Electronic Supplementary Information for

Combining gold nanoparticle antennas with single-molecule fluorescence resonance energy transfer (smFRET) to study DNA hairpin dynamics

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1, Design of DNA hairpin

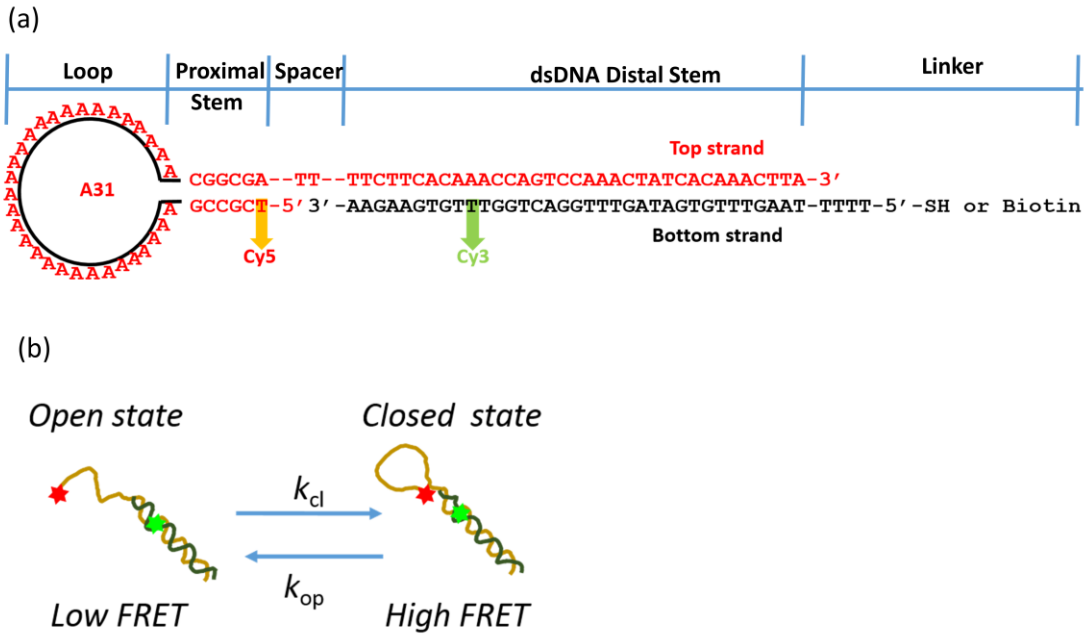

Fig. S1 (a) Schematic of the DNA hairpin structure. The hairpins were designed to minimize underlying interference interactions between the Cy3 (donor) and Cy5 (acceptor) when the hairpins are closed. (b) Schematic illustrations for the conformational fluctuation of the DNA hairpin between closed and open states. The open state generated low FRET signals while the closed state yielded high FRET signals. Here, k_{op} and k_{cl} are the transition rates between the open and closed state.

2, Numerical simulations of Fluorescence Enhancement

In the absence of Gold Nanoparticles Antennas (GNAs), the fluorescence decay rate of a single molecule can be expressed as:

$$k^0 = k_r^0 + k_{nr}^0 \qquad (1)$$

Where k_r^0 and k_{nr}^0 stand for the fluorescence radiative and non-radiative decay rates, respectively.[1]

The fluorescence intrinsic quantum efficiency is defined as:

$$\phi^0 = \frac{k_r^0}{k^0} = \frac{k_r^0}{k_r^0 + k_{nr}^0} \qquad (2)$$

The superscript ^{0} denotes the absence of the nano-antenna.

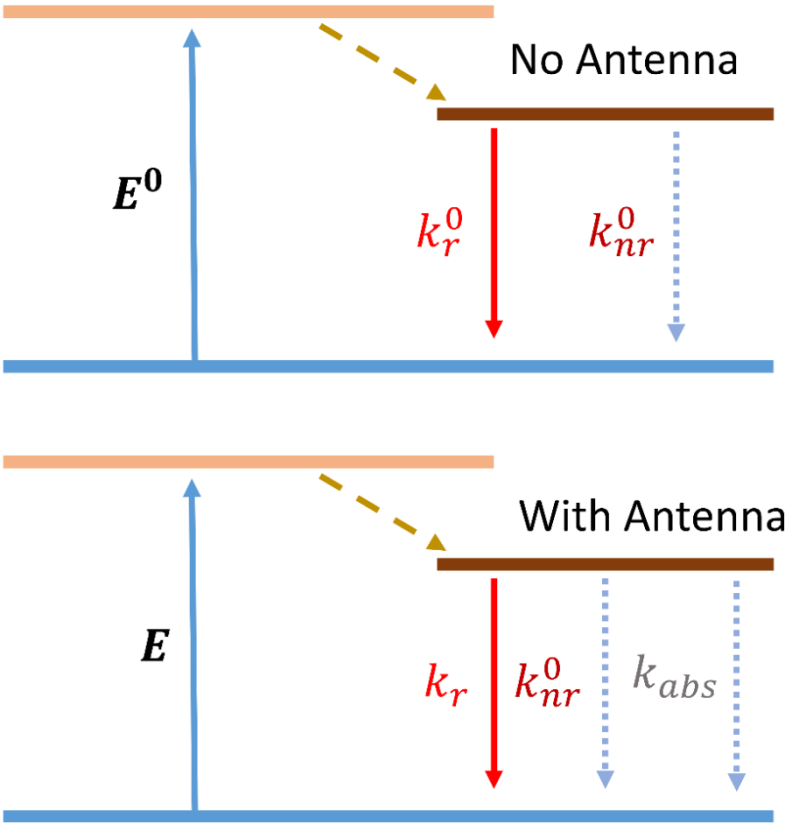

Fig. S2 Simplified Jablonski diagram describing the fluorescence decay rate of a single molecule without (top) and with (bottom) nano-antenna.

When the fluorescent molecule is coupled to the GNAs, an additional non-radiative decay rate k_{abs} is introduced due to the energy dissipation by the metallic nanostructure.[2-4] As a result, the new decay rate changes to $k = k_r + k_{nr} + k_{abs}$ and the quantum efficiency or quantum yield is modified to be

$$\phi = \frac{k_r}{k_r + k_{nr} + k_{abs}} \qquad (3)$$

Assuming that the GNAs does not influence the intrinsic non-radiative rate, i.e. $k_{nr} = k_{nr}^0$, and introducing the Purcell factor F (defined as $F = k_r/k_r^0$) and the antenna efficiency ϕ_a (defined as $\phi_a = k_r/(k_r + k_{abs})$), then the quantum efficiency modified by the GNAs can be rewritten as

$$\phi = \frac{\phi^0}{(1-\phi^0)/F + \phi^0/\phi_a} \qquad (4)$$

For the excitation power below saturation, the fluorescence intensity I^0 is proportional to

$\eta^0\phi^0|\boldsymbol{d}\cdot\boldsymbol{E^0}|^2$,[5] where η^0 represents the light collection efficiency, $\boldsymbol{d}$ is the electric transition dipole moment, $\boldsymbol{E^0}$ represents the excitation field at the position of the fluorescent molecule, ϕ^0 is the quantum efficiency. Similarly, when the emitter is placed in the vicinity of the GNAs, the fluorescence signal I will be proportional to $\eta\phi|\boldsymbol{d}\cdot\boldsymbol{E}|^2$.

Assuming that the light collection efficiency is not changed, i.e. $\eta=\eta^0$, the fluorescence signal enhancement can then be expressed as

$$\frac{I}{I^0}=\frac{\phi|\boldsymbol{d}\cdot\boldsymbol{E}|^2}{\phi^0|\boldsymbol{d}\cdot\boldsymbol{E^0}|^2} \qquad (5)$$

Notice that the fluorescence intensity enhancement is attributed to two contributions: i) an increase in the local electric field for the excitation; ii) a modification in the quantum yield of the fluorophores.

In this work, we calculated the fluorescence enhancement of Cy3 using the finite-difference time-domain (FDTD) method. Results are shown in Fig. S3. The maximum fluorescence enhancement is found when Cy3 is located about 10 nm from GNAs.

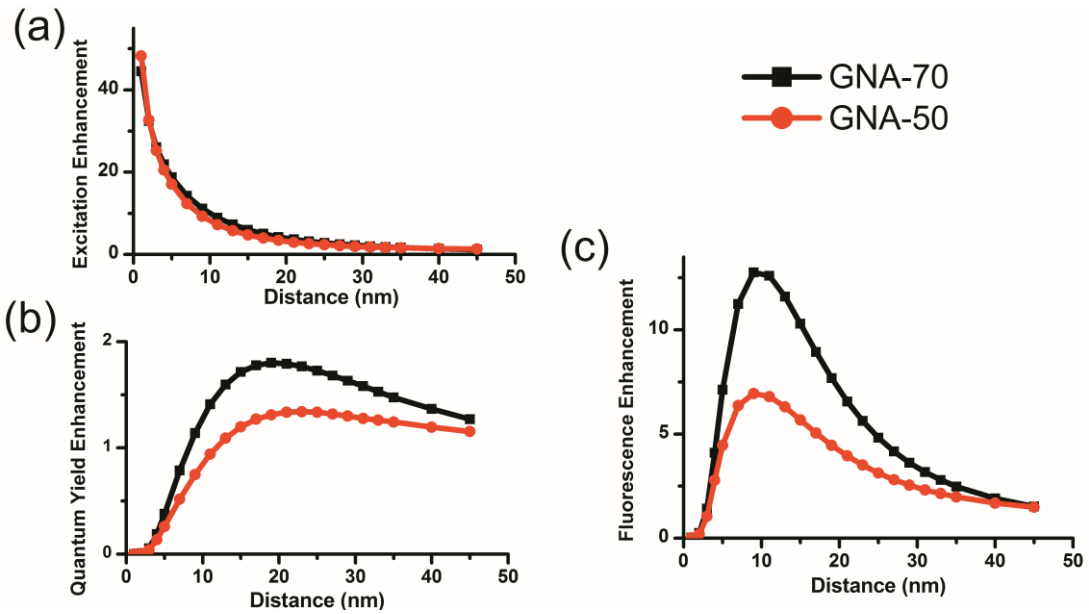

Fig. S3 FDTD calculations for Cy3 coupled to Gold Nanoparticles Antennas at different distances. (a) Excitation enhancement. (b) Quantum yield enhancement. (c) Fluorescence enhancement. The nanoparticle size is 50 nm (red) and 70 nm (black). The emission wavelength of Cy3 is set to 570 nm and the excitation wavelength of the laser is set to 532 nm. The quantum efficiency ϕ^0 of Cy3 in free space at 570 nm is fixed at 0.15 in our simulation. As the orientation of the Cy3 in the GNA is assumed to be random, we perform the calculation with a mean angle of 45° between the molecular transition dipole and the axis of the GNA.

3, The ratio-metric approach for the FRET efficiency determination

Although the ratio-metric approach has been routinely used for FRET efficiency calculation in the conventional FRET experiments, its validity in the presence of GNAs has not been clarified. In the following, we prove that such a method still remains valid even though the photo-dynamics of the fluorophores has been significantly modified.

We first consider the FRET efficiency in the absence of nano-antennas.

When the acceptor is absent, the decay rate of the donor (k_D^0) can be expressed as:

$$k_D^0 = \frac{1}{\tau_D^0} = k_{Dnr}^0 + k_{Dr}^0 \qquad (6)$$

When the acceptor molecule is present, as shown in Fig.S4, a long-range dipole–dipole interaction results in an additional relaxation term due to the energy transfer (k_{FRET}^0), thus the decay rate of the donor changes to,[6]

$$k_{DA}^0 = \frac{1}{\tau_{DA}^0} = k_{Dnr}^0 + k_{Dr}^0 + k_{FRET}^0 = k_D^0 + k_{FRET}^0 \qquad (7)$$

The superscript "0" indicates the absence of the nano-antenna.

So the FRET efficiency E^0 (quantum efficiency for energy transfer from the donor to the acceptor) is defined as the ratio of the energy transfer rate to the sum of all the donor de-excitation rates and can be calculated by

$$E^0 = \frac{k_{FRET}^0}{k_D^0 + k_{FRET}^0} = k_{FRET}^0 * \tau_{DA}^0 = 1 - \frac{\tau_{DA}^0}{\tau_D^0} = 1 - \frac{\phi_{DA}^0}{\phi_D^0} \qquad (8)$$

Where τ is the lifetime, ϕ stands for the quantum efficiency. The subscript "DA" indicates the presence of the FRET process.

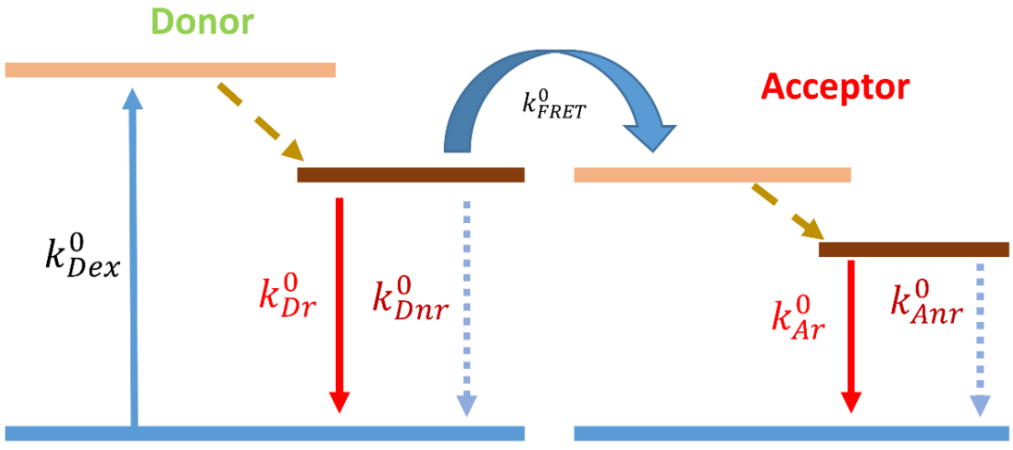

Fig. S4 Simplified Jablonski diagram describing the FRET process in the absence of the nano-antenna.

The energy transfer from the donor to the acceptor results in an excitation of the acceptor and de-excitation of the donor. In the case of without nanoantennas, the fluorescence intensity of the acceptor and donor can thus be written as：

$$I_A^0 = E^0 N_D^0 \eta_A^0 \phi_A^0 \tag{9}$$

and

$$I_{DA}^0 = (1 - E^0) N_D^0 \eta_{DA}^0 \phi_{DA}^0 \tag{10}$$

where N_D^0 is the number of donor excited states and η_A^0 and η_{DA}^0 are the detection efficiency of acceptor and donor, ϕ_A^0 and ϕ_{DA}^0 are the fluorescence quantum efficiency of the two molecules respectively.

So the FRET efficiency can be rewritten as

$$E^0 = \frac{I_A^0/\eta_A^0\phi_A^0}{I_{DA}^0/\eta_{DA}^0\phi_{DA}^0 + I_A^0/\eta_A^0\phi_A^0} = \frac{I_A^0}{(\eta_A^0\phi_A^0/\eta_{DA}^0\phi_{DA}^0)I_{DA}^0 + I_A^0} \tag{11}$$

We define $\gamma^0 = \eta_A^0\phi_A^0/\eta_{DA}^0\phi_{DA}^0$ as the correction factor. The FRET efficiency can finally be written as

$$E^0 = \frac{I_A^0}{\gamma^0 I_{DA}^0 + I_A^0} \tag{12}$$

When plasmonic nano-antennas coupling with FRET-pairs, both the fluorescence of acceptor and donor will be affected. In our case, we found that the direct excitation of Cy5 by the 532 nm laser was very weak with or without the GNAs. Therefore, in the following analysis, we only consider the contribution from FRET processes in the acceptor excitation.

We define ζ_D as the enhancement factor of the number of donor molecules in excited states due to the GNAs. Then the fluorescence intensity of acceptor can be written as

$$I_A = E\zeta_D N_D^0 \eta_A \phi_A \tag{13}$$

The donor fluorescence intensity is given by

$$I_{DA} = (1 - E)\zeta_D N_D^0 \eta_D \phi_D \tag{14}$$

Similarly, the FRET efficiency in the presence of the nano-antenna can be expressed as

$$E = \frac{I_A}{(\eta_A\phi_A/\eta_D\phi_D)I_{DA} + I_A} = \frac{I_A}{\gamma I_{DA} + I_A} \tag{15}$$

So the ratio-metric approach is still valid in calculating the FRET efficiency even in the presence of GNAs.

4, The relationship between the FRET efficiency and fluorescence enhancement of donor and acceptor

When combining plasmonic nano-antenna with FRET-pairs, the donor decay rate and energy transfer rate will be influenced by the localized surface plasmon, leading to a modification in donor and acceptor fluorescence intensity. We denote χ_{DA} and χ_A as the fluorescence enhancement factor of donor and acceptor in the presence FRET process. According to Eq. 9, 10, 13, 14, χ_{DA} and χ_A can be determined from

$$\chi_{DA} = \frac{I_{DA}}{I_{DA}^0} = \frac{(1-E)\zeta_D\eta_D\phi_D}{(1-E^0)\eta_{DA}^0\phi_{DA}^0} \qquad (16)$$

$$\chi_A = \frac{I_A}{I_A^0} = \frac{E\zeta_D\eta_A\phi_A}{E^0\eta_A^0\phi_A^0} \qquad (17)$$

Thus, the ratio of the fluorescence enhancement factor for donor and acceptor is given by

$$\frac{\chi_{DA}}{\chi_A} = \frac{1-E}{1-E^0}\frac{E^0}{E}\frac{\gamma^0}{\gamma} \qquad (18)$$

So,

$$E = \frac{E^0}{\frac{\chi_{DA}}{\chi_A}\frac{\gamma}{\gamma^0}(1-E^0)+E^0} \qquad (19)$$

Under our experimental configuration, $E^0 = \sim 0.8\ and\ \sim 0.2$ for the closed state and open state, respectively; $\frac{\gamma}{\gamma^0} = 1.1-1.3$, for the cases that DNA was immobilized on the surface of GNAs of

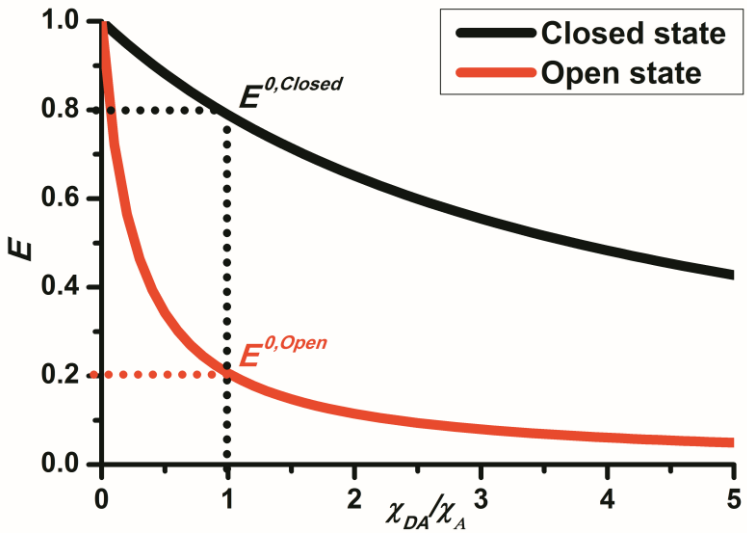

Fig.S5 The relationship between the FRET efficiency and the fluorescence enhancement for donor and acceptor. The ratio of γ/γ^0 is set as 1.

50 - 70 nm in diameter. For simplification, we choose $\gamma/\gamma^0 = 1$ for further discussion. In this case, E is solely a function of χ_{DA}/χ_A. Thus we can obtain the function curve according to Eq. 19, as shown in Fig. S5. As can be seen, when the fluorescence enhancements for the donor and acceptor are equal, i.e. $\chi_{DA} = \chi_A$, the FRET efficiency shows no changes and is equal to E^0. If the fluorescence enhancement for the donor is higher than for the acceptor, i.e. $\chi_{DA} > \chi_A$, the FRET efficiency will decrease, such as the case in our smFRET experiments. In contrast, when $\chi_{DA} < \chi_A$, the FRET efficiency will increase, which could be the case in Zhang et al's experiments.[7, 8]

5, The fraction of open state and closed state

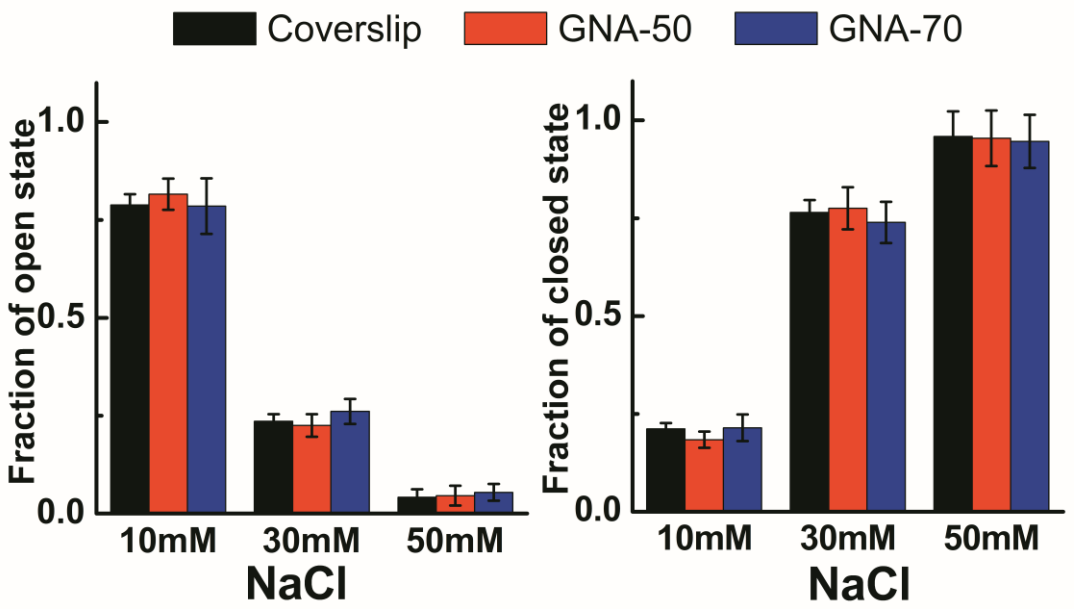

Fig. S6 The fractions of DNA hairpin molecules in the open state (left) and closed state (right) determined from FRET efficiency histograms.These fractions were basically identical for the three cases, i.e. DNA was anchored to the coverslip, GNA-50 and GNA-70, respectively.

6, Gold Nanoparticles Antennas assembly onto coverslip

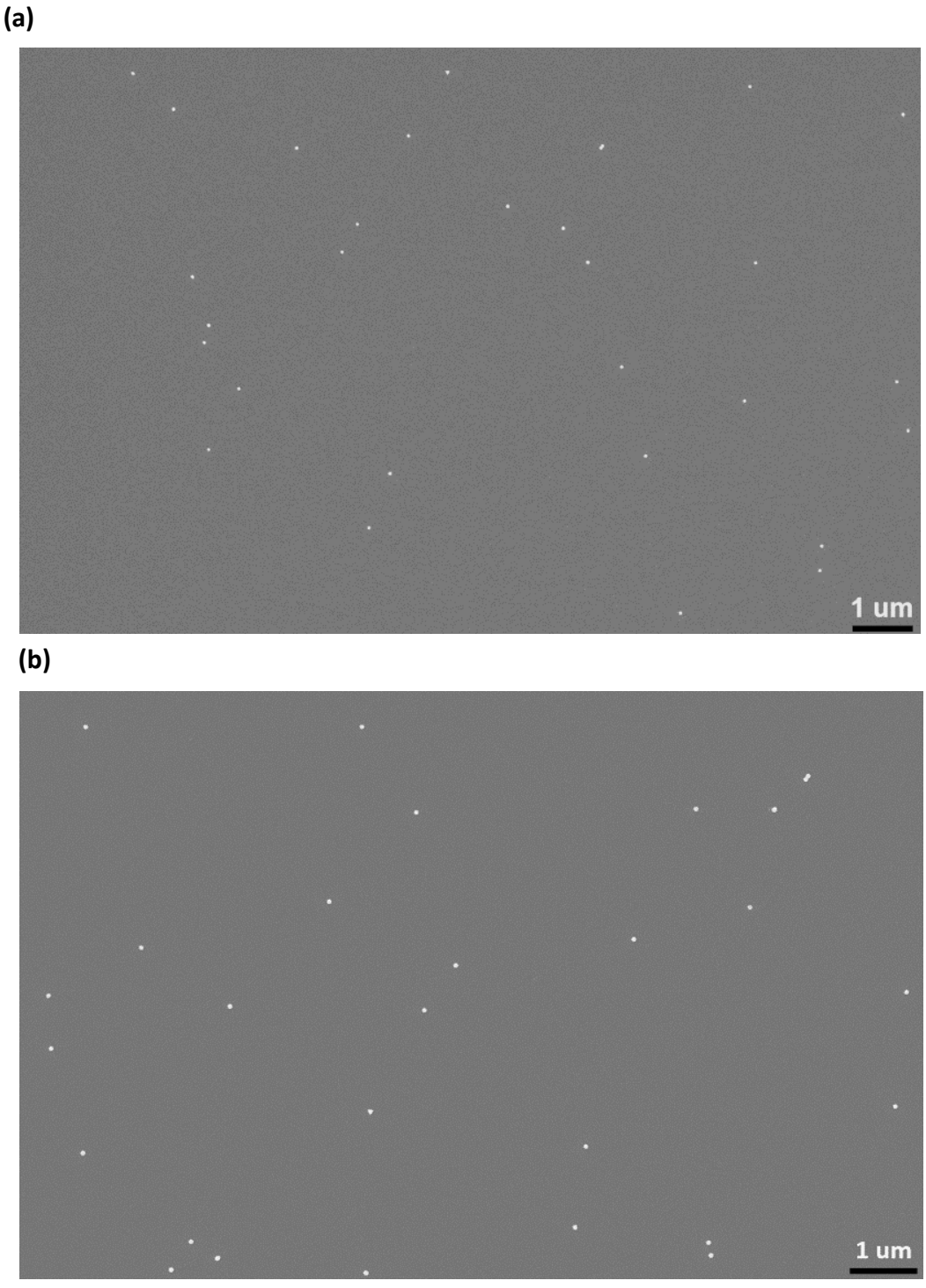

Fig. S7 Scanning electron micrograph of the (a) 50 nm and (b) 70 nm Gold nanoparticles immobilized on the silanized coverslip. The GNAs are monodispersed on the coverslip with a density of about 20 counts per 100 μm^2.

7, Exchange the position of Cy3 and Cy5 in DNA templates

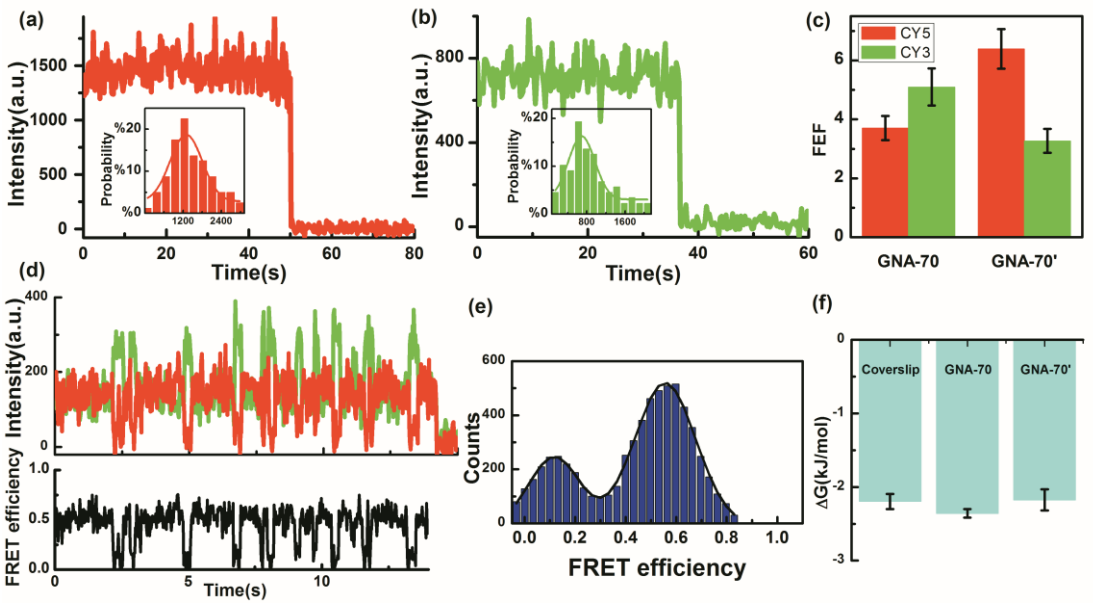

Fig. S8 (a-b) Typical single molecule fluorescence traces for (a) Cy5 (excited at 637 nm) and (b) Cy3 (excited at 532 nm) with single-step photo-bleaching on the GNA-70 when swapping the position of Cy3 and Cy5 in the DNA hairpin template. The inset represents the histogram of the measured fluorescence intensity. (c) The measured fluorescence enhancement factor (FEF) for Cy3 and Cy5 in the presence of GNA-70 and GNA-70′ (the superscript ′ indicates the exchange of the position of Cy3 and Cy5 in DNA hairpin). (d) The representative smFRET time trajectories (excited only at 532 nm) and the corresponding FRET efficiency for DNA hairpins immobilized on GNA-70′ at a buffer condition of 30 mM NaCl. The Cy3 (green) and Cy5 (red) fluorescence trajectories exhibit clearly anti-correlated behavior and the corresponding FRET efficiency is then calculated using the ratio-metric approach (black trace). (e) The histograms of FRET efficiency for DNA hairpins immobilized on GNA-70′ at a buffer condition of 30 mM NaCl. The FRET efficiency still decreases in comparison with the case that the GNAs is absent. (f) The measured Gibbs free energy differences (ΔG) between the closed and open states under different experimental configuration. They are in good agreement with each other.

Table S1 The measured fluorescence enhancement factors (FEFs) for the closed and open state of Cy3 and Cy5 in the smFRET experiments under the condition of GNA-70′.

	FEF- GNA-70′
Cy3- Closed state	2.93 ± 0.06
Cy5- Closed state	1.08 ± 0.02
Cy3- Open state	1.72 ± 0.06
Cy5- Open state	1.06 ± 0.12

Table S2 The measured opening rate (k_{op}) and closing rate (k_{cl}) of DNA hairpins under different experiment configuration at a buffer condition of 30 mM NaCl. They are in good agreement with each other.

	Coverslip	GNA-70	GNA-70′
k_{op}	2.00 ± 0.09	2.02 ± 0.08	2.17 ± 0.12
k_{cl}	4.87 ± 0.33	5.24 ± 0.11	5.13 ± 0.15

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