Supporting Information

A bioresponsive controlled-release bioassay based on aptamer-gated Au nanocages and its application in living cells

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Materials:

Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$) and Tris-(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma–Aldrich. Rhodamine B (RhB) was obtained from Shanghai Aladdin Chemistry Corp (China). Adenosine triphosphate (ATP), cytosine triphosphate (CTP), guanosine triphosphate (GTP), and uridine triphosphate (UTP) were obtained from Sigma. ATP-binding aptamer (5'-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3') was purchased from SBS Genetech. Co. Ltd. (China). Unless stated otherwise, all reagents were purchased from commercial sources and used without additional purification. Silver nanocubes were prepared according to literature procedures[1]. Ramos cells and HCT 116 colon cancer cells were obtained from Chinese Academy of Medical Sciences and Ocean University of China, respectively.

Cell Culture:

Ramos cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU mL^{-1} of penicillin-streptomycin. The cells were maintained at 37°C in a humidified atmosphere (95% air and 5% CO_2). The amount of cells was determined by using a hemocy-tometer prior to each experiment. The cells were collected and separated from RPMI cell medium buffer by centrifugation at 2000 rpm for 5 min, followed by washing twice with a sterile phosphate buffer solution (10 mM, pH 7.4 PBS). The sediment was resuspended in PBS (1.0 mL) containing Ca^{II} (0.1 mM) and Mg^{II} (0.1 mM) to obtain a homogeneous cell suspension (approximately 1.0×10^5 cells mL^{-1}).

Apparatus:

F-4600 fluorescence spectrophotometer (Hitachi, Japan); UV-visible spectra were taken with a Cary 50 UV-vis-NIR spectrophotometer (Varian, Agilent). Transmission electron microscopy (TEM) image was taken with JEM-2000EX/ASID2 instrument (HITACHI, Japan). Fluorescence images were obtained on a laser confocal microscope (Leica TCS SP5 II). Magnetic nanoparticles (MNPs) modified with sulfhydryl groups (3.0~4.0 μm, 10 mg mL^{-1}) (SH-MNPs) and a magnetic rack were obtained from BaseLine Chrom Tech Research Centre (Tianjin, China).

Synthesis of AuNCs

AuNCs were synthesized by the galvanic replacement reaction between Ag nanocubes and chloroauric acid ($HAuCl_4$).[2] Briefly, 400 μL of silver nanocubes was dispersed in 10

mL water containing 1 mg mL^{-1} PVP in a 50 mL flask under magnetic stirring. The dispersion was then refluxed for 10 min before 6.0 mL of 0.2 mM $HAuCl_4$ aqueous solution was added to the flask at a rate of 0.8 ml of solution per minute. The mixture was refluxed for another 10 min until its LSPR peak had reached about 780 nm. After cooling down to room temperature, the sample was centrifuged and washed with saturated NaCl solution before characterization by TEM. The obtained AuNCs were finally redispersed in deionized water for further use. UV-visible-near-IR absorbance spectrum of AuNCs (Fig. S1) was provided here to show the corresponding surface plasmon resonance peak of the AuNCs. The result proved that the synthesized AuNCs had a near-infrared LSPR peak at 782.94 nm, indicating that the hollow structure of AuNCs.

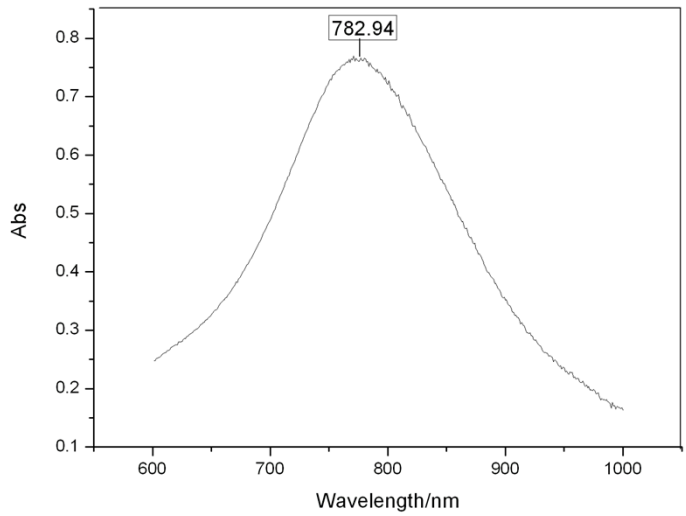

Fig. S1 UV-visible-near-IR absorbance spectrum of AuNCs.

Assembly of the AuNCs onto SH-MNPs

To improve the separation process, magnetic nanoparticles (MNPs) modified with sulfhydryl groups (SH-MNPs) were used to combine with the as-prepared AuNCs. After washing 10 μL SH-MNPs (10 mg mL^{-1}) with 200 μL of 0.01 M PBS buffer (pH 7.4), magnetic separation was performed. 200 μL of the as-prepared AuNCs suspension was added into the washed SH-MNPs, and the mixture was agitated overnight at room temperature.

Modification of the AuNCs with poly(diallyl dimethylammonium) chloride (PDDA)

Magnetic separate and wash the obtained AuNCs-MNPs with 200 μL PBS. 200 μL of PDDA solution (5.832 mg/mL) was added, and incubated in constant temperature oscillator at 37°C for 12 h.

Loading the positive charges-modified AuNCs with RhB

Magnetic separate and wash the obtained complexes DNAs-AuNCs-MNPs with 200 μL of 0.01 M PBS buffer (pH 7.4). Then, the washed complexes were soaked in 100 μL of PBS solution (pH 7.4) containing 1.0×10^{-5} M RhB for loading cargo molecules RhB. The complexes were incubated in constant temperature oscillator at 37°C for 12 h.

Fabrication of the controlled-release bio-system

In order to encapsulate the loaded RhB inside the AuNCs, ATP aptamers were used to cap the holes of the RhB-loaded AuNCs. 10 μL of ATP aptamers was added into the above solution. The complexes were incubated in constant temperature oscillator at 37°C for 12 h. After that, the obtained conjugates were washed with PBS. The controlled-

release bio-system is completed. Fig. S2 shows the fluorescent signals of RhB released from the hollow interiors of AuNCs blocked with 1.0 × 10^{-4} M ATP aptamers. Curve b shows the fluorescent signal of released RhB in the absence of ATP. As indicated in Fig. S2, significantly enhanced fluorescent signal was indeed observed in the presence of 5.0 × 10^{-4} M ATP (curve a).

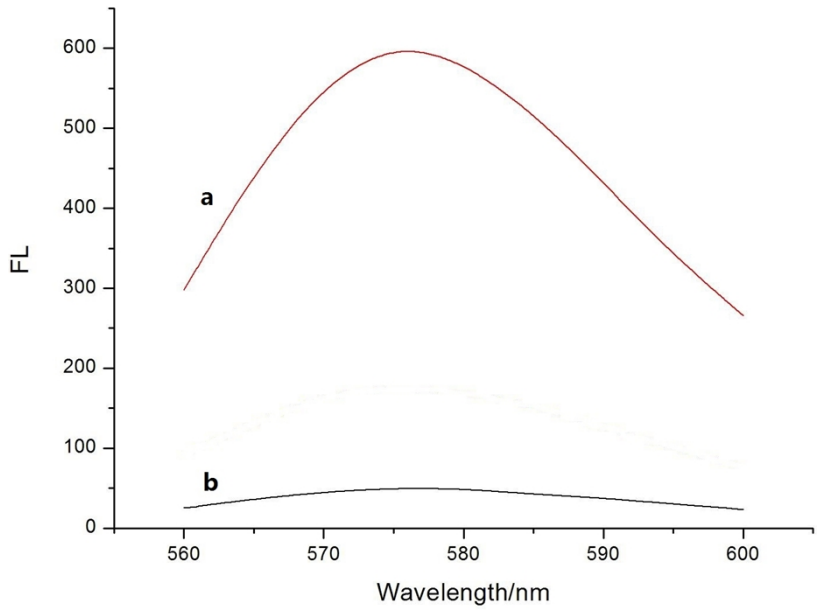

Fig. S2 Fluorescent signal of the solution in the absence (b) and in the presence of 5.0 × 10^{-4} M of ATP (a).

In order to confirm that positive charge PDDA has been bound on the surface of AuNCs, a control experiment was implemented. The AuNCs modified without PDDA was used as a control under the same experimental conditions. The results indicated that the fluorescence intensity of RhB released from the AuNCs modified with PDDA was

significantly higher than that of the AuNCs modified without PDDA. It confirmed that PDDA could be modified on the surface of the AuNCs.

Detection of ATP by the controlled-release bioassay

To test the ability of *in vitro* probing of the controlled-release bioassay, we performed the detection of ATP by employing the fabricated system blocked with 1.0×10^{-5} M ATP aptamers. ATP solution was added to the bio-system and the obtained mixture was subjected to incubation for 1 h at 37℃ to ensure the full reaction between the aptamers and ATP. Because of the specificity recognition reaction of ATP and ATP aptamers, the ATP aptamers could be taken away from the AuNCs surface, the pores could be opened and the cargo molecules could be released. After incubation for 1 h at 37℃, magnetic separation was performed and the supernatant was collected for fluorescence detection with an excitation wavelength at 530 nm and an emission wavelength at 573 nm.

Fig. S3 shows the fluorescence intensity of RhB released from the hollow interiors of AuNCs toward different concentrations of ATP (0, 1.0×10^{-10}, 5.0×10^{-10}, 8.0×10^{-10}, 1.0×10^{-9}, 2.0×10^{-9}, 5.0×10^{-9}, 6.0×10^{-9}, 8.0×10^{-9}, 1.0×10^{-8}, 2.0×10^{-8}, 5.0×10^{-8} M). As shown in Fig. S3, the fluorescence signal increased with the increase of the concentration of ATP, and then reached equilibrium at 1.0×10^{-8} M ATP or more.

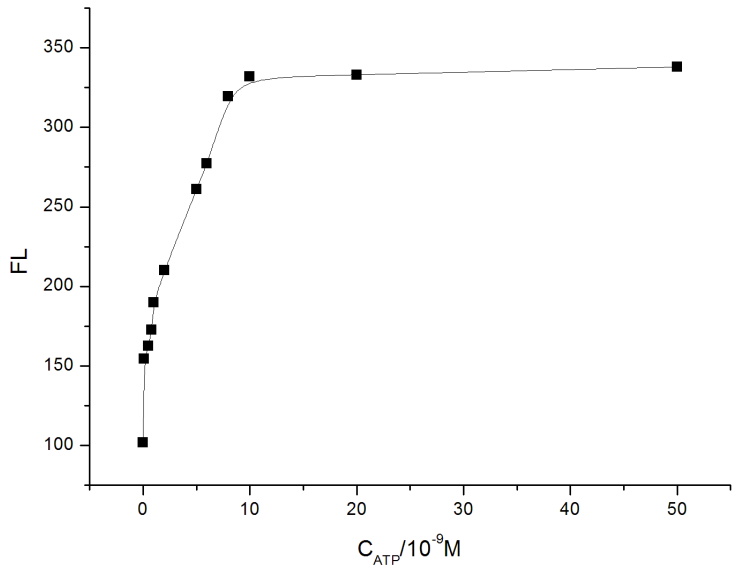

Fig. S3 The fluorescence intensity toward different concentrations of ATP (0, 1.0×10^{-10}, 5.0×10^{-10}, 8.0×10^{-10}, 1.0×10^{-9}, 2.0×10^{-9}, 5.0×10^{-9}, 6.0×10^{-9}, 8.0×10^{-9}, 1.0×10^{-8}, 2.0×10^{-8}, 5.0×10^{-8} M).

Determination of ATP in a Cultured Cell Extract

Ramos cells were applied to evaluate the capability of the proposed bioassay in biological samples. A suspension of 1.0×10^5 Ramos cells dispersed in 1.0 mL of RPMI cell media buffer was centrifuged at 2000 rpm for 5 min and washed with 200 μL of PBS (0.01 M, pH 7.4) some times and resuspended in 200 μL of PBS. Finally, the cells were disrupted by sonication for 20 min at 0°C. To remove the homogenate of cell debris, the lysate was centrifuged at 18 000 rpm for 20 min at 4°C. The cell supernatants were collected for intracellular ATP detection after centrifugation. 10 μL of the cell lysate was added into the bio-system, and the mixture was diluted to 200 uL with PBS (0.01 M, pH 7.4). The obtained solution was subjected to incubation at 37°C for 1 h to ensure the full reaction between the aptamer and ATP. After magnetic separation, the supernatant

was collected for fluorescence detection with an excitation wavelength at 530 nm and an emission wavelength at 573 nm. Fig. S4 shows the fluorescence intensity of RhB released from the hollow interiors of AuNCs toward the Ramos cell lysate (a) and PBS (b). The experimental results demonstrated that the fluorescence signal was significantly enhanced as compared with PBS control.

Fig. S4 The fluorescent signal of RhB released from the hollow interiors of AuNCs toward Ramos cell lysate (a) and PBS (b).

Fluorescence microscopy imaging of HCT 116 colon cancer cells

The fluorescence microscopy imaging was performed by incubating HCT 116 colon cancer cells with the bio-system in growth media for 1 h at 37°C. The AuNCs modified with random DNAs was used as a control under the same conditions. A laser confocal microscope (Leica TCS SP5 II) was employed for fluorescence microscopy imaging.

References

(1) Y. C. Wang, J. B. Xu, X. H. Xia, M. X. Yang, S. Vangveravong, J. Y. Chen, R. H. Mach and Y. N. Xia, *Nanoscale*, 2012, **4**, 421.

(2) W. Wang, T. Yan, S. Cui, and J. Wan, *Chem. Commun.,* 2012, **48**, 10228.