# Supporting Information

**Core-Shell Gold Nanocubes for Point Mutation Detection Based on Plasmon Enhanced Fluorescence**

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## Experimental details

*Preparations of Au NCs and Au NSs*

We adopted a seed-growth method which contains a two-step process for synthesis of Au NCs. [1] Au seeds are produced by reducing $Au^{3+}$ with $NaBH_4$. Firstly, CTAB (9.75 mL, 0.1 M) and $HAuCl_4 \cdot 4H_2O$ (0.25 mL, 10 mM) were mixed under stirring in a flask (50 mL). The freshly prepared, ice-cold aqueous solution of $NaBH_4$ (0.01 M, 0.6 mL) was rapidly added, generating a brownish solution. The seed solution was mixed further for 5 min and kept undisturbed for 1 h in a 30 °C water bath to decompose the remaining

unreacted NaBH$_4$ in the solution. Then the seeds solution was diluted 10 times by water before further use. The growth solution was prepared by the addition of HAuCl$_4$ (0.8 mL, 10 mM), CTAB (6.4 mL, 0.1 M) into 32 mL of H$_2$O while stirring, then AA aqueous solution (3.8 mL, 100 mM) was added and the growth solution turned colorless. Finally, 0.02 mL of diluted seed solution was added into the growth solution. The mixture solution was mixed by gentle inversion for 10 s and then left undisturbed overnight in a 30 °C water bath. The resulting solution was centrifuged at 12500 rpm for 13 min to remove excess CTAB. The precipitate was redispersed in deionized water and centrifuged twice at 12500 rpm for 13 min to acquire pure Au NCs. Finally, the Au NCs suspended in the same volume of deionized water.

The Au NSs were synthesized in the same process, except for adding double the amount of diluted seed to growth solution.


*Synthesis of Core-Shell Au NC@SiO$_2$ NPs*

Silica coating on the surface of Au NC was carried out by a modification of the Stöber method.[2] CTAB concentration is an important factor affect the thickness of the shell. By controlling the concentration of CTAB, six different thickness of the silicon layer were synthesized in this work. Before the modification, the Au NCs solutions were centrifuged again at 12500 rpm for 13 min to ensure the concentration of CTAB was less than 0.01 mM. We prepared six vials of 10ml redispersed Au NCs aqueous solution, labeled 1 to 6. Then, the different amounts of CTAB (0.1 M, 40 μL, 70 μL, 90 μL, 120 μL, 180 μL and

210 μL) were added to six vials respectively. The solutions were stored at 30 °C without stirring overnight to make the CTAB uniform distribution on the surface of Au NCs. Next, NaOH (80 μL, 0.1 M) was added to each vial under agitation. After 30 min, three 30 μL of TEOS methanol solution with a concentration of 20% was added into the mixed reactants at intervals of 30 min with stirring. The solutions were further mixed for 24 h at room temperature. Au NC@SiO$_2$ NPs were collected via centrifugation at 12500 rpm for 12 min and washed with deionized water and ethanol twice. The obtained purified Au NC@SiO$_2$ NPs were redispersed in 10 mL ethanol for further use.

*Preparation of Au NC@SiO$_2$@5-FAM NPs*

In order to facilitate the fluorescent molecular with carboxylic groups connections on silica shell, the Au NC@SiO$_2$ NPs were modified with amino groups in the first. Ammonium hydroxide (100 μL, 25%) was first added into 10 mL of Au NC@SiO$_2$ ethanol solution under stirring. After 30 min, 50 μL of APTES solution was injected. The mixture reacted 3h at 35 °C followed by heated at 65 °C for 1 h. The amino-modified Au NC@SiO$_2$ NPs were collected by centrifugation and washed with water and ethanol respectively. The final products were redispersed in 10 mL water before use. Au NC@SiO$_2$@5-FAM NPs were prepared by a condensation reaction between 5-FAM and amino group functionalized Au NC@SiO$_2$. 0.5 mL of 5-FAM solution was mixed 4 mL H$_2$O, NHS (5 μL, 0.1 M) and EDC (5 μL, 0.1 M) was added with stirring. After 30 min, 1.0 mL of the amino-modified Au NC@SiO$_2$ NPs were injected into the mixture and

stirred overnight. The resulting Au NC@SiO$_2$@5-FAM NPs were collected by centrifugation and washed with water several times to remove excess 5-FAM molecules. The obtained products were dispersed in water.

*Preparation of Au NS@SiO$_2$@5-FAM NPs*

The synthesis method of Au NP@SiO$_2$@5-FAM NPs is same as the produce of Au NC@SiO$_2$@5-FAM NPs.

*Conjugation of 5-FAM with SiO$_2$*

The synthesis method of SiO$_2$@5-FAM NPs is similar to the Au NC@SiO$_2$@5-FAM NPs.

## S1. Measurements and Calculation of the Concentration of Au NCs

Accroding the Beer`s Law A =εbc, the concentration of Au NCs was measured. A is the absorbance, ε is the molar extinction coefficients, b is the cuvette path length in cm and c is the concentration in mol L$^{-1}$. We calculation the concentration of Au NCs by combining the number of Au atoms measured by inductively coupled plasma mass spectrometry (ICP-MS). The edge length of Au NCs is 50 nm from TEM. The density is 19.3 g cm$^{-3}$ according to previous report.[3] Then, the concentration of Au NCs was calculated combine with UV–vis spectroscopy. From above measurements, Au NCs prepared in this paper consisted of $3.86 \times 10^6$ Au atoms. The concentration of Au NCs was

estimated to be 0.02 nM.

**S2. Calculation of the number of 5-FAM connected to the Au NC@SiO$_2$ NP.**

The number of connected 5-FAM to per Au NC (N) is estimated utilizing the absorption

spectra according to literature.[4]

N = The number of connected 5-FAM / the number of Au NCs = $N_{5\text{-FAM}}$ / $N_{Au\ NC}$

The number of Au NCs in the solution ($N_{Au\ NC}$) is given by

$N_{Au\ NC}$ = Au NCs concentration × Solution volume × Avogadro's constant

$\quad$ = $c_{Au\ NCs}$ V $N_A$

The concentration of 5-FAM before connected ($c_0$)=absorbance/ ($\varepsilon_{5\text{-FAM}}$× cuvette path

length)=$A_0$/($\varepsilon_{5\text{-FAM}}$× b)

The total number of 5-FAM in solution before connected ($N_0$) is given by

$N_0$ = The concentration of 5-FAM in solution × solution volume × Avogadro's constant =

$\quad$ $c_0 V_0 N_A$

The concentration of 5-FAM in the collected supernatant after immobilization ($c_1$)

$c_1$ = absorbance/ ($\varepsilon_{5\text{-FAM}}$×cuvette path length) = $A_1$/($\varepsilon_{5\text{-FAM}}$× b)

The number of 5-FAM in collected supernatant ($N_{supernatant}$) is given by

$N_{supernatant}$ = Concentration of 5-FAM in supernatant ×volume of collected supernatant ×

Avogadro's constant= $c_1$ × $V_1$×$N_A$

The number of connected 5-FAM

$N_{5\text{-FAM}}$ = $N_0$ - $N_{supernatant}$

$$= c_0 V_0 N_A - c_1 \times V_1 \times N_A$$

$$= [A_0/(\varepsilon_{5\text{-FAM}} \times b)]\, V_0 N_A - [A_1/(\varepsilon_{5\text{-FAM}} \times b)]\, V_1 \times N_A$$

$$= N_A (A_0 V_0 - A_1 V_1)/ (\varepsilon_{5\text{-FAM}} \times b)$$

Therefore, the number of connected 5-FAM to per NP is given by

N = The number of connected 5-FAM / the number of Au NCs

$$= N_{5\text{-FAM}} / N_{Au\ NC}$$

$$= (N_0 - N_{supernatant})/ N_{Au\ NC}$$

$$= [N_A(A_0 V_0 - A_1 V_1)/ (\varepsilon_{5\text{-FAM}} \times b)\,] / c_{Au\ NCs}\, V N_A$$

$$= ( A_0 V_0 - A_1 V_1) / (c_{Au\ NCs}\, V \varepsilon_{5\text{-FAM}} \times b)$$

$A_0$, and $A_1$ is absorbance of 5-FAM, $V_0$ and $V_1$ is the solution volume of 5-FAM. Extinction coefficients of 5-FAM ($\varepsilon_{5\text{-FAM}}$) is $7.38 \times 10^4$ $M^{-1}cm^{-1}$, which can be get from the product specifications. The cuvette path length b=1 cm. According the above formula, the number of connected 5-FAM to per Au NC is estimated to 3726.
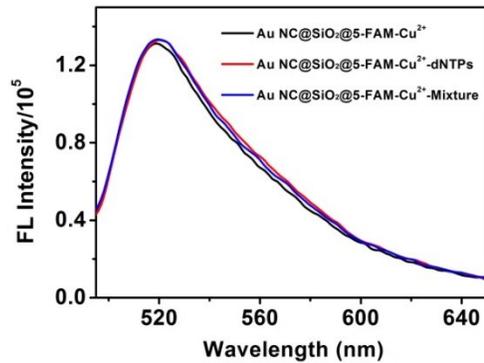


**Fig.S1** Fluorescence spectra of Au NC@SiO$_2$@5-FAM−Cu$^{2+}$, Au NC@SiO$_2$@5-FAM−Cu$^{2+}$ with the addition of dNTPs, as well as the unreacted solution.
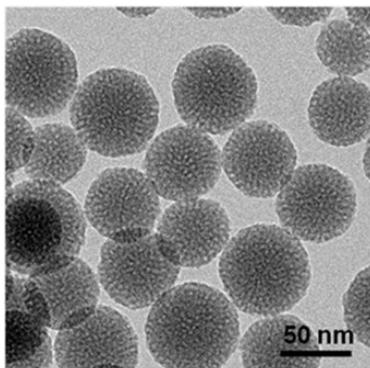
**Fig.S2** TEM image of SiO$_2$@5-FAM NPs.
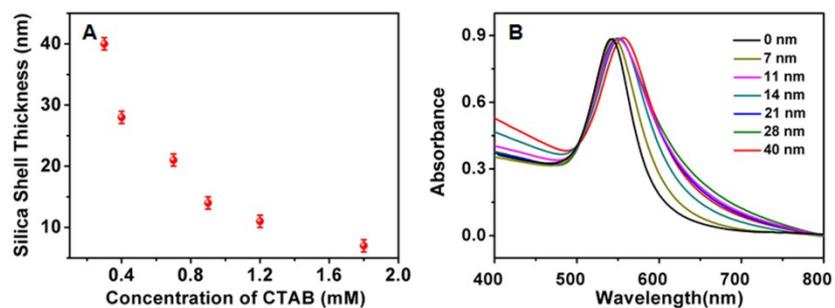


**Fig.S3** (A) CTAB concentration corresponds to the silica shell thickness. (B) UV–vis spectra of Au NCs and Au NC@SiO$_2$ NPs with different silica shell thicknesses.
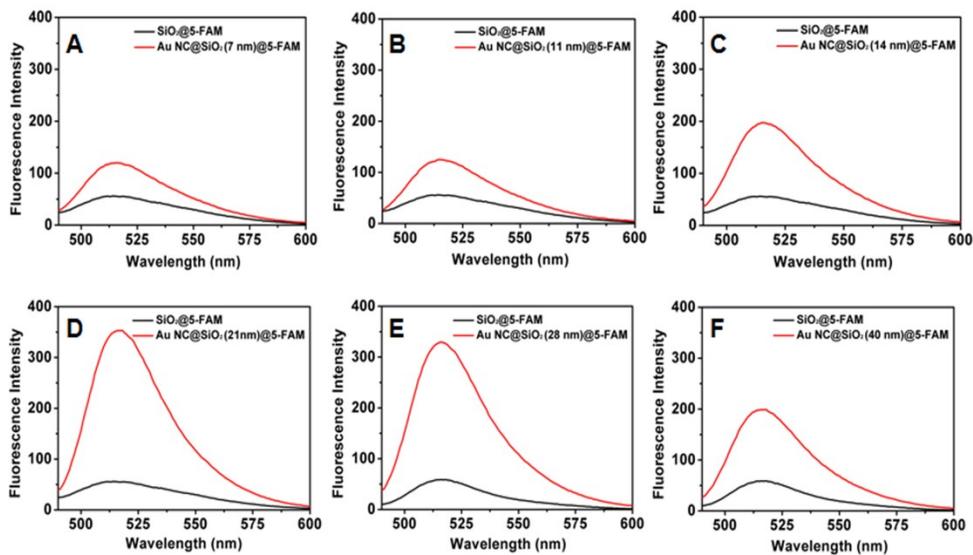
**Fig.S4** The fluorescence spectrograms of Au NC@SiO$_2$@5-FAM with various silica shell thicknesses (A-F. 7, 11, 14, 21, 28 and 40 nm). SiO$_2$@5-FAM was used as the control.
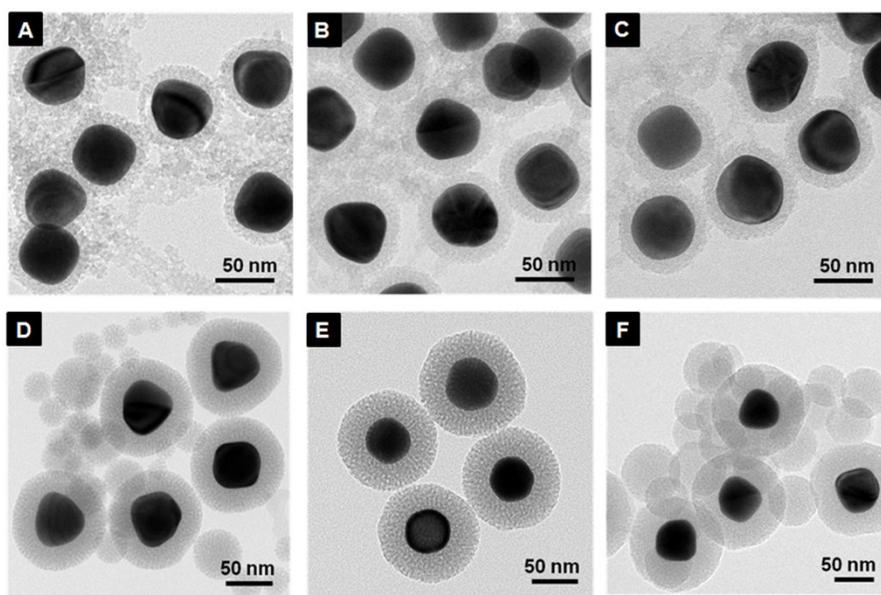


**Fig.S5** TEM images of Au NS@SiO$_2$ NPs with different silica shell thicknesses ranging from 7 to 40 nm.
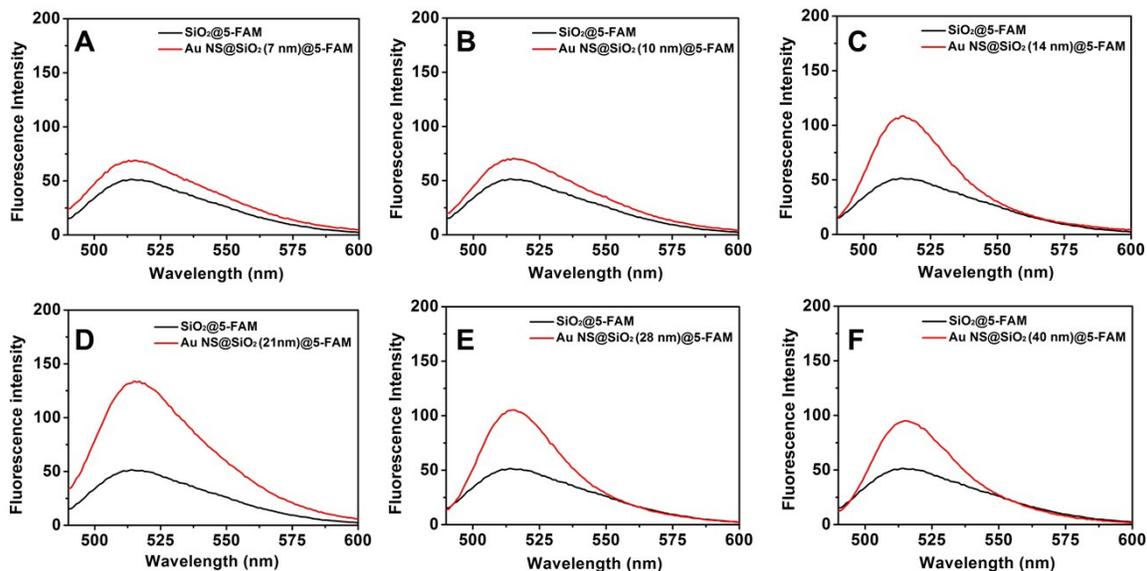
**Fig.S6** The fluorescence spectrograms of Au NS@SiO$_2$@5-FAM with various silica shell thicknesses (A-F.7, 10, 14, 21, 28 and 40 nm). SiO$_2$@5-FAM was used as the control.
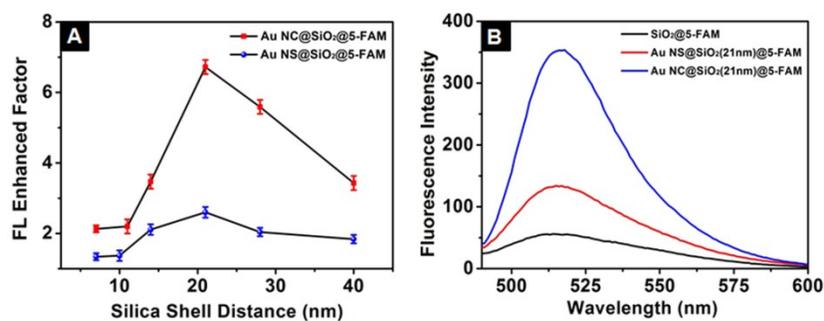


**Fig.S7** (A) Direct comparison of distance-dependent of the fluorescence enhancement factors of Au NC@SiO$_2$@5-FAM and Au NS@SiO$_2$@5-FAM. (B) Fluorescence spectra of Au NC@SiO$_2$ (21 nm)@5-FAM, Au NS@SiO$_2$ (21 nm)@5-FAM, and SiO$_2$@5-FAM.
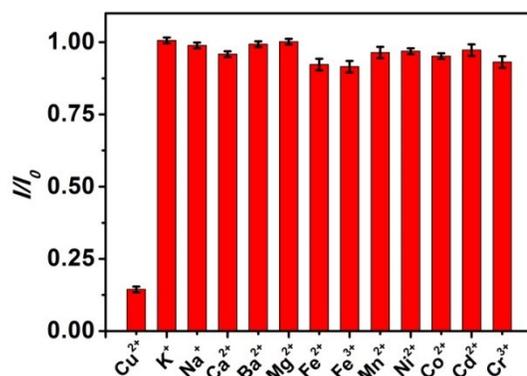
**Fig.S8** Fluorescence intensity ratios ($I_0$ was the fluorescence intensity of Au NC@SiO$_2$@5-FAM NPs and $I$ was the corresponding fluorescence intensity in the presence of metal ions) for the Au NC@SiO$_2$@5-FAM solution in addition of various cations with a final concentration of 20 μM.
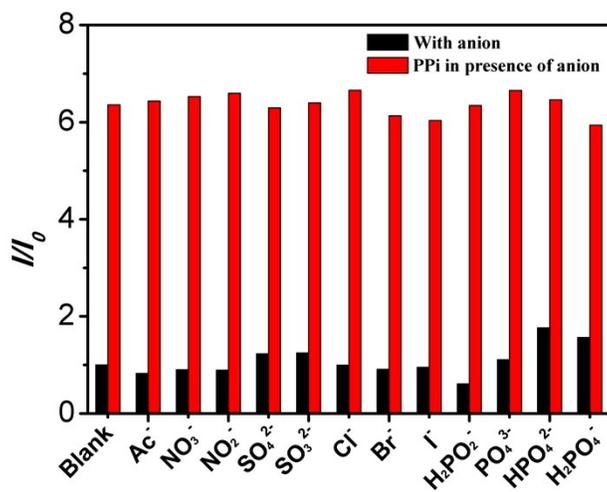


**Fig.S9** Fluorescence intensity of Au NC@SiO$_2$@5-FAM$-$Cu$^{2+}$ solution in addition of various anions. Black bars display the addition of foreign anions alone. Red bars display subsequent addition of PPi.
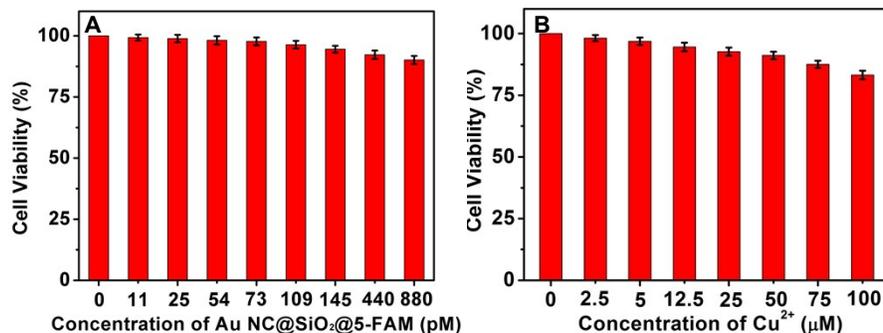
**Fig.S10** MTT assay in HeLa cells incubated with different concentrations of (A) Au NC@SiO$_2$@5-FAM NPs and (B) Cu$^{2+}$.

**Table S1. The oligonucleotides used in this work.**

| Name | Sequence(5'to3') |
|---|---|
| mutant DNA | TTTGTGCCTGTCCTGGGAGAGAC**T**GGCGCACAGAGGAA GAGAATCT |
| wild DNA | TTTGTGCCTGTCCTGGGAGAGAC**C**GGCGCACAGAGGAA GAGAATCT |
| mutant DNA -specific padlock probe | phosphate- GTCTCTCCCAGGACAGGCTTTTGATCACAGTTTACGGTT TAGCATAACTCTACTATCATTTACTTTACGATTTCGGCT CTTCCTCTGTGCGCC**A** |
| wild DNA -specific padlock probe | phosphate- GTCTCTCCCAGGACAGGCTTTTGATCACAGTTTACGGTT TAGCATAACTCTACTATCATTTACTTTACGATTTCGGCT CTTCCTCTGTGCGCC**G** |
| primer | TAAACCGTAAACTGTGATCA |

The underlined bold letters indicate the base at mutant site in mutant DNA and wild DNA, respectively.

**Table S2. Comparison of the present method with other reported methods for mutation detection.**

| Method | Detection limit | Reference |
|--------|-----------------|-----------|
| Fluorescence | 10 pM | Lu et al.[5] |
| Colorimetric | 1 μM | María et al.[6] |
| Fluorescence | 2 μM | Yeh et al.[7] |
| Atomic force microscopy | 5 mM | Subramanian et al.[8] |
| Fluorescence | 1 nM | Zhang et al.[9] |
| Laser intensity | 250 mM | Lee et al.[10] |
| Thermal resistance | 600 pM | Van grinsven et al.[11] |
| SPR | 1 nM | Knez et al.[12] |
| PEF | 1.3 pM | This work |

**References**

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