Fluorinated smart micelles as enzyme-responsive probes for ^{19}F magnetic resonance

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Supporting information

Table of contents

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 I and Avance III 400MHz (^{1}H) and 100MHz (^{13}C) spectrometers. 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. **^{19}F NMR:** spectra were collected on a Bruker Avance III 376MHz spectrometer by using sodium fluoride as the internal reference. **^{19}F MRI experiments**: all measurements were conducted on an Avance-III 14.1T wide-bore NMR/MRI scanner (Bruker, Germany), equipped with a micro2.5 gradient system, capable of producing gradient pulses of 300 gauss/cm in the x, y, z-directions. **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. DMF + 25mM NH_4Ac was used as mobile phase. **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. **CMC**: all measurements were recorded on TECAN Infinite M200Pro plate reader device. **MALDI-TOF MS:** Analysis was conducted on a Bruker AutoFlex MALDI-TOF MS (Germany). DHB matrix was used. **DLS:** All measurements were recorded on a VASCO-3 Particle Size Analyzer (Cordouan).

Materials:

Esterase from porcine liver (PLE), N,N'-dicyclohexylcarbodiimide (DCC, 99%), 4-(Dimethylamino)Pyridine (DMAP, 99%) and 4-(Trifluoromethyl)Phenylacetic Acid (97%) were purchased from Sigma-Aldrich. DIPEA were purchased from Merck. O-(Benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU, 99.9%) was purchased from Chem-Impex. Trifluoroacetic acid (TFA) was purchased from Alfa Aesar. Sodium hydroxide and all solvents were purchased from Bio-Lab and were used as received. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories, Inc.

Synthesis

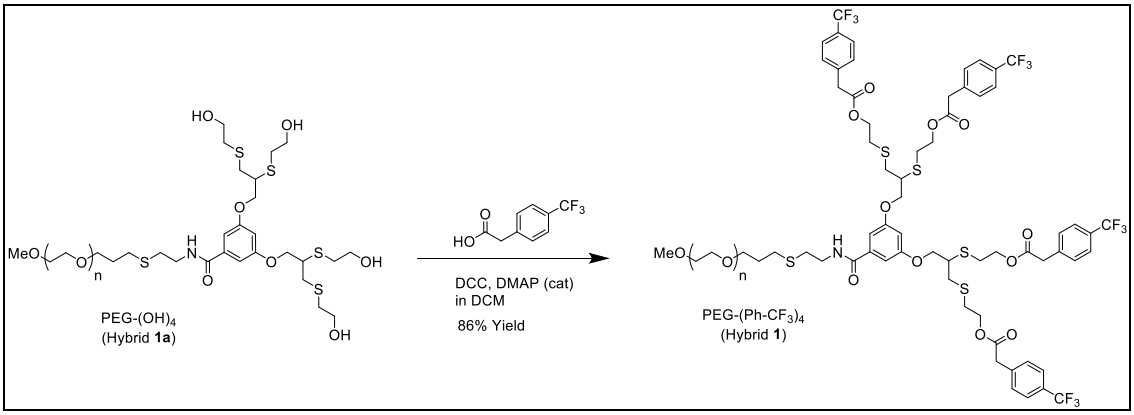

Figure S1: Preparation of hybrid **1** from previously reported hybrid **1a**.

Hybrid 1 (MeO-PEG$_{5kDa}$-(Ph-CF$_3$)$_4$):

200mg (0.04mmol) of MeO-PEG$_{5kDa}$-(OH)$_4$ (**1a**)[1] were dissolved in DCM (2mL), 2-(4-(trifluoromethyl)phenyl) acetic acid (87mg, 0.43mmol, 3eq. per OH) was added. The flask was cooled to 0°C followed by the addition of DCC (88mg, 0.43mmol, 3eq. per OH) and DMAP (0.1eq) dissolved in DCM (2mL). The reaction was stirred overnight. The crude mixture was filtered and the organic solution was evaporated to dryness. The crude mixture was purified by a silica column using 1% Acetic Acid in EtOAc followed by 20% MeOH in DCM. The fractions that contained the product were unified and the solvents were evaporated in vacuum to yield an oily residue. In order to facilitate the removal of residual solvents and solidification of the product, the oily residue was re-dissolved in DCM (2mL) followed by addition of Hexane (6mL). 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, 86% yield).

^{1}H-NMR ($CDCl_3$): δ 7.54 (d, *J* = 8.4 Hz, 8H, Ar-***H***), 7.36 (d, *J* = 8.5 Hz, 8H, Ar-***H***), 6.95 (d, *J* = 2.2 Hz, 2H, Ar-***H***), 6.82 (t, *J* = 5.6 Hz, 1H, -CH_2-N***H***-CO-), 6.55 (t, *J* = 2.3 Hz, 1H, Ar-***H***), 4.34 – 4.19 (m, 8H, -C***H***$_2$-O-CO-Ar), 4.19 – 4.04 (m, 4H, -Ar-O-C***H***$_2$-), 3.67-3.54 (m, 543H, PEG backbone), 3.35 (s, 3H, C***H***$_3$-O-PEG), 3.12 (m, 2H, -C***H***-S-), 2.97 – 2.79 (m, 8H, -CH-C***H***$_2$-S- + -CH-S-C***H***$_2$-), 2.79 – 2.65 (m, 6H, -CH-CH_2-S-C***H***$_2$- + -CH_2-S-C***H***$_2$-), 2.61 (t, *J* = 7.2 Hz, 2H, -C***H***$_2$-S-CH_2-), 1.89 – 1.76 (m, 2H, -O-CH_2-C***H***$_2$-CH_2-S-); *^{13}C-NMR($CDCl_3$): δ 170.7, 170.6, 166.8, 159.5, 137.8, 136.9, 129.8, 125.6, 106.2, 104.6, 72.0, 69.8, 69.4, 64.2, 63.8, 59.1, 45.6, 40.9, 39.3, 34.9, 31.5, 30.3, 29.7, 28.4; ^{19}F-NMR (NaF as internal reference, $CDCl_3$): 57.4 (-Ar-C*F*$_3$); FT-IR, ν (cm^{-1}): 2884, 1738, 1728, 1590, 1467, 1453, 1359, 1341, 1327, 1279, 1240, 1147, 1101, 1061, 958, 949, 842; GPC: Mn = 6.4kDa, PDI = 1.06 Expected Mn = 6.4kDa. MALDI-TOF MS: molecular ion centered at 6.4kDa.

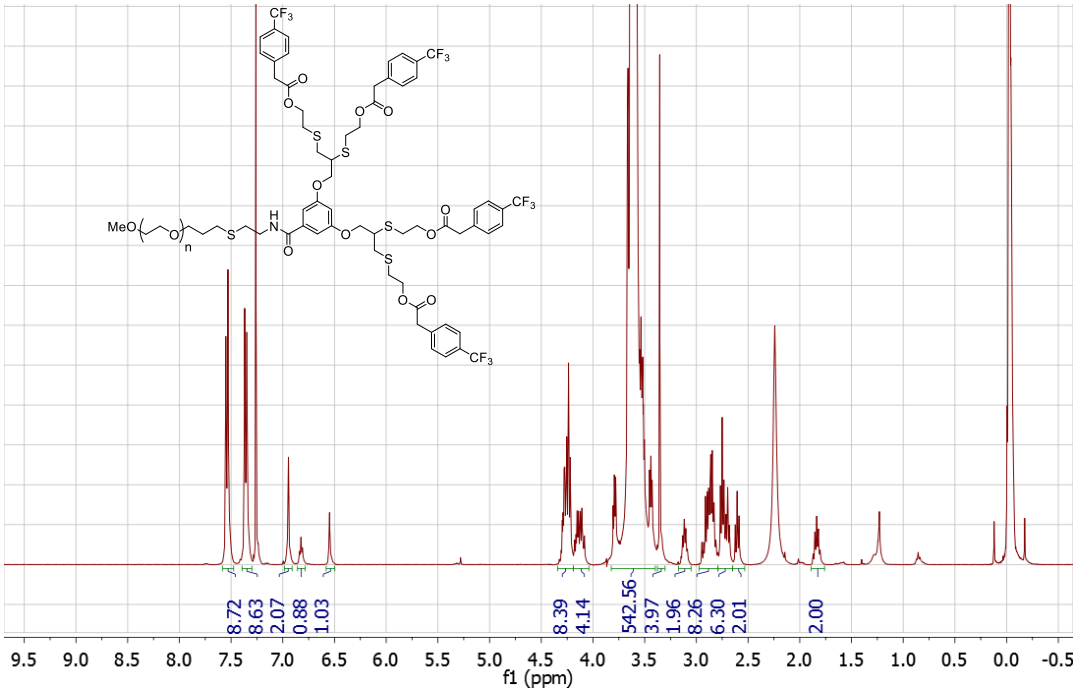

Figure S2: ^{1}H-NMR spectra of hybrid **1** in $CDCl_3$.

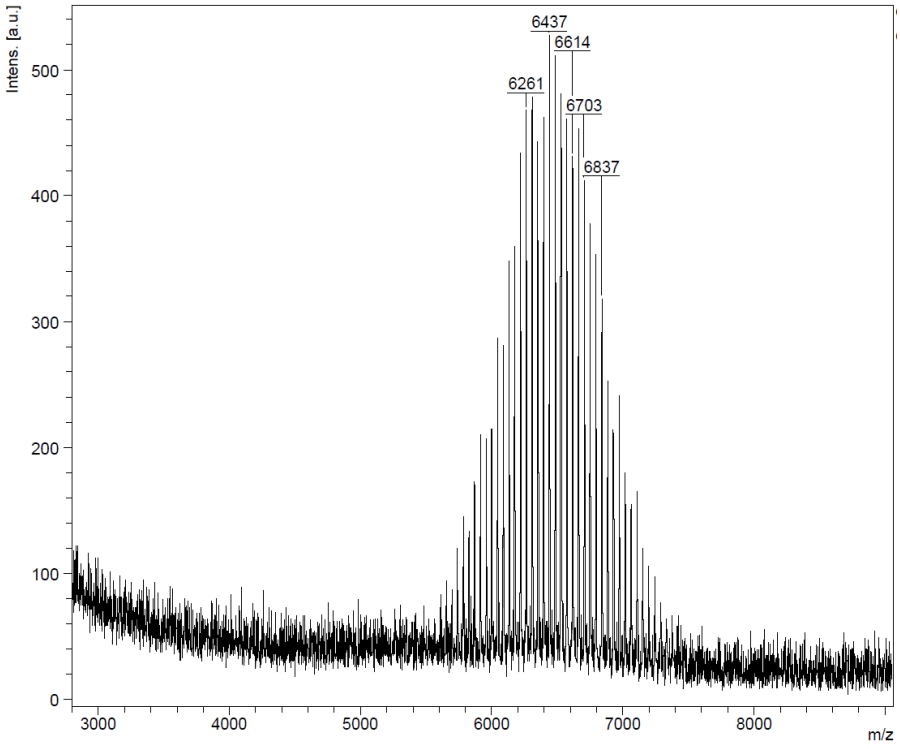

Figure S3: MALDI spectrum of hybrid **1**.

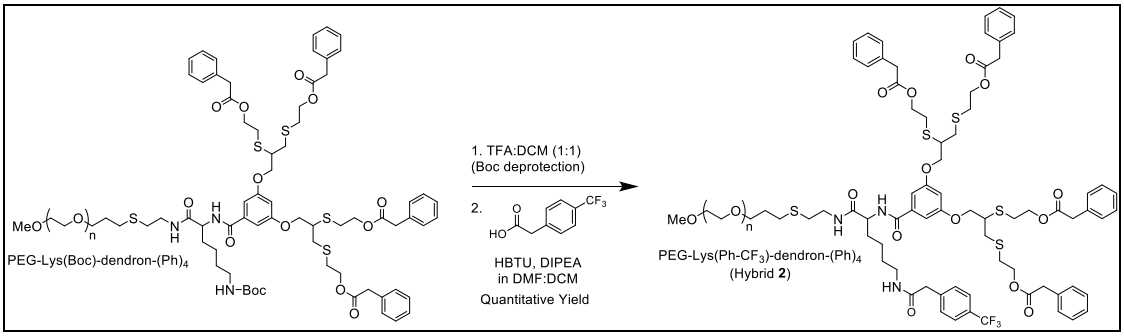

Figure S4: Preparation of hybrid **2** from previously reported hybrid **2a**.

Hybrid 2 (MeO-PEG$_{5kDa}$-Lys(Ph-CF$_3$)-dendron-(Ph)$_4$):

160mg (0.03mmol) of MeO-PEG$_{5kDa}$-Lys(Boc)-dendron-(Ph)$_4$[2] were dissolved in DCM (1.5mL) and TFA was added (1.5mL). After 30 minutes the solution was evaporated to dryness and dried in vacuum. 2-(4-(trifluoromethyl)phenyl) acetic acid (5eq.) and HBTU (5eq.) were dissolved in DCM:DMF 1:1 (2mL) followed by addition of DIPEA (20eq.) and allowed to stir for 0.5 hour. The solution was added to 160mg (0.03mmol) of the deprotected hybrid (MeO-PEG$_{5kDa}$-Lys(NH$_2$)-dendron-(Ph)$_4$) dissolved in DCM (1mL). The reaction was stirred for 1 hour. The crude mixture was purified by a silica column using 1% Acetic Acid in EtOAc followed by 20% MeOH in DCM. The fractions that contained the product were unified and the solvents were evaporated in vacuum to yield an oily residue. In order to facilitate the removal of residual solvents and solidification of the product, the oily residue was re-dissolved in DCM (2mL) followed by addition of Hexane (6mL). The white precipitate was filtered and washed twice with Ether and dried under high vacuum. The product was obtained as a white solid (160mg, quantitative yield).

^{1}H NMR ($CDCl_3$): δ 7.50 (d, *J* = 8.1 Hz, 2H, CF_3-Ar-***H***), 7.31 (d, *J* = 8.0 Hz, 2H, CF_3-Ar-***H***), 7.28 (m, 22H, Ar-***H***), 7.14 (d, *J* = 7.6 Hz, 1H, -CH-N***H***-CO-Ar), 6.98 (d, *J* = 2 Hz, 2H, Ar-***H***), 6.82 (t, *J* = 5.7 Hz, 1H, -CH_2-N***H***-CO-CH-), 6.55 (s, 1H, Ar-***H***), 6.01 (m, 1H, CF_3-Ar-CO-N***H***-), 4.51 (q, *J* = 7.3 Hz, 1H, -CO-C***H***-NH-), 4.29 – 4.17 (m, 8H, -C***H***$_2$-O-CO-), 4.14 – 3.97 (m, 4H, -Ar-O-C***H***$_2$-), 3.80-3.43(m, PEG backbone), 3.33 (s, 3H, C***H***$_3$-O-PEG), 3.17 (m, 2H, CF_3-Ar-CO-NH-C***H***$_2$-), 3.13 – 3.01 (m, 2H, -C***H***-S-), 2.92 – 2.75 (m, 8H, -CH-S-C***H***$_2$- + -CH-C***H***$_2$-S-CH_2-), 2.71 (t, , *J* = 6.8 Hz, 8H, -CH-CH_2-S-C***H***$_2$-), 2.62 – 2.51 (m, 4H, -CH_2-C***H***$_2$-S-CH_2-), 1.98 – 1.65 (m, 4H, -NH-CH_2-CH_2-CH_2-C***H***$_2$-CH- + -O-CH_2-C***H***$_2$-CH_2-S-), 1.53 – 1.41 (m, 2H, -NH-CH_2-C***H***$_2$-CH_2-CH_2-CH-), 1.35 (m, 2H, -NH-CH_2-CH_2-C***H***$_2$-CH_2-CH-); ^{13}C-NMR ($CDCl_3$) δ 171.8, 171.5, 170.3, 167.0, 159.7, 136.2, 133.8, 129.8, 129.4, 128.7, 127.3, 125.7,125.3, 106.5, 105.0, 72.0, 70.7, 69.9, 69.5, 64.2, 63.9, 59.1, 53.6, 45.6, 44.9, 43.4, 43.3, 41.3, 39.0, 38.8, 34.9, 31.8, 31.6, 30.4, 29.8, 29.7, 28.7, 28.4, 22.7; ^{19}F-NMR (NaF as internal reference, $CDCl_3$): 58.2 (-Ar-C*F*$_3$); FT-IR, ν (cm^{-1}): 2883, 1738, 1728, 1591, 1467, 1453, 1359, 1341, 1327, 1279, 1240, 1147, 1100, 1060, 960, 948, 842; GPC: Mn = 6.4kDa, PDI = 1.04. Expected Mn = 6.4kDa. MALDI-TOF MS: molecular ion centered at 6.4kDa.

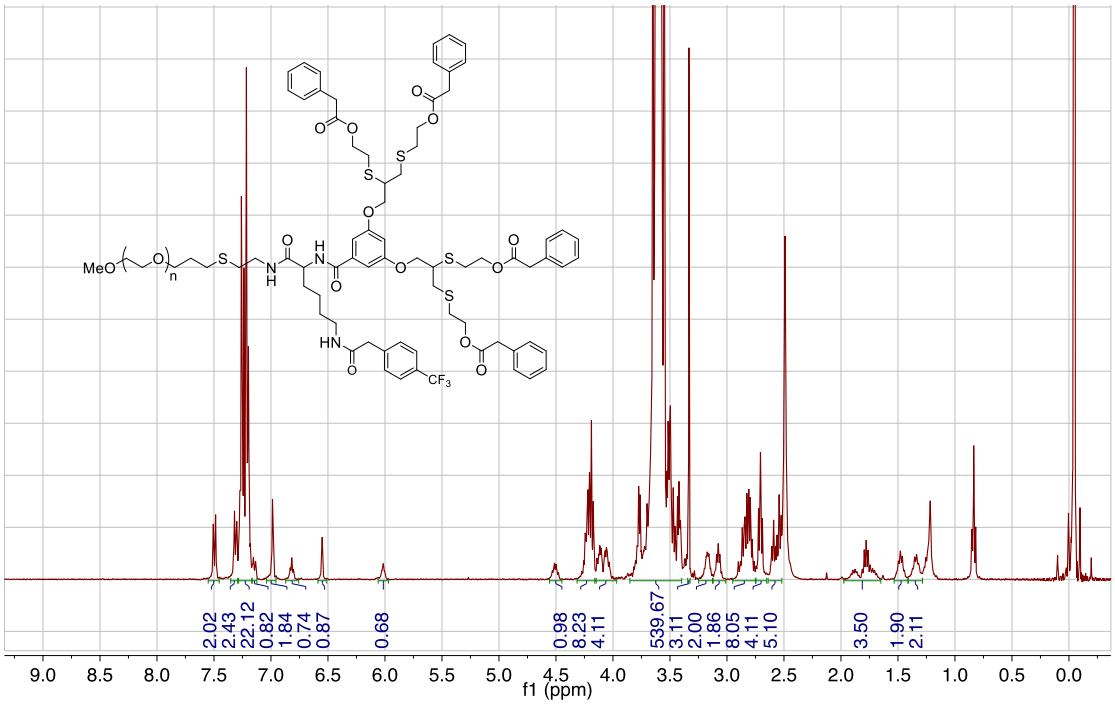

Figure S5: ^{1}H-NMR spectra of hybrid **2** in $CDCl_3$.

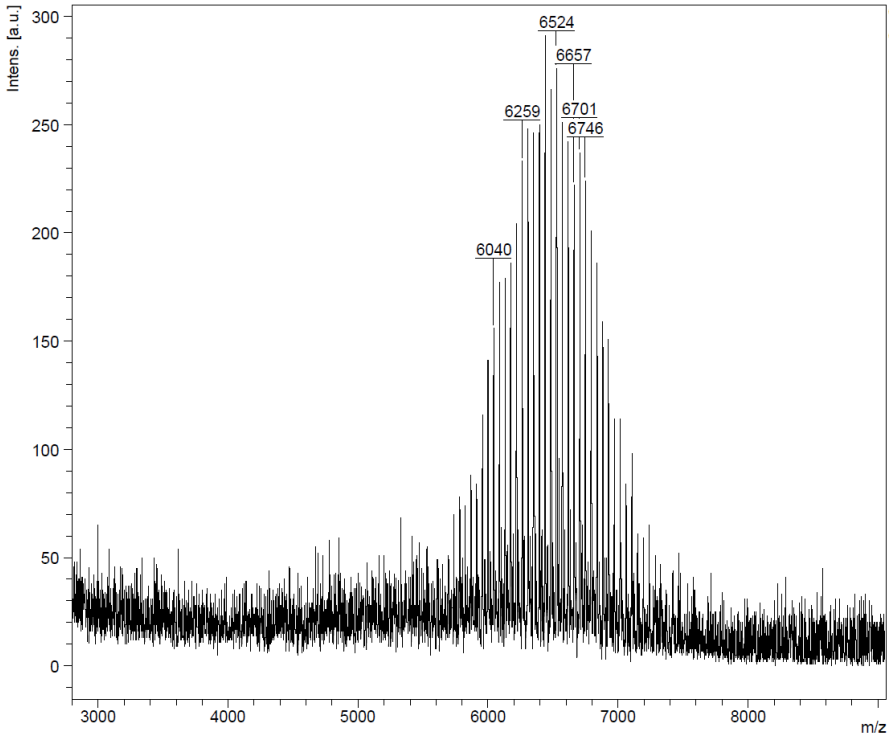

Figure S6: MALDI spectrum of hybrid **2**.

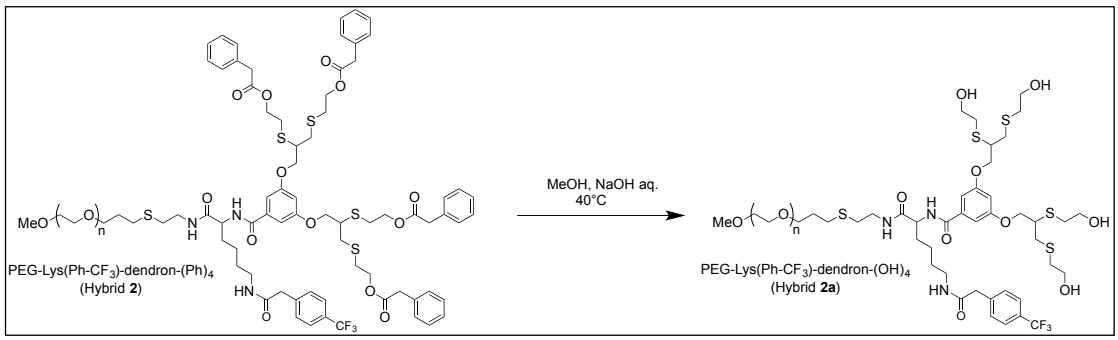

Figure S7: Preparation of hybrid **2a**.

Hybrid 2a (MeO-PEG$_{5kDa}$ -Lys(Ph-CF$_3$)-dendron-(OH)$_4$):

145mg (0.02mmol) of MeO-PEG$_{5kDa}$-Lys(Ph-CF$_3$)-dendron-(Ph)$_4$ (**2**) were dissolved in MeOH (1mL) followed by the addition of a drop of water and about 40µL of NaOH 1N (2eq.) was added. The mixture was allowed to stir over night at 40^{o}C. Complete hydrolysis was confirmed by HPLC. The pH of the mixture was neutralized (pH 7). The crude mixture was loaded on a DCM:MeOH 1:1v/v based LH20 SEC column. The fractions that contained the product were unified and the DCM and MeOH were evaporated in vacuum. In order to facilitate the removal of residual MeOH and solidification of the product, the oily residue was re-dissolved in DCM (1mL) followed by addition of Hexane (3mL). DCM and Hexane were evaporated to dryness and the obtained orange solid was dried under high vacuum. The product was obtained as an orange solid (130mg, quantitative yield).

^{1}H NMR ($CDCl_3$): δ 7.80 (s, 1H, -CH-N***H***-CO-Ar), 7.49 (d, *J* = 8.2 Hz, 2H, CF_3-Ar-***H***), 7.34 (d, *J* = 8.3 Hz, 2H, CF_3-Ar-***H***), 7.16 – 7.04 (m, 2H, -CH-N***H***-CO-Ar + Ar-***H***), 6.63-6.49 (m, 1H, -CH_2-N***H***-CO-CH- + Ar-***H***), 4.55 (s, 1H, -CO-C***H***-NH-), 4.32 – 4.10 (m, 4H, -Ar-O-C***H***$_2$-), 3.83 – 3.40(m, PEG backbone), 3.34 (s, 3H, C***H***$_3$-O-PEG), 3.30 – 3.11 (m, 5H, CF_3-Ar-CO-NH-C***H***$_2$- + -C***H***-S-), 2.92 – 2.88 (m, 2H, -CH-C***H***$_2$-S-CH_2-), 2.87 – 2.74 (m, 6H, -CH-S-C***H***$_2$- + -CH-C***H***$_2$-S-CH_2-), 2.70 (t, *J* = 6.1 Hz, 4H, -CH-CH_2-S-C***H***$_2$-) 2.64 – 2.50 (m, 4H, -CH_2-C***H***$_2$-S-C***H***$_2$-), 1.78 (m, 4H, -NH-CH_2-CH_2-CH_2-C***H***$_2$-CH- + -O-CH_2-C***H***$_2$-CH_2-S-), 1.53 – 1.32 (m, 4H, -NH-CH_2-C***H***$_2$-C***H***$_2$-CH_2-CH-); ^{13}C-NMR ($CDCl_3$) δ 171.9, 170.0, 166.7, 159.0, 139.0, 135.6, 129.2, 125.1, 125.0, 125.0, 106.0, 105.5, 98.9, 72.0, 70.7, 69.9, 63.2, 61.5, 61.4, 60.7, 58.5, 53.4, 44.8, 44.7, 42.6, 38.5, 38.4, 35.6, 34.5, 34.3, 34.3, 31.2, 31.1, 30.9, 29.1, 28.2, 27.9, 22.3; ^{19}F-NMR (NaF as internal reference, $CDCl_3$): 57.7 (-Ar-C*F*$_3$); FT-IR, ν (cm^{-1}): 2882, 1588, 1467, 1451, 1444, 1359, 1342, 1327, 1279, 1240, 1147, 1100, 1060, 960, 948, 842; GPC: Mn = 6.4 kDa, PDI = 1.16. Expected Mn = 6.0kDa. MALDI-TOF MS: molecular ion centered at 5.9kDa.

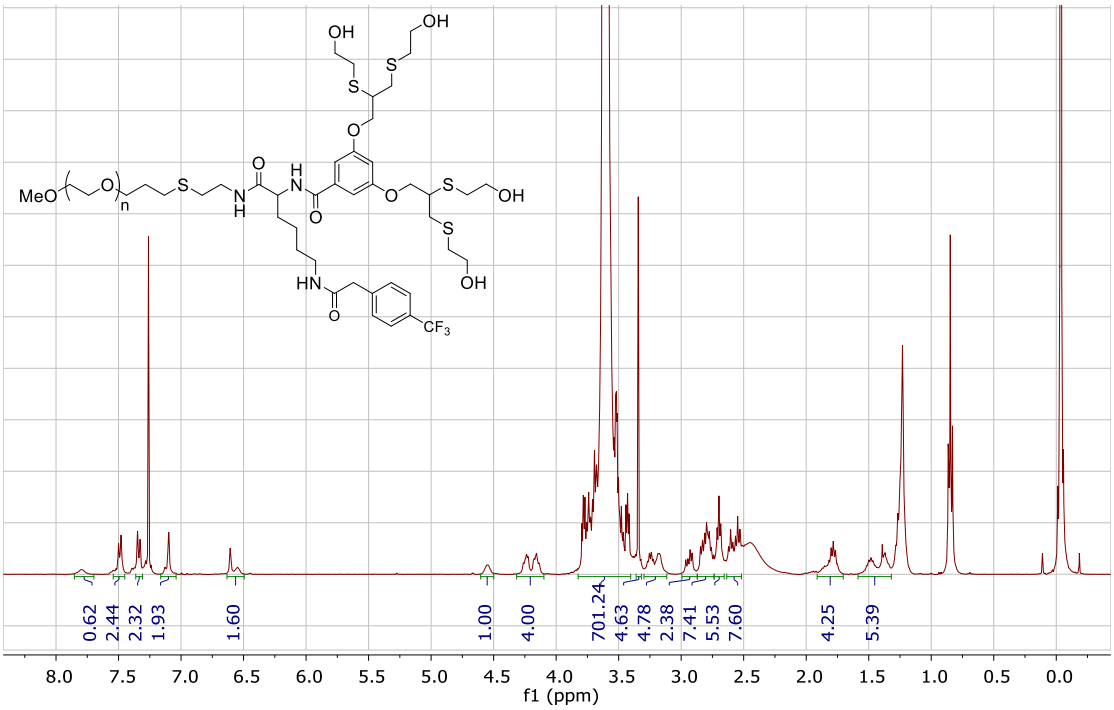

Figure S8: ^{1}H-NMR spectra of hybrid **2a** in $CDCl_3$.

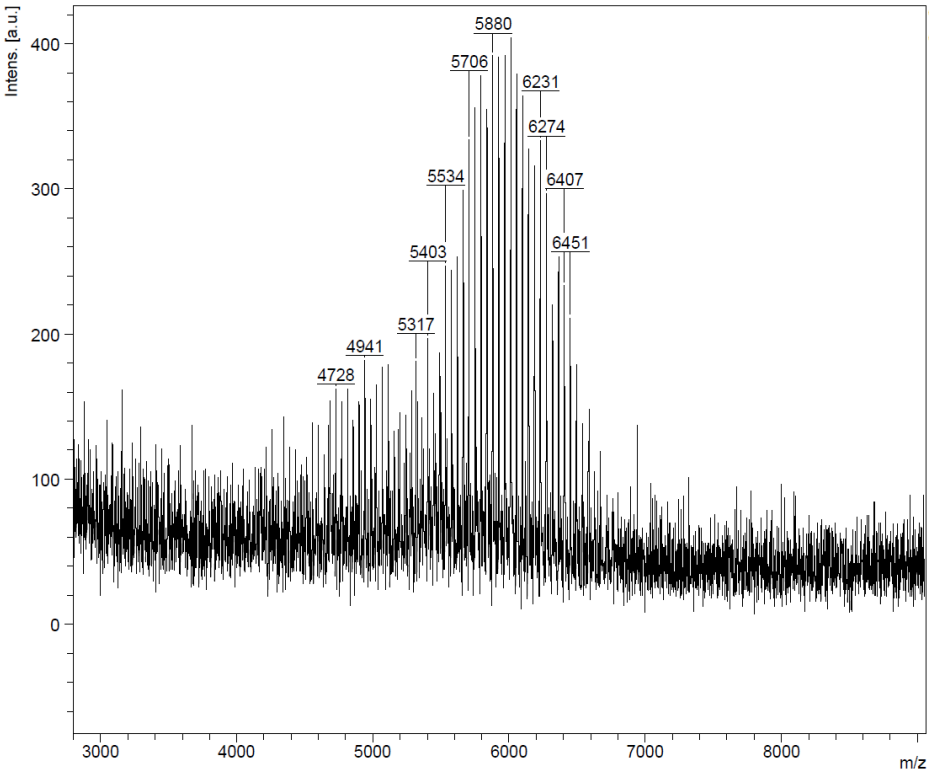

Figure S9: MALDI spectrum of hybrid **2a**.

Gel permeation chromatography (GPC):

Instrument method:

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

Columns Temperature: 50°C

Flow rate: 0.5ml/min

Mobile phase: DMF + 25mM NH_4Ac

Detector: Refractive index detector at 50°C

Injection Volume: 50µL

General sample preparation:

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

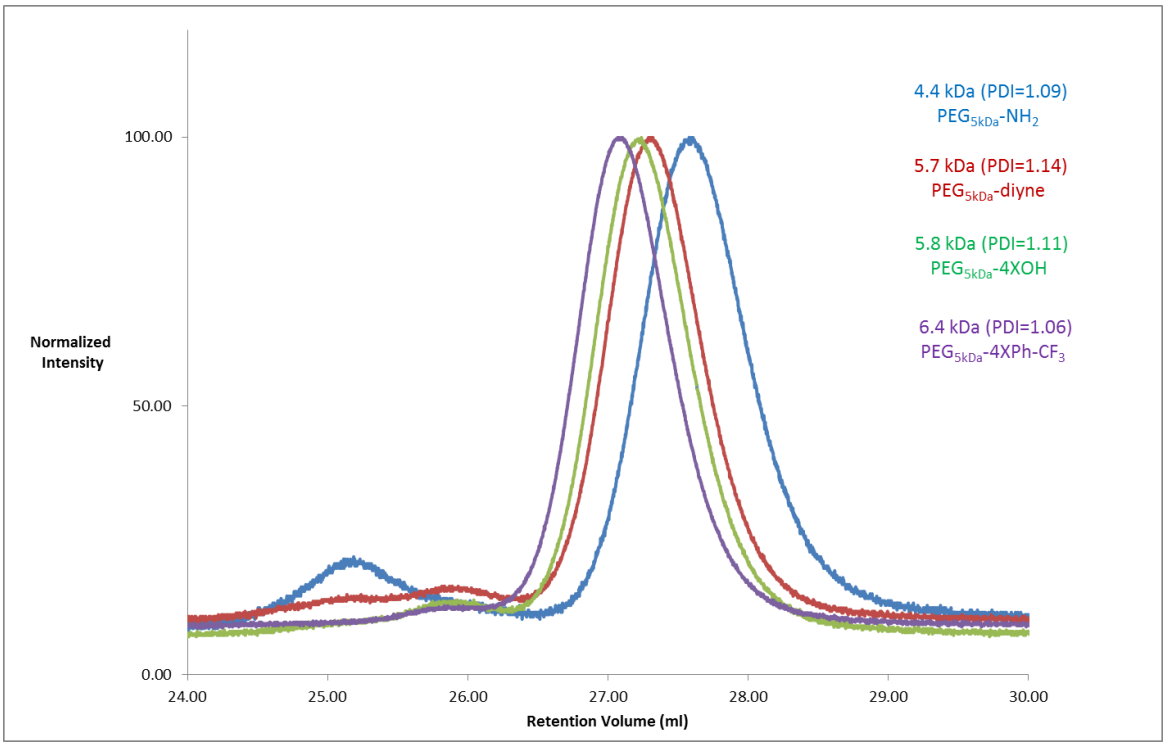

Figure S10: GPC traces of $mPEG_{5kDa}$-NH_2 (blue), $mPEG_{5kDa}$-diyne (red), $mPEG_{5kDa}$-$(OH)_4$- hybrid **1a** (green) and $mPEG_{5kDa}$-$(Ph\text{-}CF_3)_4$ - hybrid **1** (purple). (*The previously synthesized hybrids were analyzed against hybrid **1** [1]).

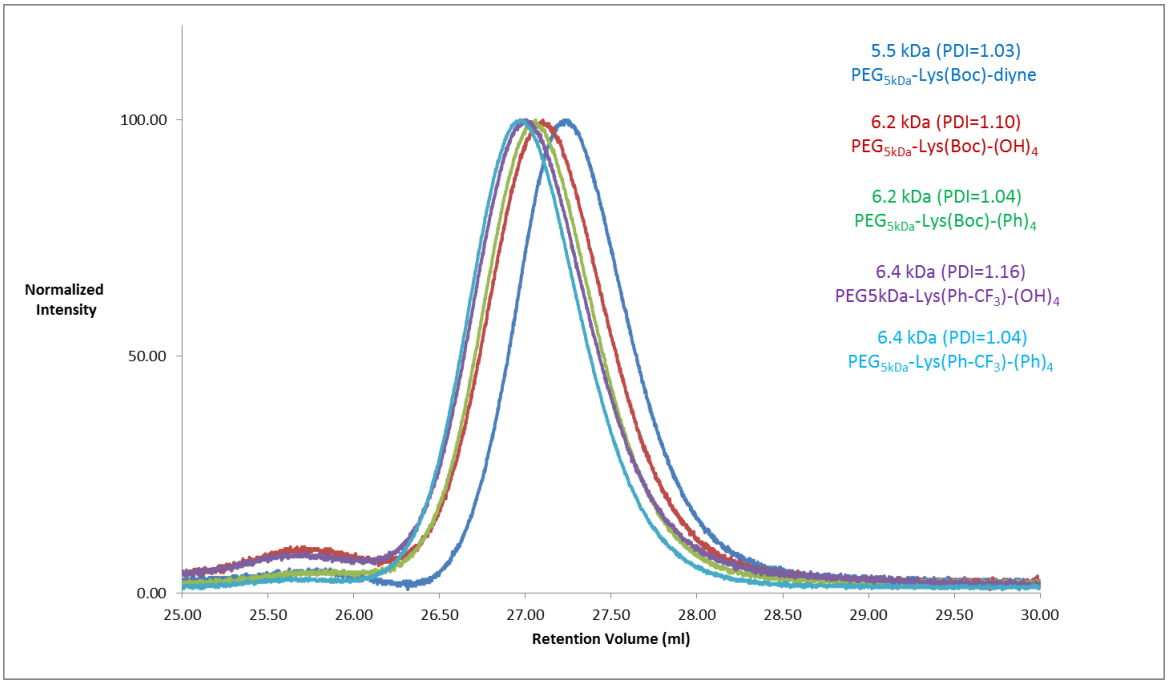

Figure S11: GPC traces of $mPEG_{5kDa}$-Lys(Boc)-diyne (blue), $mPEG_{5kDa}$-Lys(Boc)-$(OH)_4$ (red), $mPEG_{5kDa}$-Lys(Boc)-$(Ph)_4$ (green), $mPEG_{5kDa}$-Lys(Ph-CF_3)-$(OH)_4$ (purple) – hybrid **2a** (purple) and $mPEG_{5kDa}$-Lys(Ph-CF_3)-$(Ph)_4$ (light blue) - hybrid **2**. (*The previously synthesized hybrids were analyzed against hybrid **2** [2]).

Critical micelle concentration (CMC) measurements

Instrument method:

Excitation: 550nm

Emission intensity scan: 580-800nm

Diluent solution preparation:

Into 10mL Phosphate buffer solution (pH 7.4), 4.5µL 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 compounds **1** and **2**:

A 800µM solution of each hybrid was prepared in diluent and sonicated for 15 minutes. This solution was repeatedly diluted by a factor of 1.5 with diluent. 150µL 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. hybrid concentration in order to determine the CMC. All measurements were repeated 3 times.

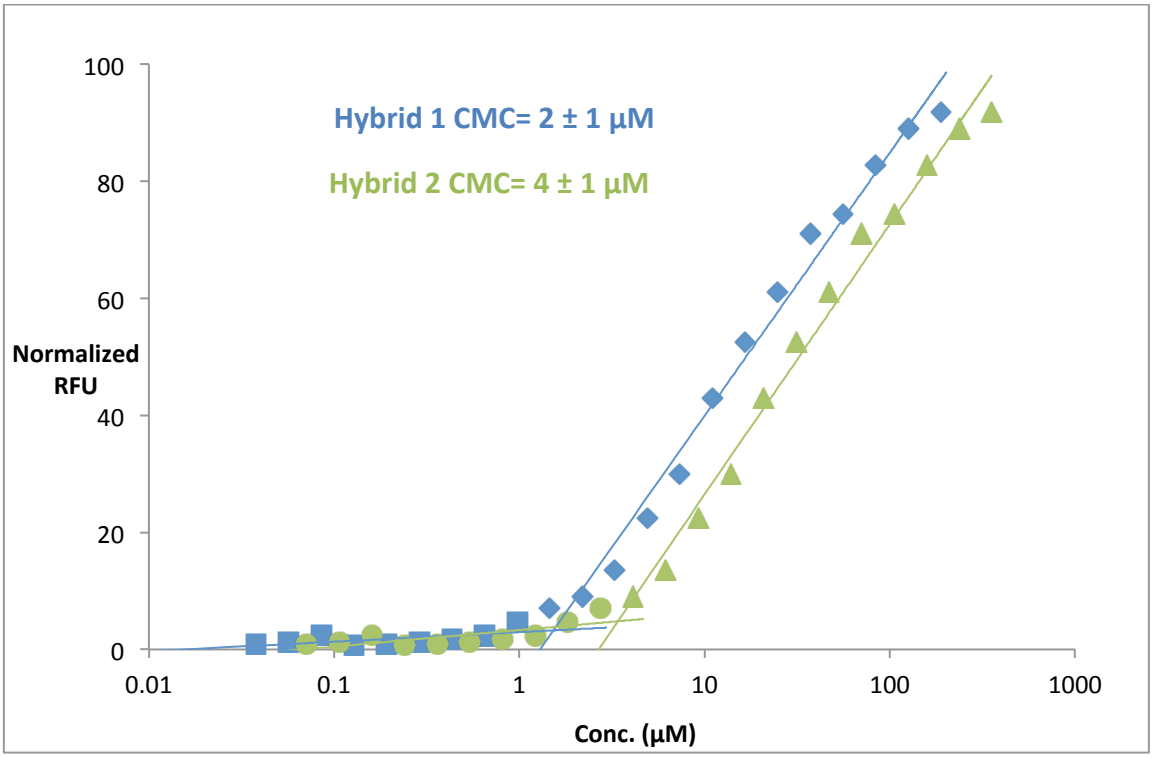

Figure S12: CMC measurements of hybrids **1** (blue) and **2** (green).

Dynamic light scattering (DLS)

General sample preparation:

Hybrids **1** and **2** were separately dissolved in phosphate buffer (pH 7.4) to give final concentrations of 160µM and 640µM respectively. The Solutions were sonicated for 15 minutes and filtered through a 0.22µm nylon syringe filter. Measurements were performed (t=0 before addition of PLE enzyme). All measurements were repeated 3 times.

For micelle degradation in the presence of 1.1µM PLE enzyme:

20.0µL of PLE enzyme stock solution (28.4µM in phosphate buffer pH 7.4) were added to 500µL solution of hybrid **1** (160µM) and also to 500µL solution of hybrid **2** (640µM). Measurements were performed after 24 hours. All measurements were repeated 3 times.

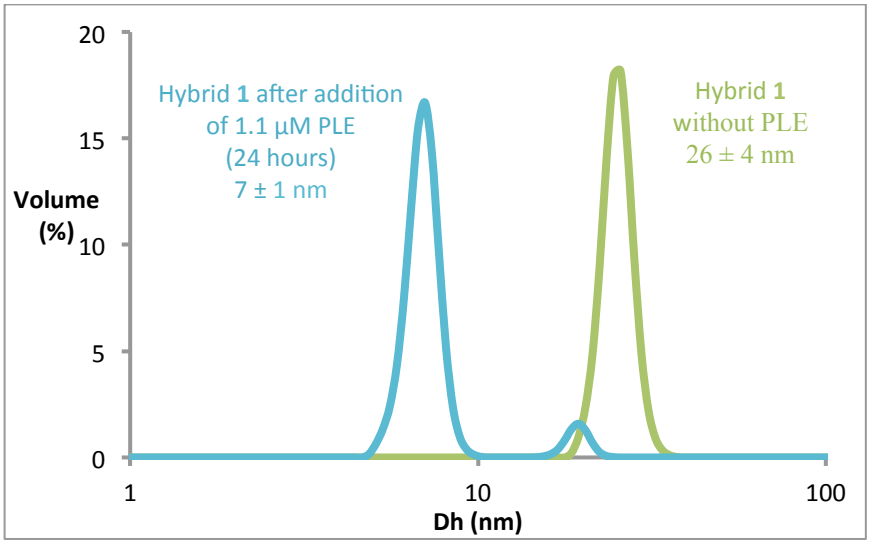

Figure S13: Micelle degradation of hybrid **1** (160µM) in presence of 1.1µM PLE enzyme.

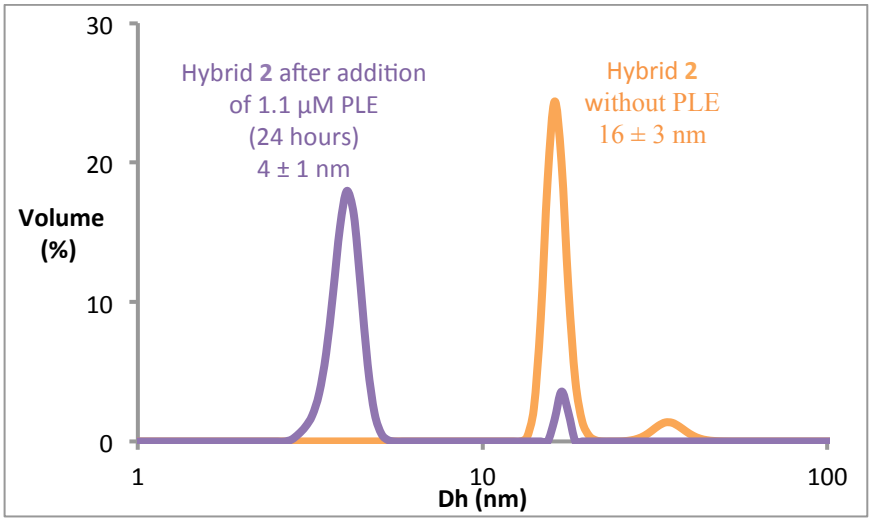

Figure S14: Micelle degradation of hybrid **2** (640µM) in presence of 1.1µM PLE enzyme.

Monitoring micelle disassembly with Nile Red fluorescence:

Instrumentation:

Monitoring of micelle disassembly rate by enzymes was performed using an Agilent Technologies Cary Eclipse Fluorescence Spectrophotometer.

Instrument Method**:**

Excitation: 550nm

Emission scan: 580-800nm

Excitation and Emission slits width: 10nm

Scan rate: 620nm/min

Temperature control: 27°C

Sample preparation and measurement:

Hybrids **1** and **2** were separately dissolved in phosphate buffer (pH 7.4) to give concentrations of 160μM and 640μM respectively. For each hybrid 4mL of the solution were accurately measured and 1.8μL of Nile Red stock solution (0.88mg/mL in Ethanol) were added to give a final concentration of 1.25μM. To each solution 40.4μL of NaF stock solution (64mM in Phosphate buffer pH 7.4) were added to give final concentration of 640μM. 700μL of each hybrid solution containing Nile Red and NaF were accurately transferred to separate quartz cuvettes for reference measurements without PLE enzyme and also for t=0 measurements.

For micelle degradation in the presence of 1.1μM PLE enzyme:

128μL of PLE enzyme stock solution (28.4μM in phosphate buffer pH 7.4) were added to 3.2mL of each solution of the tested hybrid (160μM of hybrid **1** or 640μM of hybrid **2**) containing Nile Red and NaF and mixed manually to give final PLE concentration of 1.1μM. 700μL of each solution were accurately transferred to separate quartz cuvettes (1.2mL were accurately transferred to a proper HPLC vail for monitoring enzymatic degradation by HPLC measurements and 500μL were transferred to NMR tube for ^{19}F NMR analysis). Repeating fluorescence scans were performed every 20 minutes for 18 hours (hybrid **1)** and for 8 hours (hybrid **2)**. For hybrid **1** all measurements were repeated 3 times and for hybrid **2** all measurements were repeated **2** times.

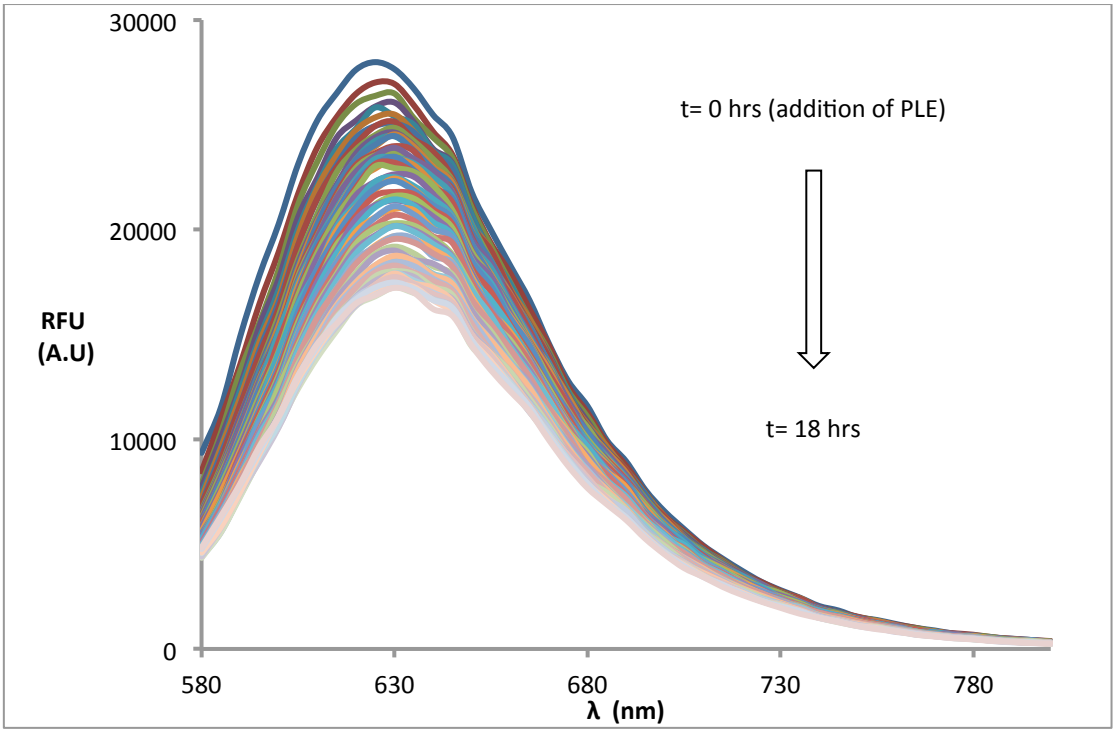

Figure S15: Fluorescence emission spectra of Nile Red (1.25µM) in the presence of hybrid **1** (160µM) as a function of time after the addition of 1.1µM PLE. A decrease in the intensity was observed as Nile Red was released into solution due to micelles degradation.

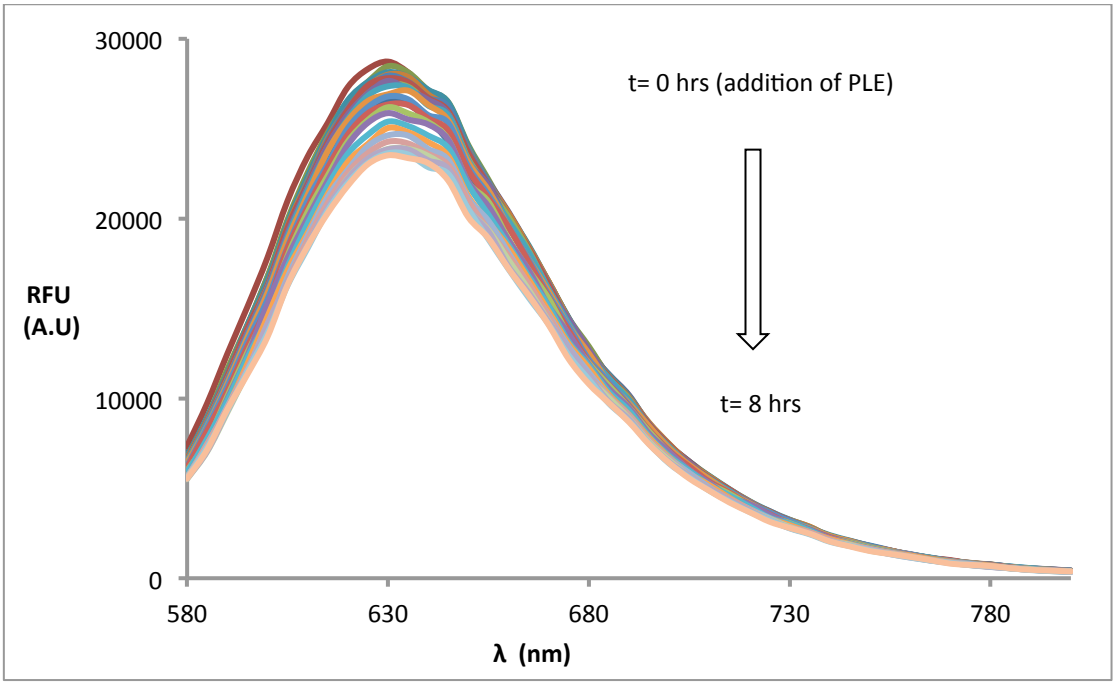

Figure S16: Fluorescence emission spectra of Nile Red (1.25µM) in the presence of hybrid **2** (640µM) as a function of time after the addition of 1.1µM PLE. A decrease in the intensity was observed as Nile Red was released into solution due to micelles degradation.

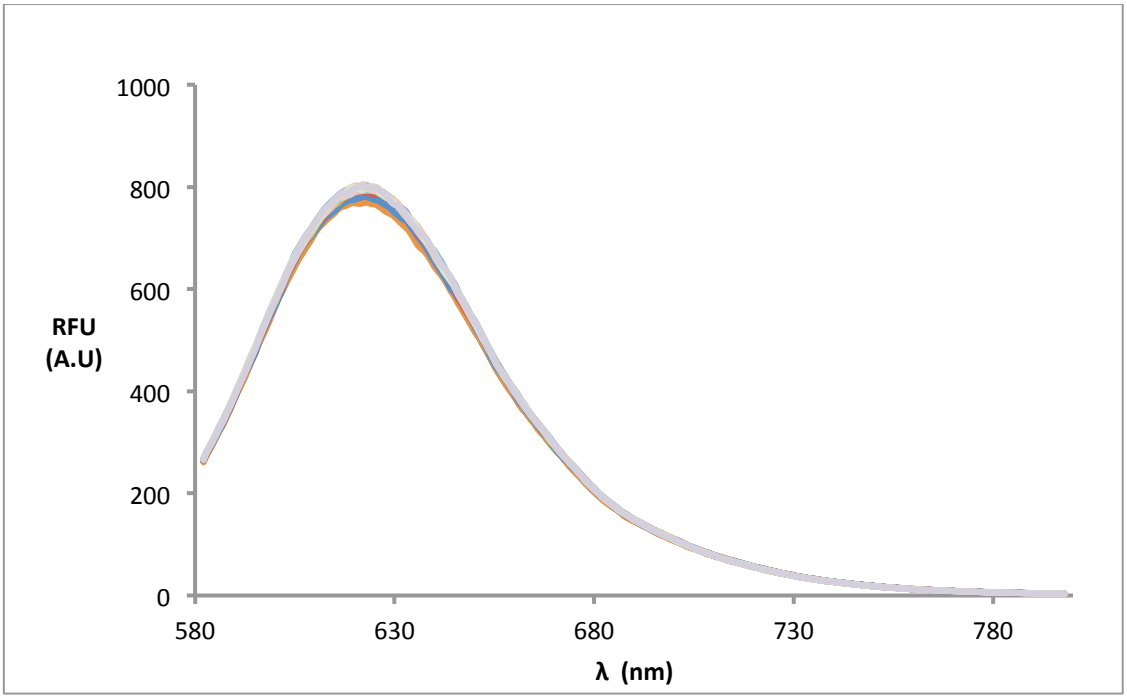

Figure S17: Fluorescence emission spectra of Nile Red (1.25µM) in the presence of hybrid **1** (160µM) in absence of PLE enzyme over 18 hours.

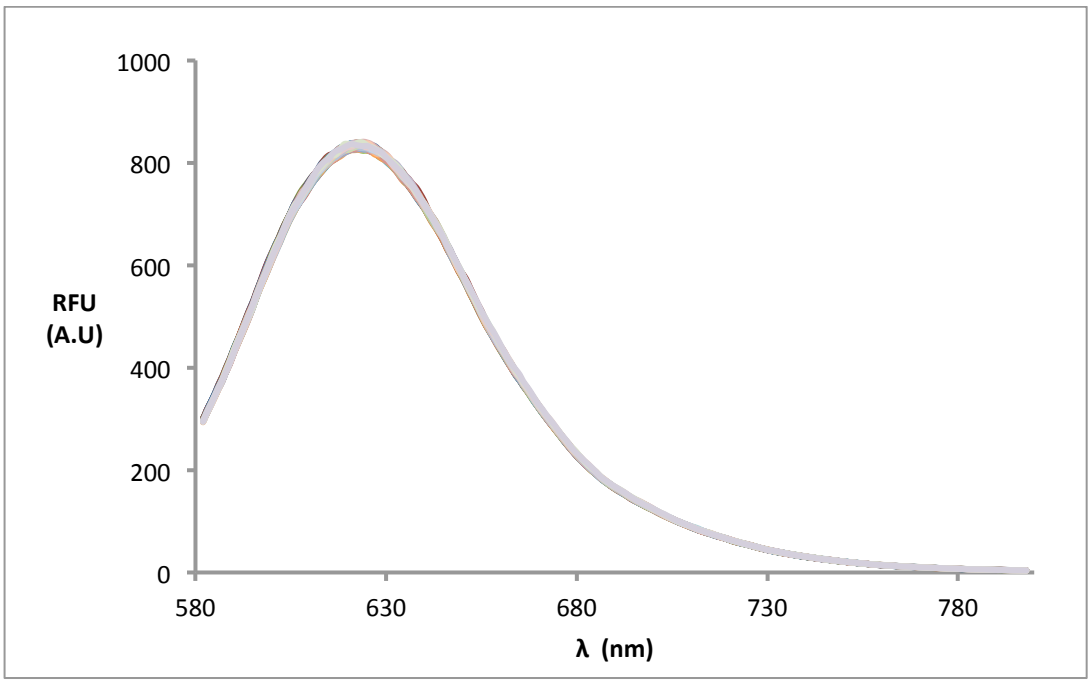

Figure S18: Fluorescence emission spectra of Nile Red (1.25µM) in the presence of hybrid **2** (640µM) in absence of PLE enzyme over 8 hours.

HPLC monitoring of enzymatic degradation:

Instrument method:

Column: Phenomenex, Aeris WIDEPORE, C4, 150x4.6mm, 3.6µm.

Column Temperature: 30°C.

Auto Sampler Temperature: 27°C.

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.

Gradient Program:

Time [min]	% Sol. A	% Sol. B	% Sol. C
0.0	95	0	5
1.0	95	0	5
20.0	0	95	5
23.0	0	95	5
23.1	95	0	5
30.0	95	0	5

Injection volume: 30µL (hybrid **1**) or 10µL (hybrid **2**).

Detector: UV at 295nm, 2Hz detection rate.

Needle Wash: MeOH.

Seal wash solution: H_2O:MeOH 90:10 V/V.

Diluent: phosphate buffer pH 7.4.

Sample preparation:

Hybrids **1** and **2** were separately dissolved in phosphate buffer (pH 7.4) to give concentrations of 160µM and 640µM respectively. For each hybrid 4mL of the solution were accurately measured and 1.8µL of Nile Red stock solution (0.88mg/mL in Ethanol) were added to give a final concentration of 1.25µM. To each solution 40.4µL of NaF stock solution (64mM in Phosphate buffer pH 7.4) were added to give final concentration of 640µM. 100µL of each hybrid solution containing Nile Red and NaF were accurately transferred to separate HPLC vials. 30µL of the hybrid **1** solution and 10µL of the hybrid **2** solution were injected to the HPLC as t=0 injection.

For micelle degradation in the presence of 1.1µM PLE enzyme:

128µL of PLE enzyme stock solution (28.4µM in phosphate buffer pH 7.4) were added to 3.2mL of each solution of the tested hybrid (160µM of hybrid **1** or 640µM of hybrid **2**) containing Nile Red and NaF and mixed manually to give final PLE concentration of 1.1µM. 1.2mL of each solution were accurately transferred to separate HPLC vials (700µL were accurately transferred to a quartz cuvette for monitoring enzymatic degradation by

fluorescence measurements and 500µL were transferred to NMR tube for ^{19}F NMR analysis). Enzymatic degradation was monitored by repeating 30µL injections from the same vail over 18 hours for hybrid **1** and for hybrid **2** by 10µL injections from the same vail over 8 hours. For hybrid **1** all measurements were repeated 3 times and for hybrid **2** all measurements were repeated 2 times.

^{19}F NMR spectroscopy:

Instrumentation:

^{19}F NMR experiments were conducted at 376MHz using a Bruker Avance III instrument equipped with a BBFO probe. NaF (640μM) was used as an internal reference added directly to the solution and its chemical shift was set to zero.

Samples preparation of hybrids **1** and **2**:

Hybrids **1** and **2** were separately dissolved in phosphate buffer (pH 7.4) and 10% D_2O (v/v) to give concentrations of 160μM and 640μM respectively. For each hybrid 4mL of the solution were accurately measured and 1.8μL of Nile Red stock solution (0.88mg/mL in Ethanol) were added to give a final concentration of 1.25μM. To each solution 40.4μL of NaF stock solution (64mM in Phosphate buffer pH 7.4) were added to give final concentration of 640μM. 500μL of each hybrid solution containing Nile Red and NaF were accurately transferred to separate NMR tubes.

For micelle degradation in the presence of 1.1μM PLE enzyme:

128μL of PLE enzyme stock solution (28.4μM in phosphate buffer pH 7.4) were added to 3.2mL of each solution of the tested hybrid (160μM of hybrid **1** or 640μM of hybrid **2**) containing Nile Red and NaF and mixed manually to give final PLE concentration of 1.1μM. 500μL of each solution were accurately transferred to separate NMR tubes for ^{19}F NMR analysis (1.2mL were transferred to a proper HPLC vial for monitoring enzymatic degradation by HPLC measurements and 700μL were accurately transferred to a quartz cuvette for monitoring enzymatic degradation by fluorescence measurements).

Sample preparation of hybrids **1a** and **2a** as reference products of enzymatic degradation:

Hybrids **1a** and **2a** were separately dissolved in phosphate buffer (pH 7.4) and 10% D_2O (v/v) to give concentrations of 160μM and 640μM respectively. For each hybrid 1.0mL of the solution were accurately measured. To solution of hybrid **1a** (160μM) 10.1μL of 4-(trifluoromethyl)phenylacetic acid stock solution (64mM in phosphate buffer pH 7.4) were added to give final concentration of 640μM. To solution of hybrid **2a** (640μM) 40.4μL of phenyl acetic acid stock solution (64mM in phosphate buffer pH 7.4) were added to give final concentration of 2.56mM. To each solution 10.1μL of NaF stock solution (64mM in Phosphate buffer pH 7.4) were added to give final concentration of 640μM. 20μL of PLE enzyme stock solution (28.4μM in phosphate buffer pH 7.4) were added to 500μL of each solution of the tested hybrid (in order to imitate the same conditions as in the micelle degradation solution). 500μL of each hybrid solution containing the degradation products, Nile Red, NaF and PLE were accurately transferred to separate NMR tubes.

^{19}F NMR monitoring of enzymatic degradation:

Monitoring of micelles disassembly rate by PLE enzyme was performed by 1D ^{19}F NMR spectra with repetition of 8 seconds of samples containing solution of hybrid **1** or hybrid **2** separately without addition of PLE enzyme (samples were prepared as described earlier) (t=0). With addition of PLE enzyme to each of the tested solutions of hybrids **1** and hybrid **2**

(samples were prepared as described earlier) repeating 1D ^{19}F NMR experiments in which a delay of 1608 sec was embedded were performed every 30 min for 18 hours for hybrid **1** and for 8 hours for hybrid **2**. Chemical shift of NaF was set to zero and its integration was set to 1. The kinetic rates were achieved by plotting the normalized integral intensities rations of the two signals in the spectra over time. All measurements for hybrid **1** were repeated 3 times and for hybrid **2** all measurements were repeated 2 times.

T_1 and T_2 measurements:

T_1 of each sample was determined using an inversion recovery pulse sequence with repetition time of 8 seconds. T_1 values were obtained by fitting the ^{19}F signal intensities vs τ according to equation $Mz = Mo\ [1 - 2\ exp(-\tau / T1)]$. The analyzed samples were separate solutions of hybrid **1** and hybrid **2** without PLE enzyme, separate solutions of hybrid **1** and hybrid **2** 24 hours after addition of PLE and separate solutions of hybrid **1a** and hybrid **2a** (all samples were prepared as described earlier). All measurements were repeated 3 times.

T_2 of each sample was analyzed using a Carr Purcell Meiboom Gill (CPMG) sequence with repetition of 8 seconds. T_2 values were obtained by fitting the ^{19}F signal integral intensities vs τ to a single exponential decay. The analyzed samples were separate solutions of hybrid **1** and hybrid **2** without PLE enzyme, separate solutions of hybrid **1** and hybrid **2** 24 hours after addition of PLE and separate solutions of hybrid **1a** and hybrid **2a** (all samples were prepared as described earlier). All measurements were repeated 3 times.

ON/OFF spectra:

The measurements were acquired using CPMG 1D sequence with repetition of 8 seconds and echo time TE=80 ms. The analyzed samples were separate solutions of hybrid **1** and hybrid **2** without PLE enzyme and 24 hours after addition of PLE enzyme (all samples were prepared as described earlier).

^{19}F MR imaging:

Sample preparation:

Hybrid **1** was dissolved in phosphate buffer (pH 7.4) to give a final concentration of 3.3mM. To 500µL of the solution 20µL of PLE enzyme stock solution (28.4µM in phosphate buffer pH 7.4) were added to give final PLE concentration of 1.1µM. The MRI experiments were preformed after 5 days.

Sample measurements:

The MRI experiments were conducted on an Avance-III 14.1T wide-bore NMR/MRI scanner (Bruker, Germany), equipped with a micro2.5 gradient system, capable of producing gradient pulses of 300 gauss/cm in the x, y, z-directions. A 5mm NMR tube containing the solution of the micelles was inserted into an 8mm NMR tube containing the same solution but after the addition of the PLE enzyme. MR images were acquired using the spin-echo MRI sequence (TE=8 ms and TR=700 ms). A 10 mm slice was acquired with a field of view (FOV) of 1×1 cm^2 and 32×32 digital resolution. Total acquisition time was about 16 hours. The EPI image was acquired with 1 segment using the above parameters and the acquisition time was 1 hour.

^{19}F MRI

^{1}H MRI

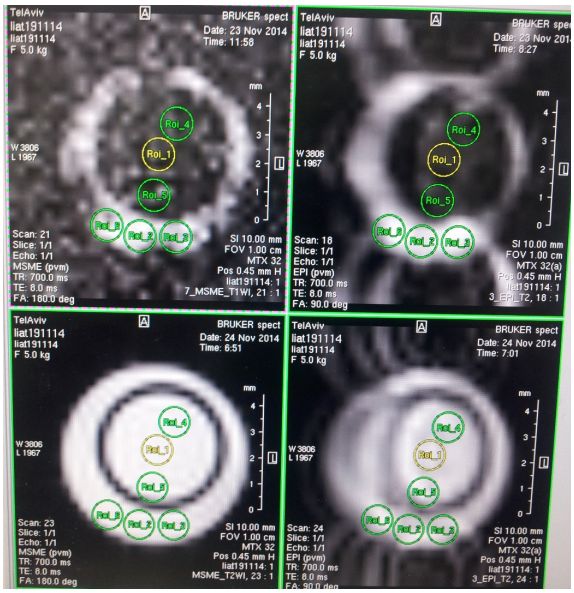

Figure S19: ^{19}F MRI and ^{1}H MRI MSME and EPI images.

	19F	1H	19F	1H
ROI	21 MSME	23 MSME	18 EPI	24 EPI
1	45	218	36	184
4	45	212	32	181
5	46	201	36	175
AVERAGE	45	210	35	180
STDEV	1	9	2	5
2	165	202	176	181
3	132	189	194	168
6	144	195	163	165
AVERAGE	147	195	178	171
STDEV	17	7	16	9
P VALUE	0.0088	0.0788	0.0033	0.2160

References

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2. M. Buzhor, A. J. Harnoy, E. Tirosh, A. Barak, T. Schwartz, R. J. Amir, *Chem. Eur. J.* **2015**, 21, 15633.