Supporting Information

Intracellular Photoactivation of Caspase-3 by Molecular Glues for Spatiotemporal Apoptosis Induction

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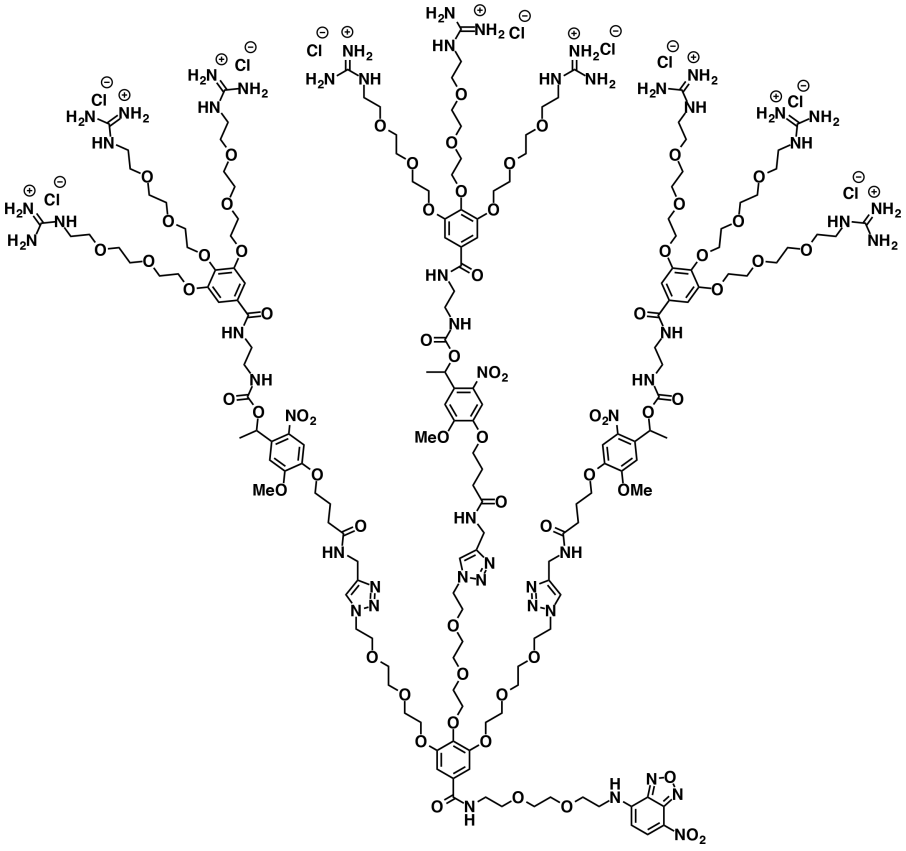
1. General

Photoirradiation was performed on an Optocode model LED-EXHD light-emitting diode (LED) light source equipped with a 365-nm LED lamp (EX-365). Differential interference contrast (DIC) and confocal laser scanning microscopies were performed on a Leica model TCS SP8 confocal laser-scanning microscope. Electronic absorption spectra were recorded on a Molecular Devices SpectraMax® Paradigm® multi-mode microplate detection platform. Zeta potential measurements were performed using a Malvern model Zetasizer Nano ZS particle size analyzer equipped with a 532-nm frequency doubled diode-pumped solid-state (DPSS) laser light source. Dynamic light scattering (DLS) analysis was performed using a Malvern model Zetasizer μV particle size analyzer equipped with an 830-nm laser light source. A Horiba type LAQUAtwin-pH-22B compact pH meter was used for pH measurements.

Unless otherwise noted, reagents and solvents were used as received from commercial sources without further purification. Human hepatocellular carcinoma Hep3B cells (HB-8064) were purchased from ATCC. Eagle's minimal essential medium (EMEM) was purchased from Thermo Fisher Scientific. Dulbecco's phosphate buffer saline (D-PBS) and glycerol were purchased from FUJIFILM Wako Pure Chemical Corporation. Fetal bovine serum (FBS) was purchased from GE Healthcare. Cell Counting Kit-8 was purchased from Dojindo. Lysotracker® Red was purchased from Lonza. Recombinant Human Caspase-3 Protein was purchased from R&D Systems. NucView® 530 Caspase-3 Substrate (1 mM in DMSO), was purchased from Biotium. Phosphate buffered saline (PBS, pH 7.4) was purchased from Nakalai Tesque.

2. Synthesis



^{PC}Glue. ^{PC}Glue was synthesized according to a method analogous to the reported procedure.[S1] In brief, azide-alkyne "click" reaction between a dendron carrying both a focal core appended with an alkyne group through a butyrate-substituted nitroveratryloxycarbonyl (^{BA}NVOC) linkage and three *tert*-butoxycarbonyl (Boc)-protected guanidinium ion (Gu^+) pendants and a dendron carrying both a nitrobenzoxadiazole (NBD)-substituted focal core and three azide (N_3) pendants, followed by the removal of the Boc groups, yielded **^{PC}Glue** as orange oil.

3. Dynamic Light Scattering (DLS) and Zeta Potential Measurements

A PBS (15% glycerol, pH 7.4) solution of a mixture of caspase-3 (Casp-3, 0.4 μM) and ^{PC}Glue (10 μM) was subjected to dynamic light scattering (DLS) and zeta potential measurements. A PBS (15% glycerol, pH 7.4) solution of Casp-3 (0.4 μM) was also measured.

4. Confocal Laser Scanning Microscopy

4-1. Cellular Uptake of ^{PC}Glue/Casp-3

Human hepatocellular carcinoma Hep3B cells (1.0×10^4 cells/well; 8-chambered glass substrate, culture area = 0.8 cm^2/well) were incubated at 37 °C under 5% CO_2 for 24 h in Eagle's minimal essential medium (EMEM, 200 μL) containing 10% fetal bovine serum (FBS). The cell sample was rinsed with Dulbecco's phosphate buffer saline (D-PBS, 200 μL × 2), supplied with EMEM (200 μL) containing ^{PC}Glue (1 μM) and Casp-3 (40 nM), and incubated at 37 °C under 5% CO_2 for 3 h. Then, the cell sample was rinsed with D-PBS (200 μL × 2) and supplied with EMEM (10% FBS, 200 μL) containing LysoTracker Red (25 nM). After incubation at 37 °C under 5% CO_2 for 30 min, the resulting cell sample was subjected to confocal laser scanning microscopy (λ_{ext} = 488 and 552 nm). An analogous cell sample incubated at 4 °C for 1 h was likewise prepared under otherwise identical conditions to the above.

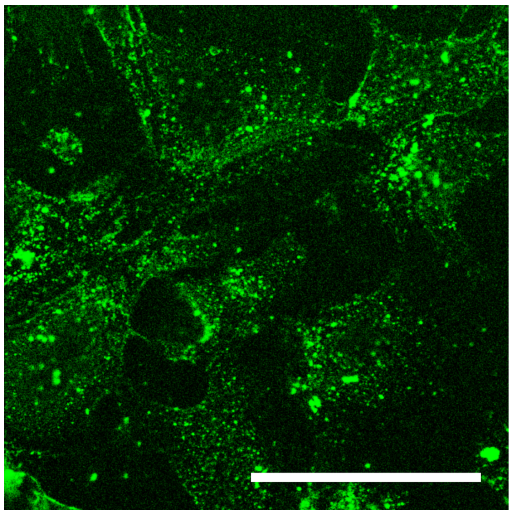


Figure S1. A confocal laser scanning micrograph (λ_{ext} = 488 nm, λ_{obs} = 500–530 nm) of Hep3B cells after 1 h incubation at 4 °C in EMEM containing ^{PC}Glue (1 μM) and Casp-3 (40 nM) followed by rinsing with D-PBS. Scale bar = 50 μm.

4-2. Quantification of Casp-3 Activity in Hep3B Cells

Hep3B cells (1.0×10^4 cells/well; 8-chambered glass substrate, culture area = 0.8 cm^2/well) were incubated at 37 °C under 5% CO_2 for 24 h in EMEM (10% FBS, 200 μL). The cell sample

was rinsed with D-PBS (200 μL × 2), supplied with EMEM (200 μL) containing ^{PC}Glue (1 μM) and Casp-3 (40 nM), and incubated at 37 °C under 5% CO_2 for 3 h. Then, the cell sample was rinsed with D-PBS (200 μL × 2), supplied with EMEM (10% FBS, 200 μL), and exposed to UV light (λ = 365 nm, 1.1 mW/mm^2) for 2 min. NucView (2 μM) was supplied to the cells followed by incubation of the resultant mixture at 37 °C under 5% CO_2 for 18 h. The resulting cell sample was subjected to confocal laser scanning microscopy (λ_{ext} = 552 nm). An analogous cell sample without UV exposure was likewise prepared under otherwise identical conditions to the above. Likewise, reference cell samples in the presence of Casp-3 (40 nM) or ^{PC}Glue (1 μM) as well as untreated cells before and after 2 min UV exposure at 365 nm were prepared under otherwise identical conditions to the above. Mean fluorescence intensities of the Hep3B cells were evaluated by using the ImageJ software.

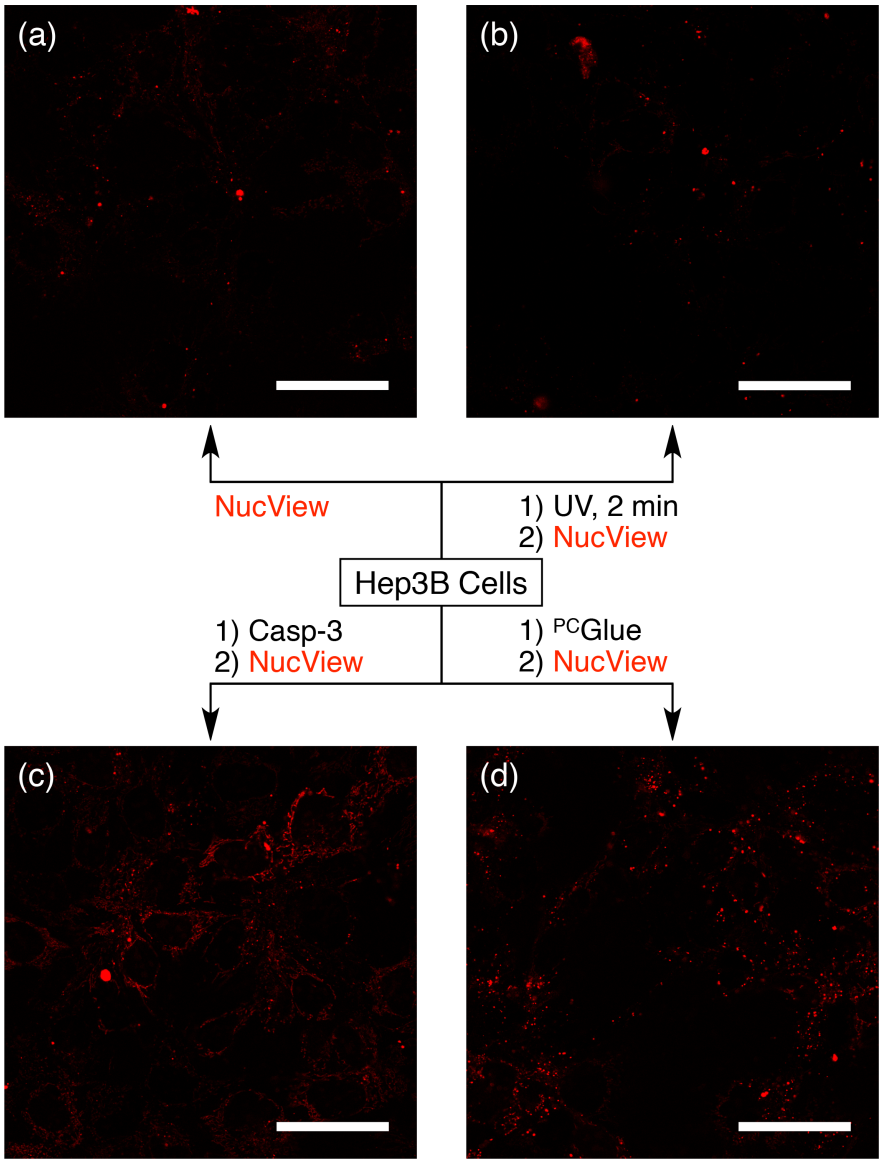


Figure S2. Confocal laser scanning micrographs (λ_{ext} = 552 nm, λ_{obs} = 565–620 nm) of Hep3B cells after 3 h incubation at 37 °C in EMEM in the absence (a, b) and presence of (c) Casp-3 (40 nM) or (d) ^{PC}Glue (1 μM), followed by rinsing with D-PBS. The Hep3B cells were then incubated at 37 °C for 18 h in EMEM (10% FBS) containing NucView (2 μM) before (a,c,d) and after (b) 2 min UV exposure at 365 nm. Scale bars = 50 μm.

4-3. Quantification of Casp-3-active Hep3B Cells

Hep3B cells (1.0×10^4 cells/well; 8-chambered glass substrate, culture area = 0.8 cm^2/well) were incubated at 37 °C under 5% CO_2 for 24 h in EMEM (10% FBS, 200 μL). The cell sample

was rinsed with D-PBS (200 μL × 2), supplied with EMEM (200 μL) containing ^{PC}Glue (1 μM) and Casp-3 (40 nM), and incubated at 37 °C under 5% CO_2 for 3 h. Then, the cell sample was rinsed with D-PBS (200 μL × 2), supplied with EMEM (10% FBS, 200 μL), and exposed to UV light (λ = 365 nm, 1.1 mW/mm^2) for 2 min. NucView (2 μM) was supplied to the cells followed by incubation of the resultant mixture at 37 °C under 5% CO_2 for 18 h. The resulting cell sample was supplied with Hoechst 33342 (2.5 μg/mL) and then subjected to confocal laser scanning microscopy. Analogous cell samples without and with ^{PC}Glue (1 μM) or Casp-3 (40 nM) were prepared under otherwise identical conditions to the above. Likewise, reference cell samples without UV exposure were prepared under otherwise identical conditions to the above.

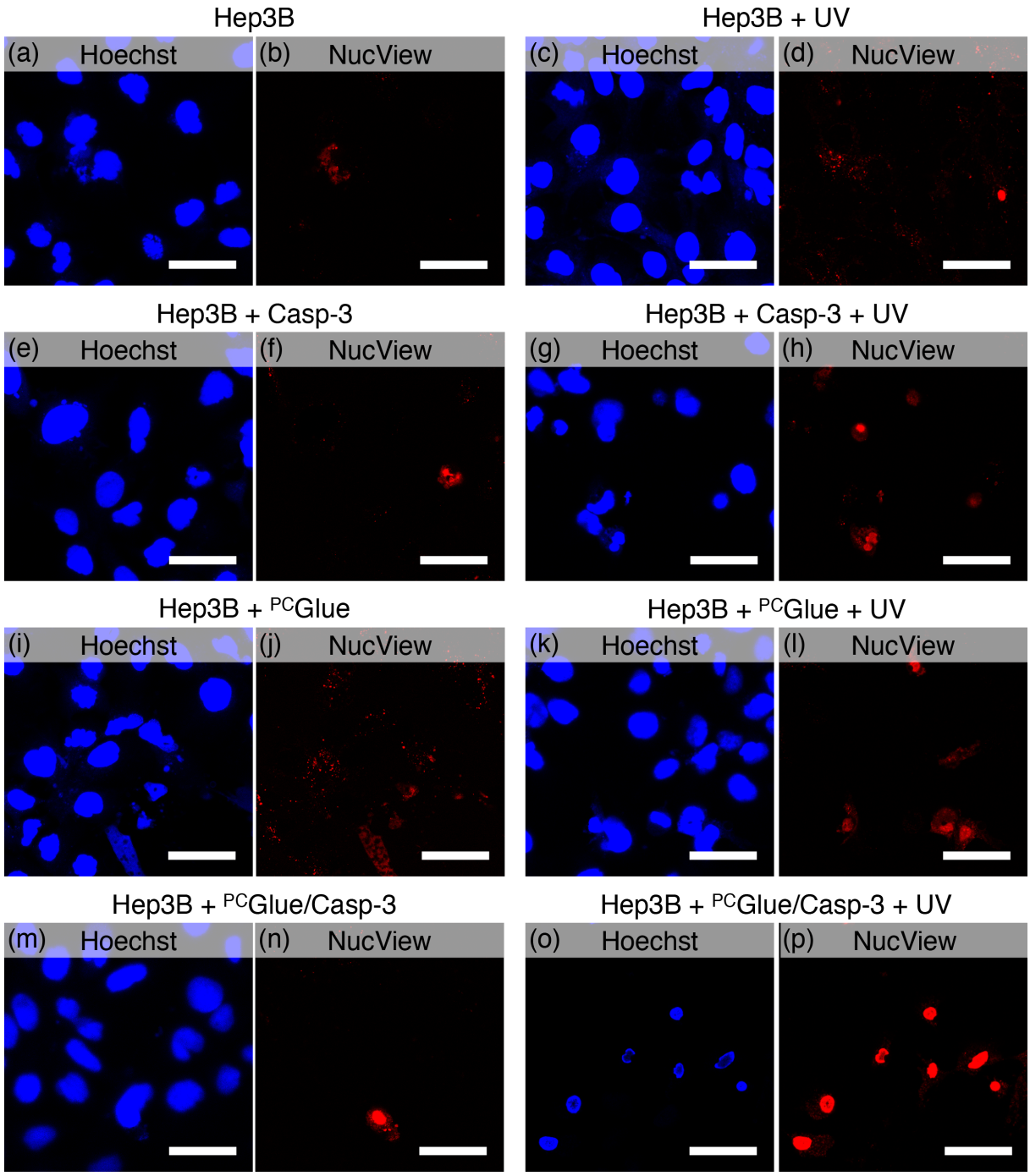


Figure S3. Confocal laser scanning micrographs of Hep3B cells after 3 h incubation at 37 °C in EMEM in the absence (a–d) and presence of Casp-3 (40 nM; e–h), ^{PC}Glue (1 μM; i–l), or ^{PC}Glue/Casp-3 ([^{PC}Glue] = 1 μM, [Casp-3] = 40 nM; m–p), followed by rinsing with D-PBS. The Hep3B cells were incubated at 37 °C for 18 h in EMEM (10% FBS) containing NucView (2 μM) before (a, b, e, f, i, j, m, n) and after (c, d, g, h, k, l, o, p) 2 min UV exposure at 365 nm. Micrographs

recorded upon excitation at 405 nm (λ_{obs} = 420–470 nm; a, c, e, g, i, k, m, o) and 552 nm (λ_{obs} = 570–660 nm; b, d, f, h, j, l, n, p). Scale bars = 50 μm.

Table S1. Rates of apoptotic Hep3B cells estimated from confocal laser scanning micrographs as the ratio of the number of Casp-3 active cells to the number of total cells.[S2]

Cell Sample	Apoptotic Cells (%)
Hep3B	7
Hep3B + UV	6
Hep3B + Casp-3	13
Hep3B + Casp-3 + UV	29
Hep3B + ^{PC}Glue	9
Hep3B + ^{PC}Glue + UV	20
Hep3B + ^{PC}Glue/Casp-3	13
Hep3B + ^{PC}Glue/Casp-3 + UV	77

4-4. Photoactivation of Casp-3 by Two-photon NIR Light in Hep3B Cells

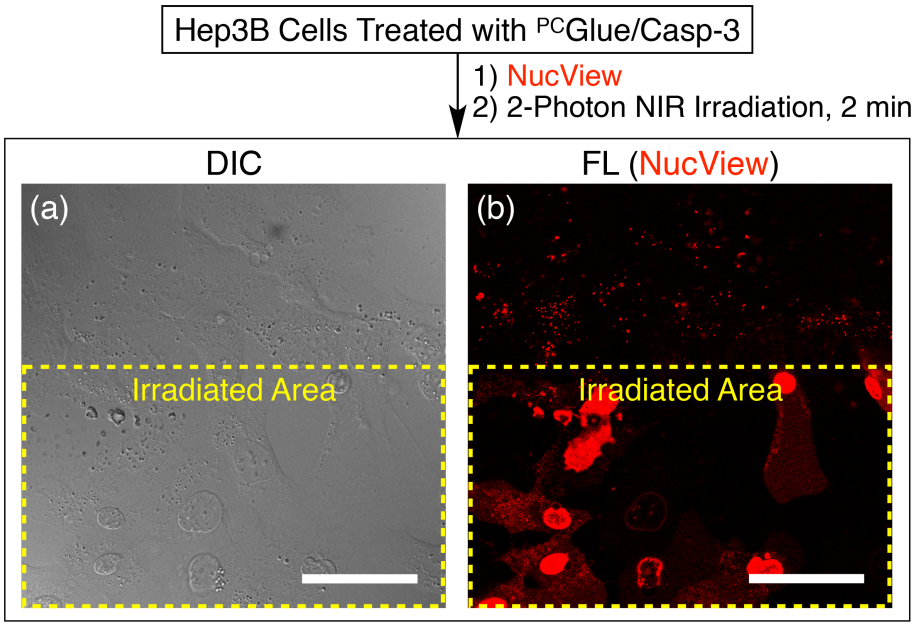


Figure S4. (a) Differential interference contrast (DIC) and (b) confocal laser scanning micrographs upon excitation at 514 nm (λ_{obs} = 540–660 nm) of Hep3B cells. Hep3B cells were incubated at 37 °C for 3 h in EMEM containing a mixture of ^{PC}Glue (1 μM) and Casp-3 (40 nM), rinsed with

D-PBS, and then incubated at 37 °C for 18 h in EMEM (10% FBS) containing NucView (2 μM), followed by two-photon irradiation at 710 nm for 2 min. The yellow dashed boxes in (a) and (b) represent the irradiated area. Scale bars = 50 μm.

5. Cell Viability Assay

Hep3B cells (5.0×10^3 cells/well; 96-well culture plate, culture area = 0.33 cm^2/well) were incubated in EMEM (10% FBS, 100 μL) at 37 °C under 5% CO_2 for 24 h. The cell sample was rinsed with D-PBS (100 μL × 2) and supplied with EMEM (10% FBS, 100 μL) containing ^{PC}Glue (1 μM) and Casp-3 (50 nM). After incubation at 37 °C under 5% CO_2 for 13 h, the cell sample was rinsed with D-PBS (100 μL × 2) and supplied with EMEM (10% FBS, 100 μL), which was exposed to UV light (λ = 365 nm, 1.1 mW/mm^2) for 2 min prior to incubation. After incubation at 37 °C under 5% CO_2 for 90 min, Cell Counting Kit-8 reagent (10 μL) was supplied to the cells followed by incubation of the resultant mixture at 37 °C under 5% CO_2 for 1 h. An analogous cell sample without UV exposure was likewise prepared under otherwise identical conditions to the above. The cell samples thus prepared were subjected to absorption spectroscopy (λ = 450 nm).

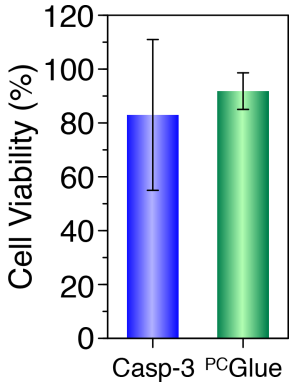


Figure S5. Viabilities of Hep3B cells after 12 h incubation at 37 °C in EMEM (10% FBS) containing Casp-3 (50 nM, blue) or ^{PC}Glue (1 μM, green).

6. References

S1. Mogaki, R.; Okuro, K.; Ueki, R.; Sando, S.; Aida, T. Molecular Glue that Spatiotemporally Turns on Protein–Protein Interactions. *J. Am. Chem. Soc.* **2019**, *141*, 8035–8040.

S2. Prieto, A.; Diaz, D.; Barcenilla, H.; Garcia-Suárez, J.; Eduardo, R.; Monserrat, J.; Antonio, E. S.; Melero, D.; de la Hera, A.; Orfao, A.; Alvarez-Mon, M. Apoptotic Rate: A New Indicator for the Quantification of the Incidence of Apoptosis in Cell Cultures. *Cytometry, Part A* **2002**, *48*, 185–193.