Supporting Information for:

Two Birds with One Stone: Dendrimer Surface Engineering Enables Tunable Periphery Hydrophobicity and Rapid Endosomal Escape

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MATERIALS AND METHODS

Materials

Tris(hydroxymethyl)aminomethane (Tris) was from Sigma-Aldrich (Beijing, China). 1-propylpiperazine-dihydrobromide was from J & K Scientific Ltd. (Tianjin, China). 4-dimethylaminopyridine (DMAP) was from Aladdin (Shanghai, China). Succinic anhydride was from Institute of Guangfu Fine Chemical Research (Tianjin, China). Fluorescein isothiocyanate (FITC) and 1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride (EDC • HCl) were sourced from Medpep Ltd. (Shanghai, China). 4',6-diamidino-2-phenylindole (DAPI) and LysoTracker® Red DND-99 were purchased from KeBaiao biological reagents Ltd. (Tianjin, China). All other chemicals were obtained from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China.)

Synthesis of OPPBA

1-propylpiperazine-dihydrobromide (2.90 g, 10 mmol) was dissolved in 100 mL water containing sodium hydroxide (0.80 g, 20 mmol) and then extracted by excess dichloromethane (DCM). The organic layer was treated with anhydrous sodium sulphate, followed by solvent evaporation to get 1-propylpiperazine. Freshly prepared 1-propylpiperazine together with excess triethylamine (TEA) was mixed in 50 mL DCM followed by the addition of succinic anhydride (1.50 g, 15 mmol); the reaction was maintained at ambient temperature (25^{o}C) for 5 h. Then DCM was evaporated and the remaining was precipitated in diethyl ether, followed by recrystallization in ethanol, and vacuum drying for 24 h to get 4-oxo-4(4-propylpiperazin-1-yl)butanoic acid (OPPBA) (**Figure S1**).

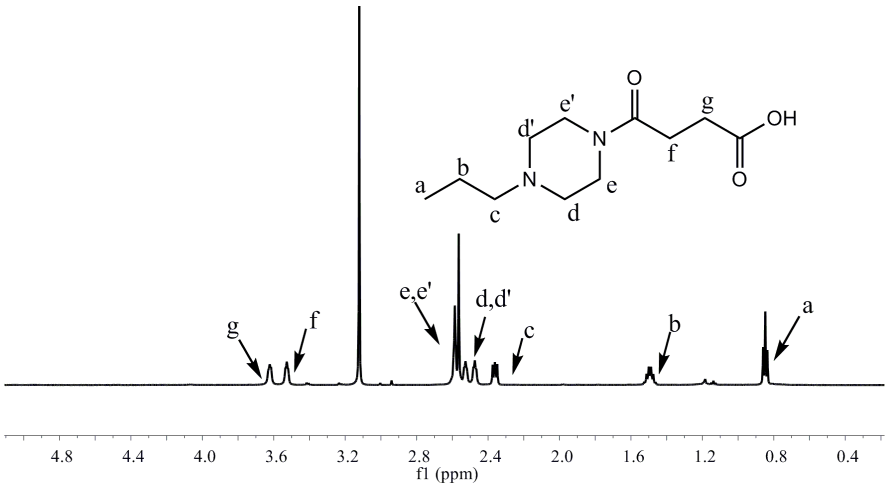

Figure S1**. ^{1}H NMR spectrum of OPPBA in $CDCl_3$.**

Synthesis of $PAMAM_{Tris}$-OPPBA and FITC-$PAMAM_{Tris}$-OPPBA

Then Tris-modified PAMAM (i.e. $PAMAM_{Tris}$) was obtained using the protocol in our previous report.[1] Then the esterization reaction between OPPBA and $PAMAM_{Tris}$ produced surface-modified thermosensitive $PAMAM_{Tris}$-OPPBA. In brief, OPPBA (1.90 g, 8.32 mmol), EDC·HCl (1.71 g, 8.32 mmol), and DMAP (1.01 g, 8.32 mmol) was dissolved in 50 mL dimethyl sulfoxide (DMSO) ; the mixture was maintained for 1 h, followed by slow addition of G3.5 $PAMAM_{Tris}$ (381.0 mg, 43.3 μmol) under nitrogen protection at ambient temperature. After 72 h, DMSO was evaporated and the crude product was dialyzed against water for 48 h with a dialysis bag (molecular weight cut-off/MWCO: 3500 Da) to get $PAMAM_{Tris}$-OPPBA (G3.5) (**Figure S2**). FITC labelled dendrimer was generated as follows. G3.5 $PAMAM_{Tris}$-OPPBA (245 mg, 12.1 μmol) was dissoved in 20 mL dimethyl formamide (DMF) containing 20 μL TEA. Then FITC (188 mg, 0.48 mmol) dissolved in 20 mL DMF was mixed with the above solution under nitrogen protection. The reaction was maintained at 80^{o}C for 72 h, followed by DMF removal and then dialysis (MWCO: 3500 Da) for 48 h. The final freeze-dired product was FITC-$PAMAM_{Tris}$-OPPBA (**Figure S3**).

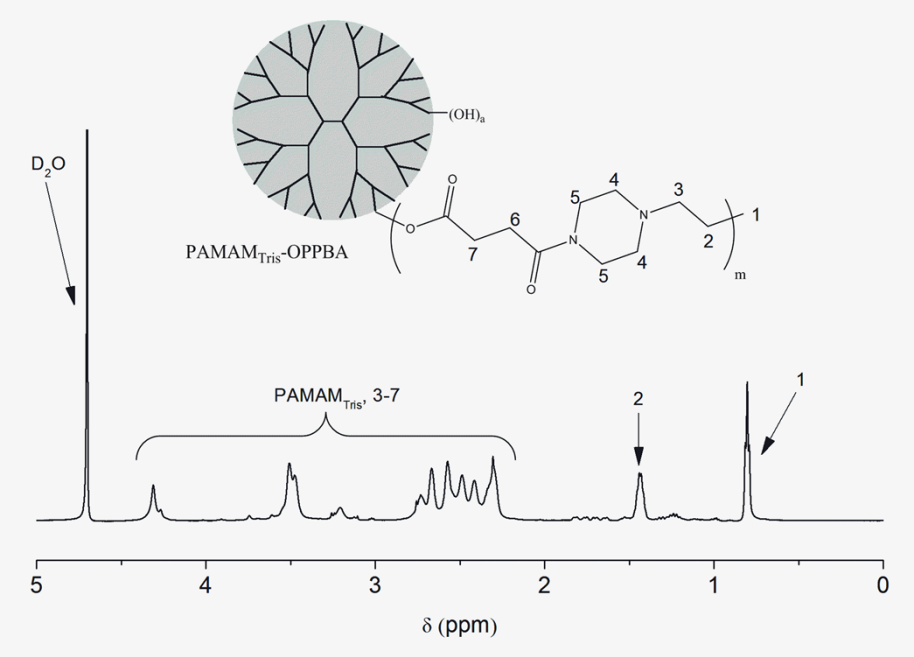

Figure S2. **^{1}H NMR Spectrum of PAMAM$_{Tris}$-OPPBA in D$_2$O.**

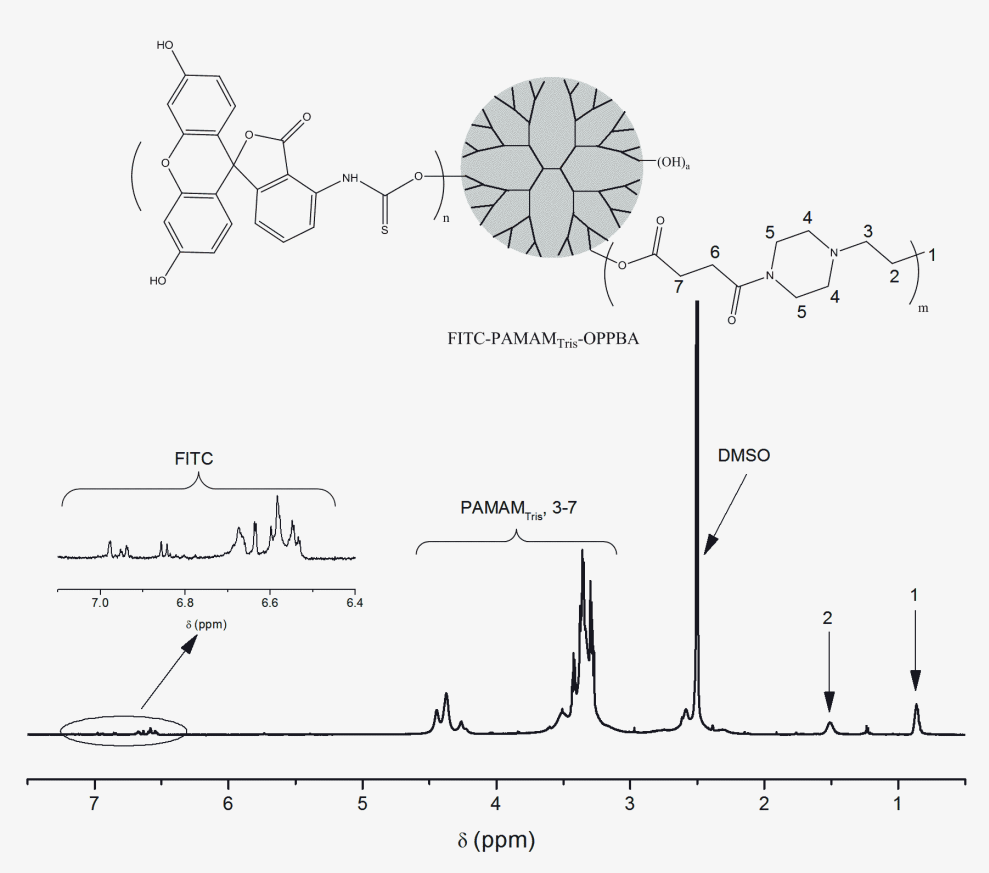

Figure S3. **^{1}H NMR Spectrum of FITC-PAMAM$_{Tris}$-OPPBA in DMSO.**

Cloud Point Determination

The thermosensitivity of $PAMAM_{Tris}$-OPPBA at different phosphate buffer (10 mM, pH 6.4-8.0) was assessed by the measurement of cloud point, the temperature at which the optical transmittance at 500 nm reached 50% of the maximum value.[1]

Cellular Uptake Study

MCF-7 cells provided by Institute of Biomedical Engineering (Chinese Academy of Medical Sciences & Peking Union Medical College) were seeded to a CLSM plate at a density of 2.5×10^4 cells per well in 1 mL DMEM. After 24 h' incubation at 37°C and 5% CO_2, the medium was removed and the cells were washed with PBS, followed by the addition of 200 μL fresh medium containing FITC-$PAMAM_{Tris}$-OPPBA (50 μg/mL) for further incubation. At predesigned time point (1 h, 2 h, 4h, and 6 h), the medium was discarded, followed by PBS washing and fresh medium supplement. Then 20 μL DAPI (1 μg/mL) and LysoTracker® Red DND-99 (1 μg/mL) was added to stain nuclei and lysosomes, respectively. After 15 min, the unbound dyes were removed by PBS and fresh medium was then added. The localization of dendrimers, nuclei, and lysosomes was observed by confocal laser scanning microscope with an excitation wavelength at 358 nm, 488 nm and 577 nm, respectively. Surface unmodified FITC-$PMAM_{Tris}$ was employed as the control.

Cytotoxicity Study

MCF-7 cells were seeded to 96-well plates containing 200 μL DMEM medium the cell density was 5×10^3 per well. After 24 h' incubation at 37°C and 5% CO_2, the medium was removed and the cells were washed with PBS, followed by the addition of $PAMAM_{Tris}$-OPPBA at different concentration (1-100 μg/mL). After further incubation for 48 h, 20 μL cell counting kit-8 reagent

(1 μg/mL) from Dojindo Laboratories (Shanghai, China) was added, followed by another 4 h's incubation. Then the spectrophotometric measurement at 450 nm was recorded and fresh medium was employed as the control (n = 6).

Dendrimer Aggreation Analysis

Dynamic light scattering technique was employed the aggregation behavior upon heating. The concentration of $PAMAM_{Tris}$-OPPBA dendrimer was fixed at 0.1 mM with the pH at 8.0. The analysis was performed in triplicate at 25°C (below cloud point) and 40°C (above cloud point), respectively.

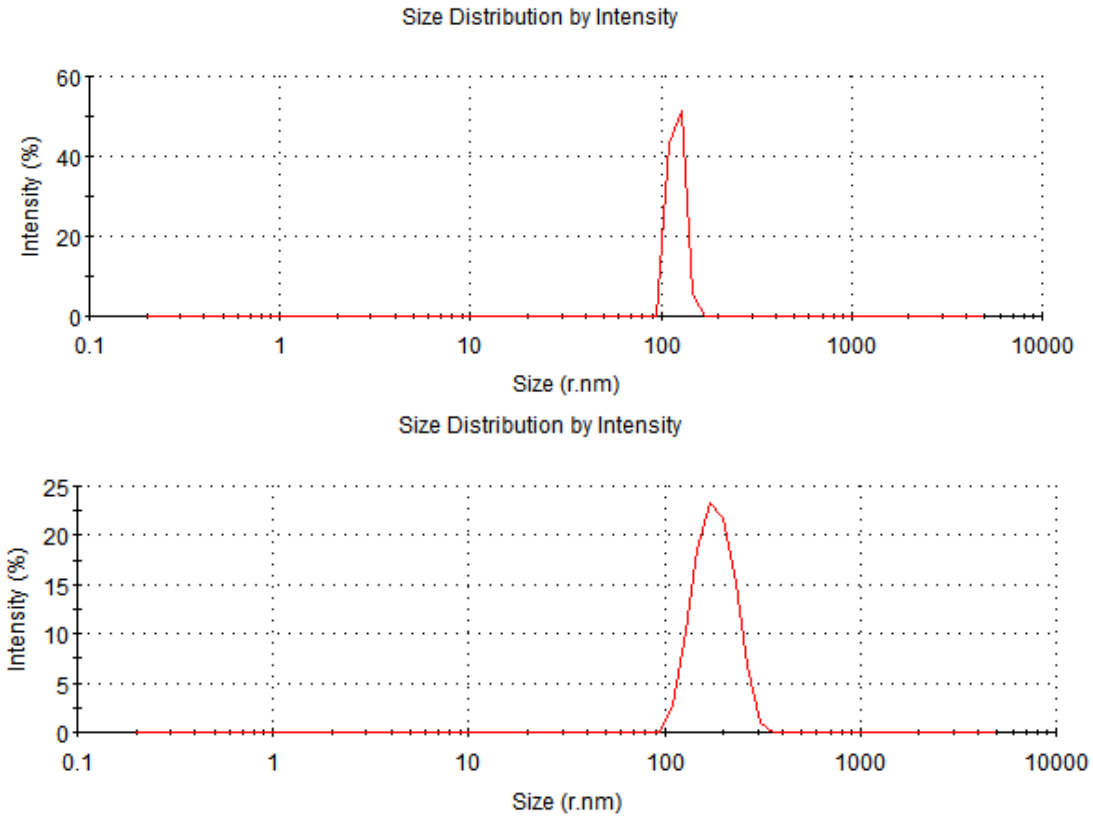

Figure S4. Particle size analysis of $PAMAM_{Tris}$-OPPBA in aqueous buffer (pH 8.0) at 25°C (top, 193 nm) and 40°C (below, 384 nm).

REFERENCES

1. Zhao, Y.; Shen, L.; Wan, Y.; Zhu, X. X.; Wang, Z. Thermosensitivity of low generation poly(amidoamine) dendrimers with enriching peripheral functional groups. Colloids Surf., A 2012, 403, 164-168.