Table S1. Mean size and Zeta-potential of different CAT-Aco NPs.

	non-cl PEG-DGL/ CAT-Aco	*non-cl* PGP-PEG-DGL/ CAT-Aco	*cl* PEG-DGL/ CAT-Aco	*cl* PGP-PEG-DGL/ CAT-Aco
Mean size (nm)	103.3±12.6	109.5±13.4	97.21±11.6	99.56±12.7
Zeta potential (mV)	4.96±0.9	5.45 ± 1.3	5.41±1.1	5.6±1.4

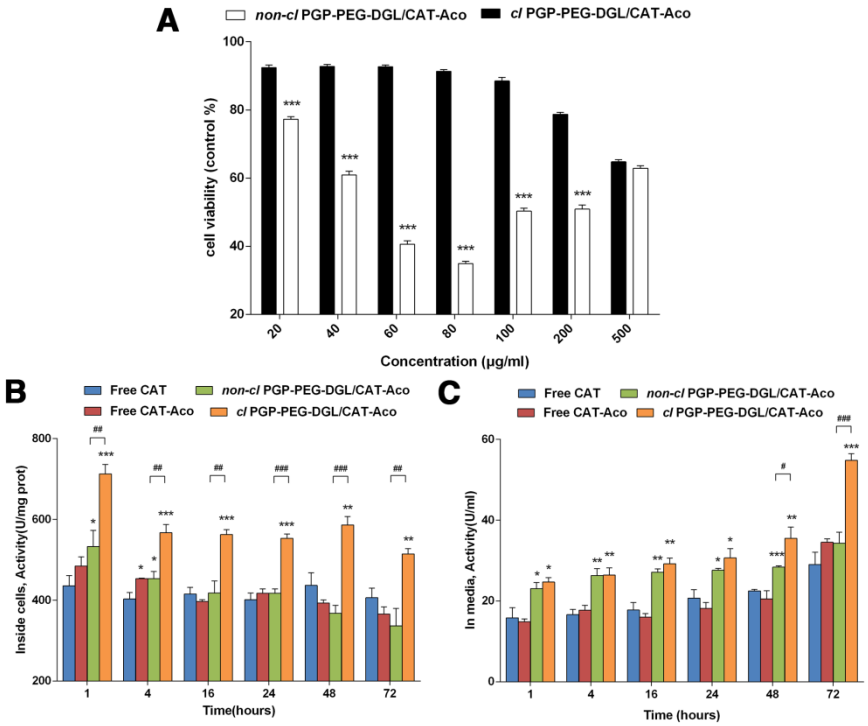

Figure S1. (A) Viability of HL-60 cells treated with different NPs at a polymeric concentration range from 20 to 500 μg/mL after 3 h incubation at 37 ℃ (mean ± SD, n=3). ***P < 0.001 compared with *cl* PGP-PEG-DGL/CAT-Aco group. (B&C) Quantification of catalase activity released into media and retained in cells by

spectrophotometry (mean ± SD, n=3). *P < 0.05, **P < 0.001 ***P < 0.001 compared with free CAT group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

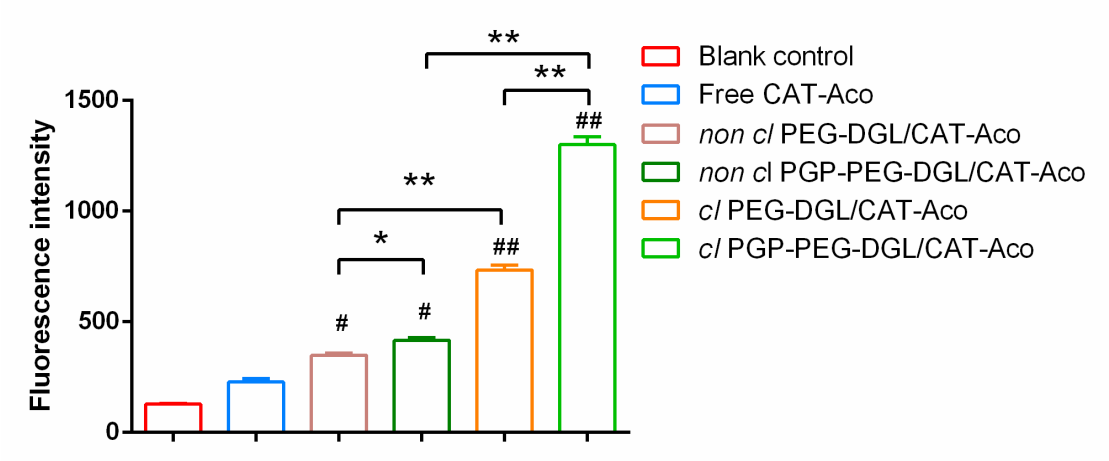

Figure S2. Cellular uptake of FITC-labeled free CAT-Aco and various DGL vector/CAT-Aco NPs (mean ± SD, n=3). $^{\#\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Free CAT-Aco group. *$P < 0.05$, **$P < 0.01$.

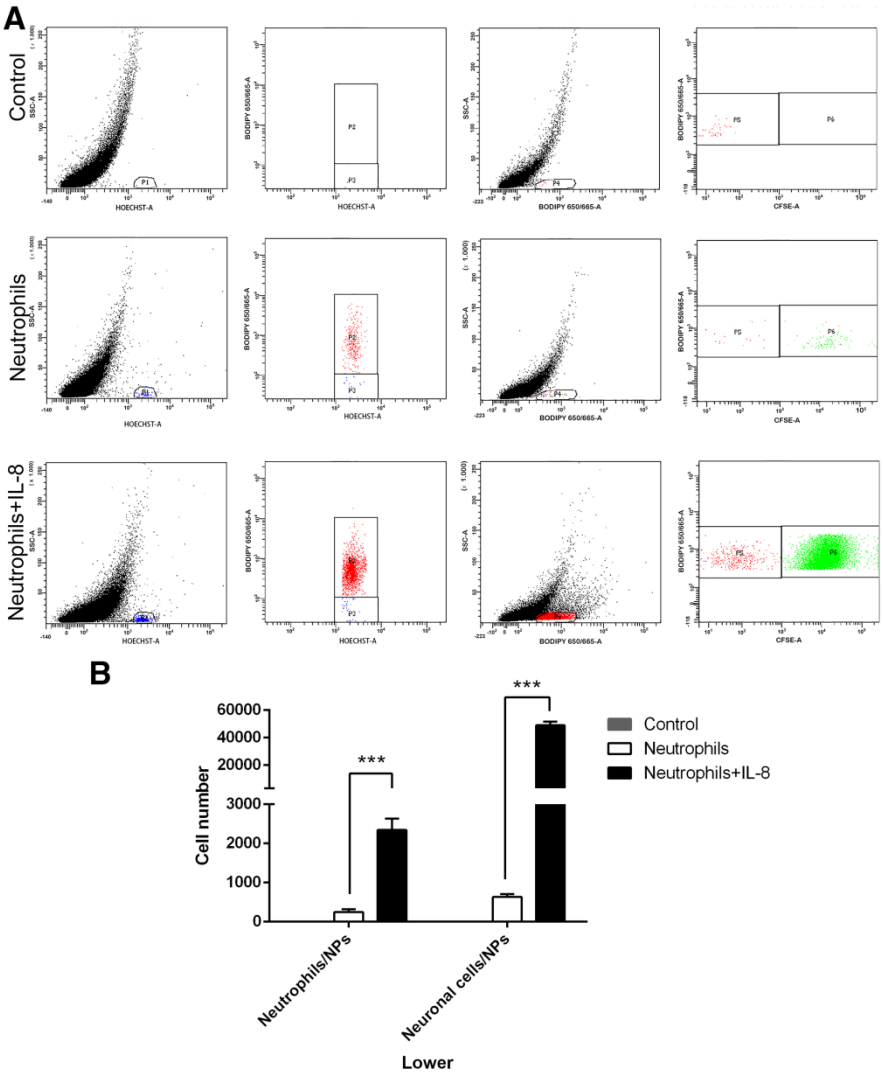

Figure S3. (A) The transmigration across endothelial cells (HUVEC) of neutrophils and transfer NPs from neutrophils to neuronal cells by flow cytometric analysis. P1 represents neutrophils labeled with HOECHST. P2 represents neutrophils with NPs. P4 represents total cells containing NPs in lower chamber. P6 represents neuronal cells with NPs. (B) Quantitative data of migrated neutrophil-like differential HL-60 cells with NPs and PC12 neuronal cells with NPs in the lower chamber. Neutrophils stained with HOECHST were loaded with BODIPY 650/665-labeled NPs, and then added to upper chamber followed by collecting all cells in the lower chamber after 12 h. After co-incubation with HUVECs, PC 12 cells alone in the lower chamber were used as control. PC12 neuron cells were labeled with CFSE. Mean ± SD, n=3; ***$P <$ 0.001.

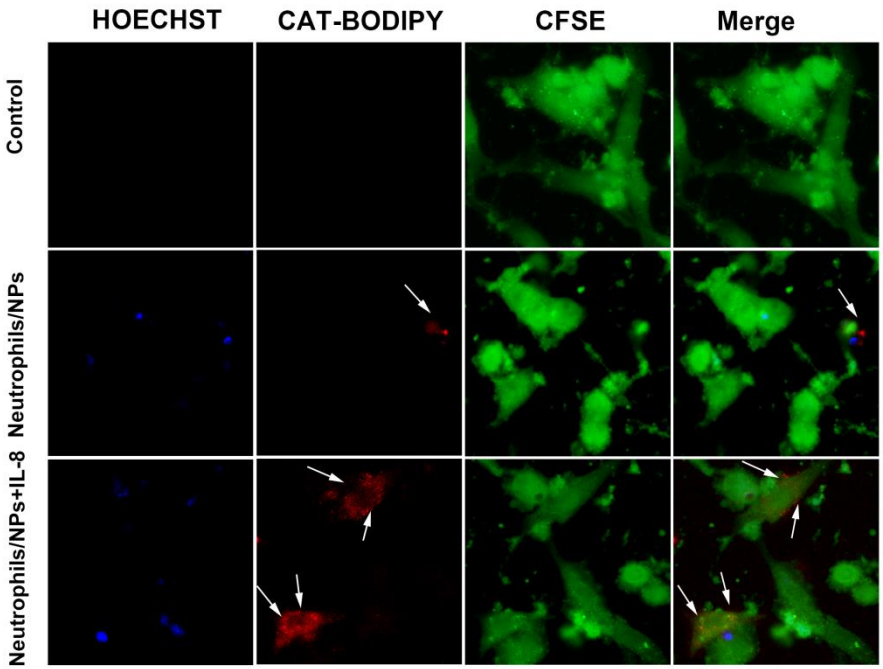

Figure S4. The transfer of NPs from migrated HL-60 cells to PC12 cells by confocal microscopy. The fluorescence signal of NPs overlapped with that of PC12 (white arrow). Neutrophil-like differential HL-60 cells and PC12 neuron cells were labeled with HOECHST (Blue) and CFSE (green), respectively. Red represents BODIPY 650/665-labeled NPs.

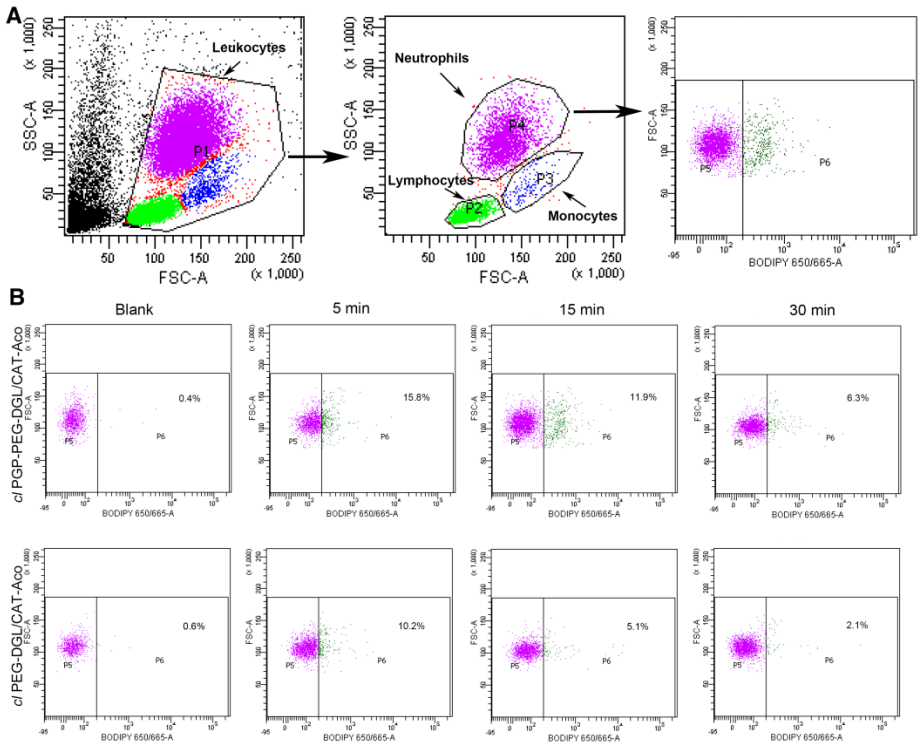

Figure S5. (A) The classification of leukocyte and the percentage of BODIPY 650/665 positive neutrophils from MCAO rats blood using flow cytometry assay. (B) The NPs uptake by neutrophils in MCAO rats at different times using flow cytometry.

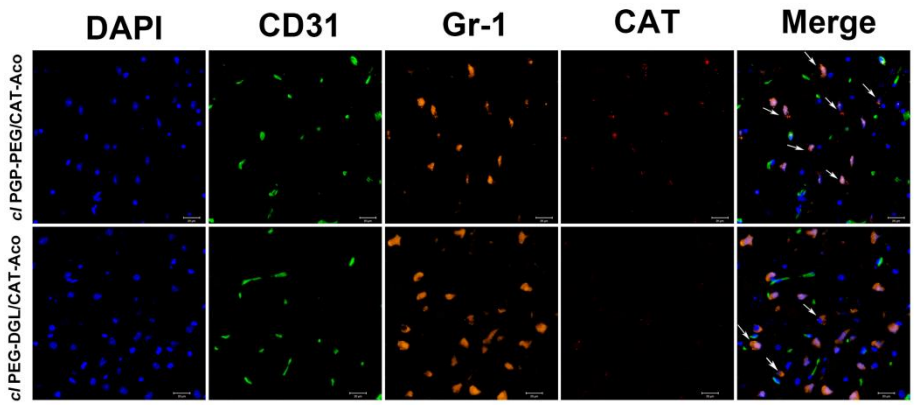

Figure S6. Co-localization of BODIPY 650/665-labeled CAT with neutrophils in ischemic brain after the administration of different *cl* dendrimer/CAT-Aco NPs. Blood vessels were stained with anti-CD31 (green). Neutrophils were labeled with anti-Gr-1 antibody (anti-granulocyte receptor-1) (Orange).

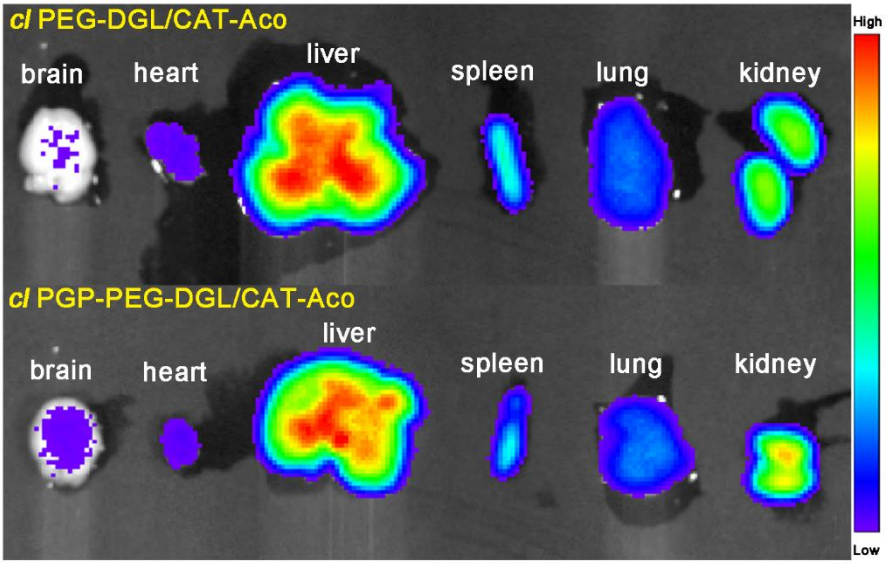

Figure S7. *Ex vivo* near-infrared imaging of main organs 1 h post-injection.

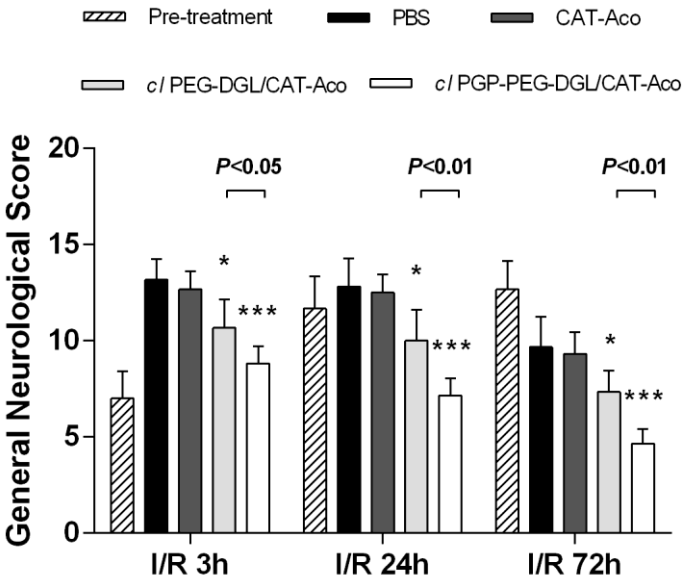

Figure S8. Effect of PBS, CAT-Aco alone (250 U/mouse), *cl* PEG-DGL/CAT-Aco and *cl* PGP-PEG-DGL/CAT-Aco on neurological deficit induced by MCAO. Data are expressed as mean ± SD (n=6). **P* < 0.05, ****P*< 0.001 vs. PBS group.

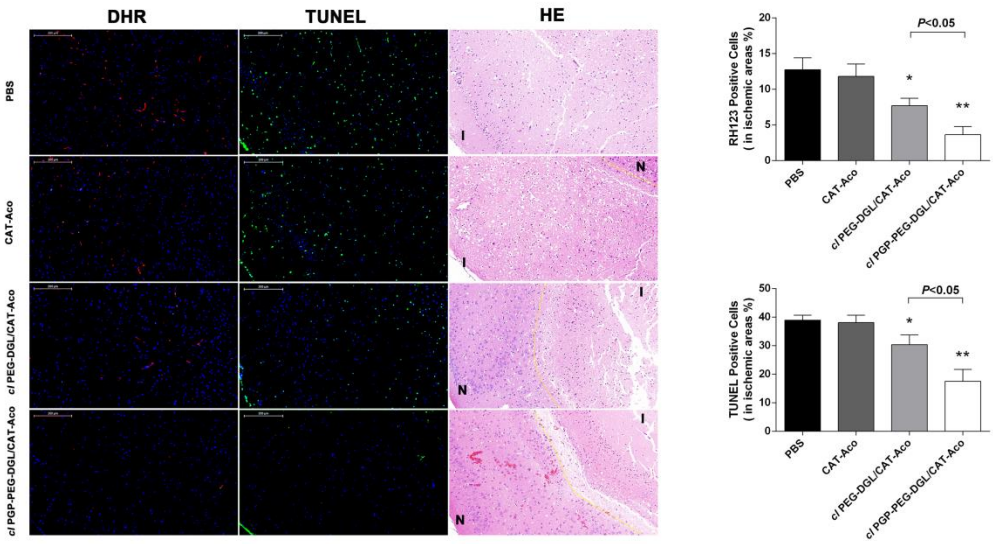

Figure S9. The RH123 and TUNEL as well as HE microscopic images of brain sections 24 h after treatments. The yellow dashed line indicates the boundary between ischemic area (I) and normal area (N). Bar=200 μm. Data are mean ± SD, n=3. **P* < 0.05, ***P* < 0.01 vs. PBS group.

Methods:

Determination of CAT-Aco complexation efficiency. The CAT-loaded NPs were separated from the aqueous suspension medium by ultracentrifugation at 3000000 g for 30 min at 4 ℃. Absorbance of free CAT-Aco in the supernatant was recorded at 404 nm using Tecan Infinite 200 microplate reader and the concentration of free CAT-Aco in the supernatant was calculated from a standard curve. CAT-Aco complexation efficiency was calculated using the following formula:

$$\text{Complexation efficiency (\%)} = \frac{\text{Total amount of CAT-Aco} - \text{Free CAT-Aco}}{\text{Total amount of CAT-Aco}} \times 100\ \%$$

Cytotoxicity Assay. HL-60 cells (1×10^6 cells) plated in 2 cm^2 dish were incubated with different NPs in various concentrations (20, 40, 60, 80, 100, 200, 500 μg/ml, calculated with DGL) for 3 h. Then the cells were washed and suspended with staining solution containing a fluorescent dye, propidium iodide (PI, 668.4 Da, red

fluorescent). PI cannot penetrate through the membrane of viable cells, but stains cells with compromised plasma membrane integrity. A total of 10,000 cells were analyzed per sample. Cell viability was expressed as a percentage of the control culture.

Preservation of Enzymatic Activity against Degradation in HL-60 cells. HL-60 cells were incubated with catalase alone or different NPs (DGL to CAT-Aco 2.5:1, w/w) respectively at 37 ℃ in FBS-free medium for 1 h. After being washed with HBSS twice, the cells were collected and supplemented with fresh media. Over different time intervals, the activity of CAT released from HL-60 cells and retained in cells were examined at 25 ℃ using catalase assay kit (Beyotime Institute of Biotechnology, Shanghai) according to the manufacturer's instructions. The pre-loaded cells were lysed in a sufficient volume of cell lysis buffer with 1% protease inhibitor and then centrifuged at 14500 rpm for 15min. One half of the supernatant was used to investigate the activity of catalase, while the other half was used to determine the protein concentration by BCA Protein Assay Kit. The activity units were normalized to protein amounts and expressed in activity units per mg of protein.

Transmigration Assay. Human umbilical vein endothelial cells (HUVECs) were grown to confluency on porous membranes (1×10^5 cells per sq. cm, Millicell Hanging Cell Culture Insert with pore size of 8-μm used in a 6-well plate) for 9 days at 37℃. Then PC12 cells were plated in 6-well plate (2×10^5 cells/well), and co-cultured for 3 days with HUVECs. Meanwhile, HL-60s were differentiated into PMN-like cells by adding 96-h 1.3% DMSO. In addition, HUVECs were stimulated with TNF-α (200 U/ml, Recombinant Human TNF-α, PeproTech) for 4 h at 37 ℃ to induce the

expression of ICAM-1, which plays a key role in neutrophil transmigration as previously reported [1]. PC12 neuronal cells were incubated with cell tracer (5μg/ml, carboxyfluorescein diacetate, succinimidyl ester, CFSE. Beyotime Biotechnology) for 30 min in HBSS and then incubated with fresh medium. Differentiated HL-60s were added into 6-well plates (1×10^7 cells/well) and then pre-treated with HOECHST (5 μg/ml) for 30 min in HBSS. After staining the nuclear, the cells were incubated with *cl* PGP-PEG-DGL/CAT-Aco NPs labeled with CAT-BODIPY 650/665 (0.25 mg/ml DGL) for 1 h in HBSS. After being washed with HBSS twice, HL-60 cells were dispersed in culture medium and added into the upper chamber (1×10^5/chamber). All cells were incubated in culture medium containing 10% FBS with or without IL-8 as a well-known neutrophil chemotactic factor for 12 h. 10^{-8} M IL-8 (Human IL-8, Sino Biological Inc.) was added into the lower chamber to establish a chemotactic gradient for neutrophil migration across a monolayer of endothelial cells (HUVECs). After incubation, cells in the lower chamber were observed with confocal microscopy and were then collected. The cells were washed three times with HBSS and determined by flow cytometry. All cells were analyzed for each sample.

Drugs-loaded NPs uptaken by Neutrophils in ischemic brain: Immunofluorescence assay was performed as described before, with minor modifications. Brain sections were blocked with 5 % goat serum at 37 °C for 1 h. Microvessels were labeled with anti-CD31 antibody. Neutrophils were stained with anti-Ly6G mAb (RB6-8C5, Thermo Fisher Scientific, Rat monoclonal antibody). DAPI was used for nuclear counterstain. The distribution of fluorescence was observed by confocal microscopy.

Inflammation Facilitated Brain-targeting and Neutrophils Depletion. Neutrophils were depleted by a single i.p. injection of 0.5 mg anti-Gr-1 antibody (Ly6G and Ly6C, RB6-8C5, Bioxcell) in nude mice three days prior to intravenous NPs administration, which has been used extensively to deplete neutrophils in mice [2, 3]. Nude male mice (6-8 weeks) MCAO models were carried out according to protocol described before two days following neutrophil depletion. BODIPY 650/665-labeled *cl* PGP-PEG-DGL/CAT-Aco NPs were intravenously administered to IS mice 24 hours after reperfusion. At defined time points, brain fluorescence images were conducted using an *in vivo* imaging system (IVIS Spectrum, Caliper, USA).

Evaluation of Neurological Function. All animals were scored for neurological deficit according to a 28-point general scoring system (general neurological scale) prior to MRI image [4]. Scoring was performed independently by two observers, blinded to groups, and averaged for final score on each mouse. In the general score, these 6 general deficits were measured: (a) hair conditions (0-2), (b) position of ears (0-2), (c) eyes conditions (0-4), (d) posture (0-4), (e) spontaneous activity (0-4), and (f) epileptic behavior (0-12).

Detection of ROS by DHR Assay. The mice were administrated with PBS, CAT alone (250 U/mouse) and *cl* dendrimer/CAT NPs respectively 3 h post-establishment of ischemia model. To monitor oxidative stress in brains, 200 μl DHR (1.0 mg/ml in PBS) was injected via i.v. 24 h after administration. The mice were scarified and perfused 2 h after DHR injection. The brains were fixed, dehydrated and cyro-sectioned as described previously. After staining the nuclear with DAPI, the

slides were imaged by confocal microscopy. The fluorescence count of oxidation product rhodamine 123 (RH123) was mounted and normalized to the number of nuclei in cerebral ischemia area by an image analysis program (Image-Pro Plus 6.0).

TUNEL Assay. Apoptosis associated DNA fragmentation was evaluated by TUNEL Assay Kit according to the manufacturer's instructions. Brain tissue sections were permeabilized with 0.1% TritonX-100 in 0.3% citric acid for 8 min and incubated with mixed reagent (10% (v/v) tube 1 and 90% (v/v) tube 2) for 60 min at 37 °C. Brain tissue sections were then treated with DAPI for 5 min. The fluorescence count was mounted using the following forum.

$$\text{TUNEL positive cells (\%)} = \frac{\text{amount of TUNEL positive cells in ischemic areas}}{\text{amount of total cells in ischemic areas}} \times 100\ \%$$

HE Staining. Infarct areas after brain ischemia were assessed by HE staining [5]. Brain tissue sections were stained with HE as a method described previously [6], and then recorded by camera.

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