Electronic Supplementary Information

Polypyrrole Nanoprobes with Low Non-specific Protein Adsorption for Intracellular mRNA Detection and Photothermal Therapy

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Experimental Section

Chemicals and Materials. Pyrrole (≥98%) was purchased from Alfa Aesar. Polyvinylpyrrolidone (PVP, MW=29,000, AR) and Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥99%) were purchased from Sigma-Aldrich. DNA sequences were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). RNA sequence was purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Japan). All other reagents of analytical reagent grade were purchased from Sinopharm Chemical Reagent Co., Ltd. (China), and were used without further purification. The water used in all the preparation was ultrapure, which obtained from a Millipore water purification system (resistivity 18.2 MΩ).

Instruments. Ultraviolet-visible-near-infrared light (UV-vis-NIR) absorption spectra were collected in a standard 1-cm path length quartz cuvette on an Infinite M200 PRO Microplate Reader (Tecan Trading Co., Ltd, Switzerland) in the range of 400-1000 nm. Fluorescence spectra were recorded using a Hitachi Model F-4600 Fluorescence Spectrophotometer (Hitachi Co., Ltd., Japan). Transmission electron microscopy (TEM) images were obtained by using a FEI Tecnai G20 microscope (FEI NanoPorts, USA) operated at an accelerating voltage of 200 kV. The fluorescence images of cells were taken on a Confocal Laser Scanning Microscope (CLSM) (Nikon, Japan).

Preparation of PPy NPs. PVP (1g) was dissolved in ultrapure water (25 mL) in a 50 mL sealed container by stirring at room temperature for 30 min. 130 μL pyrrole was added to the solution. After 10 min, 1 mL iron (III) chloride hexahydrate (0.75 g/mL)

was quickly added to the reaction mixture. The polymerization was carried out for another 3 hours. After that, the products were purified by washing with copious amounts of ethanol-acetone (v:v=1:1) mixture and centrifuged at 40000 rpm for 20 min for several times, and finally redispersed in water. PPy NPs solution with a good dispersion was obtained and its initial concentration was ~2.4 mg/mL. The obtained solution was stored in darkness at 4 °C for further use.

The initial concentration of PPy NPs was calculated by using the gravimetric analysis. The concentration of PPy NPs is calculated by the formula: $c=(m_1-m_0)/V$, where c is mass concentration of PPy NPs, m_0 is the mass of measuring flask, V is the volume of PPy NPs solution, and m_1 is the total mass of measuring flask and PPy NPs after drying.

UV-vis-NIR Absorption Spectrum Measurements. To investigate the absorption of PPy NPs, 1 mL of PPy NPs aqueous solution with different concentrations were first prepared. Then, the UV-vis-NIR absorption spectra of PPy NPs were collected by an Infinite M200 PRO Microplate Reader (Tecan Trading Co., Ltd, Switzerland) in the range of 400-1000 nm.

Preparation of the Nanoprobes. In the fluorescence quenching assays, FAM-labeled hairpin ssDNA were incubated with different concentrations of PPy NPs in the phosphate buffered saline buffer (PBS, 100 mM NaCl, 10 mM $MgCl_2$, pH=7.4), and the fluorescence was measured after 5 min by a Hitachi Model F-4600 fluorescence spectrophotometer with excitation at 480 nm and emission range from 500 to 650 nm. The fluorescence emission maximum was at 520 nm.

Hybridization Experiment. PPy NPs (with a final concentration of 50 μg/mL) were first incubated with 50 nM FAM-labeled hairpin DNA in the PBS buffer (100 mM NaCl, 10 mM $MgCl_2$, pH=7.4) for 5min.Then the target mRNA with different concentrations were added into this mixture respectively, and fluorescence measurements were performed after 30 min.

The quenching efficiency (QE, [%]) of the PPy NPs was calculated by using the formula: (1-β)*100%, where β is the ratio of fluorescence of the quenched-to-completely dequenched state.

Cell Culture and Cytotoxicity Assay. MCF-7 (human breast-cancer cells), MCF-10A (human normal mammary epithelial cells), HeLa (human cervical cancer cells) and HepG2 (human liver cancer cells) cell lines, were cultured in Dulbecco's modified Eagle's medium (DMEM, Wisent) supplemented with 10% fetal bovine serum (FBS, Sigma), 100 μg/mL penicillin and 100 μg/mL streptomycin at 37 °C in a 5% CO_2 incubator.

Cell viability was measured with CCK-8 according to the manufacture's protocol. For the cytotoxicity assay, the MCF-7, HeLa and HepG2 cells were first cultured in 96-well plates (100 μL, 1×10^4 cells per well) for 24 h. And cultured for another 24 h after the culture medium was replaced with 100 μL of DMEM supplemented with 10% FBS containing 20 μL of the PPy NPs at different doses. Then, followed by removal of the culture medium, 10 μL of CCK-8 solution was added to each cell well which was washed with PBS buffer twice and contained 90 μL of culture medium. The cells were further incubated for 1 h. The optical density (OD) of the mixture was

measured at 450 nm with an Infinite M200 PRO Microplate Reader. The cell viability was estimated according to the following equation:

Cell viability (%) = (OD treated-OD blank) / (OD control-OD blank) *100%

Where OD control was obtained in the absence of PPy NPs, OD treated obtained in the presence of PPy NPs, and OD blank obtained in the presence of culture medium and CCK-8 solution but without cells. Each result was the average of three wells, and 100% viability was determined from untreated cells.

Fluorescence Imaging in Living Cells. In comparative experiment of cancer cells and normal cells, MCF-7 cells and MCF-10A cells were seeded on 35-mm confocal dishes for 24 h (500 μL, 1×10^4 cells per dish), respectively. Before the experiments, cells were washed with PBS buffer for three times, and then the cells were incubated with PPy-DNA nanoprobes (containing 50 μg/mL PPy NPs and 50 nM DNA probe) at 37 °C for 4 h. Cell imaging was then carried out after washing the cells with PBS. The confocal fluorescence images in living cells were obtained by a Nikon C2 confocal microscope (Nikon, Japan) equipped with an oil immersion 60 objective. The cells were examined by confocal laser scanning microscopy (CLSM) with different laser transmitters. The cellular myelocytomatosis oncogene (c-myc) mRNA was recorded by FAM in green channel with excitation of 488 nm, and the thymidine kinase 1 (TK1) mRNA was recorded by Cy3 in red channel with excitation of 543 nm.

Photothermal Performance. PPy NPs with different concentrations were suspended in quartz cuvette (total volume of 1mL) and exposed to a NIR laser at 808 nm (BWT

Beijing LTD, China) with an output power of 1 W/cm^2 for 10 min. The temperature of the solutions was recorded by a digital thermometer with a thermocouple probe.

MCF-7 cells seeded in 35-mm confocal dishes were incubated with 100 μg/mL PPy NPs for 4 h. After exposed to NIR laser (808 nm, 1 W/cm^2) for certain time, the cells were stained with propidium iodide (PI) and imaged by CLSM.

To quantitatively evaluate the photothermal cytotoxicity of PPy NPs, MCF-7 cells were incubated in 96-well plates (100 μL, 1×10^4 cells per well) at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. The PPy NPs with different concentrations were added and the cells were further incubated for 4 h. After with or without irradiated by a NIR laser for 5 min, the cells were allowed to incubate for another 24 h. The standard CCK-8 assays were carried out to evaluate the cell viabilities.

Table S1 The sequences employed in this work.

name	**Sequence(5'-3')**
FAM-DNA	5'-CCGGGTTTGGTGAAGCTAACGTTGAGGACCCGG-FAM-3'
c-myc target	5'-CCUCAACGUUAGCUUCACCAA-3'
Control sequence	5'-CAAGGAGCUGGAAGGCUGGGA-3'
Cy3-DNA	5'-CCGGGTGCGAGTGTCTTTGGCATACTTACCCGG-Cy3-3'

Underline denotes base pairs in the stem.

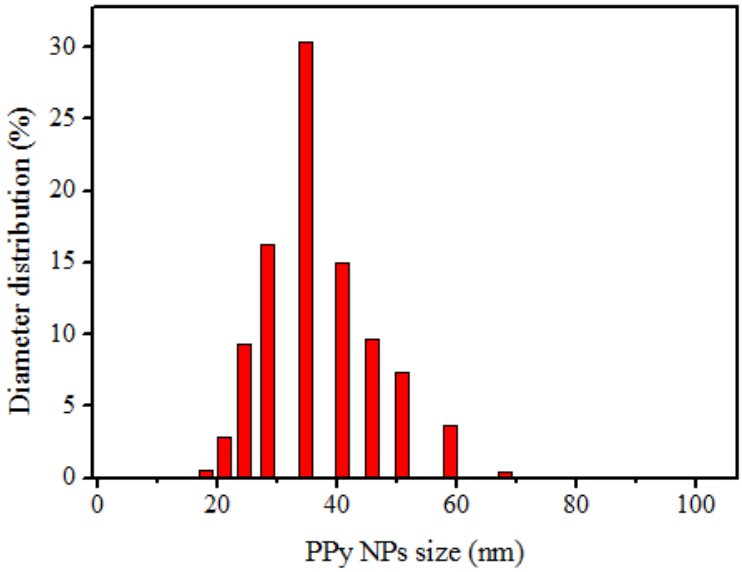

Fig. S1 The diameter distribution of PPy NPs by DLS test.

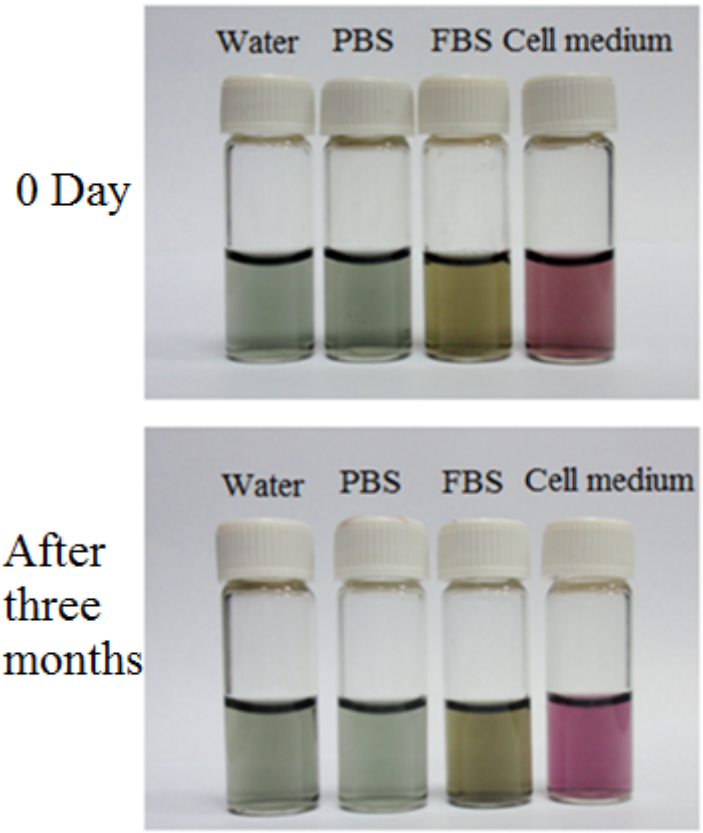

Fig. S2 Photos of PPy NPs dispersed in different solutions including water, PBS, FBS and cell medium.

Fig. S2 showed that the as-obtained PPy NPs still dispersed well (without showing obvious aggregation) in water and physiological solutions over three months.

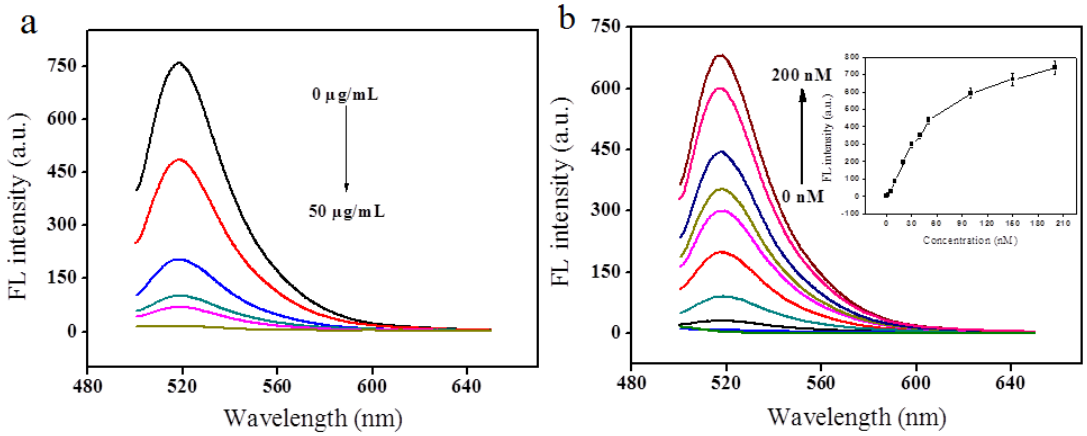

Fig. S3 (a) Fluorescence emission spectra of 50 nM FAM-DNA treated with different concentrations of PPy NPs (from top to bottom: 0, 10, 20, 30, 40 and 50 μg/mL). (b) Fluorescence emission spectra of PPy NPs-DNA nanoprobes incubated with different concentrations of target mRNA (from bottom to top: 0, 2, 5, 10, 20, 50, 100, 150 and 200 nM). Inset: Calibration curves for the fluorescence intensity versus corresponding target concentrations (0, 2, 5, 10, 20, 50, 100, 150 and 200 nM). Excitation: 480 nm, emission: 520 nm. The data are from at least three independent experiments.

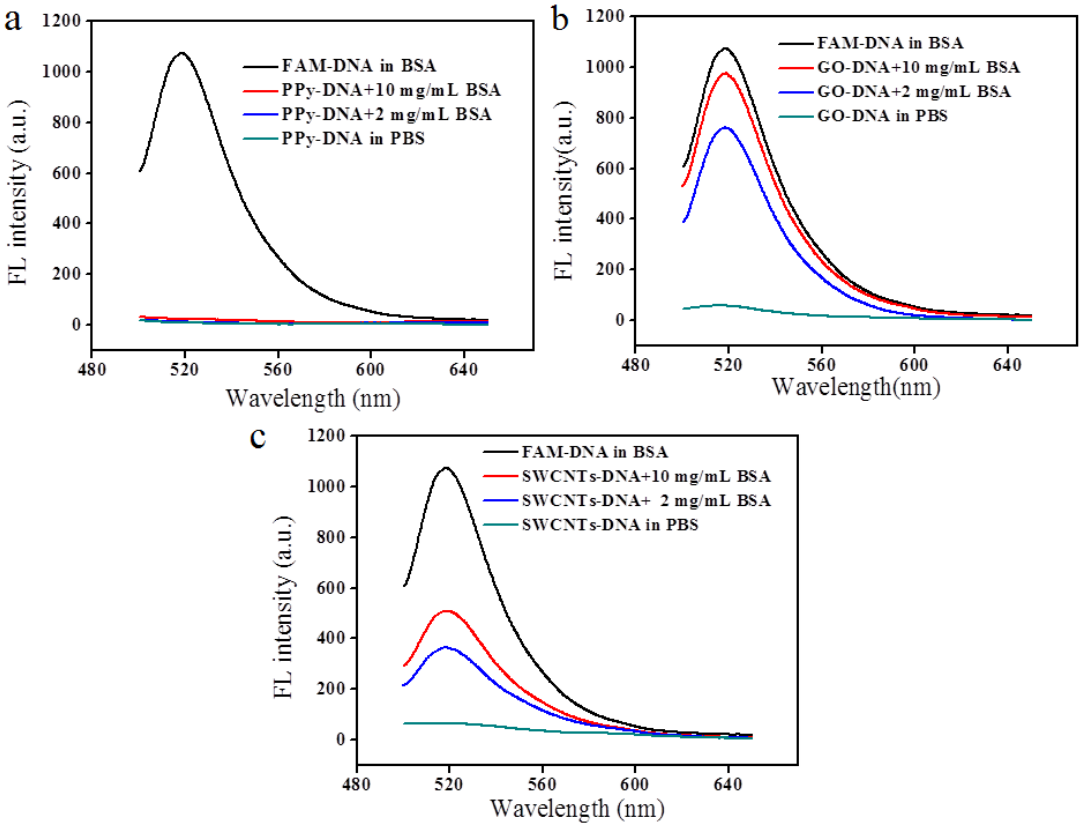

Fig. S4 (a) Fluorescence emission spectra of PPy NPs-DNA nanoprobes (containing 50 μg/mL PPy NPs and 50 nM FAM-DNA) dispersed in BSA (2 mg/mL or 10 mg/mL) or PBS solution. (b) Fluorescence emission spectra of GO-DNA nanoprobes (containing 50 μg/mL GO and 50 nM FAM-DNA) dispersed in BSA (2 mg/mL or 10 mg/mL) or PBS. (c) Fluorescence emission spectra of SWCNTs-DNA nanoprobes (containing 50 μg/mL SWCNTs and 50 nM FAM-DNA) dispersed in BSA (2 mg/mL or 10 mg/mL) or PBS. Excitation: 480 nm, emission: 520 nm.

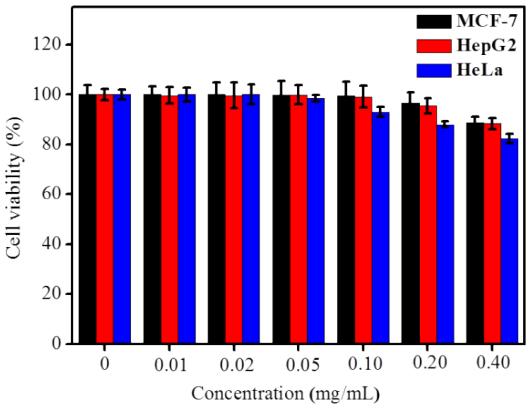

Fig. S5 Cell viability of MCF-7, HepG2, and HeLa cells incubated with different concentrations of PPy NPs (0, 0.01, 0.02, 0.05, 0.10, 0.20 and 0.40 mg/mL) for 24 h.