Supporting Information (SI)

Coating Carbon Nanosphere with Patchy Gold for Production of Highly Efficient

Photothermal Agent

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1. Experimental section

1.1 Preparation of patchy gold on Silica nanospheres.

Preparation of silica nanospheres (NPs): Silica NPs were synthesized by the Stöber method. Briefly, 15 mL of ethanol and 5 mL of water were put in a 250 mL flask, followed by adding 0.6 mL of ammonia (28% w/w). After adding 0.6 mL of tetraethyloxysilane (TEOS), the mixture was left under stirring for 10 h at room temperature. After the reaction, silica NPs were collected and washed with water by centrifugation. Finally, the resulting particles were dispersed in ethanol with the concentration of $1 \text{ mg} \cdot \text{mL}^{-1}$.

Surface modification of silica NPs: To 5 mL of the above ethanol solution with $1 \text{ mg} \cdot \text{mL}^{-1}$ of silica NPs, 100 μL of 3-aminopropyltriethoxysilane (APTS) was added. Then, the resulting reactant mixture was stirred for 24 h at room temperature. Finally, the resulting APTS-coated silica NPs were separated from the mixture by centrifugation, washed with ethanol for two times, and dispersed in water with the concentration of $1 \text{ mg} \cdot \text{mL}^{-1}$. In order to facilitate the linkage of branched polyethylenimine (PEI, MW=600), glutaraldehyde, as an amine-reactive homobifunctional crosslinker, was further covalently attached to the surface of APTS-coated silica NPs through the formation of Schiff base. Firstly, 5 mL of APTS-coated silica NPs aqueous solution was mixed with 200 μL 25% (wt) glutaraldehyde under stirring. After incubation at 37 °C for 2 h, the solution was washed three times with carbonated buffer solution (pH=8) and resuspended in 5 mL of carbonated buffer solution. Next, 200 μL of 30% (wt) PEI/water solution was

added into the above solution under stirring. After incubation at 37 °C for 2 h, these PEI-coated silica NPs were washed with water by centrifugation for three times. Finally, the resulting particles were dispersed in water with the concentration of 1 mg·mL⁻¹.

Preparation of patchy gold on silica sphere: The procedure to prepare both patchy gold on silica NPs and silica NP@Au nanoclusters were similar to the way used in the preparation of PG/CNSs and CNS@Au nanoclusters by replacing PEI-modified CNSs with PEI-modified silica NPs.

1.2 Selective functionalization of PG/CNSs.

The surface of carbon domain can be firstly tagged with FITC by the reaction of amine group from PEI and the isothiocyanate group from FITC. Considering the fact that just like other carbon-based material CNSs are also capable of quenching of fluorescence, the linker HOOC-PEG-COOH (MW=20 kDa) with long chain was used to connect the PEI group with FITC. Firstly, 0.1 g of HOOC-PEG-COOH was dissolved in 2 mL of MES buffer (pH =6.0). To the above solution, 1 mg of EDC and 1 mg of NHS were added separately under room temperature, and the activation reaction lasted for 20 min. Then, 0.2 mL of the obtained PG/CNSs was added into the above solution, and the reaction was kept for 24 h under 4 °C. Finally, the resulting PG/CNSs were collected and washed with water by centrifugation. After functionalization with HOOC-PEG-COOH, the resulting PG/CNSs-PEI-PEG-COOH was grafted with PEI (MW=600 Da) again with the help of EDC activation. Next, FITC was grafted to the surface of PG/CNSs-PEI-PEG-PEI by direct dissolution of

FITC (20 μL of 1 $\text{mg}\cdot\text{mL}^{-1}$ FITC/DMSO) into resulting PG/CNSs-PEI-PEG-PEI aqueous solution under stirring at the room temperature according to the literature.¹ Free FITC was washed away with water by centrifugation.

The selective functionalization of the Au domain can be then achieved by incubating an aqueous solution of resulting NPs with thiol modified 54-mers MUC1 aptamer based on the method reported by Mirkin and co-workers with some modifications.² The sequences of 54-mers MUC1 aptamer was 5' -CACTACAGAGGTTGCGTCTGTCCACGTTGTCATGGGGGGTTGGCCTGTT TTTT-SH-3'. Firstly, HS-MUC1 aptamer (final concentration is 5.0 μM) and PG/CNSs-FITC solution (final concentration is 0.1 $\text{mg}\cdot\text{mL}^{-1}$) were mixed together, and allowed to stand for 24 h under 4 $^{\circ}\text{C}$. After that, excess reagents were removed by centrifuging at 12 000 rpm for 20 min. The precipitate was then washed using 0.1 M PBS and re-centrifuged twice. Finally, the modified NPs were dispersed in a 400 μL solution (10 mM PBS) for the following experiments.

Figure S1. TEM image (a) and the corresponding size distribution of CNSs (b).

Figure S2. FI-IR spectra of CNS and CNS-PEI

As shown in Figure.S2, for CNS, the bands at 3420 cm^{-1} can be assigned to the stretching vibration of O-H bond, while the band at 1708 cm^{-1} is due to the stretching vibration of C=O bond. All of those bands reveal that there are large numbers of functional groups such as -OH and C=O on the surface of CNS.

Figure S3. TEM images of PG/CNSs obtained from CNSs modified with various MW PEI: (a) 600 Da; (b) 10 KDa; (c) 25 KDa; (d) the product obtained from CNSs modified with 1,6-Diaminohexane.

Figure S4. UV-vis-NIR absorbance spectra of aqueous solution containing various samples obtained from CNSs modified with various MW PEI or 1,6-Diaminohexane.

Figure S5. Large-scale (a) and enlarged (b) TEM images of patchy gold on silica NPs; TEM image of the product (silica NP@Au nanoclusters) obtained by using NaBH_4 as reductant (c); their corresponding UV-vis-NIR absorbance spectra (d).

The calculation of photothermal conversion efficiency

According to the literatures^{3,4}, we have calculated the photothermal conversion efficiencies of both PG/CNSs and Au NRs using the following equations:

$$\eta = \frac{hS(T_{\max} - T_{\text{surr}}) - Q_{\text{dis}}}{I(1 - 10^{-A_{808}})}$$

Where h is heat transfer coefficient, S is the surface area of the container, and the value of hS is obtained from the following Figures S6 and S7. The $T_{\max}$ is the equilibrium temperature, T_{surr} is ambient temperature of the surroundings, and $(T_{\max} - T_{\text{surr}})$ can be calculated according to Figure S6 and S7. The Q_{dis} expresses heat dissipated from light absorbed by the quartz sample cell itself, and it was measured independently to be 0.102 W/°C using a quartz cuvette cell containing pure water without a nanoparticles. I is incident laser power (5 W/cm²), A_{808} is the absorbance of particles at 808 nm. Thus, the 808 nm laser heat conversion efficiencies of PG/CNSs and Au NRs are 31.6% and 23.1%, separately.

Figure S6. (a) Photothermal effect of the irradiation of the aqueous dispersion of Au NRs with the NIR laser (808 nm, 5 W/cm²), in which the irradiation lasted for 9 min, and then the laser was shut off. (b) Time constant for heat transfer from the system is determined to be $\tau_s = 309.41$ s by applying the linear time data from the cooling period versus negative natural logarithm of driving force temperature. (c) Absorption spectrum of the aqueous dispersion of Au NRs.

Figure S7. Photothermal effect of the irradiation of the aqueous dispersion of PG/CNSs with the NIR laser (808 nm, 5 W/cm²), in which the irradiation lasted for 9 min, and then the laser was shut off. (b) Time constant for heat transfer from the system is determined to be $\tau_s = 273.69$ s by applying the linear time data from the cooling period versus negative natural logarithm of driving force temperature. (c) Absorption spectrum of the aqueous dispersion of PG/CNSs.

Scheme S1. Schematic illustration of the surface modification of PG/CNS with mPEG-COOH.

Figure S8. FI-IR spectra (a) and zeta potential (b) of PG/CNSs and PG/CNSs-PEG.

Figure S9. Dynamic light scattering (DLS) of PG/CNSs-PEG.

Scheme S2. Schematic illustration of the surface modification of PG/CNSs with FITC and MUC1 aptamer separately.

Figure S10. Photos of breast cancer-bearing mice at different time after the treatments.

PG/CNSs-PEG group (a); Laser (injected with saline) (b); Laser + PG/CNSs-PEG group (injected with PG/CNSs-PEG) (c) during 3 min irradiation of $2.5 \text{ W} \cdot \text{cm}^{-2}$ NIR every day.

Reference:

- (1) Martini, M.; Perriat, P.; Montagna, M.; Pansu, R.; Julien, C.; Tillement O.; Roux, S. How Gold Particles Suppress Concentration Quenching of Fluorophores Encapsulated in Silica Beads. *J. Phys. Chem. C*. **2009**, *113*, 17669-17677.
- (2) Taton, T. A.; Mirkin ; Letsinger, R. L. Scanometric DNA Array Detection with

- Nanoparticle Probes. *Science* **2000**, *289*, 1757–1760.
- (3) Chen, H. J.; Shao, L.; Ming, T.; Sun, Z. H.; Zhao, C. M.; Yang, B. C.; Wang, J. F. Understanding the Photothermal Conversion Efficiency of Gold Nanocrystals. *Small* **2010**, *6*, 2272-2280.
- (4) Tian Q. W.; Jiang, F. R.; Zou, R. J.; Liu, Q.; Chen, Z. G.; Zhu, M. F., Yang, S. P.; Wang, J. L.; Wang, J. H.; Hu, J. Q. Hydrophilic Cu₉S₅ Nanocrystals: A Photothermal Agent with a 25.7% Heat Conversion Efficiency for Photothermal Ablation of Cancer Cells in Vivo. *ACS Nano* **2011**, *5*, 9761-9771.