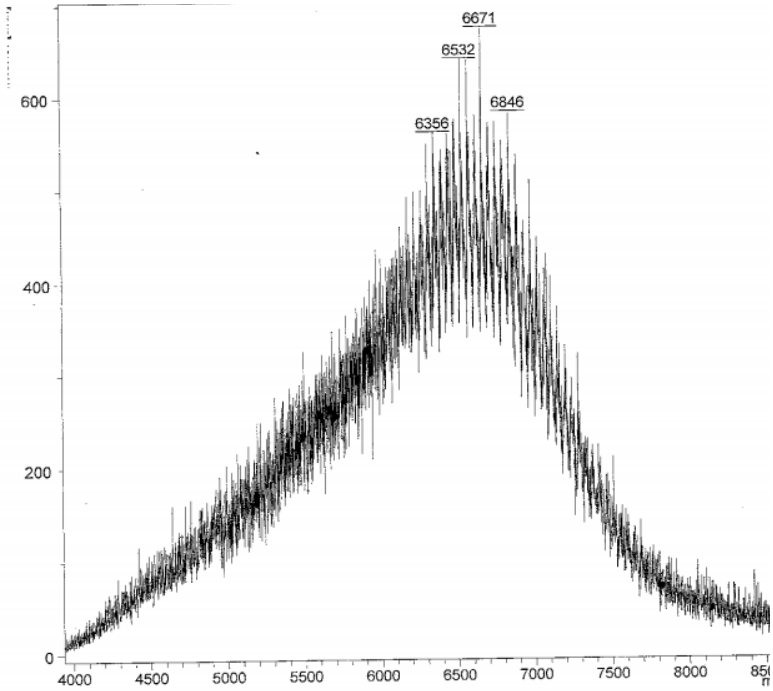

Figure S7: MALDI spectrum of hybrid **2**

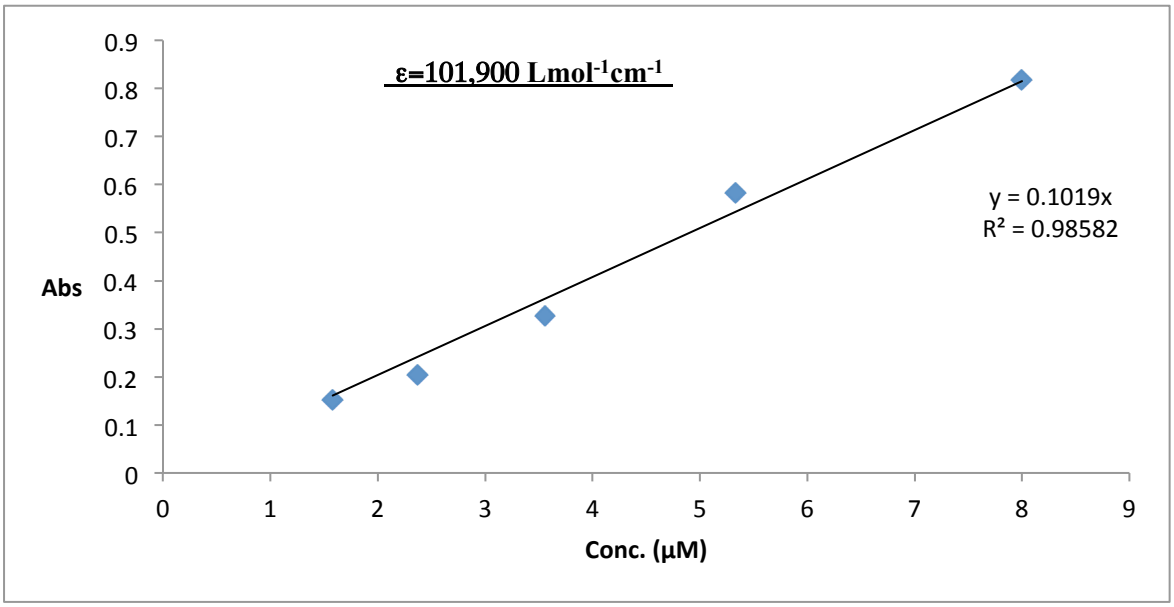

Figure S8: Beer-Lambert graph of hybrid **2**

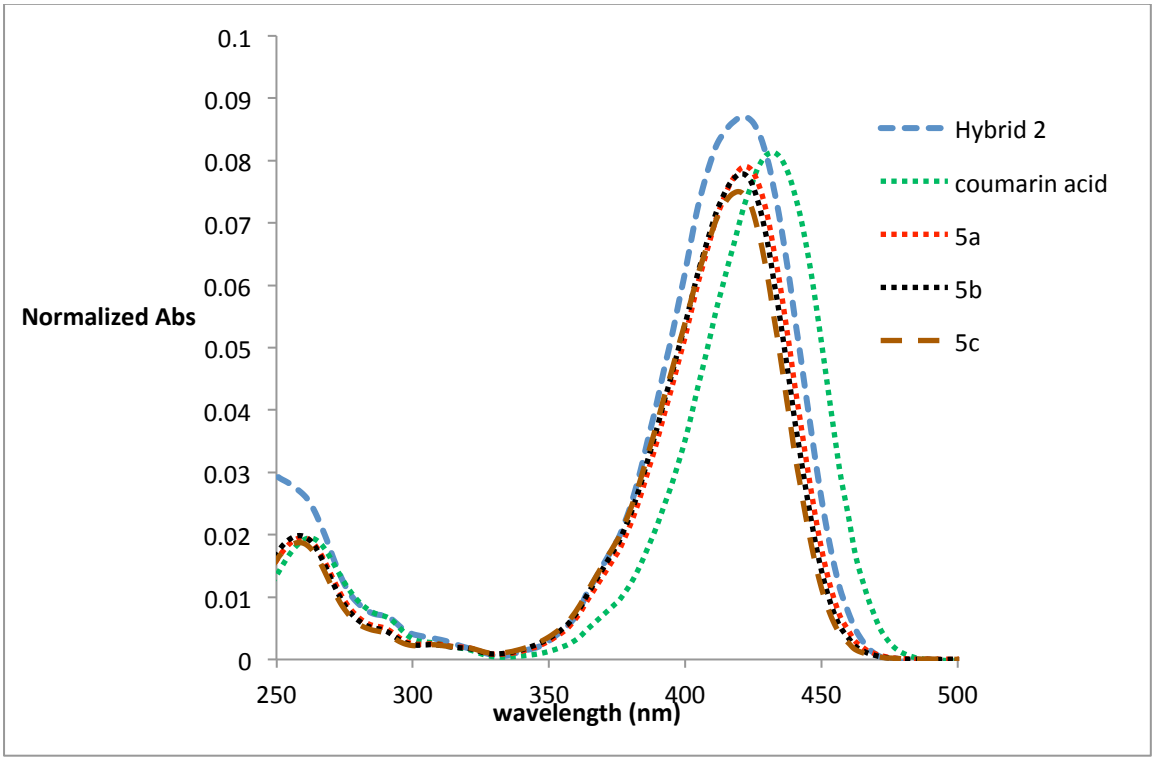

Figure S9: UV spectra of hybrid **2**, Coumarin acid, **5a**, **5b** and **5c**.

Gel Permeation Chromatography (GPC) Data

Instrument method:

Columns: 2 x PSS GRAM 1000Å + PSS GRAM 30Å

Columns Temperature: 50°C

Flow rate: 0.5ml/min

Mobile phase: DMF + 50mM LiBr

Detector: Refractive index detector at 50°C

Injection Volume: 50µL

General sample preparation:

Polymers were dissolved in the mobile phase to give a final concentration of 10mg/ml. Solution was filtered through a 0.22µm PTFE syringe filter.

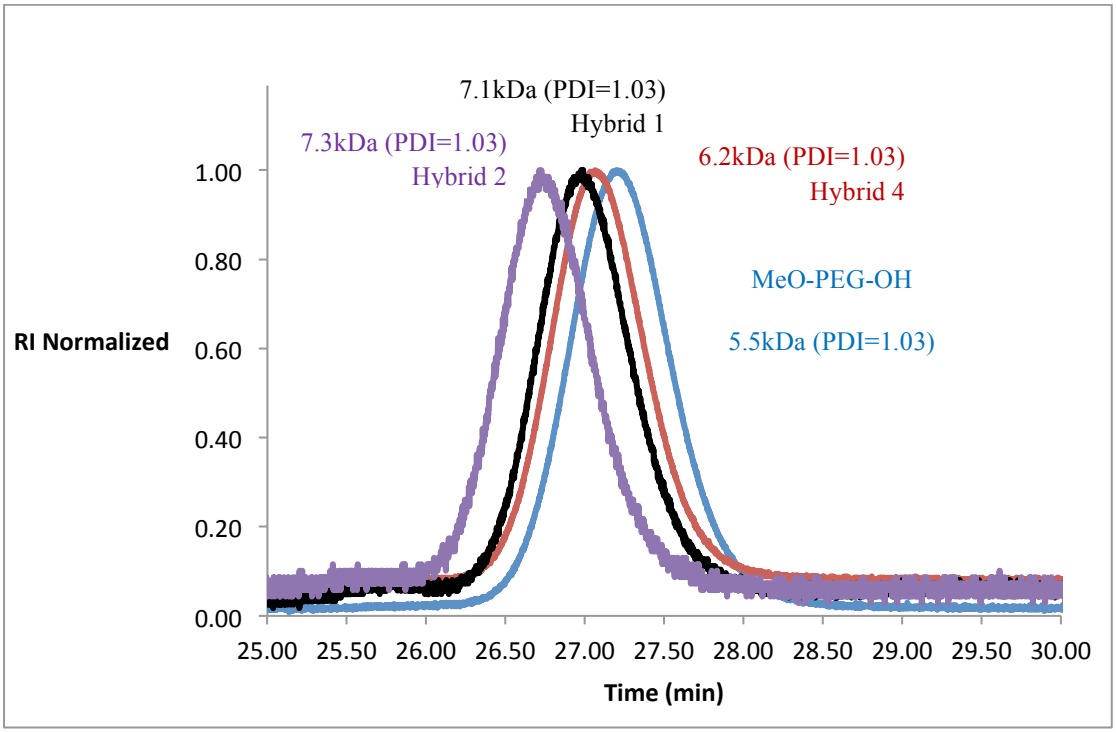

Figure S10: GPC data of commercial available 5kDa Poly (Ethylene glycol) methyl ether (blue), hybrid **4** (red), hybrid **1** (black) and hybrid **2** (purple).

Critical Micelle Concentration (CMC) Measurements

Instrument: Fluorescence Spectrometer using 96 wells plate.

Excitation: 550nm

Emission intensity scan: 580-800nm

Diluent solution preparation:

Into 100ml phosphate buffer solution (pH 7.4), 45µL of Nile red stock solution (0.88mg/ml in Ethanol) were added and mixed to give a final concentration of 1.25µM.

CMC measurement for hybrids **1** and **2**: A 800µM solution was prepared in diluent. Solution was sonicated for 15 minutes. This solution was repeatedly diluted by a factor of 2 with diluent. 100µL of each solution were loaded onto a 96 wells plate. The fluorescence emission intensity was scanned for each well. Maximum emission intensity was plotted vs. concentration in order to determine the CMC.

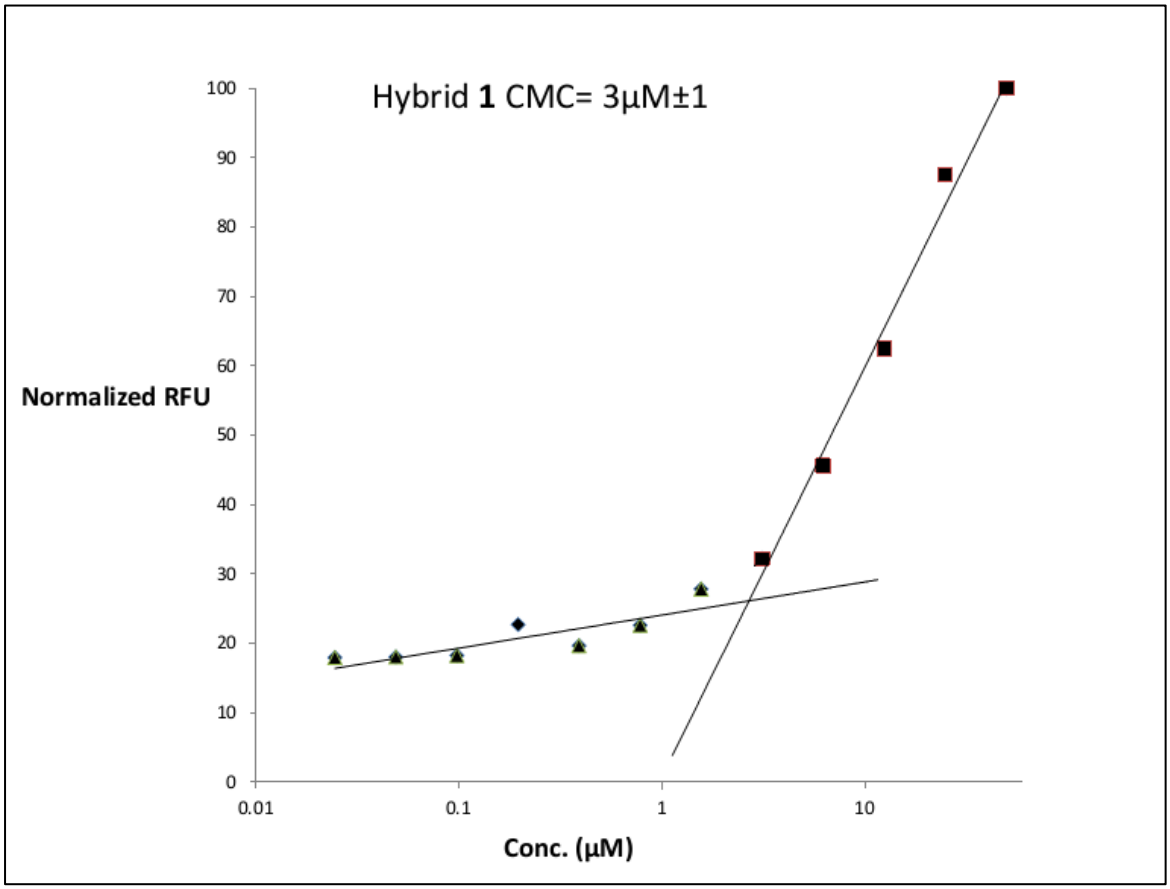

Figure S11: CMC measurement of hybrid **1**.

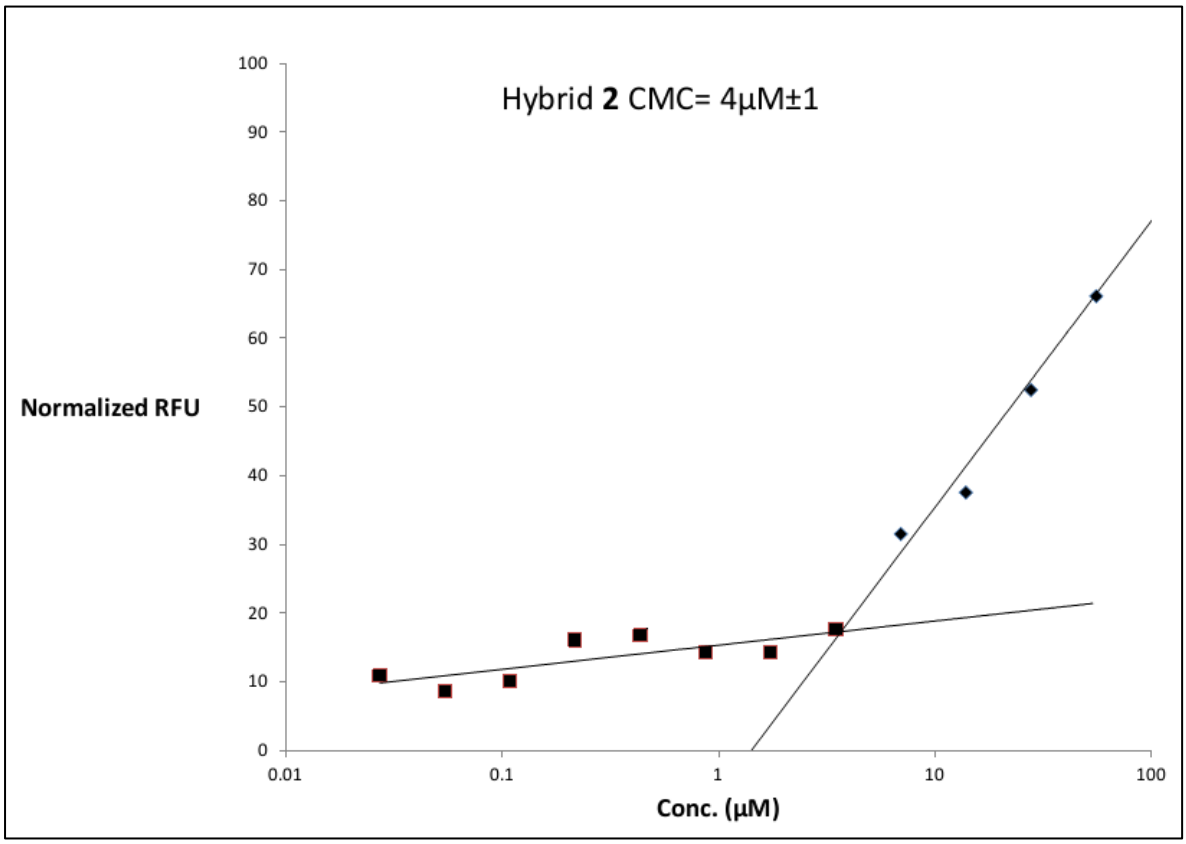

Figure S12: CMC measurement of hybrid **2.**

Dynamic Light Scattering (DLS)

General sample preparation:

Hybrid **1** was dissolved in phosphate buffer (pH 7.4) to give a final concentration of 160µM. Hybrid **2** was dissolved in phosphate buffer (pH 7.4) to give a final concentration of 40µM. Solutions were sonicated for 15 minutes and filtered through a 0.22µm nylon syringe filter. 800µL of these solutions were accurately transferred into a polystyrene cuvette and a measurement was performed (t=0), all measurements were repeated 3 times.

For micelle degradation in the presence of 0.23µM PLE enzyme: 2.4µL of PLE enzyme stock solution (80µM in phosphate buffer pH 7.4) were added. Measurement was performed after 2 hours.

For micelle degradation in the presence of 2.3µM PLE enzyme: 24µL of PLE enzyme stock solution (80µM in phosphate buffer pH 7.4) were added. Measurement was performed after 24 hours.

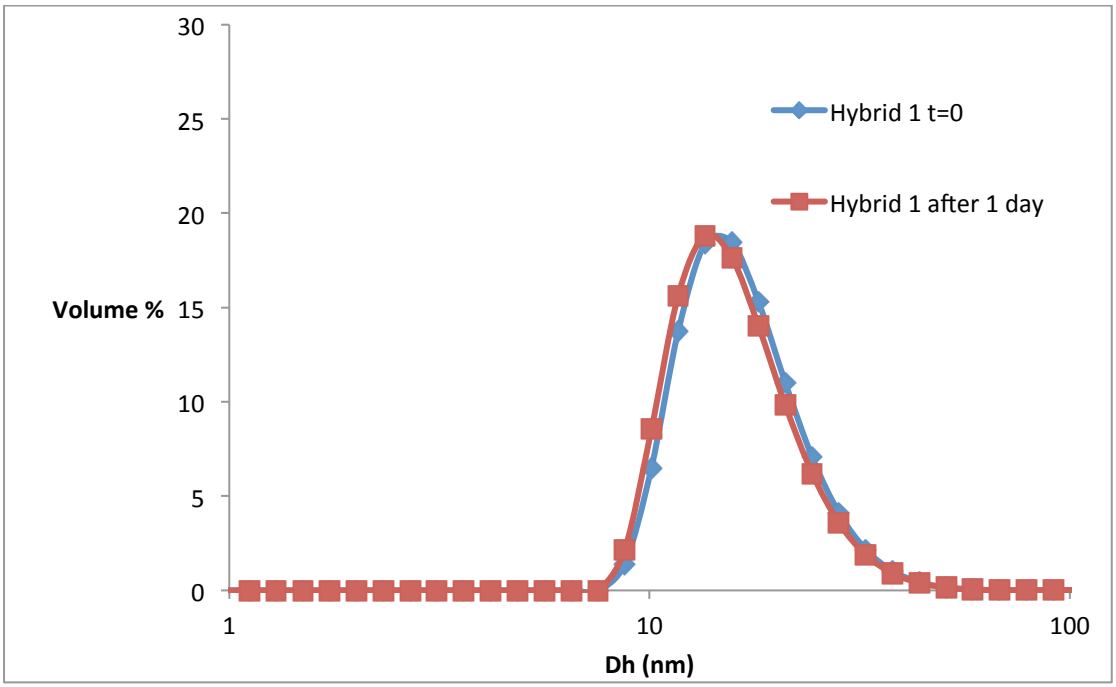

Figure S13: DLS data show the size of the micelles based on hybrid **1** is unaffected in the absence of activating enzyme PLE after 24 hours.

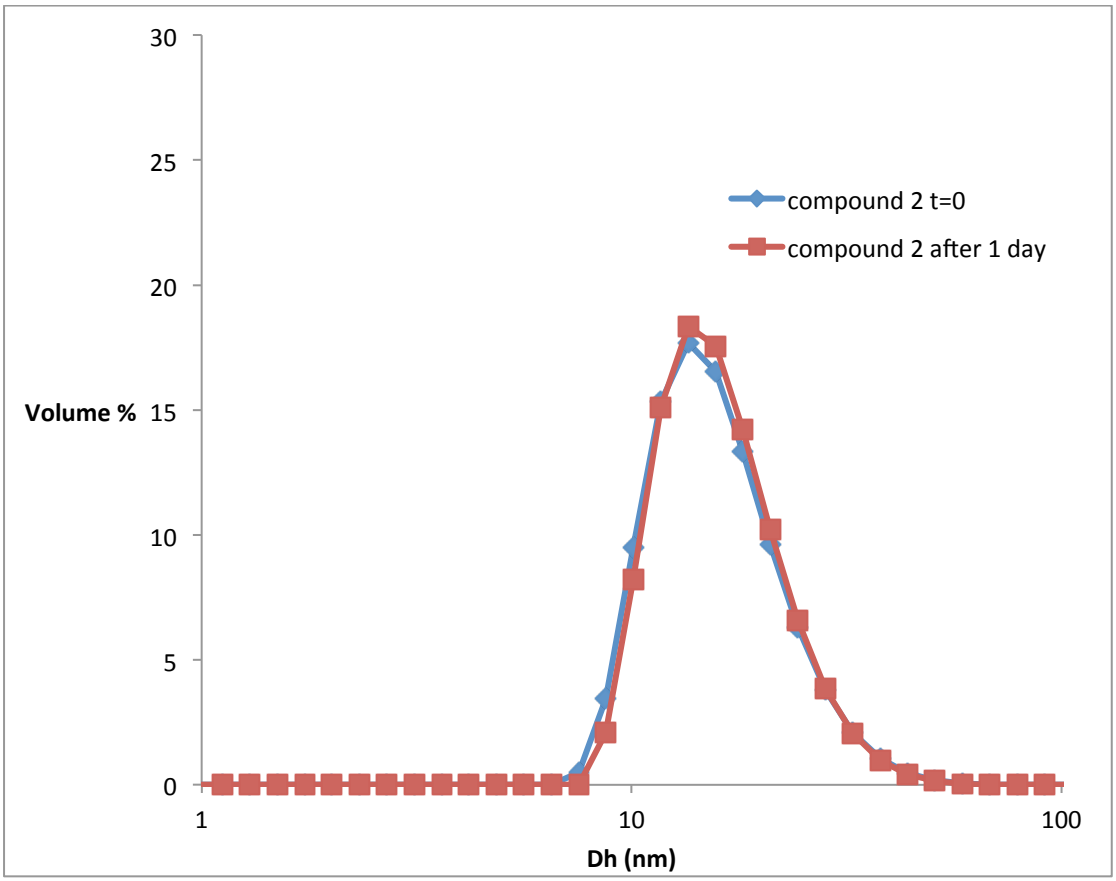

Figure S14: DLS data show the size of the micelles based on hybrid **2** is unaffected in the absence of activating enzyme PLE after 24 hours.

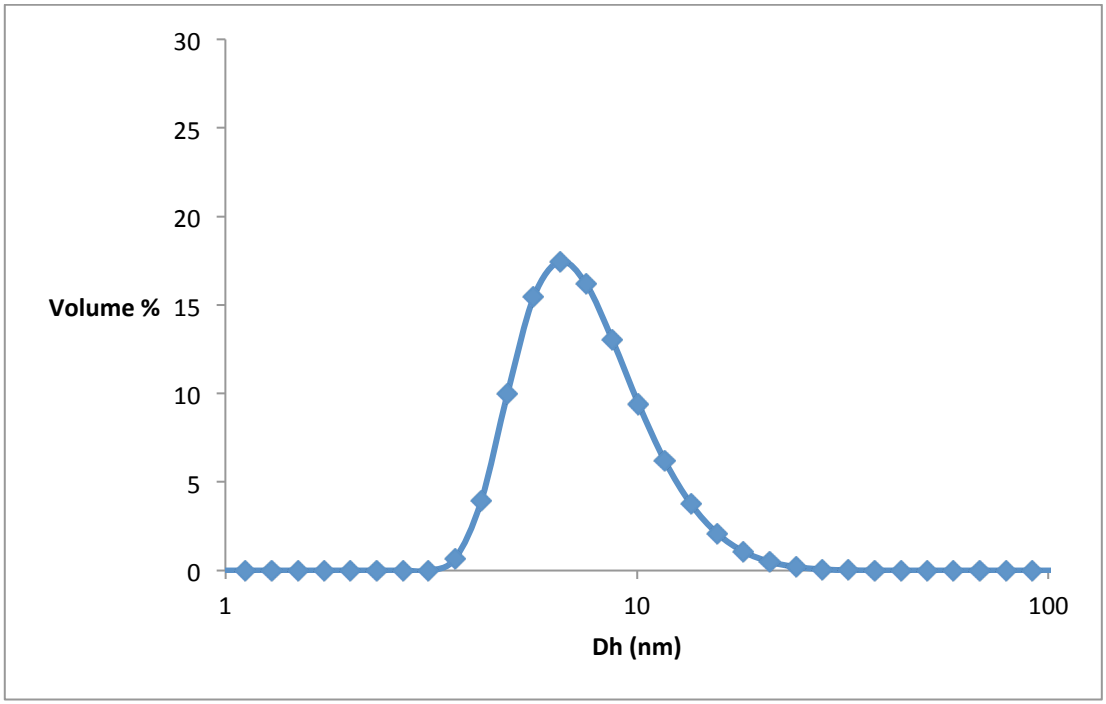

Figure S15: DLS data show the size of the PLE at 2.3µM.

Transmission Electron Microscopy (TEM)

General sample preparation:

5 µL sample solution were dropped cast onto carbon coated copper grids and inspected in a transmission electron microscope (TEM), operated at 200 kV (Philips Tecnai F20). The excessive solvent of the droplet was wiped away using a solvent-absorbing filter paper after 1 min and the sample grids were left to dry in air at room temperature for 5 minutes. This procedure was repeated 3 times. After the third cycle the sample grids were left to dry in air at room temperature overnight.

^{1}H-NMR in D_2O for hybrids 1 and 2

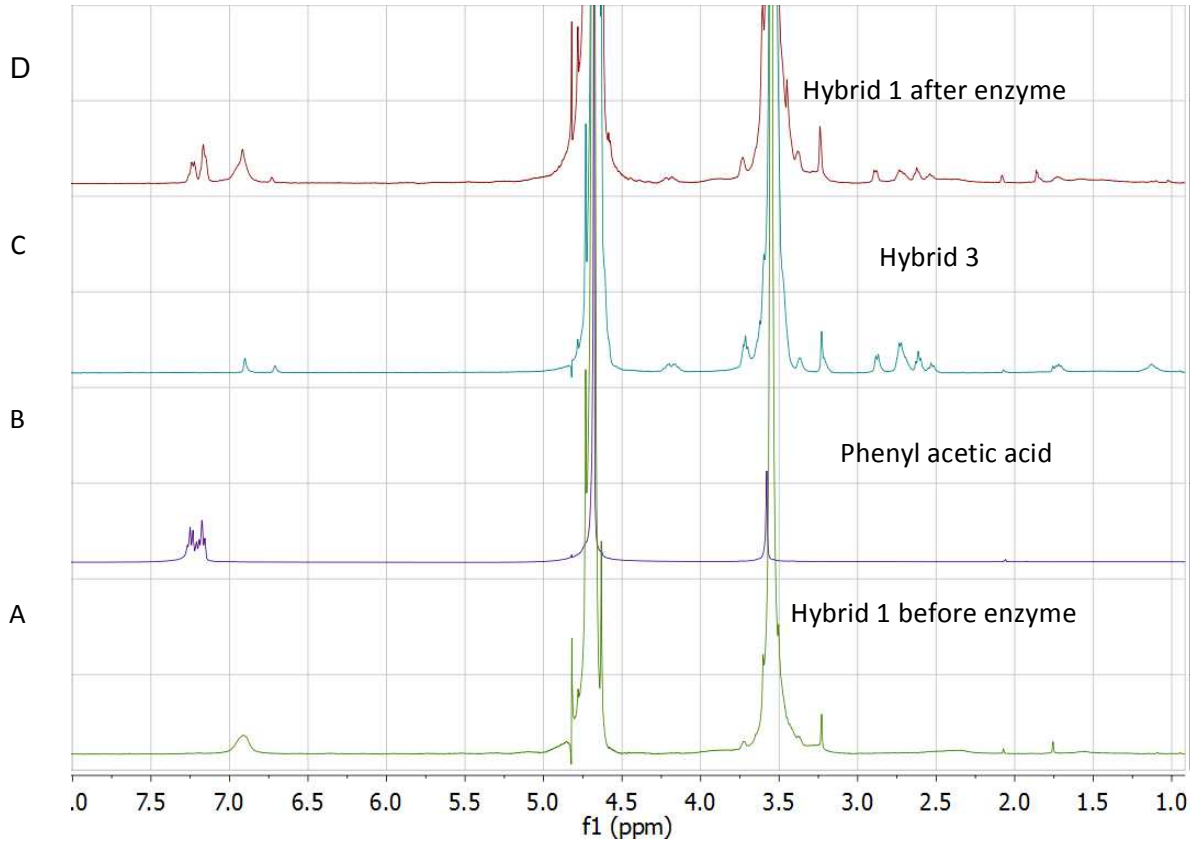

Figure S16: ^{1}H-NMR spectra in D_2O of (A) hybrid **1** (160 µM), showing only PEG protons before the addition of PLE; (B) Phenyl acetic acid (640 µM); (C) Hybrid **3** (160 µM); (D) hybrid **1** (160 µM) after incubation with the activating enzyme, PLE (0.76 µM).

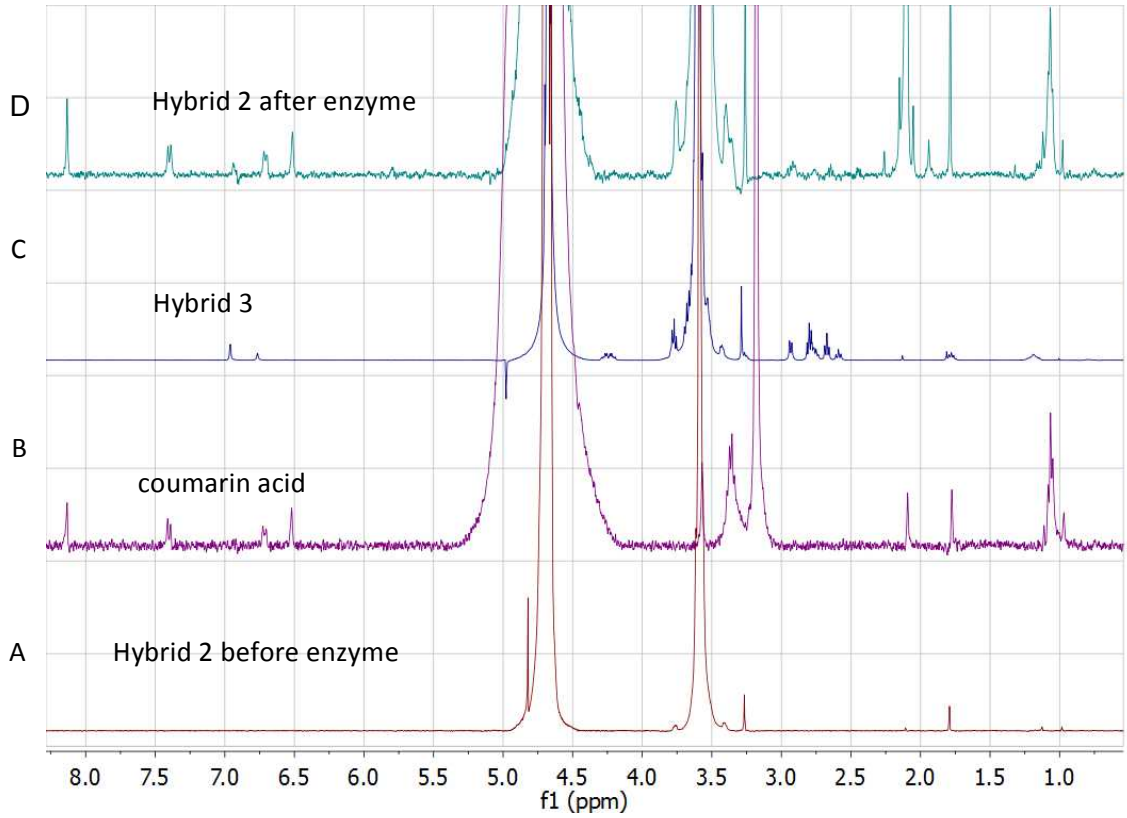

Figure S17: ^{1}H-NMR spectra in D_2O of (A) hybrid **2** (160 µM), showing only PEG protons before the addition of PLE; (B) Coumarin acid (640 µM); (C) Hybrid **3** (160 µM); (D) hybrid **2** (160 µM) after incubation with the activating enzyme, PLE (0.76 µM).

Fluorescence spectroscopy

Monitoring micelle disassembly with Nile Red fluorescence

Instrument method:

Excitation: 550nm

Emission scan: 575-800nm

Excitation and emission slits width: 20nm

Scan rate: 520nm/min

Sample preparation and measurement:

Hybrid **1** was dissolved in phosphate buffer (pH 7.4) to give a concentration of 160μM. Solution was sonicated for 15mintues. 2.0mL of this solution was accurately transferred to a quartz cuvette .A fluorescence emission scan was performed (t=0) and then 6μL of PLE stock solution (80μM in phosphate buffer pH 7.4) were added to give a final PLE concentration of 0.23μM. Repeating fluorescence scans were performed every 10 minutes for 6 hours.

Monitoring fluorescence of hybrid 2

Instrument method:

Excitation: 420nm

Emission scan: 450-800nm

Excitation and Emission slits width: 20nm

Scan rate: 520nm/min

Sample preparation and measurement:

Hybrid **2** was dissolved in phosphate buffer (pH 7.4) to give a concentration of 40μM. Solution was sonicated for 15 minutes. 2.0mL of this solution was accurately transferred to a quartz cuvette .A fluorescence emission scan was performed (t=0) and then 60μL of PLE stock solution (80μM in phosphate buffer pH 7.4) were added and mixed for 5 seconds (vortex mixer) to give a final PLE concentration of 2.3μM. Repeating fluorescence scans were performed every 20 minutes for 5 hours.

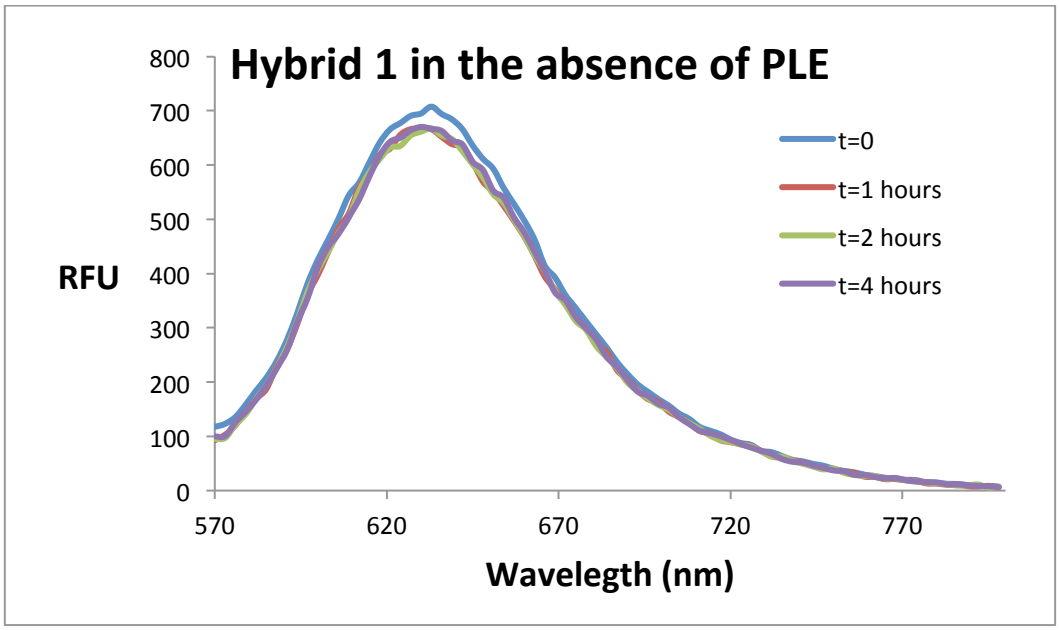

Figure S18: Fluorescence emission spectra of encapsulated Nile red encapsulated in micelles of hybrid **1**, is unaffected in the absence of the activating enzyme PLE after 4 hours in buffer.

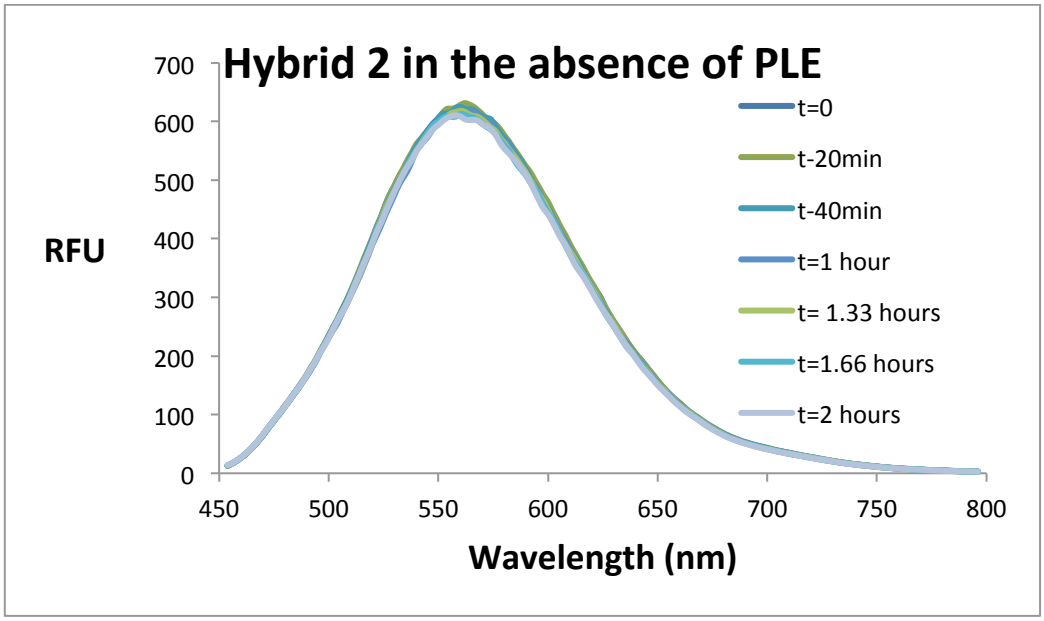

Figure S19: Fluorescence emission spectra of hybrid **2** is unaffected in the absence of the activating enzyme PLE after 2 hours in buffer.

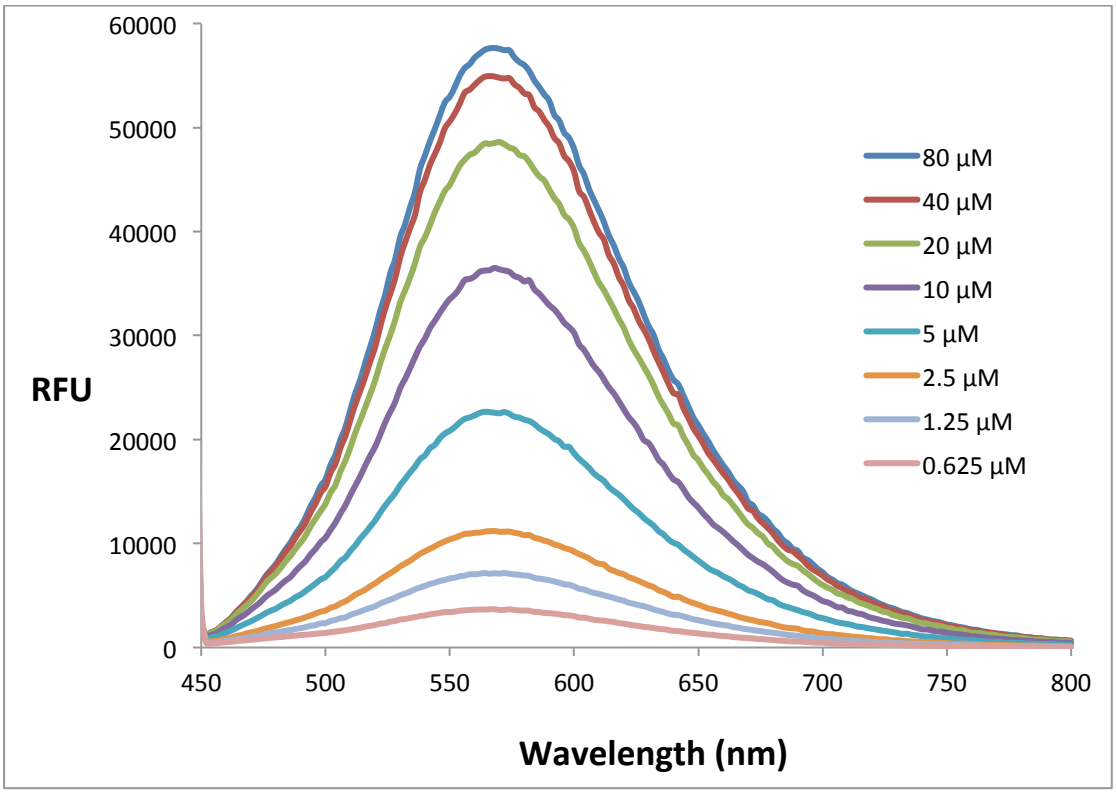

Figure S20: Fluorescence emission spectra of hybrid **2** at different concentrations.

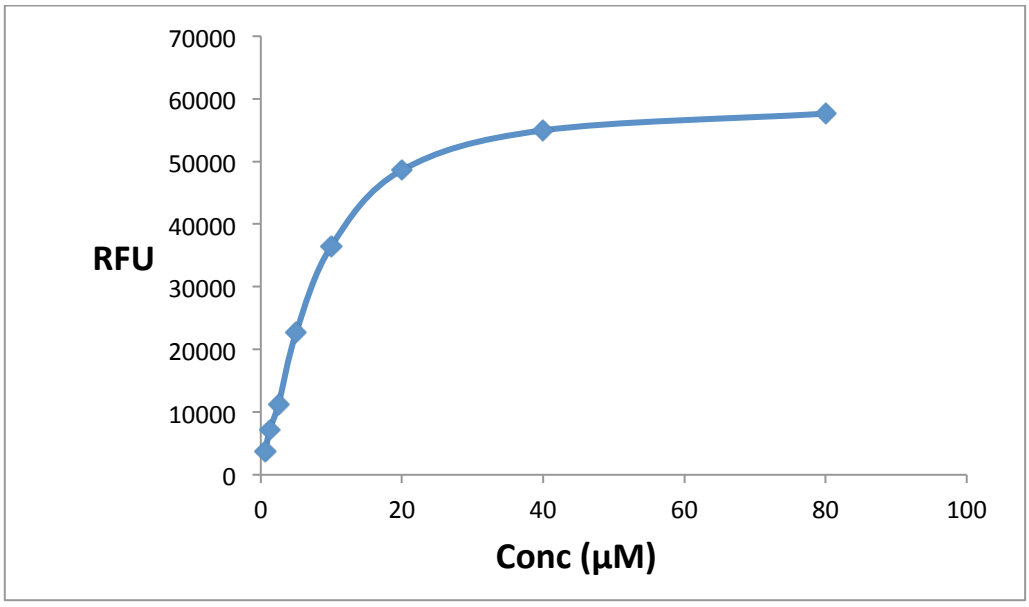

Figure S21: Change in fluorescence emission intensity of hybrid **2** at different concentrations.

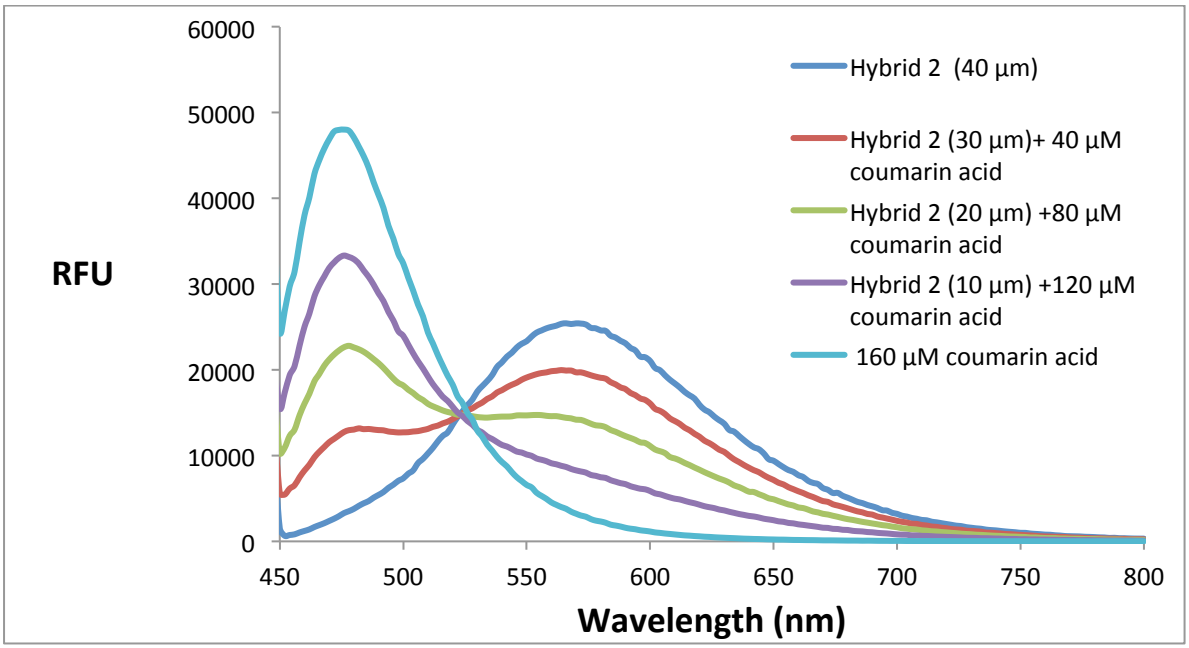

Figure S22: Fluorescence emission spectra of hybrid **2** and coumarin in different concentrations.

Hybrid **2** con. (µM)	coumarin acid con. (µM)	Total con. of coumarin (µM)
40	0	160
30	40	160
20	80	160
10	120	160
0	160	160

HPLC Monitoring of Enzymatic Degradation

Instrument method:

Column: Phenomenex, Luna, C18, 150x4.6mm, 5µm. Column temperature: 30°C.

Detector: UV at 215 nm or 295 nm, 2Hz detection rate for degradation experiments and UV at 420 nm, 2Hz detection rate for dye release (dialysis) experiments.

Needle wash: 0.1% concentrated H_3PO_4 in MeOH, Seal wash solution: H_2O:MeOH 90:10 V/V.

Diluent: phosphate buffer pH 7.4.

Gradient program A:

Time [min]	% Sol. A	% Sol. B	% Sol. C
0.0	95	0	5
20.0	0	95	5
23.0	0	95	5

Mobile phase: Solution A: 0.1% TFA in H_2O:Acetonitrile 95:5 V/V.

Solution B: 0.1% TFA in H_2O:Acetonitrile 5:95 V/V.

Solution C: THF.

Gradient Program B:

Time [min]	% Sol. A	% Sol. B
0.0	100	0
20.0	0	100
23.0	0	100
23.1	100	0
30.0	100	0

Mobile phase: Solution A: 0.1% TFA in H_2O:Acetonitrile 95:5 V/V.

Solution B: 0.1% TFA in H_2O:Acetonitrile 5:95 V/V.

Gradient program C:

Time [min]	% Sol. A	% Sol. B	% Sol. C
0.0	95	0	5
20.0	0	95	5
23.0	0	95	5

Mobile phase: Solution A: 0.1% $HClO_4$ in H_2O:Acetonitrile 95:5 V/V.

Solution B: 0.1% $HClO_4$ in H_2O:Acetonitrile 5:95 V/V.

Solution C: THF.

General sample preparation:

Hybrids **1** and **2** were dissolved in phosphate buffer pH 7.4 to give a concentration of 160µM (hybrid **2** was also dissolved in diluent to give a concentration of 40µM). Solutions were sonicated for 15 minutes.

For enzymatic cleavage in the presence of 0.23μM PLE enzyme (Figures 3a, 3c and 5b):

1200μL of a solution of hybrid **1** (160μM) or hybrid **2** (160μM) were transferred to a proper vial. 30μL were injected to the HPLC as t = 0 injection. 3.5μL of PLE stock solution (80μM in phosphate buffer pH 7.4) were added to give a final concentration of 0.23μM. Enzymatic degradation was monitored by repeating 30μL injections from the same vial over time (HPLC gradient program B for hybrid **1** and gradient program C for hybrid **2**).

For enzymatic cleavage in the presence of 2.3μM PLE enzyme (Figures 3b and 3c):

1200μL of a solution of hybrid **1** (160μM) or hybrid **2** (160μM) were transferred to a proper vial. 30μL were injected to the HPLC as t = 0 injection. 35μL of PLE stock solution (80μM in phosphate buffer pH 7.4) were added and mixed for 5 seconds (vortex mixer). Enzymatic degradation was monitored by repeating 20μL injections from the same vial over time (HPLC gradient program C).

For enzymatic cleavage in the presence of 2.3μM PLE enzyme (figure 6b):

1200μL of a solution of hybrid **2** (40μM) were transferred to a proper vial. 30μL were injected to the HPLC as t = 0 injection. 35μL of PLE stock solution (80μM in phosphate buffer pH 7.4) were added and mixed for 5 seconds (vortex mixer). Enzymatic degradation was monitored by repeating 20μL injections from the same vial over time (HPLC gradient program A).

Sample preparation of coumarin ester and phenyl acetic ester experiment:

21 μL of Hybrid **5a** (8mM stock solution) and 70 μL of phenyl acetic ester (2.56mM stock solution) were added to 188μL. Solution was sonicated for 15 minutes. 30μL were injected to the HPLC as t = 0 injection. 5.0μL of PLE stock solution (8μM in phosphate buffer pH 7.4) were added to give a final PLE concentration of 0.15μM. Enzymatic degradation was monitored by repeating 30μL injections from the same vial over time (HPLC gradient program B).

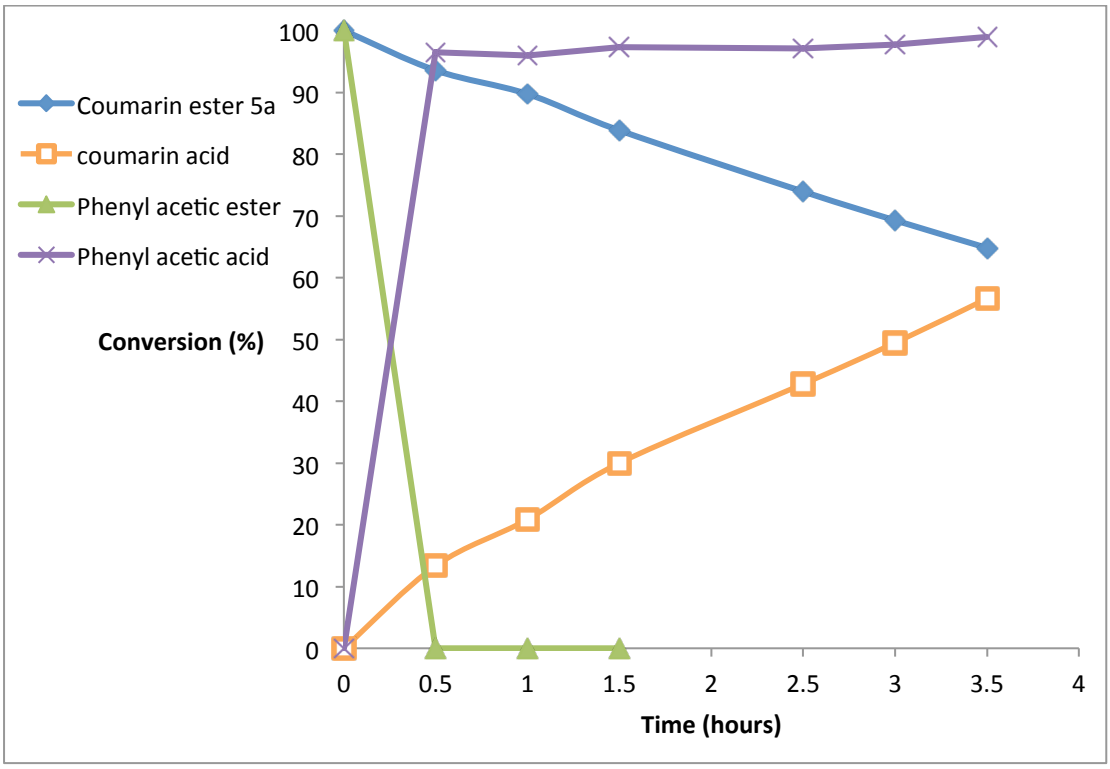

Figure S23. Degradation of coumarin ethyl ester **5a** and phenyl acetic ester in the presence of 0.15µM of PLE.

Sample preparation of coumarin esters experiment:

2.6 µL of compound **5a** (9.2 mM stock solution), 1.1 µL of compound **5b** (22 mM stock solution), and 5.5 µL of compound **5c** (5.1 mM stock solution) were added to 1200µL of 40µM solution of compound **1** to give a concentration of 20µM of each dye. Solution was sonicated for 15 minutes. 30µL were injected to the HPLC as t = 0 injection. 3.5µL of PLE stock solution (80.4µM in phosphate buffer pH 7.4) were added to give a final concentration of 0.23µM. Enzymatic degradation was monitored by repeating 30µL injections from the same vial over time.

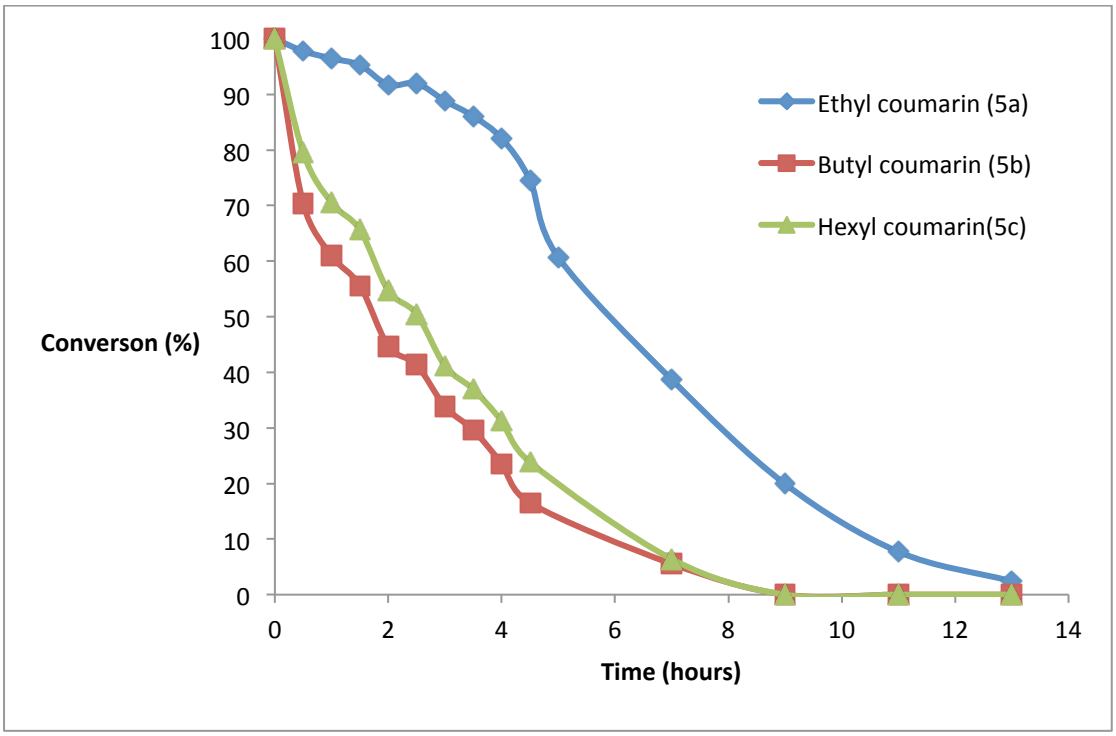

Figure S24. Degradation of coumarin ester derivatives **5a**, **5b** and **5c** in the presence of 0.23µM of PLE.

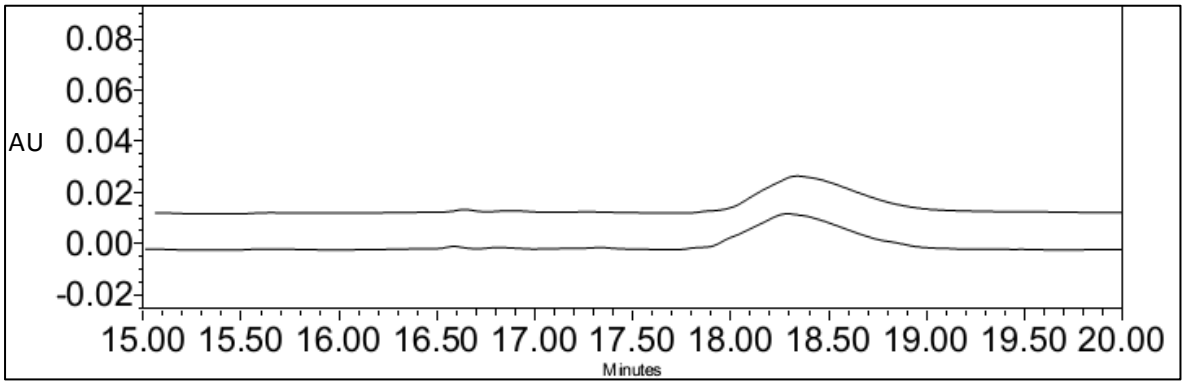

Figure S25. HPLC chromatographs of hybrid **1** at t = 0 and after 24 hours in the absence of PLE (HPLC gradient program A).

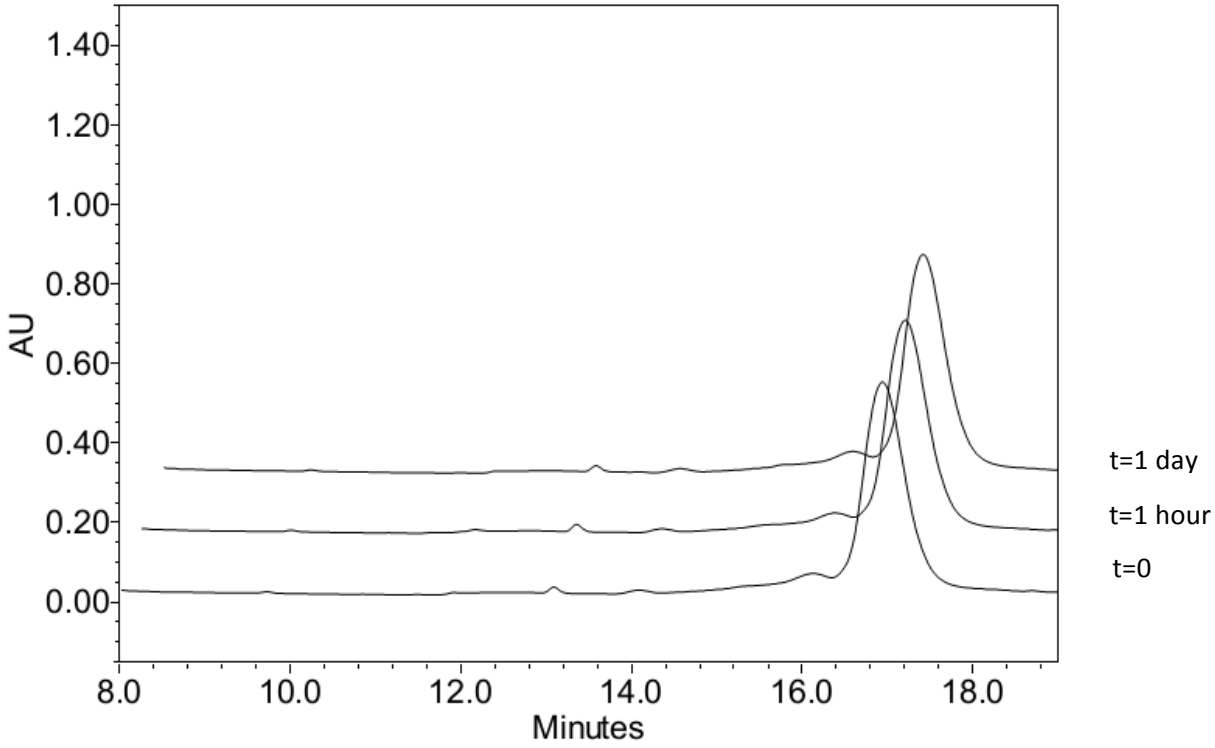

Figure S26. HPLC chromatographs of hybrid **1** at t = 0, 1 hour and after 1 day in mildly acidic pH 5.5 (HPLC gradient program C).

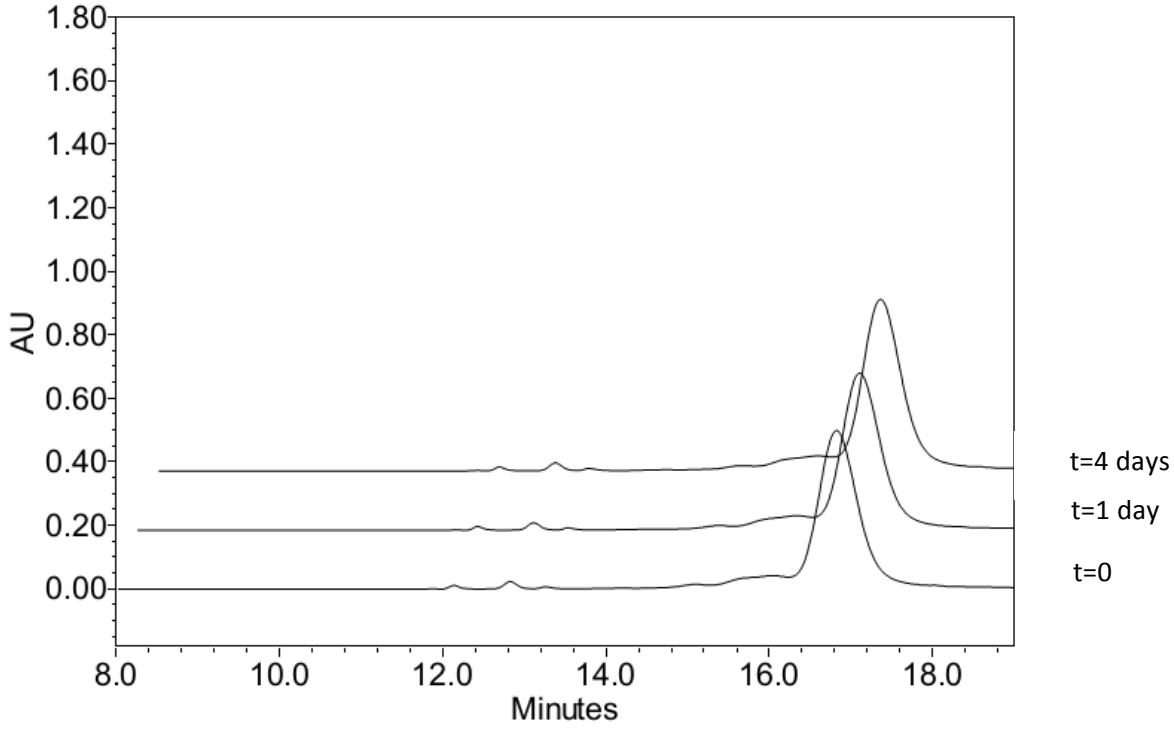

Figure S27. HPLC chromatographs of hybrid **2** at t = 0, 1 day and after 4 days in mildly acidic pH 5.5 (HPLC gradient program C).

Encapsulation and release experiments

General sample preparation:

Hybrid **1** was dissolved in phosphate buffer (pH 7.4) to give a concentration of 40µM. 800 µL of solution were accurately measured and couamrin ester derivatives were added from DMSO stock solution to give a final dye concentration of 160µM. For ethyl ester (**5a**) 16 µL were added (8mM stock solution), for butyl ester (**5b**) 17.5 µL were added (6.88mM stock solution) and for hexyl ester 22µL were added (5.8mM stock solution). In the case of hexyl ester (**5c**), due to the higher hydrophobicity of the dye, some precipitation occurred and therefore, the solution was filtered to remove any non-soluble aggregates. Analyzing the filtrate by HPLC revealed that the effective concentration of the hexyl-coumarin derivate 7c was decreased to around 30µM. Solutions were sonicated for 15 minutes, 700 µL of solution were placed in a dialysis tube (internal tube). The tube was placed in an external tube containing 14mL of phosphate buffer (in total 21 times dilution of the original solution). The plastic tube was placed under constant shaking. Samples of 200µL were taken from the external tube every hour and was measured using HPLC (HPLC gradient program C), followed by addition of 200µL of fresh buffer to keep total volume of the external tube. Hybrid **2** was dissolved in phosphate buffer (pH 7.4) to give a concentration of 40µM and was measured in similar procedure. All measurements were repeated 3 times.

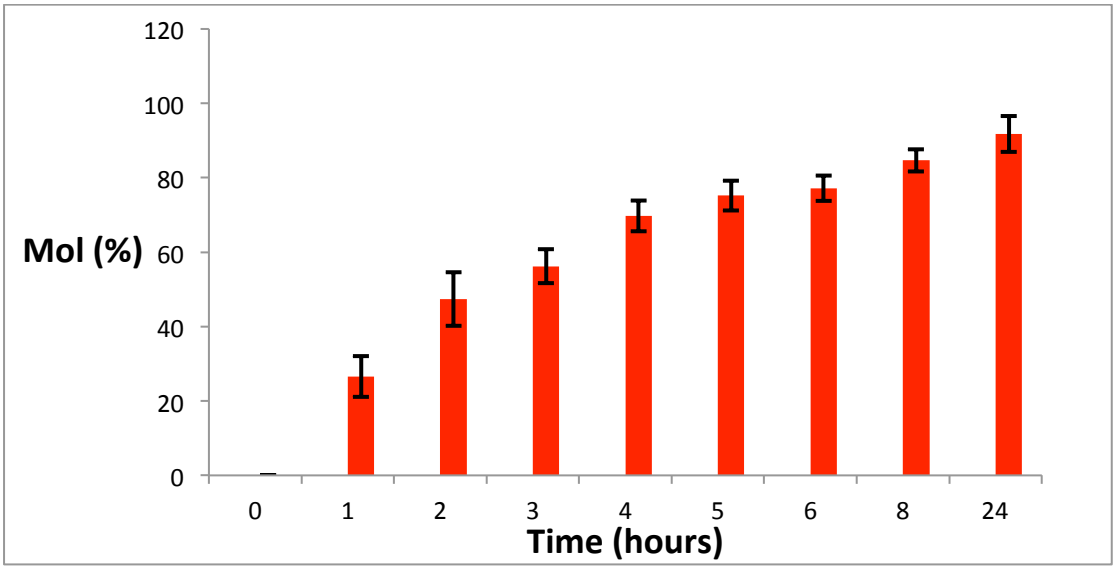

Figure S28. Analysis of the relative concentrations of coumarin acid (in this case the coumarin ester was not observed) that was released from micelles based on hybrid **1**, which were loaded with dye **5c** in the presence of the activating enzyme PLE (0.23µM).

References:

1. Harnoy, A. J. .; Rosenbaum, I .; Tirosh, E.; Ebenstein, Y.; Shaharabani, R.; Beck, R.; Amir, R. J, *J.Am. Chem*. Soc. **2014**, 136, 21.
2. G. He, D. Guo, C. He, X. Zhang, X. Zhao, C. Duan, *Angew. Chem., Int. Ed.* **2009**, 48, 6132.