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Supporting Information

A Novel Fluorescent Probe for the Detection of Apoptosis

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Experimental Section

Materials and Measurements: All chemicals were purchased from Acros, Aldrich Chemical Company or Alfa-Aesar and used as received. All organic solvent was purchased from Beijing Chemical Works and used as received. Methylthiazolyldiphenyl-tetrazolium bromide (MTT) was obtained from Xinjingke Biotechnology Co., Ltd (Beijing, China). Jurkat T-leukemia cells were purchased from cell culture center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Fetal Bovine Serum (FBS) was purchased from Sijiqing Biological Engineering Materials (Hangzhou, China). Modified RPMI 1640 was purchased from HyClone/Thermofisher (Beijing, China). LEAF™ Purified anti-human CD95 (Fas) was purchased from Biolegend/Dakewei Biotech Company Limited (clone: EOS9.1, Beijing, China). The cell apoptosis detection kit (including both labeled Annexin V and binding buffer) was obtained from KeyGen Biotech Company Limited (Nanjing, China). The ^{1}H NMR spectrum was recorded on a Bruker Avance 400 MHz spectrometer. The ^{13}C NMR spectrum was recorded on a Bruker Avance 600 MHz spectrometer. Fourier transform infrared (FT-IR) spectrum was measured on a Bruker Tensor

27 spectrometer. The UV-Vis absorption spectrum was taken on a JASCO V-550 spectrophotometer. The fluorescence spectrum was measured on a Hitachi F-4500 fluorometer equipped with a Xenon lamp excitation source. The absorbance for MTT analysis was recorded on a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 490 nm. Confocal laser scanning microscopy (CLSM) characterization was conducted by a confocal laser scanning biological microscope (FV1000-IX81, Olympus, Japan). Flow cytometric analysis was taken by a flow cytometry (FACSCalibur, Becton, Dickinson and Company, USA).

Synthesis of polymer PPV-NMe_3^+: A solution of monomer **1** (55 mg, 0.15 mmol), monomer **2** (117 mg, 0.15 mmol), $Pd(OAc)_2$ (6 mg, 0.026 mmol), tri-o-tolyphosphine (9 mg, 0.03 mmol), triethylamine (53 μL) in DMF (3.5 mL) was refluxed at 100 °C under nitrogen for 42 h. The hot solution was poured into 200 mL of acetone, and then the precipitate was collected by centrifugation. The precipitate was dissolved in doubly distilled water, filtered, and the solution was dialyzed using a membrane with a 3500 cut-off for 2 d (8 water changes). The water was removed under vacuum to afford a dark red solid (70 mg, 52 %). ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.8-8.0 (br, 8H; Ar*H*, -C*H*=C*H*-), 3.4-4.5 (br, 34H; -OCH_2-, -OCH_3), 2.8-3.3 (br, 22H; -N-CH_2-, -N-CH_3). ^{13}C-NMR (150 MHz, CD_3OD, ppm): 152.7 (br, 4C; -OAr*C*), 128.9 (br, 4C; -CH=CH-Ar*C*), 125.9 (br, 4C; -*C*H=*C*H-), 113.2 (br, 4C; Ar*C*), 73.0 (2C;-O*C*H_2-), 69.0-72.0 (br, 8C;-O*C*H_2-), 67.0 (2C; -O*C*H_2-), 66.0 (2C; -O*C*H_2-), 59.2 (2C; -O*C*H_3), 54.9 (8C; -N-*C*H_2-, -N-*C*H_3). FT-IR (KBr pellet, cm^{-1}): 2873, 1595, 1493, 1414, 1355, 1242, 1202, 1122, 1056, 954, 875. $C_{40}H_{64}Br_2N_2O_{10}$: calcd C 53.81, H 7.23, N 3.14;

found C 52.27, H 6.88, N 2.37. Generally speaking, it is difficult to determine the molecular weight of water-soluble conjugated polymers.[1] According to previous reports about PPV prepared by Heck coupling, we estimated that the number average molecular weight (M_n) for PPV-1 was in the range of 10000-30000 which corresponded to 15-40 repeat units in the backbone and the polydispersity index was between 1.5 and 3.[2,3]

Cell culture: Jurkat T-leukemia cells were routinely grown in modified RPMI 1640 medium supplied with 10 % FBS. Cells were harvested through centrifugation (1800 rpm for 5 min) and cultured at 37 °C in a humified atmosphere containing 5 % CO_2.

In vitro cell viability assay: Cells were seeded in 96-well U-bottom plates at a density of 1~3 $\times10^4$ cells/well, and then were incubated with PPV-1 with varying concentrations followed by further culture for 24 h. The culture media were discarded and MTT (0.5 mg mL^{-1} in RPMI 1640, 100 μL/well) was added to the wells followed by incubation at 37 °C for 4 h. The supernatant was abandoned, and 110 μL DMSO per well was added to dissolve the produced formazan. After shaking the plates for 10 min, absorbance values of the wells were read with a microplate reader at 490 nm. The cell viability rate (VR) was calculated according to the following equation:

$$VR = \frac{A}{A_0} \times 100\%$$

where A is the absorbance of the experimental group treated by PPV-1 and A_0 is the absorbance of the control group without any treatment.

Procedure for inducing apoptosis in Jurkat cells and staining with PPV-1 or Annexin V:

In order to induce apoptosis in a large extent, the cells were suspended in fresh medium to a concentration of 1~3 × 10^6 cells ml^{-1} and treated with 0.7 μg ml^{-1} anti-Fas mAb for 48 h (PPV-1) or 22 h (Annexin V). As a negative control, untreated cells (without anti-Fas mAb) were also incubated under exactly the same conditions. After harvesting the cells by centrifugation at 1800 rpm for 5 min, both cells induced with and without anti-Fas mAb were incubated with 40 μM PPV-1 in PBS (10 mM, pH=7.4) or Annexin V in binding buffer at 37 °C for 15 min, respectively. The cells were centrifuged again (1800 rpm for 5 min) and washed once with PBS (PPV-1) or binding buffer (Annexin V). Finally, the cell pellet was resuspended in PBS (PPV-1) or binding buffer (Annexin V) and preserved on ice for further test.

Confocal laser scanning microscopy (CLSM) characterization: Individual aliquots of 10μL of the pre-prepared cell suspensions were added to clean glass slides followed by slightly covering coverslips for immobilization. The cells were then examined by confocal laser scanning microscopy using a 488 nm laser (FV5-LAMAR). The fluorescence of PPV-1 or Annexin V was highlighted in green.

Flow cytometry (FCM): FCM was used to calculate the percentage of apoptotic cells. The pre-prepared cell suspensions were directly analyzed using a 488 nm laser. Cell fragments were excluded with forward and side-scatter gating to ensure that all detected signals originated from relatively intact cells; signals from PPV-1 or Annexin V were recorded in Channel FL-1. The flow cytometry diagrams presented were obtained from a population of 3 × 10^4 cells.

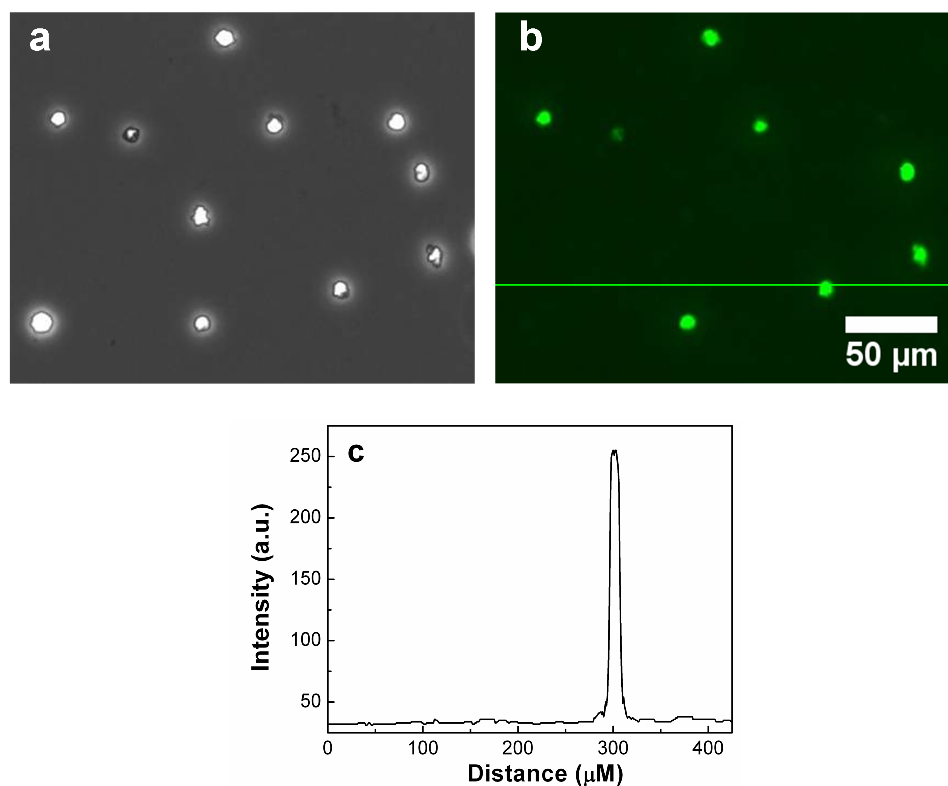

Figure S1. The contrast with respect to the fluorescence intensity between free and bound polymers. (a, b) Bright field and fluorescence images of cells induced by anti-Fas without removing free polymers. (c) The fluorescence intensity profile corresponding to the line highlighted in Figure S1b. The fluorescence image was recorded using fluorescence microscopy (Olympus 1×71) with a D455/70 nm exciter and a D525/30 nm emitter. The exposure time was 600 ms.

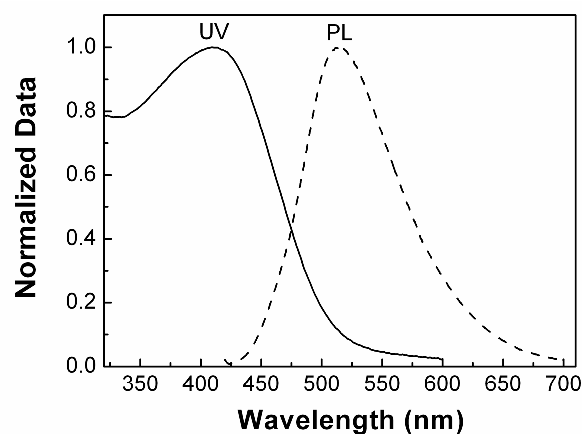

Figure S2. Normalized absorption and emission spectra of PPV-1 in water. The excitation wavelength was 410 nm.

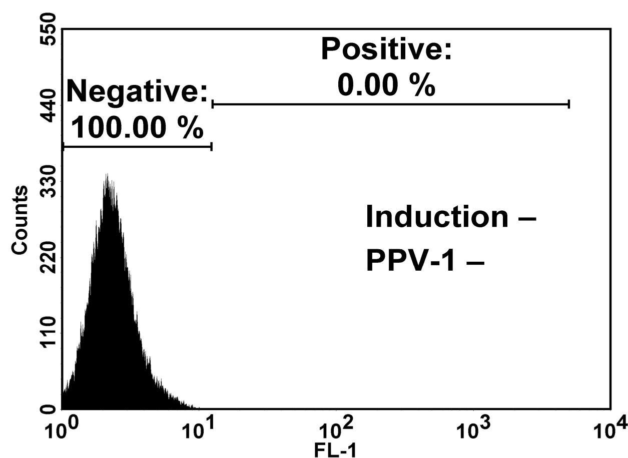

Figure S3. Flow cytometry diagram of control experiment. "Induction –" represents induction without anti-Fas, whereas "PPV-1 –" indicates incubation without PPV-1.The gates in the figure were used to determine the percentage of positive and negative cells in each panel.

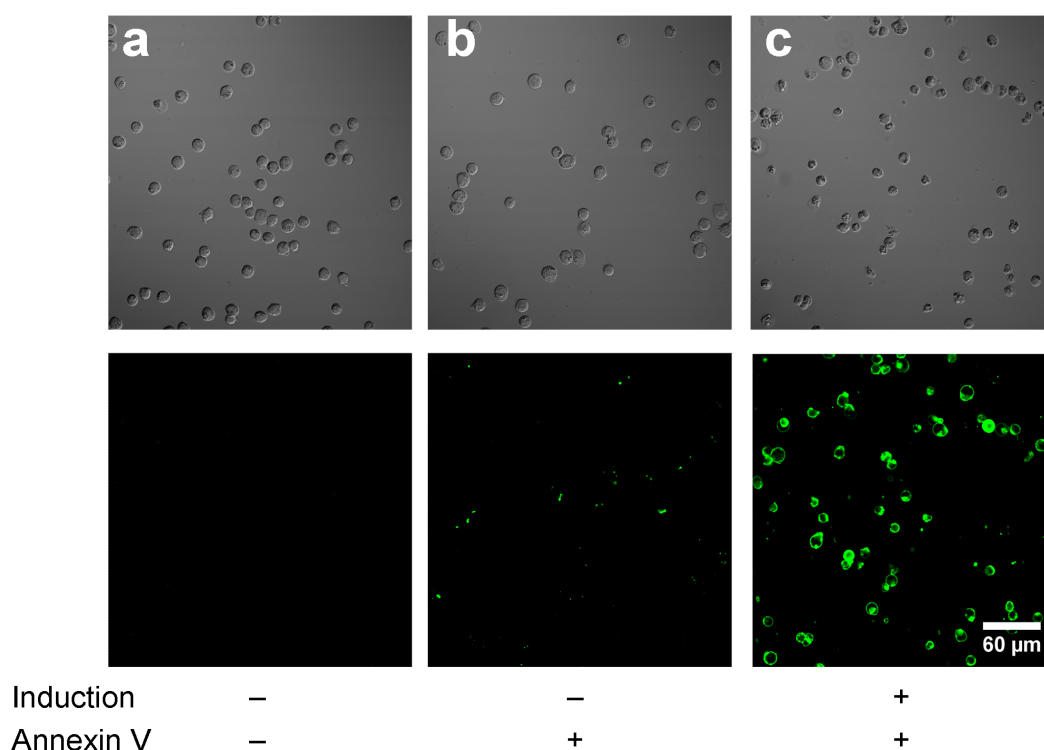

Figure S4. Fluorescence confocal microscopy images (CLSM) of Jurkat T cells staining with Annexin V. "Induction +" represents induction with anti-Fas, whereas "Induction –" represents induction without anti-Fas. "Annexin V +" indicates incubation with Annexin V, whereas "Annexin V –" indicates incubation without Annexin V. The fluorescence of Annexin V was highlighted in green.

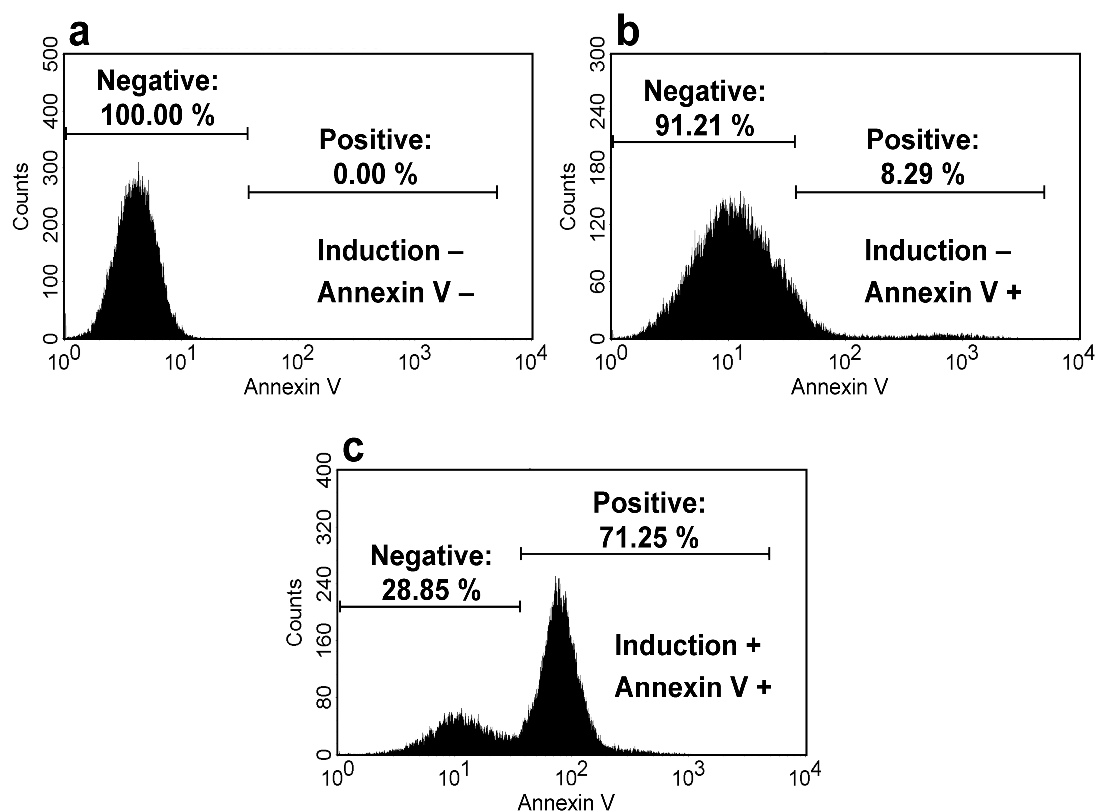

Figure S5. Detection of apoptosis by flow cytometry. "Induction +" represents induction with anti-Fas, whereas "Induction –" represents induction without anti-Fas. "Annexin V +" indicates incubation with Annexin V, whereas "Annexin V –" indicates incubation without Annexin V.

References

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2. Li, H.; Li, Y.; Zhai, J.; Cui, G.; Liu, H.; Xiao, S.; Liu, Y.; Lu, F.; Jiang, L.; Zhu, D. *Chem. Eur. J.* **2003**, *9*, 6031.

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