Supporting Information for

FRET-Based Biosensing Platform with Ultrasmall Silver Nanoclusters as Energy Acceptors

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Experimental details.

Reagents: The short peptide and all DNA strands were supplied by Sangon Biotechnology Co., Ltd. (Shanghai, China). The S1 nuclease and other proteins were purchased from Promega Corp. (Madison, WI). Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amino acids were from Ru-Ji Biotechnology Co., Ltd. (Shanghai, China). The rest of the chemical reagents were commercially available and at least analytical grade. All aqueous solutions were prepared using ultrapure water (Mill-Q, Millipore, 18.2 MΩ resistivity).

Synthesis of Ag nanoclusters: In a typical protocol, 15 μM DNA in 20 mM phosphate buffer (5 mM magnesium acetate, pH 7.0) was incubated with 90 μM $AgNO_3$ solution on ice bath for 15 minutes. Then freshly prepared sodium borohydride (90 μM) was added to the mixture and quickly vortexed for two minutes. The obtained solution was left in the dark for 12 h at 4 ℃ to form Ag NCs.

Preparation of DNA duplex-Ag nanoclusters: In the hybridization procedure, 1 μM linker DNA was first mixed with 1 μM Str-D and 1 μM Str-A. The DNA solutions were then heated at 85 ℃ for 15 minutes followed with a slow annealing treatment for 1 hour to form DNA duplex. The DNA duplexes solutions were incubated with 12 μM $AgNO_3$ solution on ice bath for 15 minutes and reduced with 12 μM $NaHB_4$ to generate Ag NCs.

Preparation of Peptide-ssDNA conjugate: The short peptide was covalently linked to the 5' end of the ssDNA (Str-A', 5′-NH_2-AAACCCTTAATCCCC-3′) following an amine-to-sulfhydryl coupling protocol. Sulfo-SMCC (144 nmol) was added to 200 μL of HEPES buffer (10 mM, pH 7.4) solution containing 14.4 nmol ssDNA. The mixture was gently shaken for 1 h at room temperature to activate the amine group. Then the unbound coupling reagents were removed by ultrafiltration using an Amicon Ultra-4 Centrifugal Filter Device with a MW cutoff of 3 kDa (Millipore Corp.) at 4 ℃. The ultrafiltration residue with molecular mass larger than 3 kDa was collected and diluted in HEPES buffer (10 mM, pH 7.4). Afterwards, 36 μL of the peptide solution (28.8

nmol) was incubated with the activated ssDNA solution with gentle shaking overnight at room temperature. The excess peptide was removed by ultrafiltration and the obtained peptide-ssDNA conjugate was finally diluted to 1 mL with ultrapure water for further use.

Preparation of Peptide-ssDNA-Ag NCs: $AgNO_3$ solution (4.32 mM, 5 μL) was added to aliquots of ssDNA or the Peptide-ssDNA conjugate (14.4 μM, 500 μL). After 15 min of incubation on ice bath, freshly prepared sodium borohydride (4.32 mM, 5 μL) was added to the mixture and quickly vortexed for two minutes. Then the solution was left in the dark for 12 h at 4 ℃. For simplicity, the concentration of the as-prepared ssDNA-Ag NCs or peptide-ssDNA-Ag NCs was designated as 1×.[1]

Sensing of S1 nuclease: A 1.5 μM DNA duplex-Ag NCs complex or 10 μM peptide-ssDNA-Ag NCs complex solution was incubated with various concentrations of S1 nuclease in aqueous buffer (5 mM CH_3COONa, 28 mM NaCl, 0.45 mM $ZnSO_4$, pH 4.5) at 37 ℃ with gentle shaking for 0.5 h. Thereafter, the mixtures were diluted to 500 μL with phosphate buffer for subsequent fluorescence measurements. To examine the specificity of the peptide-ssDNA-Ag NCs biosensor towards S1 nuclease, some other biomolecules and inorganic ions were added in place of S1 nuclease with the same experimental conditions and procedures.

Gel electrophoresis: The 18% polyacrylamide gel electrophoresis (PAGE) analysis of the DNA and hydrolysis products of DNA-silver nanoclusters by nuclease was carried out in 1×Tris-Borate-EDTA at a constant voltage of 100 V for about 1 h. After ethidium bromide staining, the gel was scanned using a Pharos FX Molecular Imager (Bio-Rad, USA).

Instrumentation: The size and morphology of Ag nanoclusters were characterized by a JEM-2010 transmission electron microscope with an acceleration voltage of 200 kV. The UV-vis absorption spectra were recorded using a UV-2550 UV-vis spectrometer (Shimadzu, Japan). The fluorescence measurements were performed with a RF-5301 PC fluorometry (Shimadzu, Japan). The time-resolved fluorescence was measured by using a FLS920 Lifetime Spectrometer (Edinburgh, England).

Supplementary Tables and Figures

Table S1. Sequences of the oligonucleotides used in the experiemnts.

	Sequence (5'- 3')		
Names	Str-D	Str-A	Str-L
DNA duplex 1	*GAGTTACCGTAGTCA	GCTGGTATTTTTTCCCCCCCCCCCCC	TGACTACGGTAACTCTTAAAAATACCAGC-TTAYTCAATT
DNA duplex 2	*GAGTTACCGTAGTCA	CCCCCCCCCCCCTTTTTGGTGT	TGACTACGGTAACTCACACCAAAAA
DNA duplex 3	TGCCATTGAG*	CCCCCCCCCCCCTTTTTGGTGT	CTCAATGGCAACACCAAAAA
DNA duplex 4	AGTATACTGATGCCATTGAG*	CCCCCCCCCCCCTTTTTGGTGT	CTCAATGGCATCAGTATACTACACCAAAAA

The asterisks (*) refer to the fluorophore (JOE) labeling site.

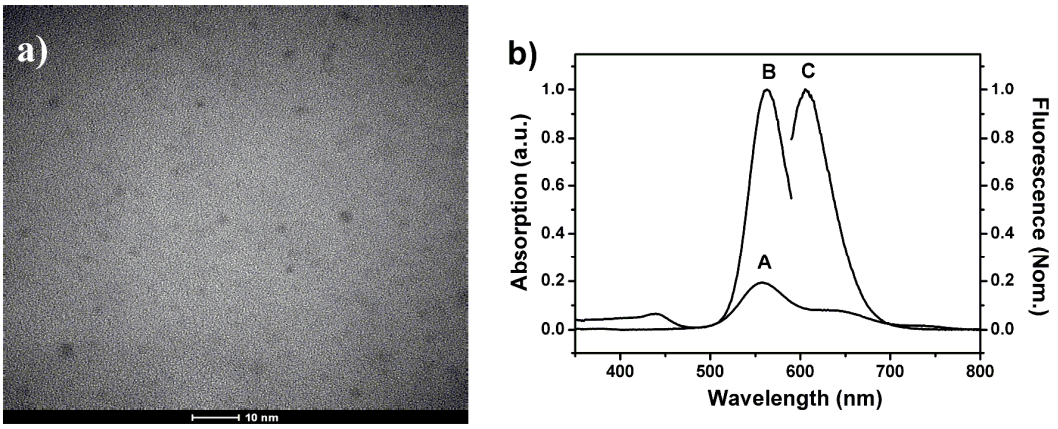

Figure S1. (a) TEM image of the as-synthesized Str-A-Ag NCs. (b) UV−vis absorption (A) and normalized fluorescence excitation (B) and emission (C) spectra of the Str-A-Ag NCs.

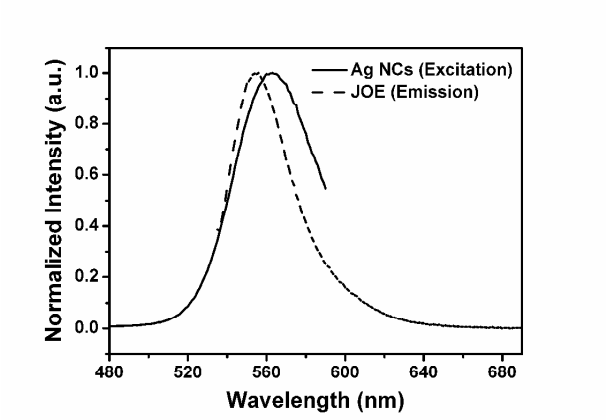

Figure S2. The spectral match between the excitation of Str-A-Ag NCs (solid line) and the fluorescence emission of JOE (dash line).

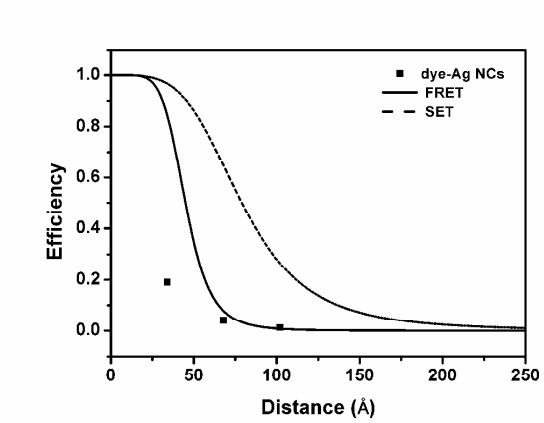

Figure S3. Energy transfer efficiency plotted versus separation distance between JOE and Ag NCs. The black squares are the experimental values of dye-Ag NCs complexes, while the dash line and solid line are the theoretical curves for FRET and SET, respectively.

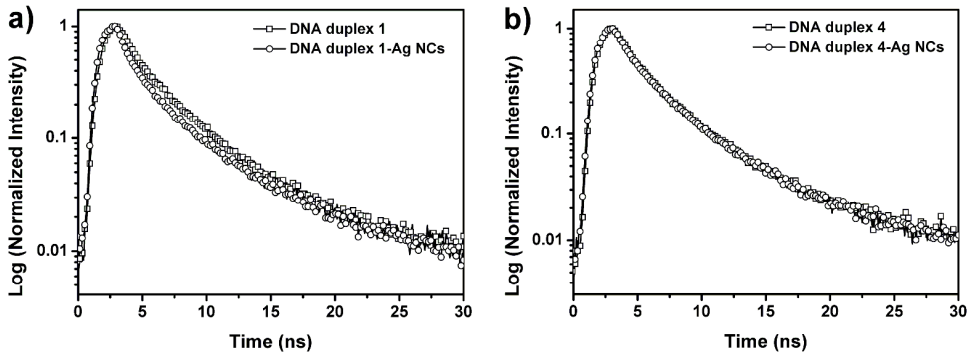

Figure S4. Time-resolved fluorescence decay curves of JOE (excited at 375 nm) without and with Ag NCs in DNA duplex 1 (a) and DNA duplex 4 (b). The concentrations of all samples were 0.2 μM.

Table S2. Decay Parameters for JOE without and with Ag NCs in DNA duplex 1 and DNA duplex 4.

Fitting parameters				
Names	τ_1/ns	τ_2/ns	τ_3/ns	χ^2
DNA duplex 1	1.3492	4.0775	16.9596	1.098
DNA duplex 1-Ag NCs	0.9126	3.6609	13.5171	1.029
DNA duplex 4	1.3496	3.9434	17.0377	1.155
DNA duplex 4-Ag NCs	1.2489	3.9135	16.0129	1.010

All data were fitted by a three exponential decay function.

Figure S5. The polyacrylamide gel electrophoresis analysis of (a) 10 μM Str-A, (b) 10 μM Str-A-Ag NCs and (c) 10 μM Str-A-Ag NCs with 70 U/mL S1 nuclease after reacting for 30 min at 37 ℃.

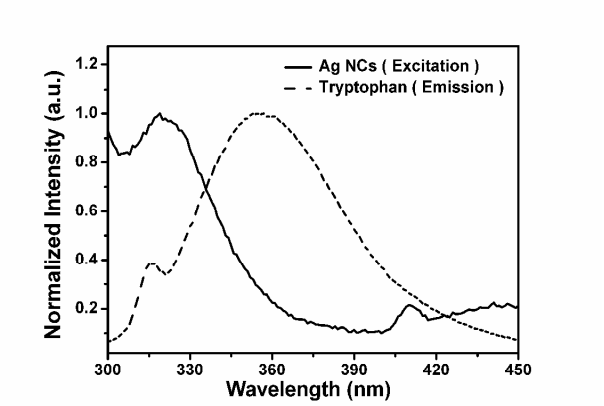

Figure S6. The spectral match between the excitation of Str-A'-Ag NCs (solid line) and the fluorescence emission of tryptophan (dash line).

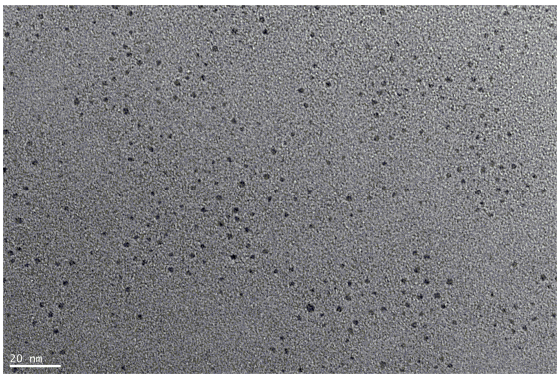

Figure S7. TEM image of the as-synthesized Str-A'-Ag NCs.

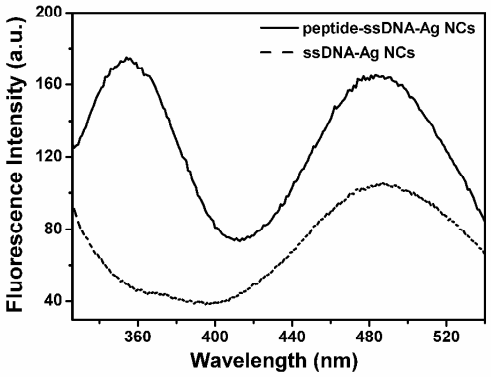

Figure S8. The fluorescence spectra of the peptide-ssDNA-Ag NCs complex (solid line) and ssDNA-Ag NCs complex (dash line) with the same ssDNA-Ag NCs concentration.

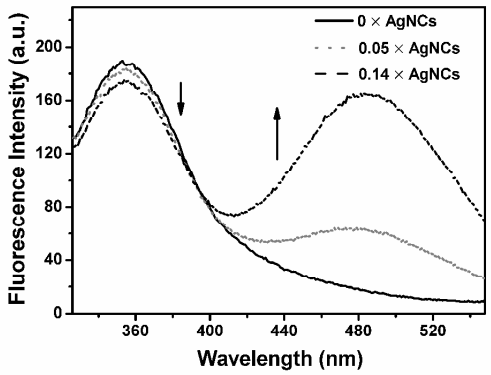

Figure S9. Emission spectra of the peptide-ssDNA-Ag NCs complex with increasing concentration of Ag NCs. The concentration of the peptide was 1.4 μM. The definition of self-consistent Ag NCs concentration (×) was described in experimental part of the supporting information.

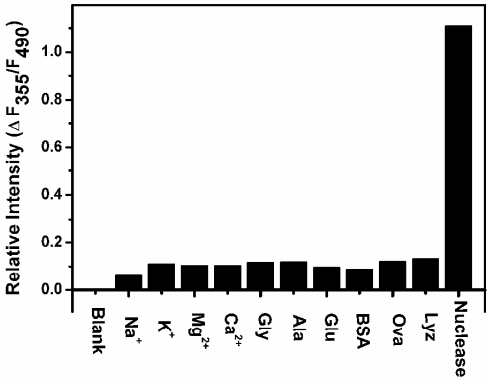

Figure S10. Relative fluorescence intensity (F_{355}/F_{490}) of the S1 nuclease biosensor in the presence of different substances. The concentration of S1 nuclease was 40 U/mL, and the concentration of other species was 1 μM.

References

[1] Chen, W. Y.; Lan, G. Y.; Chang, H. T. *Anal. Chem.* **2011**, *83*, 9450.