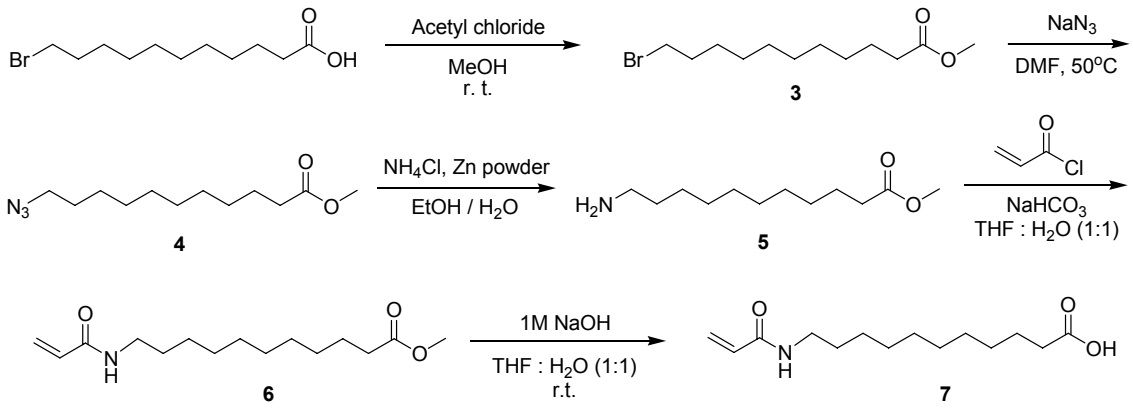
Supporting Information

Bioactive Molecular Sheets from Self-Assembly of Polymerizable Peptides

Kyung-Soo Moon, Eunji Lee, Yong-beom Lim, and Myongsoo Lee*
Center for Supramolecular Nano-Assembly and Department of Chemistry, Yonsei University, Seoul 120-749, Korea.
E-mail: mslee@yonsei.ac.kr

Materials. 11-Bromoundecanoic acid (99%), acetyl chloride (98%), sodium azide (98%) and acryloyl chloride (99%) from Aldrich was used as received. Unless otherwise indicated, all starting materials were obtained from commercial suppliers (Aldrich, Lancaster, etc.) and were used without purification. Visualization was accomplished with UV light and iodine vapor. Flash chromatography was carried out with Silica Gel 60 (230-400 mesh) from EM Science. The compounds were synthesized according to the procedure described scheme S1.



Scheme S1. Synthesis of 11-(acrylamido)undecanoic acid.

Synthesis of compound 3

To a 100 mL round-bottom flask was added 11-bromoundecanoic acid (11.3 mmol), MeOH (50 mL) and AcCl (56.5 mmol). The solution was stirred for 3 hr at rt and concentrated. The resulting white solids were resuspended in CH_2Cl_2 (100 mL), washed with $NaHCO_3$ (2 × 100 mL) and brine (100 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (silica gel) using hexane : ethyl acetate (9:1 v/v) as eluent to yield 3.2 g (99 %) of pale yellow liquid. ^{1}H-NMR (250 MHz, $CDCl_3$, ppm): δ = 3.67 (s, 3H), 3.40 (t, 2H, *J* = 6.9 Hz), 2.30 (t, 2H, *J* = 7.4 Hz), 1.88-1.80 (m, 2H), 1.64-1.27 (m, 14H).

Synthesis of compound 4

To a 100 mL round-bottom flask was added **3** (11 mmol), anhydrous dimethyl formamide (DMF, 40 mL) and sodium azide (56 mmol). The solution was stirred overnight at 50 ^{o}C, cooled to rt and concentrated. The crude material was resuspended with CH_2Cl_2 (100 mL), washed with water (2 × 100 mL) and brine (100 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (silica gel) using hexane : ethyl acetate (9:1 v/v) as eluent to yield 3.1 g (99 %) of pale yellow liquid. ^{1}H-NMR (250 MHz, $CDCl_3$, ppm): δ = 3.66 (s, 3H), 3.25 (t, 2H, *J* = 6.9 Hz), 2.30 (t, 2H, *J* = 7.4 Hz), 1.64-1.27 (m, 16H).

Synthesis of compound 5

To the solution of **4** (5.1 mmol) and ammonium chloride (12.7 mmol) in ethyl

alcohol (20 mL) and water (7 mL), zinc powder (7.7 mmol) was added, the mixture was stirred vigorously at refluxing. After the reaction is over (monitored by TLC), ethyl acetate (200 mL) and aqueous ammonia (10 mL) was added. The mixture was filtered, and the filtrate was washed with brine, dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was purified by column chromatography (silica gel) using dichloromethane : methanol (10:1 v/v) as eluent to yield 628 mg (57 %) of white solid. ^{1}H-NMR (250 MHz, $CDCl_3$, ppm): δ = 3.65 (s, 3H), 2.66 (t, 2H, *J* = 6.8 Hz), 2.29 (t, 2H, *J* = 7.4 Hz), 1.62-1.26 (m, 16H).

Synthesis of compound 6

To the solution of **5** (2.7 mmol) in THF (15 mL) and saturated $NaHCO_3$ (15 mL), acryloyl chloride (2.7 mmol) was slowly added at 0 ^{o}C, the mixture was stirred at rt for 1 h. After the reaction is over, ethyl acetate (100 mL) was added and organic layer was washed with 1N HCl (100 mL), saturated $NaHCO_3$ (100 mL), brine (100 mL) and dried over $MgSO_4$. After removal of solvent under reduced pressure, the residue was purified by column chromatography (silica gel) using hexane : ethyl acetate (1:1 v/v) as eluent to yield 577mg (78 %) of liquid. ^{1}H-NMR (250 MHz, $CDCl_3$, ppm): δ = 6.31-6.23 (dd, 1H, *J* = 16.9, 1.6 Hz), 6.13-6.02 (dd, 1H, *J* = 16.9, 10.1 Hz), 5.64 (s, 1H), 5.60-5.59 (dd, 1H, *J* = 10.1, 1.6 Hz), 3.66 (s, 3H), 3.35-3.27 (m, 2H), 2.29 (t, 2H, *J* = 7.4 Hz), 1.63-1.22 (m, 16H).

Synthesis of compound 7

To a 100 mL round-bottom flask were added **6** (2.1 mmol), THF (10 mL) and water (10 mL). 1M NaOH (10.7 mmol) was then added dropwise and reaction was

stirred overnight at rt. The reaction mixture were diluted with ethyl acetate (50 mL), washed with 10% HCl (25 mL), washed with water (2 × 50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$ and concentrated to afford the corresponding compound **7** of white solid in good yield (511 mg, 94 %). ^{1}H-NMR (250 MHz, $CDCl_3$, ppm): δ = 6.33-6.25 (dd, 1H, *J* = 16.9, 1.6 Hz), 6.13-6.02 (dd, 1H, *J* = 16.9, 10.1 Hz), 5.66 (s, 1H), 5.66-5.61 (dd, 1H, *J* = 10.1, 1.6 Hz), 3.36-3.28 (m, 2H), 2.34 (t, 2H, *J* = 7.4 Hz), 1.64-1.20 (m, 16H).

Synthesis of peptides 1 and 2

Tat CPP and arginine rich peptides were synthesized on Rink amide MBHA resin (Anaspec) using standard Fmoc protocols on Applied Biosystems model 433A peptide synthesizer. Then Fmoc-L-lysine(Fmoc)-OH in which both α- and ε-amines are protected with Fmoc was coupled for the dendritic growth of the peptide. Compound **7** was coupled manually following deprotection of Fmoc group from the resin-bound peptide. The resin-bound peptide (37 μmol) was suspended in *N*-methyl-2-pyrrolidone (NMP) (1 mL) and then compound **7** (48 mg, 187 μmol), *N*,*N*-diisopropylethylamine (DIPEA; 374 μmol), and HBTU (64 mg, 168 μmol) dissolved in 1 mL of NMP were added. The reaction continued overnight with shaking at room temperature. The resin was then washed with NMP (×5), THF (×5), and dried *in vacuo*. The dried resin was treated with cleavage cocktail (trifluoroacetic acid : triisopropylsilane; 96 : 4) for 3 h, and was triturated with *tert*-butyl methyl ether (TBME)/hexane. The peptide **1** was purified by reverse phase HPLC on C_4 column (Vydac) using linear gradient of water (0.1 % TFA) and acetonitrile (0.1 % TFA). The peptide **2** was also purified by reverse phase HPLC on C_4 column (Vydac) using linear gradient of water (0.1 % HFBA) and

acetonitrile (0.1 % HFBA). Peptide molecular weight was confirmed by MALDI-TOF mass spectrometry. The purity of the peptides was >95% as determined by analytical HPLC. Concentration of the peptides was determined spectrophotometrically in water / acetonitrile (1 : 1) using a molar extinction coefficient of tryptophan (5500 M^{-1} cm^{-1}) at 280 nm.

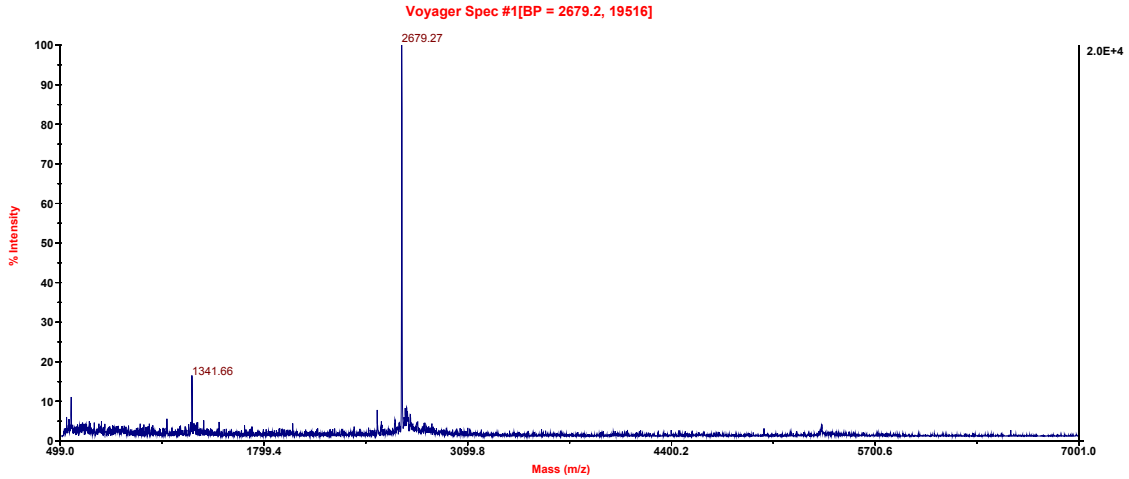


Figure S1. MALDI-TOF data of peptide **1**.

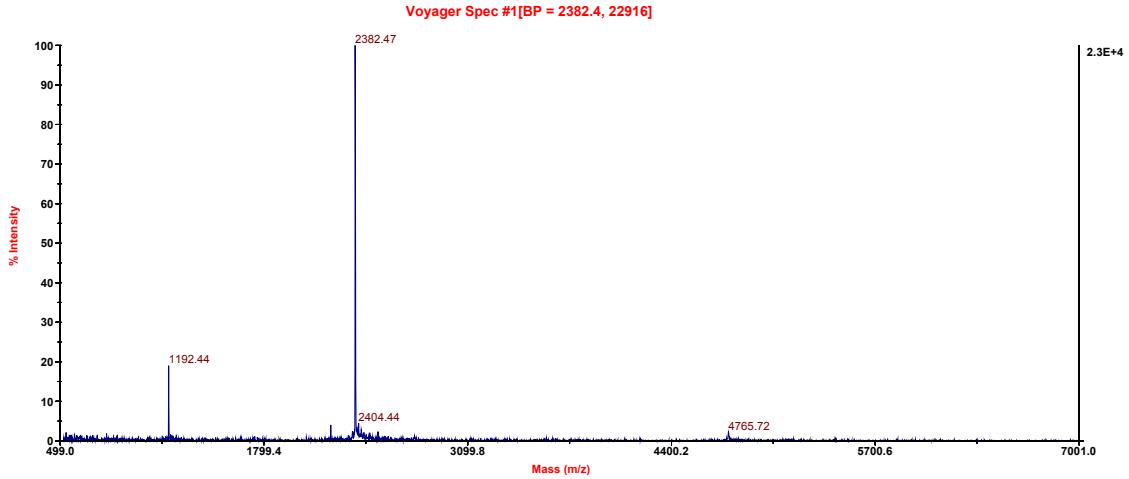


Figure S2. MALDI-TOF data of peptide **2**.

Photo-polymerization of self-assembled 1 and 2

1,2-Ethanedithiol (1 μmol) was added to a aqueous solutions of **1** and **2** (1 mL in quartz cuvette). After purged with argon, the mixture was irradiated with UV light (254 nm, VL-4.LC hand UV from VILVER LOURMAT) for 2 h. The polymer was characterized by ^{1}H-NMR spectroscopy and dynamic light scattering (DLS).

Nile Red encapsulation experiment

Typically, to the dye Nile Red (133 ng, 0.45 nmole) dissolved in acetone (2 μL) in microcentrifuge tube was added 300 μL of polymer of **1** (9 nmole) in water and the solution was sonicated. The acetone was evaporated by opening the microcentrifuge tube cap overnight and the volume of the solution became about 280-290 μL. The solution was then lyophilized to dryness to remove any trace of acetone. The dried residue was redissolved in water at the desired concentration and sonicated. The solution was then centrifuged for 5 min at maximum speed (16,110 × g) in tabletop centrifuge to remove Nile Red molecules not encapsulated in nanostructures of **1**.

DLS, TEM, IR and AFM experiments

Dynamic light scattering experiment was performed at room temperature with ALV/CGS-3 Compact Goniometer System equipped with He-Ne laser operating at 632.8 nm. The scattering angle was 90°. For TEM experiment, 3 μL of an aqueous solution of sample was placed onto a holey carbon-coated copper grid, and 3 μL of 2 % (w/w) uranyl acetate solution was added for negative stain. The sample was deposited for 1 min, and excess solution was wicked off by filter paper. The dried specimen was observed with a JEOL-JEM 2010 instrument operating at 120 kV. The data were

analyzed with Digital Micrograph software. For IR experiment, several drops of an aqueous solution of sample were placed onto a ZnSe pellet and dried. The dried specimen was recorded on BRUKER Equinox 55 FT-IR spectrophotometer. For AFM experiment, one drop of an aqueous solution of sample was placed onto a holey carbon-coated copper grid, and the sample was deposited for 1 min. Excess solution was wicked off by filter paper. The dried specimen was observed with a Nanoscope IIIa Multimode AFM instrument.

Cell culture and assays

For MTT assay,[1] HeLa cells (1×10^4) were seeded in 96-well plate and grown in DMEM with 10 % FBS to reach 60-70% confluence. Following exposure of the cells with Tat peptide, peptide **1** and polymer of **1** for 3 h, the cells were washed with phosphate-buffered saline (PBS), and 100 μL of culture medium and 25 μL of 5 mg/mL stock solution of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were added to each well. After 2 h of incubation at 37 °C, 100 μL of extraction buffer (20% w/v of SDS in a solution of 50% DMF, pH 4.7) were added. Absorbance was measured at 570 nm after an overnight incubation at 37 °C.

For microscopic observation of intracellular delivery of Nile Red, HeLa cells (5×10^4) were seeded on 8-well Lab-tek II chambered coverglass system (Nunc) in DMEM with 10% FBS and cultured overnight. The cells were treated with Nile Red-encapsulated polymer of **1** for 3 h. Concentration of polymer sheets of **1** was 10 μM and the encapsulated Nile Red was 5 mole percent relative to that of **1**. The cells were then washed three times with PBS. Live cell images were observed with Nikon Eclipse TE2000-U inverted fluorescence microscope equipped with Y-2E/C filter set (Nikon).

For confocal images, Nikon Eclipse TE2000-U inverted microscope equipped with Perkin Elmer UltraVIEW RS confocal scanner was used.

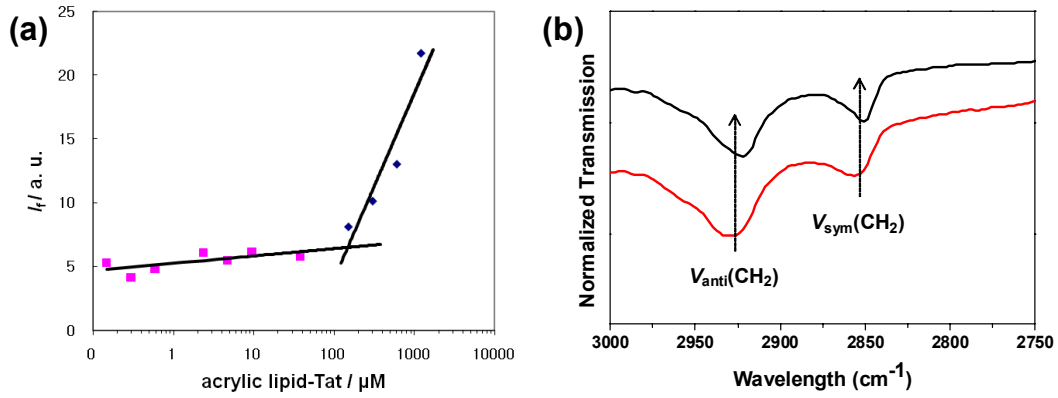


Figure S3. (a) Critical micelle concentration (CMC) determination. Fluorescence emission intensities of Nile Red at 635 nm were plotted against concentrations of **1**. Excitation was 550 nm. I_f = fluorescence intensity. (b) IR spectrum of peptide **1** (red line) and polymer of **1** (black line) indicative of alkyl crystallization.

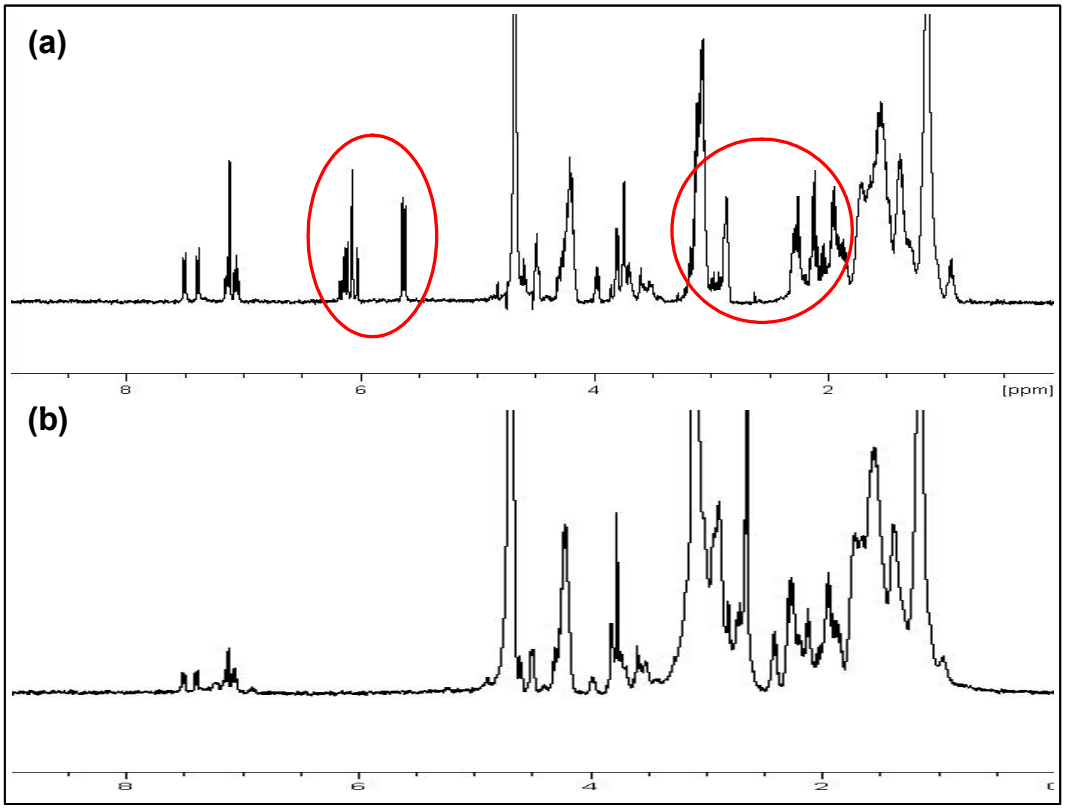


Figure S4. ^{1}H-NMR spectra of (a) **1** and (b) polymer of **1** in D_2O. The ^{1}H-NMR spectrum of polymer of **1** reveals complete disappearance of the acrylic double bond at around 6 ppm as well as appearance of a thioether peak at around 3 ppm, which indicates that all the acryl groups of **1** have been successfully converted to thioether linkages upon formation of polymer.

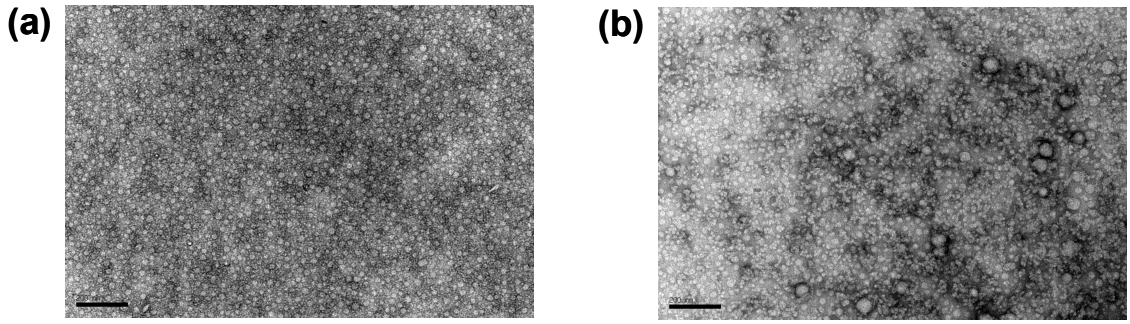


Figure S5. TEM images of (a) **2** and (b) polymer of **2** in aqueous solution with negative staining (scale bar = 200 nm).

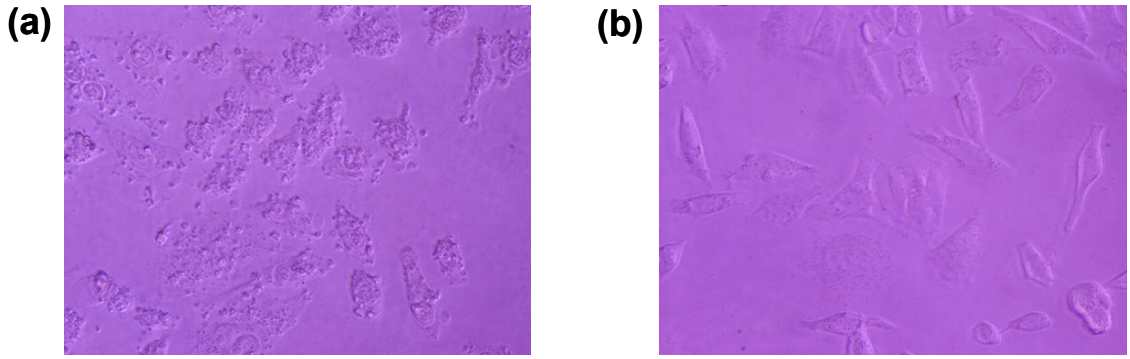


Figure S6. Microscope images (400×) of Hela cells treated with (a) **1** (25 μM) and (b) polymer of **1** (25 μM) for 3 h.

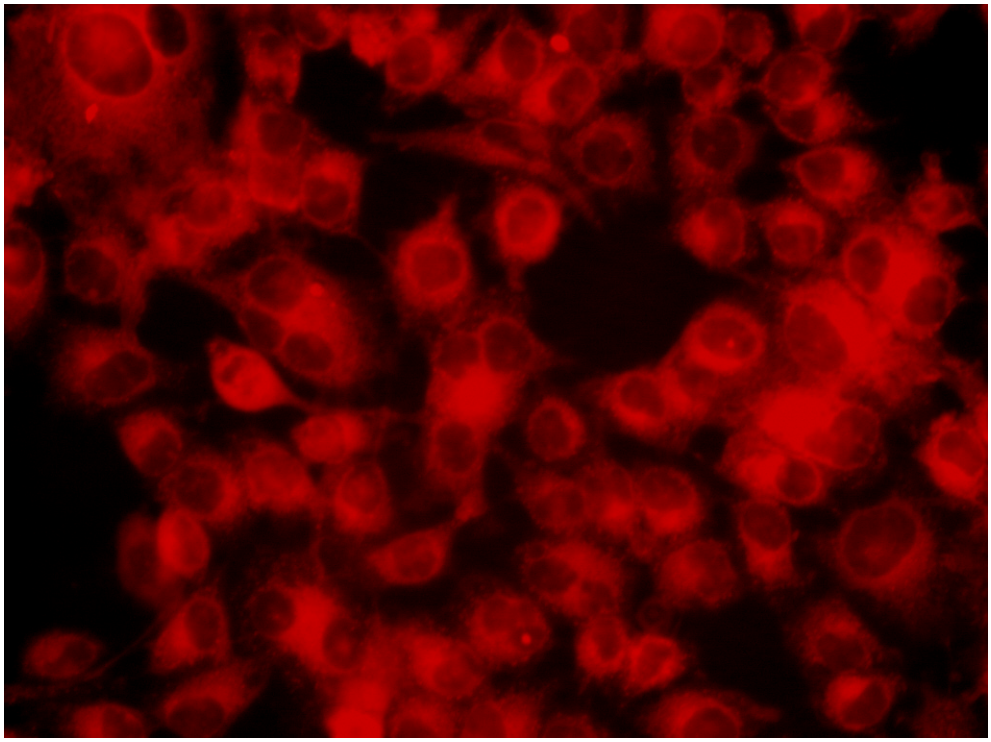


Figure S7. Fluorescence microscope image (400×) of Hela cells treated with Nile Red encapsulated polymer of **1** for 3 h.

Reference

1. M. B. Hansen, S. E. Nielsen, K. Berg, *J. Am. Chem. Soc*., 2000, **122**, 6524.