Supporting Information

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Experimental Section

Chemicals and Materials

All oligonucleotides were synthesized and HPLC purified by Generay Biotech (Shanghai, China). The stock solution of each nucleic acid (10 mM) was prepared with TE buffer containing 10 mM Tris-HCl, 1 mM EDTA, 12.5 mM $MgCl_2$ (pH 7.4). The sequences were shown in Table S1. Tris(2-carboxyethyl) phosphine hydrochloride (TCEP) was purchased from Sigma Aldrich (St. Louis, MO). 40% acrylamide mixed solution, ammonium persulfate (APS), 1,2-bis(dimethylamino)-ethane (TEMED), 5×TBE buffer consisting of Tris (40 mM), acetic acid (20 mM), and EDTA (1 mM) (pH 8.3), DNA loading buffer were purchased from Sangon Bio Inc. (Shanghai, China). Ethylene glycol (EG), sodium hydrosulfide (NaHS), chloroauric acid ($HAuCl_4$), ascorbic acid ($C_6H_8O_6$), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), hydrochloric acid (HCl) and ascorbic acid (AA) were purchased from Sinopharm Chemical Reagent Co., LTD. (China). Silver trifluoroacetate ($C_2AgF_3O_2$) and malachite green (MG) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (China). Phosphate buffer salt solution (PBS, (10 mM, pH 7.4), fetal bovine serum (FBS), DMEM medium and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) were purchased from Jiangsu Keygen Biotechnology Co., LTD. poly (vinyl pyrrolidone) (PVP, Mw=55000) was purchased from Shanghai Biyuntian Biotechnology Co., LTD. All the chemicals were of analytical grade and used as received without further purification. RNase-free water was used in all experiments.

Instruments

Transmission electron microscope (TEM) images were taken on JEOL-2100 Transmission

electron microscope (Electron optics Laboratory, Japan). Scanning electron microscope (SEM) images were taken with an S-4800 scanning electron microscope (Hitachi LTD., Japan). UV-vis spectroscopy was performed by UV-3600 spectrometer (Shimadzu, Japan). A Shimadzu RF-5301PC fluorescence spectrometer equipped with xenon lamp was used for fluorescence detection (Shimadzu, Japan). Polyacrylamide gel electrophoresis (PAGE) was imaged using a Bio-rad ChemiDoc XR imager. Confocal laser scanning microscopy (CLSM) imaging was performed on a Leica TCS SP8 (Germany). Surface-enhanced Raman spectroscopy (SERS) was obtained by Renishaw Invia-Reflex Microscopic Raman spectrometer (Renishaw, UK). A Helium-neon laser at 633 nm was used for excitation, and spectra were acquired using a 50× working objective lens on a sample at laser power of 17 mW and exposure time of 10 s. The Zeta potential was measured by Brookhaven 90Plus Dynamic Light Scatterer (Brookhaven, USA).

Preparation of gold nanoflowers (AuNFs)

The silver nanocubes (AgNCs) was first synthesized following the previously reported protocol. Briefly, 20 mL of EG was preheated in 100 mL flask at 150 °C for 1h under magnetic stirring and N_2 bubbling. Then, 0.25 mL of NaHS solution (3 mM) was added, after 3 min, 1.5 mL of HCl (3 mM), 5 mL of PVP (20 mg/mL) were added to the mixture in turn. After another 5 min, 1.5 mL of CF_3COOAg solution (282 mM) was added. During the entire process, all the reagents were dissolved in EG and the flask was only transitory open when the reagent was added to ensure an anhydrous and oxygen-free environment. After 30 min, the AgNCs were obtained when the suspension had reached a brown color through stopping the reaction in the ice-water bath. The AgNCs were precipitated with acetone from the mixture and washed with water for three times, and redispersed in 1 mL of EG for further use.

Subsequently, AuNFs were fabricated through galvanic replacement processes with AgNCs as sacrificial template. Specifically, 100 μL of colloidal AgNCs was added to 10 mL of deionized water containing 1 mg/mL PVP in a 25 mL beaker with vigorous magnetic stirring for 10 min. When 400 μL $HAuCl_4$ (0.5%) and 400 μL (40 mM) AA was successively added, the mixture changed to blue immediately. The products were centrifuged and washed with water for three times and finally redispersed in 2 mL of H_2O. The concentration of AuNFs was 1.45 nM by using ICP-AES.

Preparation of Capture RNA-Modified AuNCs (CP-AuNCs)

10 μL thiolated-capture RNA (SH-CP, 100 μM) was activated by 2 μL TCEP (10 mM, pH 5.2) for 1 h, then mixed with 200 μL AuNFs. After incubation for 16 h at room temperature with gentle shaking, the resulting solution was salted with 24 μL of NaCl (3 M), and the mixture was allowed to stand for 24 h at room temperature. The excess SH-CP was removed by centrifugation and washed with water for three times. Finally, the CP-AuNCs redispersed in PBS with a final concentration of 1 nM and stored at 4 ℃ for later use.

MG Loading and Release of CP-AuNFs

To confirm the loading capacity of CP-AuNFs, different concentration (0, 0.5, 1, 2, 5, 8, 10, 20, and 30 μM) of MG were mixed with 100 μL of CP-AuNFs (1 nM). The final volume was supplemented to 150 μL with ultrapure water. After incubation for 24 h at room temperature with gentle shaking, the signal of was monitored by SERS. Next, the release of MG was monitored by SERS as well. In brief, 1.2 mL of MG/CP-AuNFs was divided into two groups of six tubes and the supernatant was removed by centrifugation. These groups were added with PBS buffer of pH 7.4 and 5.5, respectively, and incubated for different time (0, 2, 4, 6, 12 and 24 h). Then, the

supernatant was removed by centrifugation and the sediment was redispersing in PBS buffer (10 mM, pH 7.4) for the SERS signal determination of MG/CP-AuNFs.

Preparation of Nanodevice

This nanodevice utilized the AuNFs as delivery carrier, the two major modules of RNA amplifier, TN and FN which were modified on the AuNFs via the CP strand. In brief, After the thermal annealing 10 μL of SH-A (100 μM), 10 μL of C (100 μM) and 10 μL of MGA (100 μM), the TN was obtained. Then, 10 μL of TN (100 μM) and 10 μL of FN (100 μM) were mixed with 500 μL of MG/CP-AuNFs (1 nM). After incubation at 37 °C for 2 h, the mixture was then centrifugated to remove the free RNAs and washing three times with PBS (10 mM, pH 7.4), the TN/FN/MG/CP-AuNFs (RNA amplifier/MG/CP-AuNFs), that is nanodevice was obtained. The resulting nanodevice was finally redispersed in PBS and stored at 4 °C for further use. The ratio of TN/FN/AuNFs was calculated as 69/85/1 using our previously reported method.

Photothermal Release of RNA Amplifiers from Nanodevice

We utilized the photothermal effect of AuNFs for controlled release of RNA amplifiers. The nanodevice was irradiated with 660 nm laser at a power density of 0.8 W cm^{-2} for 10 min to release the RNA amplifier. This result was confirmed by using the PAGE, UV-vis and fluorescence spectra.

Native PAGE Characterization

The 12% PAGE was first prepared by 40% acrylamide mixed solution (2.4 mL), 5×TBE buffer (1.6 mL), ultrapure water (4 mL), ammonium persulfate (APS, 56 μL), 1,2-bis(dimethylamino)-ethane (TEMED, 8 μL). Then, the RNA amplifier was analyzed by this PAGE in 1×TBE buffer at 150 V for 40 min, after staining with ethidium bromide (EB) and water

elutreaction, the resulting gel was imaged by BIO-RAD ChemiDoc XRs.

Fluorescence Measurements

For detection of TK1 mRNA in the buffer solution, different concentrations of TK1 mRNA were added to 200 μL of PBS buffer containing 1 nM nanodevice. Then the mixture was irradiated with 660 nm laser at a power density of 0.8 W cm^{-2} for 10 min to release the RNA amplifier and incubated at 37 °C for 2 h. The fluorescence spectra were collected from 630 to 800 nm with 620 nm excitation. All experiments were repeated three times.

Cell Culture

Human breast cancer cells (MCF-7 cells), human cervical cancer cells (HeLa cells) and human normal liver cells (L02 cells) were obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). These cells were respectively cultured in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% FBS and penicillin streptomycin (100 U/mL) at 37 °C in a humidified 5% CO_2 incubator. The cells at the end of the log phase were selected for the following experiments.

Cytotoxicity

The cytotoxicity of nanodevice was assessed by the MTT assay. MCF-7 cells (1×10^5 cells for each well) were cultured in 96-well plate and incubated at 37 °C for 24 h to reach 85% confluency, and then cells were incubated with different treatment for 24 h. Subsequently, the cells were washed with PBS buffer for three times. Then 10 % (v/v) of MTT/DMEM medium were added to each well with incubation at 37 °C for 4 h. Finally, DMSO was added and then absorbance at 490 nm was measured.

CLSM Analysis

The MCF-7 cells with TK1 mRNA overexpression were used as target cells, while the L02 cells and Hela cells were used as negative control cells. To test the fluorescent response of nanodevice in MCF-7 cells with and without 660 nm laser illumination, the two group of MCF-7 cells (1×10^5 cells) were incubated with nanodevice (0.2 nM) for 2 h at 37 °C, respectively. One group was then irradiated with 660 nm laser for 10 min (0.8 W cm^{-2}) and the other was left untreated, followed by incubation another 4 h in incubator. Finally, washing twice with 1 mL of PBS (10 mM, pH 7.4) and adding fresh medium for CLSM observation.

To investigate the response of nanodevice in different cells, MCF-7 cells, HeLa cells and L02 cells (1×10^5 cells for each) were incubated with nanodevice (0.2 nM) for 2 h at 37 °C, followed by incubation another 4 h in incubator after irradiation with 660 nm laser for 10 min (0.8 W cm^{-2}), then washing twice with 1 mL of PBS (10 mM, pH 7.4) and final adding fresh medium for CLSM observation. In the same way, the response of different nanodevices in MCF-7 cells was monitored. After incubation with different nanodevices (0.2 nM) that unequipped MG, MGA and response to target, respectively, the results were observed by CLSM. All the excitation wavelength is 620 nm and the emission filter is (660 ± 20) nm.

Table S1. Sequences of oligonucleotides used in this work.

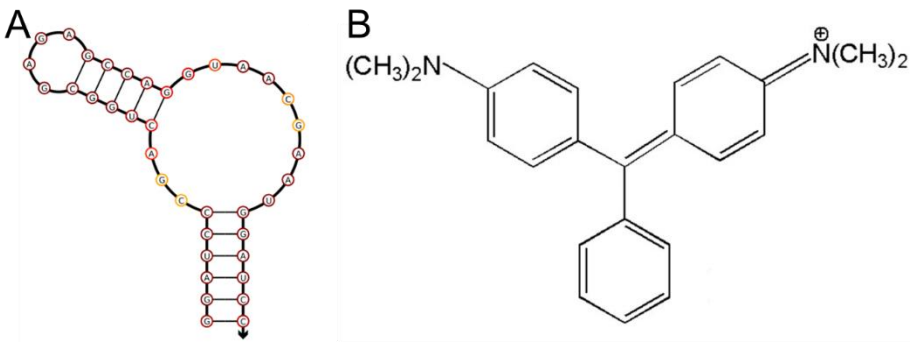
Oligonucleotides	Sequence (5'-3')
A	GCG AGU GUC UUU GGC AUA CUU GAG GGG GAU CCA UUC GUU AC C UGG CUC
FN	GUG GAU CCC GAA GGU AAC GAA UGG AUC CCC CUC AAG UAU GCC AA A GAC
C	CCC UCA AGU AUG CCA AAG AC
MGA	GUGGA UCC CGA CUG GCG AGA GCC AGG UAA CGA AUG GAU CC
TK1 mRNA	CAA GUA UGC CAA AGA CAC UCG C
C-myc mRNA	GGU AGU GGA AAA CCA GCA GCC U
survivin mRNA	UCA AGG AGC UGG AAG GCU GGG
single base-mismatched TK1 mRNA	CAA GUA UGC CAU AGA CAC UCG C
Capture RNA (CP)	SH-AAA UCG GGA UCC AC

U is the mismatched base of TK1 mRNA.

Additional Results and Discussion

Optimal Ratio of MGA to MG

The secondary structures of MGA and the structural formula of MG were shown in Scheme S1. To confirm the optimal ratio of MGA to MG, different concentrations (0, 0.5, 1, 2, 2.5, 5, 10, 20 and 40 μM) of MG (50 μL) were mixed with 50 μL of MGA (1 μM). The final volume was 100 μL. After incubation at room temperature for 10 min, the fluorescence spectra were monitored. From Figure S1, with increasing the concentration of MG, the fluorescence of MG was gradually increased and reached the maximum at 10 μM. Thus, the ratio of MG/MGA at 10/1 was used in the subsequent studies.



Scheme S1. The secondary structures of MGA (A) and the structural formula of MG (B).

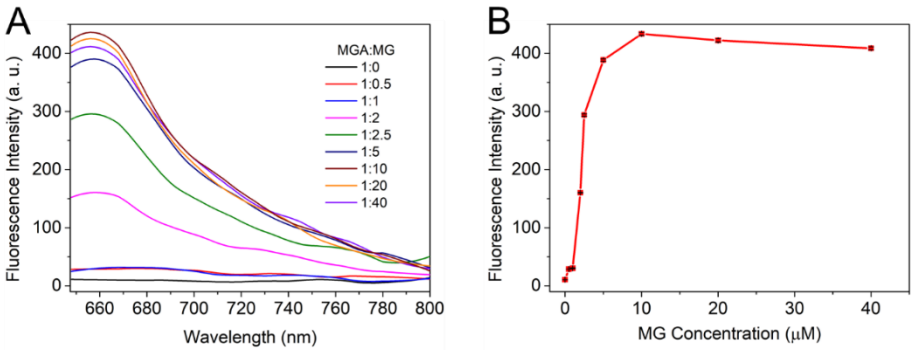


Figure S1. Optimal Ratio of MGA to MG. (A) Fluorescence spectra of different MG and MGA composite. (B) The corresponding fluorescence intensity at 660 nm in Figure S1 (A), error bars represent standard deviations from three repeated measurements.

Characterization of AgNCs

The AgNCs of ~50 nm edge lengths were observed in SEM with a well-defined localized surface plasmon resonance peak at around 430 nm (Figure S2), which was consistent with previous report.[1]

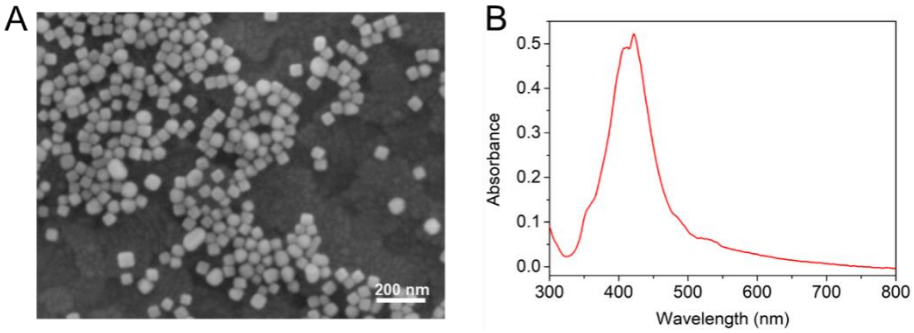


Figure S2. (A) SEM image and (B) corresponding UV-vis absorption spectra of AgNCs. Scale bar: 200 nm.

Characterization of AuNFs

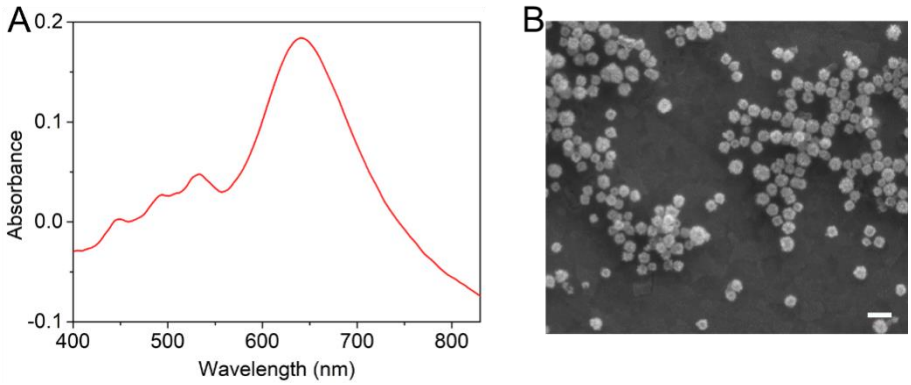


Figure S3. (A) The UV-vis absorption spectra of AuNFs. (B) SEM image of AuNFs. Scale bar: 200 nm.

Determination of MG Loaded in Nanodevice

Briefly, the CP-AuNFs (2 nM) was mixed with different concentrations of MG in PBS buffer (10 mM, pH 7.4). After incubation at room temperature for 24 h, the SERS spectra were monitored (Figure S4). The peak intensity at 1617 nm was gradually increased with increasing the concentration of MG from 0 to 10 μM, however, when the concentration of MG continually increased to 30 μM, the SERS intensity of MG was unchanged, indicating MG load reached saturation at 10 μM and the ratio of MG/CP-AuNFs was 5000/1.

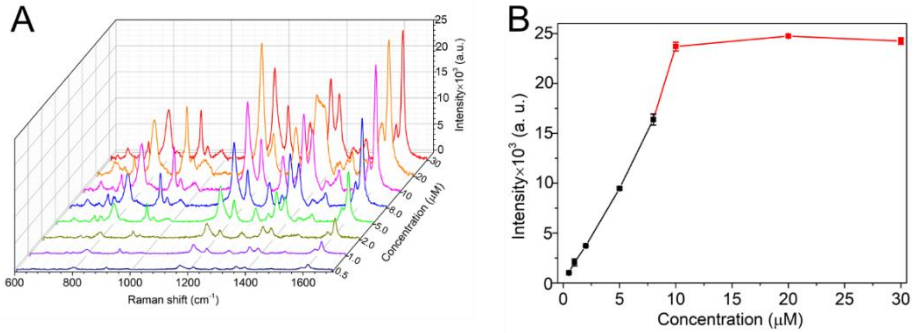


Figure S4. (A) SERS spectra of loading different amounts of MG into the nanodevice. (B) The corresponding SERS intensity at 1617 nm in Figure S4 (A). The error bars represent standard deviations from three repeated measurements.

Release of MG in Acidic Environment

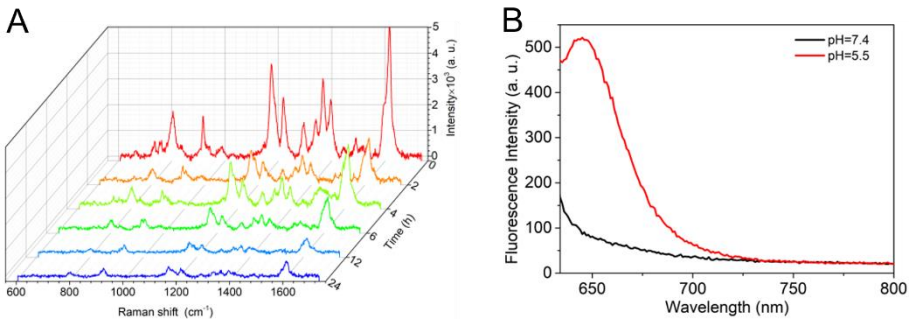


Figure S5. (A) SERS spectra of MG-AuNFs incubated with acidic buffer (pH 5.5) for different times. (B) Fluorescence spectra of MG/AuNFs binding with MGA in different buffer.

Photothermal Effect of Nanodevice

The photothermal effect of AuNFs (0.2 nM) was tested by illumination with 660 nm laser at a power density of 0.8 W cm^{-2} for different time (from 0 to 10 min), the PBS buffer (10 mM, pH 7.4) as control. From Figure S6, the PBS buffer, almost no increase in temperature was observed after illuminated. Whereas, the AuNFs exhibited a significantly elevated temperature and was over 45 °C. Thus, the optimum irradiation time was 10 min for the subsequent studies.

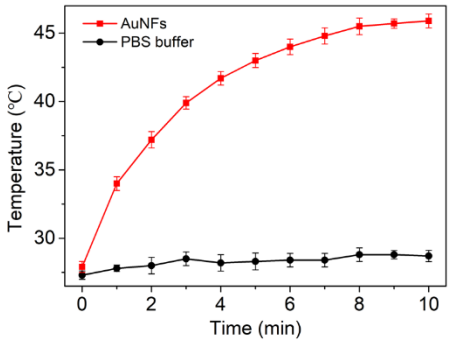


Figure S6. Temperature-time curves of AuNFs and PBS buffer under 660 nm laser irradiation. The error bars represent the standard deviations from three repetitive measurements.

Release of RNA Amplifiers from Nanodevice

Briefly, after incubation the nanodevice (2 nM, 50 μL) in 37 °C and 45 °C for 10 min, respectively. Then centrifuged immediately to collect the sediment and supernatant, and measured

the RNA by PAGE and NanoDrop 2000c Spectrophotometer (Thermo). As shown in Figure S7A, TN and FN were as control (lanes 1 and 2, respectively). The supernatant from 37 °C treatment showed no obvious band (lane 3), in contrast, there was distinct bands of TN and FN can be observed in 45 °C treated supernatant (lane 4). This result was consistent with that of UV-vis characterization (Figure S7B).

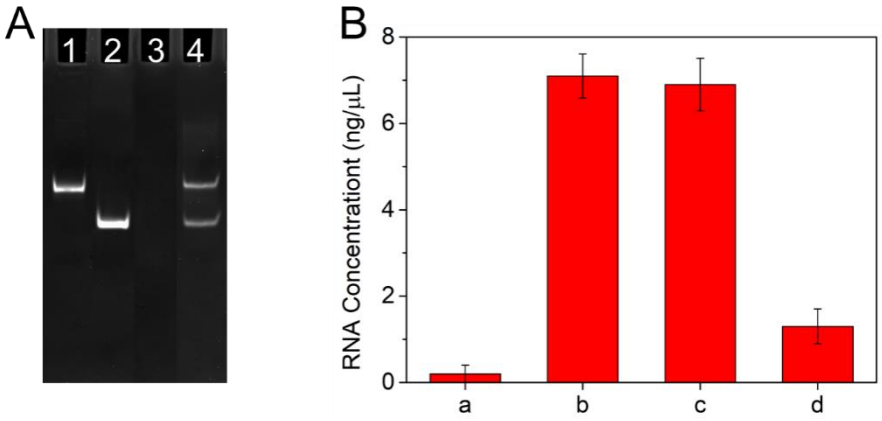


Figure S7. (A) PAGE to analyze the release of RNA amplifier. (B) RNA concentrations in supernatant (a, c) and sediment (b, d) after incubation the nanodevice in 37 °C (a, b) and 45 °C (c, d). The error bars represent the standard deviations from three repetitive measurements.

Calculation of Detection Limit

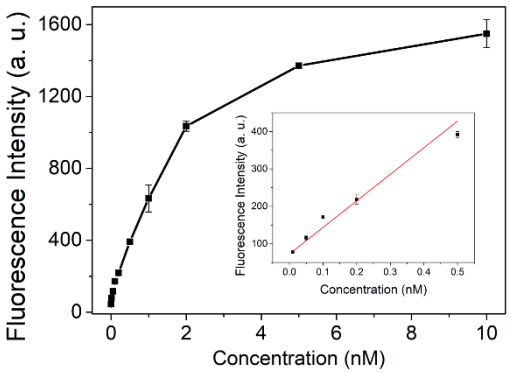


Figure S8. The relationship between fluorescence intensity and TK1 mRNA concentration. Inset is the responses of the nanodevice to TK1 mRNA at the low concentration and the regression equation is FL = 616.97$C_{TK1\ mRNA}$ + 89.03 (R^2=0.98).

Selectivity and Stability of Nanodevice

The nanodevice was incubated with different analytes for selectivity assay, including C-myc mRNA, survivin mRNA, single-base mismatched TK1 mRNA and TK1 mRNA. As shown in Figure S9A, the TK1 mRNA group exhibited an effective fluorescence enhancement. In contrast, a negligible fluorescence increase was observed from the other groups compared to the blank (Free MG). Moreover, the stability of the nanodevice was investigated by incubation the FAM-labelled nanodevice with 10% fetal bovine serum (FBS) at 37 °C for 0, 2, 4, 8, 10 and 12 h, respectively (Figure S9B). As the incubation time prolonged, the fluorescence intensity of FAM showed only a little increase which was much lower than that of the free FAM at the same concentration. Obviously, the complexes of MGA and MG exhibited good stability even in cell lysates (Figure S10). All these results indicated the excellent selectivity and stability of the nanodevice, which would hold great promise for accurate imaging in living cells.

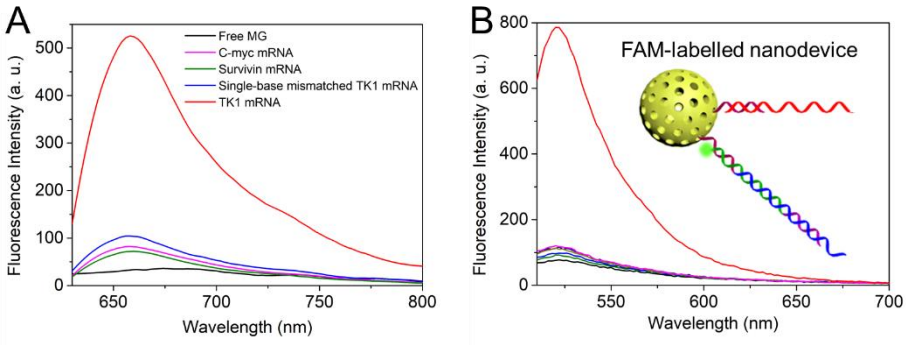


Figure S9. (A) Fluorescence spectra of the nanodevice in response to different species, including C-myc mRNA, survivin mRNA, single-base mismatched TK1 mRNA and TK1 mRNA. The concentration of TK1 mRNA is 1 nM, while the other interferent mRNAs are 10 nM. (B) The fluorescence spectra of FAM-labelled nanodevice after incubation with 10% FBS at 37 °C for different times (From bottom to top: 0 h, 2 h, 4 h, 8 h, 10 h, 12h). Insert is the scheme of FAM-labelled nanodevice.

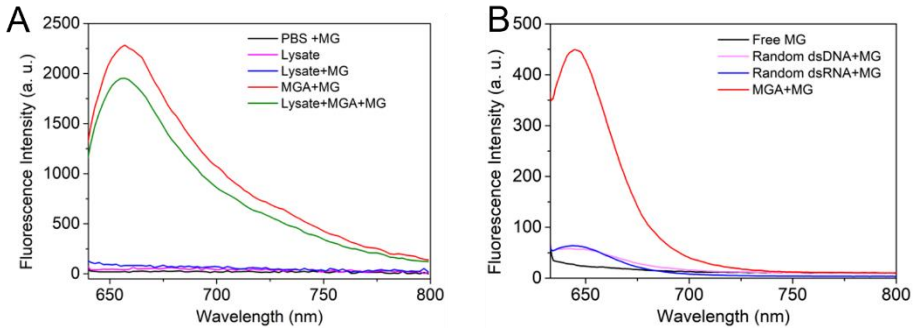


Figure S10. (A) The stability of MGA-MG complex in MCF-7 cell lysate. (B) Fluorescence spectra of random dsDNA, random dsRNA and MGA binding with MG.

Cytotoxicity Study of Nanodevice

The cytotoxicity of nanodevice and its component against the MCF-7 cells and were studied by MTT assay. As shown in Figure S11, after incubation for 24 h, the free MG, AuNFs carrier showed no obvious toxicity for MCF-7 cells compared with control group. The nanodevice treated group exhibited the same result, even after 660 nm laser irradiation for 10 min (0.8 W cm^{-2}), the viability of MCF-7 cells was more than 80%, revealing the excellent biocompatibility of the nanodevice.

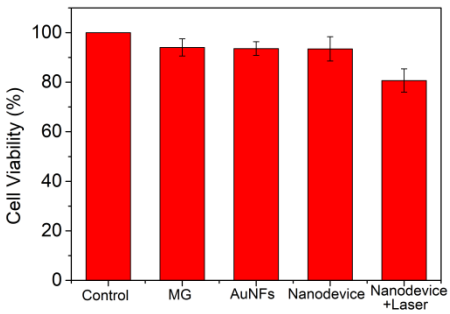


Figure S11. The viability of MCF-7 cells untreated and treated with MG (1 μM), AuNFs (0.2 nM), nanodevice (0.2 nM), nanodevice (0.2 nM) with 660 nm laser. The error bars represent the standard deviations from three repetitive measurements (0.8 W cm^{-2} for 10 min).

Stability Study of Nanodevice in Living Cells

The biostability of nanodevice in MCF-7 cells was explored by CLSM images. After

incubation the FAM-labelled nanodevice with MCF-7 cells for 4, 8 and 12 h, respectively, there were no obvious green fluorescence in all groups, indicating the excellent stability of nanodevice in living cells (Figure S12).

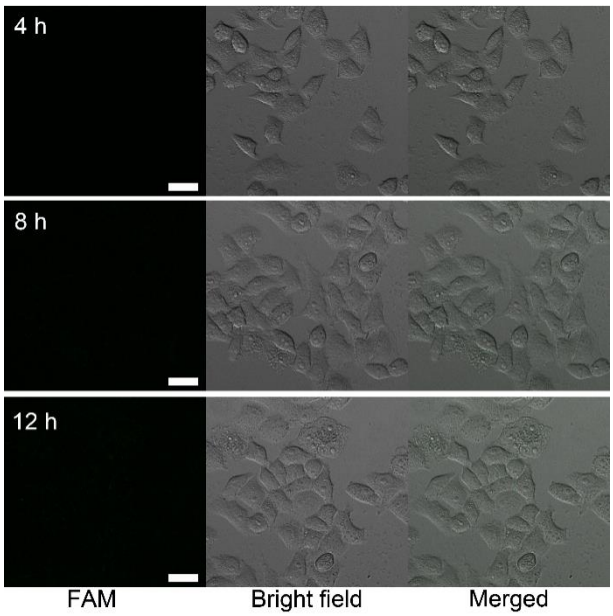


Figure S12. CLSM images of MCF-7 cells incubated with FAM-labelled nanodevice for 4 h, 8 h and 12 h. Scale bar: 25 μm.

Optimization of Incubation Time

The optimal incubation time of nanodevice and MCF-7 cells was investigated by CLSM imaging (Figure S13). After laser irradiation and incubation for 1 h, the red fluorescence was hardly observed, indicating the nanodevice has not been activated. After incubation for 2 h, a slightly red fluorescence was observed. Until incubation for 4 h, a strong fluorescence was observed in MCF-7 cells and its fluorescence intensity was equivalent to 6 h, demonstrating that the nanodevice has been successfully activated and the 4 h was chosen as the optimal incubation time in the subsequent studies.

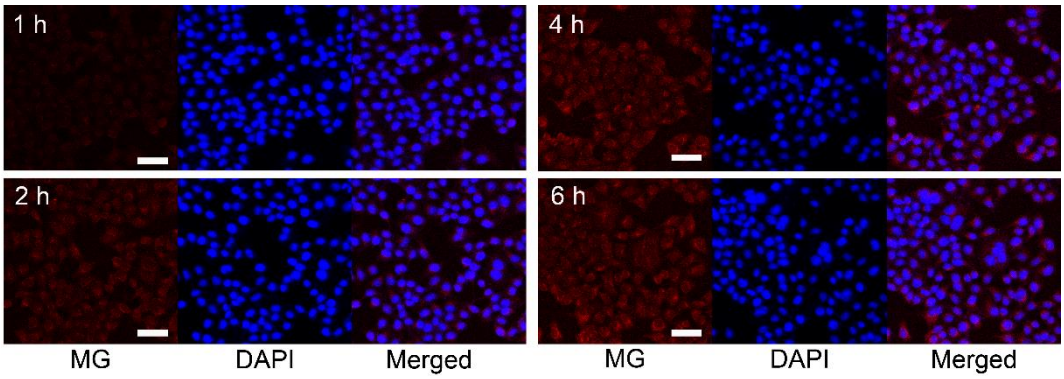


Figure S13. CLSM imaging of MCF-7 cells treated with nanodevice with different incubation time. Scale bar: 50 μm.

Specific Response of Nanodevice in MCF-7 Cells

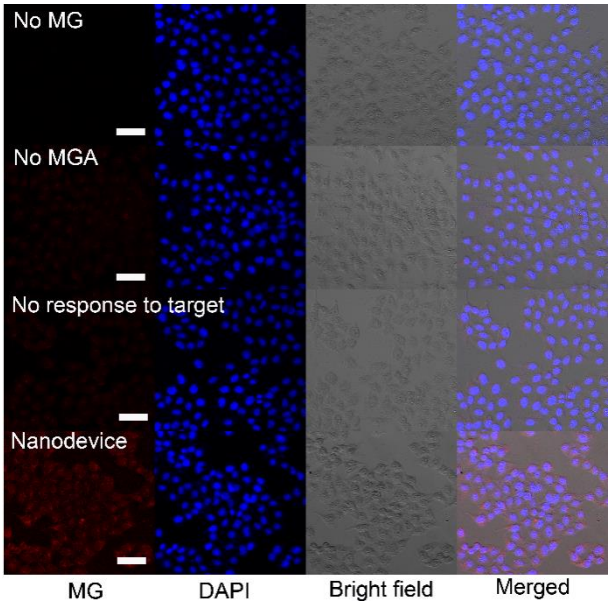


Figure S14. CLSM images of MCF-7 cells incubated with nanodevice that equipped without MG, MGA and response to target, the intact nanodevice as control. Scale bars: 50 μm.

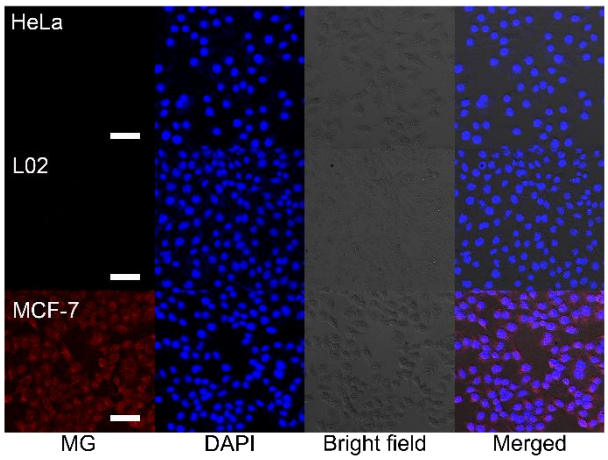


Figure S15. CLSM images of HeLa cells, L02 cells and MCF-7 cells incubated with nanodevice. Scale bars: 50 μm.

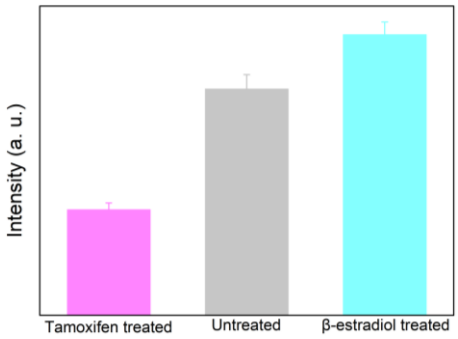


Figure S16. Fluorescence intensity of MG in different treated MCF-7 cells calculated by using Image J.

References

1. Y. Su, Q. Zhang, X. Miao, S. Wen, S. Yu, Y. Chu, X. Lu, L.-P. Jiang, and J.-J. Zhu, *ACS Appl. Mater. Interfaces*, 2019, **11**, 41979-41987.