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Electronic Supplementary Information

Multifunctional nanoparticles *via* host-guest interactions: a universal platform for targeted imaging and light-regulated gene delivery†

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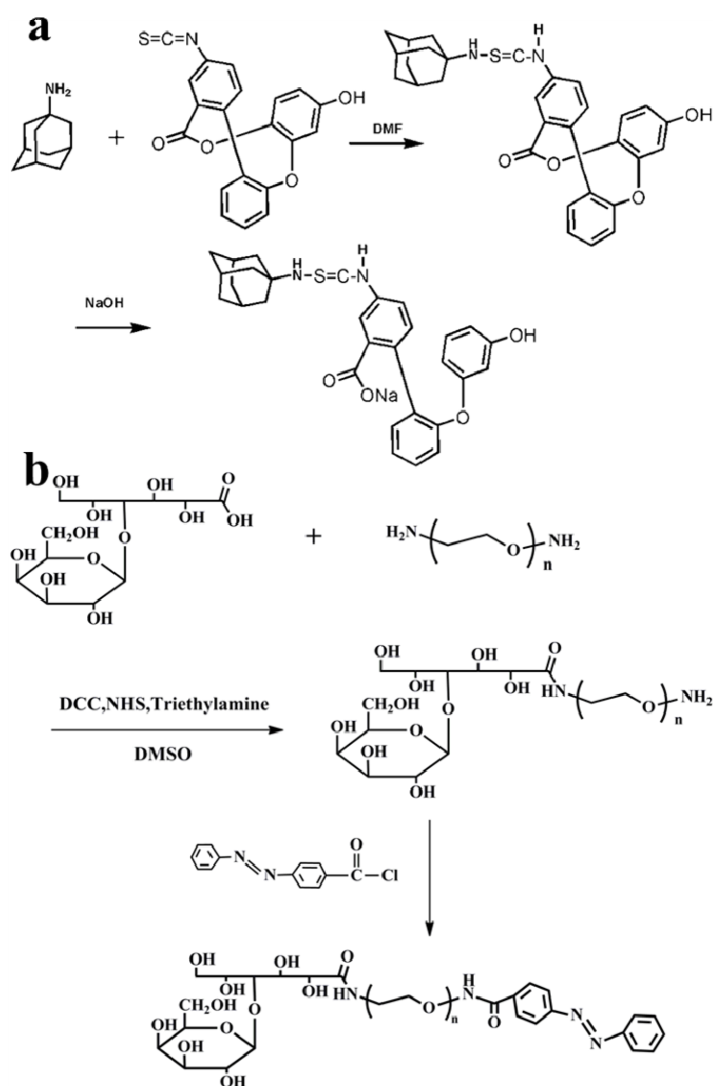
Materials

Branched polyethylenimine (PEI, 25kDa) was purchased from Sigma-Aldrich. 4-Phenylazbenzoyl chloride (AzCOCl) was obtained from Tokyo Chemical Industry (Shanghai, China) Development Co., Ltd. Polyoxyethylene bis(amine) (NH_2-PEG-NH_2, 4kDa), lactobionic acid (LA), fluorescein isothiocyanate (FITC), adamantanamine (AD-NH_2), N-hydroxy succinimide (NHS), and N, N'-dicyclohexylcarbodiimide (DCC) were purchased from Aladdin (Shanghai, China). Deoxyribonucleic acid (DNA, fish sperm, sodium salt) and N-[2-hydroxyethyl] piperazine-N' -[2-ehtanesulfonic acid] (HEPEs, free acid, high pure grade) were obtained from AMRESCO. Cy3-labelled DNA and heparin (>150 IU /mg) was purchased from Sangon Biotech (Shanghai, China). Plasmid DNA pGL-3 as transfection agent was kindly supported by the group of Professor Guping Tang in Zhejiang University. 4', 6-diamidino-2-phenylindole (DAPI) was obtained from J &K Chemical. 3-(4, 5-dimethylthiAzl-2-yl)-2, 5-diphenyltetrAzlium bromide (MTT) was purchased from Bio Basic Inc.

Synthesis and characterization of PEI-CD , AD-FITC and Az-PEG-Gal

PEI-CD: β-CD modified with branched polyethylenimine (PEI-CD) was prepared according to the previous study with some modifications.[1] Briefly, 6-deoxy-(p-toluenesulfonyl) -β-CD (6-OTs-β-CD) was firstly synthesized by reaction of 4-toluene sulfonyl chloride with β-CD. Then PEI-CD was prepared by reaction of 6-OTs-β-CD with the amines of PEI under 70℃ for 3 days and dialysis against water for 7 days.



Scheme S1 The synthetic progress of AD-FITC (a) and Az-PEG-Gal (b).

AD-FITC: The synthetic progress was shown in Scheme S1a. Briefly, Adamantanamine ($AD\text{-}NH_2$, 26 mg) and fluorescein isothiocyanate (FITC, 40 mg) were separately dissolved in 1 mL of dimethylformamide (DMF). Then sodium hydroxide (NaOH, 12 mg) dissolved in 1 mL DMF/H_2O (1:1 by volume) was poured into the above mixture and stirred for 24 h in the dark at room temperature. Next, the mixture was precipitated by 1 N HCl and the product was collected by centrifugation (12,000×g for 10 min). After, the product was rinsed by water until the supernatant had no absorbance of FITC detected by UV-vis spectrometer. After that, the product was dissolved in 0.1 M $NaHCO_3$/$NaCO_3$ buffer (pH=8.0) and the concentration of FITC and AD was inferred by UV spectra according to standard curve of FITC. Then the product was used without any purification.

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Az-PEG-Gal: The synthetic steps were shown in Scheme S1b. Firstly, NH_2-PEG-Gal was synthesized as the following steps: lactobionic acid (LA, 37 mg), N-hydroxy succinimide (NHS, 18 mg), and N, N'-dicyclohexylcarbodiimide (DCC, 31 mg) dissolved in 20 ml of anhydrous N, N-dimethylformamide (DMF) were added into a three-necked flask and stirred under nitrogen for 1 h. polyoxyethylene bis(amine) (NH_2-PEG-NH_2, 400 mg) and 70 μL of triethylamine dissolved in 5 mL of anhydrous DMF was added into the above mixture. The reaction mixture was stirred at room temperature for another 24 h. The resulting product was isolated by centrifugation to remove insoluble byproducts, purified by dialysis using a membrane (MWCO 2000 Da) for 3 days, and followed by freeze-drying. Then Az-PEG-Gal was synthesized according to the previous methods.[2] Briefly, NH_2-PEG-Gal (200 mg) was dissolved in 20 ml of anhydrous DMF. AzCOCl (120 mg) and excess amount of MgO were added into the above solution and stirred for 24 h under nitrogen environment at room temperature. Then the product was filtrated, precipitate from ether, washed repeatedly with ether and dialyzed for 4 days.

These products were analyzed by ^{1}H NMR (300MHz, Varian Spectrometer, USA) and Ultraviolet-visible spectrophotometer (UV-2550, Shimadzu, Japan). The spectra of ^{1}H NMR of PEI-CD and Az-PEG-Gal were shown in Figure S1. The CD-grafting level was calculated by the equation (1).

$$\textbf{CD-grafting level (\%)} = \frac{Ia/7}{Ib/4} * 100 \qquad \textbf{Equation (1)}$$

Herein, Ia and Ib represent integration area of peak a and b, respectively. The results indicated that the CD-grafting level was around 1.5%, which mean that every PEI chain had about 9 CD molecules. For Az-PEG-Gal, the proton peaks of benzene and LA appeared at 7.0-8.0 ppm and 4.5-4.6 ppm, which suggested that Az-PEG-Gal was successfully synthesized. UV spectrum (dissolved in H_2O) of the product showed that the absorption peak were at 325 nm, which was consistent with the previous report. [2]

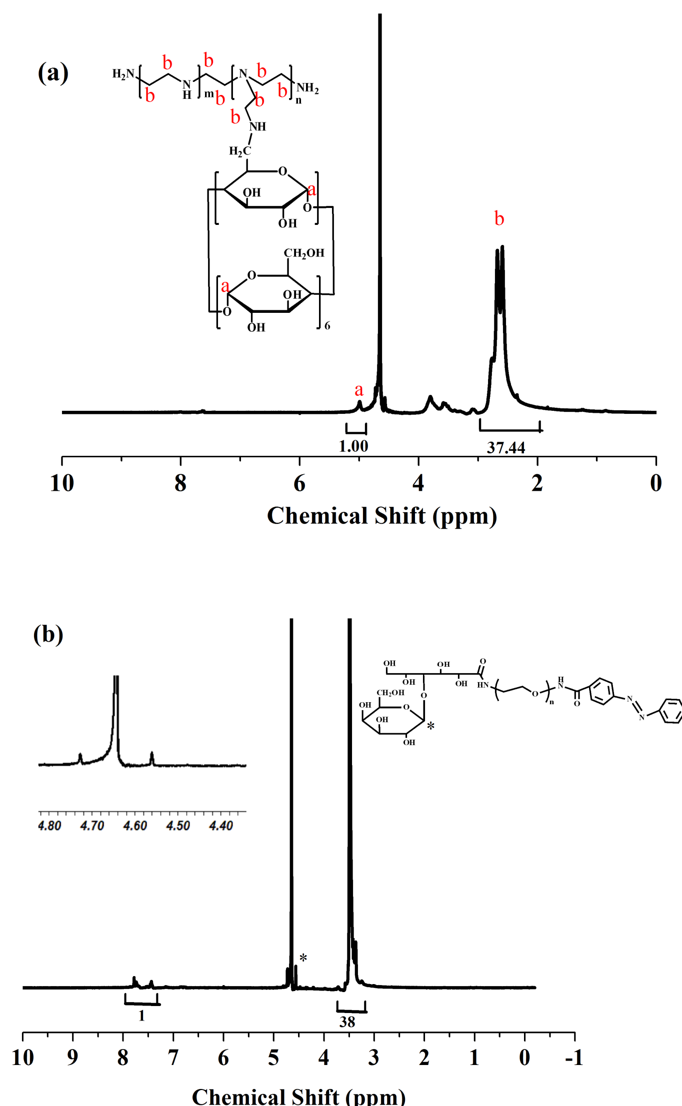

Figure S1 The ^{1}H NMR spectra of PEI-CD (a) and Az-PEG-Gal (b).

Formation and characterization of nanoparticles

Formulation of SP/DNA and SPG-Gal/DNA nanoparticles: AD-FITC (0.265mg/mL) was added into the PEI-CD (1mg/mL in 20 mM HEPEs buffer solution) solutions and with sonication for 15 min by the molar ratio (AD to CD on PEI-CD) of 1:3, formed supramolecular polymer AD-FITC/PEI-CD and designated as SP. Then SP/DNA nanoparticles were prepared by vortexing equal volume of SP solution with DNA solution (0.1 mg/ml in 20 mM HEPEs buffer solution) at the appropriate N/P ratio. SPG-Gal/DNA nanoparticles were prepared as follows: Az-PEG-Gal solution and SP solution were ultrasonically agitated for 30 min at various molar ratios (Az/RC) of Az to CD remaining on PEI-CD. Then the above mixture was added to the equal volume of DNA solution by

vortexing for 30 s. All the nanoparticles were prepared freshly and incubated for 30 min before analysis.

Particle size and zeta (ζ-) potential measurements: Particle size and zeta potentials of the nanoparticles of SP/DNA and SPG-Gal/ DNA nanoparticles at various Az/RC ratios were determined by Malvern Zetasizer (Malvern Inst. Ltd. UK) equipped with either a four-side clear cuvette for particle size analysis or ZET 5104 cell for zeta-potential measurement. For particle size analysis, the samples were carried out in 4 serial measurements at 173° angle and determined at 25℃. For samples treatment with light irradiation for 15 min, the samples were determined as above. As shown in Figure S2c and S2d, the particle size increased from around 70 nm to above 100 nm after light irradiation. Similarly, the ζ-potential changed from around 6 mV to 18 mV. These results suggested the PEG detachment caused by light irradiation.

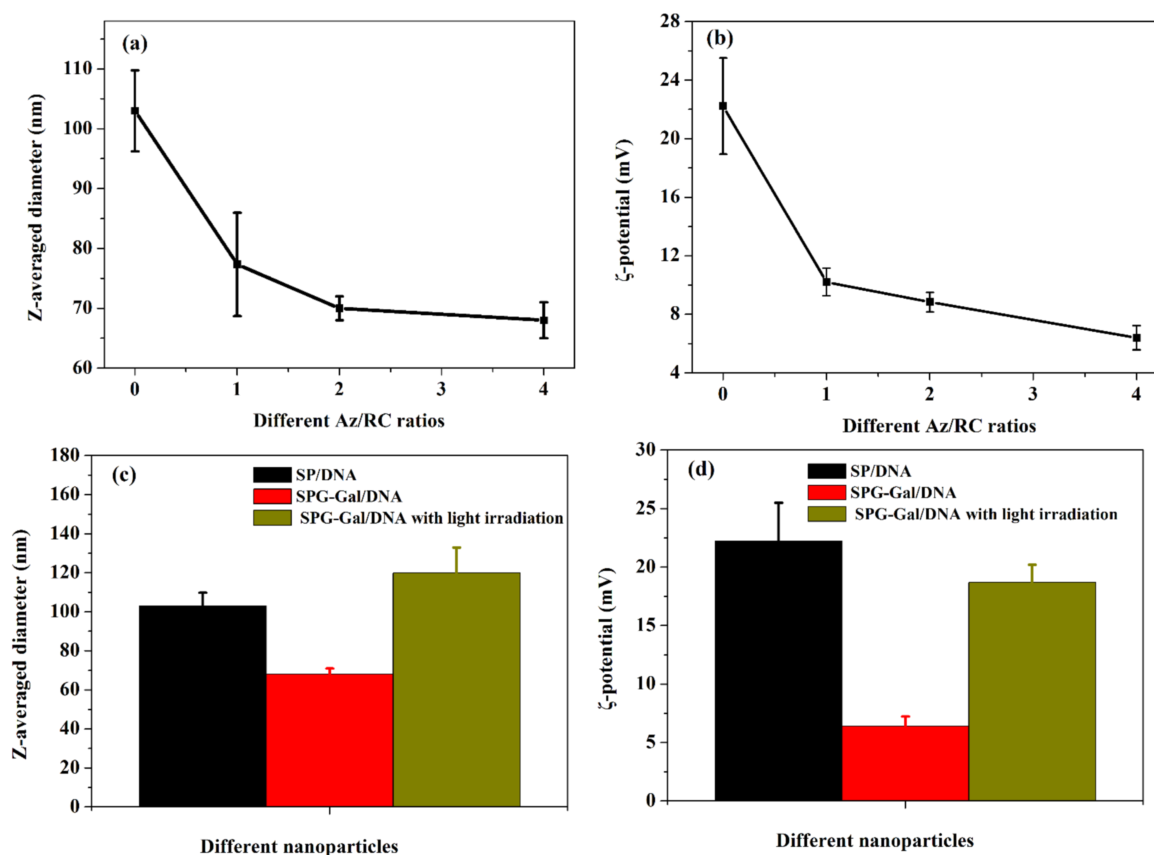

Figure S2 The particle sizes (a) and ζ-potentials (b) of SPG-Gal/DNA nanoparticles at N/P ratio of 10 with various Az/RC ratios. The particale size (c) and ζ-potential (d) changes after light irradiation for 15 min.

Gel retardation assay: The DNA condensation capability was examined by gel retardation assay. The nanoparticles containing 300 ng pDNA were prepared as above, mixed with

loading buffer (5:1 by volume) , loaded to each well of an agarose gel (1% by weight in 0.5× TBE buffer) and performed at 100 V for 50 min. Then the gel was immersed in ethidium bromide solution (0.5 μg/ml) for 30 min, observed by UV illuminator (Gel Doc, Bio-Rad, USA). The results were shown in Figure S2. Similar to covalent PEGylated polyplexes, the influence of PEG on DNA condensation mainly depended on the PEG-conjugation level. In our experiment, PEG-incorporation level was very small, so that little influence was observed.

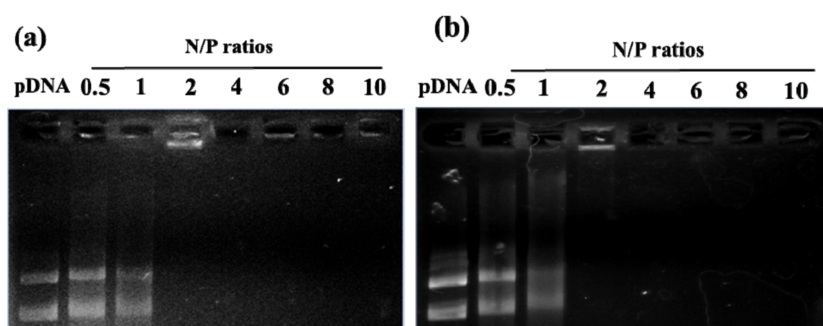

Figure S3 Agarose gel electrophoresis assay images of SP/pDNA and SPG-Gal/pDNA nanoparticles at various N/P ratios.

Transmission Electron Microscopy (TEM): TEM images were performed on transmission electron microscope (JEM-1200EX，NEC, Tokyo, Japan) operated at 80 kV. 20μL of the nanoparticles was deposited onto 200-mesh carbon-coated copper grid for 10 min. In order to obtain enough particles on the grid, the above processes were repeated three times.

The stability of SPG-Gal/DNA nanoparticles

Aggregation stability: The nanoparticles were prepared as mentioned above. Then the salt concentration was adjusted to 150 mM. For stability in DMEM with 10% FBS, 1 volume of nanoparticles were added to 3 volume of DMEM with 10% FBS. The nanoparticles were incubated for different time. The stability profile was detected by DLS and shown in Figure S4.

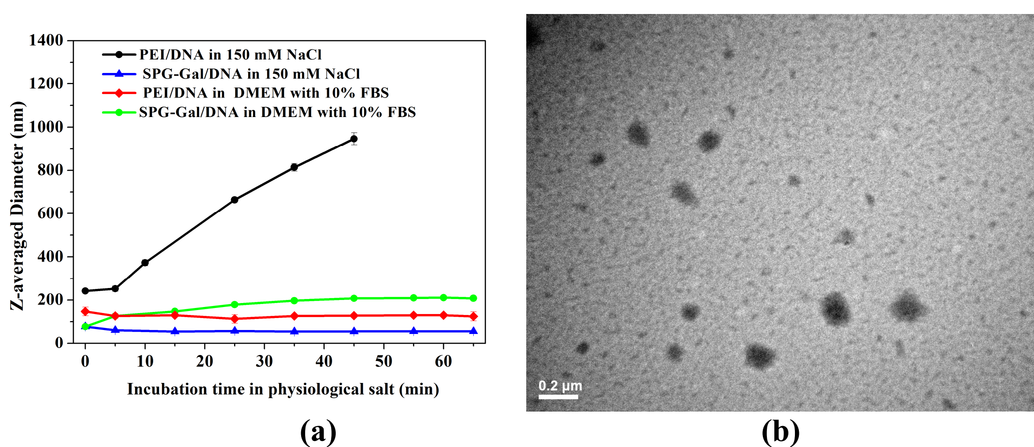

Figure S4 (a) The aggregation stability of different nanoparticles in physiological salt or culture media with 10% FBS detected by DLS (0 min means at prepared condition); (b) TEM image of SPG-Gal/DNA nanoparticles in physiological salt conditions for 2 h.

Competition stability assay **:** Competition stability was detected by gel retardation assay. The nanoparticles containing 300 ng pDNA were prepared as described above at N/P ratio of 10, then mixed with heparin at final concentration of 0, 10, 20, 30, 40, 60 and 80 μg/mL, respectively. The mixture was incubated for 20 min and then gel electrophoresis gel was performed as the above method.

The influence of light irradiation on DNA release ability: The DNA release ability of multifunctional nanoparticles after 15 min light irradiation was investigated. After mixing PEGylated polyplexes at N/P ratio of 10 and heparin, the mixture was exposed with 365 nm UV light for 15 min. The results were shown in Figure S5. Ethidium bromide (EtBr) is weakly fluorescent but exhibits strong fluorescence upon intercalation into the DNA duplex. So DNA bands would be revealed under UV illuminator when polyplexes become loose or dissociate to release free DNA. The result showed that after 15 min light irradiation, fluorescent intensity of DNA bands was improved in the agarose gel chamber. It indicated that the tight complexes changed to loose structure after light irradiation.

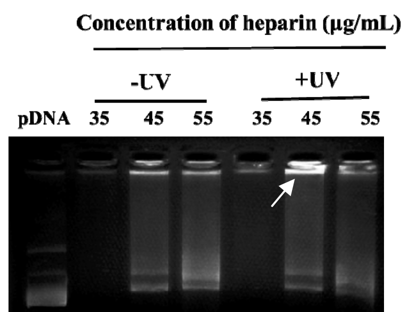

Figure S5 Agarose gel electrophoresis of SPG-Gal/pDNA in the presence of heparin with and without 365 nm UV light irradiation.

Cell culture experiment

HepG2 cells (Human Hepatoblastoma cell line) were cultured in DMEM (Dulbecco's modified Eagle medium) with 10% FBS and 1% penicillin-streptomycin. The cells were maintained under humidified air containing 5% CO_2 at 37 °C.

Cell cytotoxicity assay : HepG2 cells were seeded into 96-well plates at a density of 1×10^4 cells/well. The cells were cultured for 24 h. The medium was replaced with 100 μl of fresh medium. Different nanoparticles containing 1 μg DNA were added into the cells. After incubation for 4.5 h, cells were exposed to 365 nm light for 15 min. Followed by incubation for another 43.5 h, 20 μl of MTT (5 mg/ml, dissolved in PBS) and 100 μl of fresh DMEM were added. The cells were incubated for 4 h at 37 °C. Then the medium was removed, 200 μl of DMSO was added and incubated for additional 15 min at 37 °C. The absorbance of 100 μl of the above mixture at 570 nm was measured by microplate reader (550, Bio-Rad, USA). All experiments were performed in quintuplicate. The results were shown in Figure S5.

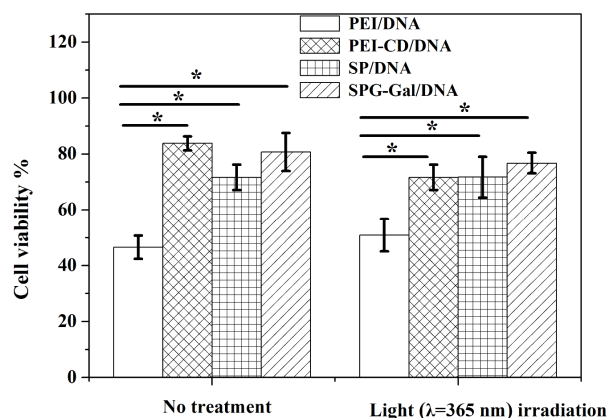

Figure S6 Cell cytotoxicity of HepG2 cells exposed to different nanoparticles for 48 h. * denotes statistically significant difference at $p < 0.05$.

The cellular uptake efficiency: The cellular uptake efficiency of nanoparticles was performed in HepG2 cells. The cells were seeded into 24-well plates at a density of 1×10^5 cells/well and incubated for additional 24 h. Before the nanoparticles addition, the medium was replaced with 0.5 ml of fresh media. SP/DNA, SPG/DNA, and SPG-Gal/DNA nanoparticles containing 2 μg DNA were prepared as described above. The nanoparticles were added and incubated for 0.5 h or 4.5 h. Then the cells were washed three times with PBS in order to detach surface-associated nanoparticles, trypsinized and analyzed by flow cytometry. All experiments were performed in triplicate.

Bioimaging and intracellular trafficking of multifunctional nanoparticles: HepG2 cells with initial density of 5×10^4 cells/dish in glass base dishes and incubated overnight in 0.5 mL of medium. Before experiment, the media were replaced with 0.5 mL of fresh media and different nanoparticles containing 2 μg Cy3-labelled DNA at N/P ratio of 10 were added and incubated for 4.5 h. Then, the cells were exposed to 365 nm light for 15 min, washed three times with PBS and incubated for another 12 h. After that, the cells were fixed with 4% (w/v) paraformaldehyde for 30 min and stained with DAPI (2.5μg/ml) for 20 min. The bioimaging and intracellular trafficking of multifunctional nanoparticles was analysed by confocal laser scanning microscope using a 63× objective. (CLSM, Leica TSSP5, Germany).

***In vitro transfection efficiency*：** HepG2 cells were seeded in 24-well plates at a density of 1×10^5 cells/well and cultured for 24 h in 0.5 ml of medium. Different nanoparticles containing 2 μg pGL-3 were added and incubated for 4.5 h. Then one group cells treated with SPG-Gal/pGL-3 nanoparticles were exposed to 365 nm light, with the lamp approximately 15 cm

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from the cells. Then the media were replaced with 500 μl of fresh medium. Luciferase expression was quantified 48 h later using a Promega luciferase assay system. Luciferase activity was measured in relative light units (RLU) using luminometer. Results were normalized to total cell protein as determined using a KEYGEN BCA protein assay. All transfection experiments were performed in triplicate.

Statistical analysis: Data are expressed as mean ± standard deviation (SD). Statistical analysis was performed by ANOVA. The significant level was set as $p < 0.05$.

References

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