

Electronic Supplementary Information for:

NanoPARCEL: A method for controlling cellular behavior with external light

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Methods

Materials.

Tetra-poly(ethyl glycol)-amine (SUNBRIGHT PTE-050PA; Mn, 5328 g/mol) was purchased from NOF Corporation (Tokyo, Japan). Tetraethylmethylene diamine (TEMED), dichloromethane (DCM), methacryloyl chloride (MC), triethylamine (TEA), ammonium persulfate (APS), tris(hydroxymethyl) aminomethane, hydrochloric acid, methanol, acetone, 1-butyl alcohols, diethyl ether, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride *n*-hydrate (DMT-MM), sodium bicarbonate, trypsin(**porcine**) and caspase-3 (**human recombinant**) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Photocleavable linker, 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenyl] butyric acid, was purchased from Sigma-Aldrich (St. Louis, MO). Water was purified with a Milli-Q apparatus (Millipore, Bedford, MA). BODIPY-casein and lipofectamine 2000 were purchased from invitrogen (California, U.S.A). **CR(DEVD)₂ was purchased from Enzo life sciences (Pennsylvania, U.S.A).** The plasmid of EB1 (end-binding 1)-GFP (green fluorescence protein) was prepared by the procedure reported previously.^[S1]

Encapsulation of proteins in photodegradable nanoparticles

Proteins were encapsulated in photocleavable nanoparticles by combining PEG-Photo-MA (**2**) (20 μ L), protein (**2 mg/mL trypsin or 10 mg/mL BODIPY-casein or 10 μ g/mL caspase-3**) solution (20 μ L), 0.1 M ammonium persulphate (10 μ L), and 0.1 M *N,N,N',N'*-tetramethylethylene diamine in 1 M Tris/HCl buffer (pH 7.0, 10 μ L) in that order at a temperature below 4 ° C and then stirring the mixture for 20 min to afford a dispersion of nanoparticles with diameters of ~150 nm.

DLS measurement of nanoparticle size distribution

A DLS-8000 spectrophotometer (Otsuka Electronics Co., Osaka, Japan) was used to measure the size distribution of the nanoparticles in the dispersion. The dispersion was diluted 100 fold with water for DLS measurements. We also measured the dispersion after UV irradiation using a UV curing system (Aicure UV20, Panasonic, Osaka, Japan) as a light source (UV=365 nm). All DLS measurements were carried out at 25 ° C with a scattering angle of 90° . The autocorrelation function, $g(\tau)$, was analyzed by means of the cumulant method.^[S2]

TEM measurement of nanoparticle size

TEM images were obtained with an H-7000 electron microscope (Hitachi, Tokyo, Japan) operating at 75 kV. Copper grids (400 mesh) were coated first with a thin film of collodion and then with carbon. The nanoparticle dispersion (1 μL) was placed on the coated copper grids, and the surface of the sample-loaded grid was stained with a drop of a 50% aqueous solution of ethanol containing 2 wt% uranyl acetate. The stained surface was dried at room temperature before observation.

The size of the nanoparticle was measured by ruler. The ratio of nanoparticle larger than 60 nm were shown in Supporting Figure 2.

In vitro fluorescence assay of the activity of nanoparticle-encapsulated trypsin

A dispersion of trypsin-containing nanoparticles (10 μL) and 190 μL of 10 $\mu\text{g mL}^{-1}$ BODIPY-casein substrate were added to each well of a 96-well microassay plate. The plate was incubated at 37 ° C for 2 h, and the fluorescence signals (Ex 485 nm, Em 535 nm) were measured with a multiplate reader (SH-9000, Hitachi, Tokyo, Japan). The plate was then subjected to UV irradiation at 365 nm for 10 s (0.57 W cm^{-2}) using Aicure UV20, after which the fluorescence signals were measured again.

Cell culture

The human hepatocyte carcinoma cells (Huh-7) were cultured in a humidified atmosphere of 95% air and 5% CO_2 at 37 ° C in Dulbecco's Modified Eagle's Medium (DMEM Sigma-Aldrich) supplemented with 1% (v/v) penicillin-streptomycin solution (x 100) (Wako) and 10% (v/v) fetal bovine serum (FBS, invitrogen). For each experiment, cells at 80-90% confluence were harvested by trypsin/EDTA (Wako) digestion, washed and re-suspended in fresh growth medium with FBS at a cell concentration of 10^6 viable cells/culture flask (75 cm^2).

Cell based assay of BODIPY casein using HuH-7 cells

Nanoparticle-encapsulated BODIPY casein was prepared as described above. The resulting dispersion was filtered through an Ultrafree-MC centrifugal filter (0.22 μm GV Durapore; Millipore, Billerica MA, USA). After the addition of dextran-Texas Red (25 μL of 20 mg/mL) to the filtrate, the solution was injected into HuH-7 cells with an InjectMan NI 2 micromanipulator (Eppendorf, Hamburg, Germany). Half the cells were irradiated with UV light (Aicure UV20) for 10 s (0.25 W/cm^2), and then all the cells were incubated at 37 ° C for 3 h. CLSM images of the cells were obtained with an LSM510 microscope (Carl Zeiss AG., Oberkochen, Germany) using W-PI 10X/ 23 eye peaces (Carl Zeiss AG.), and a C-Apochromat 40X/1.2W UV-VIS-NIR objective (Carl Zeiss AG.).

Activation of caspase-3 within HuH-7 cells

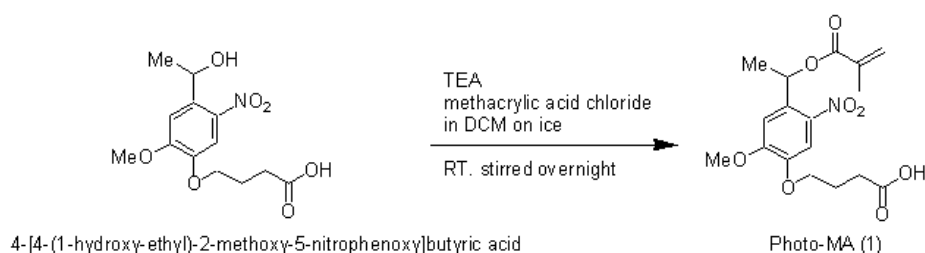
Free activated caspase-3 was injected into HuH-7 cells to confirm that it induced cell death. Then nanoparticle-encapsulated activated caspase-3 was prepared as described above and injected into HuH-7 cells along with dextran-Texas Red(25 μL of 20 mg/mL). Half the cells were irradiated with UV light (Aicure UV20) for 10 s (0.25 W/cm^2), and then all the cells were incubated at 37 ° C for 1 day. CLSM and PCM images of the cells were obtained with the LSM510 microscope.

Transfection of an EB1-GFP plasmid to HuH-7 cells

2×10^5 HuH-7 cells in 2 mL of DMEM were seeded in a 36-mm-diameter dish one day before transfection. Solutions of EB1-GFP (1 μg) plasmid and Lipofectamine 2000 (4 μL) in Dulbecco's Modified Eagle Medium (DMEM) without serum (250 μL for each solution) were prepared and allowed to stand for 5 min at room temperature. The two solutions were then mixed and allowed to stand for 30 min. After the HuH-7 cells were washed with DMEM without serum, the transfection complex was added dropwise to the dish containing the cells. After 40 min incubation, the medium was changed to DMEM containing serum, and incubation was continued for an additional 12 h.

Local release and activation of caspase-3 by irradiation

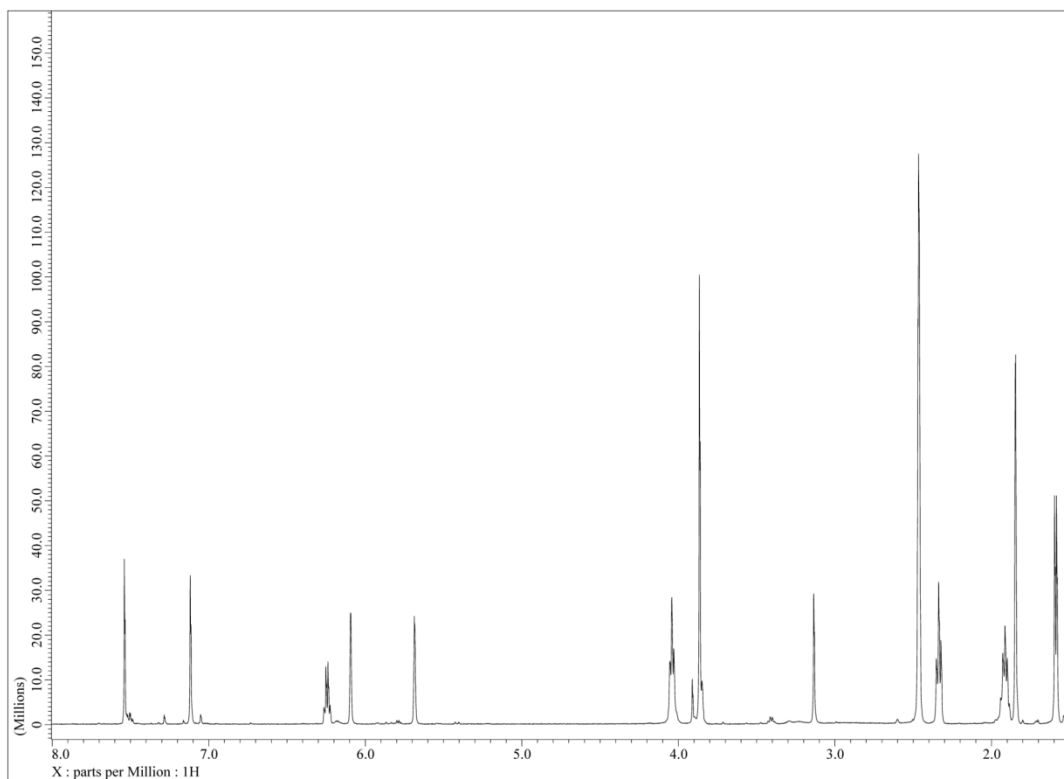
Nanoparticle-encapsulated activated caspase-3 (3.3 $\mu\text{g}/\text{mL}^{-1}$) was injected into a transfected HuH-7 cell. A portion of the injected cell was irradiated with a 405-nm laser (200 μW 20 sec) attached to the LSM510 microscope, and then CLSM and PCM images of the cell were obtained.

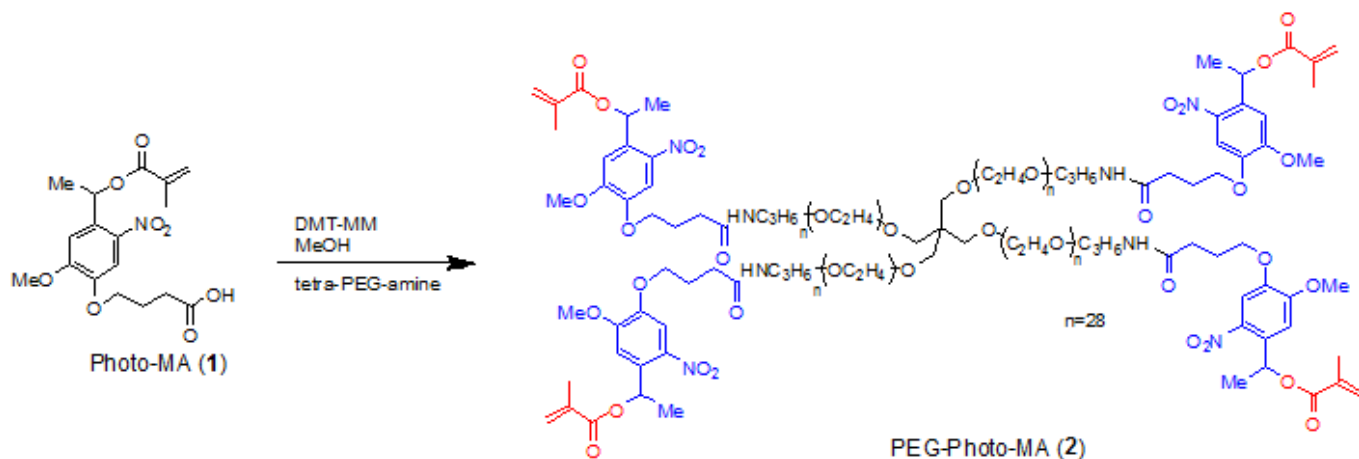


Supporting Scheme 1

Synthesis of Photo-MA(1). The photolabile molecule 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitro-phenoxy] butyric acid (1 mmol) was dissolved in dry DCM and stirred in a lightproof vial purged with N₂ gas. TEA (3 mmol) and MC (2.5 mmol), both dissolved in dry DCM, were added dropwise at 0 ° C. The reaction was stirred at room temperature overnight. The reaction solution was washed with sodium bicarbonate (5 w/v % aq), dilute hydrochloric acid (1 v/v % aq), and water. The solution was evaporated, and the liquid product was dissolved in aqueous acetone (50 v/v % aq). This reaction mixture was stirred at room temperature overnight and then extracted with DCM to recover the liquid acrylated monomer. The DCM layer was washed with dilute hydrochloric acid (1 v/v % aq) and water, dried over magnesium sulfate, and evaporated to yield the photocleavable methacrylate monomer referred to as Photo-MA (1). The structure of the compound was confirmed by ¹H NMR. ¹H NMR (500 MHz DMSO-*d*₆): δ=7.53 (s, Aromatic-H), δ=7.12 (s, Aromatic-H), δ=6.25 (q, Aromatic-CH(CH₃)OC(=O)C(CH₃)=CH₂), δ=6.10, 5.68 (s, s, OC(=O)C(CH₃)=CH₂), δ=4.05 (t, Aromatic-OCH₂CH₂CH₂COOH), δ=3.85 (s, Aromatic-OCH₃), δ=2.32 (t, Aromatic-OCH₂CH₂CH₂COOH), δ=1.91 (m, Aromatic-OCH₂CH₂CH₂COOH), δ=1.84 (q, Aromatic-CH(CH₃)OC(=O)C(CH₃)=CH₂), and δ=1.60 (d, Aromatic-CHCH₃).

^1H NMR spectrum of Photo-MA (1)



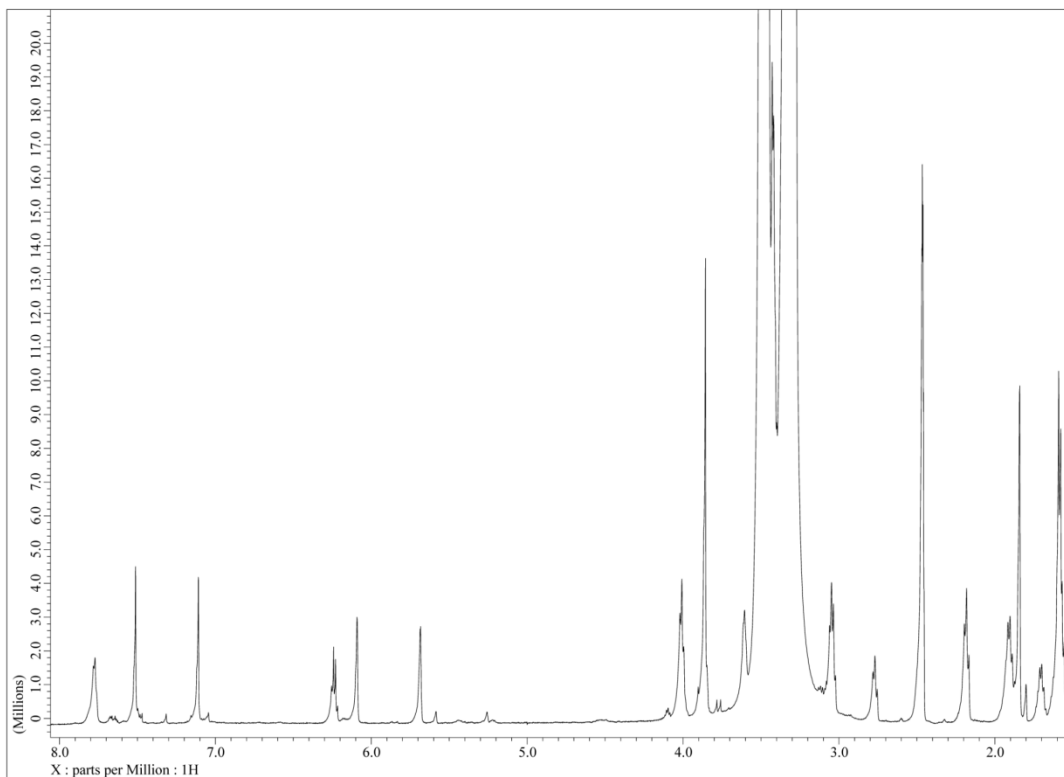


Supporting Scheme 2

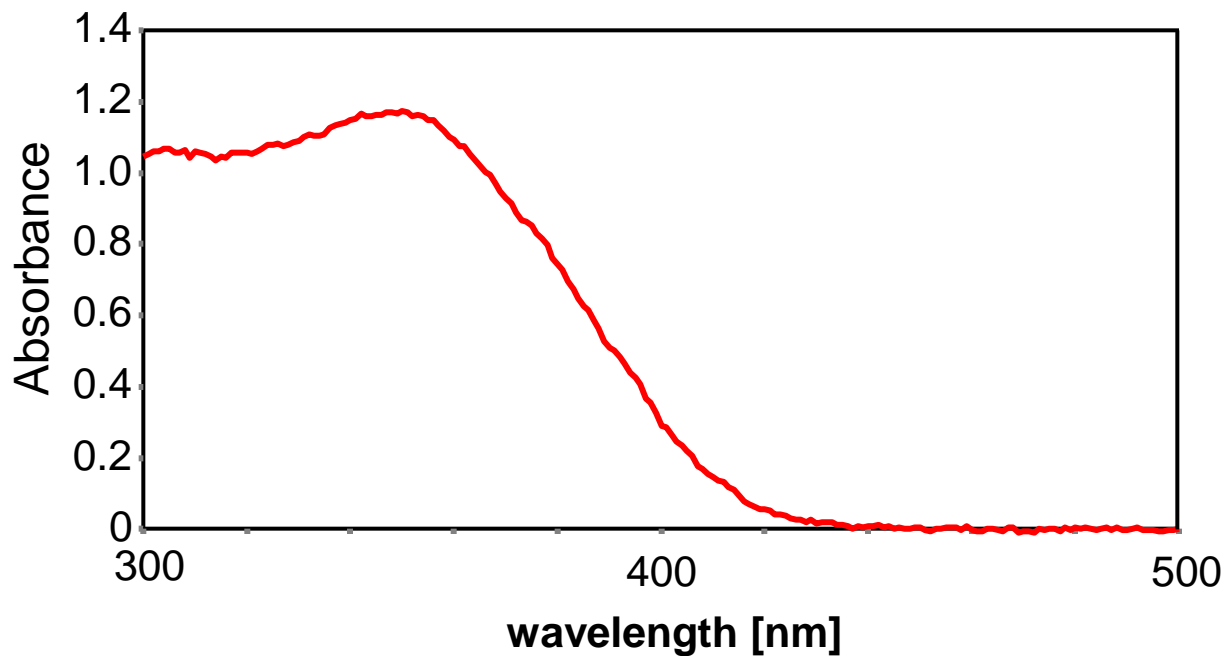
Synthesis of PEG-Photo-MA (2). Photo-MA(1) (0.85 mmol) was dissolved in methanol and stirred. Then, tetra poly (ethylene glycol)-amine (tetra-PEG-amine; 0.042 mmol) was added and stirred until all reactants were dissolved. After that, DMT-MM was added to start the synthesis without stirring.^[S3] The reaction was done at room temperature by overnight. The product was precipitated in diethyl ether on ice and filtered. The collected substance was washed with diethyl ether and dissolved in water. The aqueous solution was dialyzed (SpectraPor6, CO 1000 $gmol^{-1}$) and freeze-dried to yield the tetra-acrylated PEG referred to as PEG-Photo-MA. The structure of the linker was confirmed by 1H NMR.

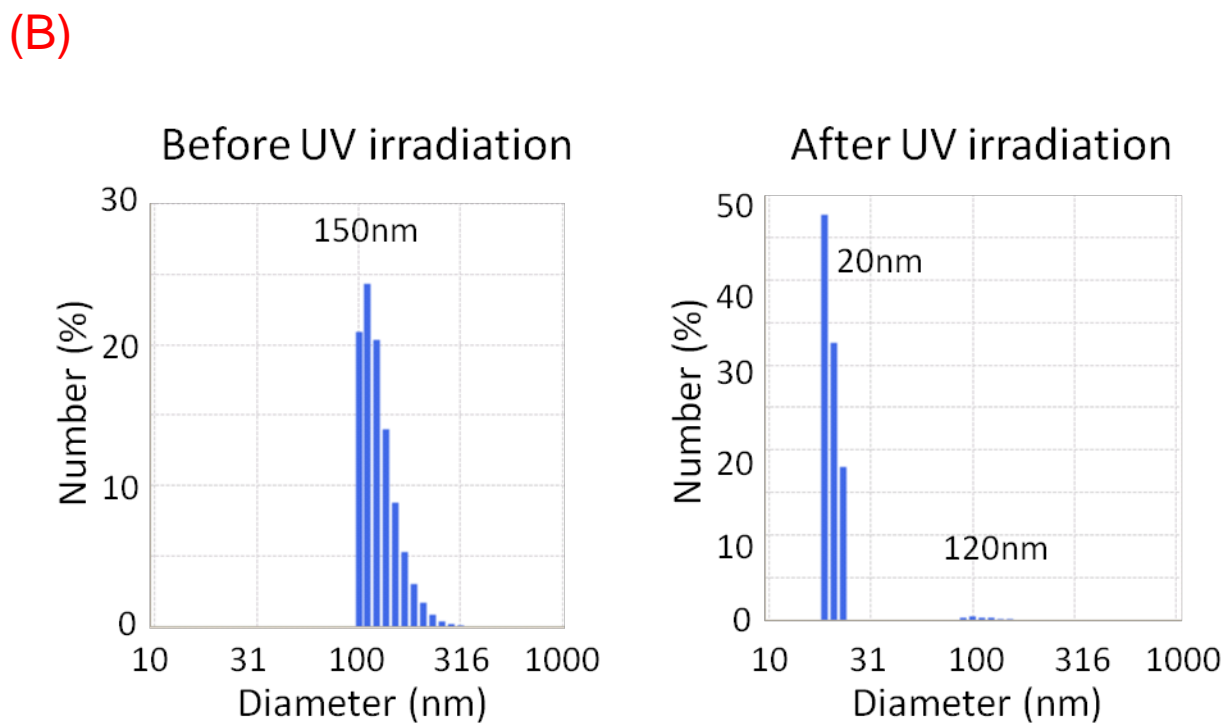
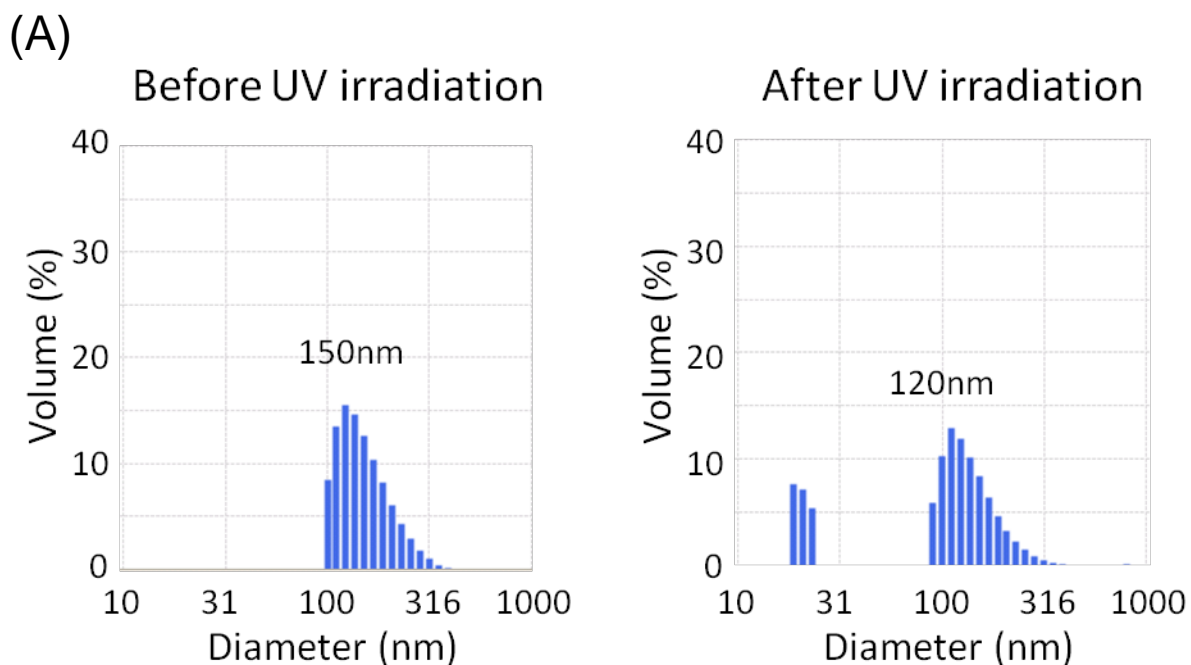
1H NMR (DMSO- d_6): $\delta=7.78$ (br, C(=O)NHCH₂CH₂O), $\delta=7.51$ (s, Aromatic-H), $\delta=7.10$ (s, Aromatic-H), $\delta=6.23$ (q, Aromatic-CH(CH₃)OC(=O)C(CH₃)=CH₂), $\delta=6.10$, 5.70 (s, s, OC(=O)CH=CH₂), $\delta=4.02$ (m, Aromatic-OCH₂CH₂CH₂COOH), $\delta=3.9$ (s, Aromatic-OCH₃), $\delta=3.61$ (m, NHCH₂CH₂CH₂O), $\delta=3.5$ (br, [CH₂CH₂O]_n, $n \approx 28$), $\delta=3.08$ (s, OCH₂C), $\delta=2.80$ (m, NHCH₂CH₂CH₂O), $\delta=2.20$ (m, Aromatic-OCH₂CH₂CH₂COONH), $\delta=1.93$ (m, Aromatic-OCH₂CH₂CH₂COONH), $\delta=1.83$ (s, Aromatic-CH(CH₃)OC(=O)C(CH₃)=CH₂), $\delta=1.72$ (m, NHCH₂CH₂CH₂O), and $\delta=1.60$ (d, Aromatic-CHCH₃).

^1H NMR spectrum of PEG-Photo-MA (2)



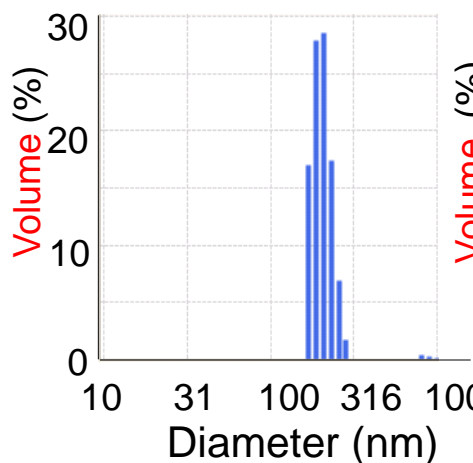
Absorption spectrum of PEG-Photo-MA (2)





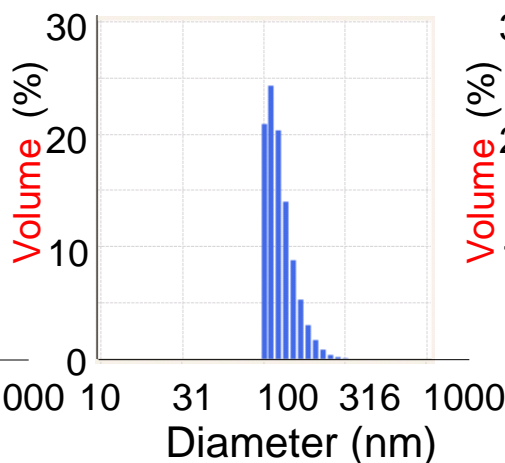
Supporting Figure 1. Effect of UV irradiation of nanoparticles. Size distribution of photodegradable nanoparticle-encapsulated trypsin before and after UV irradiation, measured by means of DLS (A):size distribution by volume, (B):size distribution by number

0.01 M APS



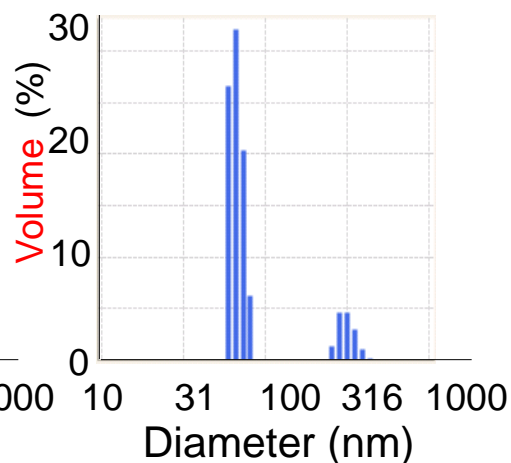
Average 200nm

0.1 M APS



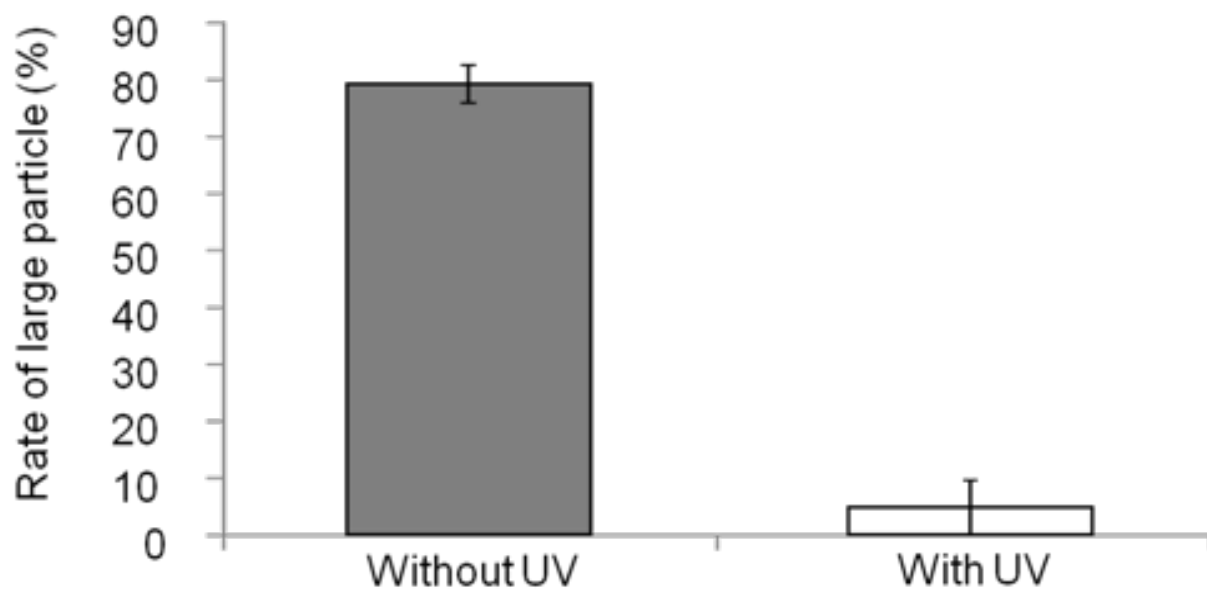
Average 150nm

1 M APS

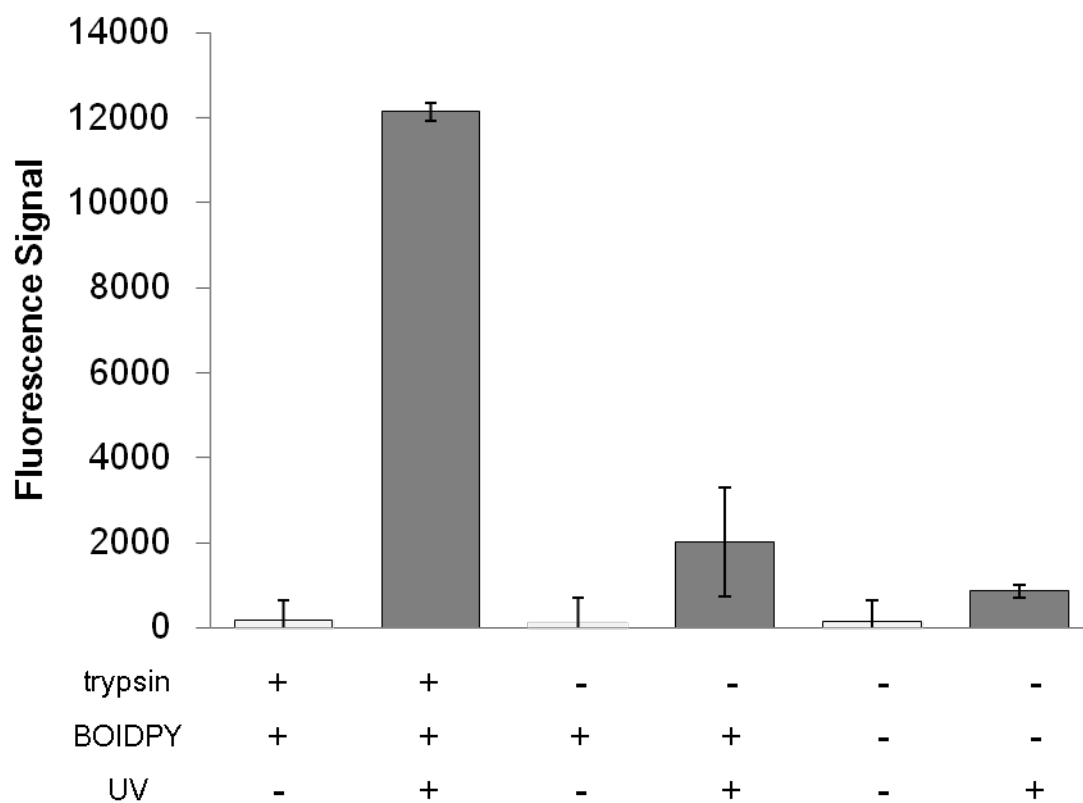


Average 70nm

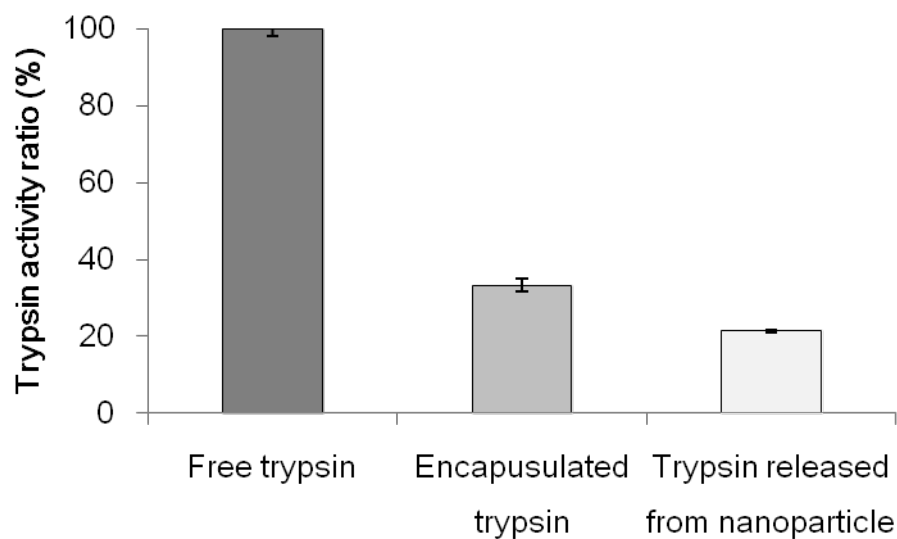
Supporting Figure 2. Effect of APS concentration on the size distribution **by volume** of the nanoparticle measured by means of DLS.



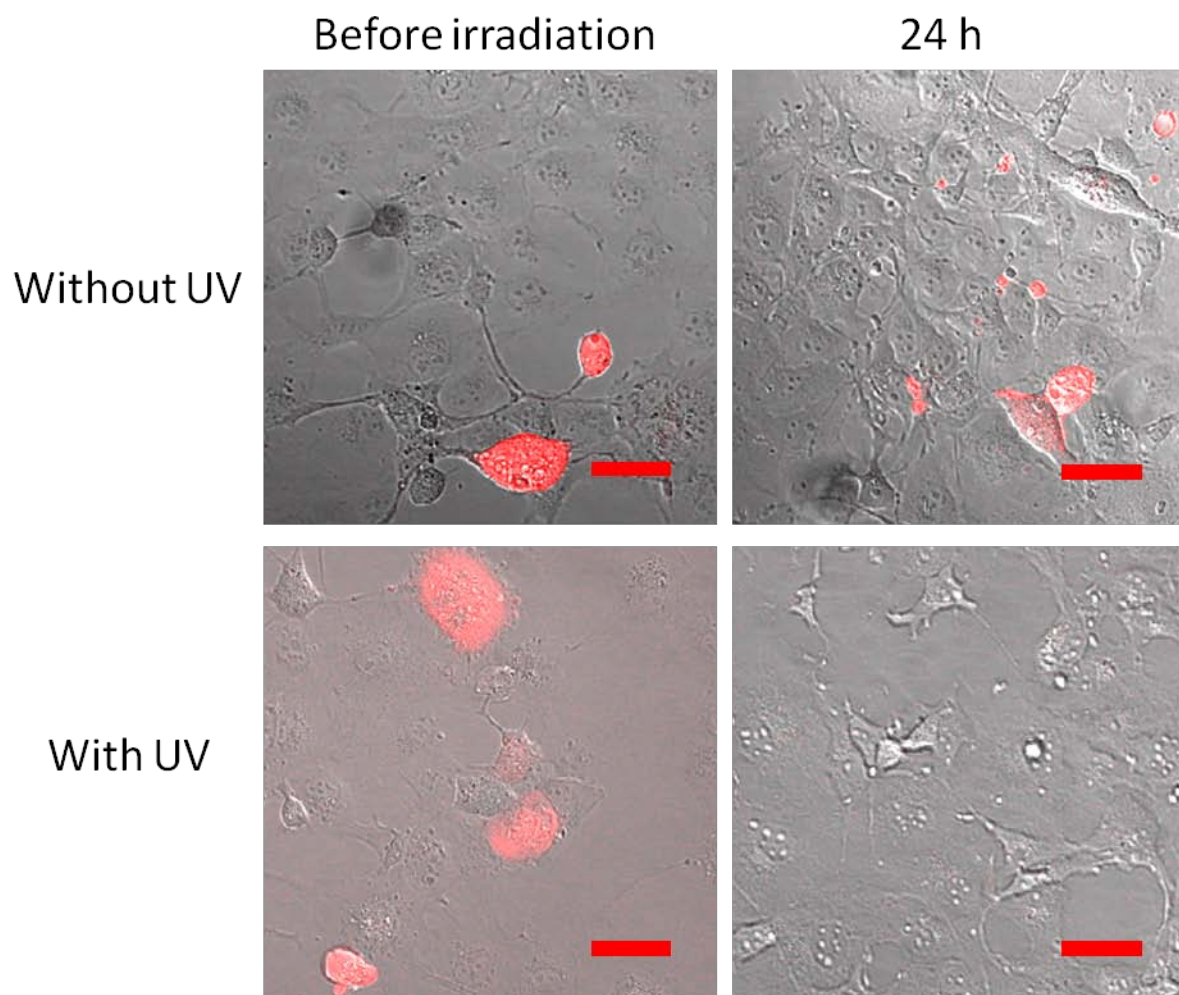
Supporting Figure 3. Ratio of nanoparticle larger than 60 nm before and after irradiation (20 s) measured by TEM. Three samples were measured and error bars indicate standard deviations.



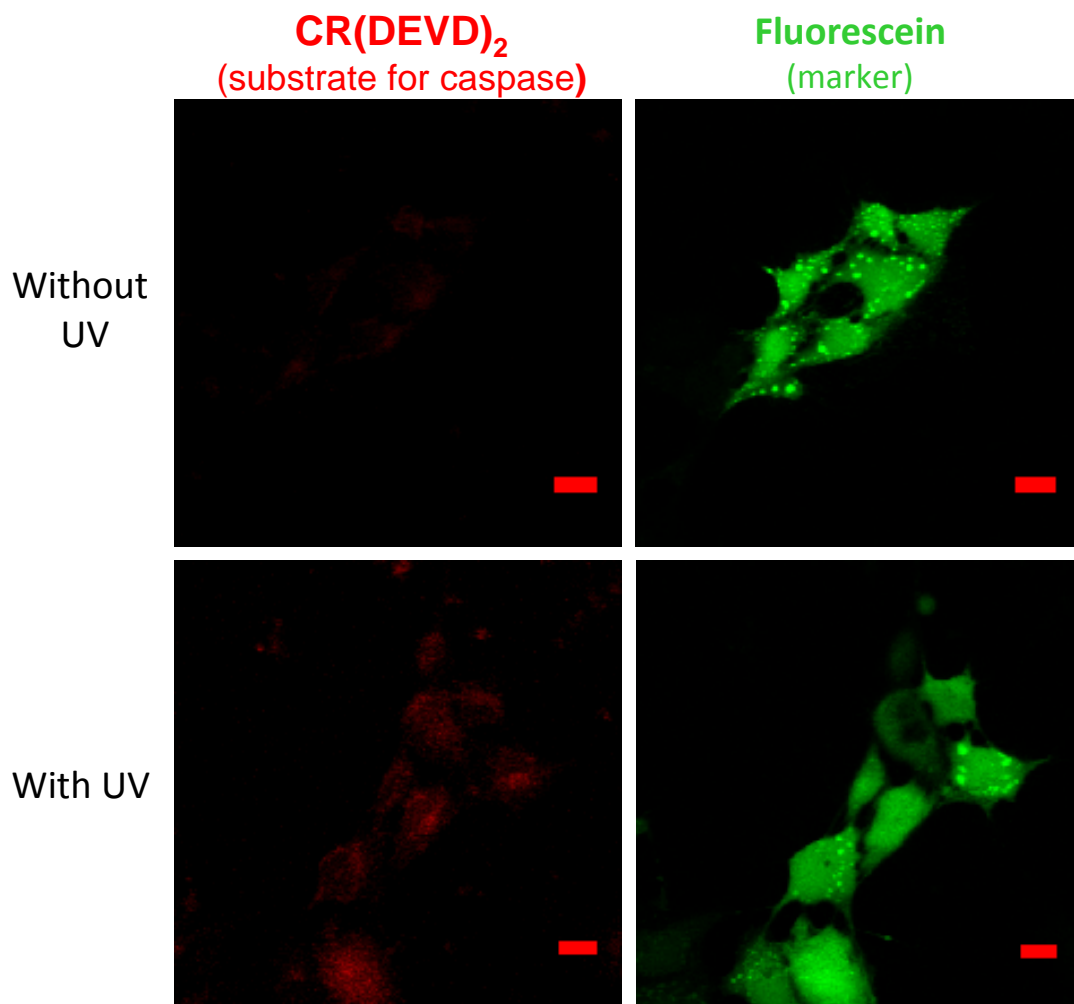
Supporting Figure 4. The control experiments or nanoparticle-encapsulated trypsin. All samples were measured in triplet and error bars indicate standard deviations. The results of nanoparticle-encapsulated trypsin (two results of the left side) were from Fig. 1.



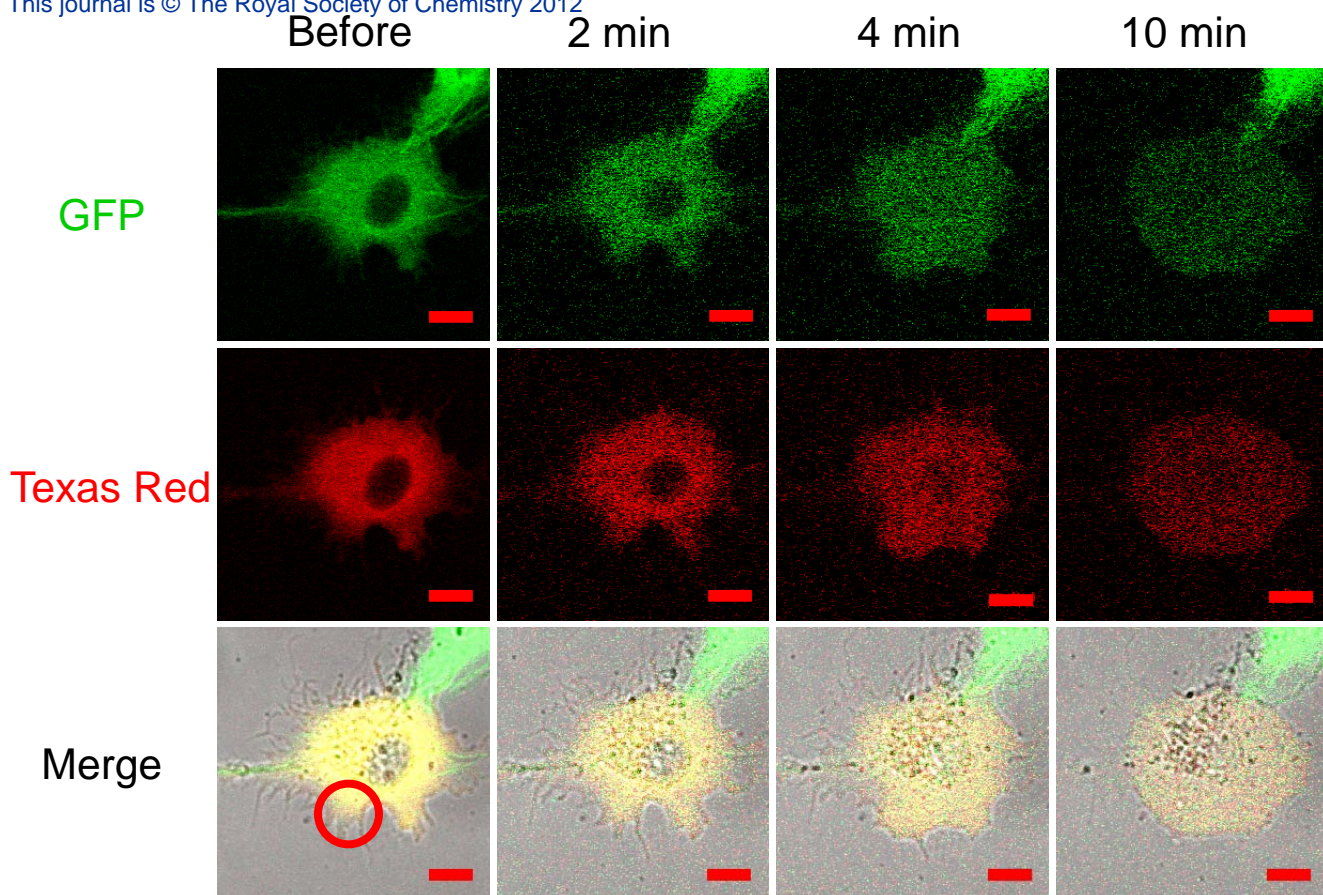
Supporting Figure 5. Comparison of tryptic activities between free solution, encapsulated and released trypsins. All samples were measured in triplet and error bars indicate standard deviations. The activity of the encapsulated trypsin was estimated from the following equation; activity of the encapsulated trypsin = activity of the free trypsin – activity of the non-encapsulated trypsin.



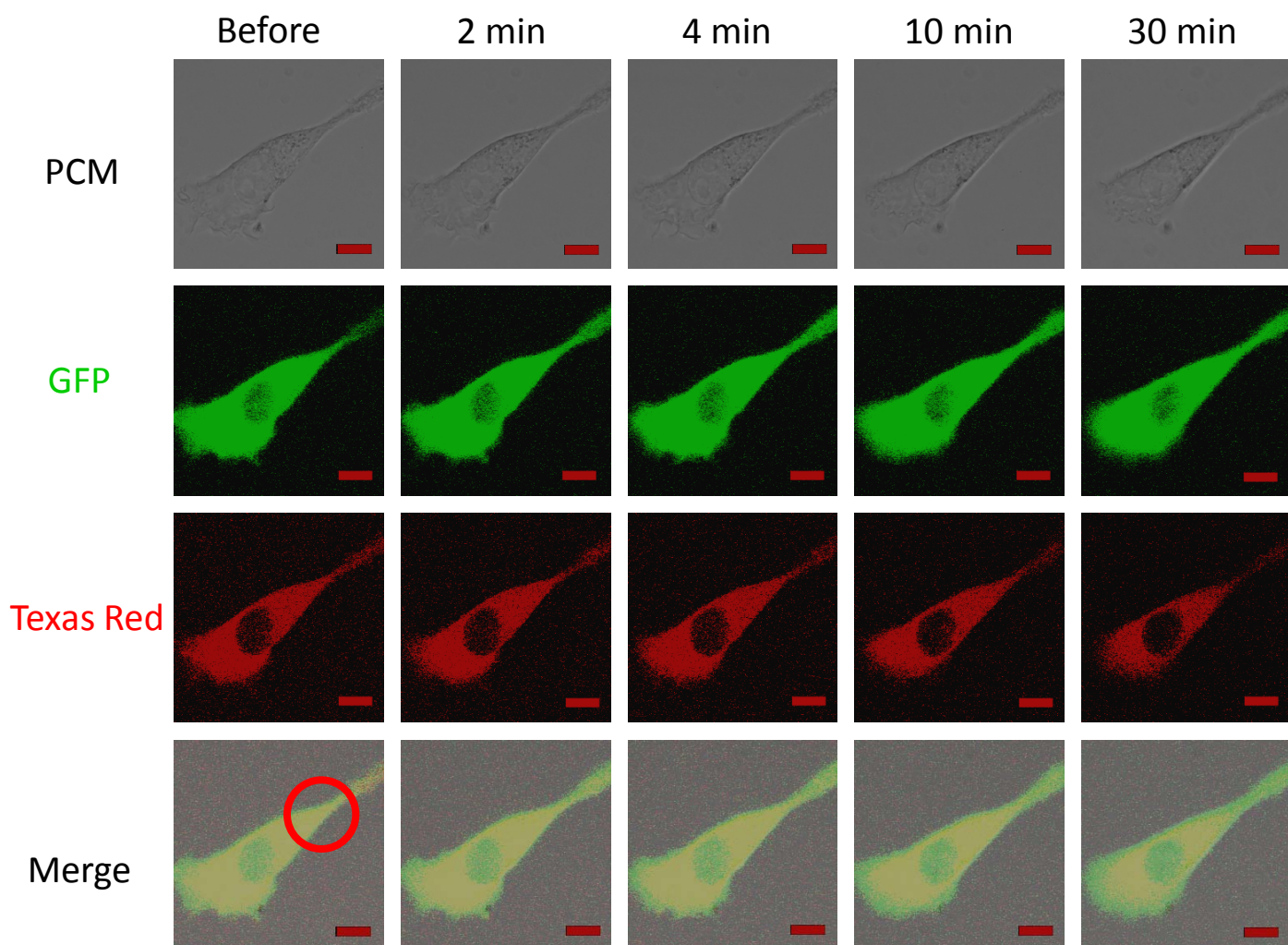
Supporting Figure 6. Photo induced release of activated caspase-3 within cell. Overlaid CLSM and PCM images of HuH-7 cells injected with nanoparticle-encapsulated activated caspase-3. All scale bars are 50 μm .



Supporting Figure 7. Photo induced release of activated caspase-3 within cell. CLSM images of Cos-7 cells induced with nanoparticle-encapsulated activated caspase-3 and then treated with CR(DEVD)₂ that were observed at 15 min after irradiation. All scale bars are 20 μm.



Supporting Figure 8. Local activation of caspase-3 by focused UV irradiation. CLSM, and merged images showing the progress of cell death induced by focused light: before irradiation and 2, 4, and 10 min after irradiation. The red circle indicates the irradiation point. Green, GFP-labeled microtubules; red, dextran-Texas Red; yellow, merged green (GFP) and red (dextran-Texas Red). All scale bars are 20 μm .



Supporting Figure 9. Effect of nanoparticle and focusing light.

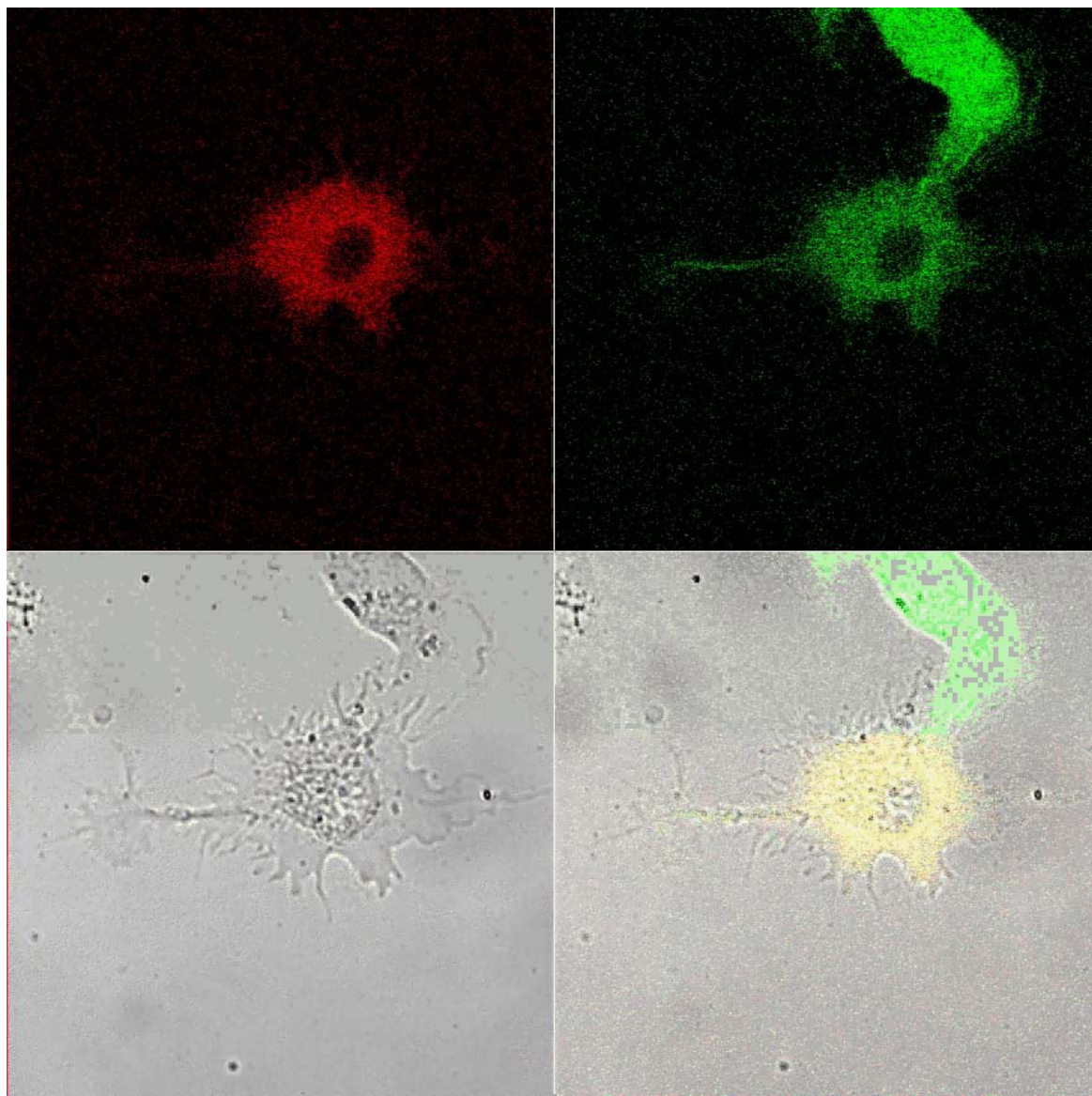
Nanoparticles without caspase-3 were injected in a cell. Focusing light and collapse of nanoparticle did not induce cell death: before irradiation and 2, 4, and 10 min after irradiation. The red circle indicates the irradiation point. Green, GFP-labeled microtubules; red, dextran-Texas Red; yellow, merged green (GFP) and red (dextran-Texas Red). All scale bars are 20 μm .

Supporting Reference

[S1] Y. Mimori-Kiyosue, N. Shiina, S. Tsukita. *Curr Biol.* **2000** *10*, 865-868.

[S2] A. Harada, K. Kataoka, *Macromolecules* **1995**, *28*, 5294.

[S3] M. Kunishima, C. Kawachi, F. Iwasaki, K. Terao, S. Tani, *Tetrahedron Lett.* **1999**, *40*, 5327-5330



Supporting Movie. Movie of a PCM image superposed on two CLSM images showing cell death induced by focused light. Green, GFP-labeled microtubules; red, dextran-Texas Red; yellow, merged green (GFP) and red (dextran-Texas Red).