Supporting information

Progressively targeted gene delivery system with pH triggered surface charge-switching ability to drive angiogenesis in vivo

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Experimental Section

Materials

Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Gly-Pro-Lys-Lys-Lys-Arg-Lys-Val-Gly-Cys (YGRKKRRQRRR-G-PKKKRKV-G-C, TAT-G-NLS-G-C), and Cys-Ala-Gly-Trp (CAGW) peptides were purchased from GL Biochem. (Shanghai) Ltd. Octa-ammonium polyhedral oligomeric silsesquioxanes (POSS-(Pr-$NH_3^+Cl^-$)$_8$) was purchased from Hybrid Plastics (Hattiesburg, MS). Diallylcarbamyl chloride (DAC) was purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai). 2,2-Dimethoxy-2-phenylacetophenone (DMPA) and succinimidyl-3-(2-pyridyldithio)propionate (SPDP) were purchased from J&K Scientific Ltd (Beijing). ε-Poly(L-lysine) (PLL, Mn = 4200 Da) was purchased from Yuan Ye Biotechnology Co., Ltd. (Shanghai). Cis-aconitic anhydride was purchased from Alfa Aesar (China) Chemical Co., Ltd. 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was obtained from Ding Guo Chang Sheng Biotech. Co., Ltd. (Beijing, China). Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Corporation (Carlsbad, CA). A BCA protein assay kit was supplied by Solarbio Science and Technology Co., Ltd (Beijing, China). Rabbit anti-human ZNF580 polyclonal antibody, goat anti-rabbit IgG and amiloride hydrochloride were purchased from Abcam Ltd. (Shanghai, China). Rabbit anti-beta-

actin antibody was obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Cy5 labeled oligonucleotide (Cy5-oligonucleotide) was purchased from Sangon Biotech Co., Ltd. (Shanghai, China). LysoTracker Green (DND-26, Life Technologies) and Hoechst 33342 were obtained from Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). Chlorpromazine hydrochloride was purchased from Sigma-Aldrich (St. Louis, USA). Filipin III was purchased from Cayman Chemical (Michigan, USA). EA.hy926 cells were obtained from the Cell Bank of Typical Culture Collection of Chinese Academy of Sciences (Shanghai, China). The pEGFP-ZNF580 plasmid (pZNF580) was supplied by department of physiology and pathophysiology, Logistics University of Chinese People's Armed Police Force.

Characterization

^{1}H NMR spectrum was recorded on an ECA-400 (400 MHz) spectrometer in deuterated chloroform ($CDCl_3$), deuterated water (D_2O) or deuterated hydrochloric acid (DCl) at 25 ℃. Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) was performed on a Bruker Daltonics Inc. Autoflex Tof/TofIII MALDI-TOF mass spectrometer with DHB matrix. Transmission electron microscope (TEM) was performed on a Japanese model JEM-2100F with an accelerating voltage of 200 kV. The size and zeta potential were detected via Zetasizer Nano ZS (Malvern Instrument, Inc., Worcestershire, UK).

Preparation and characterization of POSS-$(DA)_8$

The preparation was carried out according to the typical Schotten-Baumann procedure[1]. In detail, POSS-(Pr-$NH_3^+Cl^-$)$_8$ (1.5 g, 1.28 mmol) was dissolved in pH 8.5 phosphate buffers (PBS, 10 mL) and cooled to 0 ℃. DAC (9.80 g, 61.38 mmol) was dissolved in anhydrous chloroform (40 mL) and added dropwise to the aqueous solution. In addition, pH of the reaction mixture was kept at 8.5 using 1 N sodium hydroxide (NaOH) aqueous solution. The reaction mixture was stirred for another 12 h at RT after the chloroform solution was completely added. The chloroform phase was separated, sequentially washed (HCl solution, saturated NaCl solution and deionized water), dried (anhydrous Na_2SO_4), filtered, vacuum evaporated, washed (anhydrous ether), vacuum dried, and then the crude product was obtained. Eventually, the crude product was purified by reversed-phase column chromatography of C_{18} (0.5% TFA in water/acetonitrile) to obtained POSS-(DA)$_8$ as an off-white powder. The graft ratio of DAC to POSS-(Pr-$NH_3^+Cl^-$)$_8$ was determined to be 8 by the area ratio of peak d to c' in ^{1}H NMR spectrum (Figure S3, B). POSS-(DA)$_8$ showed M = 1887.072 $(M + Na)^+$ by MALDI-TOF-MS (Figure S4) , which agreed with the ^{1}H NMR result.

Preparation and characterization of POSS-(C-G-NLS-G-TAT)$_{16}$

POSS-(DA)$_8$ (2 mg, 1.07×10^{-3} mmol), DMPA (1 mg) and TAT-G-NLS-G-C (55 mg, 20.82×10^{-2} mmol) were added into 3 mL THF. Ultra purified water (1.5 mL) was introduced to form transparent and uniform mixture solution. The solution was stirring and continuously treated by UV-light for 10 min under nitrogen atmosphere, then dialyzed and lyophilized. POSS-(C-G-NLS-G-TAT)$_{16}$, named as PP1, was obtained as

a light yellow powder. The graft ratio of TAT-G-NLS-G-C to POSS-$(DA)_8$ was 16 because of the absolute disappearance of peak e and f from ^1H NMR spectrum (Figure S3, C).

Preparation and characterization of PLL-g-CAGW$_m$-g-Aco$_n$

PLL (20 mg, 1.56×10^{-1} mmol NH_2 group) was dissolved in ultra purified water (15 mL), and a methanol solution of SPDP (5 mg/mL, 2 mL) was gradually dropped into the aqueous solution. The reaction mixture was stirred for 4 h in dark condition, then CAGW (17 mg, 3.90×10^{-2} mmol) was added to the reaction solution while keeping stirring for another 6 h. Directly, the solution was cooled to 0 ℃ and first adjusted to pH 8.5 using 1 N NaOH aqueous solution. Under this condition, CA (60.90 mg, 3.90×10^{-1} mmol) was gradually added into the solution. Meanwhile, the reaction solution was kept pH = 8-9 by supplying 1 N NaOH solution. When pH was stable, the reaction was continued for overnight. The solution was dialyzed (against diluted NaOH solution with pH 8-9) and lyophilized to obtain PLL-g-CAGW$_m$-g-Aco$_{n3}$ (PP2-3). By adjusting the feeding amount of CA (18.30 mg, 1.17×10^{-1} mmol; 14.80 mg, 9.48×10^{-2} mmol) PLL-g-CAGW$_m$-g-Aco$_{n2}$ (PP2-2) and PLL-g-CAGW$_m$-g-Aco$_{n1}$ (PP2-1) were respectively obtained with the same procedure. To quantify the graft ratio of CAGW peptide to the PLL side chain, we have prepared the intermediate substance PLL-g-PDP$_m$-g-Aco$_{n3}$ with the similar procedure, just without addition of CAGW peptide. For PLL-g-PDP$_m$-g-Aco$_{n3}$, the graft ratio of PDP group to the PLL side chain was confirmed to be about 21% from the peak area of methine proton in the PLL main chain

(δ = 4.10 ppm) to the pyridyl protons (δ = 7.2-8.3 ppm) by ^{1}H NMR spectrum (Figure S5, A). Entirely substituted by CAGW peptide, the pyridyl proton peaks of PDP group in PP2-1, PP2-2 and PP3-3 completely disappeared in ^{1}H NMR spectra (Figure S5, C, D, E), which illustrated the same graft ratio of CAGW peptide as PDP to the PLL side chain. CAGW exhibited the methine proton peaks corresponding to alanine and glycine residues at 4.0-4.2 ppm (D_2O, 25℃). Thereby, for PLL-g-$CAGW_m$-g-Aco_n, the area of methine proton peaks at 4.0-4.2 ppm has become about 1.42 times as high as that of PLL main chain (Figure S5, C, D, E). Therefore, the conversion of primary amine in the PLL side chain to cis-aconitic amide in PP2-1, PP2-2 and PP2-3 was respectively calculated to be about 47%, 57% and 64% from the peak area ratio of the methine protons from cis-aconitic moiety (-$COCHC(COO^-)CH_2COO^-$, δ ≈ 6.0 ppm) to the methine protons (δ = 4.0-4.2 ppm).

Preparation and characterization of binary gene complexes (BCPs) and ternary gene complexes (TCPs) with different weight ratios.

PP1 was dissolved in pH 7.4 PBS under ultrasound condition to prepare a homogeneous solution (0.20 mg/mL). Afterwards, BCPs with different weight ratios of w/w (PP1/pZNF580 plasmid) were prepared by gradually dropping corresponding amount of pZNF580 plasmid solution (50 μg/mL) into PP1 solution. TCPs with different w/w/w (PP1/pZNF580 plasmid/PP2) ratios were further prepared by slowly adding appropriate amount of PP2 solution (0.15 mg/mL) to BCPs solution. The morphology of PP1 was analyzed by TEM (Figure S6). The size and zeta potential of BCPs with w/w ratios of

0.5/1, 1/1, 2/1, 3/1, 4/1, 5/1 (Figure S8) and TCPs with w/w/w ratios of 5/1/0.5, 5/1/1.25, 5/1/2.5, 5/1/5 were detected via Zetasizer Nano ZS. Besides, the ability of BCPs to load pZNF580 plasmid was analyzed by Agarose gel electrophoresis assay (Figure S7). TCPs were also measured by Agarose gel electrophoresis assay for evaluating whether the addition of anionic PP2 led to pZNF580 plasmid release from TCPs or not.

Hydrophobic POSS was covalently modified by highly hydrophilic TAT-G-NLS-G-C peptide to construct 16-armed star shaped amphipathic polymer PP1. According to previous report[2], high molecule weight, hydrophilic to hydrophobic ratio, as well as multi-arm architecture contributed to the fabrication of stable\, low-aggregated micelles with small size (Figure S6). From electrophoresis result, PP1 could completely inhibit pZNF580 plasmid migration at low weight ratio of 2 (Figure S7), which illustrated that PP1 had strong ability to load gene.

For efficient gene delivery, physicochemical properties of gene complexes play a fundamental role, such as size and zeta potential. In Figure S8, BCPs with different w/w ratios presented suitable value. The size of BCPs was in the range of 100 nm to 200 nm, which was suitable for cell transfection. The zeta potential of BCPs showed an increasing trend with the increase of w/w ratio. When w/w ratio was higher than 1/1, the zeta potential values of BCPs were positive, which was consistent with electrophoresis results. With appropriate zeta potential (24.1 ± 0.5 mV) and particle size (127.2 ± 0.3 nm), BCPs with w/w ratio = 5/1 were selected to prepare TCPs.

Preparation and characterization of PLL-g-Aco

The primary amines in PLL side chain reacted with 2.5 times amount of CA powder to obtain PLL-g-Aco. The conversion of primary amines in the PLL side chain to cis-aconitic amide was calculated to be about 78% from the peak area of e and g in ^{1}H NMR spectrum (Figure S5, B).

Study of the pH responsive hydrolysis reaction of cis-aconitic amide

^{1}H NMR spectrum was employed to monitor the hydrolysis reaction. To clearly observe the change of the characteristic peak in ^{1}H NMR, PLL-g-Aco was selected as the reactant model rather than PP2. In detail, PLL-g-Aco was dissolved in D_2O, and then the solution pH was respectively adjusted to pH 5.5 and 7.4 by DCl. According to the predetermined time (0.5 h, 2.0 h, 4.0 h and 24.0 h), the ^{1}H NMR spectrum of samples was recorded.

After the cis-aconitic amide was hydrolyzed, the characteristic peak of the methine protons of the cis-aconitic moiety moved from a (-NHCOCHC(COO^-)CH_2COO^-) to a' ($^-$OOCCHC(COO^-)CH_2COO^-). Therefore, the location and intensity change of characteristic peak were used to evaluated the hydrolysis reaction. At pH 7.4, the location and intensity of peak a kept basically unchanged within 4 h, and peak a' with very low intensity appeared in slightly lower field after 24 h. This result illustrated that cis-aconitic amide was basically stable within 24 h at pH 7.4. However, at pH 5.5, the peak a' was quickly observed within half an hour and became obviously stronger and stronger accompanying significant decrease of peak a within 4 h, eventually the peak a

disappeared in 24 h. These results illustrated that cis-aconitic amide could be completely hydrolyzed in 24 h at pH 5.5. These results agreed with the previous report[3] and confirmed the pH responsive hydrolysis reaction of cis-aconitic amide.

pH responsive zeta potential change of TCPs

TCPs with w/w/w ratio of 5/1/1.25 were selected to determine pH responsive zeta potential changes. In short, the same amount of PP2-1, PP2-2 and PP2-3 were respectively added to the BCPs (5/1 ratio) under the condition of pH 5.5 acetate buffer (ABS) and pH 7.4 PBS, named TCPs-1, TCPs-2 and TCPs-3. At predetermined time intervals (1 h, 2 h, 4 h, 7 h, 10 h, 13 h, 24 h), Zetasizer Nano ZS was performed to measure the zeta potential changes at pH 5.5 and 7.4 of TCPs-1, TCPs-2 and TCPs-3, respectively.

Hemolysis Assay

1 mL human blood sample was freshly obtained from a healthy human donor in Hospital of the Armed Police and centrifuged at 3000 rpm for 5 min, and the supernatant was poured off. After washing until supernatant was clear, the packed cells were resuspended in 10 ml 0.01M PBS (pH =7.4). 20 μL of the diluted RBCs suspension was added to 1 mL of 0.5 mg/mL gene delivery system suspension in PBS, and the mixture was incubated at 37 °C for 24 h. Meanwhile, a 20 μL amount of diluted RBC suspension incubated with 1 mL of PBS or 1 mL of water was used as the negative or positive control, respectively. The absorbance value of the hemoglobin at 577 nm was measured

with the reference wavelength of 655 nm. The percent of hemolysis was calculated as follows:

$$\text{Hemolysis}(\%)=\frac{\text{sample absorbance - negative control}}{\text{positive absorbance - negative control}}\times 100\%$$

The non-viral gene delivery system carrying positive charge tended to interact with blood components during blood circulation, which could lead to low biocompatibility and low transfection efficiency, and then restricted the biomedical application in vivo. Here, the hemocompatibility of gene delivery materials and systems was evaluated through hemolysis assay using fresh human RBCs. In Figure S10, according to the images of RBCs morphology, compared with PBS negative control, all experiment groups exhibited very low RBCs destruction. Besides, we observed that TCPs possessed lower hemolysis rate than BCPs (5/1 ratio), because PP2 decreased their zeta potential. Meanwhile, BCPs (5/1 ratio), TCPs-1, TCPs-2 and TCPs-3 displayed gradually low hemolysis mainly due to their gradually decreased zeta potential. Importantly, the hemolysis rate of BCPs (5/1 ratio) was less than 5% which was the medical standard. TCPs especially TCPs-3 (hemolysis rate was about 1%) could be considered to be highly hemocompatible, which was the foundation of gene delivery system in vivo.

Cell culture and transfection

HUVECs were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin solution at 37 °C in 5% CO_2. When cultured to reach 80-90% confluence, cells were first seeded into 24-well plate at a density of 1×10^5 cells per well and

cultured for 24 h. Then the cells were starved with serum-free medium for 12 h. The gene delivery systems with free medium were added into each well (3 μg pZNF580 per well). After 4 h of transfection, the medium was replaced with fresh DMEM containing 10% FBS and the cells were cultured for another 24 h. The expressed green fluorescent proteins (GFPs) were observed via an inverted fluorescent microscope.

Cell viability assay

3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) powder was dissolved in 0.01M PBS at 5 mg/mL concentration, and filtered through sterilized syringe filter (0.22 μm pore diameter). The prepared MTT stock solution was stored at 4 ℃. HUVECs (1×10^4 cells/well) were plated in 96-well plates and cultured in DMEM containing 10% FBS for 24 h to achieve 80% confluence. Then the medium was replaced by serum-free medium and cultured overnight. The cells were treated with appropriate concentration of gene delivery materials and gene delivery systems, and then incubated for 48 h. Then 100 μL of serum free medium with 0.5 mg/mL MTT was added, and followed by additional incubation of 4 h until purple color developed to detect the metabolically active cells. The medium was discarded and the cells were rinsed with PBS to remove remaining MTT. Finally, 100 μL of DMSO was added to each well to dissolve water insoluble formazan salt. The optical densities of each well were measured at 490 nm wavelength. Mean and standard deviation of triplicated were calculated and plotted. The relative cell viability was calculated as:

$$\text{relative cell viability}(\%)=\frac{OD_{490}(\text{sample})}{OD_{490}(\text{control})}\times 100\%$$

Cellular uptake and transmembrane mechanism study

Different Cy5-oligonucleotide delivery systems were prepared to quantitatively evaluate their cellular uptake in HUVECs by a flow cytometry (Beckman MoFlo XDP, USA). Briefly, cells (3×10^5 cells/well) were plated in 6-well plate and transfected with these Cy5-oligonucleotide complexes. After 4 h incubation, cells were washed three times with 0.01 M PBS (pH =7.4) and trypsinized with 0.25% trypsin. And then, cells were centrifuged and resuspended in 300 μL PBS (pH = 7.4), then the suspension was analyzed with a flow cytometer. To investigate the pathways of cellular uptake, three endocytosis inhibitors were used, i.e., Chlorpromazine (CPZ, 30 μM), Amiloride hydrochloride (Amil, 50 μM) and Filipin Ⅲ (Filip, 5 μg/ml), to inhibit clathrin-mediated endocytosis, macropinocytosis and caveolae-mediated endocytosis, respectively. HUVECs were pretreated with each of the endocytosis inhibitors for 1 h and incubated with Cy5-oligonucleotide delivery systems for 4 h at 37 ℃. The untreated cells were used as a control. Subsequently, the cells were washed three times with 0.01M PBS (pH = 7.4) and resuspended in 300 μL PBS (pH = 7.4) after trypsinization and centrifugation. The transfected cells were analyzed by a flow cytometry.

Intracellular location study

The cells were seeded at 1×10^5 cells per well into a confocal dish and cultured for 24 h. Afterward, the cells were incubated with gene delivery systems for 4 h in free serum medium to facilitate the cellular uptake. After that, the cells were further incubated with

10% serum-containing DMEM for another 24 h. Cells were washed with PBS twice, and added with prewarmed medium containing 0.5 mM Lyso Tracker Green to continue culture for another 1 h. Finally, the cells were washed twice with prewarmed PBS and stained with 2 μg/mL of Hoechst 33342 at room temperature for 20 min. The cells were then washed twice with PBS and observed by a confocal laser scanning microscopy (CLSM) at excitation wavelengths of 649, 504 and 350 nm for Cy5 (red), Lyso Tracker Green (green) and Hoechst 33342 (blue), respectively. The co-localization rate (CLR) was calculated by Image-Pro Plus 6.0 software according to the following equation.

$$\text{Co-localization ratio}=\frac{\text{Number of yellow or pink pixels}}{\text{Number of yellow, red and pink pixels}}\times 100\%$$

Quantitative real-time PCR

Total RNA was extracted from transfected cells using TRIzol reagent and then reverse-transcribed into cDNA using the HiFiScript cDNA Synthesis Kit. The resulting cDNAs as templates were quantified via Ultra SYBR Mixture (low ROX) on 7500 Real Time PCR system according to the manufacturer's instructions. GAPDH was used as the reference gene for normalization of target gene. The forward and reverse primer sequences were 5'-GAGGTTACTGCCTTACCCTGG-3' and 5'-ACCCAGTTCCGACTGGTTC-3' for ZNF580, 5'-AGGTGAAGGTCGGAGTCAAC-3' and 5'-CGCTCCTGGAAGATGGTGAT-3' for GAPDH, respectively. All results are expressed as the means ± SD of three independent experiments.

Western blotting

Total protein was extracted from transfected cells using RIPA lysis buffer containing 1% volume of PMSF and quantified by BCA protein assay kit. Before measuring, the protein was denatured by adding 6 × SDS. All samples were separated by SDS-polyacrylamide gel electrophoresis and subjected to western blot. Membranes were incubated with rabbit anti-ZNF580 polyclonal antibody in TBST overnight and horseradish peroxidase conjugated anti-rabbit secondary antibody for 1 h. Membranes were scanned with the Tanon-5200 automatic chemiluminescence image analysis system and analyzed.

Cell proliferation assay

At 12, 24, 48 and 72 h after transfection, cell culture medium was removed and 100 μL of free serum medium containing 10% Cell Counting Kit-8 assay (CCK-8) was added to each well of a 96-well plate. After incubation for another 4 h at 37°C in 5% CO_2, the absorbance was measured at a wavelength of 450 nm using an ELISA microplate reader (Thermo Scientific). Assays were repeated at least three times.

Cell migration assays

The wound healing assay and transwell migration assay were performed to estimate the migration capability of the transfected cells. The operations of HUVECs with the treatment of different complexes in 24-plate were described before. For wound healing

assays, wound/scratch was created along the diameter of each well using a 200 μl pipette tip after transfected 24 h. Cell debris were removed by washing once with D-Hanks, followed by addition of fresh medium. The sites of cells were monitored and recorded by an inverted microscope at 0, 12 and 24 h. The relative recovered area at 24 h was calculated using Image J software with the following equation to expose the migration ability.

$$\text{Relative recovered area (\%)} = \frac{\text{Recovered area}}{\text{Wounded area}} \times 100\%$$

For transwell migration assay, the cells transfected 24 h were centrifuged, resuspended and then added to the upper chambers of inserts with a density of 1×10^5 cells per well. After 6 h of incubation, the migrated cells across the transwell were stained with violet crystal, photographed and counted to calculate the migration rate. By contrast, cells without any treatment were performed as negative control.

In vitro angiogenesis assay

The formation ability of capillary-like structures was detected in a 96-well plate using growth factor-reduced Matrigel. After the treatment according to the Corning Matrigel Matrix instruction, HUVECs transfected with gene delivery systems (5×10^4 cells/well) were plated onto Matrigel (50 μl/well) and were cultured for another 6 h. The tubes were observed using a bright-field microscope and the number was quantified using Image J software.

In vivo angiogenesis assay

The cells transfected by various gene delivery systems for 24 h were mixed with 800 μL Matrigel, and then were injected into male mice (6 weeks old, 20-25 g). Four days later, matrigel implants were removed and sliced into thick sections after being fixed with formalin and embedded in paraffin. The sections were stained with hematoxylin and eosin (H&E) and the luminal structures were observed using a microscope. In addition, the sections were immunohistochemically stained with mouse antiCD31 antibody for 1 h and followed with goat anti-mouse IgG H&L secondary antibody. The cell nuclei were stained with DAPI. The stained sections were observed by a fluorescence microscope to further determine the formation of micro-vessel structure.

Reference

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(2) P. Zhang, Z. Zhang, X. Jiang, L. Rui, Y. Gao and W. Zhang, *Polymer*, 2017, **118**, 268-279.

(3) S. Kang, Y. Kim, Y. Song, J. Choi, E. Park, W. Choi, J. Park and Y. Lee, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 2364-2367.

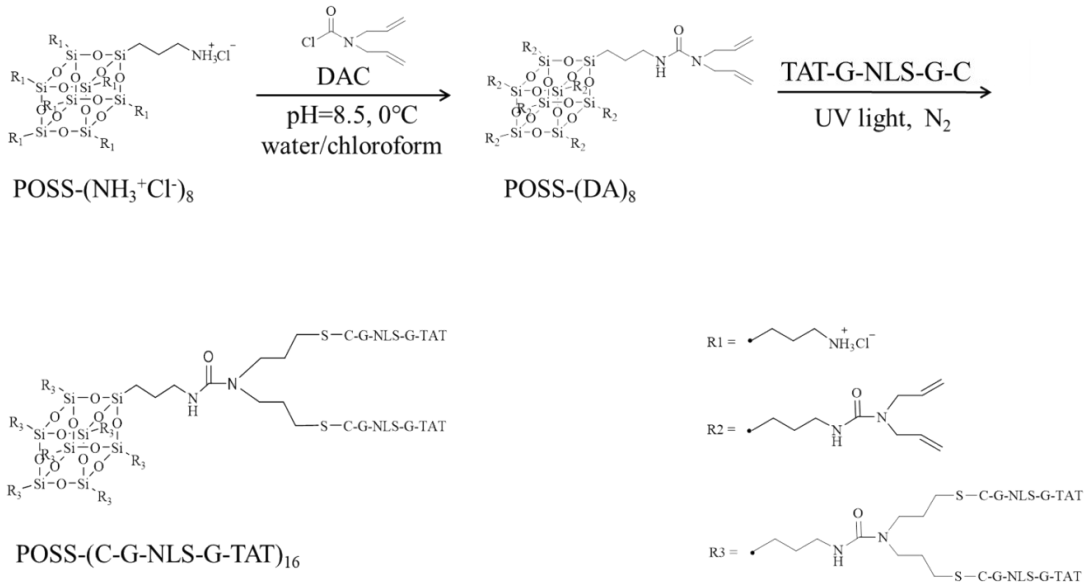

Figure S1. Synthesis pathway of POSS-(C-G-NLS-G-TAT)$_{16}$.

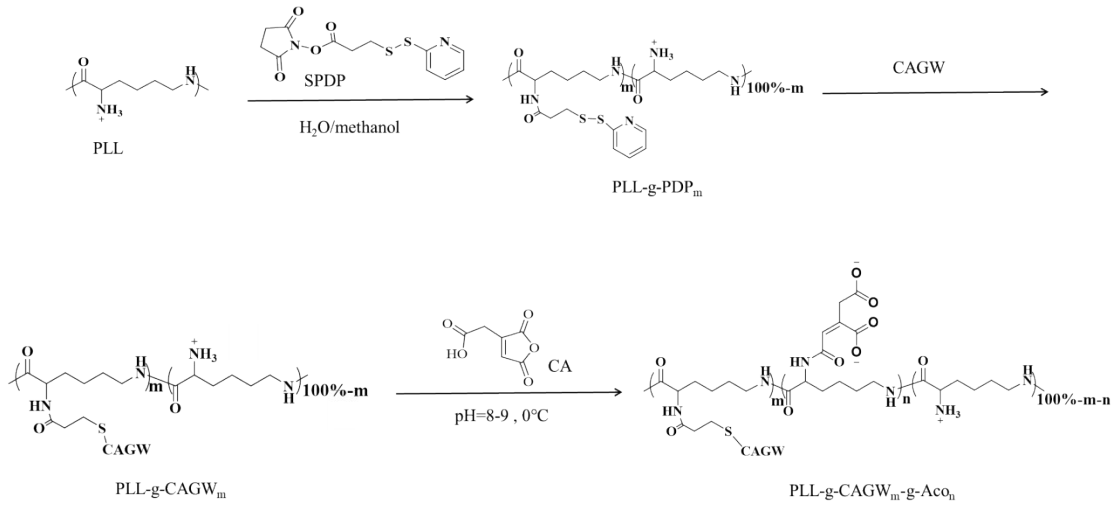

Figure S2. Synthesis route of PLL-g-CAGW$_m$-g-Aco$_n$.

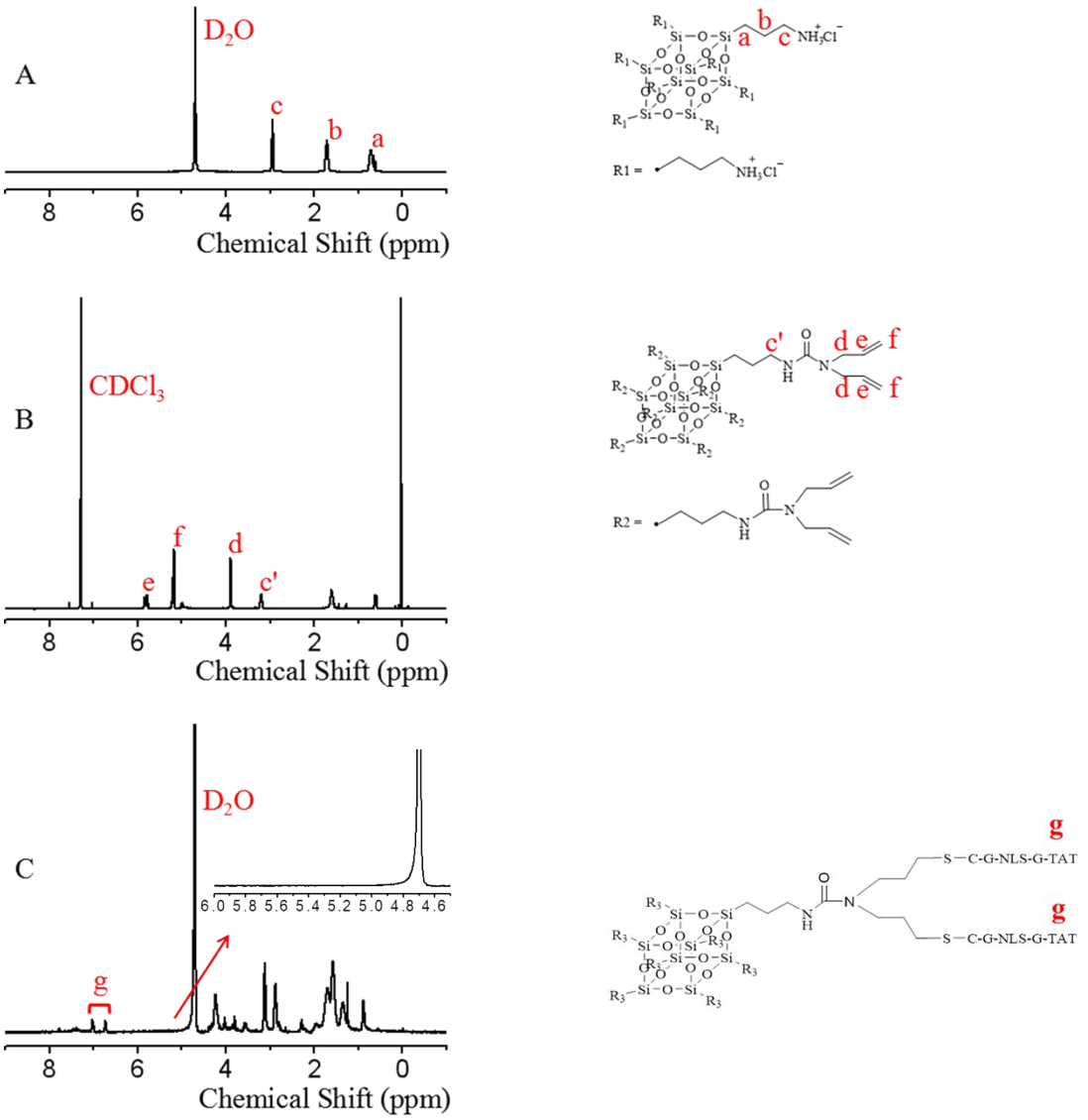

Figure S3. ^{1}H NMR spectra of (A) POSS-(Pr-$NH_3^+Cl^-$)$_8$ in D_2O, (B) POSS-(DA)$_8$ in $CDCl_3$ and (C) POSS-(C-G-NLS-G-TAT)$_{16}$ in D_2O.

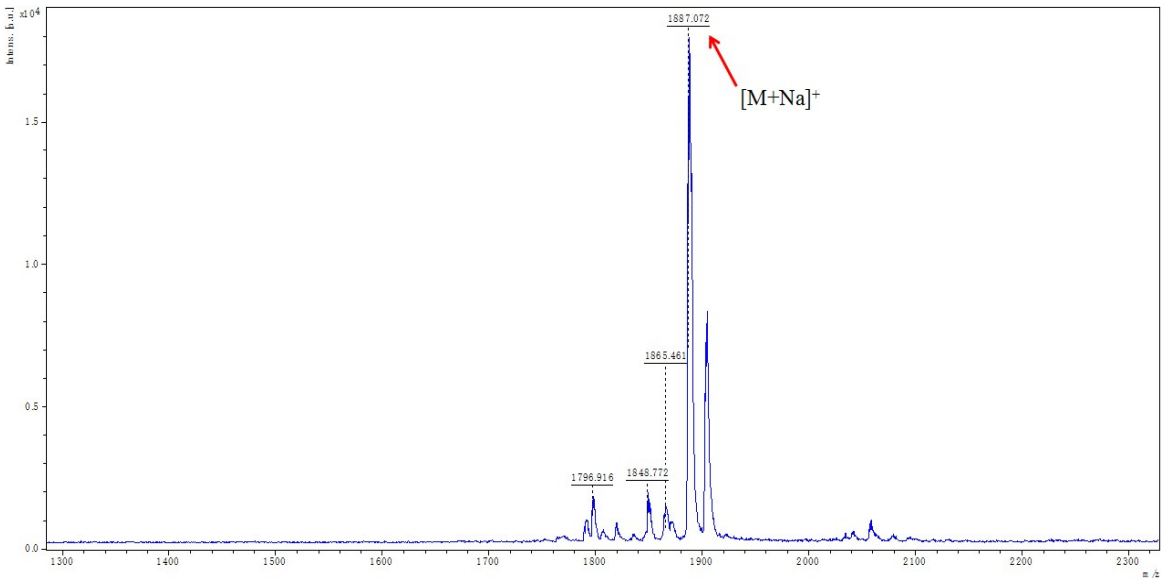

Figure S4. MALDI-TOF mass spectrum of POSS-$(DA)_8$ using DHB as the matrix.

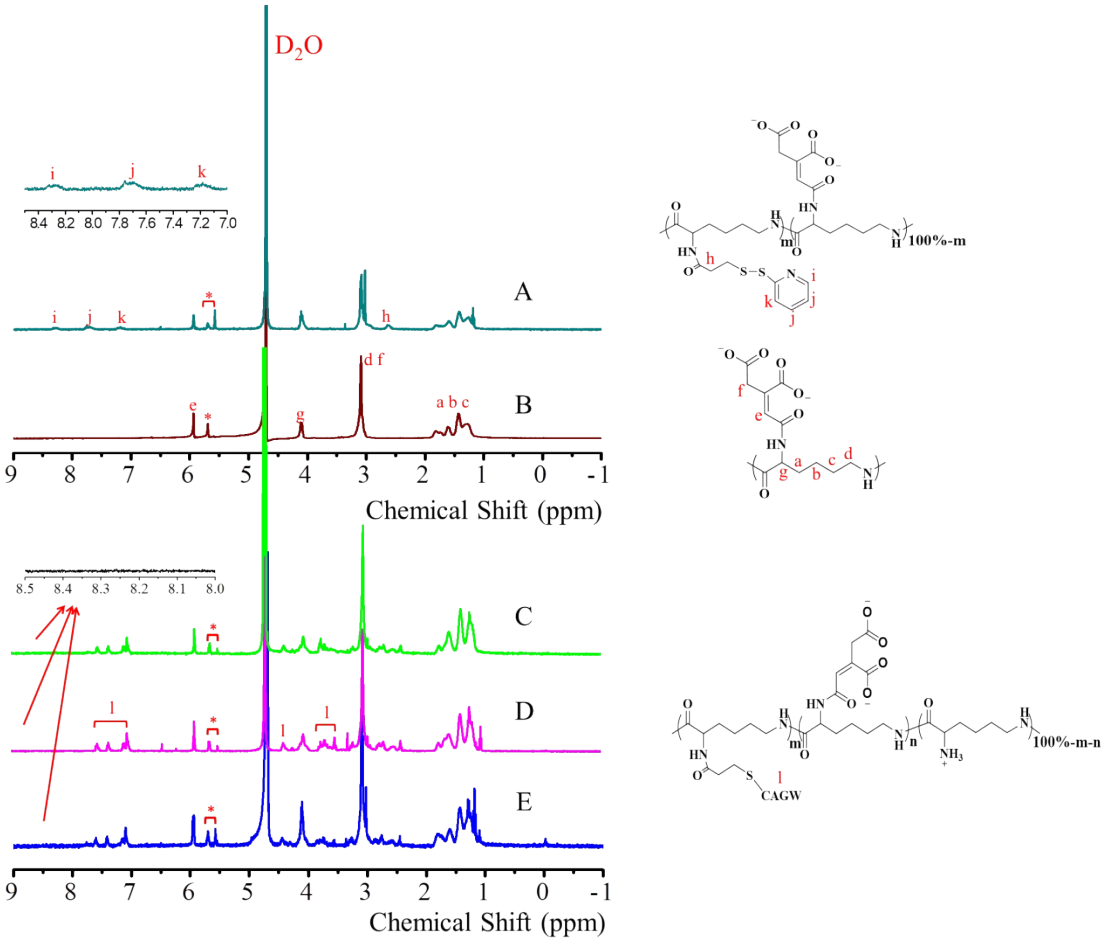

Figure S5. ^1H NMR spectra of synthesized polymers in D_2O. (A) PLL-g-PDP_m-g-

Aco_{n3}, (B) PLL-g-Aco, (C) PLL-g-CAGW_m-g-Aco_{n1}, (D) PLL-g-CAGW_m-g-Aco_{n2}, and (E) PLL-g-CAGW_m-g-Aco_{n3}. * protons arising from a decarboxylated byproduct.

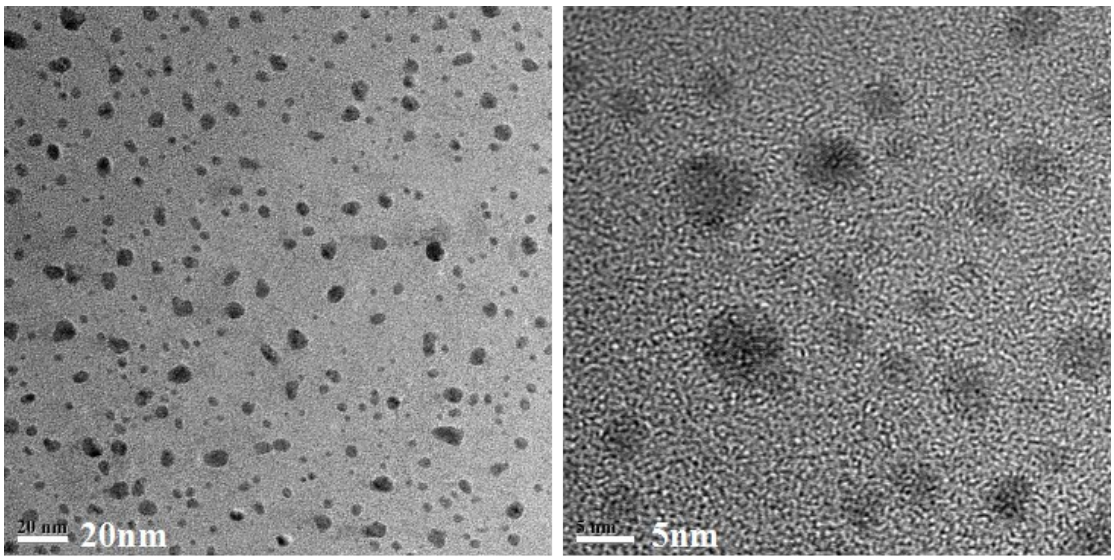

Figure S6. Morphology of PP1 with different magnification detected by TEM.

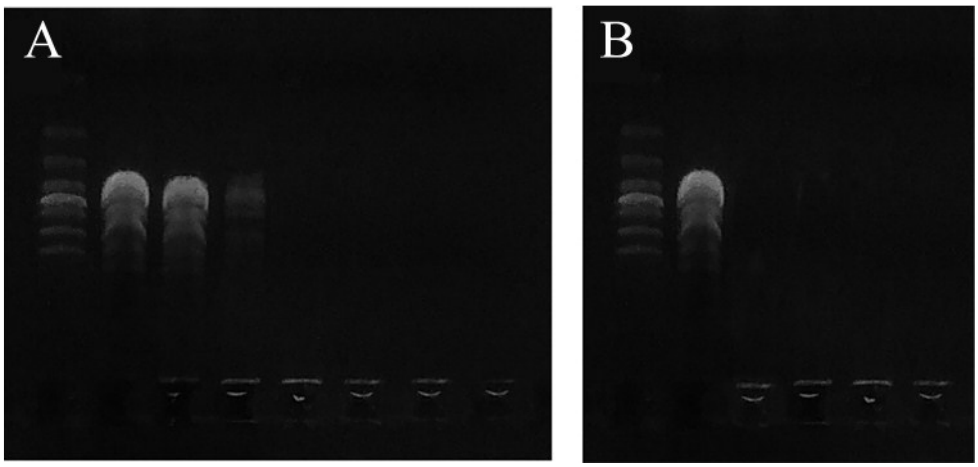

Figure S7. Agarose gel electrophoretic results of BCPs with different w/w ratios, Lane 1, marker; Lane 2, naked pZNF580 plasmid; Lane 3-8, BCPs with 0.5/1, 1/1, 2/1, 3/1, 4/1, 5/1 ratios (A) and TCPs-3 with different w/w/w ratios, Lane 1, marker; Lane 2, naked pZNF580 plasmid; Lane 3-6, TCPs-3 with 5/1/0.5, 5/1/1.25, 5/1/2.5, 5/1/5 ratios (B).

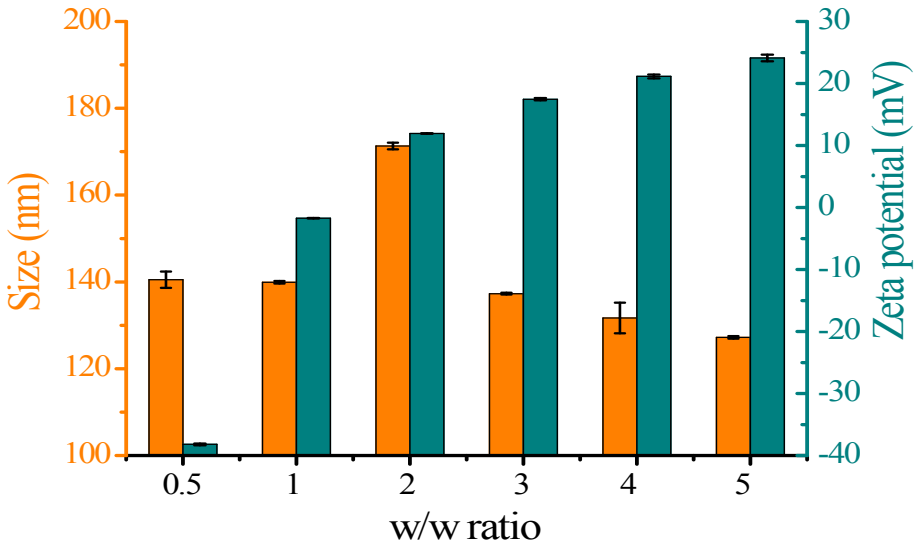

Figure S8. The size and zeta potential of BCPs with different weight ratios.

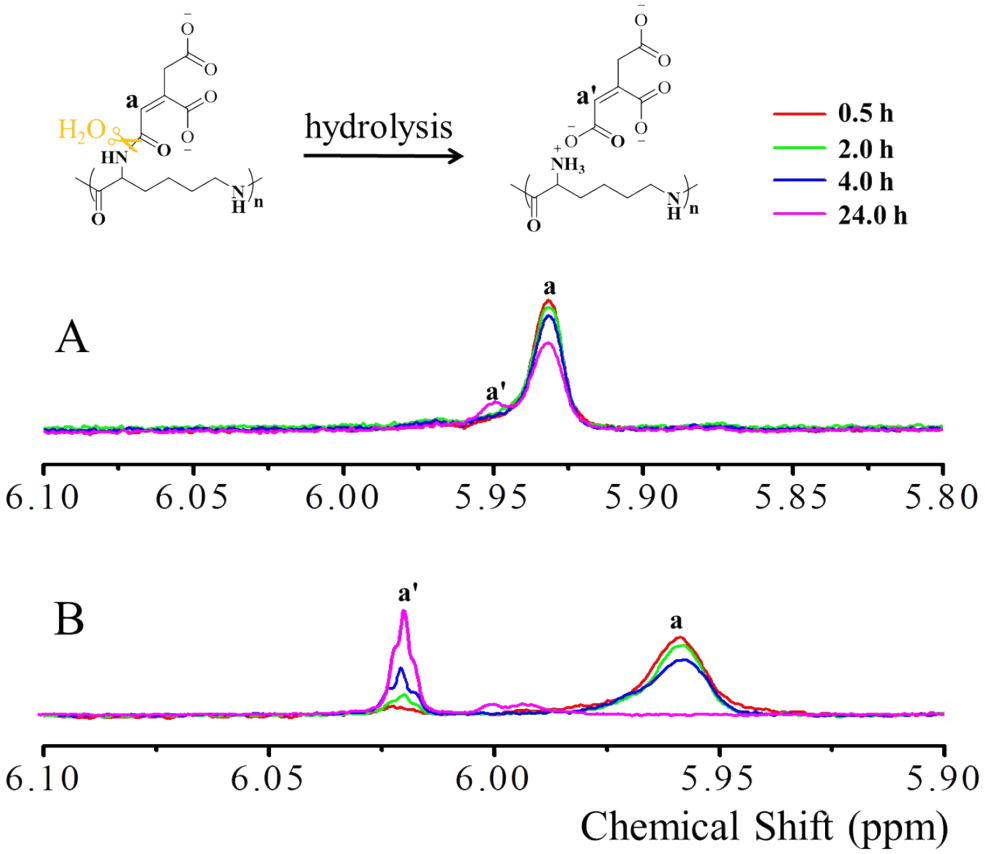

Figure S9. The pH responsive hydrolysis reaction of PLL-g-Aco at pH 7.4 (A) and pH 5.5 (B) recorded by ^{1}H NMR spectrometer.

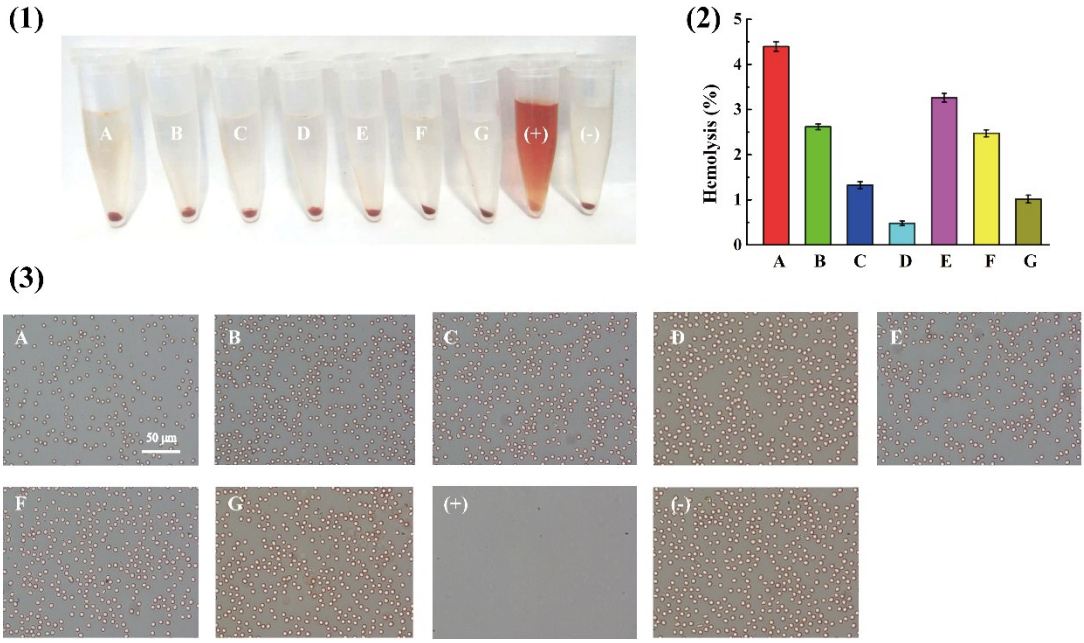

Figure S10. Photographs of hemolysis assay (1), hemolysis rate (2) and microscope images of RBCs in the presence of gene delivery materials and systems. (A) BCPs (5/1 ratio), (B) PP2-1, (C) PP2-2, (D) PP2-3, (E) TCPs-1, (F) TCPs-2 and (G) TCPs-3. Ultra-purified water (+) and PBS (-) mean positive and negative controls respectively. Data were shown as mean ± SD (n = 3).

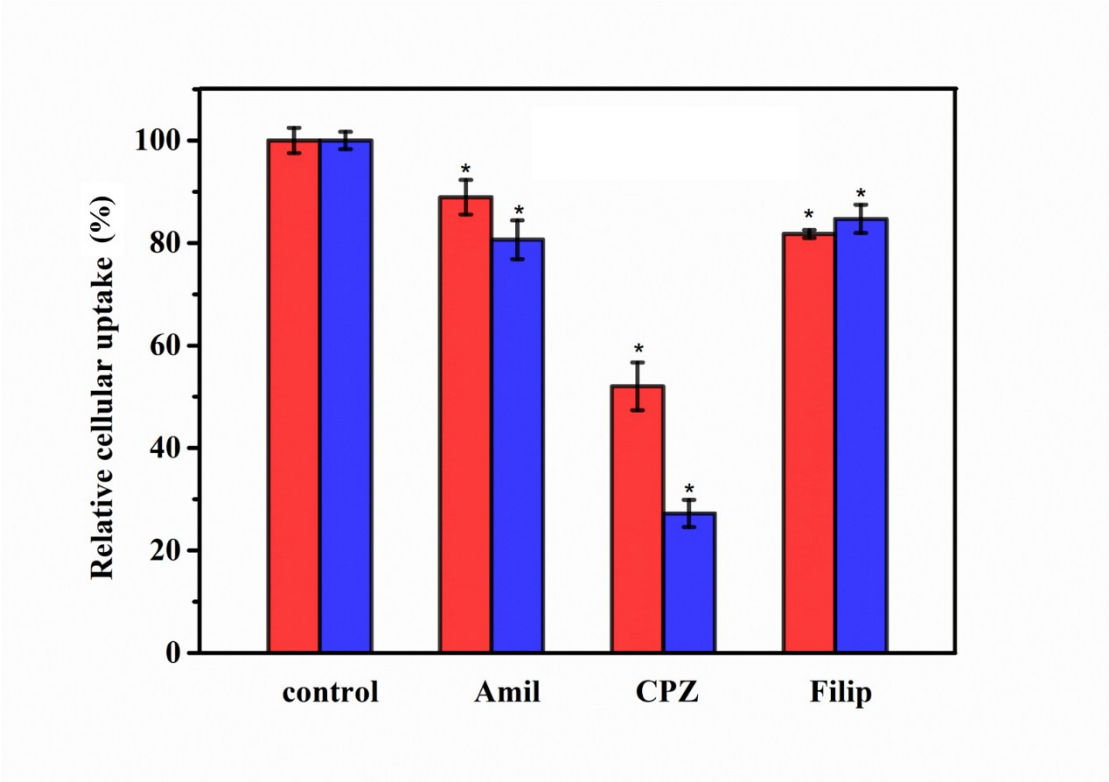

Figure S11. Quantitative influence of inhibitors on cellular uptake of BCPs (5/1 ratio) (red column) and TCPs-3 (blue column). (A) Cy5-oligonucleotide control group, (B) BCPs (5/1 ratio), (C) TCPs-1, (D) TCPs-2, (E) TCPs-3. (n = 3, mean ± SD, *p < 0.05, statistically significant difference between these two groups).

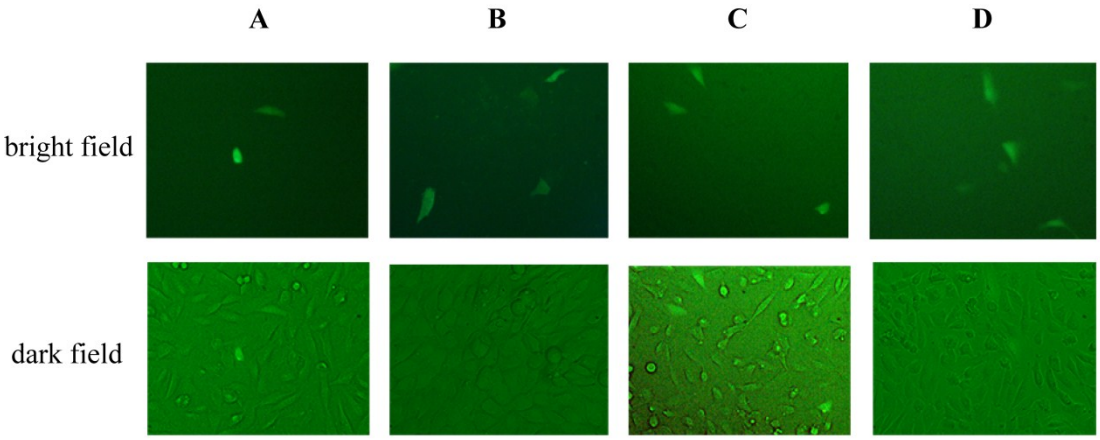

Figure S12. In vitro transfection image about GFP expression of HUVECs transfected for 24 h by (A) BCPs (5/1 ratio), (B)TCPs-1 (5/1/1.25 ratio), (C)TCPs-2 (5/1/1.25

ratio), (D) TCPs-3 (5/1/1.25 ratio).