Electronic Supplementary Information

Visual Detection of Single-Nucleotide Polymorphisms and DNA Methyltransferase Based on cation-exchange of CuS nanoparticles and Click Chemistry of Functionalized Gold Nanoparticles

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EXPERIMENTAL SECTION

Materials. Nuclease S1 and 5×S1 buffer (pH 4.5) were purchased from Thermo Scientific (USA). Dam MTase (Escherichia coli, 8000 U•mL^{-1}), 10 × dam MTase buffer, S-adenosyl-Lmethiolnine (SAM, 32 mM) and Dpn I endonuclease (20000 U·mL^{-1}) were ordered from New England Biolabs Inc. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid was obtained from Solarbio (Beijing, China). TBTA were purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Strepavidin modified magnetic beads (MBs, 1.0~2.0 μm) were purchased from Tianjin Baseline ChromTech Research Centre (China). Hydrogen tetrachloroaurate(III) tetrhydrate ($HAuCl_4$•$4H_2O$) and trisodium citrate were ordered from Sigma-Aldrich. 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and Sodium L-ascorbate (SA) was purchased from J&K Technology Co., Ltd. (Beijing, China). DNA oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and used without further purification. The DNA sequences are listed in Table 1. All other reagents were of analytical reagent grade and were used without further purification.

Table S1. Sequences of Oligonucleotides Used in the Experiments

Name	Sequence（5' to 3'）
Capture DNA1	biotin – GGC ACA AAC ACG CAC
Report DNA1	CTC AAA GCT GTT CCG TCC-C_6-NH_2
PolyA 1	AAAAAAAAAA-CA TA GCT CAT C –N=N=N
PolyA 2	AAAAAAAAAA-CA TA GCT CAT C – C≡CH
Wild DNA	GGA CGG AAC AGC TTT GAG GTG CGT GTT TGT GCC
Mutant C	GGA CGG AAC AGC TTT GAG GTG CCT GTT TGT GCC
Mutant T	GGA CGG AAC AGC TTT GAG GTG CTT GTT TGT GCC
Mutant A	GGA CGG AAC AGC TTT GAG GTG CAT GTT TGT GCC
Random DNA	GAA GAG ATG CAT CCC AGT GTA TAC AAC CTA CTA
Capture DNA2	biotin-GGACGGAACAGCTTTGATCGCGAACCGTTCCTTCGGTTC GCGATCAAAGCTGTTCCG
Report DNA2	NH_2- C_6 – CTC AAA GCT GTT CCG TCC

Synthesis of AuNPs

AuNPs were synthesized according to a previously described procedure.[1,2] Briefly, 50 mL of

0.01% $HAuCl_4$ solution was brought to reflux while stirring, and then 1 mL of a 1% trisodium citrate solution was added quickly. After refluxing for another 20 min, the color of the solution changed from pale yellow to deep red. Then, the solution was cooled to room temperature with stirring, and Au nanoparticles with a diameter of approximately 20 nm were obtained.

Preparation of CuS NPs.[3]

The general procedure for the synthesis of mercaptopropionic acid-stabilized CuS NPs was as follows. Typically, 15 μL 3-MPA was added to a solution of $Cu(NO_3)_2$ (50 mL, 0.4 mM), and the pH of the mixture was adjusted to 7.0 with 0.5 M NaOH solution. Then, this mixture was bubbled with N_2 for 30 min, and 50 mL of a 1.34 mM Na_2S solution was added dropwise. The reaction was continued for 24 h under a N_2 atmosphere until a dark-green solution was obtained. After dialyzing against distilled water for 48 h using a dialysis membrane with a MWCO of 7000, water-soluble CuS NPs were obtained.

Preparation of azide- and alkynyl- functionalized AuNPs[4,5]

40 μL of alkynyl modified **PolyA 2** (2.0×10^{-5} M) was added to 1 mL AuNP solutions (prepared according to the methods in the supporting information). After thorough mixing, the solution was left at room temperature for 10 min. Then, 22 μL of 500 mM citrate•HCl buffer (pH 3) and 38 μL of ultrapure water were added to reach a final concentration of 50 mM citrate. The sample was reacted for 3 min at room temperature followed by the addition of 66 μL HEPES buffer (500 mM, pH 7.6). The mixture was incubated for approximately 5~10 min at room temperature to produce **PolyA 2**-modified AuNPs. Finally, the crude product was centrifuged at 10000 rpm for 30 min at 4°C, and the supernatant was discarded. The resulting conjugates were washed three times with HEPES buffer (5 mM, pH 7.4) to remove excess DNA and were resuspended in 200 μL ultrapure water to give alkyne-functionalized AuNPs. Azide-functionalized AuNPs was prepared in the same way, except that 40 μL of azide **PolyA 1** (2.0×10^{-5} M) was used instead.

7 μL of the azide-functionalized AuNPs and 7 μL of alkyne- functionalized AuNPs were mixed. Then, 2 μL of 5.0×10^{-3} M SA and 1 μL of 1.0×10^{-3} M TBTA were added to give **solution A**.

Preparation of report DNA-CuS NPs conjugates.

A total of 200 μL of imidazole solution (0.1 M, pH 6.8) was added to 2 mL of 3'-amino group modified with report DNA (2.0×10^{-6} M), and the mixture was incubated for 30 min. Then, 100 μL of 0.1 M EDC and 3.0 mL of CuS NPs (prepared according to the methods in the supporting information) were added, and stirred at room temperature for 12 h. After centrifugation at 10000 rpm for 30 min, the obtained solution of report DNA-modified CuS NPs was stored at −4°C in for the further use.

SNP detection

First, 200 μL of streptavidin-modified MBs were washed three times with 400 μL of PBS and then resuspended in 200 μL of PBS. Then, 20 μL of 1.0×10^{-5} M biotin-labeled **capture DNA 1** was added and incubated at room temperature for 30 min. The resulting **capture DNA 1** conjugated MBs were washed three times with PBS, resuspended in 200 μL of PBS and stored at 4 °C for further use.

To the solution of **capture DNA 1,** MB conjugate (5 μL), different concentrations of 5 μL mutant DNA (Mutant C, T, A) and 20 μL of **report DNA1-**modified CuS NPs were added. After reacting at 37 °C for 30 min and washing three times with PBS, the resulting mixture was resuspended in 20 μL of PBS. Then, 6 μL 5×S1 buffer (pH 4.5), 0.2 μL nuclease S1 (20 U) and pure water were added to reach a final volume of 30 μL. The mixture was incubated at room temperature for 30 min, and then the S1 nuclease was denatured by adding 2 μL of 0.5 M EDTA and heating to 70 °C for 10 min. After magnetic separation, the supernatant was subjected to ultrafiltration (3000 Dollon) to remove salt ions. The pellet was redispersed in 10 μL of water, and then 10 μL of 4.0×10^{-4} M $AgNO_3$ was added. The mixture was reacted at room temperature for 10 min, giving **solution B**.

2 μL of different concentrations of Cu^{2+} or 2 μL of **solution B** was added to 17 μL of **solution A**. Color changes were observed directly by the naked eye. UV-vis spectroscopy was carried out using a NanoPhotometer P300 (Implen, Germany).

Analysis of Dam MTase

The reaction of streptavidin-modified MBs with biotin-labeled capture MB DNA was carried out as described above. 20 μL of the reaction mixture consisting of 5 μL **capture DNA 2-**modified MBs, 2 μL of 10 × Dam MTase buffer, 20 units of Dpn I endonuclease, 80 μM SAM and different

concentrations of Dam MTase was reacted at 37 °C for 2 h and then washed three times with PBS. The supernatant was discarded and 20 μL of the **report DNA2**-modified CuS NPs were added, followed by incubation at 37 °C for 30 min. Then, the mixture was washed with PBS three times and washed with deionized water two times. Finally, 10 μL of deionized water and 10 μL of 4.0×10^{-4} M $AgNO_3$ were added and reacted at room temperature to give **solution C**.

2 μL of **solution C** was added to 17 μL of **solution A**. Color changes were observed directly by the naked eye and recorded using a NanoPhotometer P300.

RESULTS AND DISCUSSION

DNA-functionalized AuNPs is one of the most important bionanomaterials, and they have been widely used in the field of biosensors, materials chemistry and nanomedicine. The most commonly used method for the preparation of this nanoconjugate is based on the reaction of thiolated DNA with AuNPs through a salt aging process, which takes 1−2 days to form a stable conjugate. Recently, Liu's group reported that by adjusting the pH of the reaction buffer, poly adenine (polyA) could be adsorbed on the surface of AuNP in a few minutes.[4,6] Here, we used this novel method to prepare DNA-functionalized AuNPs. The obtained conjugates were characterized by UV-visible spectroscopy. As shown in **Fig. S1**, curve A was the characteristic absorbance of DNA at approximately 260 nm. Curve B exhibited the characteristic absorbance of AuNPs at approximately 527 nm. Curve C exhibited both the characteristic absorbance of DNA and AuNPs, which indicated that the AuNPs were successfully labeled with DNA. This method is fast, reproducible as well as low cost and stable DNA loading.

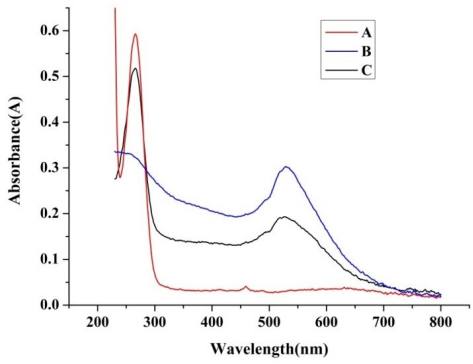

Fig. S1 The UV-visible spectra of DNA and gold nanoparticles. (A) Unmodified DNA, (B) gold nanoparticles, (C) DNA-functionalized gold nanoparticles

Exchange efficiency of cation-exchange reaction

From the ICP-MS measurement shown in Table S2, we could calculate the exchange efficiency of cation-exchange reaction. Total concentration of Cu^{2+} in CuS NPs can be obtained by the nitric acid digestion. The samples were prepared as follows: for the first group samples, 5 μL CuS was dissolved in 10 mL deionized water. Then 5 μL 0.016 M HNO_3 or 12.5 μL 8×10^{-5} M $AgNO_3$ was added, respectively. For the second group samples, 10 μL CuS was dissolved in 10 mL deionized water. Then 10 μL 0.016 M HNO_3 or 25 μL 8×10^{-5} M $AgNO_3$ was added, respectively. For the third group samples, 20 μL CuS was dissolved in 10 mL deionized water. Then 20 μL 0.016 M HNO_3 or 50 μL 8×10^{-5} M $AgNO_3$ was added, respectively. Three replicate determinations at different concentration levels exhibited the average exchange efficiency was 63.89%.

Table S2 ICP-MS measurement of Cu^{2+} obtained by nitric acid digestion and cation-exchange reaction

Sample	Total [Cu^{2+}] determined by the nitric acid digestion (μg/L)	[Cu^{2+}] determined by the cation-exchange reaction (μg/L)	Exchange efficiency (%)	Average Exchange efficiency (%)
1	5.721	3.779	66.05	63.89
2	11.607	7.132	61.45	
3	23.170	14.870	64.18	

* Each data represents the average value of three independent measurements.

Quantification of Cu^{2+} by using functionalized gold nanoparticles and click chemistry

When different concentrations of Cu^{2+} were added to solution A, which contained azide-functionalized AuNPs, alkyne- functionalized AuNPs, SA and TBTA, a click reaction was initiated, and aggregation of AuNPs occurred in a few seconds. As shown in insert of **Fig. S2**, the color of the mixture change from wine-red to blue–purple with increasing Cu^{2+} concentrations (0, 2.0×10^{-5}, 3.0×10^{-5}, 4.0×10^{-5}, 6.0×10^{-5}, 8.0×10^{-5} and 1.0×10^{-4} M). When 1.0×10^{-5} Cu^{2+} was added, almost no obvious color change was observed. From the UV/Vis spectra shown in Fig. S1, we could see that the absorbance at 527 nm decreased and the absorbance at 565 nm increased

with increasing Cu^{2+} concentrations. The absorption ratio between A565 and A527 was linear with the logarithm of Cu^{2+} in the range from 1.0×10^{-5} M to 1.0×10^{-4} M (**Fig. S3**). The correlation equation was $A_{565}/A_{527} = 2.66 + 0.384$ lgC (C was the concentration of Cu^{2+}, R^2=0.8564). This sensitivity was the same as the naked-eye-based Cu^{2+} detection reported previously,[7] but compared to the literature, the detection time is greatly shortened from overnight to a few seconds under the catalysis of TBTA.

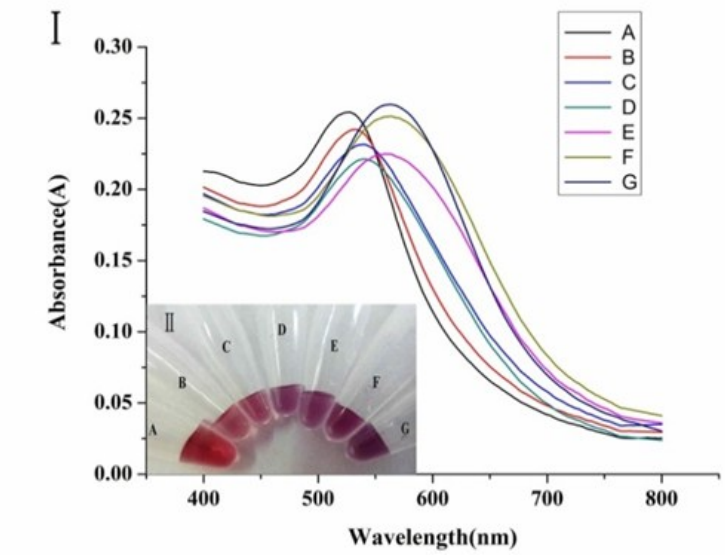

Fig. S2 (I) Absorption spectra and (II) photographs of the AuNPs in response to different concentrations of Cu^{2+}. A, 0; B, 2.0×10^{-5} M; C, 3.0×10^{-5} M; D, 4.0×10^{-5} M; E, 6.0×10^{-5} M; F, 8.0×10^{-5} M; G, 1.0×10^{-4} M.

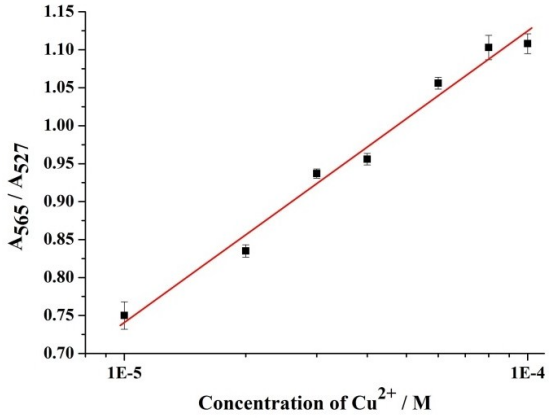

Fig. S3 The relative of absorption ratio of A_{565}/A_{527} is log−linear correlation with the amount of Cu^{2+} in the range from 1.0×10^{-5} M to 1.0×10^{-4} M

Gel electrophoresis assay for the detection of SNPs

To test the feasibility of our methods for the detection of SNP, a gel electrophoresis assay was carried out (**Fig. S4**). The reaction was performed in solution. In lane 1, when equal amounts of capture DNA, wild-type DNA and **report DNA1** were mixed, a sandwich structure was formed. When nuclease S1 was added to the mixture, the band remained unchanged (lane 2), since the perfect complementary product cannot be recognized by nuclease S1. Once mutant C DNA instead of wild-type DNA was added into the mixture, two lower bands were appeared due to the cutting at the mismatch site by nuclease S1.

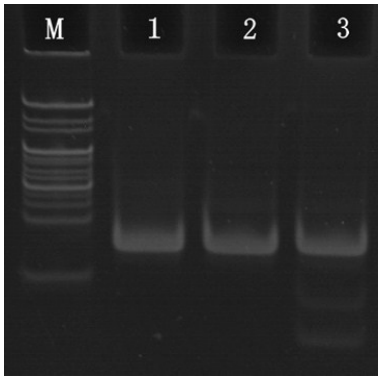

Fig. S4 The verification of enzyme cleavage by PAGE. M：marker，1：capture DNA + wild DNA + report DNA1，2：capture DNA + wild DNA + report DNA1+ S1 nuclease；3：capture DNA + mutant C DNA + report DNA 1+ S1 nuclease.

Study on the location of SNP site

To study the generality of the proposed method for SNP detection, the capacity to discriminate against different type of base-pair mismatch at a different position has been investigated in **Fig. S5**. The sequence of new mutant DNA is Mutant DNA = GGA CGG AAC AGC TTT GAG GTC CGT GTT TGT GCC. From the result we could see that our proposed method showed excellent applicability for the detection of SNP present at a different position. This can attribute to the nonspecific recognition of S1 nuclease.

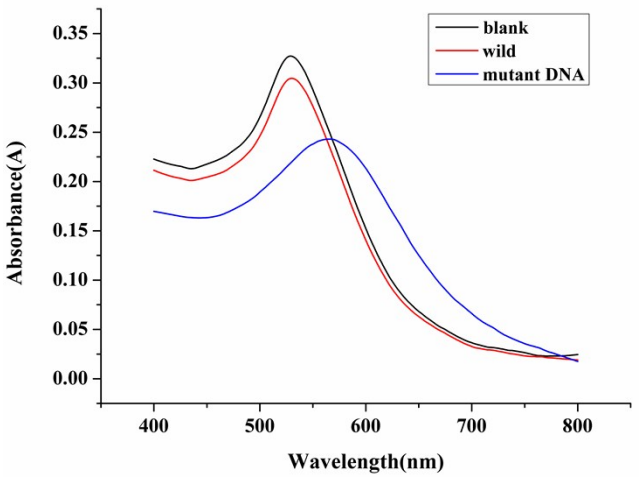

Fig. S5 Absorption spectra on the different SNP location site. Concentration of mutant DNA is 1×10^{-8} M.

Gel electrophoresis assay for the detection of DNA methyltransferase

DNA methylation was verified by polyacrylamide gel electrophoresis experiments, as shown in **Fig. S6**. Only one band was found in lane 1, which belongs to the hairpin probe. When restriction endonuclease Dpn I was added to the solution of hairpin probe, the band remain unchanged (lane 2), indicating that Dpn I cannot cleave the hairpin probe without DNA methylation. Once Dam MTase and restriction endonuclease Dpn I were both introduced into the solution of hairpin probe, two new bands whose molecule weights were smaller than the hairpin probe appeared (lane 3), indicating the cutting of methylated hairpin probe.

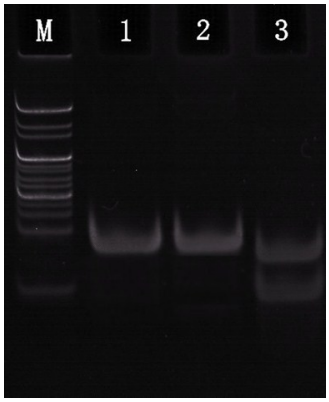

Fig. S6 Verification of DNA methylation by PAGE. M：marker，1：capture DNA，2：capture DNA + DpnI，3：capture DNA + dam + DpnI .

Optimization of the incubation time of DAM

To achieve the best performance, the incubation time for endonuclease Dpn I and DAM was

optimized using 60 U·mL^{-1} DAM and 1000 U·mL^{-1} DpnI. As shown in **Fig. S7**, the absorbance value of A_{565}/A_{527} increases rapidly with the reaction time from 10 to 120 min and reaches a plateau beyond 120 min. Therefore, the reaction time of 120 min was used in the following experiments.

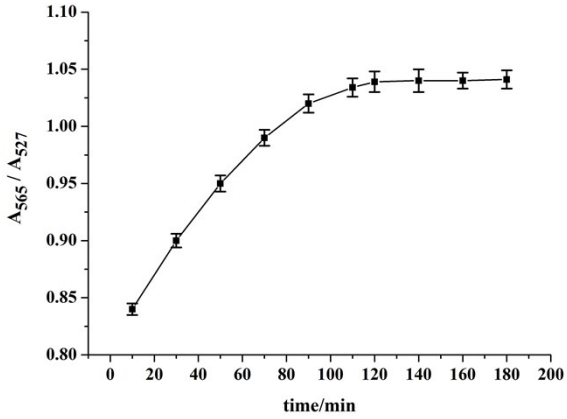

Fig. S7 Influence of the incubation time of DAM on the signal responding

Analyzing in real samples

In order to demonstrate the real clinical applicability of the developed SNP sensor, the implemented methodology was applied to analyze SNP in different human cell lines. Human cell lines of HT29 and NCI-H358 were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum. Cells were cultured at 37°C in 5% CO_2 humidified environment. The harvested cells (about 10^7) were used to extract genomic DNA using the Genomic DNA Isolation Kit (Beijing Dingguo Biological Technology Co., Ltd.) according to the manufacturer protocols. PCR amplification was performed in 50μL of 10 mM Tris-HCl buffer (pH 8.3) with 10 mM KCl, 4.0 mM $MgCl_2$, 250 μM dNTPs, 1 μM forward and reverse primers. The primers used were as follows: forward=5'gggacaggtaggacctgattt3' (21bp); and reverse= ttgcggagattctcttcctc (20bp). Amplification was achieved by thermal cycling for 40 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were purified by the ethanol precipitation method and redissolved in deionized water. From the results shown in **Fig. S8**, we can see that the signal obtained from the HT29 sample was much higher than that from the NCI-H358 sample, implying higher content of mutated TP53 R273H in HT29 cell than in NCI-H358 cell, as indicated by the previous report.[8]

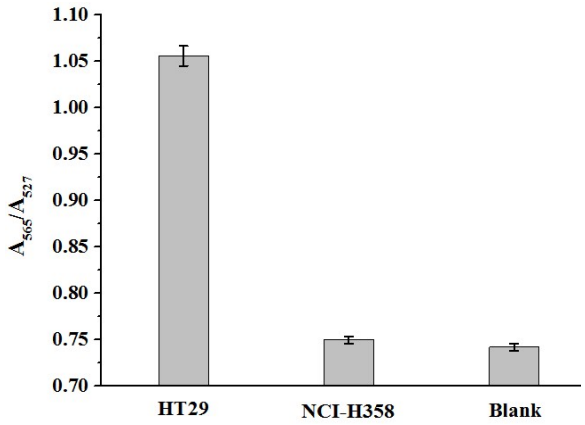

Fig. S8 Signal response of the present biosensor for the detection of these PCR amplicons

Next, to evaluate the practicality of this method for the detection of DNA methyltransferase in biological fluids, the performance in 5% human serum was implemented. Different concentrations of Dam MTase were spiked into PBS and 5% human serum. As shown in **Fig. S9**, the signals for different concentration of Dam MTase in 5% human serum were almost the same or a little higher as that in buffer, and the signal increased with the increase of Dam MTase concentration, indicating the developed sensor can perform well in real biological sample.

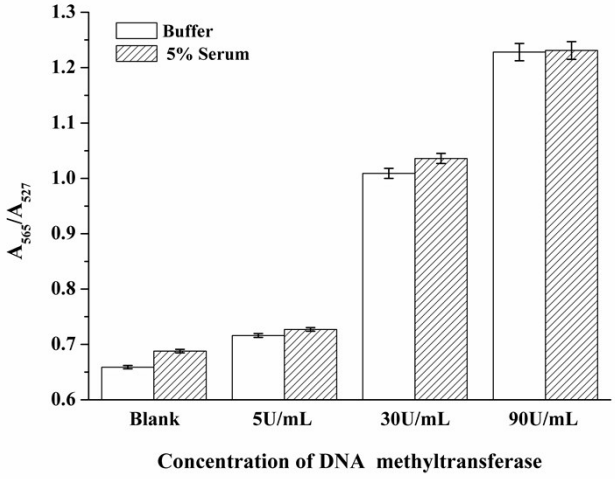

Fig. S9 Signals for different concentration of Dam MTase in buffer and in 5% human serum.

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