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Supporting Information

Silver Nanoparticles Plasmonic Enhanced Förster Resonance Energy Transfer (FRET) Imaging of Protein-Specific Sialylation on Cell Surface

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Oligonucleotide sequences noted in text

Apt-2 : 5'-SH-$(CH_2)_6$-CCTGACTCATCTGATCTGATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-3'

Apt-3 : 5'-SH-$(CH_2)_6$-CCTGACTCATCTGATCTGAACTCCCCCAGAGAGAGAATCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGA-3'

Random: 5'-SH-$(CH_2)_6$-CCTGACTCATCTGATCTGAACTCCCCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'

Cy3-2 : 5'-Cy3-CAGATCAGATGAGTCAGG-3'

Cy3-3 : 5'-Cy3-TCTCTCTCTGGGGGAGTTCAGATCATATGAGTCAGG-3'

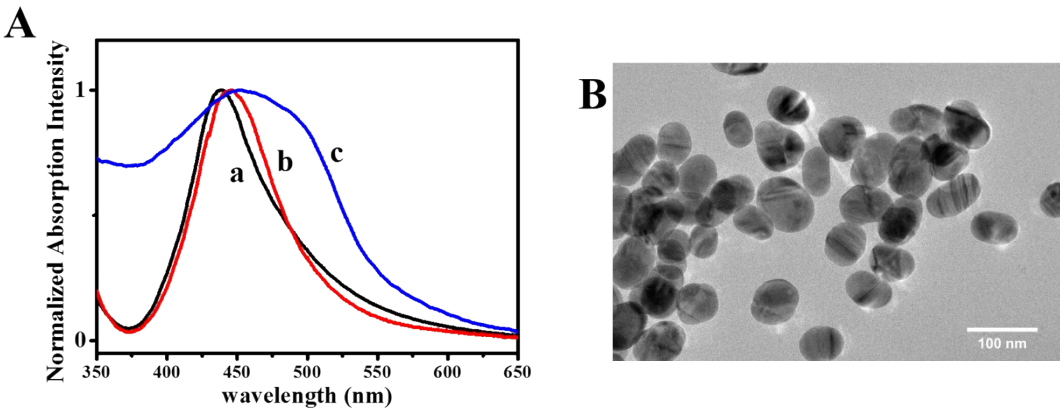
Measurements of DNA density on AgNPs surface

The amount of oligonucleotide connected to one AgNPs surface was calculated by the difference value between the total DNA amount and the amount of supernatant DNA. Specifically, the supernatant was obtained by the preparation of Apt-AgNPs and the following centrifugation. According to Eq. S1, the number of DNA connected to one AgNP was about 28.

$$DNA\ per\ AgNPs = \frac{c(DNA)_{total} - c(DNA)_{supernatant}}{c(AgNPs)} \quad (1)$$

Characterization of AgNPs

Fig. S1 shows the UV-Vis spectra (A) and transmission electron microscope (TEM) images (B) of AgNPs. The prepared AgNPs presented a size ~ 70 nm. After



modification with oligonucleotide strands of Apt-1, the maximum absorption peaks of the AgNPs shifted from 438.5 nm to 446 nm (Fig. S1A a, b). When the AgNPs were conjugated to the CCRF-CEM cell surface via aptamer-protein recognition, the Fig .S1 c showed that extinction band of AgNPs was extended and partly overlap with the characteristic emission band of Cy3.

Fig. S1 (A) The normalized UV−vis spectra. (a): AgNPs, (b): Apt-1 modified AgNPs, (c): the conjugated AgNPs to CCRF-CEM cells. (B) TEM image of AgNPs.

Fluorescence spectra and FRET enhancement ratio of CCRF-CEM cell suspension solutions by AgNPs Enhanced FRET method using different AgNPs probes (lengths between AgNPs and Cy3 are 18bp, 27bp, 36bp)

Different AgNPs probes were prepared by different lengths complementary Apt strands and Cy3 modified strands. Metabolically labeled SiaNAz was modified by Cy5 via bioorthogonal chemistry on cell surface, and these CCRF-CEM cells were incubated with different AgNPs probes (18bp, 27bp, 36bp). Then, the fluorescence emission spectra excited at 543 nm were collected (Fig. S2B，Fig.S3B and Fig. S4B). The results show that the Apt-Cy3-AgNPs with 27 bp DNA sequences presents the strongest FRET signal intensity enhancement (Table S1), which was due to the aptamer and dyes enriching on the AgNPs and the appropriate distance between

AgNPs and dyes in the Apt-Cy3-AgNPs with 27 bp DNA sequences. (Inside figures indicated AgNPs enhanced FRET coming with similar amount of FRET donors.)

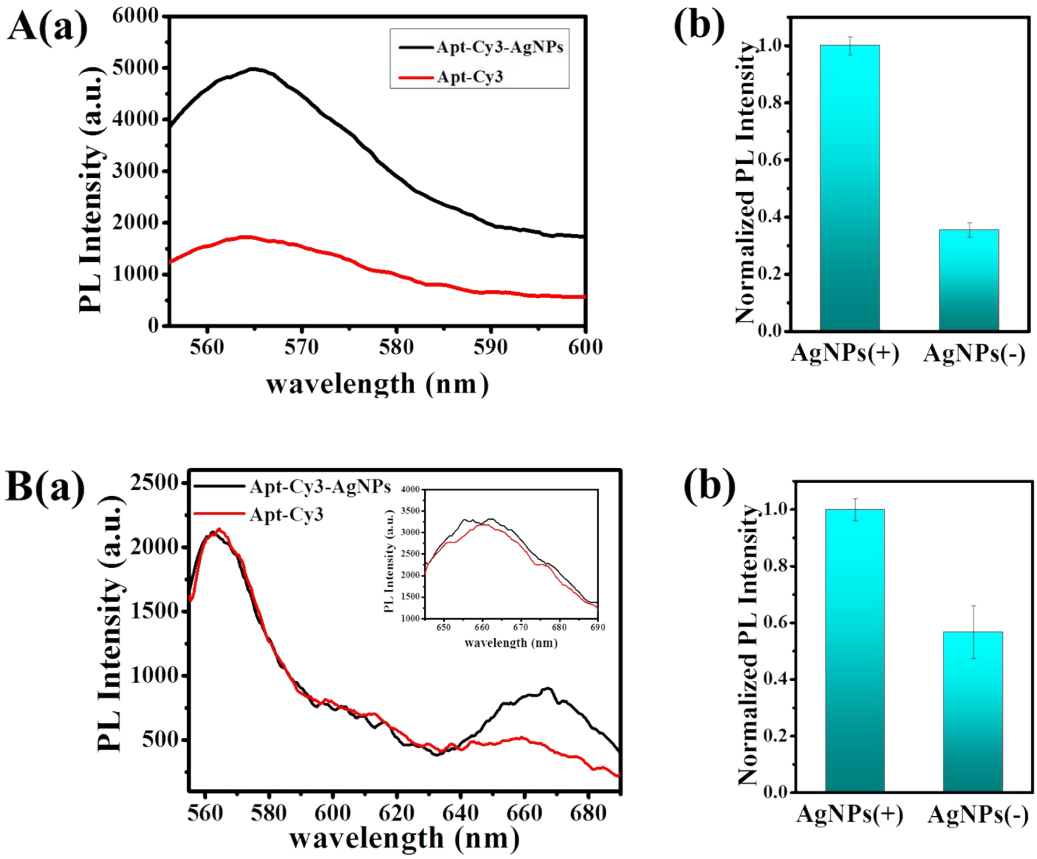


Fig. S2 (A) Fluorescence spectra of Apt-Cy3-AgNPs and Apt-Cy3 in solution (a) and the normalized fluorescence intensity of Cy3 in solution (b). (B) Fluorescence spectra of CCRF-CEM cells incubated with Apt-Cy3-AgNPs and dissolving AgNPs treated as Apt-Cy3 probe after labeled with Cy5 fluorophore to SiaNAz (Inside figure was the fluorescence spectra excited at 633 nm) (a) and the normalized fluorescence intensity of Cy5 based FRET on the cell surface (b). (λ_{ex} = 543 nm)

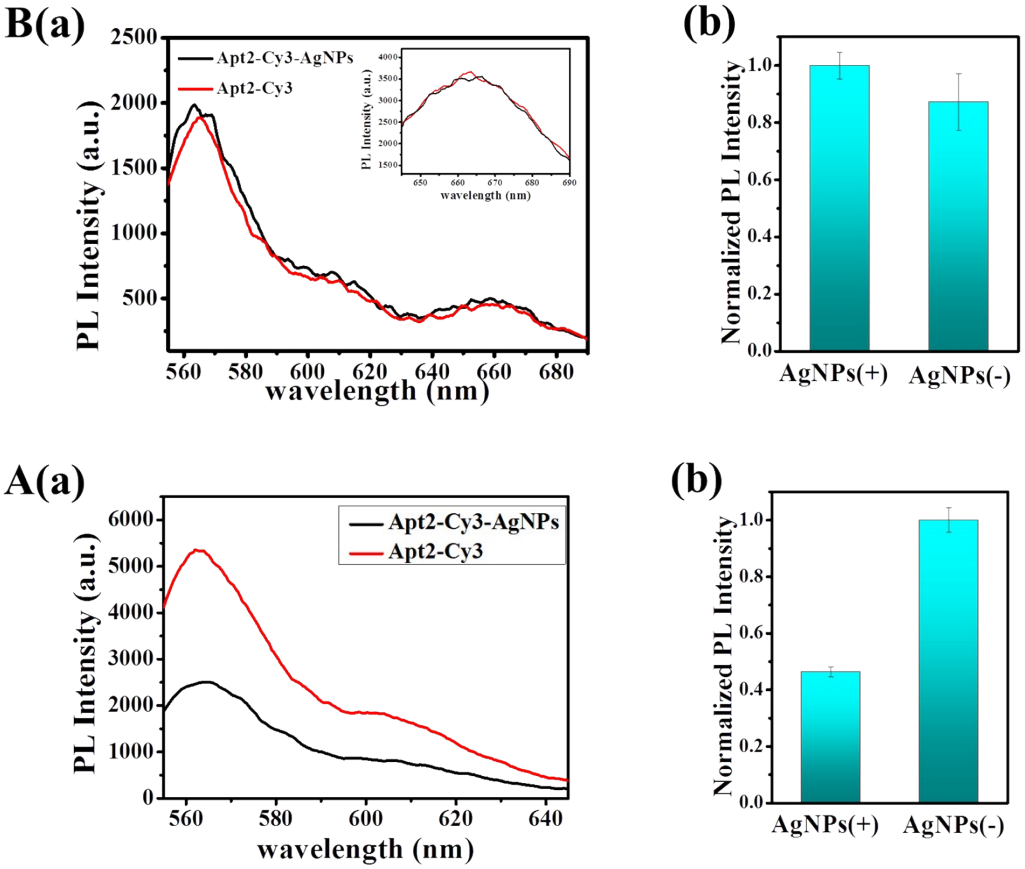
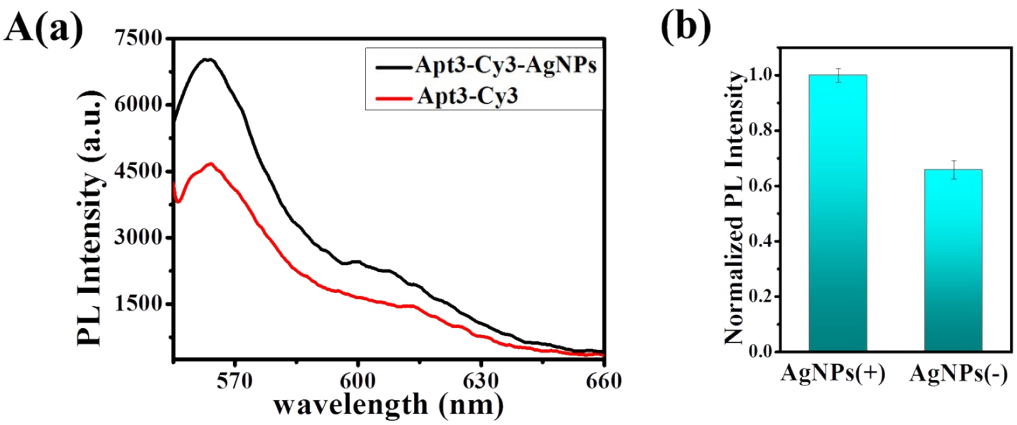


Fig. S3 (A) Fluorescence spectra of Apt2-Cy3-AgNPs and Apt2-Cy3 in solution (a) and the normalized fluorescence intensity of Cy3 in solution (b). (B) Fluorescence spectra of CCRF-CEM cells incubated with Apt2-Cy3-AgNPs and dissolving AgNPs treated as Apt2-Cy3 probe after labeled with Cy5 fluorophore to SiaNAz (Inside figure was the fluorescence spectra excited at 633 nm) (a) and the normalized fluorescence intensity of Cy5 based FRET on the cell surface (b). (λ_{ex} = 543 nm)



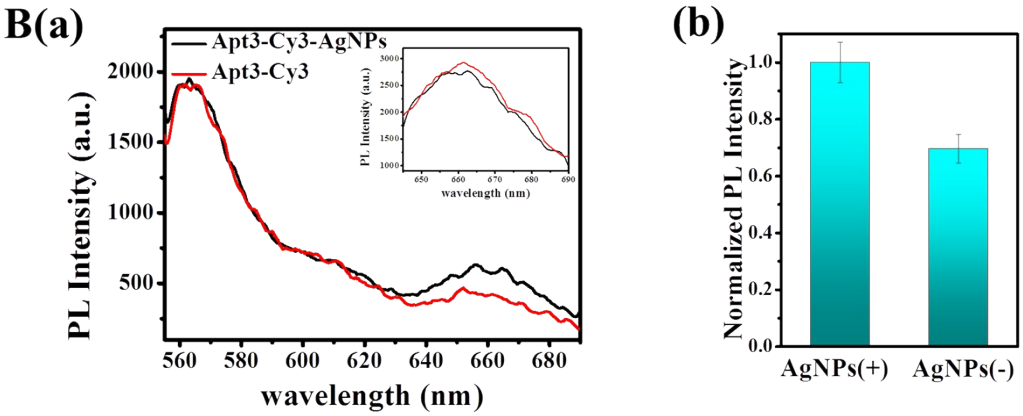


Fig. S4 (A) Fluorescence spectra of Apt3-Cy3-AgNPs and Apt3-Cy3 in solution (a) and the normalized fluorescence intensity of Cy3 in solution (b). (B) Fluorescence spectra of CCRF-CEM cells incubated with Apt3-Cy3-AgNPs and dissolving AgNPs treated as Apt3-Cy3 probe after labeled with Cy5 fluorophore to SiaNAz (Inside figure was the fluorescence spectra excited at 633 nm) (a) and the normalized fluorescence intensity of Cy5 based FRET on the cell surface (b). (λ_{ex} = 543 nm)

Distance between AgNPs and Cy3	PL intensity enhancement ratio of Cy3 in solution (λ_{ex} = 543nm)	PL intensity enhancement ratio of Cy5 on cell surface based FRET(λ_{ex} = 543nm)
27 bp (Apt-Cy3-AgNPs)	182%	76%
18 bp (Apt2-Cy3-AgNPs)	-54%	15%
36 bp (Apt3-Cy3-AgNPs)	52%	43%

Table S1 The fluorescence intensity enhance ratio of Cy3 dye based different probes in solution and Cy5 fluorophore based AgNPs plasmonic FRET on CCRF-CEM cells surface.

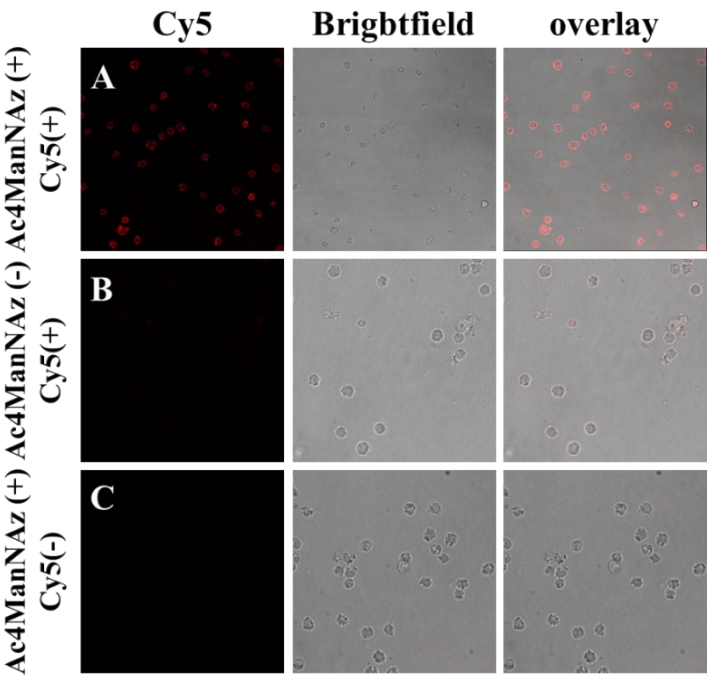


Fig. S5 Fluorescence, bright field, and merged images of CCRF-CEM cells incubated with or without $Ac_4ManNAz$ upon introduced Cy5 fluorophore by bioorthogonal chemistry. (λ_{ex} = 633 nm)

Determination of the FRET efficiency

The FRET efficiency was calculated according the Eq. S2 based on the acceptor photobleaching method.

$$FRET\ efficiency = 1 - \frac{I_B}{I_A} \tag{2}$$

where the I_B and I_A were the Cy3 fluorescence intensities excited at 543 nm before and after the photobleaching of Cy5, respectively. As shown in the Fig. S6, the FRET efficiencies were 21% and 37% in the absence and presence of AgNPs, respectively.

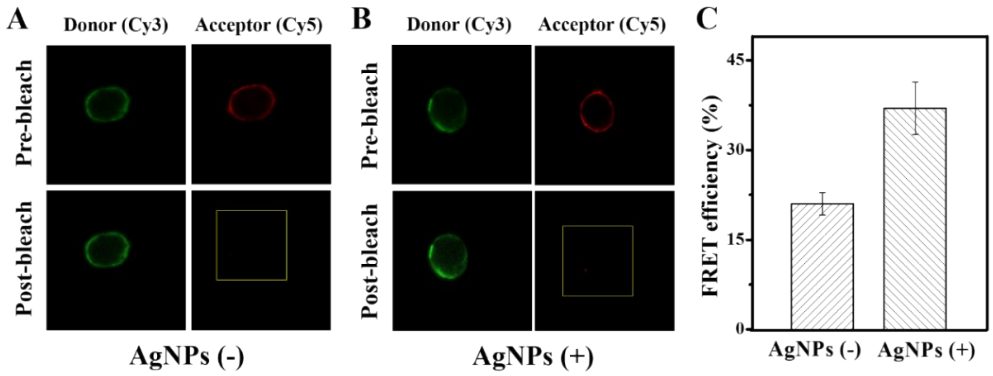


Fig. S6 Acceptor photobleaching fluorescence images (A, B) and the FRET efficiency (C) of CCRF-CEM cells incubated with Ac_4ManNAz upon introduced Cy5 fluorophore by bioorthogonal chemistry in the absence and presence of AgNPs.

Optimization of the incubation time for SiaNAz metabolic labeling on CCRF-CEM cells surface

CCRF-CEM cells were incubated for different times in culture medium containing the metabolic labeling reagents at 50 μM to metabolic label the Cell-surface SiaNAz. Cells were washed three times with PBS containing 1% FBS, incubated in ice bath for 30 min in PBS containing 1% FBS. And then incubated with the reaction PBS buffer including Cy5 alkyne (15 μM), CuSO4 (25 μM), THPTA (125 μM), and sodium ascorbate (2.5 mM) at 4 ^{o}C for 5 min. After washing with PBS containing 1% FBS, the cells were fixed with 4% paraformaldehyde in PBS buffer for 10 min followed by washing three times and imaged by CLSM. Through the results of imaging, Cells would incubate for 48h with AC_4ManNAz in this strategy to obtain stable and adequate fluorescence signal (Fig. S7).

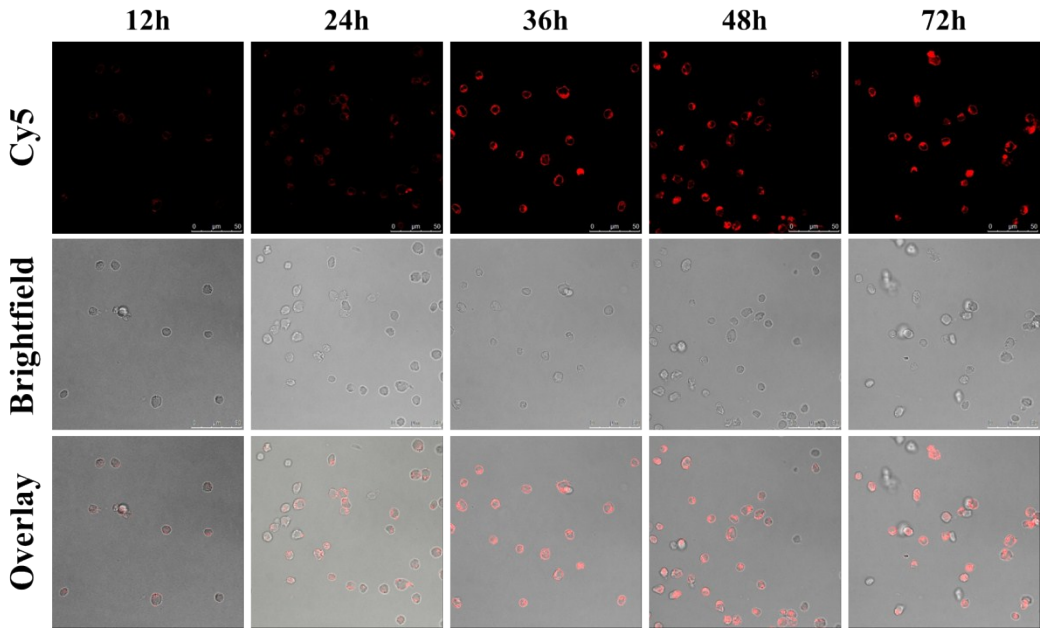


Fig. S7 Fluorescence, bright field, and merged images of CCRF-CEM cells introduced Cy5 fluorophore by bioorthogonal chemistry after different metabolic incubation time of Ac_4ManNAz. (λ_{ex} = 663 nm)

Optimization incubation time of Apt-Cy3-AgNPs with CCRF-CEM cells

Due to the specific recognition of Sgc8 to cell-surface PTK7, Apt-Cy3-AgNPs was incubated with CCRF-CEM cells for different times. After washing three times with PBS to remove medium and free probes and imaged with CLSM. Then 1h incubation time was selected to get abundant fluorescence signal for this AgNPs enhanced FRET strategy (Fig. S8).

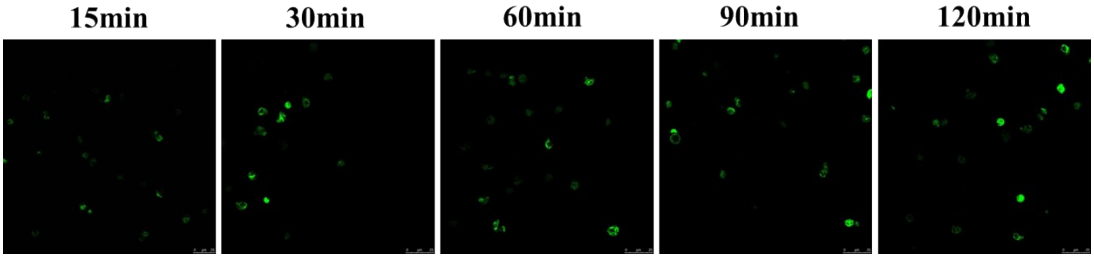


Fig. S8 Confocal fluorescence images of CCRF-CEM cells incubated with Apt-Cy3-AgNPs probes for different times. (λ_{ex} = 543 nm)

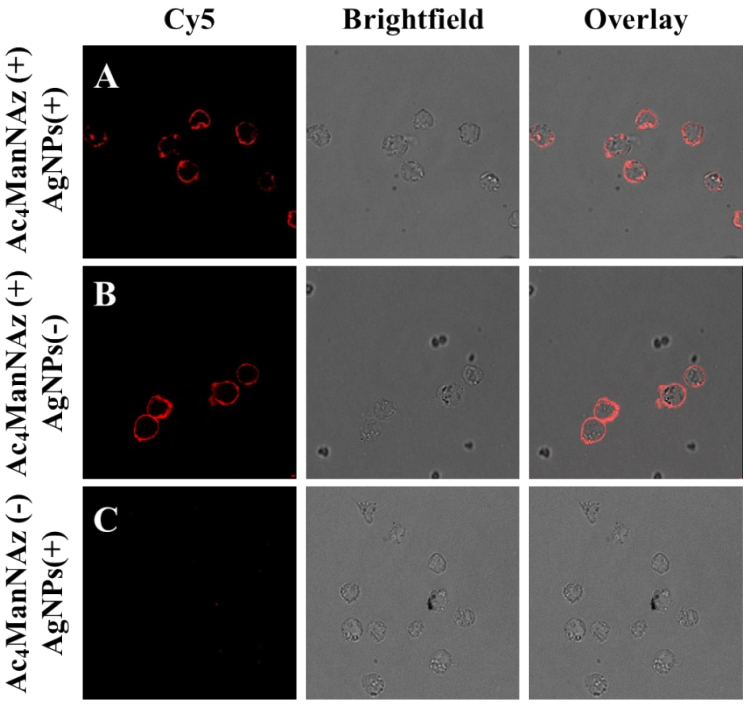


Fig. S9 Fluorescence, bright field, and merged images of CCRF-CEM cells introduced Cy5 fluorophore by bioorthogonal chemistry after incubated with $Ac_4ManNAz$ and AgNPs (A), with $Ac_4ManNAz$ and without AgNPs (B) and without $Ac_4ManNAz$ and with AgNPs (C). AgNPs would have no effect on the fluorescence intensity of Cy5 fluorophore in confocal fluorescence image. (λ_{ex} = 633 nm)

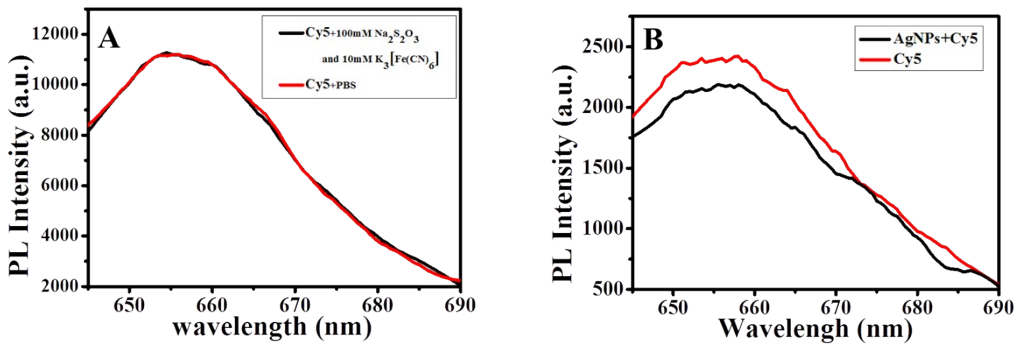


Fig. S10 (A) Fluorescence spectra of Cy5 fluorophore with PBS and 100 mM $Na_2S_2O_3$, 10 mM $K_3[Fe(CN)_6]$ solution. The mixing of $Na_2S_2O_3$ and $K_3[Fe(CN)_6]$ would have no effect to the fluorescence intensity of Cy5. (B) Fluorescence spectra of Cy5 fluorophore with AgNPs and without AgNPs in solution. (λ_{ex} = 633 nm) The fluorescence intensities of Cy5 in these two different solutions were almost

unchanged, and AgNPs would have no obvious effect to the fluorescence intensity of Cy5.