Electronic Supplementary Information

Isotopic Core-Satellites Enable Accurate and Sensitive Bioassay of Adenosine Triphosphate

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EXPERIMENTAL SECTION

Instruments

A NexION 350X inductively coupled plasma mass spectrometer (ICPMS, PerkinElmer, Inc.) was used in this study. The ICPMS experimental conditions were optimized according to the instrument manual and summarized in Table S1. The adjustable pipettors (Dragon Laboratory Instruments, China) were employed for transferring solutions. A JEM-2010 transmission electron microscopy (TEM) was utilized for observing the morphology of the synthesized Au NPs and core-satellites structure.

Reagents

Deionized water (DIW, 18.2 MΩ cm^{-1}) was acquired from a Milli-Q Integral water purification instrument. All glass ware was firstly soaked in aqua regia solution, rinsed with DIW, and dried in a vacuum oven. Surface activated magnetic beads (carboxylic acid) were acquired from Baseline ChromTech Research Centre (Tianjin, China). The oligonucleotides (DNA1: 5'-NH_2-T_{10}-ATTAAAGCTCGCCATCAAATAGCTGC-3', DNA2: 5'-SH-T_{10}-GCAGCTATTT-3' and T_{10} SH-DNA: 5'-SH-TTTTTTTTTT-3') were provided by Shanghai Sangon Inc. ATP Bioluminescence Detection Kit (LF101) was obtained from Tianjin Yuanping Biotechnology Co. Ltd. (Tianjin, China). Adenosine triphosphate, bovine serum albumin (BSA), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 3-(N-morpholino)-propane sulfonic acid (MOPS), and imidazole were obtained from Beijing Solarbio Science and Technology Co. Ltd. (Beijing, China). Chloroauric acid hydrate ($HAuCl_4 \cdot 4H_2O$) and Tris-(hydroxymethyl) methyl amino methane were obtained from Aladdin Biochem Technology Co. Ltd. (Shanghai, China). Tris(2-carboxyethyl)phosphine and other reagents were all purchased from Changzheng Chemical Reagent Inc. (Chengdu, China). 0.1 M imidazole (PH=7.0), 10 mM MOPS (2 mM $MgCl_2$, 150 mM NaCl,

PH=7.6), 10 mM PBS (100 mM NaCl, PH=7.4), 20 mM Tris-HCl (PH=7.4) and washing buffer (7 mM Tris, 0.17 M NaCl, 0.05% Tween20, PH=8.0) were used in this work.

Human serum samples were obtained from Chengdu Seventh People's Hospital; A549 cells, L929 cells and Hela cells were obtained from the Cell Resource Center, IBMS, CAMS/PUMC.

Preparation of Au NPs

Briefly, after boiling 100 mL 0.01% (m/v) $HAuCl_4 \cdot 4H_2O$ with 2.0mL, 1.0 mL, 0.5mL, and 0.3mL of 1% (m/v) trisodium citrate for 30 min in aqueous solution, the resulted AuNPs suspension was cooled down and stored in 4 ^oC. The diameters of AuNPs were averagely 14 nm, 18 nm, 24 nm, 44 nm, as confirmed by TEM and dynamic light scattering.

Preparation of Au NPs-DNA2 Conjugates

Au NPs-DNA2 conjugates were prepared by immobilizing the thiolated DNA on Au NPs. Briefly, 31 μL of thiolated DNA2 (100 μM) was activated by 3.1 μL 10 mM TCEP for 5 min and then incubated with 1 mL Au NPs solution and 62 μL of thiolated T_{10} DNA (100 μM) at room temperature for 12 h. 50 μL 1% Tween-20 was added in the solution to stabilize Au NPs. During the salt aging progress, the concentration of NaCl was increased to 0.3 M by slowly adding 2 M NaCl into the bottom of glassware for three times in 24 h. The unreacted DNA strands were removed from mixtures by centrifugation. The resulted AuNPs-DNA2 conjugates were washed with PBS, and redispersed in 0.5 mL PBS.

Preparation of MBs-DNA1 Conjugates

50 μL of 10 mg mL^{-1} carboxyl-modified MBs was transferred into a 2 mL centrifuge tube. The MBs were washed with 100 μL of 0.1 M imidazole-HCl buffer (pH=7.0) for three times, suspended to a final volume of 1100 μL in 0.1 M imidazole-HCl buffer solution containing 0.1 M EDC, and incubated at 37 ^oC for 30 min. Then 140 μL 10 μM DNA1 was added and incubated for 2 h. The MBs-captured

probe was washed with washing buffer for three times, and incubated with 1 mL 10% BSA for 1 h to minimize the nonspecific adsorption. The resulting capture DNA labeled MBs were magnetically separated, washed, resuspended in 100 μL 10 mM MOPS buffer, and kept in 4 ^{o}C.

Assay Procedure

For the construction of the core-satellites structure (Fig. 1), 3 μL of core MBs-DNA1 and 6 μL satellites AuNPs-DNA2 were mixed with 7 μL 10 mM PBS (PH=7.4, 150 mM NaCl) and 33 μL 20 mM Tris-HCl (PH=7.4, 100 mM NaCl). After several minutes gentle shaking, 20 μL various concentrations of ATP was added to the solutions, respectively. After the incubation for around 4 h with gentle shake, the supernatant was magnetically separated from the solution. The supernatant was digested by 50 μL aqua regia for 20 min and diluted to 4 mL by 1.5 ng mL^{-1} In element solution in DIW. Finally, the $^{197}Au/^{115}In$ signal ratio was recorded by ICPMS for the supernatant.

Cell Culture

A549 cells, L929 cells and Hela cells were cultured in DMEM medium supplemented with 100 IU/mL penicillin-streptomycin and 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells were passaged by trypsinization with 0.25% trypsin in PBS buffer.

Preparation of Cellular Lysate.

6 x 10^6 cells were washed with PBS buffer and harvest by 0.25% trypsin (2.2 mM EDTA, 1 x sodium bicarbonate). Then, cells were centrifuged for 5 min, 1200 rpm at 4 ºC and washed with DMEM medium. After the cell pellet was suspended in 400 μL of lysis buffer, the cell suspension was incubated at room temperature for 15 minutes. Then, the lysed cell suspension was mixed and centrifuged at 12,000 rpm for 15 minutes at 4 °C, and the supernatant was collected.

RESULTS AND DISCUSSION

Optimization of Experimental Conditions

The experimental parameters, including the diameter of AuNPs satellites, the reaction buffer, the ratio of AuNPs satellites to MBs core, and reaction temperature, were investigated in detail to realized high performance ATP assay.

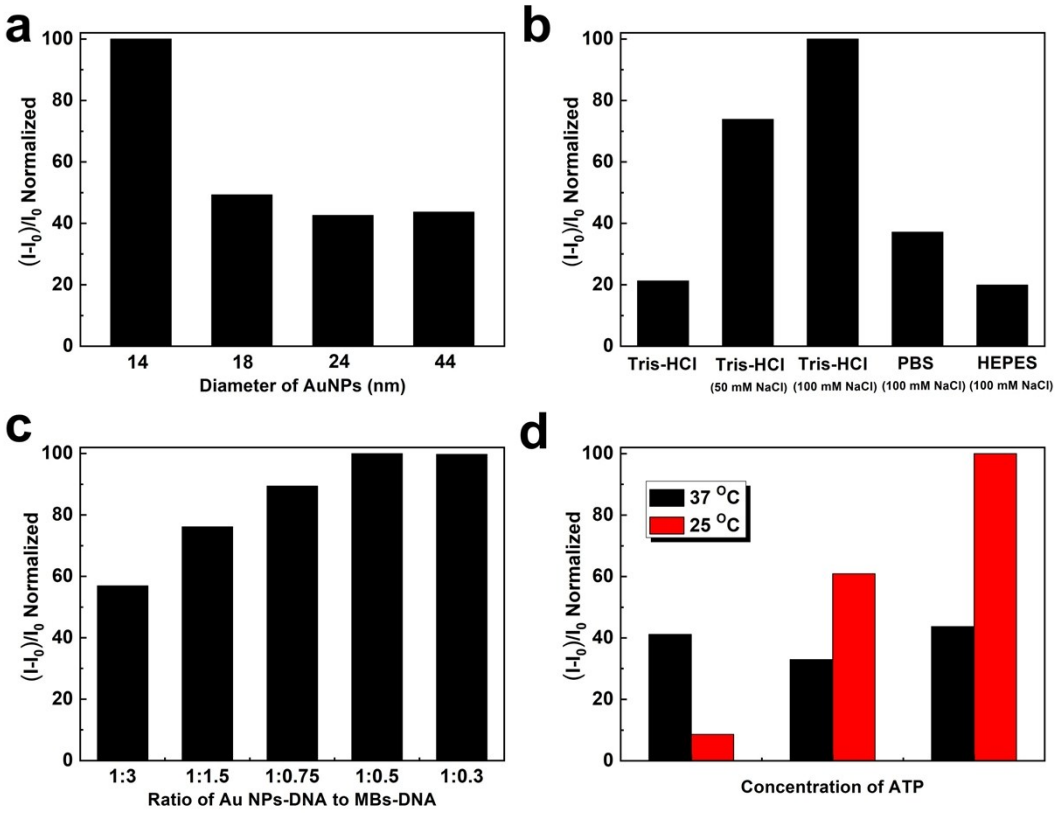

Fig. S1. Optimization of experimental parameters. The effect of the diameter of AuNPs satellites (a), the reaction buffer (b), the ratio of AuNPs satellites to MBs core (c), and reaction temperature (d).

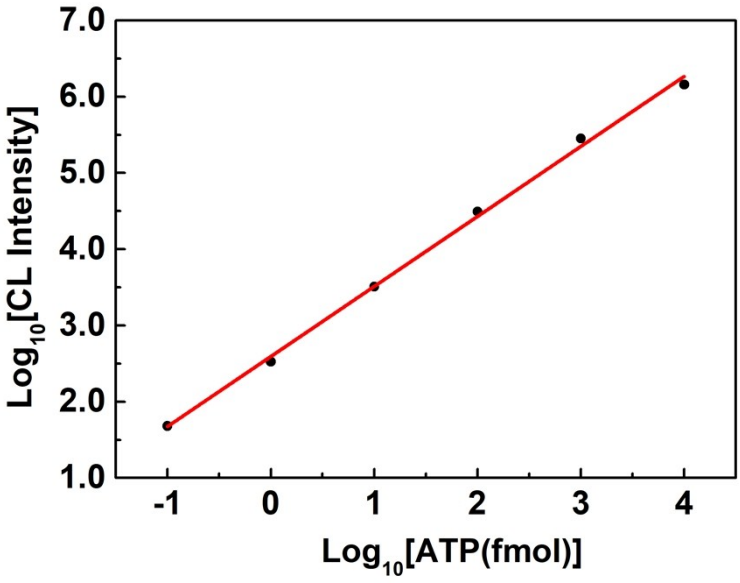

Fig. S2 the calibration curve between bioluminescence and amount of ATP.

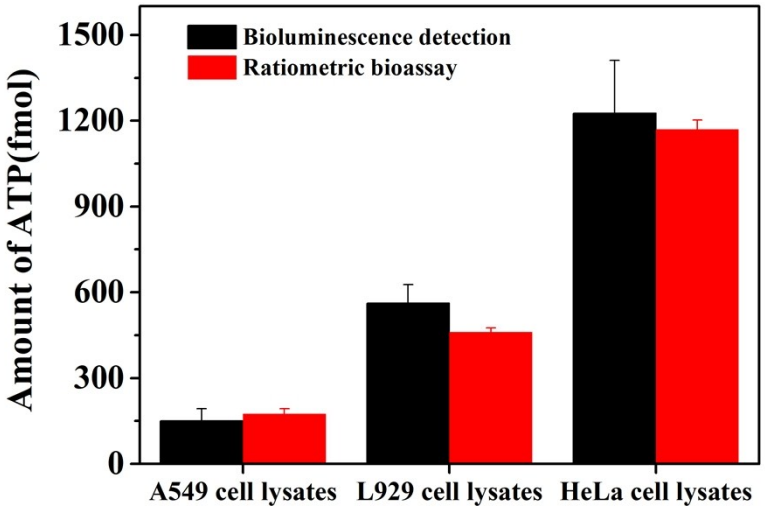

Fig. S3 The amount of ATP in the three cell lysates detected by the bioluminescence and ratiometric bioassay.

As shown in Fig. S4, the maximum absorption wavelength of AuNPs-DNA is red-shifted, which proves that DNA is successfully conjugated with AuNPs.

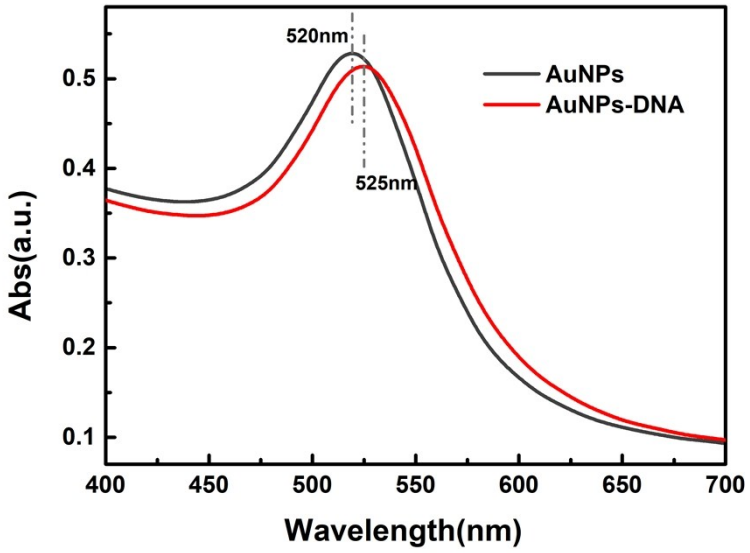

Fig. S4 The UV-vis absorption spectrum of AuNPs (black line) and AuNPs-DNA (red line).

As shown in Fig. S5, the zeta potential of MBs is -32 mV because the surface of the magnetic beads is modified by a large number of carboxyl groups. After reacting with DNA, the zeta potential of MBs-DNA is more negatively caused by negative charge of DNA. Fig. S5 and Fig. 2b demonstrate that DNA is successfully conjugated with MBs.

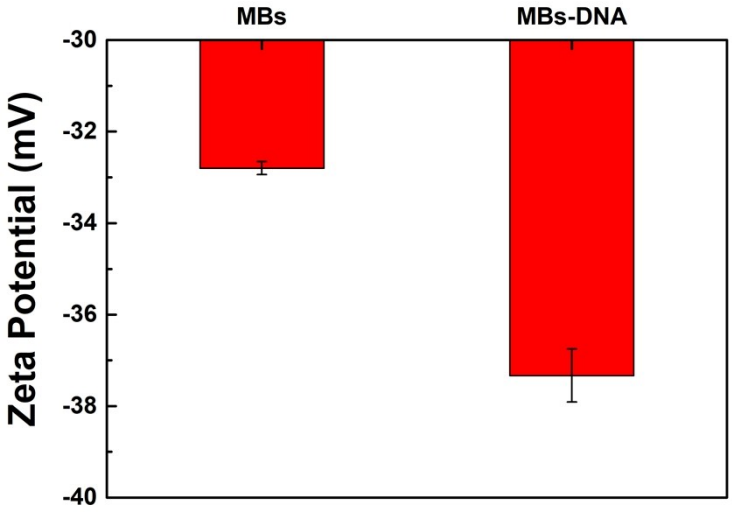

Fig. S5 The zeta potential of MBs and MBs-DNA at the same concentration.

Table S1. The ICPMS operating parameters.

Parameters	**Settings**
Radiofrequency power	1300 W
Plasma gas flow	18 L min^{-1}
Auxiliary gas flow	1.2 L min^{-1}
Nebulizer gas flow	0.94 L min^{-1}
Analog stage voltage	-1700 V
Pulse stage voltage	800 V
Deflector voltage	11.8 V
Cell entrance voltage	-4 V
Isotope monitored	^{197}Au and ^{115}In

Table S2. The comparison of the analytical performance of ratiometric bioassays for ATP detection.

Method	**Settings**	**Linear range**	**Detection limit**	**Ref.**
Fluorescent	$F_{575–720}/F_{725–900}$	0 –90 μM	1 nM	[1]
Fluorescent	F_D/F_A	0.1–100 nM	0.08 nM	[2]
Fluorescent	I_{580}/I_{530},	0.1 – 10 μM	0.1 μM	[3]
Fluorescent	$\Phi_{control}/\Phi_{test}$	100 μM – 2 mM	69 μM	[4]
ICPMS	^{197}Au /^{115}In	0.7–100 nM	0.1 nM (2 fmol)	This work

References

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