Supporting Information

Single Nanoparticle Analysis-based CRISPR/Cas12 Bioassay for Amplification-Free HIV Detection

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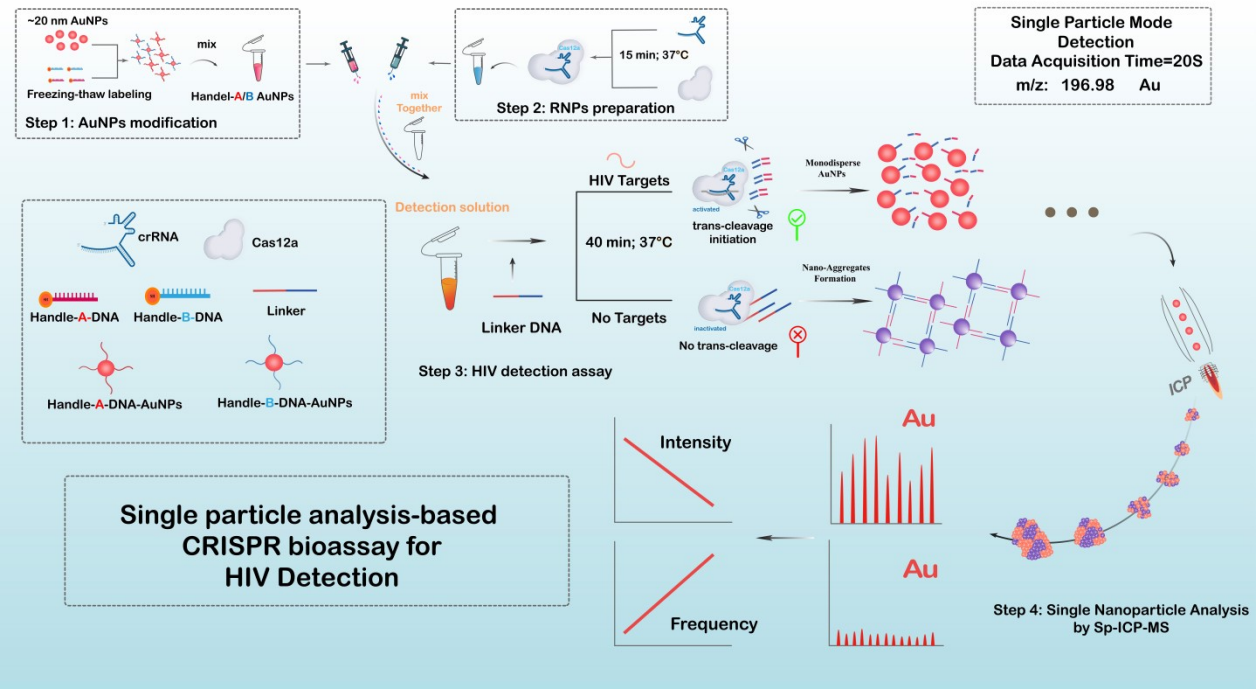
Regents

All oligonucleotides (shown in Table S1) were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China). The Cas12a was supplied by Tolo Biotech Co., Ltd (Shanghai, China). Sodium chloride solid (NaCl, for acs, ≥99.9%), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$, AR, ≥99.9%), sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$, ≥ 99.5%), sodium Citrate (98%, Adamas) , and Chloroauric acid hydrate ($HAuCl_4 \cdot xH_2O$) were obtained from Titan Scientific Co. Ltd (Shanghai, China). Tris-HCl stocking solution (1 M, pH=7.5, sterile), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$, molecular biology grade), and 10% Tween-20 were all supplied by Sangon Biotechnology Co. Ltd. (Shanghai, China). Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP). The Cas12a proteins and NEBuffer 2.1 buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 100 μg/mL BSA, pH 7.9) were purchased from New England Biolabs (Ipswich, MA, USA). Ultrapure water with 18.24 MΩ cm-1 was utilized from the UPURE Sichuan water purification system. Cell culturing high glucose medium (Dulbecco's Modified Eagle Mediumg), fetal bovine serum (FBS), penicillin-streptomycin solution, and insulin were purchased from Thermo Fisher Scientific Inc (Fremont, USA). Water used in the RNA-related application was DEPC-treated nuclease-free water purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Serum samples were provided by Seventh People Hospital Chengdu, Sichuan, China. Human embryonic kidney (HEK293) cell lines were provided by West China Hospital Chengdu, Sichuan, China.

Instruments.

A PerkinElmer NexION 350 quadrupole inductively coupled plasma mass spectrometer was applied throughout the experiment. The detailed working conditions and parameters of the ICPMS apparatus are included in Table S2. The annealing process of preparation of hairpin DNAs was performed with the assistance of K960 Thermal Cycler (Heal Force Inc., China). Transmission electron microscopy (TEM) characterization was conducted by Tecnai G2 F20 S-TWIN (FEI., USA). The UV-vis spectra for noble metal nanoparticle characterization were carried out by Cytation 5

Cell Imaging Multi-Mode Reader (BioTek Instruments, Inc). Dynamic light scattering (DLS) and zeta potential for the conformation of noble metal nanoparticle labeling processes were operated by Malvern Zetasizer Nano ZS90 (Malvern PANalytical Ltd., Shanghai, China). The Fluochem M (Cell Biosciences, Santa Clara, CA) was introduced for the electrophoresis imaging experiment. Countstart Biotech cell analyzer (Alit Biotech Co., Ltd, Shanghai, China) was applied for counting cell numbers.



Scheme.S1. The detailed illustration of single nanoparticle analysis-associated CRISPR bioassay for HIV detection.

Synthesis of 25 nm Gold nanoparticles.

Based on the previously reported synthesizing approaches of the citrated-protected AuNPs with slight modification, we successfully synthesized gold nanoparticles with diameters of 25 nm. [1,2] All glass apparatus was thoroughly washed with ultrapure water, then sank into aqua regia for 1 h. Later, 50 ml 0.01% $HAuCl_4$ (w/v) was heated until boiling in a three-necked flask. After boiling it for 20 min, 1.2 mL 1% (w/v) trisodium citrate solution was quickly injected into the boiling $HAuCl_4$ solution. After boiling for 30 min, a deep magenta color solution was obtained very quickly. The solution was allowed to slowly cool down to room temperature and stored under 4 °C for later use.

Gold nanoparticle concentrations calculation.

An external standard method was applied for measuring the metal-ion concentrations of the above synthesized and AuNPs-DNAs after digesting by aqua regia (v_{HNO3}: v_{HCl} = 3:1). According to our previous work and reported literature, the molar concentrations of AuNPs and AuNPs-DNAs were calculated by using equation listed below[3]:

$$C_{AuNPs} = \frac{6C_{ion}}{\pi \rho_{Au} N_A D^3}$$

C_{ion} means ion concentration after digestion of nanoparticles after digestion. C_{AuNPs} represented the concentration of DNA-AuNPs, N_A is the Avogadro constant, ρ_{Au} is the density of gold, and D represents the diameters of AuNPs which charactered by TEM.

Serum Samples Preparation and Spiked Recovery Test.

The first step involves subjecting the serum to centrifugation at 14000 rpm under 4°C for 20 minutes. After that, the supernatant was carefully collected and subsequently diluted by a factor of 100. Next, samples containing varying concentrations of HIV (0 pM, 1000 pM, and 5000 pM) were spiked to the serum matrix. Next, the as-proposed method was used to assess HIV levels within the serum.

Surface Modification of gold nanoparticles.

The handle-DNA modified (thiol and biotin-labeled) $Handle_A$/$Handle_B$-DNAs were employed to prepare $Handle_A$/$Handle_B$-DNA-AuNPs through a freeze-thaw labeling approach with mild modification.[4] In brief, 1 nmol $Handle_A$/$Handle_B$-DNA were treated with 20-fold excess molar ratio Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) for 1 h. The TCEP-treated $Handle_A$/$Handle_B$-DNA were added to 1 mL synthesized AuNPs. Later, the as-prepared $Handle_A$/$Handle_B$-DNA-AuNPs were set under -20°C overnight. Next, the $Handle_A$/$Handle_B$-DNA-AuNPs were allowed to wash three times with PBST (10 mM PB; 150 mM NaCl; 0.01% Tween-20; pH 7.4) through centrifugation (10 minutes at 4°C, 10000 rpm) to eliminate any unconjugated $Handle_A$/$Handle_B$-DNA. The collected AuNPs was resuspended in the PBST for later use.

Detection of HIV target by Nanoparticle analysis Enhanced CRISPR bioassay.

The same amount of Cas12a protein and crRNA were added in 1× NEBuffer r2.1, and the mixture was then incubated at 37°C for 20 minutes to facilitate the synthesis of Cas12a/crRNA ribonucleoprotein complexes (RNPs). Subsequently, 100 nM of the Cas12a-based RNPs, 1.5 μM of linker DNA substrates, and HIV targets with different concentrations were mixed together to initiate the trans-cleavage activities of Cas12a-based RNPs. After allowing the cleavage reaction to proceed for 30 minutes at 37°C, the temperature was then increased to 85°C for 5 minutes to terminate the trans-cutting activity. After that, the same concentrations of $Handle_A$/$Handle_B$-DNA-AuNPs were added and incubated at 50°C for 25 minutes (1500 rpm). After thorough dilution, the resulting AuNP products in different states are ready for single-particle analysis.

Cell Resuscitation.

Pre-heat a water bath to 37°C and prepare 15 mL centrifuge tubes and culture flasks. Prepare complete culture medium for HEK293 cells by mixing 45 mL DMEM medium, 5 mL FBS, 50 μL of 10 mg/mL insulin, and 500 μL of penicillin-streptomycin solution (5,000 U/mL). Mild agitate the thawed cell cryovial in the water bath until the freezing medium is fully dissolved, then transfer the cells to a 15 mL centrifuge tube containing 3 mL of complete culture medium, mixing thoroughly. Centrifuge at 1200 rpm for 5 minutes, discard the supernatant, add 1 mL of complete culture medium, mix

thoroughly, and then transfer to a culture dish containing 7 mL of complete culture medium. Set the above cells in an incubator (incubation conditions are 37 °C, 5% CO_2).

Medium Change.

Change the culture medium every 48 h while observing cell density under a microscope. Use a pipette to discard the old culture medium, then wash the cells several times by adding calcium- and magnesium-free PBS (DPBS) along the edge of the dish. Subsequently, 8 mL of fresh complete culture medium was added and returned to the 37°C, 5% CO_2 incubator for continued culture.

Cell Passaging.

When cell density reaches approximately 80%, proceed with passaging. Prepare 0.25% trypsin-0.53 mM EDTA, complete culture medium, PBS, three culture dishes, and 15 mL centrifuge tubes in advance. First, remove the old culture supernatant by using pipettes. The cells were washed with 3 mL of DPBS (3 times), then 1 mL of trypsin was added and incubated in the incubator for 3 minutes. The digestion process can be observed under a microscope. Subsequently, digestion could be terminated by adding 3 mL of complete culture medium. Next, centrifuge at 1200 rpm for 5 minutes, discard the supernatant, and add 1 mL of complete culture medium, mixing thoroughly. Disperse the cell suspension into three culture dishes containing 10 mL of complete culture medium (1:3 passage ratio), mix well, and return to the incubator for continued culture.

Cell Cryo-preservation.

Prepare cryovials and prepare the freezing medium (90% FBS + 10% DMSO) in advance. First, digest the adherent cells into a single-cell suspension as described above. Then, count the cell numbers by using the Counter star analyzer. Finally, centrifuge the cell suspension, add an appropriate volume of above medium to the cell pellet, and transfer to cryovials. Place the cryovials in a programmed freezing container and store at -80°C for 6 hours before transferring them to liquid nitrogen for long-term storage.

Table S1. Sequences of all oligonucleotides used in this work

Names	Sequences（5' to 3'）
HIV	ACTGCTAGAGATTTTCCACAT
Linker	CACAAATCCTAAACGCACAACGAACATCAT
$Handle_A$-DNA	HS-(CH2)6-T10-ATGATGTTCGTTGTG
$Handle_B$-DNA	CGTTTAGGATTTGTG-T10-(CH2)6-HS
crRNA	UAAUUUUCUACUAAGUGUAGAUAUGUGGAAAAUCUCUAGCAG
HAV	HAVGGACTTGATACCTCACCGCC
HBV	ATACCACATCATCCATATAACTGAAAGCCA
HCV	ATCTCCAGGCATTGAGCGGGTTTATCCAGGA
HPV	AATATGTCATTATGTGCTGCCATATCTACTTCAGAAACT

Table S2. Working Conditions of sp-ICP-MS

Instrument Parameters.

Parameters	Values
Au (m/z)	197 (100%)
ICP RF Power (W)	1300
Plasma Gas Flow (L/min)	18
Auxiliary Gas Flow (L/min)	1.20
Nebulizer Gas Flow (L/min)	0.96
Deflector Voltage (V)	11.78
Pulse Stage Voltage (V)	1181
Analog Stage Voltage (V)	1917
Sample Uptake Rate (mL/min)	0.25
Dwell Time (μs)	200
Detecting Time (s)	20

Table S3. Comparison of the analytical performances of different approaches for HIV targets.

Strategy	Cas effectors	Amlification	Target	Linear range	LOD	Virus Positive Serum Analysis	Ref.
Fluorescence	N/A	Yes	HIV	2.7 nM-200 nM	2.7 nM	N/A	[5]
CHA-based electrochemiluminescence	N/A	Yes	HIV	1 fM-100 fM	300 fM	N/A	[6]
SERS	N/A	N/A	SARS-Cov-2	N/A	20 virus/μL	Demonstrated	[7]
Label-free Colorimetry	N/A	N/A	HIV	1 nM-1 μM	1.86 pM	Demonstrated	[8]
RT-RPA-based Fluorescence	Cas12a	Yes	HIV	N/A	63.9 copies/μL	Demonstrated	[9]
Sp-ICP-MS	N/A	N/A	miR-21; miR-155; miR-16	10 pM -300 pM 10 pM-300 pM 10 pM-200 pM	49 amol 51 amol 55 amol	Demonstrated	[10]
Sp-ICP-MS	Cas12a	Yes	miR-21	0.5 fM-100 fM	0.5 fM	N/A	[11]
Sp-ICP-MS	Cas12a	N/A	HIV	1 pM-10 nM	600 fM	N/A	This work

Table S4. Spiked recoveries in two biological matrixes

Samples (n=5)	Spiked (pM)	Detected (pM)	Recovery (%)	RSD (%)
#1 Serum sample	0	Not found	N/A	3.70
	1000	1071	107.1 (96.8-118.1%)	1.98
	5000	5011	100.2 (98.8-102.1)	0.130
# 2 Cell sample	0	Not found	N/A	2.70
	1000	1202	120.2 (119.5-121.2%)	1.08
	5000	4955.5	99.10 (97.5-100.1%)	0.178

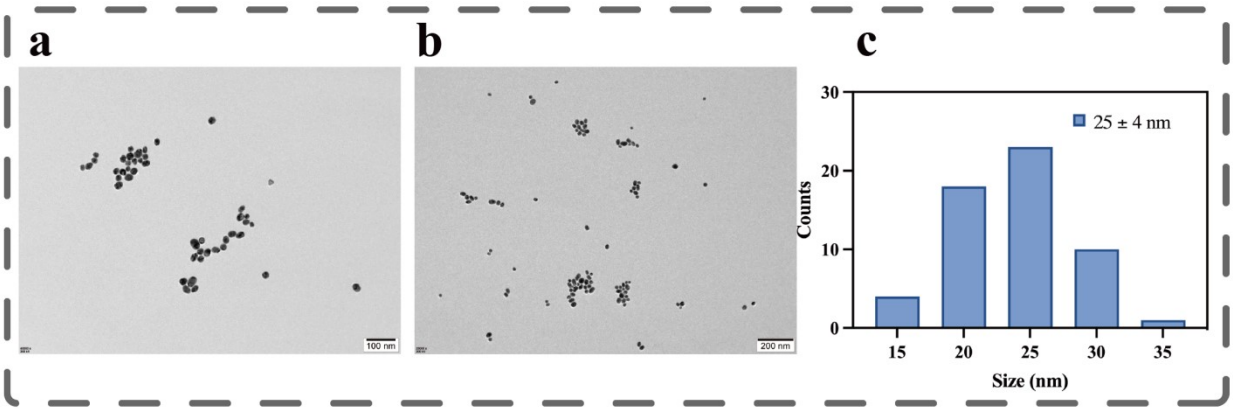


Fig.S1. (a)-(b) are the TEM characterization for Bare AuNPs in the different scales used during this work. (c) demonstrates the size evaluation of AuNPs processed by ImageJ software (1.53a version)

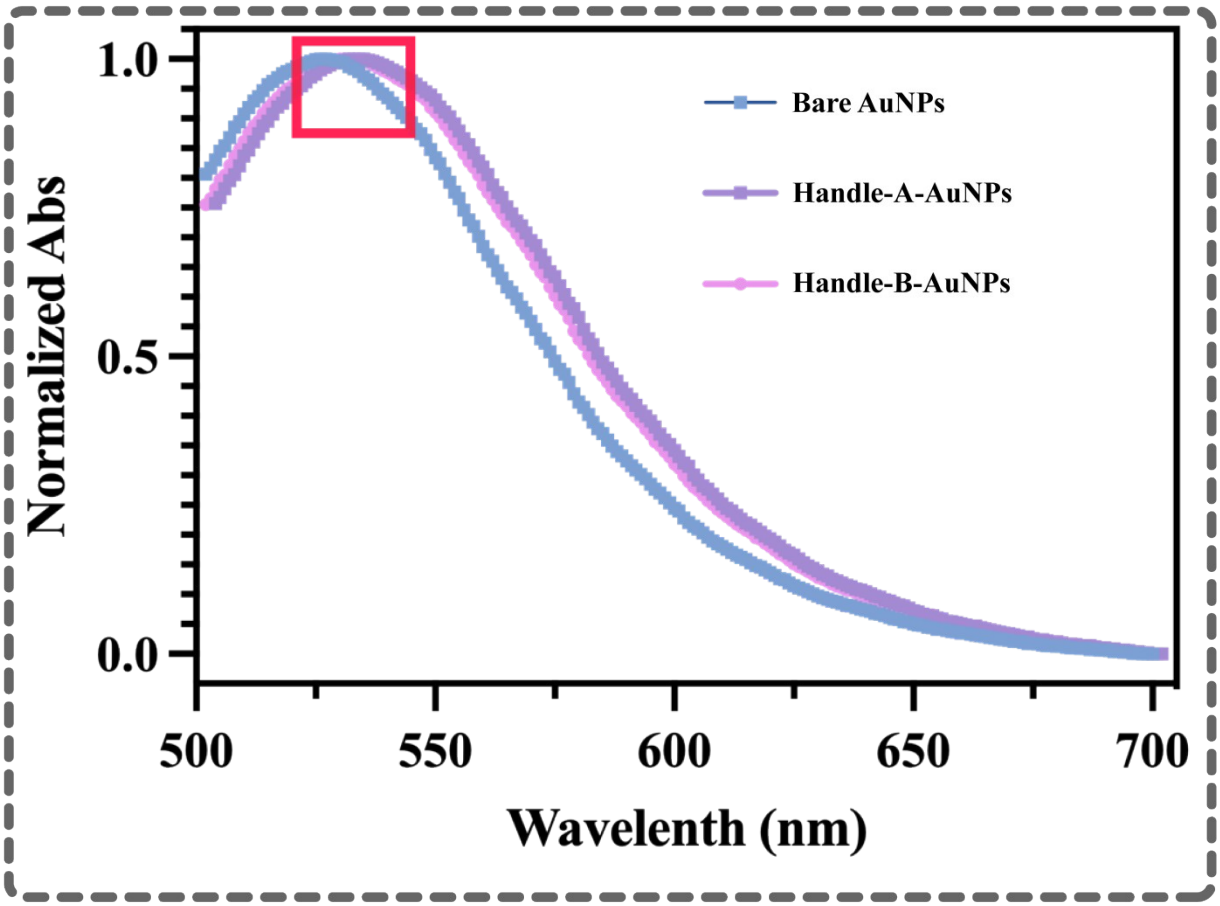


Fig.S2. The Uv-vis spectra characterization of labeling of $Handle_A$/$Handle_B$-DNA.

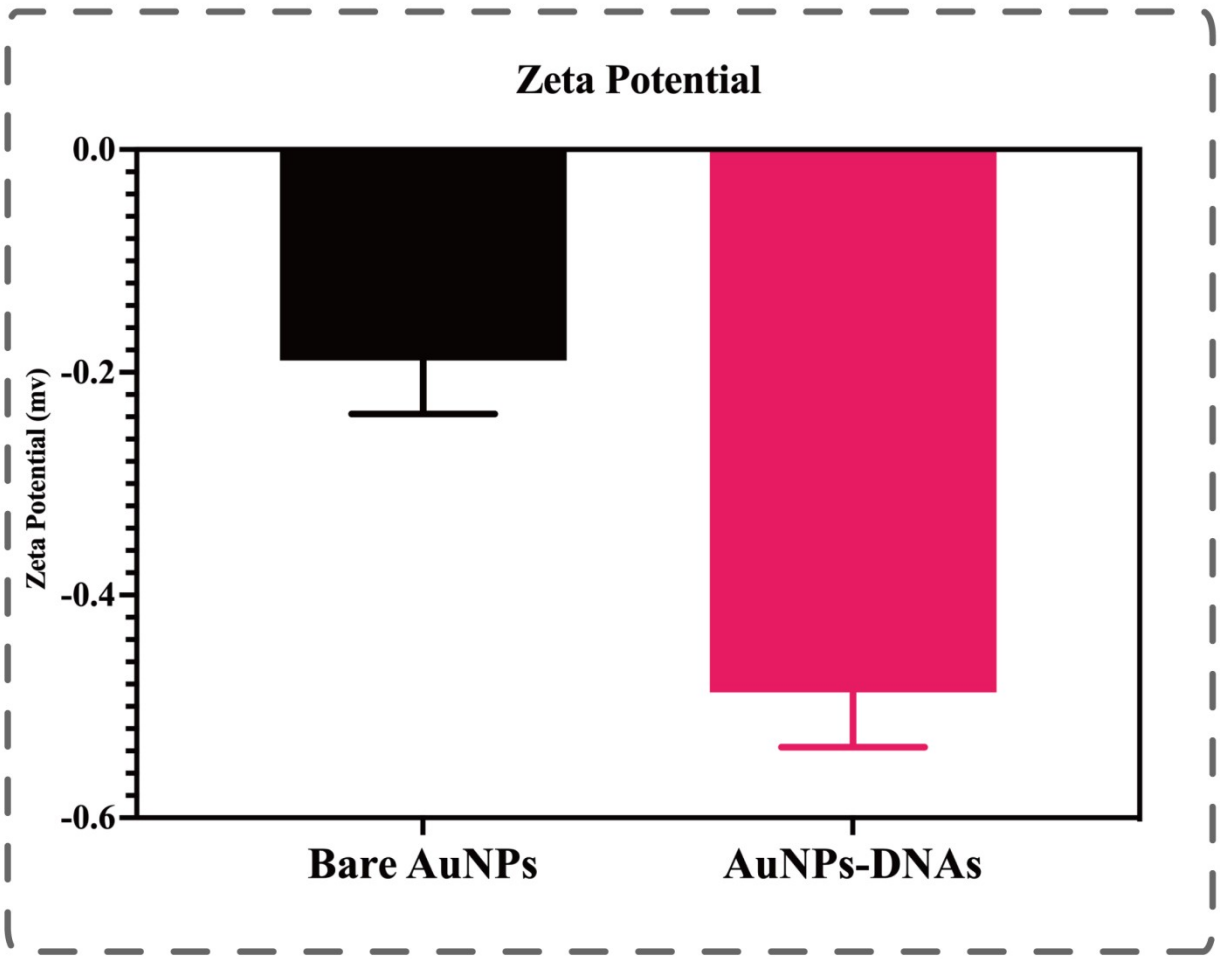


Fig.S3. Zeta potential characterization of labeling of AuNPs with $Handle_A$/$Handle_B$.

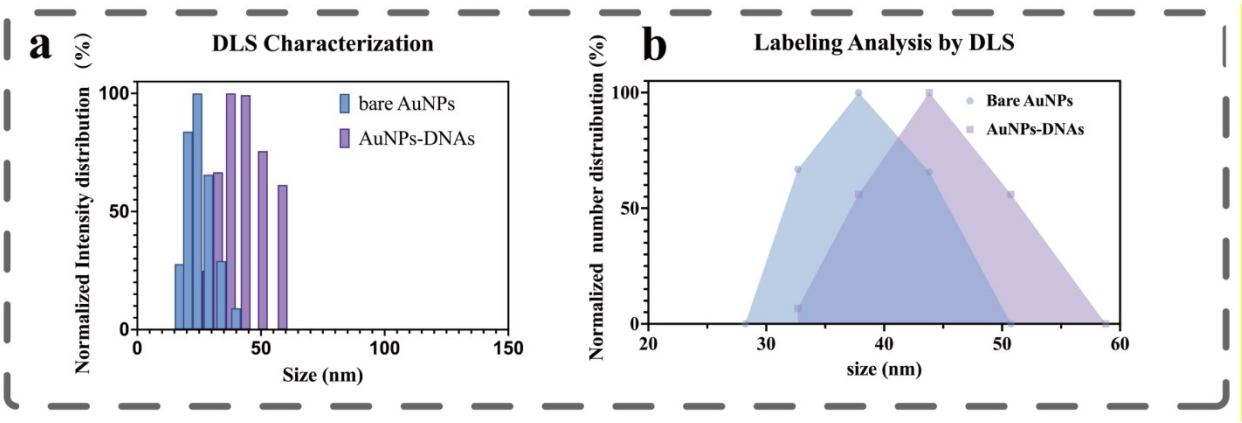


Fig.S4. Characterization of labeled AuNPs with $Handle_A$-DNA and $Handle_B$-DNA by DLS intensity distribution (left) and number distribution (right).

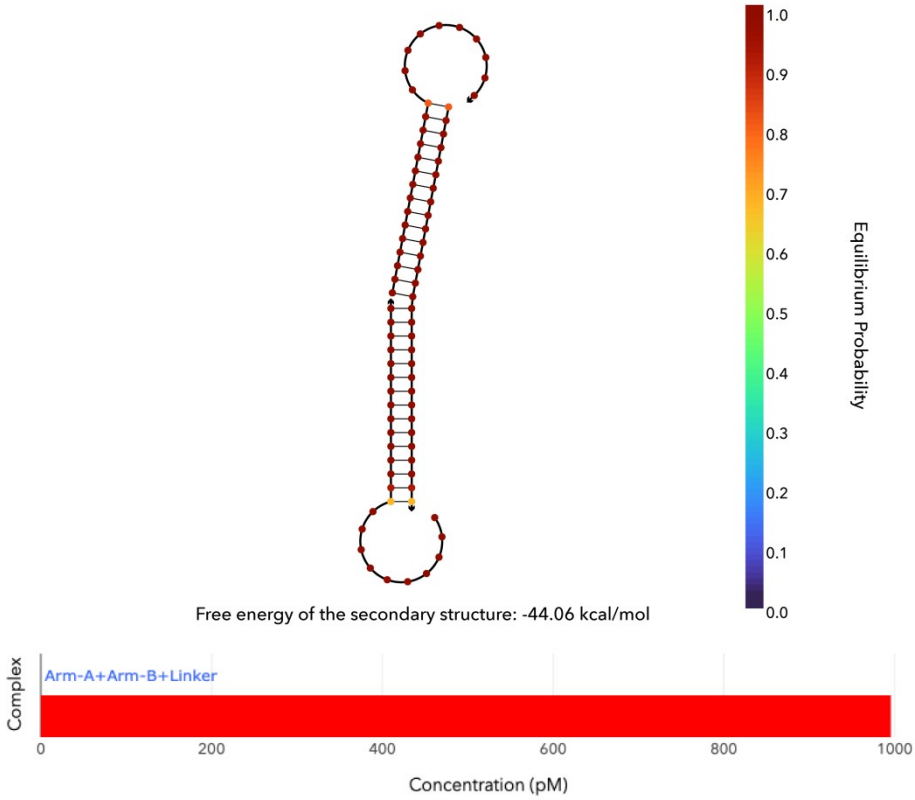


Fig.S5 NUPACK stimulation analysis of the cross-linking reaction upon $Handle_{A/B}$ and Linkers.

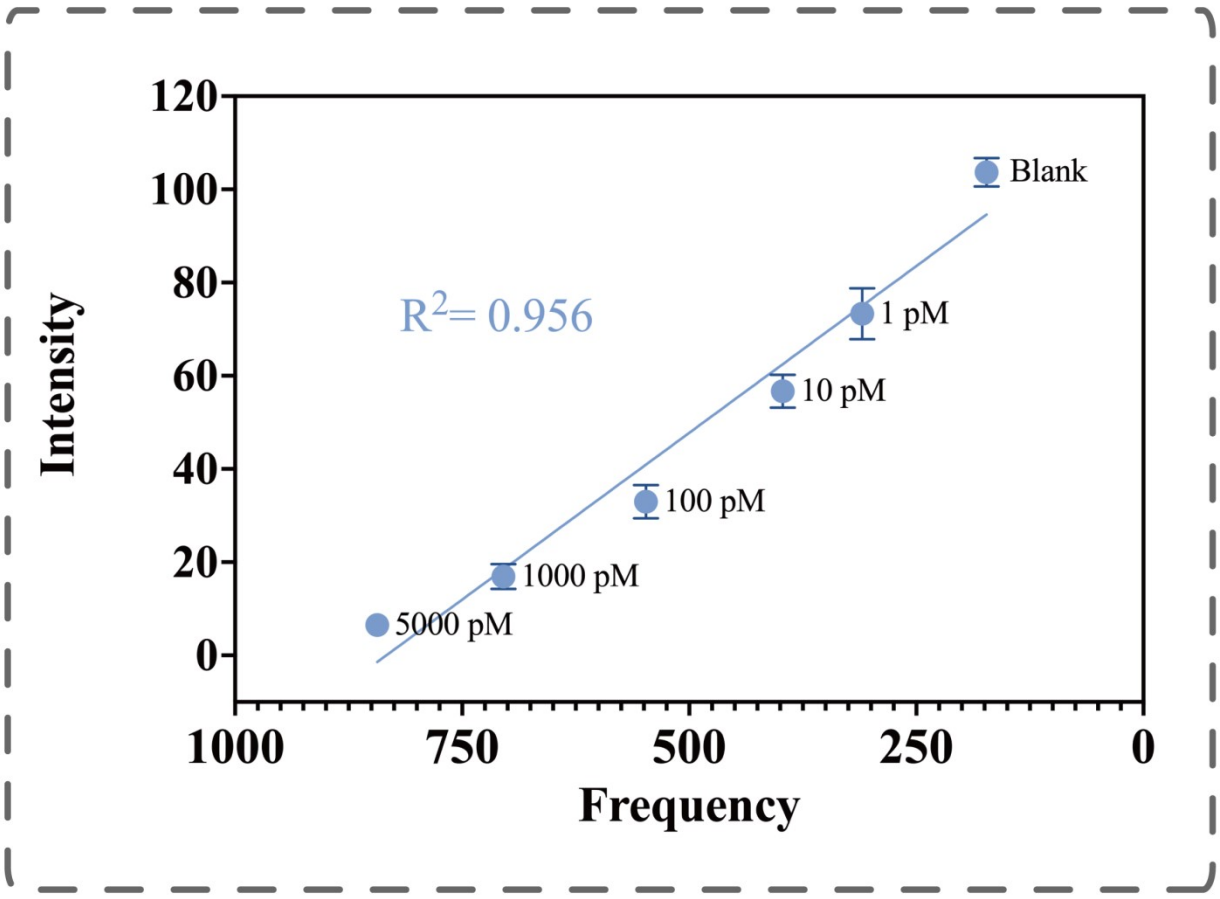


Fig.S6. The correlation of results obtained by two modes (Intensity Vs vs. Frequency).

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