Supplementary Information

Precise and controllable synthesis of ultra-stable gold nanoparticles based on polymer templates for miRNA detection

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Experimental

Materials

Polyamidoamine (PAMAM, in methanol, Sigma-Aldrich), poly (ethylene oxide) methyl ether (mPEO, Ponsure Biological, Mn = 5000), Tertbutylamine-borane complex (TBAB, Sigma-Aldrich, 97%), L-Glutamic acid-α-benzyl ester (H-Glu(OBzl)-OH, GL Biochem, 98%), were used as received. Other agents, unless otherwise specified, were purchased from Energy Chemical. DNA was purchased from Sangon Biotech.

Instruments

The ^{1}H NMR spectra was recorded on Bruker 500 MHz spectrometer (AVANCE NEO 500). Gel permeation chromatography (GPC) analysis was performed on an Agilent 1260 infinity with a Mix-B 300×7.5 mm column (flow 1 mL/min, eluent: DMF), and the number-average molecular weight (Mn), polymer dispersity index (PDI), determined by GPC, calibrated by PS standard. The particle size of the complexes were measured by Malvern Zetasizer Nano ZS90 (Malvern, Britain). Transmission electron microscopy (TEM) images were observed on a transmission electron microscope (Thermo Fisher Scientific, Tecnai G2 SpiritBiotwin). Scanning electron microscopy (SEM) images were observed on a Field emision Scanning Electron Microscopy (Zeiss GeminiSEM 500). The spherical nucleic acid probes uptake was examined by confocal microscope (CLSM) (Leica STELLARIS 5).

Synthesis of Monomer BLG-NCA

Under magnetic stirring, add H-Glu(OBzl)-OH (1.0 eq), THF (150 mL), and epichlorohydrin (4.0 eq) successively. Finally, add triphosgene (0.5 eq) in one portion. Stir the reaction at room temperature for about 2 hours, then add 70 mL of cold water in an ice bath and stir for 2 minutes to quench the excess triphosgene. Extract the mixture at room temperature with ethyl acetate (EA, 50 mL × 2). Wash the combined organic phase with brine, and dry over anhydrous Na_2SO_4. Afterward, recrystallize the product three times using THF and n-hexane to obtain the monomer BLG-NCA.

Synthesis of Gold Nanoparticles Using Polymer Template

PAMAM was used to initiate the polymerization of BLG-NCA, the monomer concentration was controlled at 0.05 M, and the ratio of monomer to initiator was controlled according to the desired degree of polymerization. BLG-NCA was dissolved in DCM, PAMAM was added under rapid stirring, and after 5 hours of reaction, triethylamine was added and BIBB (2 times molar ratio) was directly added dropwise under ice bath conditions for bromination, and the reaction was carried out at 0 °C for 2 hours and at room temperature for 22 hours. After the reaction, the dichloromethane was spun dry, and after DMF was redissolved, cold methanol water and ether were used for precipitation three times in sequence.

The brominated polymer was dissolved in DMF, sodium azide (10 times molar ratio) was added, and after 24 hours

of reaction at room temperature, it was precipitated once with cold methanol water and once with ether.

The azide-modified polymer was dissolved in DMF, and alkynyl PEG (1.2 times molar ratio), $CuSO_4 \cdot 5H_2O$ (2 times molar ratio), and ascorbic acid (2 times molar ratio) were added. After nitrogen bubbling for 15 minutes to remove oxygen, the mixture was reacted at 40 °C for 24 hours, dialyzed using a 10000 Da dialysis bag, and freeze-dried.

The PEG-modified polymer was dissolved in TFA, and 1/3 volume of HBr was added. After reacting at room temperature for 4 hours, deionized water was added to precipitate the product, and the product was repeatedly rinsed with deionized water for 3 times to obtain a polymer template.

10 mg of the polymer was dissolved in 10 mL of solvent (DMF : Benzyl alcohol = 8:2), and the precursor chloroauric acid (5 times molar ratio, relative to the carboxyl group) was added. For example, we used the G_2-100 template to prepare gold nanoparticles. We used 10 mg of polymer, 100 mg of chloroauric acid trihydrate. The mixture was stirred at 50 °C for more than 5 days, and 44 mg Tetrabutylammonium borohydride (TBAB) was added to reduce the chloroauric acid to prepare gold nanoparticles.

Synthesis of Gold Nanoparticles by Sodium Citrate Method

All glassware were rinsed with aqua regia (HCl : HNO_3 = 3:1) and deionized water in turn before use, and then dried. Prepare 50 mL of 0.01% chloroauric acid solution with deionized water, heat to 110 °C until boiling, and then quickly add 1 mL of 5% sodium citrate solution. Reflux reaction, wait until the color of the solution changes from light yellow to wine red, then stop heating, heat at residual temperature for 5 minutes, and naturally cool to room temperature, and store in the dark.

Synthesis of spherical nucleic acid probes

SH-DNA: SH-TTTTTCCAATCAACATCAGTCTGATAAGCTA

FAM-DNA: TATCAGACTGATGTTGATTGG-FAM

miRNA21: TAGCTTATCAGACTGATGTTGA

Place SH-DNA (3 nmol) and FAM-DNA (3 nmol) in a 200 μL centrifuge tube, dilute with Tris buffer to a concentration of 40 μM, and perform PCR at 95 °C for 5 minutes. Then, slowly cool down to room temperature to obtain double-stranded DNA.

Reduce the double-stranded DNA using TCEP at room temperature for 1 hour, then incubate it with gold nanoparticles. After reacting in the dark for 24 hours, centrifuge at 8000 rpm to remove the supernatant, and finally resuspend in ultrapure water. Add mercaptoethanol to the resuspended gold nanoparticle solution, react for 24 hours, then centrifuge and measure the fluorescence intensity in the supernatant.

Response of the Au-DNA1/DNA2 Nanoprobe to miRNA21

Incubate the Au-DNA1/DNA2 nanoprobe (1 nM) with different concentrations (0, 2.5, 5, 10, 20, 40 nM) of miRNA21 at 37 °C for 45 minutes, collect the fluorescence signal intensity of the nanoprobe at each concentration, and plot the fluorescence intensity change curve using the lowest effective concentration (10 nM).

Confocal testing of spherical nucleic acid probes in cells

Dilute the Au-DNA1/DNA2 nanoprobe in the culture medium to a final concentration of 1 nM, and incubate with HeLa (high miRNA21 expression) and CHO (low miRNA21 expression) cells for 6 hours. After incubation, wash the cells three times with PBS (pH = 7.4) to remove any remaining nanoprobe and reduce background fluorescence interference. Place the confocal culture dish on a laser scanning confocal microscope, select a 488 nm wavelength as the excitation wavelength, and measure the fluorescence intensity of FAM, which represents the expression of miRNA21 in HeLa and CHO cells.

Supporting Figures

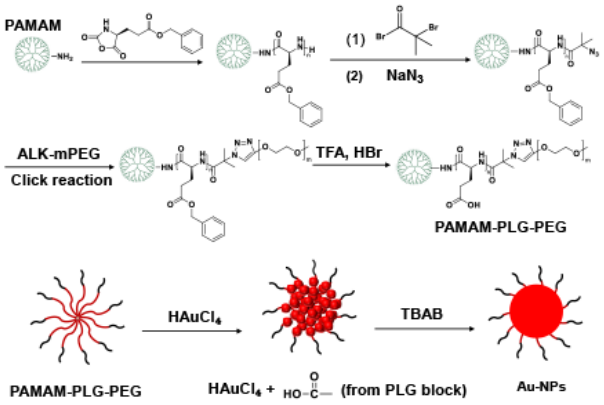

Fig. S1 The reaction of preparing gold nanoparticles by polymer template method.

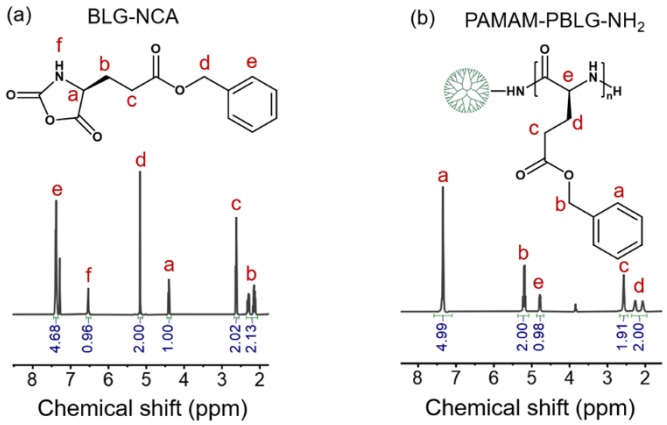

Fig. S2 ^{1}H NMR spectrum of monomer BLG-NCA in $CDCl_3$ and polymer PAMAM-PBLG in CF_3COOD.

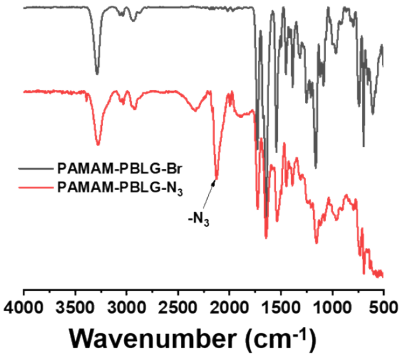

Fig. S3 FT-IR spectrum of polymer before and after terminal azide modification.

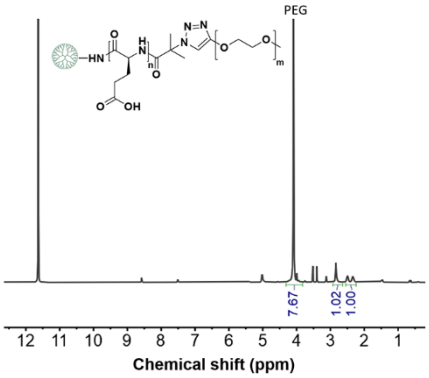

Fig. S4 ^{1}H NMR spectrum of PAMAM-PLG-PEG in CF_3COOD.

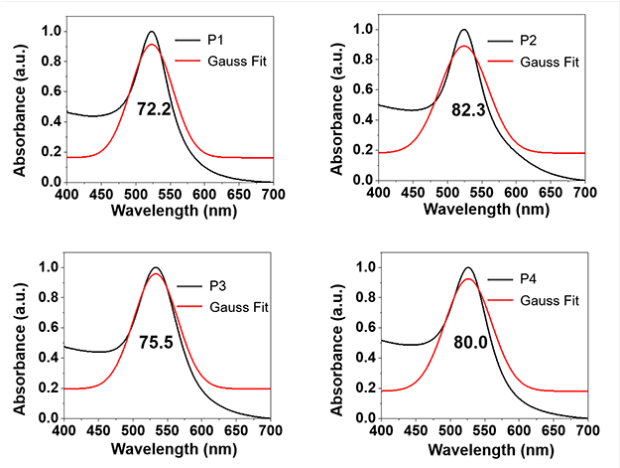

Fig. S5 The full width at half maximum (FWHM) of the absorption peak in the UV-Vis spectrum of gold nanoparticles prepared by the polymer template method.

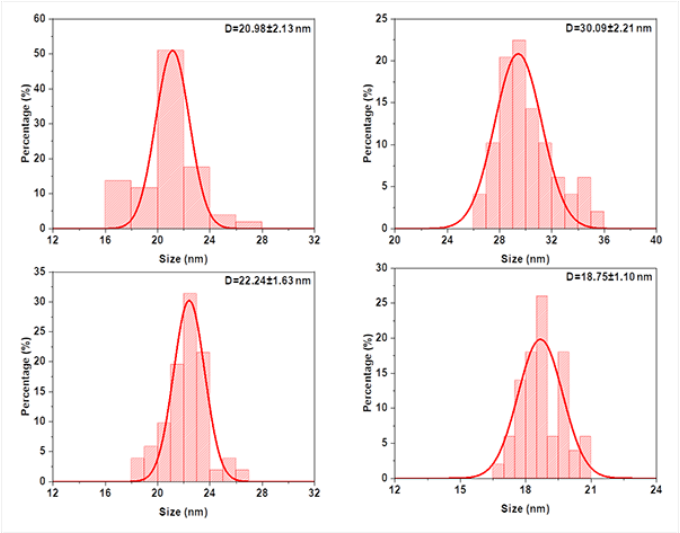

Fig. S6 The histogram of the average size distribution of gold nanoparticles prepared by the polymer template method. (a) P1: 20.98±2.13 nm, (b) P2: 30.09±2.21 nm, (c) P3: 22.24±1.63 nm, (d) P4: 18.75±1.10 nm

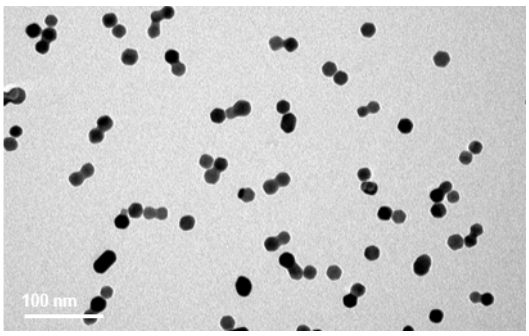

Fig. S7 TEM image of gold nanoparticles prepared by P4.

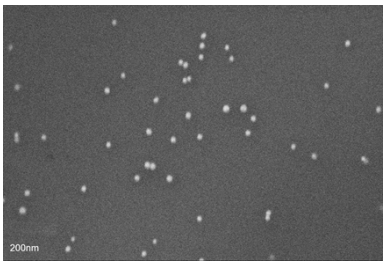

Fig. S8 SEM image of gold nanoparticles prepared by sodium citrate method.

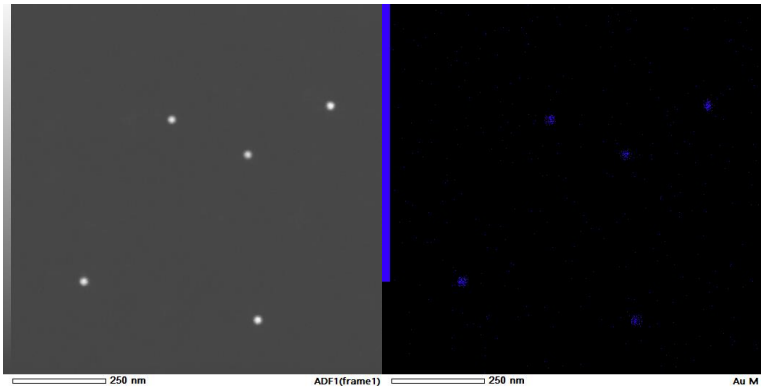

Fig. S9 Gold nanoparticles prepared by polymer template method and elemental analysis.

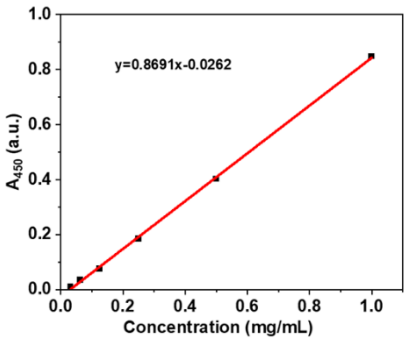

Fig. S10 The relationship between the absorbance of gold nanoparticles prepared by the polymer template method at 450 nm and concentration (mg/mL).

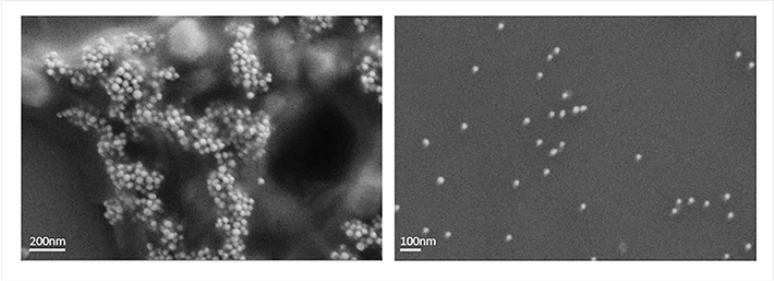

Fig. S11 SEM images of gold nanoparticles after long-term storage, citric acid method (left), polymer template method (right).

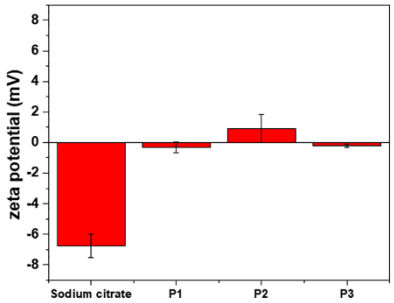

Fig. S12 Zeta potential of gold nanoparticles prepared by polymer template method and citric acid method.

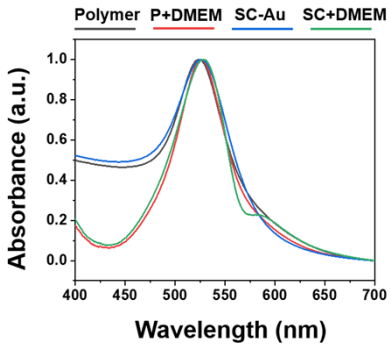

Fig. S13 UV-Vis spectra before and after DMEM added to demonstrate their suitability in biological systems.