Electronic Supporting Information

One-Pot Synthesis of DNA-Anchored SERS Nanoprobe with Simultaneously Nanostructural Tuning and Raman Reporter Encoding

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1. Experimental details:

1.1 Chemicals and equipment

All oligonucleotides used in current study were purchased from Sangon Biotech Co., ltd. 13 nm gold nanospheres (GNSs) seed solutions were self-made. Gold(Ⅲ) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, ACS reagent; Sigma-Aldrich), Hydroxylamine hydrochloride ($NH_2OH \cdot HCl$, >99%; Sigma-Aldrich), Sodium hydroxide (NaOH > 97%; Sigma-Aldrich), sodium citrate tribasic dehydrate (Na_3CA > 99%; Sigma-Aldrich), 3-Mercaptopropionic acid (MPA, >99%; Sigma-Aldrich) were used without further purification.

In our experiment, most of the TEM photographs were taken by FEI Tecnai G2 F20 S-TWIN, meanwhile part of the TEM photographs were taken by Hitachi HT7700. The ultraviolet-visible spectrometer was UV-Vis 1750 bought from Shimadzu Co.

1.2 Preparation of Gold seed solutions

First of all, 13nm gold nanospheres were synthesized with the boiling method. First, we boiled the 1mM HAuCl4 solution, then 38.8mM sodium citrate (NaCA) solution was added and kept boiling for 15-20min. During the process, the color of solution changed from light yellow, then to colorless, black, purple and finally dark red in just half a minute. The transformation process reflected that the color of GNP solution changed with the growth of size of gold nanoparticles. The synthesized 13nm GNSs were the seeds of the subsequent experiments.[1]

1.3 Synthesis of the gold nanoparticles mediated by DNA

In the probe synthesizing process, the purified GNSs were firstly incubated with deoxynucleoside for 20-30 minutes. Then 10 μM Rhodamine B were mixed with 300 uL seed solution for about 2 hours. After the incubation, 15 uL NH_2OH (HA) solution (400 mM) and 2.1 uL $HAuCl_4$ solution (1%wt) was added into the solution and shaken vigorously for about 1 hour. Then the Raman probes were purified by centrifugation to remove the unattached Raman dye molecules or reduced agents in the supernatant.

1.4 the influence of Rhodamine B on the morphology

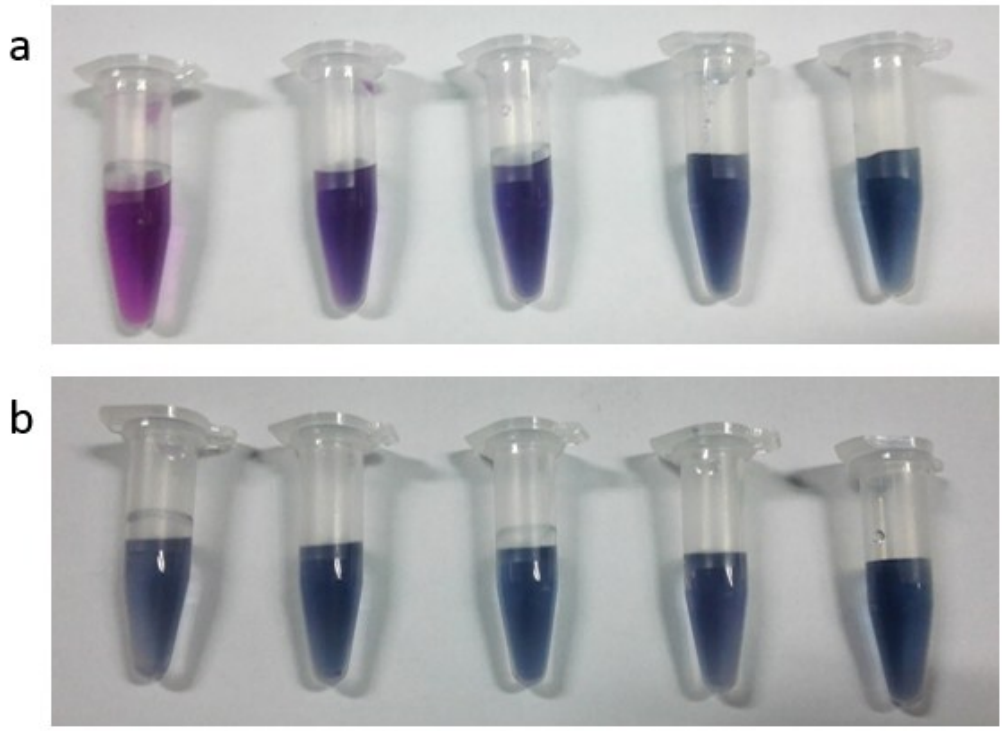

Figure S1. (a) Photographs showing the different colors generated after the growth of 13 nm GNSs with 100 μM, 50 μM, 20 μM, 10 μM, 2 μM Rhodamine B added into the solution before growth. (b) Photographs showing the color of (a) after twice centrifugation.

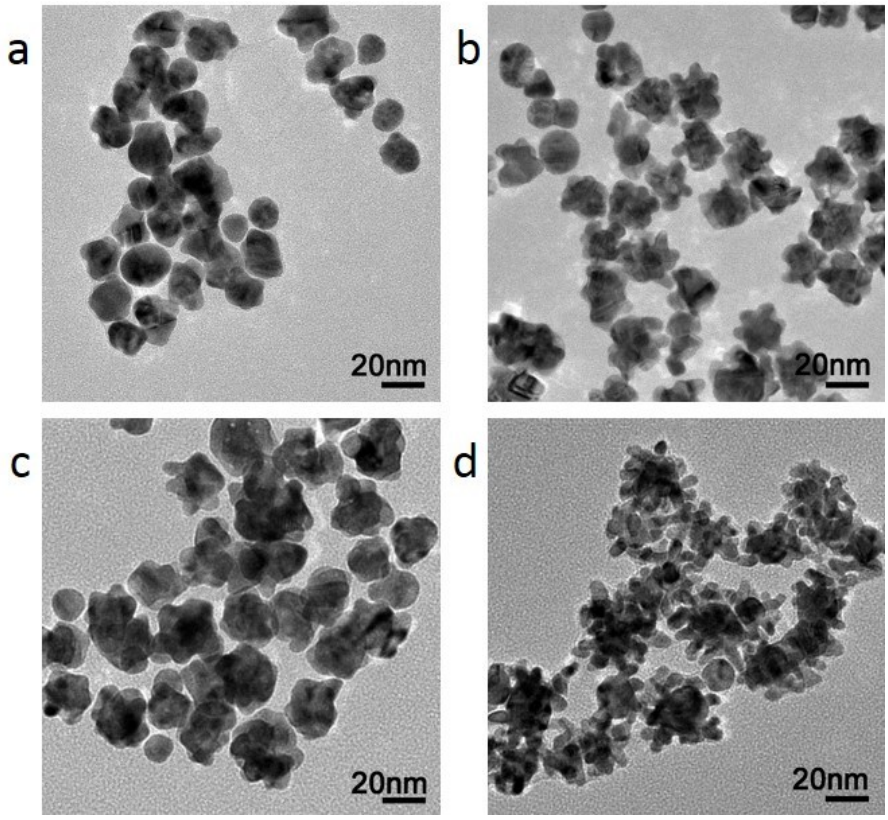

Figure S2. The shape of Raman probes synthesized with (a) no dye, (b) 1 μM dye, (c) 10 μM dye and (d) 100 μM dye.

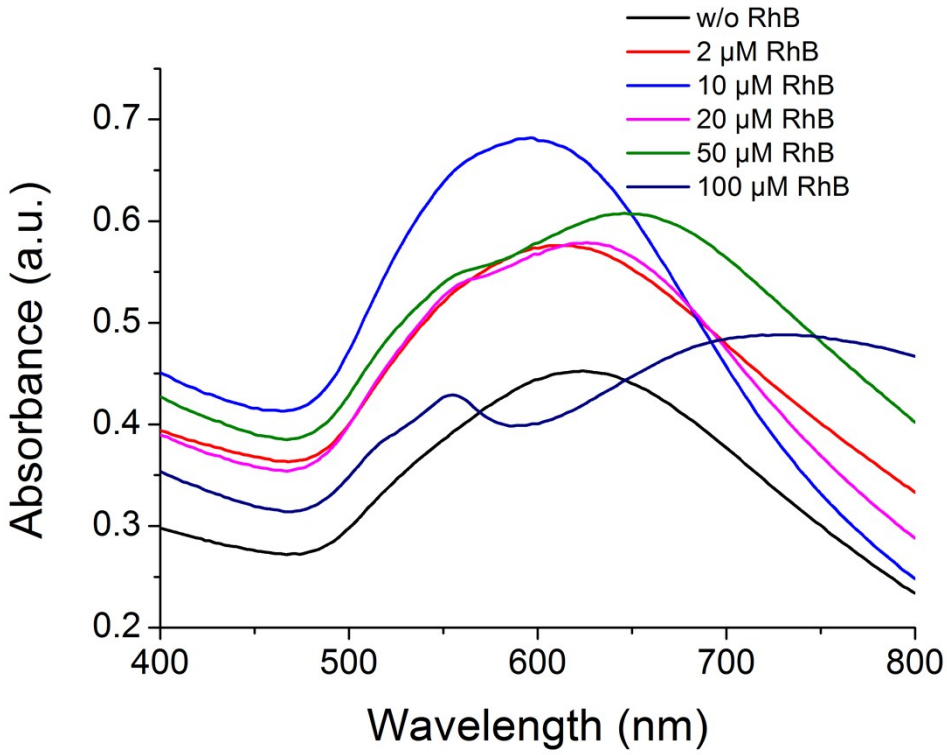

Figure S3. UV-Vis spectra of GNF nanotag solution mediated with DNA and (black)without, (red)2 μM, (blue)10 μM, (pink)20 μM, (green)50 μM, (dark blue)100 μM Rhodamine B.

1.5 Quantification of the number of Raman dye molecules on GNF

According to Beer-Lambert Law:

$A = kbc$

A was the measured absorbance, k was the wavelength-dependent molar absorptivity coefficient, b was the path length, c was the analyte concentration.

In our experiment, the path length b was 1cm, which is the distance between the input facet and output facet of the cuvette. According to previous stduy[2], the molar absorptivity coefficient of 13nm GNS was 2.7×10^{8} $M^{-1}\cdot cm^{-1}$. For GNS solution with absorbance of 0.5 a.u., according to Beer-Lambert Law, the concentration of 13nm GNS is 1.85 nM. It was measured that Rhodamine B had a fluorescence peak at the wavelength of 580 nm.

The number of Raman reporters on every nanoparticles could be calculated by:

$$N = \frac{N_{total} - M_{sp} \cdot V}{N_{NP}}$$

According to the proportional relation of fluorescence intensity and concentration, we could get the number of Raman reporters absorbed on every GNP. From the result, we could conclude that the number of Raman dye molecules adsorbed on the $GNF_{A20+RhB}$ nanotags was more than that of the GNF_{A20}_RhB nanotags, which meant that the former Raman probes carried more signal molecules when Raman dye was added into the system before growth (Figure S4).

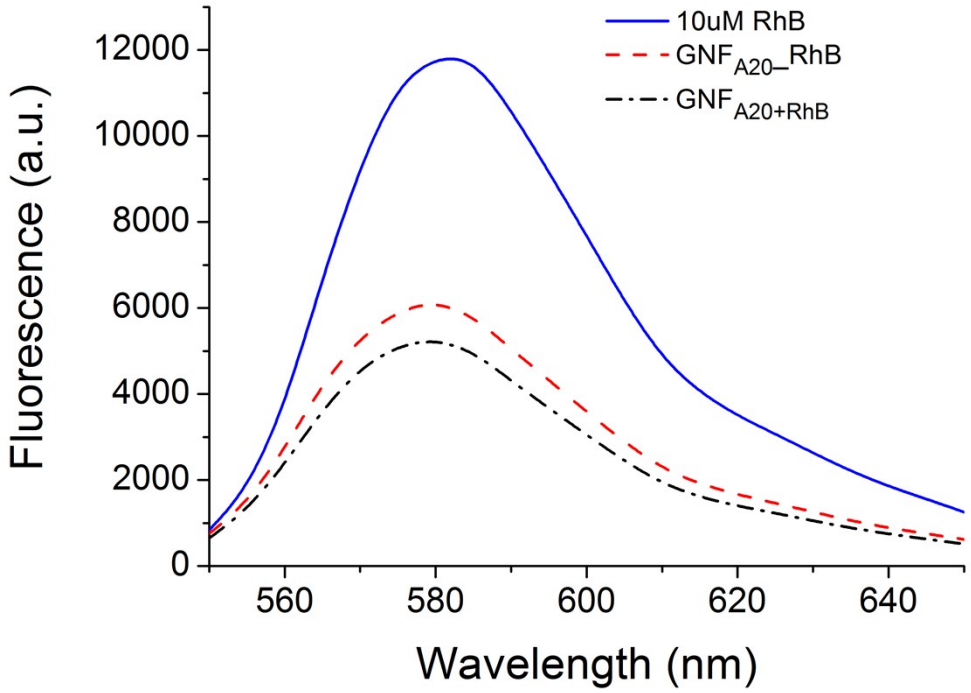

Figure S4. The fluorescence spectra of the supernatant of (blue) 10 μM Rhodamine B solution; (red) GNF_{A20}_RhB nanotag solution; (black) $GNF_{A20+RhB}$ nanotag solution.

1.6 Stability study of Raman dye molecules adsorbed on GNSs

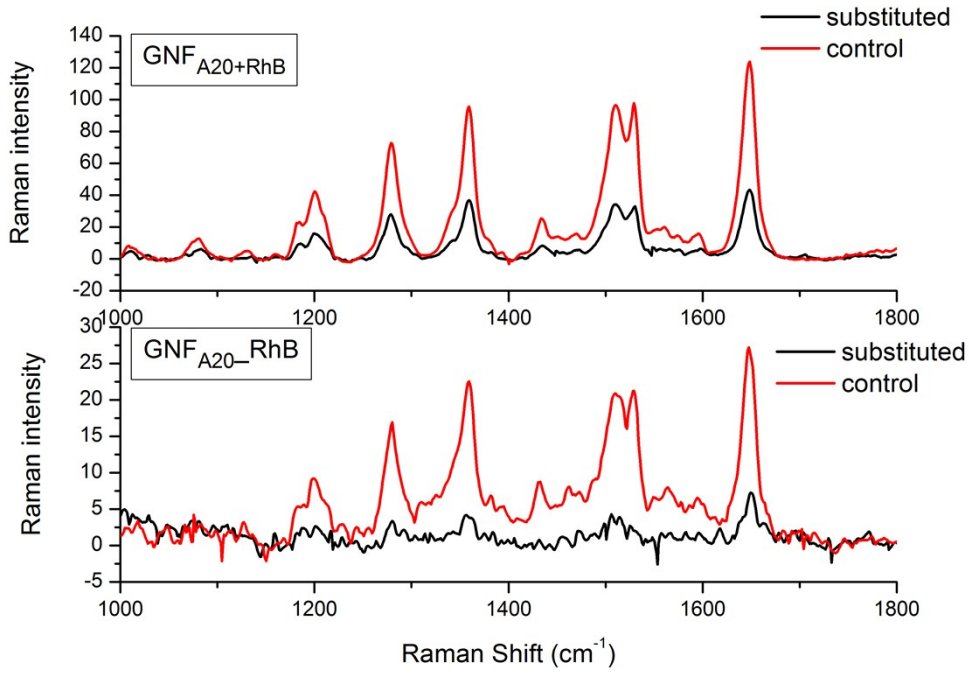

Figure S5. The Raman spectra of $GNF_{A20+RhB}$ nanotag solution and GNF_{A20}_RhB nanotag solution under the condition of adding 10 μM MPA which is more adsorptive than Rhodamine B on gold to test the stability of synthesized nanotag.

The spectra shows that after the addition of 10 μM MA, the $GNF_{A20+RhB}$ nanotag solution maintains 35.0% its original Raman intenstiy while the GNF_{A20}_RhB nanotag solution only gets 26.7%. Thus, it proves that the dye molecules adsorbed on our synthesized functionalized nanotag is more stable, which may be explained by the embedment of dye molecules into the nanotag.

1.7 Enhancement factor calculation

The enhancement factor can be calculated by the following equation:

$$EF = \frac{I_{SERS} \cdot N_{solid}}{I_{solid} \cdot MV}$$

In liquid, the molar concentration is 10 μM. The excitation volume in liquid is regarded as a sphere, whose volume can be calculated by the radius of light spot (λ is the excitation wavelength, N.A. is the numerical aperture)

$$r = \frac{1.22\lambda}{2N.A.}$$

In solid Rhodamine B, the number of excitated dye molecules can be calculated by the following equation (ρ is the density of Rhodamine B which is 0.79g/cm^3, V is the volume of excitated solid, M is the molar mass which equals 479.01g/mol)

$$N_{solid} = \frac{\rho V}{M}$$

Thus the enhancement factor of $GNF_{A20+RhB}$ solution is 1.47×10^5.

1.8 Biorecognition ability

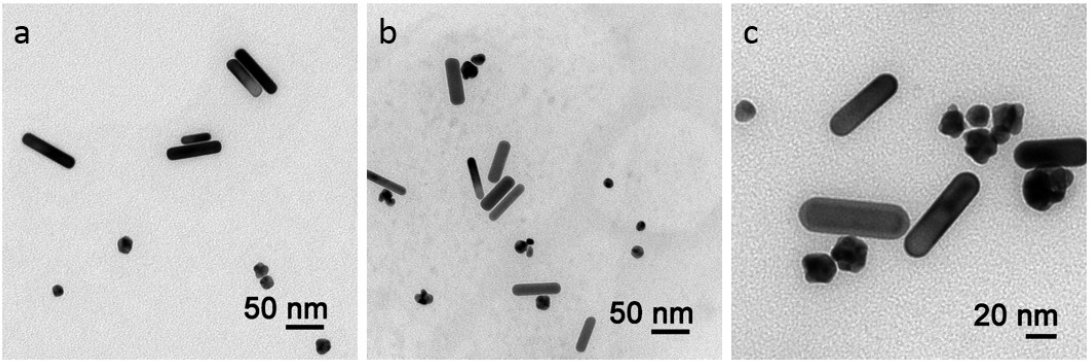

Figure S6. (a-c) TEM imaging shows the incubation of A20-enchored GNFs nanoprobes ($GNF_{A20+RhB}$) with non-complementary A20-modified gold nanorods (A20-GNR).

1.9 hybridization experiment

To study the hybridization activity of the DNA assay on the probe, we applied a sandwich structure.[3]

Having demonstrated that the probes are easy to biofuntionalize and have excellent capability for biorecognition, we developed a sandwich analytical strategy for meaningful DNA assay, like HIV Target DNA. SERS nanoprobes were prepared via the modification of HIV Detection DNA encoded by Rhodamine B, while HIV Capture DNAs were coassembled on the magnetic beads

(MBs) anchor via a avidin-biotin bridge. Figure S7 presents the results of the sequence selective analysis for HIV Target DNAs. First, the magnetic beads were incubated with overdue capture DNA. Then BSA was used to block the unbinded avidin. Then we checked the SERS effect of the mixture with or without target DNA. And the result confirmed the capabilities of their DNA anchored nanoparticles in real DNA hybridization assays.

Target DNA assay has complementary assay part for both capture and detection DNA. From figure S8, in the existence of 10 nM target DNA, the spectra shows much higher enhancement effect.

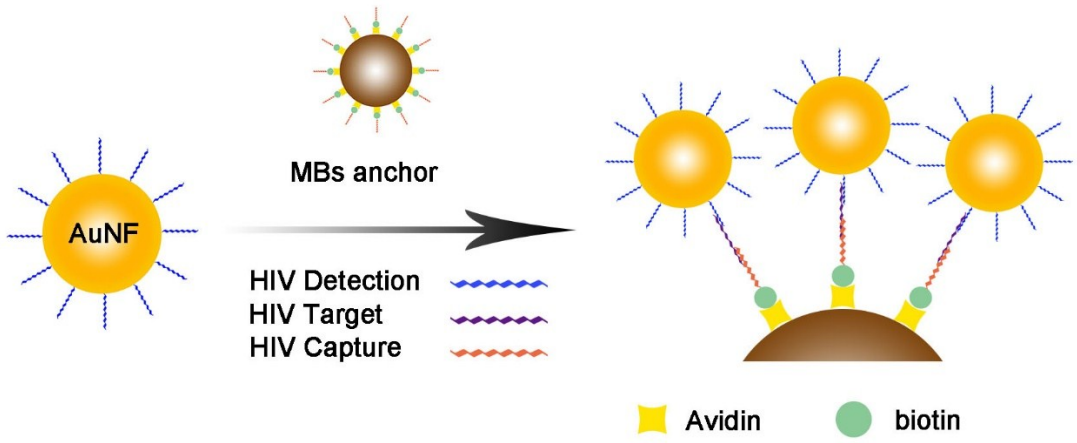

Figure S7. Hybridization activity analytical strategy for HIV Target DNA. The target DNA was captured by the corresponding capture DNA followed by hybridization with detection nanoprobes, leading to the formation of a sandwich complexes.

HIV Target	5'-AGAAGATATTTGGAATAACATGACCTGGATGCA-3'
HIV Detection	5'-AAAAAAAAAAAAAAATGCATCCAGGTCATG-3'
HIV Capture	5'-TTATTCCAAATATCTTCTAAAAAAAAAA-(Biotin)-3'

Table S1. The DNA assay used in the experiment.

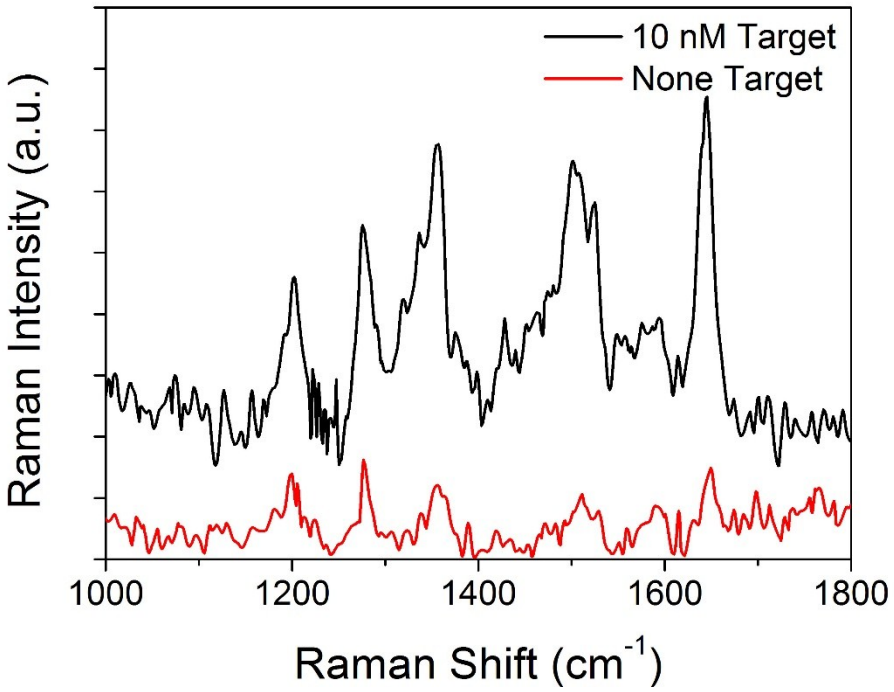

Figure S8. SERS spectra of the hybridization mixture w/wo the existence of target DNA.

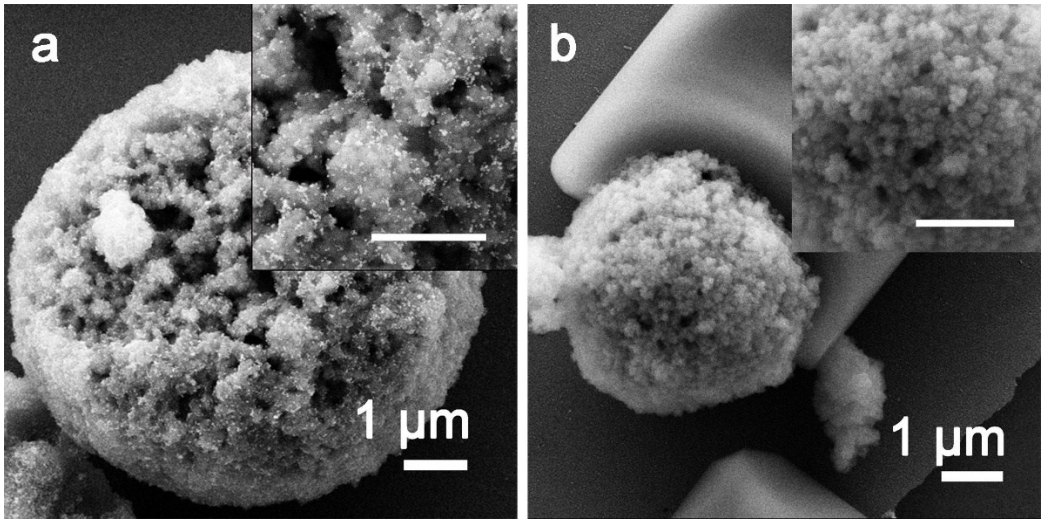

Figure S9. SEM photograph of the hybridization group and the blank contrast group without target DNA. The inset shows that there are a large amount of probes attached onto the surface MBs when target DNA was added and few probes are found in the lack of target DNA. (the scale bar in the photographs are 1 μm)

References

1. K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal Chem*, 1995, **67**, 735-743.
2. W. Haiss, N. T. K. Thanh, J. Aveyard and D. G. Fernig, *Anal Chem*, 2007, **79**, 4215-4221.
3. B. Zhao, J. L. Shen, S. X. Chen, D. F. Wang, F. Li, S. Mathur, S. P. Song and C. H. Fan, *Chem Sci*, 2014, **5**, 4460-4466.