

## Supporting Information

**Label-free and enzyme-free plasmon-enhanced single molecule  
fluorescence detection of HIV DNA fragment based on catalytic  
hairpin assembly**

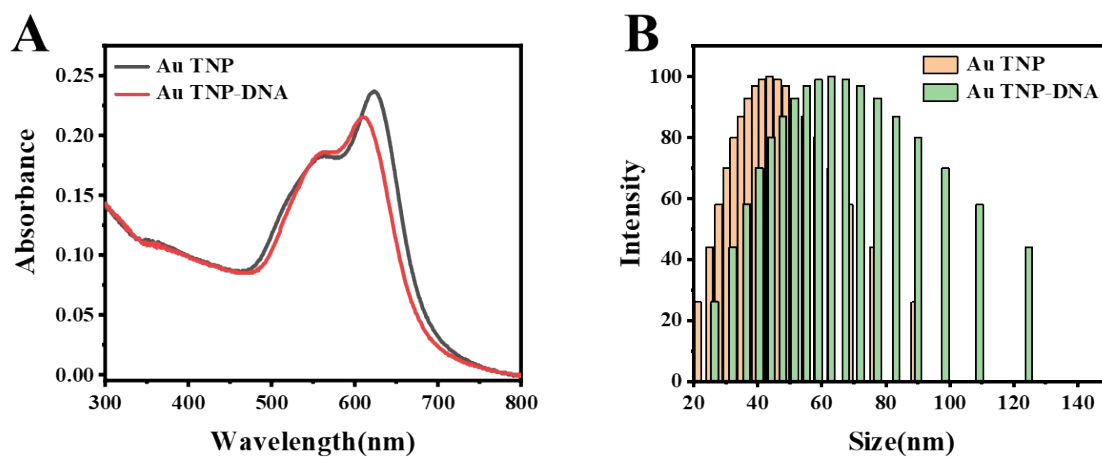
*Ke Shi, Na Na, Jin Ouyang\**

Key Laboratory of Theoretical and Computational Photochemistry, Ministry of  
Education, College of Chemistry, Beijing Normal University, Beijing, 100875 (P. R.  
China)

\*Corresponding author: Tel: +86-10-58805373; Fax: +86-10-62799838

E-mail: jinoyang@bnu.edu.cn

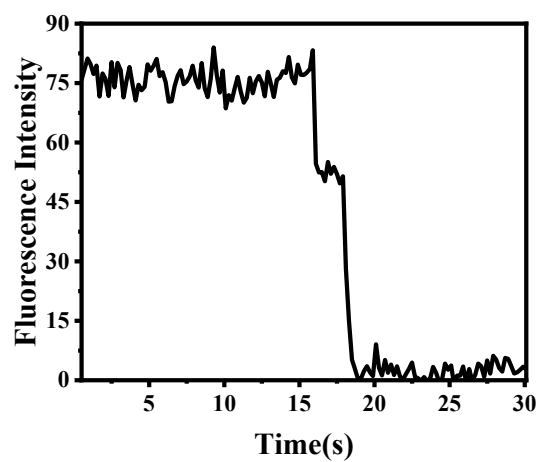
## Results and Discussion



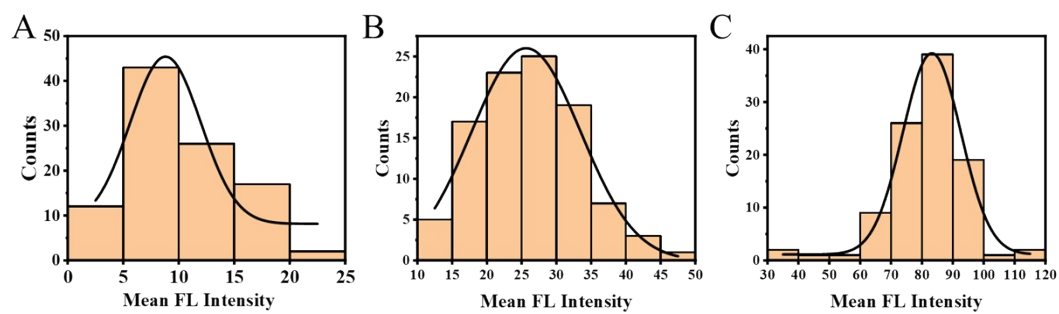
**Fig. S1** (A) UV-vis spectra of solutions containing unmodified Au TNP (black) and Au TNP-DNA (red). (B) Dynamic light scattering (DLS) characterization of the unmodified Au TNPs (orange) and Au TNP-DNA (green).

**Table S1.** Sequences of the oligonucleotides used in this work

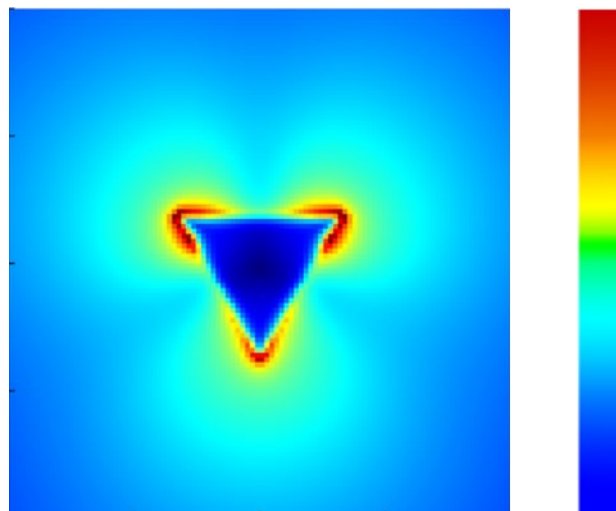
| Note       | Sequence                                                                                                              |
|------------|-----------------------------------------------------------------------------------------------------------------------|
| Target DNA | 5'-ACT GCT AGA GAT TTT CCA CAT-3'                                                                                     |
| HP1        | 5'-AT GTG GAA AAT CTC TAG CAG TTT TCG ACA<br>TCT GGC ATG CAT TTG TAC TGGGC AGGGC TGG<br>GC TAGGG AT-3'                |
| HP2        | 5'-GTA CAA ATG CAT GCC AGA TGT CGA AAA C<br>TGCTA GAG ATT TTC CAC AT-3'                                               |
| 1MT        | 5'-ACT GCT AGA GCT TTT CCA CAT-3'                                                                                     |
| 2MT        | 5'-ACT GAT AGA GAT TTT CGA CAT-3'                                                                                     |
| 3MT        | 5'-ACA GCT AGA GCT TTT CCA TAT-3'                                                                                     |
| NCT        | 5'-TAG CAT CGC TAC GGA TCT ACC-3'                                                                                     |
| HP1-12nt   | 5'-AT GTG GAA AAT TGGGC AGGGC TGGGC TAG<br>GG AT-3'                                                                   |
| HP1-24nt   | 5'-AT GTG GAA AAT CTC TAG CAG TTT TGGGC A<br>GGGC TGGGC TAGGG AT-3'                                                   |
| HP1-36nt   | 5'-AT GTG GAA AAT CTC TAG CAG TTT TCG ACA<br>TCT GGC TGGGC AGGGC TGGGC TAGGG AT-3'                                    |
| HP1-48nt   | 5'-AT GTG GAA AAT CTC TAG CAG TTT TCG ACA<br>TCT GGC ATG CAT TTG TAC TGGGC AGGGC TGG<br>GC TAGGG AT-3'                |
| HP1-60nt   | 5'-AT GTG GAA AAT CTC TAG CAG TTT TCG ACA<br>TCT GGC ATG CAT TTG TAC CAT CGC TAC GGA<br>TGGGC AGGGC TGGGC TAGGG AT-3' |



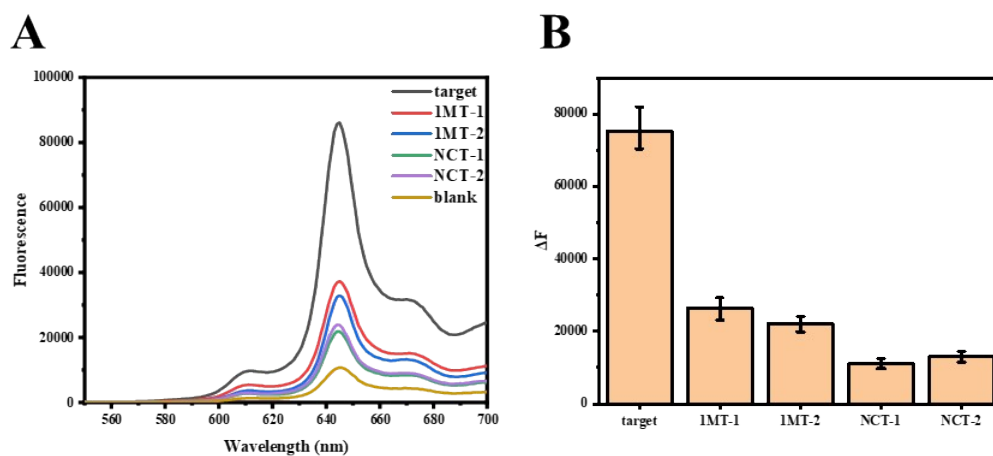
**Fig. S2** A typical two-step photobleaching behavior of fluorescence time traces of the NMM@G-quadruplex attached to the Au TNP.



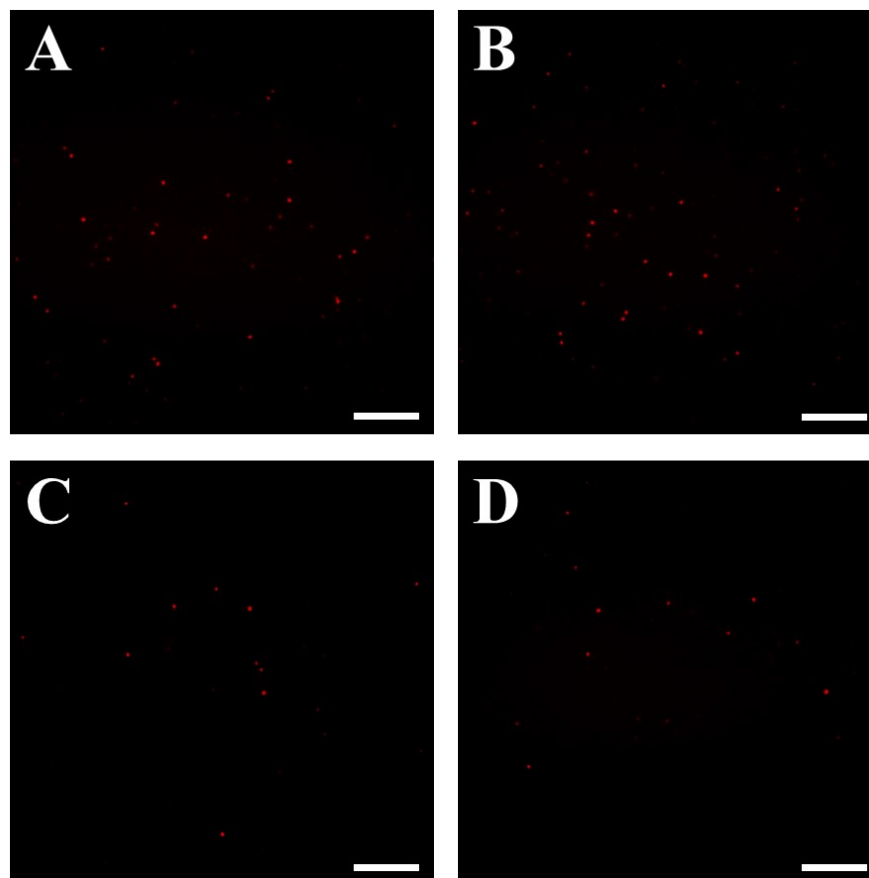
**Fig. S3** Fluorescence intensity histograms of (A)NMM, (B) G-quadruplex@NMM, (C)Au TNP@G-quadruplex@NMM.



**Fig. S4** The electric fields of Au TNP were simulated with FDTD model.

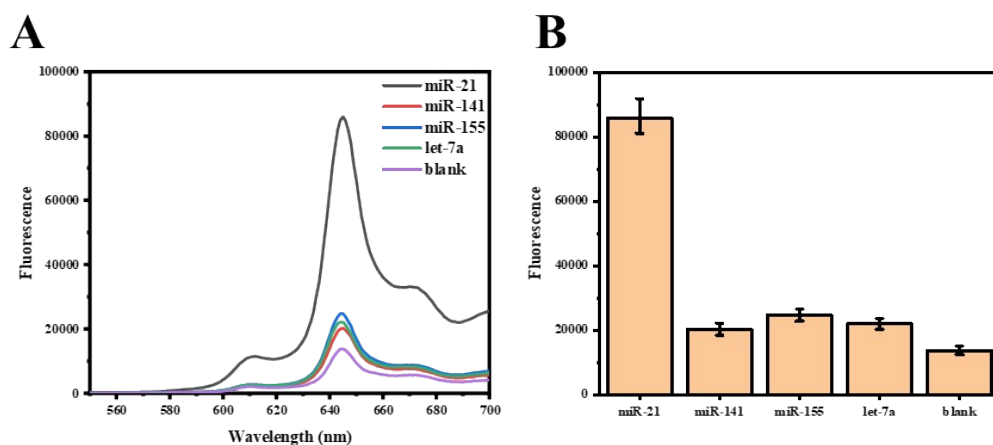


**Figure S5.** (A) Fluorescence spectrum and (B) Histogram of fluorescence intensity change of one-base mutation and completely non-complementary target.

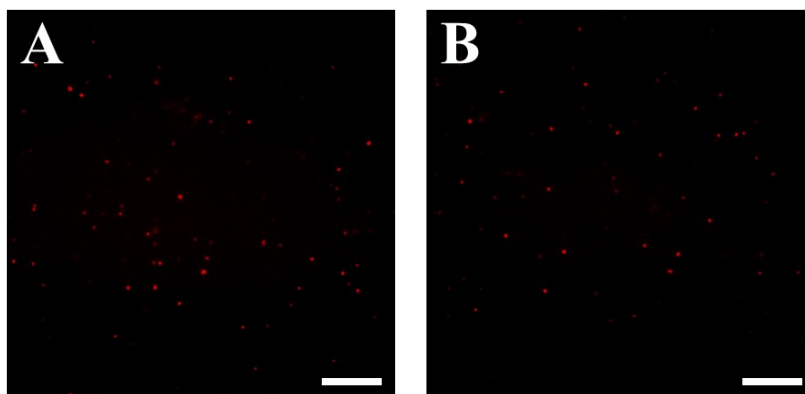


**Figure S6.** Representative single-molecule fluorescence image of one-base mutation target (A) 1MT-1 (B) 1MT-2 and completely non-complementary target (C) NCT-1 (D) NCT-2. Scale bar = 5  $\mu\text{m}$ .





**Figure S7.** Verification of the feasibility of this strategy for microRNA-21 detection and specificity investigation for microRNA-21 against microRNA-141, microRNA-155, let-7a.



**Figure S8.** Representative single-molecule fluorescence image of target in HeLa and A549 cell lysates.

**Table S2.** Comparison of HIV DNA detection between this work and other strategy

| Detection strategy                                    | Methods          |                              | Linear range     | Detection limit | Ref       |
|-------------------------------------------------------|------------------|------------------------------|------------------|-----------------|-----------|
| G-quadruplex-based fluorescence strategy              | Fluorescence     |                              | 0.1 nM-50.0 nM   | 13 pM           | 1         |
| CRISPR-based biosensor                                | ECL              | Electrochemiluminescence     | 100 fM-1 $\mu$ M | 30 fM           | 2         |
| fluorescent lateral flow assay strips                 | flow             | Fluorescence                 | 1 pM-10 nM       | 11 pM           | 3         |
| DNA-stabilized based probe                            | AgNCs-           | Fluorescence                 | 0.2-700 nM       | 33 pM           | 4         |
| DNA nanomachine on gold nanoparticles (AuNPs)         | Fluorescence     |                              | 5 fM-1.67 pM     | 1.46 fM         | 5         |
| Exo I Hydrolysis-Assisted hpDNA Biosensors            | Electrochemistry |                              | 0.1 pM-10 nM     | 150 fM          | 6         |
| Multiple Amplified Biosensor                          | ECL              | Electrochemiluminescence     | 0.05 pM-50 nM    | 39.81 fM        | 7         |
| upconversion luminescence nanoprobe                   | luminescence     | luminescence                 | 0.02-30 nM       | 10.2 pM         | 8         |
| G-quadruplex-based TNP enhanced fluorescence strategy | Au               | Single-molecule fluorescence | 0-1000 fM        | 0.83 fM         | This work |

**References:**

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3. Deng, X.; Wang, C.; Gao, Y.; Li, J.; Wen, W.; Zhang, X.; Wang, S. *Biosensors and Bioelectronics* 2018, 105, 211-217.
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6. Gao, X.; Wang, X.; Li, Y.; He, J.; Yu, H. *Analytical Chemistry* 2018, 90 (13), 8147-8153.
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**Table S3.** DNA and miRNA sequences used in the additional experiments

| Names     | Sequences (5' to 3')                                                      |
|-----------|---------------------------------------------------------------------------|
| SH-HP1    | ATGTGGAACATCCTGCCCATAGACTTCAACATCAGTCTGAT<br>AAGCTATGGGCAGGGCTGGGCTAGGGAT |
| HP2       | ATAGACTTCAACAATAGCTTATCAGACTGATGTTGAAGTCT<br>ATGGGAGG                     |
| miRNA-141 | U AACACUGUCUGGUAAAGAUGG                                                   |
| miRNA-155 | U UAAUGCUAAUCGUGAUAGGGGU                                                  |
| miR-21    | U AGCUUAUCAGACUGAUGUUGA                                                   |
| let-7a    | U GAGGUAGUAGGUUGUAUAGUU                                                   |

**Table S4.** One-base mutation and non-complementary target sequences used in the additional experiments.

| Names | Sequences (5' to 3')   |
|-------|------------------------|
| 1MT-1 | ACTGCTAGAGCTTTTCCACAT  |
| 1MT-2 | ACTGCTAGAGATTTTTCGACAT |
| NCT-1 | TAGCATCGCTACGGATCTACC  |
| NCT-2 | ACAGGGATGAGCTTTTCGACAT |

**Table S5.** Relative standard deviation of this detection strategy.

| Target concentration | Average fluorescence intensity change | RSD%(n=5) |
|----------------------|---------------------------------------|-----------|
| 1 pM                 | 15033.63                              | 5.35      |
| 10 pM                | 29745.13                              | 3.70      |
| 100 pM               | 45921.47                              | 4.91      |
| 1 nM                 | 70422.53                              | 5.64      |
| 10 nM                | 90628.90                              | 4.25      |