Electronic Supplementary Information (ESI)

for

Nanosurface Energy Transfer Indicating Exo III-Propelled Stochastic 3D DNA Walker for HIV DNA Detection

Na Wang, [a] Yong Jian Jiang, [a] Xu Zhang, [a] Hua Rong Lin, [a] Feng Cheng, [a] Qian Li, [a] Chun Mei Li, [†a] and Cheng Zhi Huang [ab]

[a]Key Laboratory of Luminescence Analysis and Molecular Sensing (Southwest University), Ministry of Education, College of Pharmaceutical Sciences, Southwest University, Chongqing 400715, P. R. China.

[b]Key Laboratory of Luminescent and Real-Time Analytical System (Southwest University), Chongqing Science and Technology Bureau, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China.

[†]Corresponding Author: licm1024@swu.edu.cn; Fax: +86 2368367257; Tel: +86 2368254059

Experimental section

Characterization of agarose gel electrophoresis. In the gel electrophoresis assay, a test sample containing 10 μL different reaction products and 2 μL loading bu□er is subjected to 12% polyacrylamide gel. The gel was run at 120 V for 35 min in 1 × TBE bu□er, and stained with 4S Red Plus nucleic acid stain for 15 min and imaged the gel.

Single-particle scattering analysis for the operation of 3D stochastic DNA walkers. At first, positively charged surfaces of glass slides were obtained sequentially cleaned by chromic acid and distilled water, then dried by nitrogen. To make nanoparticles distinguishable under the DFM system, they were kept immobilized on the glass slides through two steps. Second, a cross label was marked on each glass slide, and the diluted 3D nanomachine solution was placed into the glass slide for 15 min to prepare the control group. One region nearby the cross label was chosen and captured under the DFM system. Lastly, the same slide was placed the diluted 3D nanomachine with HIV DNA and Exo III solution for 10 min to prepare the probe group, in which PRET would occur. The same region under the DFM system was recognized based on the outline of the cross label, and then its DFM image was taken again.

Kinetic analysis experiments. Add 10 microliters of HIV-DNA (final concentration: 1nM) to a mixture of 200 μL, add 100 μL of hairpin DNA 3D nanomachine (final concentration: 0.1 nM) and 120 U of Exo III. The solutions were transferred to 96-well plates, and the fluorescence intensities were monitored at different time by microplate reader. Control group in the absence of Exo III was carried out for hairpin DNA 3D nanomachine in the same way. In addition, linear DNA modified 3D nanomachine in the absence and presence of HIV DNA were carried out as the above protocol, respectively.

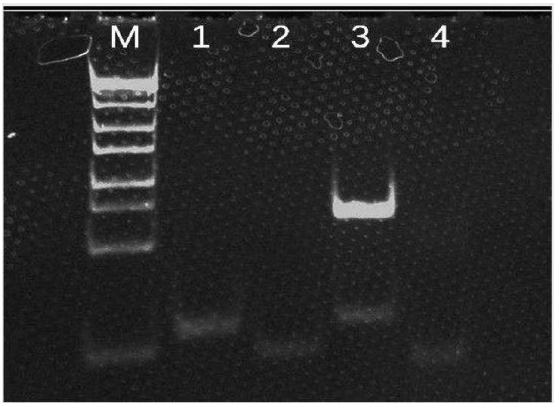

Figure S1. Performances of nucleic acid reaction for HIV DNA detection. 12% PAGE. M: DNA ladder (25-500 bp); Lane 1: Hairpin DNA strand; Lane 2: HIV DNA strand; Lane 3: Hairpin DNA+HIV DNA; Lane 4: Hairpin DNA+HIV DNA+Exo III.

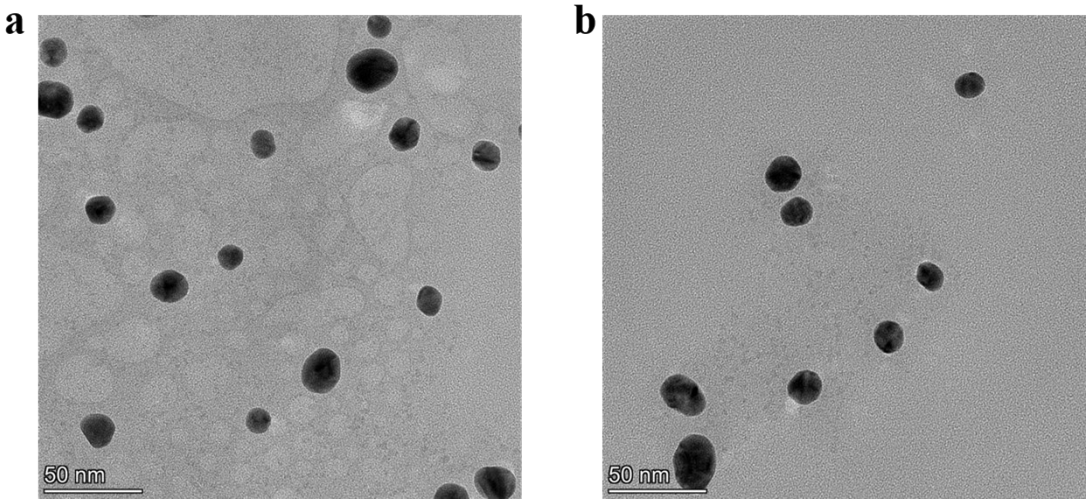

Figure S2. TEM images of the prepared AuNPs (a) and 3D DNA nanomachine(b).

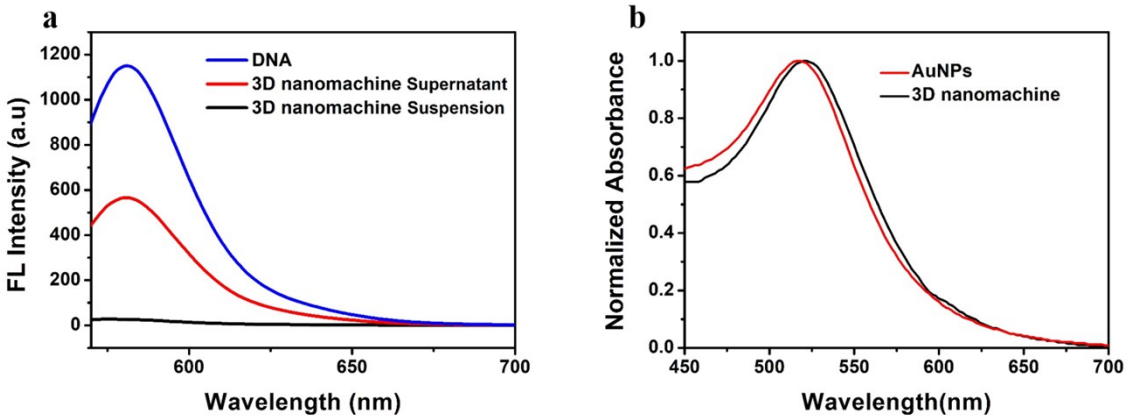

Figure S3. Characterization for the modification of DNA on AuNPs. (a) fluorescence emission spectra of the centrifuged solution; (b) normalized UV-vis absorption spectra of AuNPs and 3D nanomachine. The maximum absorption of the AuNPs was at 518 nm, which was red-shifted to 522 nm after modification with DNA.

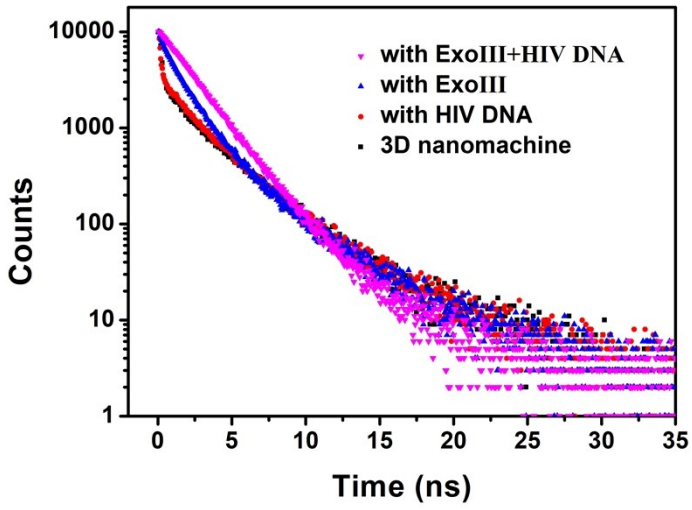

Figure S4. FL lifetime measurements of 3D nanomachine in the presence of Exo III (blue), HIV DNA (red), Exo III and HIV DNA (pink).

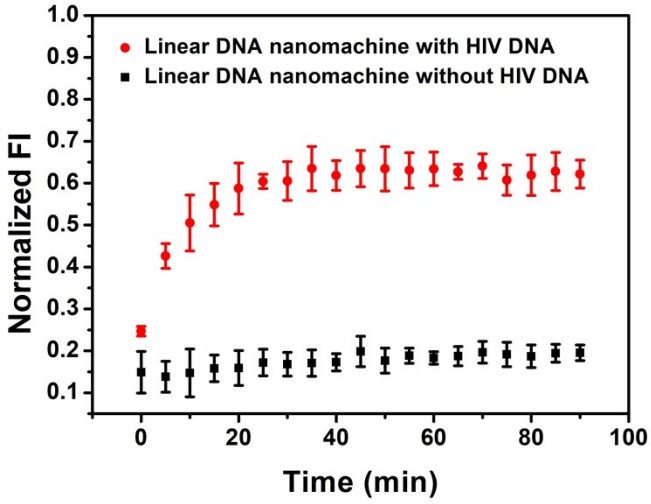

Figure S5. Kinetic analysis for HIV DNA detection with linear DNA nanomachine.

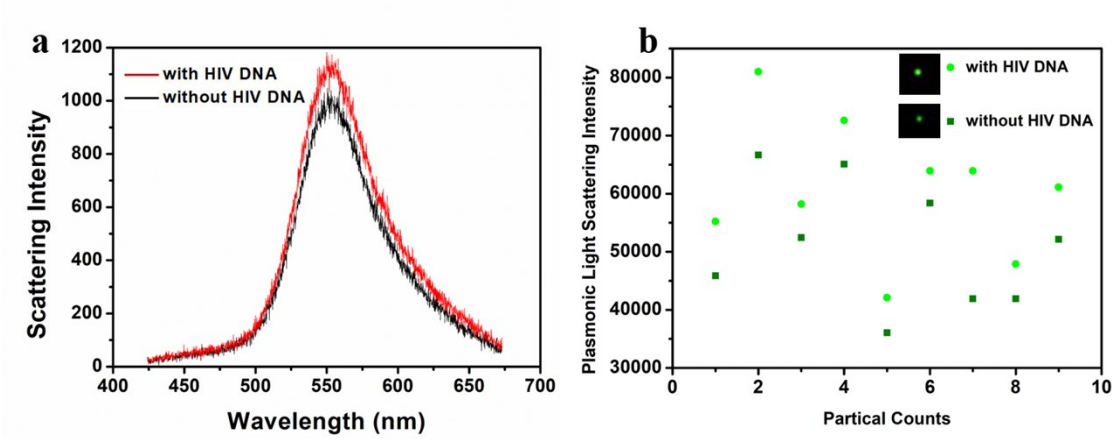

Figure S6. (a) Dark-field light scattering spectra of single of 3D DNA nanomachine with or without HIV DNA. (b) Statistics on the light scattering intensity of 9 particles in the field of vision under DFM.

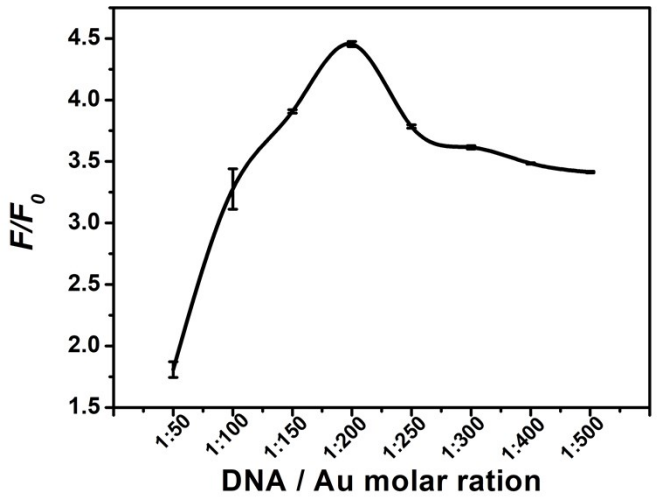

Figure S7. Molar ratio of DNA and AuNPs optimization for the detection of HIV DNA with 3D nanomachine. Target HIV DNA: 1.0 nM; Exo Ⅲ: 120U; 3D nanomachine: 0.1nM; Time: 2.5h; Temperature: 37 ^{o}C.

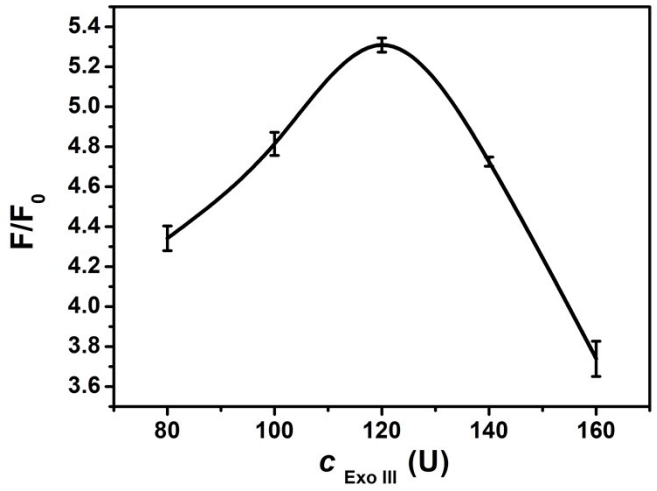

Figure S8. The optimization of concentration of Exo Ⅲ for the detection of HIV DNA with 3D nanomachine. Target HIV DNA: 1.0 nM; 3D nanomachine: 0.1nM; Time: 2.5h; Temperature: 37 ^{o}C.

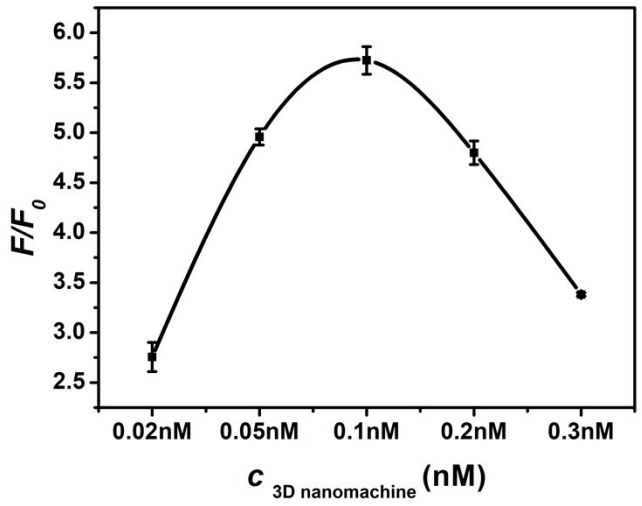

Figure S9. The concentration of 3D nanomachine optimization for the detection of HIV DNA with 3D nanomachine. Target HIV DNA: 1.0 nM; Exo Ⅲ: 120U; Time: 2.5h; Temperature: 37 ºC.

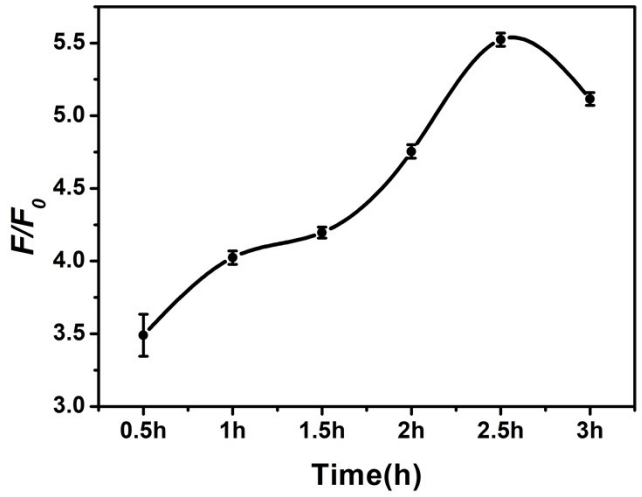

Figure S10. The optimization of incubation time for the detection of HIV DNA with 3D nanomachine. Target HIV DNA: 1.0 nM; Exo Ⅲ: 120U; 3D nanomachine: 0.1nM; Temperature: 37 ºC.

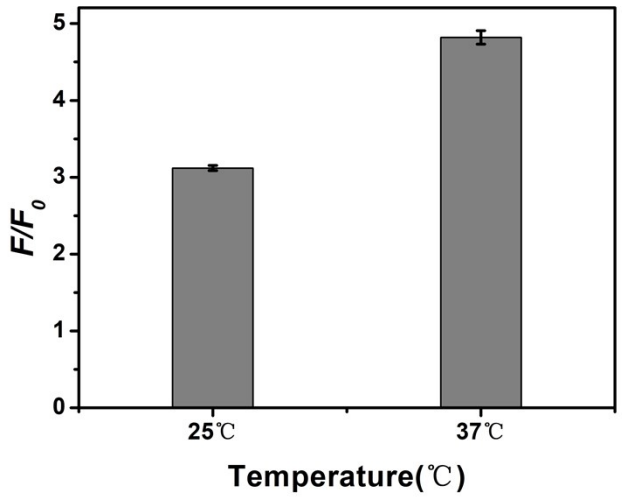

Figure S11. Temperature optimization for the detection of HIV DNA with 3D nanomachine. Target HIV DNA: 1.0 nM; Exo Ⅲ: 120U; 3D nanomachine: 0.1nM; Time: 2.5h;

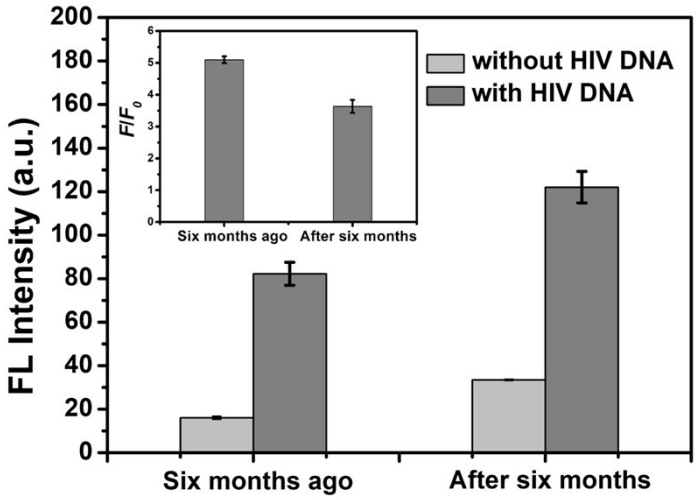

Figure S12. Stability of 3D nanomachine for the detection of HIV DNA before (left) and after (right) six months. Concentrations: 3D nanomachine, 0.1 nM; HIV DNA , 1.0 nM; Exo III, 120 U; Temperature: 37 ^{o}C.

Table S1 The DNA sequences information.

Names	Sequence (from 5' to 3')
Hairpin	SH-TTTTTTTTTTATTTGGTGTTATTCCAAATATCTTCT-TAMRA
Linar	SH-TTTTTTTTTTTAAACCTGTTATTCCAAATATCTTCT-TAMRA
HIV	AGAAGATATTTGGAATAACATGACCTGGATGCA
MT-1	AGAAGATATTTCGAATAACATGACCTGGATGCA
MT-3	AGAAGTTATTTCGAATAAGATGACCTGGATGCA
HBV	TGGGAGGAGTTGGGGGAGGAGATTAGGTTAAAGGT
HIV'	GCTAGAGATTTTCCACACTGACT

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

Probe	Methods	Linear range	Detection limit	Ref
UCNPs-DNA-AuNPs	Fluorescence	0-80.0 nM	3 nM	1
ENs-DNA-MB	Electrochemiluminescence	0.05 pM-50.0 nM	39.81 fM	2
CNP-DNA-AgNCs	Fluorescence	1-50.0 nM	0.4 nM	3
ThT-G-triplexes-DNA	Fluorescence	0.4-110.0 μM	33 pM	4
ThT-G-triplexes-DNA	Fluorescence	0.1-50.0 nM	13 pM	5
BHQ_2-DNA-QDs	Fluorescence	0-20.0 nM	0.5 nM	6
FAM-DNAzyme-DABCYL	Fluorescence	0.001-2 nM	1 pM	7
TAMRA-DNA-AuNPs	Fluorescence	0.05-1.2 nM	12.7 pM	This work

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