Electronic Supplementary Information (ESI)

One-pot synthesized Cu/Au/Pt trimetallic nanoparticles with enhanced catalytic and plasmonic properties as a universal platform for biosensing and cancer theranostics

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Experimental Section

Materials and Chemicals. DNA probes were custom-designed and synthesized by Sangon Biotech. Co., Ltd. The sequence of 3'-thiol aptamer Sgc8c (Sgc8c-SH) is 5'-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-SH-3'. The sequence of 3'-thiol DNA library (Lib-SH) is 5'-$(NNN)_{13}$NN-SH-3'. Glucose oxidase (GOD, from Aspergillus niger), K_2PtCl_4 (>99.9%), Calcein-AM, Propidium iodide (PI), sodium citrate and Bovine serum albumin (BSA) were purchased from Sigma-Aldrich Chemicals. $HAuCl_4 \cdot H_2O$ (>99.5%), $CuSO_4 \cdot 5H_2O$, 3,3,5,5-tetramethylbenzidine (TMB) and $NaBH_4$ were gained from Ding Guo Biotech Co., Ltd. (Beijing, China). Glucose, R-lactose, D-fructose and maltose were obtained from Aladdin Industrial Inc. (Shanghai, China). Yeast tRNA was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Binding buffer was prepared by mixing 0.1 mg/mL yeast tRNA and 1 mg/mL BSA with the D-PBS solution containing 5 mM $MgCl_2$ and 4.5 g/L glucose.

Preparation and functionalization of Cu/Au/Pt TMNPs. Generally, $CuSO_4$ solution (0.36 mM) was added with 25 μL of sodium citrate (0.1 M) and 10 mL of water. Afterward, the system was rapidly mixed with 500 μL of freshly prepared $NaBH_4$ solution (1.9 mg in 2 mL of H_2O). About 15 min later, the mixed solution was added with the mixture of $HAuCl_4$ (0.24 mM) and K_2PtCl_4 (0.24 mM) and continuously stirred for 20 minutes. Finally, the ultimate Cu/Au/Pt TMNPs was kept at 25 °C for storage and further application. Specified for condition optimization experiments, Cu/Au/Pt TMNPs were prepared with various concentrations of $CuSO_4$ and $HAuCl_4$.

To obtain cancer targeting function, aptamers were assembled on the surface of Cu/Au/Pt TMNPs by the “Au-S” interaction. Cu/Au/Pt TMNPs were mixed with 1 μM thiolated DNA probes and 1 μM BSA, and incubated for 12 h. Then, DNA-modified Cu/Au/Pt TMNPs were purified through centrifugation at 12,000 rpm for 5 min.

Characterization of Cu/Au/Pt TMNPs. UV-vis spectrum analysis was performed using a Beckman Counter DU800 spectrophotometer. High Response Transmission Electron Microscopy (HRTEM) was carried out on a JEOL-3010 microscope working at a 200 kV accelerating voltage. All of the TEM samples were prepared by depositing on carbon-covered copper grids and then dried overnight before determination. The elements of the TMNPs were analyzed using energy-dispersive spectroscopy (EDS, Titan G2 60-300) at a 300 kV accelerating voltage through depositing nanoparticles solution on Mo-

supported film. Size distribution of the TMNPs was analyzed by dynamic light scattering (DLS, Malvern Zetasizer 3000 HS).

Heating ability of Cu/Au/Pt TMNPs was determined using a 780 nm laser as the irradiation source at 0.54 W/cm^2 for 5 min. Catalytic activity of Cu/Au/Pt TMNPs was tested as follows: 50 μL of native Cu/Au/Pt TMNPs solution was mixed with 25 μL of H_2O_2 (0.5 mM), 50 μL of H_2SO_4 (0.5 M), 50 μL of TMB (1.7 μM) and 75 μL of H_2O, and then incubated for 10 min in a 45 °C water bath. Subsequently, the absorbance at 450 nm of TMB^+ was recorded by a multifunctional micro-plate reader (Infinite M1000) through adding 150 μL of the final reaction solution into a 96-well plate.

Detection of H_2O_2 and glucose based on Cu/Au/Pt TMNPs with high catalytic activity. For H_2O_2 detection: 50 μL of Cu/Au/Pt TMNPs (0.05 mg mL^{-1}), 50 μL of H_2SO_4 (0.05 M) and 50 μL of TMB (1.7 μM) were added with 100 μL of various concentrations of H_2O_2, and then incubated for 10 min in a 55 °C water bath. Subsequently, photographs were immediately taken, and the spectra or the absorbance at 450 nm were recorded by a multifunctional micro-plate reader (Infinite M1000) through adding 150 μL of the final reaction solution into a 96-well plate.

For glucose sensing: 45 μL of glucose with different concentrations in 10 mM phosphate buffer solution (pH 7.4) was mixed with 5 μL of 5.0 mg/mL GOD, and then incubated for 15 min in a 37 °C water bath. Next, 50 μL of Cu/Au/Pt TMNPs (0.05 mg mL^{-1}), 50 μL of H_2SO_4 (0.05 M), 50 μL of TMB (1.7 μM) and 50 μL of H_2O solution were added to the above reaction solution. After incubation for 10 min at 55 °C, the absorbance at 450 nm of TMB^+ was recorded by a multifunctional micro-plate reader (Infinite M1000) through adding 150 μL of the final reaction solution into a 96-well plate.

Cells. Ramos cells (CRL-1596, Blymphocyte, human Burkitt's lymphoma) and CCRF-CEM cells (CCL-119, T lymphoblast, human acute lymphoblastic leukemia) were acquired from the Cell Lab of our Experiment Center. All of the cells were incubated in the medium of RPMI 1640 containing 100 IU/mL streptomycin-penicillin and 12% FBS, cultured in a moistened incubator with 5% weight/volume CO_2 at 37 °C. The concentration of cells was monitored by a hemocytometer.

Colorimetric analysis of cancer cells with Sgc8c-Cu/Au/Pt TMNPs. Generally, Sgc8c-Cu/Au/Pt TMNPs (2 mg/mL) was added into 50 μL of the suspension of CCRF-CEM cells dispersed in binding buffer, and incubated for an hour at 37 °C. Then the mixed solution was centrifuged for 5 min at 1,500 rpm to get rid of the Sgc8c-Cu/Au/Pt TMNPs unbound to cells. Next, the cells binding with Sgc8c-

Cu/Au/Pt TMNPs were added with the detection solution containing 25 μL of H_2O_2 (1 mM), 50 μL of H_2SO_4 (0.05 M), 50 μL of TMB (1.7 μM) and 125 μL of H_2O, and incubated for 10 min at 55 °C. Finally, the absorbance at 450 nm of TMB^+ was recorded by a multifunctional micro-plate reader (Infinite M1000) through adding 150 μL of the final reaction solution into a 96-well plate.

Specified in the feasibility investigation, 5,000 cells were used. Ramos cells were used as the control cells and Lib-Cu/Au/Pt TMNPs were used as the control probes. The detected cell numbers for quantitative analyzing of target cells were listed as follows: 0, 200, 500, 1,000, 2,500, 5,000, 6,000, 8,000 and 10,000 cells.

Selective photothermal ablation of cancer cells using Sgc8c-Cu/Au/Pt TMNPs. 50 μL of Ramos or CCRF-CEM cells suspension (10^5 cells) was incubated with Cu/Au/Pt TMNPs, Sgc8c-Cu/Au/Pt TMNPs or Lib-Cu/Au/Pt TMNPs (2 mg/mL) for 60 min at 37 °C, and then centrifuged for 5 min at 1500 rpm to remove nanoparticles unbound to cells. After re-dispersing in binding buffer, cells suspension was added into 96-well cell culture plates for 5 min irradiation at 0.54 W/cm^2 by a 780 nm laser. The viability of cells was carried out by flow cytometry analysis through staining cells with 5.5 μM PI for half an hour at 37 °C. Moreover, As an alternative, co-stained assay was performed by incubating cells with 5.5 μM PI and 5.5 μM calcein-AM for half an hour at 37 °C. And then the stained cells were imaged on an inverted microscope (Nikon, Te300).

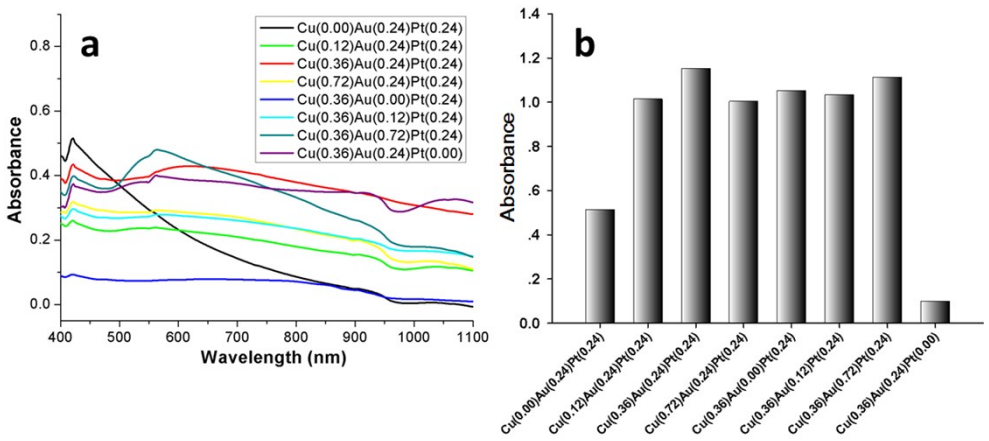

Fig. S1 Comparison of the plasmonic and catalytic properties of Cu/Au/Pt TMNPs that were prepared with different concentrations (mM) of $CuSO_4$, $HAuCl_4$ and K_2PtCl_4. (a) Absorption spectra of metallic nanoparticles. (b) Background-subtracted absorbance at 450 nm of the TMB-H_2O_2 reaction system catalyzed by metallic nanoparticles.

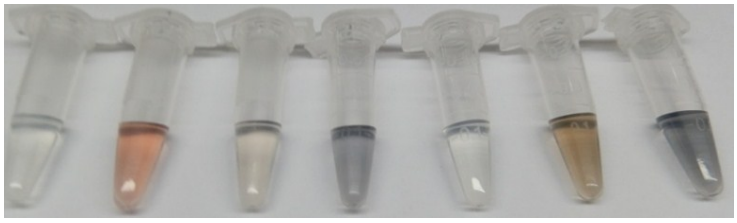

Fig. S2 A photograph of different metallic nanoparticle samples in an aqueous solution under ambient light. (From left to right: Cu nanoparticles, Au nanoparticles, Pt nanoparticles, Cu/Au bimetallic nanoparticles, Cu/Pt bimetallic nanoparticles, Au/Pt bimetallic nanoparticles and Cu/Au/Pt TMNPs).

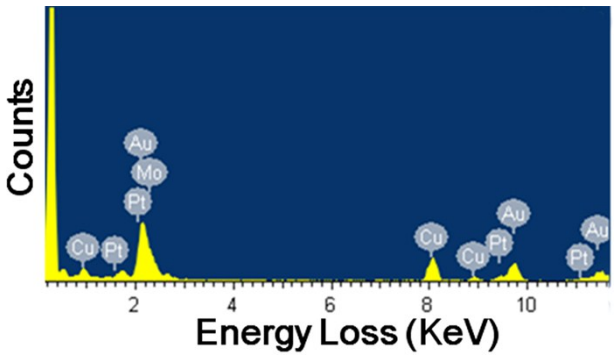

Fig. S3 EDS characterization result of Cu/Au/Pt TMNPs.

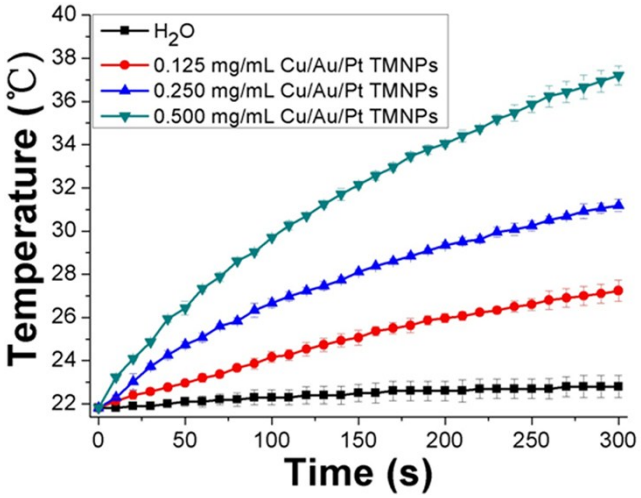

Fig. S4 Heating curves of water and Cu/Au/Pt TMNPs solutions with different concentrations under 780 nm laser irradiation.

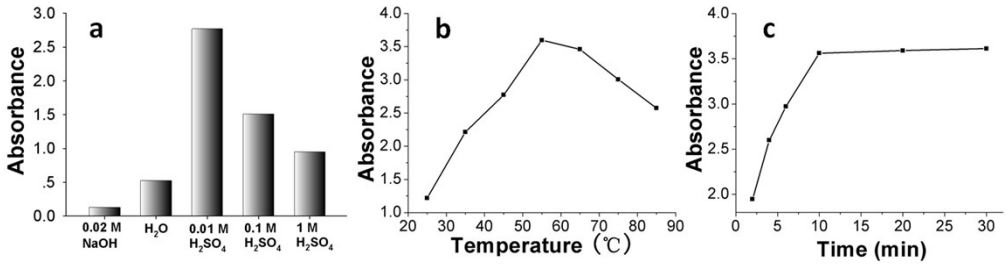

Fig. S5 Optimization of parameters affecting the catalysis of Cu/Au/Pt TMNPs on the TMB-H_2O_2 reaction system. (a) pH; (b) reaction temperature; (c) reaction time.

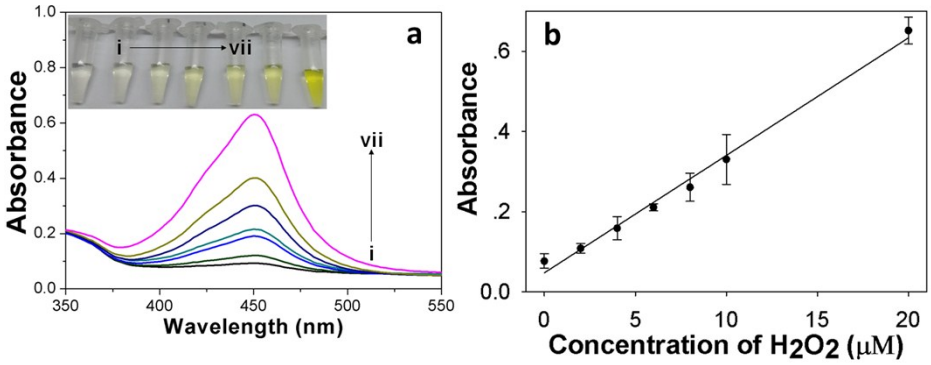

Fig. S6 The dependence of colorimetric signal on the concentration of H_2O_2 based on Cu/Au/Pt TMNPs-mediated TMB-H_2O_2 reaction. (a) Photographs and UV-vis absorption spectra of TMB-H_2O_2 reaction samples with different concentrations of H_2O_2, catalysed by Cu/Au/Pt TMNPs (from i to viii: 0, 2, 4, 6, 8, 10, and 20 μM). (b) The relationship between the absorbance at 450 nm and the concentration of H_2O_2.

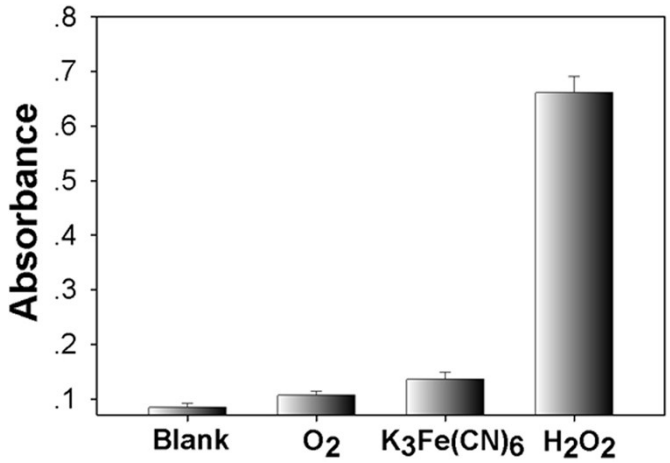

Fig. S7 Selectivity determination of H_2O_2 based on Cu/Au/Pt TMNPs-mediated TMB-H_2O_2 reaction, by using 200 μM $K_3Fe(CN)_6$ and dissolved O_2 as controls (H_2O_2: 20 μM).

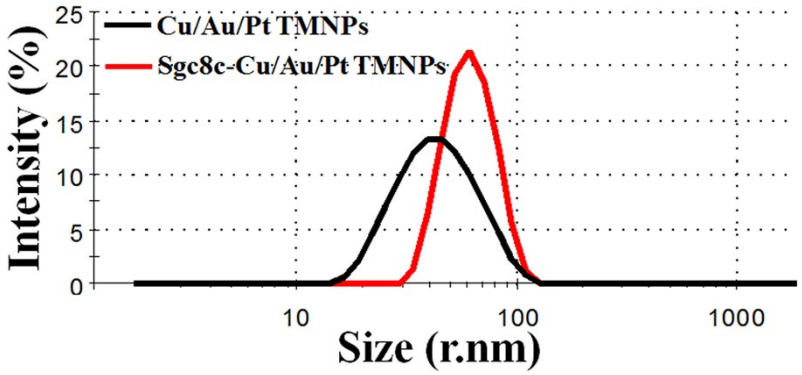

Fig. S8 Hydrodynamic radius characterization of Cu/Au/Pt TMNPs and Sgc8c-Cu/Au/Pt TMNPs. The concrete values of Cu/Au/Pt TMNPs and Sgc8c-Cu/Au/Pt TMNPs were 38.01 ± 18.78 nm and 58.06 ± 15.80 nm, respectively.

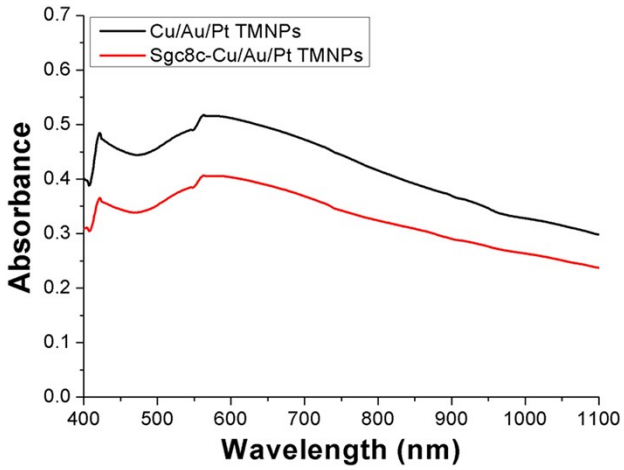

Fig. S9 Absorption spectral characterization of Cu/Au/Pt TMNPs and Sgc8c-Cu/Au/Pt TMNPs.

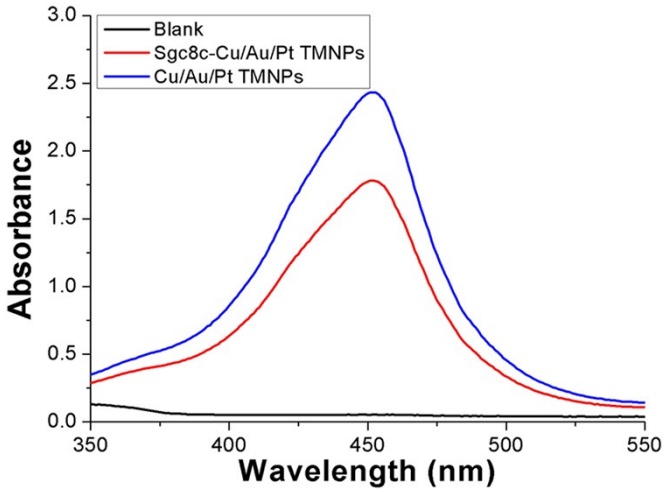

Fig. S10 Catalytic activity characterization of Cu/Au/Pt TMNPs and Sgc8c-Cu/Au/Pt TMNPs on the TMB-H_2O_2 reaction.

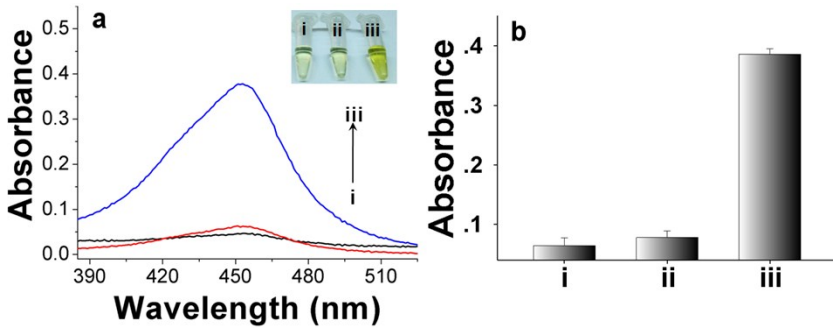

Fig. S11 Feasibility analysis of the Sgc8c-Cu/Au/Pt TMNPs-based strategy for colorimetric detection of cancer cells. (a) Photographs and the characterization of background-subtracted absorption spectrum. (b) Histogram of the relevant background-subtracted absorbance at 450 nm in (a). (i) Lib-Cu/Au/Pt TMNPs incubated with CCRF-CEM cells; (ii) Sgc8c-Cu/Au/Pt TMNPs incubated with Ramos cells; (iii) Sgc8c-Cu/Au/Pt TMNPs incubated with CCRF-CEM cells. (Bar graph data are expressed as mean values ± s.d.; n=3.).

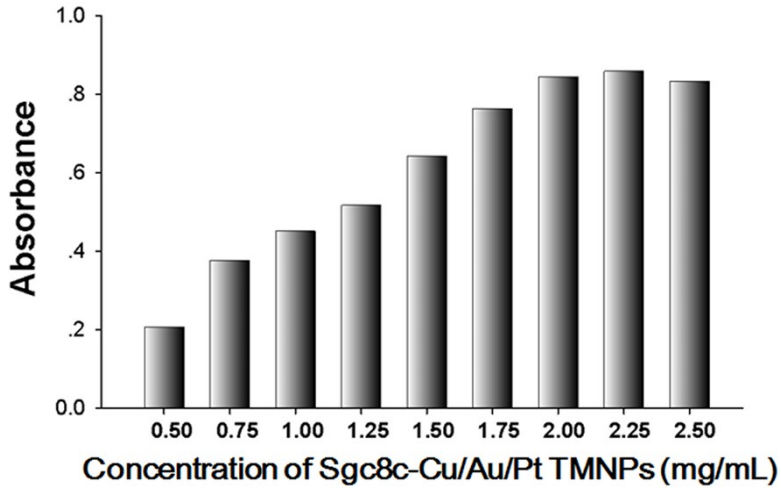

Fig. S12 Optimization of the concentration of Sgc8c-Cu/Au/Pt TMNPs used for colorimetric analysis of target cells.

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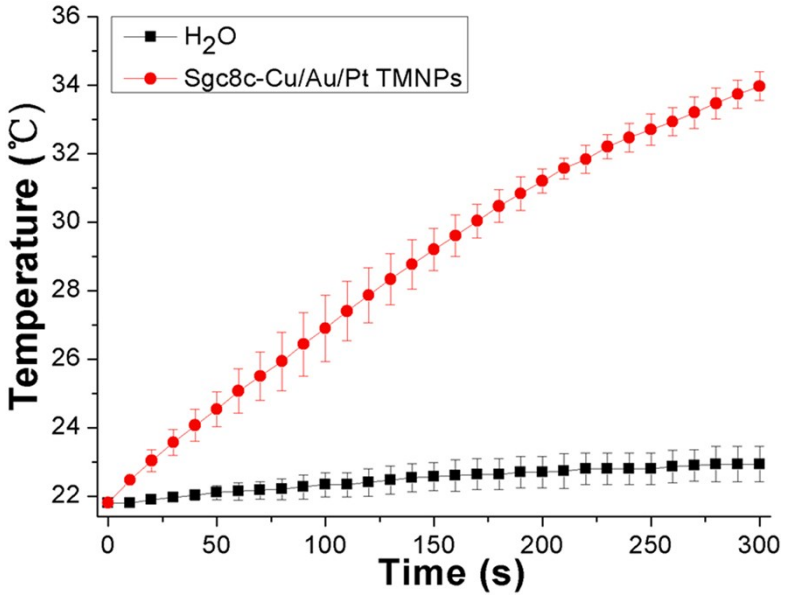

Fig. S13 Heating curves of water and Sgc8c-Cu/Au/Pt TMNPs solution under 780 nm laser irradiation.

Table S1 The comparison of different colorimetric methods for detection of glucose

Materials	Linear range	Detection limit	Reference
Cu/Au/Pt TMNPs	0-0.2 mM	25 μM	This work
Carbon dots	0.2-2.5 mM	60 μM	[1]
Silver fabric	0.1-2 mM	80 μM	[2]
Multilayer paper	0.02-4 mM	14 μM	[3]
NiFe nanosheets	0.05-2 mM	23 μM	[4]
Brominated graphene	40–100 mM,	28.41 mM	[5]
Graphene quantum dots	0.025-0.375 mM	16 μM	[6]
Fe_3O_4 Nanoparticles	0.05-1 mM	30 μM	[7]

Reference

[1] B. Wang, F. Liu, Y. Wu, Y. Chen, B. Weng and C. M. Li, *Sensor. Actuat. B-Chem*., 2018, **255**, 2601-2607.

[2] M. N. Karim, S. R. Anderson, S. Singh, R. Ramanathan and V. Bansal, *Biosens. Bioelctron*., 2018, **110**, 8-15.

[3] X. Wang, F. Li, Z. Cai, K. Liu, J. Li, B. Zhang and J. He, *Anal. Bioanal. Chem*., 2018, **410**, 2647-2655.

[4] T. Zhan, J. Kang, X. Li, L. Pan, G. Li and W. Hou, *Sensor. Actuat. B-Chem*., 2018, **255**, 2635-2642.

[5] S. Singh, K. Mitra, R. Singh, A. Kumari, S. K. S. Gupta, N. Misra, P. Maiti and B. Ray, *Anal. Methods*, 2017, **9**, 6675-6681.

[6] L. Lin, X. Song, Y. Chen, M. Rong, T. Zhao, Y. Wang, Y. Jiang and X. Chen, *Anal. Chim. Acta*, 2015, **869**, 89-95.

[7] H. Wei and E. Wang, *Anal. Chem.,* 2008, **80**, 2250-2254.