Electronic Supplementary Material (ESI) for Chemical Science

Supporting Information

Fluorogenic Enzyme-Responsive Micellar Nanoparticles

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General methods

All reagents were bought from Sigma-Aldrich and used without further purification. Anhydrous toluene and dichloromethane were purified using a Dow-Grubbs two-column purification system (Glasscontour System, Irvine, CA).[1] (N-Benzyl)-5-norbornene-*exo*-2,3-dicarboximide (**1**) was prepared as described in a previous report[3]. 1-{[(2S)-bicyclo[2.2.1]hept-5-en-2-ylcarbonyl]oxy}-2,5-pyrrolidinedione (**2**) was prepared as described by Pontrello *et al*.[4] $(IMesH_2)(C_5H_5N)_2(Cl)_2Ru$=CHPh was prepared as described by Sanford *et al*.[5] Polymerizations were performed under dry dinitrogen atmosphere with anhydrous solvents. MMP-9 was acquired from Calbiochem, as a solution in 200 mM NaCl, 50 mM Tris-HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.05% BRIJ® 35 Detergent, 0.05% NaN_3, at pH 7.0. HPLC analyses of peptides were performed on a Jupiter 4u Proteo 90A Phenomenex column (150 x 4.60 mm) with a binary gradient using a Hitachi-Elite LaChrom L-2130 pump equipped with UV-Vis detector (Hitachi- Elite LaChrom L-2420). Gradient: (Solvent A: 0.1% TFA in water; Solvent B: 99.0% acetonitrile, 0.9% water, 0.1% TFA; gradient: 20% B from 0-4 minutes, 20-45% B from 4-34 minutes, and 45-75% B from 34-38 minutes, Flow rate: 1 mL/min). To confirm peptide molecular weight, MALDI-TOF mass spectrometry was performed on an ABI MALDI Voyager (equipped with ThermoLaser Science, VSL-337ND) using alpha-CHC matrix (alpha-cyano-4-hydroxycinnamic acid) (Agilent technologies). Polymer polydispersity and molecular weight were determined by size-exclusion chromatography (Phenomenex Phenogel 5u **10**, 1K-75K, 300 x 7.80 mm in series with a Phenomex Phenogel 5u **10**, 10K-1000K, 300 x 7.80 mm (0.05 M LiBr in DMF)) using a Hitachi-Elite LaChrom L-2130 pump equipped with a multi-angle light scattering detector (DAWN-HELIOS: Wyatt Technology) and a refractive index detector (Hitachi L-2490) normalized to a 30,000 MW polystyrene standard. D_h was determined by DLS on a Malvern Nano-ZS90. TEM images were acquired on carbon grids (Ted Pella, INC.) with 1% uranyl acetate stain on a FEI Tecnai G2 Sphera at 200 KV. Fluorescence measurements were taken on a SPECTRAMAX GEMINI EM (Molecular Devices). Fluorescence lifetime measurements were taken on a Horiba Fluorolog-3 fluorometer system. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian Mercury Plus spectrometer. Chemical shifts (^{1}H) are reported in δ (ppm) relative to the $CDCl_3$ residual proton peak (7.27 ppm). Chemical shifts (^{13}C) are reported in δ (ppm) relative to the $CDCl_3$ carbon peak (77.00 ppm). Mass spectra were obtained at the UCSD Chemistry and Biochemistry Molecular Mass Spectrometry Facility.

Peptide Synthesis

Preparation of Peptide-1 for the synthesis of PPA-1 and PPA-2, and Peptide-2 for Dabcyl labeled control.

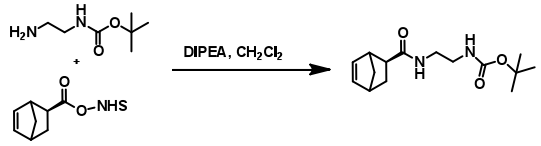
General Solid Phase Synthesis Procedure

Peptides were synthesized by Fmoc-based solid phase peptide synthesis using preloaded Wang resins. Fmoc deprotection was performed with 20 % piperidine in DMF (2×5 min) and coupling of the consecutive amino acid was carried out with HBTU and DIPEA (resin/amino acid/HBTU/DIPEA 1:3:3:4). The final peptide was cleaved from the resin by treatment with trifluoracetic acid (TFA)/Dichloromethane (DCM) (1:1) for 2 h. The resin was washed with DCM and ether and the combined organics were evaporated *in vacuo* to give an off white solid.

Peptide 1 sequence: Gly-Pro-Leu-Gly-Leu-Ala-Gly-Lys-Trp-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala-Ala-Lys HPLC (retention time = 28.8 min). MALDI-MS: Mass calcd: 1722.5; Mass obs: 1723.4.

Peptide 2 sequence: Gly-Pro-Leu-Gly-Leu-Ala-Gly-Lys(Dabcyl)-Trp-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala-Ala-Lys HPLC (retention time = 29.2 min). MALDI-MS: Mass calcd: 1973.5; Mass obs: 1974.7.

Monomer synthesis



tert-butyl-(2-((2S)-bicyclo[2.2.1]hept-5-ene-2-carboxamido)ethyl)carbamate (**3**) (shown above).
To a stirred solution of **2** (538 mg, 2.28 mmol) and mono-Boc protected ethylenediamine (500 mg, 3.42 mmol) in dry CH_2Cl_2,was added DIPEA (794 uL, 4.56 mmol). The reaction was left to stir under a dinitrogen atmosphere for 48 hrs. The reaction mixture was washed twice with 10% HCl and the organic layer dried with $MgSO_4$, filtered and concentrated to dryness to give 562 mg 88% of **3** as a white solid. ^{1}H NMR ($CDCl_3$): δ (ppm) 1.28-1.34 (m, 2H, 1 x CH_2, CH), 1.43 (s, 9H, CH_3), 1.67 (d, 1H, J = 8 Hz, CH_2), 1.86-1.91 (m, 1H, CH), 1.99-2.02 (m, 1H, CH), 2.89-2.91 (m, 2H, 2 x CH), 3.25-3.40 (m, 4H, 2 x CH_2), 5.09 (bs, 1H, NH), 6.07-6.14 (m, 2H, 2 x HC =CH), 6.40 (bs, 1H, NH). ^{13}C NMR ($CDCl_3$): δ (ppm) 28.31, 30.40, 40.22, 40.75, 41.50, 44.57, 46.30, 47.05, 79.56, 135.94, 138.10, 156.96, 176.38. LRMS (ESI), 280.84 $[M+H]^+$, HRMS, expected $[M+Na]^+$: 303.1679 , found: 303.1681.

Polymer synthesis

Backbone Copolymer (1_{21}-b-2_6-b-3_3) – Proceeds as shown in Figure below:

To a stirred solution of **1** (123 mg, 0.522 mmol) in dry CH_2Cl_2 (2 mL) cooled to –78ºC was added a solution of the catalyst ($(IMesH_2)(C_5H_5N)_2(Cl)_2Ru$=CHPh) (10 mg, 0.013 mmol) in dry CH_2Cl_2 (0.5 mL) also cooled to –78ºC. After 5 min the cold bath was removed and the reaction was left to stir under nitrogen while warming to room temperature. After 40 min a 0.30 mL aliquot was removed and quenched with ethyl vinyl ether as shown in Fig. 1 below. After 25 min the polymer was precipitated by addition to cold MeOH to give the homopolymer as an off white solid. To the remaining reaction mixture a solution of **2** (35 mg, 0.148 mmol), in dry CH_2Cl_2 (1 mL) was added. The mixture was left to stir under N_2 for 40 min and a 0.30 mL aliquot was removed and quenched with ethyl vinyl ether as shown in Fig. 1 below. After 25 min the polymer

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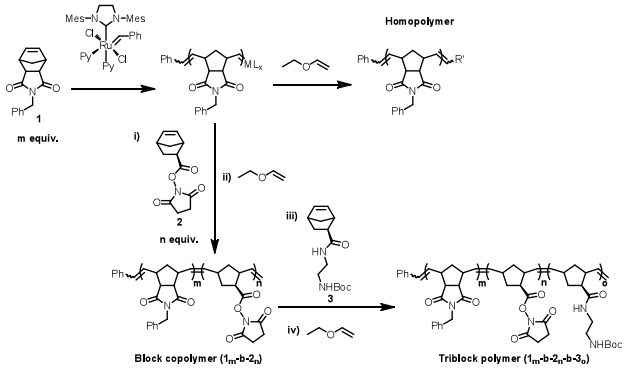
was precipitated by addition to cold MeOH to give the block coopolymer as an off white solid. To the remaining reaction mixture, a solution of **3** (9.56 mg, 0.034 mmol), in dry CH_2Cl_2 (0.6 mL) was added. The mixture was left to stir under N_2 for 40 min followed by quenching with ethyl vinyl ether (0.100 ml). After 25 min the solution was concentrated to ~ 1/3 the original volume then precipitated by addition to cold MeOH to give the copolymer as an off white solid. ^{1}H NMR of the polymer confirmed the absence of monomer (no olefin peak at 6.30 ppm) and the presence of broad trans and cis olefin peaks of the polymer backbone at 5.73 and 5.50 ppm, respectively.

SEC-MALS of polymers prior to peptide conjugation:
Homopolymer of **1:** Mn = 5253, Mw/Mn = 1.011, **1** = 21.
Copolymer of **1**-*b*-**2**: Mn = 6725, Mw/Mn = 1.050, **2** = 6.
Triblock polymer of $\mathbf{1}_{21}$-*b*-$\mathbf{2}_6$-*b*-$\mathbf{3}_3$: Mn = 7459, Mw/Mn = 1.053, **3** = 3.



General method utilized in polymerization reactions. For analysis purposes a sample of the first and second blocks in the polymer was quenched prior to addition of the second and third monomer. This is used to confirm block size and is compared with weight fraction analysis of the copolymer by SEC-MALS.

Representative synthesis of Peptide-Polymer Amphiphile (PPA)

Synthesis of PPA-1 and PPA-2

0.05 μmol of $\mathbf{1}_{21}$-*b*-$\mathbf{2}_6$-*b*-$\mathbf{3}_3$ was dissolved in 1 mL of Dimethylformamide (DMF), followed by addition of 1.2 equiv. of N,N-Diisopropylethylamine (DIPEA) and 1.2 equiv. of peptide. The reaction was stirred at room temperature for 22 hrs, followed by precipitation of the polymer by addition to cold methanol (1 mL). The precipitate was separated from the supernatant by centrifugation at 13,000 rpm. The precipitated peptide-triblock polymer product was then mixed with 12% TFA in 0.5 mL DMF for 2 hrs to remove the Boc protecting groups on the amine-functionalized block. The product was precipitated with cold ether (1 mL) followed by centrifugation at 13,000 rpm. The precipitated product was then dried and aliquoted into 0.5 mL DMF for generation of PPA-1 via addition of Fluorescein-NHS (0.54 mg, 1.1 μmol), and PPA-2 via addition of Rhodamine-NHS (1.8 mg, 3.4 μmol), each with 1.2 equiv. of DIPEA for 18 hr. The polymers were again precipitated by addition to cold ether (1 mL) followed by centrifugation at 13,000 rpm. dn/dc for the peptide-polymer conjugates is 0.179 as determined from peak analysis.

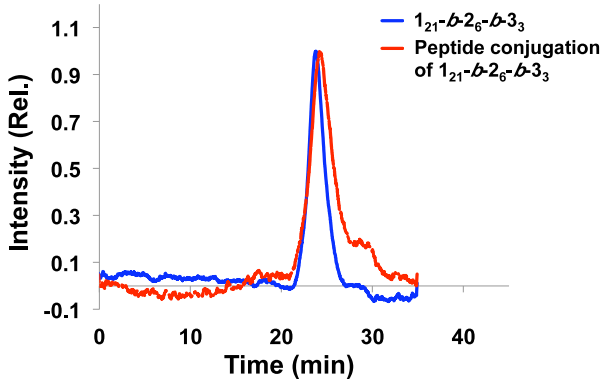
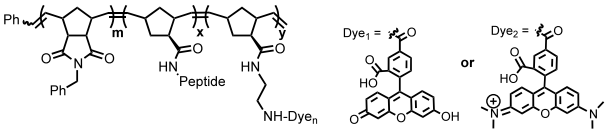


Figure 1S. SEC-MALS intensity plot of initially prepared $\mathbf{1}_{21}$-*b*-$\mathbf{2}_6$-*b*-$\mathbf{3}_3$ (blue) and following conjugation with Peptide 1 (green). SEC-MALS: $\mathbf{1}_{21}$-*b*-$\mathbf{2}_6$-*b*-$\mathbf{3}_3$; Mn = 7459 g/mol, PDI = 1.053. Peptide-conjugate of $\mathbf{1}_{21}$-*b*-$\mathbf{2}_6$-*b*-$\mathbf{3}_3$; Mn = 15270 g/mol, PDI = 1.164.

UV-Vis determination of dye conjugated efficiency to the amine block of $\mathbf{1}_{21}$-*b*-$\mathbf{2}_6$-*b*-$\mathbf{3}_3$

Dye conjugated efficiency was determined by calculating the concentration of peptides and dyes with extinction coefficient from UV-Vis measurement. The number of peptide conjugation was measured from SEC-MALS and the number of dye conjugation can be then calculated.

Table 1S. The polymers, PPAs, and resulting micelles



	Peptide substrate[a]	m[b]	n[b]	o[b]	m[b]	x[c]	y[d]	D_h[g]	PDI[g]
M1	NH_2-GPLGLAGKWA AAAKAAAAK-COOH	21	6	3	21	5	1.4[e]	37	0.267
M2	NH_2-GPLGLAGKWA AAAKAAAAK-COOH	21	6	3	21	5	1.6[f]	32	0.358
M3	NH_2-GPLGLAGKWA AAAKAAAAK-COOH	21	6	3	21	5	1.4[e] 1.6[f]	37	0.234
M4	NH_2-GPLGLAGK*W AAAAKAAAAK-COOH	21	6	3	21	6	2.8[e]	50	0.342

[a] MMP substrates are shown in blue with cleavage sites underlined, and K* is Dabcyl labelled lysine. Peptides are conjugated to the polymer through the amino termini. [b] Block sizes m, n and o (labelled in general scheme above) and [c] conjugated-peptide block size estimated from molecular weight *via* SEC-MALS.
[d] Number of conjugated Fluorescein[e] (Dye_1) and Rhodamine[f] (Dye_2) dyes measured by UV-Vis (as described above).
[g] Hydrodynamic diameter and PDI (polydispersity) of micelles were determined by DLS.
Note: M3 is the result of blending PPA-1 and PPA-2 to form the mixed dye micelle as described in the main text.

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Spherical Micelle Formation

Peptide-polymer amphiphiles (PPA-1 and -2; 0.25 mg, 16.3 μmol) were dissolved separately to generate **M1** and **M2**, each in 70 μL of DMSO/DMF (1:1 ratio) followed by addition of 100 μL of sodium phosphate buffered water (40 mM, pH 8.0). This solution was then transferred to a 3,500 MWCO dialysis tubing and left for 3 days. The buffer was changed three times, once per day. For generation of **M3** micelles, both PPA-1 and -2 (0.2 mg, 13 μmol) were mixed together and subjected to dialysis as described.

Transmission electron microscopy (TEM)

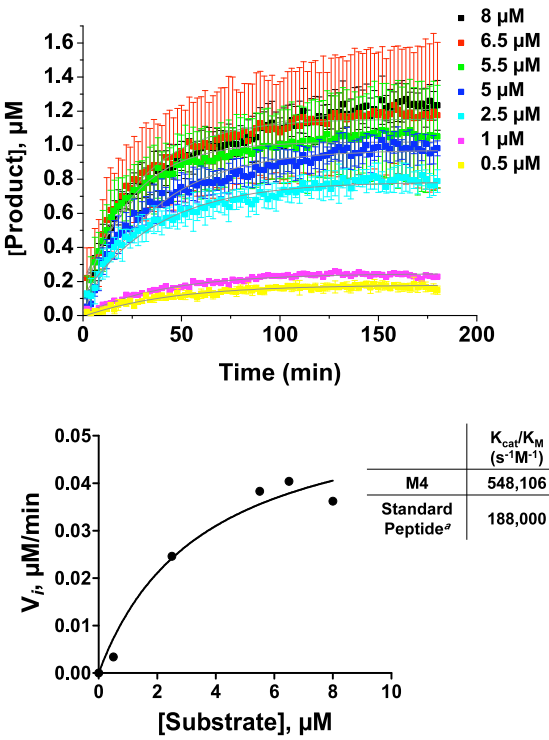
Small (5 μl) aliquots of sample were utilized for TEM *via* standard procedure. Briefly, the sample was loaded onto grids (Ted Pella Inc.) that had previously been subjected to glow discharged using an Emitech K350 glow discharge unit and plasma-cleaned for 90 s in an E.A. Fischione 1020 unit. The sample grid was then transferred into a grid holder in a FEI Sphera microscope operating at 200 keV. Micrographs were recorded on a 2K X 2K Gatan CCD camera.

Enzyme activation

To 5 μL of enzyme was added 0.4 μL of a 24 mM *p*-aminophenyl mercuric acetate solution in freshly prepared 0.1 M NaOH. The enzyme solution was heated at 37ºC for 2 hrs prior to use.

Initial study of enzyme kinetics on micellar substrates

The kinetics of the MMP-9 driven cleavage of a micelle-based substrate was carried out using **M4** (see Table 1S above), which contained a peptide substrate with a Dabcyl label, and a polymer labeled with fluorescein. The fluorescein signal increase upon cleavage of the Dabcyl label was monitored. Different substrate concentrations (0.5, 1, 2.5, 5, 5.5, 6.5, and 8 μM, Fig. 2S, top) were reacted with MMP-9 (10 nM) from which initial rates of reaction were determined. The plot of initial rate (V_i) vs substrate concentration was fit to Michaelis-Menten kinetics (using PRISM) to obtain K_{cat} and K_M showing this substrate for MMP-9 is comparable to standard substrate[2] (Fig. 2S).



	K_{cat}/K_M ($s^{-1}M^{-1}$)
M4	548,106
Standard Peptide[a]	188,000

Figure 2S. TOP: Product vs time for reactions of **M4** with MMP-9 at various substrate concentrations. BOTTOM: Initial rate vs substrate concentration. [a] The literature value of K_{cat}/K_M for standard peptide with MMP-9[2].

MMP-9 Cleavage Efficiency Assay

120 μM of **M1** (with respect to peptide) and 120 μM of Peptide-1 were treated with MMP-9 (100 μU, 1.25 μL) for 24 hrs. The control was performed using 120 μM of Peptide-1 without treatment with MMP-9. These samples were then analyzed by RP-HPLC following inactivation of MMP-9 at 65 ºC for 20 mins (Figure 3S). Peak A is the intact, full length Peptide-1 without treatment with MMP-9, and Peak B is the large fragment of Peptide-1 cleaved by MMP-9 as confirmed by MALDI-MS shown in Figure 4S. Peak C (Figure 3S) is fragment resulting from the cleavage of **M1** as confirmed by MALDI-MS (Figure 4S). MMP-9 cleavage efficiency of Peptide-1 is quantitative according to the disappearance of the peak at 29 minutes. Therefore, MMP cleavage efficiency of **M1**

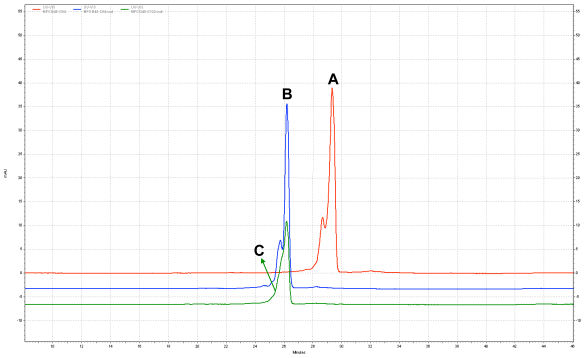


Figure 3S. MMP-9 cleavage of **M1** (green trace) and Peptide-1 (blue trace). Red trace is intact Peptide-1 without treatment with MMP-9.

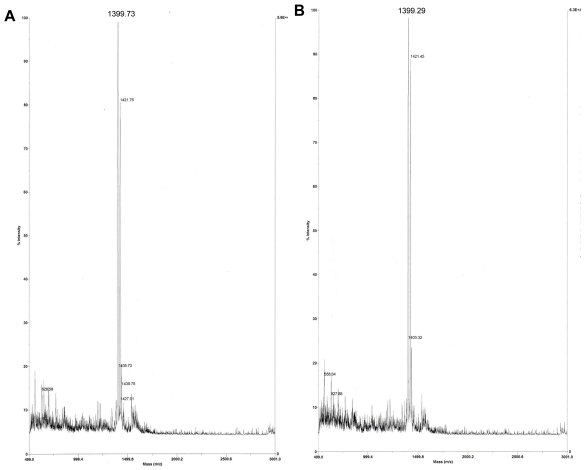


Figure 4S. MALDI-TOF mass spectrum. MALDI-MS of Peptide-1 fragment (A) and fragment from **M1** (B) cleaved by MMP-9. Mass calcd: 1398.6, Obs: 1399.7 (A) and 1399.2 (B).

is estimated to be 41% (Figure 3S) as a fraction of the area of Peak B. We note that it is possible this is a low estimate because particle aggregation may prevent all free peptide fragment from entering the RP column on HPLC. Alternatively, it is possible that steric hindrance reduces accessibility to the enzyme.

Kinetics of FRET Fluorescence by Cell-expressed MMP Enzymes (in Main text Fig. 4d)

Figure 4d of the main text shows the time course data for the development of the FRET signal from enzyme-driven **M1**/**M2** aggregation utilizing cell-expressed MMP-2 and -9, from MMP-overexpressing cell line, WPE1-NA45. MCF-7 was chosen as the control cell line. Both cells were seeded at concentrations of 1.6 x 10^4 cells/well, in 96-well clear bottom culture plates. After 24 hrs, the cell medium were transferred to another 96-well black bottom plate for fluorescence measurements in the presence of **M1** and **M2** mixtures. **M1** and **M2** at various concentrations were used in this assay. WPE1-NA45 cell-secreted MMP enzymes were quantified by ELISA assays (see below).

Enzyme-Linked Immunosorbent Assay (ELISA) for quantification of enzyme in Main text Fig. 4d

MMP-2 and MMP-9 ELISA kits were purchased from Invitrogen, inc. The procedure was carried out as per the manufacturer's standard instructions. Briefly, MMP standards from the kit and samples (cell medium from WPE1-NA45 and MCF-7 at time points of 0, 12, 24, 36 and 48 hrs) were added into the well strips and incubated for 2 hrs at room temperature. The solution was then discarded and washed four times. A solution of biotinylated MMPs was then added and reacted for an hour at room temperature. Following this, the solution was discarded and washed four times. To this was added a Streptavidin-Horse Radish Peroxidase solution for 30 min which was then discarded. The "Chromagen" solution was then added to the wells followed by another wash. The absorbance at 450 nm was measured after the addition of a "stopping" solution. Therefore, in this manner, cell-expressed MMP concentrations in the supernatant media as added to **M1** and **M2**, were calculated with calibration by the MMP standards.

Calculation of N_w^{agg} via Particle Counting.

Micelle particle counting was performed via a 20 nm gold nanoparticle (Au NP) calibration utilizing TEM image analysis. 20 μL of **M3** at a polymer concentration of 1.1 μM (as determined by UV-Vis), was mixed with 20 μL of 20 nm Au NPs at a concentration of 7 x 10^{14} particles/L. A total of 1243 **M3** particles were counted, and 158 Au NPs were counted. Figure 5S shows TEM images of particles from these solutions, with white arrows indicating the Au NPs. **M3** is clearly discernable from the high contrast Au NPs as uranyl acetate stained organic material. Using this calibration method we determine **M3** solutions contain 5.51 x 10^{15} particles/L. The aggregation number was determined to be 120, taking the particle count (5.51 x 10^{15} particles/L) and dividing by the polymer concentration in terms of molecules/L (6.624 x 10^{17}). This is on the same order as determined by SLS and via the geometrical analysis described in the main text. Alternatively, we can use this method as a confirmation of polymer concentration in solution as determined by UV-Vis. In this approach, the particle/L count (5.51 x 10^{15}) was multiplied by the weight average aggregation number (N_w^{agg}), 209 from SLS measurements, yielding 1.15 x 10^{18} surfactants/L. From this calculation we determine polymer concentration in solution to be 1.9 μM. Again, this is similar to that determined by UV-Vis (1.1 μM), which is the technique used to determine concentrations for CMC and enzymatic studies.

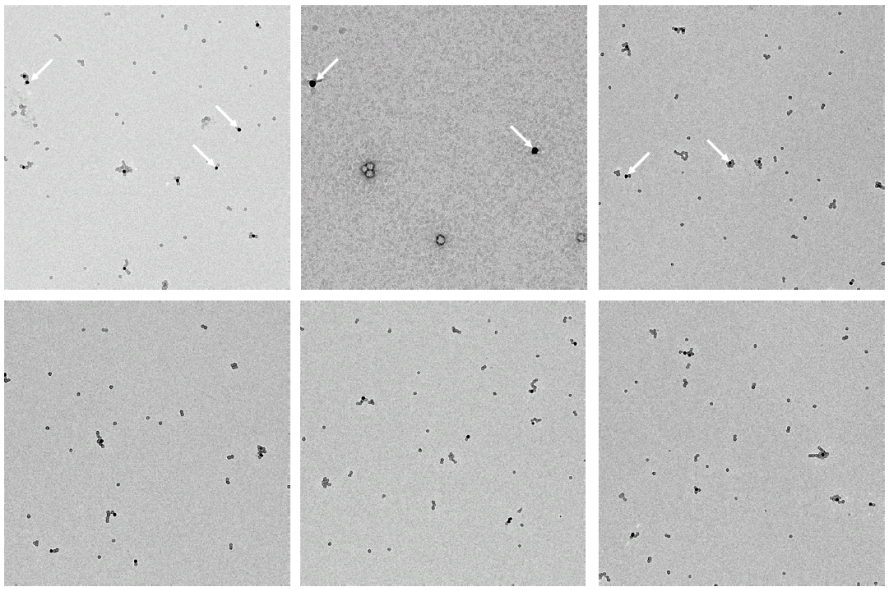


Figure 5S. Micelle counting via 20 nm Au NPs calibration visualized by TEM. 20 μL of **M3** was mixed with 20 μL of 20 nm Au NPs at concentration of 7 x 10^{14} particles/L. 1243 **M3** and 158 Au NPs were counted. TEM images shown here are representative of **M3** mixed with Au NPs. **M3** was counted as 5.51 x 10^{15} particles/L after calibration by Au NPs. Arrows indicate some representative 20 nm Au NPs visible clearly from TEM images as solid spheres, as opposed to open circles for the organic matter stained by uranyl acetate.

Micellar Molecular Weight Determination via Static Light Scattering (SLS)

M1, **M2** and **M3** micellar average molecular weight were measured on a Wyatt Dawn Heleos-II multi-angle light scattering instrument in batch mode. **M1**, **M2** and **M3** micellar molecular weights were measured as 2.428 x 10^6 g/mol, 4.646 x 10^6 g/mol, and 3.184 x 10^6 g/mol, respectively. These micellar molecular weights were then divided by polymer molecular weight (15,270 g/mol, Table 1) and micellar aggregation numbers were obtained as 159, 304 and 209 for **M1**, **M2** and **M3** micelles (Table 2S). This is further confirmation that aggregation numbers are on the order of 200 per particle for this type of surfactant, generating micelles on the scale of approximately 30-40 nm in diameter.

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Table 2S. Weight average molar mass and aggregation number (formally the weight average aggregation number, N_w^{agg}) of **M1**, **M2** and **M3** from SLS.

	M_w from SLS (g/mol) of micelles	Weight average aggregation number (N_w^{agg})
M1	2.428 x e^6 ± 5171	159 ± 0.34
M2	4.646 x e^6 ± 34426	304 ± 2.3
M3	3.184 x e^6 ± 12513	209 ± 0.82

Maximum Aggregation Number Calculated Knowing the Angle at the Vertex of Each Spherical Based Cone

The following equation was used to calculate N_{sph} and is derived from Ref: 26 cited in the main text.

$$N_{sph} = \mathrm{Int}\left(\frac{2\pi}{3\left(\arccos\left(\frac{\cos\alpha}{2\cos^2\frac{\alpha}{2}}\right)\right) - \pi}\right) \quad (1)$$

Distance Distribution via Fluorescence Lifetime Measurement (Figure 3b, Main text)

Here, we have considered a range of D–A distances where the distance is expressed as a probability function *P(r)* distributed along the *r* axis.[6] A Gaussian distribution was used to describe the distance distribution, as in the equation below:[6]

$$P(r) = \frac{1}{\sigma\sqrt{2\pi}}\exp\left[-\frac{1}{2}\left(\frac{\bar{r}-r}{\sigma}\right)^2\right]$$

In this equation $\bar{r}$ is the mean of the Gaussian with a standard deviation of σ. The distance distribution is described by two standard deviations from the mean, with the probability of finding donor and acceptor within this range is 95.4%. The donor intensity decay is a summation of the intensity decays for all accessible distances, and is written as:

$$I_{DA}(t) = \int_{r=0}^{\infty} P(r) I_{DA}(r,t)\,dr$$
$$= I_D^0 \int_{r=0}^{\infty} P(r)\exp\left[-\frac{t}{\tau_D} - \frac{t}{\tau_D}\left(\frac{R_0}{r}\right)^6\right]dr$$

This expression indicates that the intensity decay for an ensemble of flexible D–A pairs is given by the weighted average of the decays for each D–A distance. From this analysis, the distance distribution is calculated as 3.6 ± 0.61 nm shown in Figure 3 (main text) and written as *r*.

The lifetime in **M3** (τ_{DA}) was then calculated from the standard treatment of FRET efficiency (*E*):[6]

$$E = \frac{R_0^6}{R_0^6 + r^6}$$

where R_0 is the Förster distance for the fluorescein and rhodamine pair, applied as 55Å in this work given the assumption that rotation of the dyes is free and that therefore the orientation factor, $\kappa^2 = 2/3$.[6-7] The transfer efficiency can then be used to calculate the lifetime of the donor-acceptor (τ_{DA}):

$$E = 1 - \frac{\tau_{DA}}{\tau_D}$$

In this work, lifetimes of Fluorescein-Rhodamine labeled micelle (**M3**) and Fluorescein-labelled micelle (**M1**) were obtained as 0.29 ns and 3.98 ns respectively from fluorescence lifetime measurements (see Figure 3 in the Main text).

DLS of M1 and M2 with and without activated MMP-9

M1 and **M2** micelles at 500 nM each with respect to PPA, were mixed with activated MMP-9 (see above for enzyme activation details) and non-activated MMP-9 (10 nM) at 37°C for 24 hrs. DLS measurements were then taken (Figure 6S.)

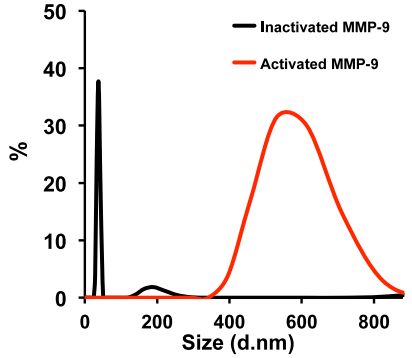

Figure 6S. DLS of **M1**/**M2** micelle mixtures mixed with non-activated MMP-9 (black) or activated MMP-9 (red). The volume % is shown on the Y-axis. X-axis is the hydrodynamic diameter.

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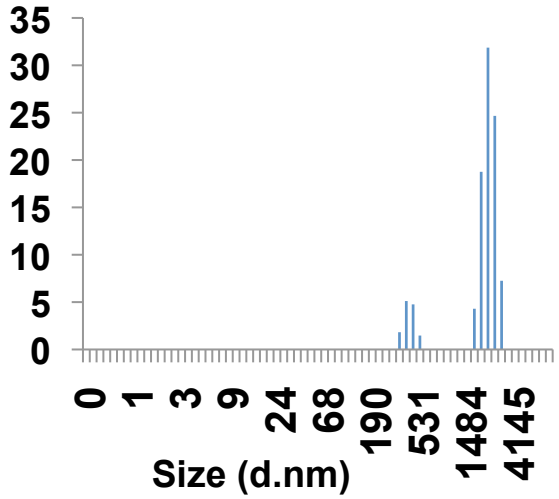
FRET efficiency for aggregated species resulting from M1/M2 plus enzyme versus M3 micelles

Relative FRET efficiency for **M3** may be expressed as the ratio of the intensity of the Rhodamine emission peak compared to the Fluorescein emission peak (shown in Main text Fig. 2c) giving 0.8:1 (Rhodamine:Fluorescein). By contrast, FRET efficiency of the aggregated species produced when **M1**/**M2** micelles were mixed with MMP-9 enzymes (Fig. 4a) is 0.43:1 (Rhodamine:Fluorescein). This can be contrasted against the efficiency of FRET from the lifetime (see equation above) of the donor for **M3** found to be 92%. This compares to the aggregates with efficiencies of 85%. These data are consistent with a higher background of donor fluorescence in the absence of acceptor.

M3 + MMP-9

This experiment confirmed that **M3** would undergo the same transformations in response to MMP-9 as **M1** and **M2**.
Micelles at 0.5 μM with respect to PPAs were mixed with MMP-9 (10 nM) for 24 hrs. Below is shown DLS and TEM data for the formation of micron scale aggregates as for **M1** and **M2**.



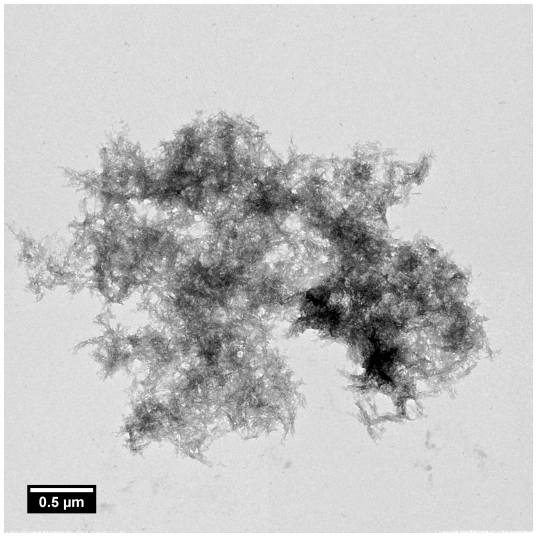


Figure 7S DLS and TEM data of **M3** mixed with MMP-9.

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