Electronic Supplementary Information

Sequence-specific detection of cytosine methylation in DNA via FRET mechanism between upconversion nanoparticles and gold nanorods

Mingmin Wu, Xiaoyong Wang*, Kun Wang, Zijian Guo*

1. Supplementary Figures and Table

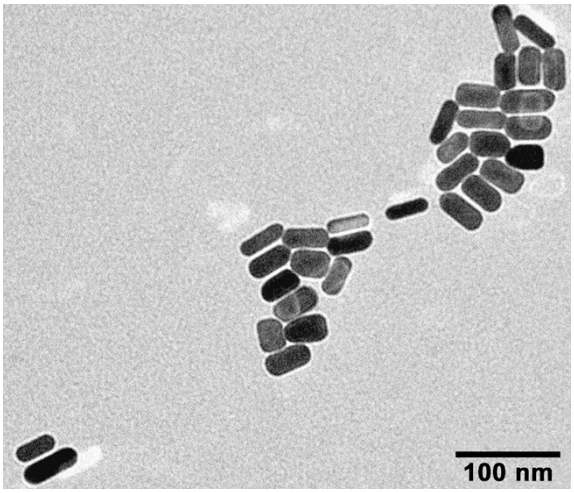


Fig. S1 TEM image of CTAB stabilized AuNRs.

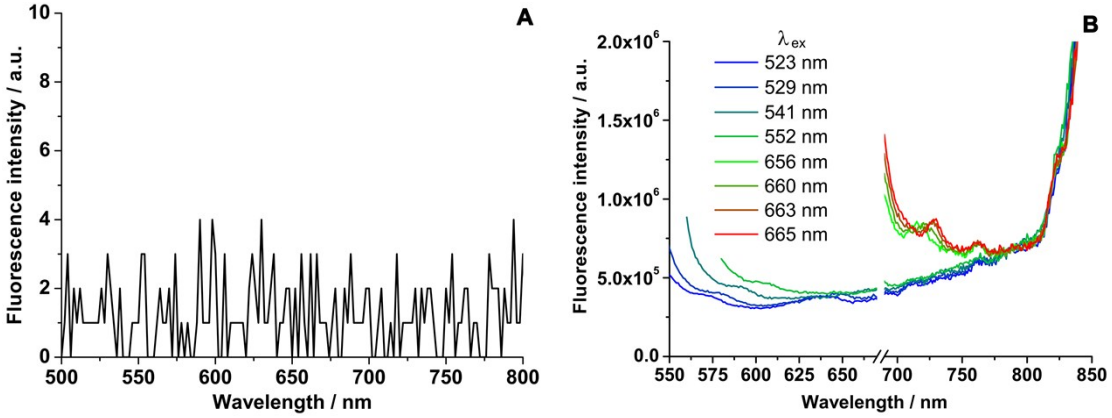


Fig. S2 The fluorescence of AuNRs at excitation wavelength of 980 nm (A) and 523–665 nm (B, characteristic λ_{em} of UCNPs).

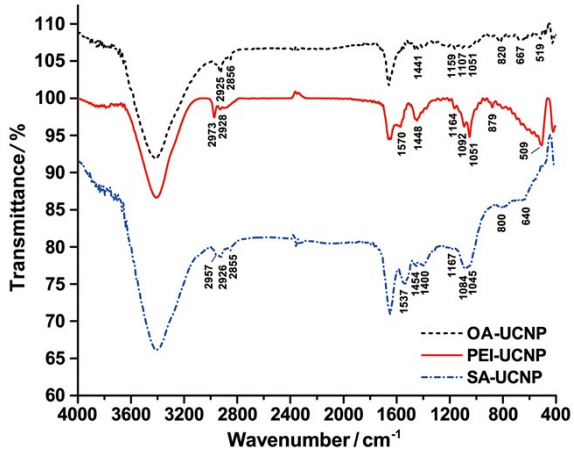


Fig. S3 FT-IR spectra of OA-UCNPs, PEI-UCNPs and SA-UCNPs.

Table S1 Concentration of rare earth in UCNPs determined by ICP-AES.

Element	Calculated	Determined
Yb	78%	75.39%
Y	20%	21.87%
Er	2%	2.78%

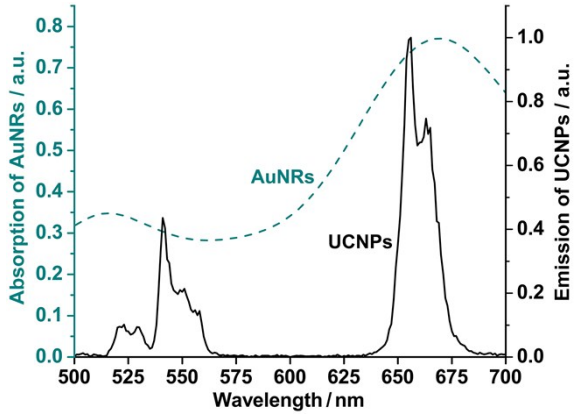
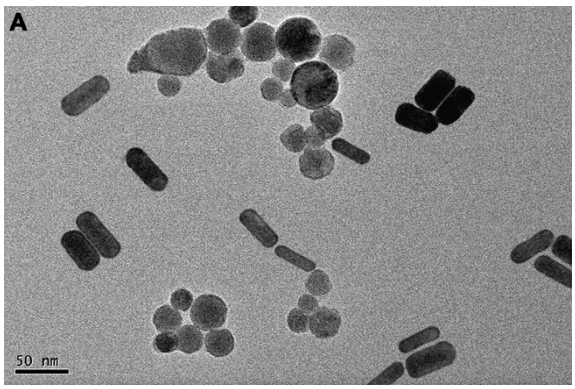


Fig. S4 Absorbance of AuNRs and UCF of PEI-UCNPs.



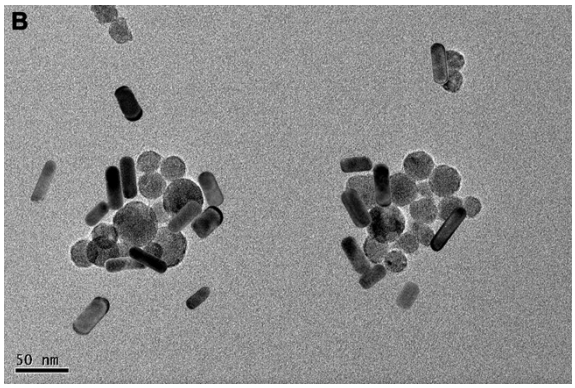


Fig. S5 TEM images of UCNPs and AuNRs in the absence (A) and presence (B) of dsDNA2. dsDNA2 = biotin-5′-ATACC^{m}GGTCTAAA-3′-S-S·3′-TATGGCCAGA-5′.

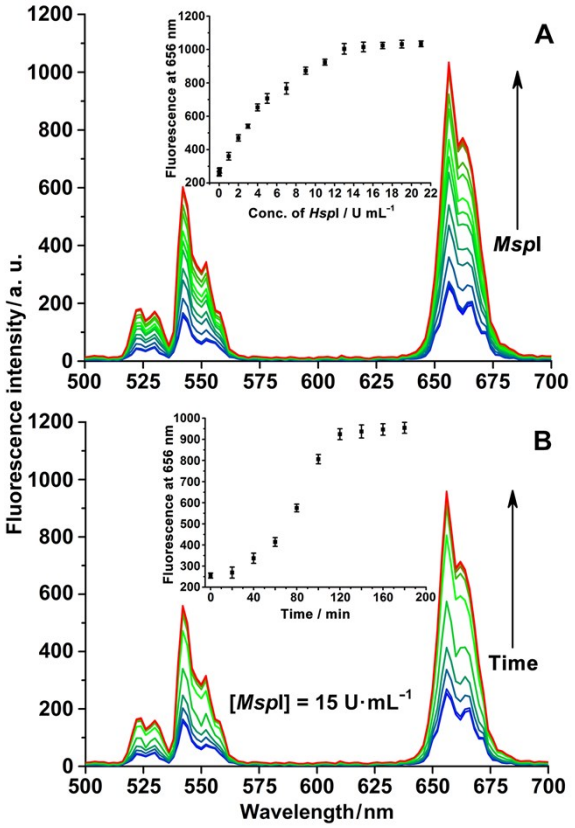


Fig. S6 Dependence of UCF restoration (λ_{ex} = 980 nm) of the AuNR-dsDNA2-UCNP system on the concentration of *Msp*I after treatment for 2 h (A) and that on the reaction time in the presence of *Msp*I (B) based on six parallel experiments.

2. Experimental

Materials

Ln_2O_3 (≥ 99.99%, Ln = Y, Yb or Er) and trifluoroacetic acid were purchased from Aladdin Reagent (Shanghai, China). Oleylamine (> 40.0%, GC) was purchased

from Tokyo Chemical Industry Co., Ltd. (Shanghai). Sodium trifluoroacetate, glycerol, *tris*(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%), poly(ethylene glycol) methyl ether thiol (mPEG-SH，Mn ≈ 6000), dithiothreitol (DTT, ~1 M in H_2O), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), bovine serum albumin (BSA) and polyethyleneimine (PEI, branched, MW = 25000) were purchased from Sigma-Aldrich Co. LLC. (Shanghai). Na_2HPO_4, NaH_2PO_4, NaCl, $MgCl_2$, HCl, NaOH, *N,N*-dimethylformamide (DMF) and ethanol were purchased from Nanjing Chemical Reagent Co., Ltd. $AgNO_3$, $HAuCl_4 \cdot 3H_2O$, ascorbic acid, $NaBH_4$ and cetyltrimethylamonium bromide (CTAB) were purchased from Sinopharm Chemical Reagent Co., Ltd. Streptavidin was purchased from Promega (Beijing) Biotech Co., Ltd. Oligonucleotides were synthesized and purified with HPLC by Thermo Fisher Scientific Inc. Restriction endonucleases *Hpa*II and *Msp*I were obtained from New England Biolabs (Beijing) Ltd. All the chemicals without specification are of analytical grade and used without further purification. Ultra-pure water (18 mΩ·cm, Milli-Q, Millipore) was used throughout the experiments.

Characterization

The size and morphology of UCNPs were observed on a transmission electron microscope (TEM, JEOL JEM-1011) at 200 kV. X-ray diffraction (XRD) data were recorded on a Simadzu XRD-6000 diffractometer using Cu K_α radiation (λ = 0.15418 nm). The upconversion fluorescence spectra were recorded at room temperature on a ZolixScan ZLX-UPL spectrometer using a 1 mL quartz cuvette (λ_{ex} = 980 nm). The UV-Vis spectra were obtained on a PerkinElmer LAMBDA 35 UV/Vis spectrophotometer using a 2 mL quartz cuvette and a 0.5 cm optical pathlength. Fourier transform infrared (FTIR) spectra were taken on KBr window using BRUKER TENSOR 27 FT-IR Spectrometer. Zeta electric potential of the nanoparticles was measured by Malvern Zetasizer Nano Z analyzer at pH 7.0.

Preparation of mPEG-SH-AuNRs

A seed-mediated growth route was used to synthesize the cetyltrimethylamonium

bromide (CTAB) stabilized AuNRs.[1, 2] Specifically, ice-cold $NaBH_4$ (0.01 M, 0.12 mL) was quickly added into the mixture of $HAuCl_4$ (0.5 mM, 1.0 mL) and CTAB (0.2 M, 1.0 mL). Brownish yellow seed solution was obtained after vigorous stirring for 2 min and standing at room temperature. Ascorbic acid (78.8 mM, 70 μL) was added into the mixture of CTAB (0.2 M, 5.0 mL), $HAuCl_4$ (1.0 mM, 5.0 mL) and $AgNO_3$ (4.0 mM, 0.10 mL) and colorless growth solution was obtained. The growth solution was mixed with the seed solution (12 μL) and kept still for 20 min, and the color was changed from colorless to dark green. The mixture was reserved overnight and the excess CTAB was removed by centrifugation at 5000 rpm and 4 °C. The concentration of the obtained AuNRs was calculated to be 2.968 nM according to the Lambert-Beer law and the extinction coefficient of AuNRs (3.1×10^9 M^{-1} cm^{-1}).[2] mPEG-SH (2.0 mM) was added to the AuNRs and incubated for 3 h to modify the surface of AuNRs.

Synthesis and modification of UCNPs

UCNPs (*β*-$NaY_{0.20}F_4$:78%Yb, 2%Er) were synthesized according to a classical method with some modifications.[3] All the rare-earth trifluoroacetates were prepared by dissolving the respective rare-earth oxide in trifluoroacetic acid and water (1/1, v/v) solution. In brief, $CF_3COONa{\cdot}3H_2O$ (2 mmol), $(CF_3COO)_3Yb{\cdot}3H_2O$ (0.78 mmol), $(CF_3COO)_3Y{\cdot}3H_2O$ (0.20 mmol), and $(CF_3COO)_3Er{\cdot}3H_2O$ (0.02 mmol) were dissolved in oleylamine (OA, 20 mL) and then passed through a filter (0.22 μm, Millipore) to remove any possible residues. Under vigorous stirring, the mixture was slowly heated to 120 °C under vacuum for 30 min to remove residual water and oxygen while purging periodically with dry Ar. The resulting pale yellow solution was heated to 340 °C in 15 min under dry Ar and the mixture was stirred at this temperature for 2 h. The resultant transparent yellowish product was allowed to cool below 60 °C. Ethanol (20 mL) was added to the solution and the precipitate was isolated via centrifugation at 10000 rpm for 20 min. The resulting nanoparticles were washed with *n*-hexane/ethanol (1/1, v/v) and water thrice respectively to remove the NaF residue. The obtained nanoparticles (OA-UCNPs) were dispersed in *n*-hexane (5

mL) for later use. OA-UCNPs (20 mg) were re-dispersed in the DMF solution (20 mL) of branched PEI (16 mg, MW = 25000) by ultrasonication for 0.5 h, and then stirred at 80 °C under the protection of Ar for 2 h. After cooling to room temperature, the products (PEI-UCNPs) were isolated by centrifugation at 10000 rpm for 20 min, purified with distilled water and ethanol and dried in a vacuum oven at 40 °C overnight.[4]

Preparation of AuNR-dsDNA

The dsDNA1 or dsDNA2 was formed by annealing biotin-5′-ATACC^{m}GGTCTAAA-3′-S-S (ssDNA, 50 μM, 20 μL) and 5′-AGACCGGTAT-3′ (S1) or 5′-AGACC^{m}GGTAT-3′ (S2, 50 μM, 20 μL) in Tris-HCl buffer (10 μL, 10 mM, pH 7.4) at 37 °C for 2 h and cooling to room temperature, respectively. The dsDNA1 or dsDNA2 (5 μL, 20 μM) was treated with TCEP solution (2 μL, 10 μM) for 1 h to reduce the disulfide bond at 3′-terminus to a free thiol group. The reduced dsDNA was incubated with mPEG-SH-AuNRs (0.5 mL, 2.968 nM) for 24 h to form an AuNR-dsDNA conjugate through the surface Au-S bond. The AuNR-dsDNA conjugate was isolated by centrifugation and washed with HEPES to remove the unbounded dsDNA.

Preparation of SA-UCNPs

Streptavidin was attached onto PEI-UCNPs according to a glutaraldehyde method.[5] Typically, PEI-UCNPs (10 mg) were dispersed in a phosphate buffer solution (PBS, 5 mL, 10 mM, pH 7.4) by ultrasonication for 20 min, and then a glutaraldehyde solution (0.55 mL, 50%, w/w) was added. The mixture was shaken slowly at room temperature for 2 h and then separated by centrifugation at 10000 rpm for 20 min. The obtained glutaraldehyde-modified nanoparticles were washed with PBS (10 mM, pH 7.4) for three times and re-dispersed in PBS (4 mL, 10 mM, pH 7.4) by ultrasonication. Streptavidin (0.4 mg) was added to the suspension and shaken slowly at room temperature for 12 h. The streptavidin-conjugated UCNPs (SA-UCNPs) were separated by centrifugation at 10000 rpm for 20 min and washed with

PBS (10 mM, pH 7.4) thrice. SA-UCNPs were dispersed in HEPES buffer solution (4 mL, 10 mM, pH 7.2) at 4 °C prior to use.

Conjugation of AuNR-dsDNA with SA-UCNP

AuNR-dsDNA1 or AuNR-dsDNA2 was added into the suspension of SA-UCNPs (0.5 mg) in Tris-HCl buffer (1 mL, 10 mM, pH 7.0, 10 mM $MgCl_2$, 100 $\mu g \cdot mL^{-1}$ BSA) and incubated for 15 min, and the conjugate AuNR-dsDNA1-UCNP or AuNR-dsDNA2-UCNP was obtained.

Identification of methylation

AuNR-dsDNA-UCNP was treated with *Hpa*II at 37 °C in Tris-HCl buffer (10 mM, pH 7.0, 10 mM $MgCl_2$, 100 $\mu g \cdot mL^{-1}$ BSA, 50% glycerol). *Msp*I was introduced and incubated for another period of time. The upconversion fluorescence intensity of the mixture was recorded before and after the addition of *Msp*I.

References

1. B. Nikoobakht and M. A. El-Sayed, *Chemistry of Materials*, 2003, **15**, 1957.
2. C. J. Orendorff and C. J. Murphy, *The journal of physical chemistry. B*, 2006, **110**, 3990.
3. G. S. Yi and G. M. Chow, *Adv. Funct. Mater.*, 2006, **16**, 2324.
4. J. Jin, Y.-J. Gu, C. W.-Y. Man, J. Cheng, Z. Xu, Y. Zhang, H. Wang, V. H.-Y. Lee, S. H. Cheng and W.-T. Wong, *ACS Nano*, 2011, **5**, 7838.
5. X. Hun and Z. Zhang, *Biosens Bioelectron*, 2007, **22**, 2743; M. V. Kiselev, A. K. Gladilin, N. S. Melik-Nubarov, P. G. Sveshnikov, P. Miethe and A. V. Levashov, *Analytical Biochemistry*, 1999, **269**, 393.