Supporting Information

Design of the DNA template for the synthesis of fluorescent silver nanoclusters

For a dual-purpose probe, the designed DNA template should not only produce fluorescent Ag NCs, but also retain the binding ability of aptamer to target protein. Poly (cytosine) template for fluorescent Ag NCs was connected with Sgc8c aptamer via a -TTTTT- linker. Meanwhile, in order to keep the aptamer secondary structure unchanged and form a T-loop, additional three bases "GGA" were added at the 5'-end of Sgc8c. The designed DNA template in this work was called "NC-Sgc8c-L5T".

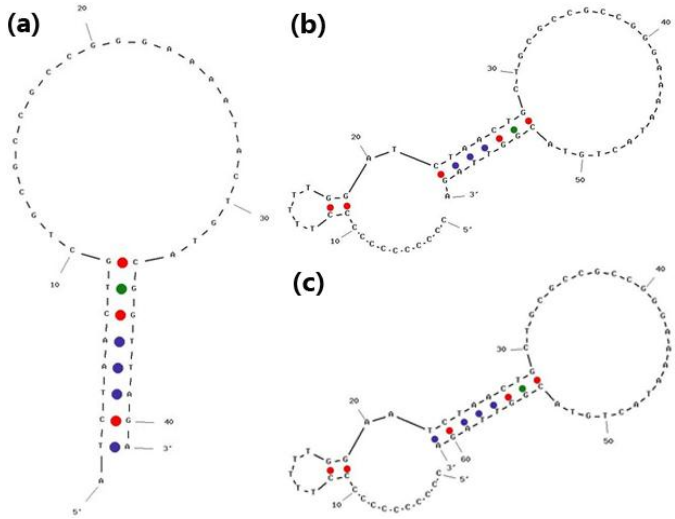

Figure S1. The secondary structures of Sgc8c a), NC-Sgc8c b), and NC-Sgc8c-L5T c), analyzed by the free software Oligo Analyzer 3.1 from IDT. Parameter settings were 50 nM oligo and 50 mM Na^+.

Characterization of the binding of Sgc8c or NC-Sgc8c-L5T to PTK 7 protein

Circular dichroism spectra were introduced to study the secondary structure of NC-Sgc8c-L5T and 4% native polyacrylamide gel was employed to test the affinity of NC-Sgc8c-L5T to the target protein. The complex band formed by FAM-NC-Sgc8c-L5T and PTK 7 protein from CCRF-CEM (Lane 2) or HeLa (Lane 3) cells showed a slower migration rate than those formed by FAM-Sgc8c (Lane 1 and Lane 4). This is because the NC-Sgc8c-L5T sequence has more nucleotides (higher molecular weight). While proteins extracted from CCRF-CEM (Lane 5) or HeLa (Lane 6) cells did not show any band, confirming bands in Lane 1 to Lane 4 were from the binding of FAM-Sgc8c or FAM-NC-Sgc8c-L5T to the target proteins. These results indicated that NC-Sgc8c-L5T retained the affinity of Sgc8c aptamer to PTK 7 protein extracted from CCRF-CEM or HeLa cells. Additionally, the binding ability of NC-Sgc8c-L5T stabilized Ag NCs with targets was measured by flow cytometry. As shown in Figure S2c, obvious fluorescence emissions from HeLa cells could be observed, although some fluorescence emissions were found in control cells which might come from the non-specific endocytosis.

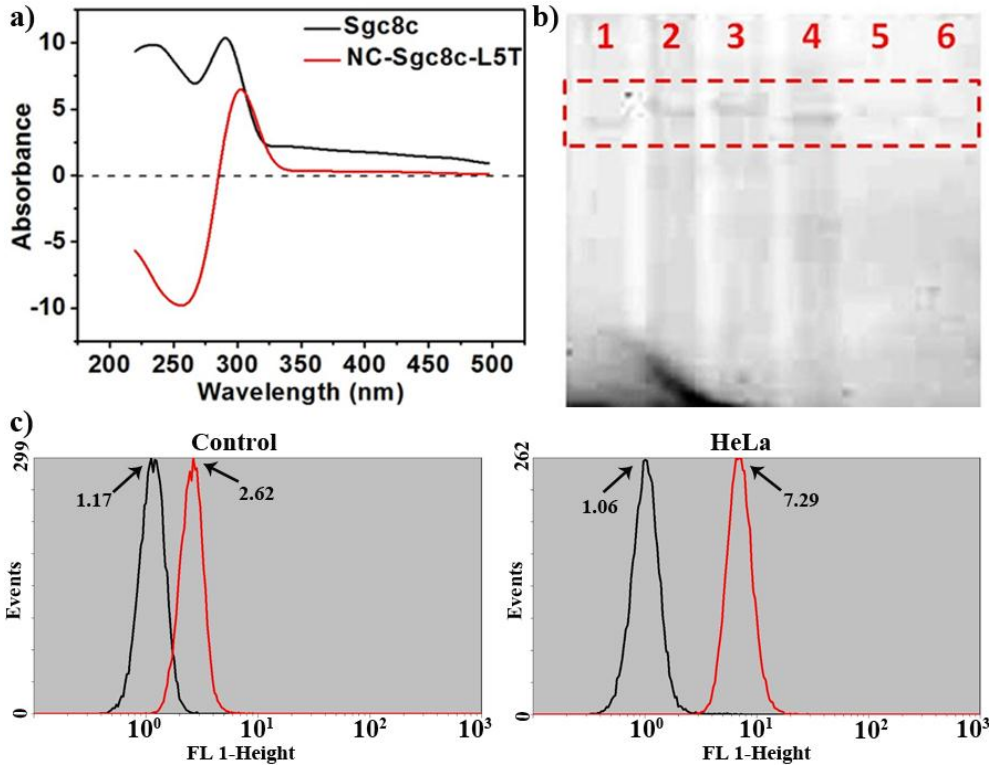

Figure S2. a) Circular dichroism (CD) spectra of Sgc8c and NC-Sgc8c-L5T with 0.1M NaCl. The concentrations of oligos were 12 μM. b) Gel-shift analysis of FAM-Sgc8c and FAM-NC-Sgc8c-L5T binding to PTK 7 protein extracted from CCRF-CEM or HeLa cells. Lane 1: FAM-Sgc8c incubated with CCRF-CEM extracts; Lane 2: FAM-NC-Sgc8c-L5T incubated with CCRF-CEM extracts; Lane 3: FAM-NC-Sgc8c-L5T incubated with HeLa extracts; Lane 4: FAM- Sgc8c incubated with HeLa extracts; Lane 5: CCRF-CEM extracts; Lane 6: HeLa extracts. c) Flow cytometry assay for the binding capacity of NC-Sgc8c-L5T stabilized Ag NCs with HeLa cells. Blank line: cells alone; red line: cells incubated with NC-Sgc8c-L5T stabilized Ag NCs. NIH-3T3 was used as the control cells. Excitation: 488nm.

Characterization of biotinylated NC-Sgc8c-L5T stabilized Ag NCs

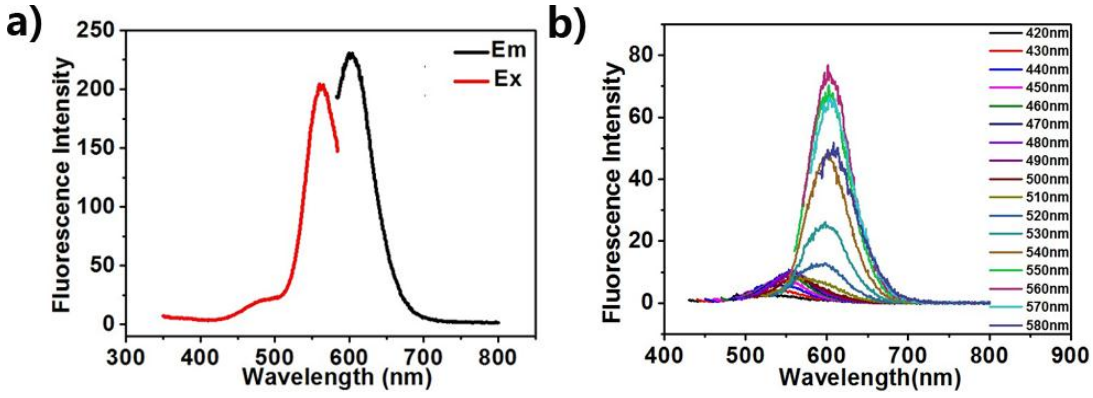

Figure S3. a) Excitation and emission spectra of biotinylated NC-Sgc8c-L5T stabilized Ag NCs and b) their emission spectra under various excitation wavelengths ranging from 420 nm to 580 nm. The maximum emission intensity was obtained with the excitation wavelength at 560 nm.

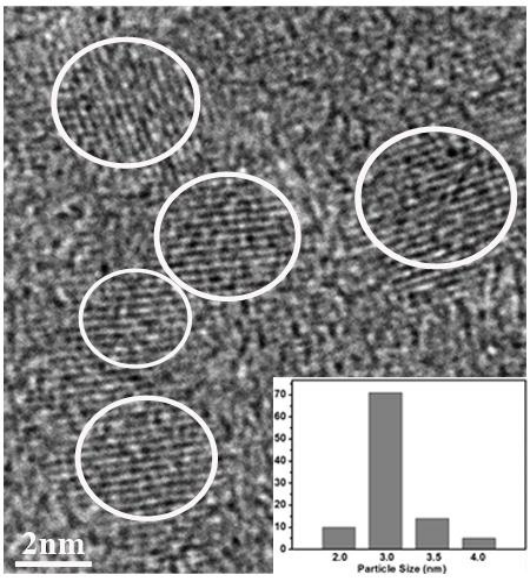

Figure S4. HRTEM image of Biotin-NC-Sgc8c-L5T stabilized Ag NCs. (Inset) Distribution of particle size.

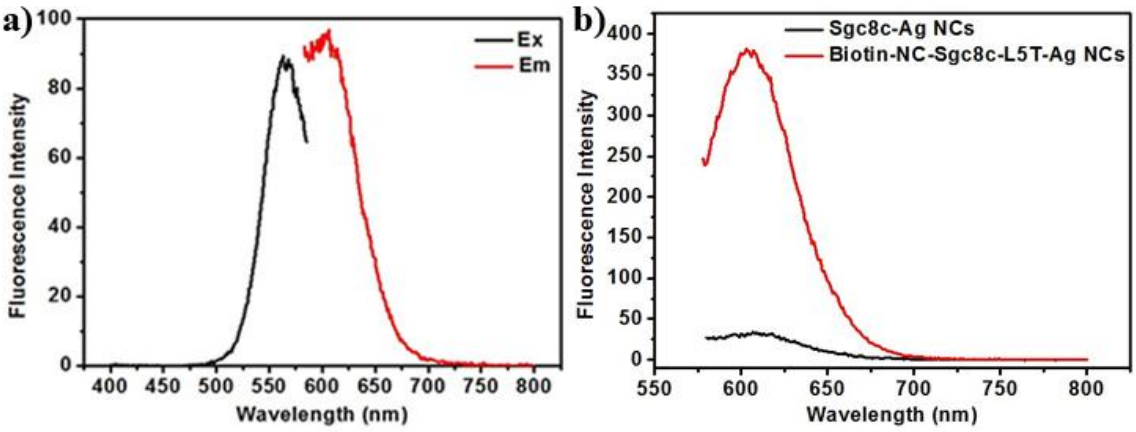

Figure S5. a) Excitation and emission spectra of Sgc8c aptamer stabilized Ag NCs and b) the comparation of fluorescence emission between Sgc8c-Ag NCs and Biotin-NC-Sgc8c-L5T-Ag NCs at the same concentration.

Optimization of the incubation ratio between aptamer-Ag NCs, streptavidin and VEGF siRNA

The successful preparation of Ag NCs-streptavidin-VEGF siRNA complex, formed by coupling biotinylated Ag NCs with biotinylated siRNA via a modular streptavidin bridge, is vital to the following gene silencing and tracking. As there are four binding sites of streptavidin to conjugate with biotin, the incubation ratio of biotinylated Ag NCs, streptavidin and biotinylated siRNA were optimized. 12% polyacrylamide gel was employed to study the formation of complex with different ratios between them. The ratios were chosen as 1:1:1, 1:1:3, 2:1:2, 3:1:1 and 2:1:1 (Ag NCs:streptavidin:siRNA), respectively. As shown in Figure S6, complex with different compositions were formed in the ratio of 1:1:1 (Lane 3) and some siRNA or Ag NCs were not conjugated with streptavidin in the case of 1:1:3 (Lane 4). The amount of complex reached maximum in the ratio of 2:1:2 (Lane 5). Additionally, the gene silencing effect of the complex with other ratios was also tested and the ratio of 2:1:2 gave the best result (shown in Figure 6).

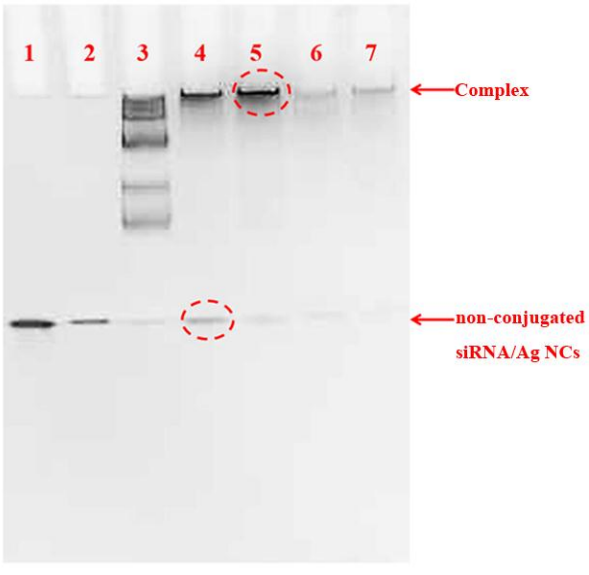

Figure S6. Gel-shift analysis of Ag NCs-streptavidin-siRNA complex formed with different incubation ratios between streptavidin, biotinylated NC-Sgc8c-L5T stabilized Ag NCs and biotinylated siRNA. Lane 1: biotinylated siRNA; Lane 2: biotinylated aptamer-Ag NCs; Lane 3: ratio of 1:1:1; Lane 4: ratio of 1:1:3; Lane 5: ratio of 2:1:2; Lane 6: ratio of 3:1:1 and Lane 7: ratio of 2:1:1.

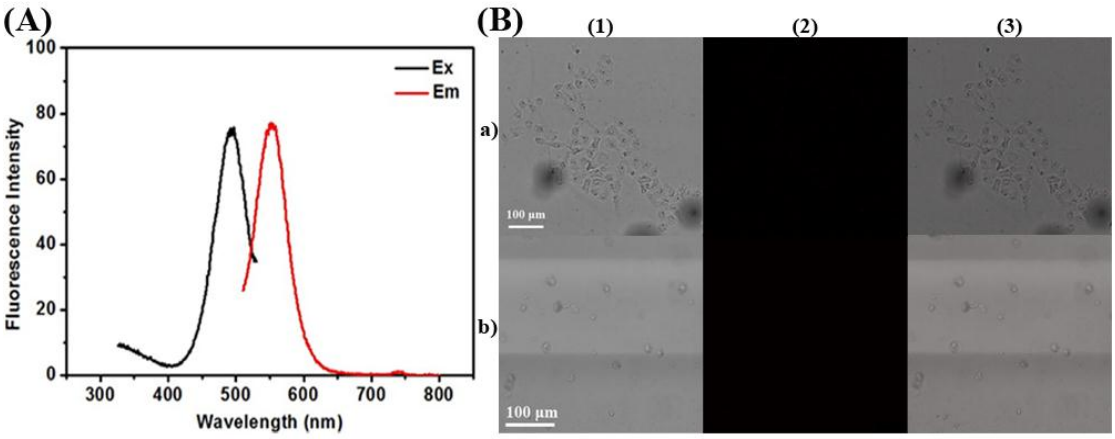

Figure S7. (A) Excitatation and emission spectra of non-binding aptamer stabilized Ag NCs. (B) Confocal laser scanning microscopic image of HeLa human cervical carcinoma cells incubated with non-binding aptamer stabilized Ag NCs (a) and Ramos human Burkitt's lymphoma cells incubated with Biotin-NC-Sgc8c-L5T templated Ag NCs under the same procedure with HeLa cells as control (b). There were no detectable emissions from the control sets. (1) bright-field images; (2) Ag NCs fluorescence images; (3) overlap of corresponding fluorescence images and bright-field images.

Z-Axis scanning and DAPI nuclear staining for the characterization of the internalization of Ag NCs–streptavidin-siRNA complex

To further confirm whether Ag NCs-streptavidin-siRNA complex was internalized, we performed vertical section scanning along the z-axis. Micrographs were taken while moving the focal plane in incremental steps from the coverslip bottom up to the top of the cell. The fluorescence images within a 15μm focal plane distance at the Z position were recorded. It demonstrated the presence of Ag NCs-streptavidin-siRNA complexes inside cells. Additonally, the nuclear DAPI staining further testified the internalized Ag NCs were mainly accumulated in nuclei eventually.

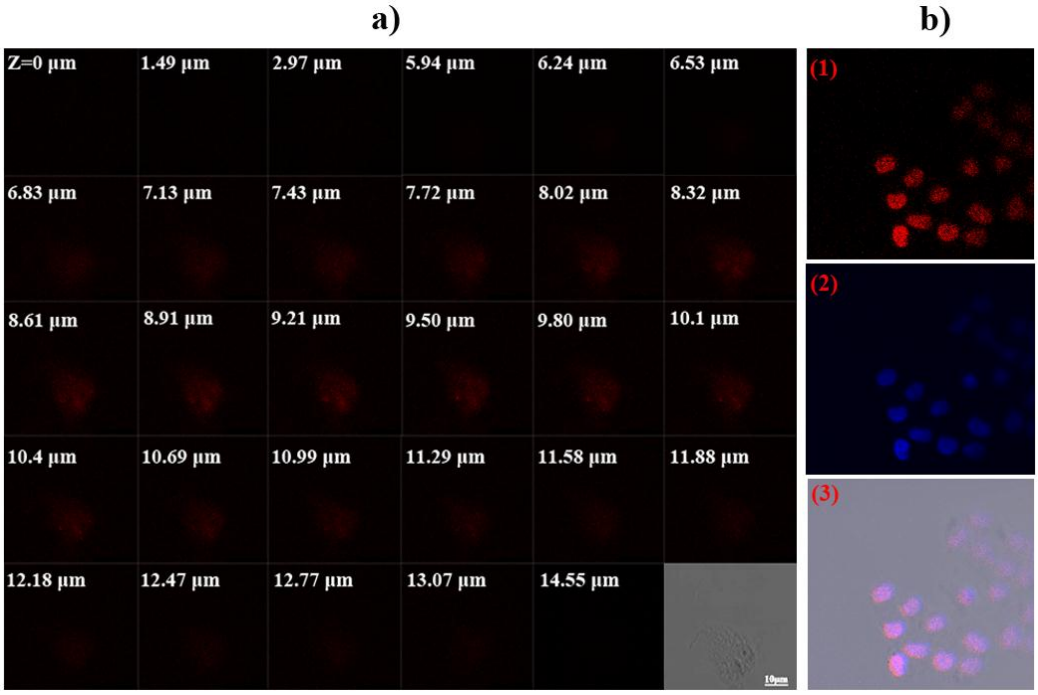

Figure S8. a) Z-Axis scanning images of internalized Ag NCs-streptavidin-siRNA complex recorded by confocal microscope. HeLa cells were cultured on the coverslip in 24-well plate for 24h before observation, and then the culture medium was replaced with complex and cocultured for 2h at 37℃. Micrographs were taken while the focal plane was moved in incremental steps from the coverslip bottom up to the top of the cells. b) Intracellular distribution of internalized aptamer-Ag NCs. (1) fluorescent image of Ag NCs (red); (2) fluorescent images with DAPI nuclear staining (blue); (3) overlap of corresponding fluorescence imaging and bright-field image.

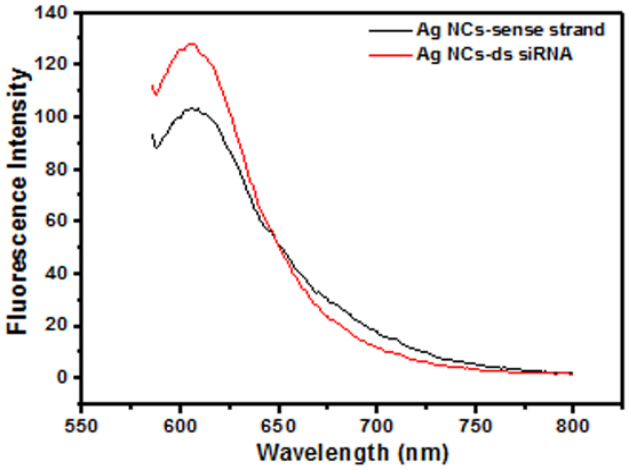

Figure S9. Fluorescence emission spectra of Ag NCs-streptavidin-sense strand and Ag NCs-streptavidin-ds siRNA. The excitation wavelength was at 565 nm.