Supporting Information

Fabrication of Positively Charged Fluorescent Polymer Nanoparticles for Cell Imaging and Gene Delivery

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Supporting Experimental Section

Spectroscopic and Microscopic Characterization of CPNPs.

Fluorescence spectroscopy analysis was conducted on a Hitachi F-7000 spectrophotometer (Hitachi, Ltd., Japan). The absolute fluorescence quantum yield of CPNPs was determined by an integrating sphere coupled to the fluorescence spectrometer. The size of the CPNPs was determined by dynamic light scattering (DLS, malvern, Nanozs90, U.K.) and transmission electron microscopy (TEM) (JEM 1230, JEOL, Japan). Single particle fluorescence imaging experiments were performed on an inverted optical microscope (Ti-U, Nikon, Japan). The mercury lamp was replaced with a solid-state diode laser (473 nm, CNI laser Changchun, China). The expended laser line was reflected with a dichroic mirror and focused onto the back port of an oil immersion objective (NA 1.49, 100 ×, Nikon, Japan). The fluorescence images were captured by an EMCCD camera (Ultra 897, Andor, UK) with an exposure time of 20 ms. All of the images were processed with ImageJ (http://rsbweb.nih.gov/ij/).

Gel Retardation Assay.

To examine the complexation of pDNA with CPNPs suspensions, we prepared CPNPs/pDNA complexes at different mass ratio from 1.25:1 to 11.25:1 (w/w,

CPNPs/pDNA) to measure the DNA binding ability. After 30 min of incubation, those complexes were electrophoretically run on a 1.0% agarose gel containing (0.2 μg/mL) ethidium bromide for DNA staining. The gel was placed in a horizontal electrophoresis apparatus (DYY-6C, Beijing Liuyi Biotechnology Co.Ltd.) containing 1x Tris acetate EDTA (TAE) buffer solution, exposed to an electric field (320 V) for 20 min, and then visualized by Gel image system (Tanon 2500, Shanghai Tianneng science and Technology Co.Ltd).

Cellular Cytotoxicity Estimation.

The cytotoxicity of CPNPs was estimated based on the commonly used MTT Method [2-7]. In this assay, Hek 293 cell line was used as the model. In brief, Hek 293 cells were plated at a density of 1.0×10^4 cells per well in a 96-well plate at 37 ˚C under 5% CO_2 atmosphere and incubated 12 h before treatment. Then Hek 293 cells were washed with PBS buffer and incubated with the complete culture medium containing different concentrations of CPNPs (0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 μg/mL) at 37 ˚C for 24 h. Then, 20 μL of 5 mg/mL MTT solution in DMEM was added to the culture dish and incubated for an additional 5 h. The MTT containing culture medium was decanted off, and 150 μL of DMSO was added to dissolve the formazan crystal formed by live cells. Cells cultured with DMEM (without CPNPs) were used as a control. Cell viability was measured using a microplate reader (BioRAD model 550, Japan) at 550 nm. The relative cell viability related to control wells containing cell culture medium without transfection agents was calculated by $[A]_{test}/[A]_{control}\times100$.

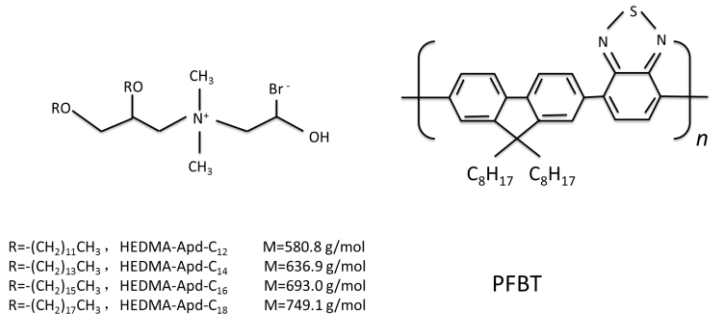

Figure S1. The chemical structure of propylene glycol amine cationic lipid [1] and PFBT.

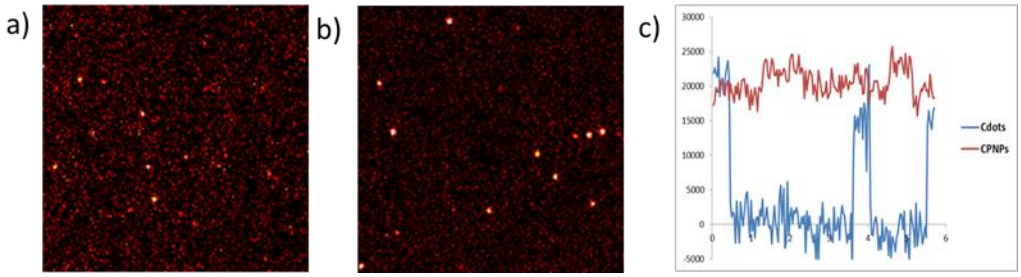

Figure S2. Representative fluorescence images of single carbon dots (Cdots) a) and CPNPs b). c) The time dependent fluorescence track of single carbon dots and CPNPs. The peak of the fluorescence from CPNPs and Cdots was corrected to the same value.

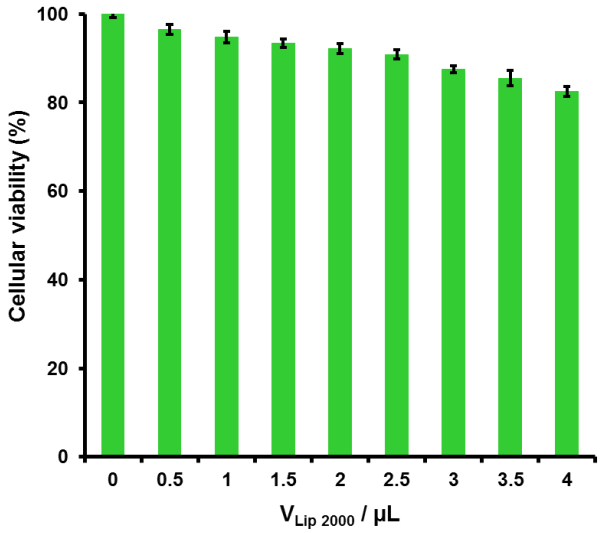

Figure S3. The cytotoxicity of lip2000 as a function of mass concentration evaluated by MTT colorimetric assay.

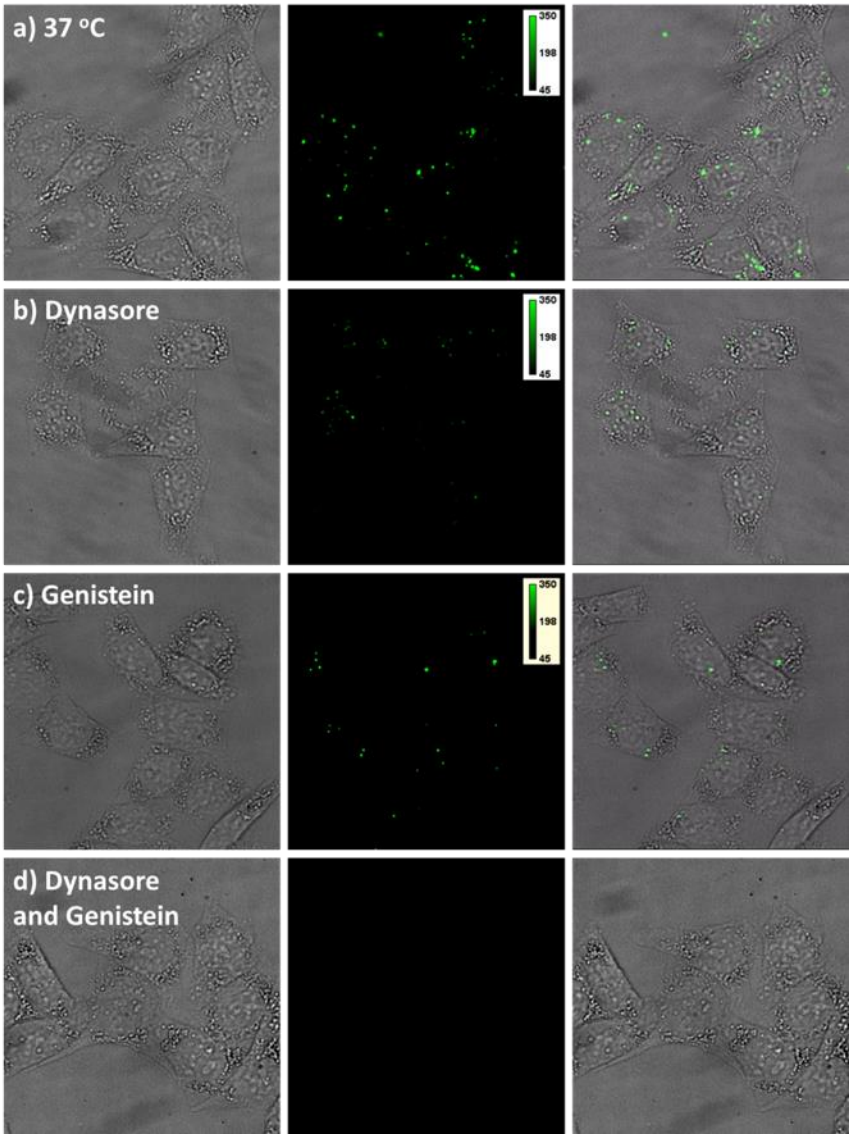

Figure S4. The fluorescence microscopic images of the cells co-cultured with CPNPs for 2 h at 37 °C a) without drug treatment, b) with Dynasore, c) Genistein, and d) both of then respectively. The images from left to right are the bright field, fluorescence and merged microscopic image respectively.

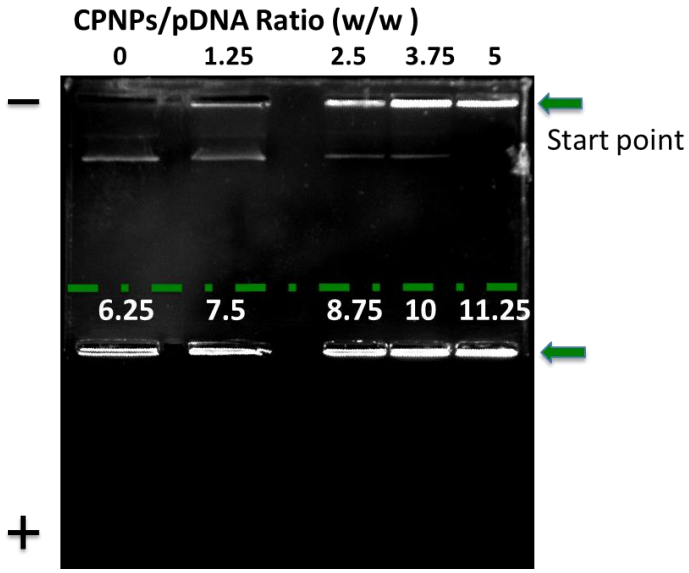

Figure S5. The pDNA binding ability assay toward the CPNPs explored by gel electrophoresis. From left to right are the gel electrophoresis results from the composite with different CPNPs/pDNA (w/w) ratios as noted in the figure.

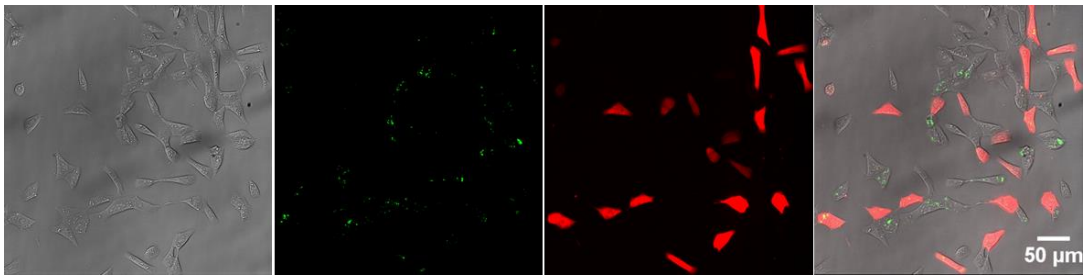

Figure S6. The gene transfection experiment on HeLa cells. From left to right are the bright field, the fluorescence image from the pdots, the red fluorescence image from the red fluorescent protein and the merged image.

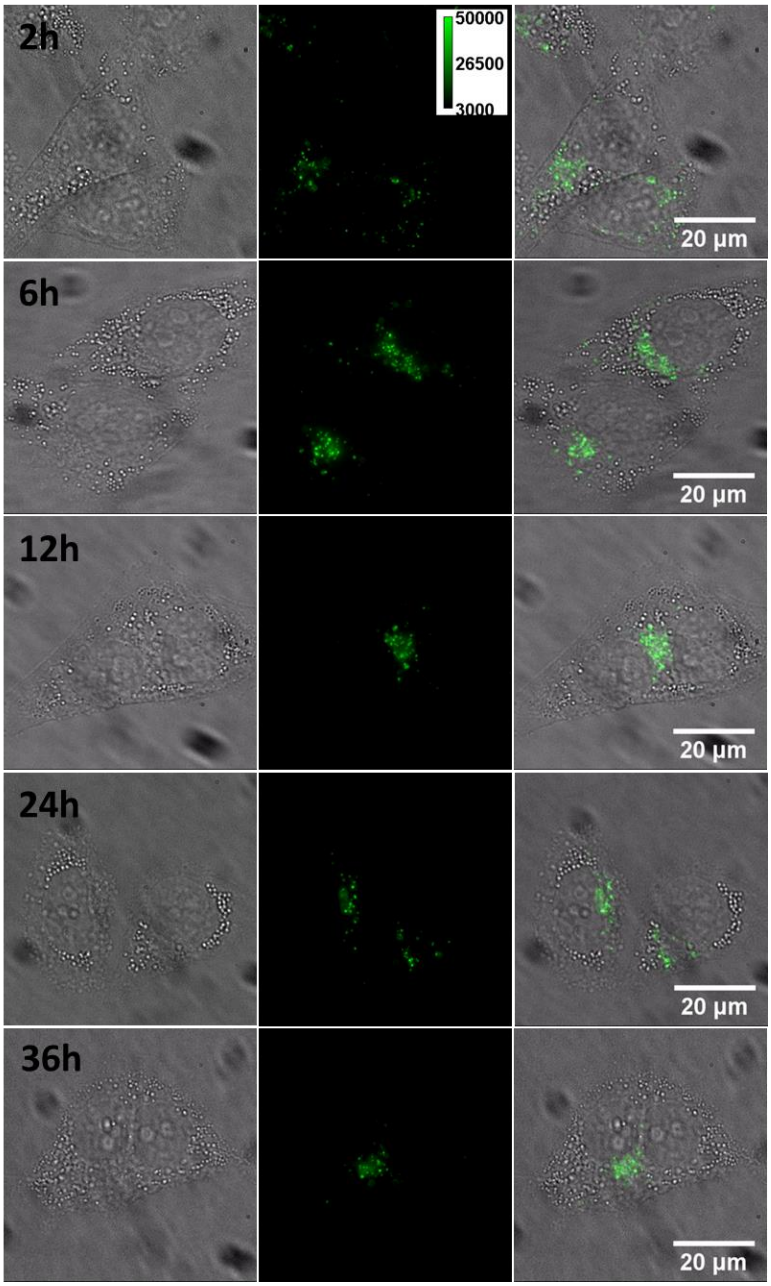

Figure S7. The magnified images of the distribution of CPNPs inside the cell. Pictures from left to right are the bright-field, fluorescence and merged microscopic images of the cells.

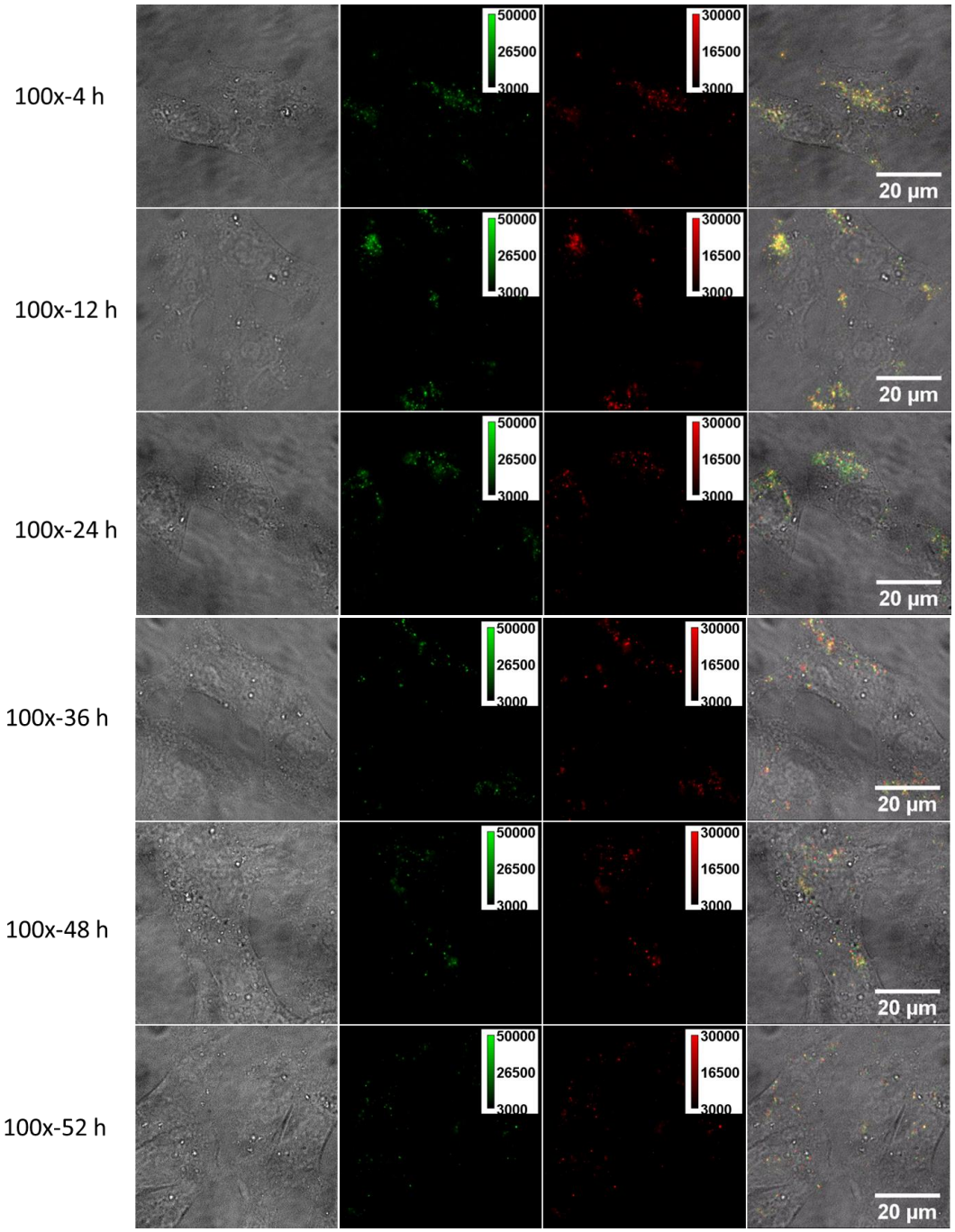

Figure S8. The fluorescence microscopic images of the cells co-cultured with the composite of CPNPs/PAA (with zeta potential of ~2.0 mV) for 2 h at 37 ºC and then incubated with fresh culture medium for different time points. The images were captured by a 100x objective. The panels from left to right are the bright field, green fluorescence, red fluorescence, and merged microscopic images respectively.

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