Supplementary Information(SI)

DNA hydrogel with aptamer-toehold based recognition, cloaking and decloaking of circulating tumor cells for live cell analysis

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Experimental Section

1. Materials and Reagents

AuNPs (10 nm) were purchased from British Biocell International Ltd.. Adenosine 5′-triphosphate (ATP) disodium salt hydrate was purchased from Sigma-Aldrich. Ultrapure water obtained from a Millipore water purification system (Milli-Q, Millipore) was used in all experiments.

Breast cancer cell line (MCF-7), human embryonic kidney (HEK293) cells and L1210 cells were purchased from Shanghai Institute of Biological Sciences. Dulbecco's Modified Eagle Medium (DMEM), RPMI Medium 1640 and trypsin were obtained from Invitrogen Inc. Fetal Bovine Serum (FBS) was obtained from Gibco. All the reagents were used without further purification.

DNA oligonucleotides were synthesized and purified by Sangon Biological Engineering Technology & Co. Ltd. (Shanghai, China) and stored in 1×PBS.

Table S1 The sequences of our designed oligonucleotides were as following:

Probe	**Sequence(5`-3`)**
H1	GATCGCGATCCTGGCTCCTGTGATTGTGCTCTAGACATCGCTAGAGCACAATCACAGG
H2	CTAGAGCACAATCACAGGAGCCAGTTTTCCTGTGATTGTGCTCTAGCGATGT
Initiator	CTAGAGCACAATCACAGGAGCCAGTTTACGTATGCCGTAAGCTTTGC
Aptamer	Cy3-CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGCCTGTTTGCAAAGCTTACGGCATACGT
Random	Cy3-TTCAGACTTAATTCCCACGTAAGTAAGTATTGGACC

N1-1	SH-TTTTTCTATGTCGTCAGGTC
1-H1	TTAACCCACGCCGAATCCTAGACTCAAAGTCTAGGATTCGGC GTG
1-H2	AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACT ACTTTG
1-I	AGTCTAGGATTCGGCGTGGGTTAATGAGCCACTGGATAC
H1-5	CTGGCTCCTGTGATTGTGCTCTAGACATCGCTAGAGCACAAT CACAGGTTTTTTTTTTCACAGATGAGT
H1-6	CTGGCTCCTGTGATTGTGCTCTAGACATCGCTAGAGCACAAT CACAGGTTTTTTTTTTCCCAGGTTCTCT
H2-4	CTAGAGCACAATCACAGGAGCCAGTTTTCCTGTGATTGTGCT CTAGCGATGT
Ap-ATP	ACTCATCTGTGAAGAGAACCTGGGGGAGTATTGCGGAGGAA GGT
I-EpCAM	CTAGAGCACAATCACAGGAGCCAGTTTACGTATGCCGTAAGC TTTGC
Apt-EpCAM	CACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTT GGCCTGTTTGCAAAGCTTACGGCATACGT

2. Assembly of DNA:

Stoichiometric amounts of DNA strands of the DNA hydrogel were added to 1×PBS buffer to give a final concentration of 1 mM for H1 and H2, 180 μM for aptamer and initiator strands. The resulting mixtures were heated to 95 °C for 5 min to homogeneously mix H1 and H2 components in solution, and subsequently cooled at 4 °C; aptamer and initiator strands mixture was cooled to rehybridize the designed structures (cy3-Aptamer-initiator bi-blocks) as the temperature slowly cooling down.

3. Cell culture and Cell cloaking via DNA hydrogel

MCF-7 cells and Hek293 cells were cultured in RPMI Medium 1640 and DMEM, respectively. The medium supplemented with fetal bovine serum (FBS; 10%), penicillin and streptomycin (1%), and L-glutamine (1%). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Before experiments, cells were washed with 1×PBS.

MCF-7 or HEK293 were cooled at 4 °C for 20 min, and then the cy3-aptamer-initiator bi-blocks (or DiO) were added into the solution containing cells. Then the solution was incubated at 4 °C for 20 min. The supernatant was removed and then cells were washed using 1×PBS for three times. (The cells were used for fluorescence microscopy imaging.) The cells were digested by trypsin for 30 seconds, then 1 ml of medium were added to stop digestion. The mixture were centrifuged at 1000 rpm for 3 min and washed with 1×PBS for three times to remove the excess medium. The cells were counted by coutstar. 20 μL of H1(1 mM), 20 μL of H2(1 mM), 2 μL of AuNPs and 4 μL of cells were mixed together and then incubated at 37 °C for 3 h.

In DNA hydrogel formation kinetics experiment, we used E. coli instead of AuNPs to investigate the hydrogel formation in real time. We used confocal microscopy to make the videos of moving *E coli*. The image J data was used to derive the velocity profile of *E coli*.

4. Cancer cells release via ATP responsive DNA hydrogel

For ATP sensitive DNA hydrogel, we mixed the H1 complexes (Group 1) which included 4 mM of H1-5(10 μL), 4 mM of H1-6 (10 μL), 4 mM of Ap-ATP (10 μL), 10×PBS (4 μL), and Milli Q water (6 μL). The initiator strands (Group 2) included 2 mM of initiator strand (I-EpCAM, 5 μL), 2 mM of Apt-EpCAM (5 μL) and 90 μL of 1×PBS. Group 3 just contained 4 mM of H2-4 strand (40 μL), 10×PBS (4 μL) and Milli Q water (16 μL). We annealed the Group 1, Group2 and Group 3 separately at 95 °C for 5 min, and then cooled to 4 °C before

use. As for MCF-7 cells induced DNA hydrogel formation, just as step 3. And then we added 500 μM of ATP to destroy the DNA hydrogel and release the cancer cells.

5. FDA stained MCF-7 in DNA hydrogel

Fluorescein diacetate (FDA) was diluted in acetone with 5 mg/mL for dyeing. MCF-7 cells were stained by FDA for 6 min and then incubate with cy3-aptamer-initiator bi-blocks for half an hour at 4 °C. The excesses FDA or cy3-aptamer-initiator bi-blocks were removed by centrifuge at 1000 rpm for 3 min. The cells were then washed with 1×PBS for three times. 5 μL of MCF-7 cells and H1, H2 with final concentration of 1000 μM (20 μL) were mixed together and then incubated in 37 °C in 5% (V/V) CO_2 95% (V/V) air for 3 hours. The final system was detected by confocal microscopy.

6. Gel analysis

For atcHCR and traditional HCR reaction (1-H1, 1-H2, 1-I), we used the same condition for gel electrophoretic migration. The final concentration of 5 μM of H1, H2 and different concentration of I was mixed together. Then the mixture was incubated at 37 °C for 3 hours. We used 5 μL of sample for gel electrophoretic migration. The 1% agarose gel was stained by GelRed. Running conditions: in 1×TBE buffer under 100 V for 1 hour.

7. Confocal Microscopy Imaging

In order to verify that the aptamers were combined with the EpCAM on the membrane of the cells, experimental cells were observed using a Leica TCS SP8 confocal microscope equipped with a live cell incubator. The same as the steps above, the cy3-aptamer-initiator bi-blocks modified cells were used for confocal microscopy measurements.

For DiO staining experiments, the cells were stained with the 3, 3′-dioctadecyloxacarbocyanine perchlorate (DiO), which was green fluorescent dye on the membrane of the cells.

8. SEM measurement

Phenom Pro was used for undried sample. LEO 1530VP was used for dried hydrogel and give a clear SEM image of the DNA hydrogel.

9. Western blot analysis

Cells were lysed in M2 buffer. Equal amounts of cell extracts were resolved by 15% SDS-PAGE and analyzed by Western blot and visualized by enhanced chemiluminescence (ECL, Amersham).

10. Real-time PCR

PCR amplification was performed using the following primers:

Forward primer for CK19, GACTACAGCCACTACTACACGACCAT, for EGFR, CCAGTGACTGCTGCCACAACCA. Reverse primer for EGFR, GAGCGGAATCCACCTCCACACT, for CK19 is CGCCGTCTTCCTCCATCTCATAGC.

For quantitative real-time PCR, the cDNA derived from MCF-7 cells were added with the Fast SYBR Green Master Mix and specific primers used above for conventional PCR. The reaction mixtures were then applied to the StepOnePlus Real-Time PCR System (Life Technologies) and thermal cycling was carried out for 40 cycles at conditions of 95 °C for 5 sec (denaturation) followed by 62 °C for 30 sec (annealing/extension). CT values were obtained using the StepOnePlus software (provided by the manufacturer).

11. Protection of gold nanoparticles (AuNPs)

To help visualize the gelling transition, we employed water-soluble SH-labeled DNA (N1-1) modified 10 nm AuNPs as "tracer agents". 1 mL of AuNPs (10 nm) were concentrated into 40 μL (0.2 μM), and incubated with DNA to Au molar ratio more than 50:1 in 1×PBS, then 1.6 μL of citrate buffer (pH 3.0) was added to change the pH value about 3.5 for the system (JACS 2012, 134, 7266−7269). The mixture was shaken with 350 rpm at 25 °C for 2 h. AuNP-DNA conjugates were washed with 1×PBS buffer to remove the extra oligonucleotides. The concentration of these AuNP-DNA conjugates was 0.64 μM measured from the optical absorbance at ~ 520 nm.

12. Ultraviolet measurements

The UV−vis spectra were obtained by a Hitachi U-3010 UV−vis spectrophotometer (Japan). With the gel-to-sol transition, 200 uL of 1×PBS was added in the tube, after 30 min, a specified amount of the upper solution of the DNA hydrogel was used for the UV-vis measurement. The UV measurements were conducted from 400 nm to 800 nm. All the cell detection experiments were repeated for three times.

13. Quantification of CTCs in blood samples

The intrinsic blood cells in the blood sample were used as indicator before the gelling process. Without cancer cells in blood sample, the hydrogel could not form and blood cells could be dispersed into the buffer solution after adding buffer solution. Upon formation of the DNA hydrogel initiated by the cancer cells, the blood cells were trapped within the hydrogel and could not be dispersed into the upper layer of buffer solution. Images were obtained with a digital single-lens reflex camera (DSLR, Canon 700d). We quantified the cancer cells by analyzing the images (the upper layer of buffer solution) through 8-bit grey scale values from histogram averages in Adobe Photoshop (Adobe systems Inc.). Histogram averages with other channel (red channel, green channel or blue channel) option of the Adobe Photoshop can also be used to quantify the cancer cells.

14. Cancer cell enrichment

The MCF-7 cells were mixed with L1210 cells (mouse lymphoblast cell line) in PBS with a ratio of 1:1000. 1 μL of cell mixture was then dropped on confocal dish for DNA hydrogel formation. After hydrogel formation, 50 μL of PBS was added into the dish for biased release of L1210. During the swelling process of DNA hydrogel, the release of L1210 cells from DNA hydrogel to PBS were observed.

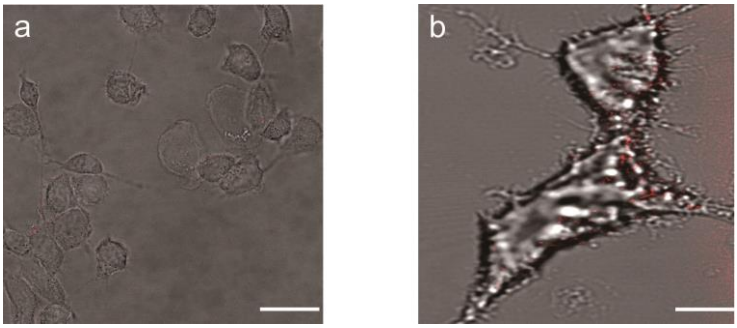

Figure S1. (a) Confocal image of MCF-7 cells with the binding of cy3 labeled random DNA (scale bar: 25 μm). (b) Confocal image of HEK-293 cells with the binding of cy3 labeled aptamer-initiator bi-blocks (scale bar: 10 μm).

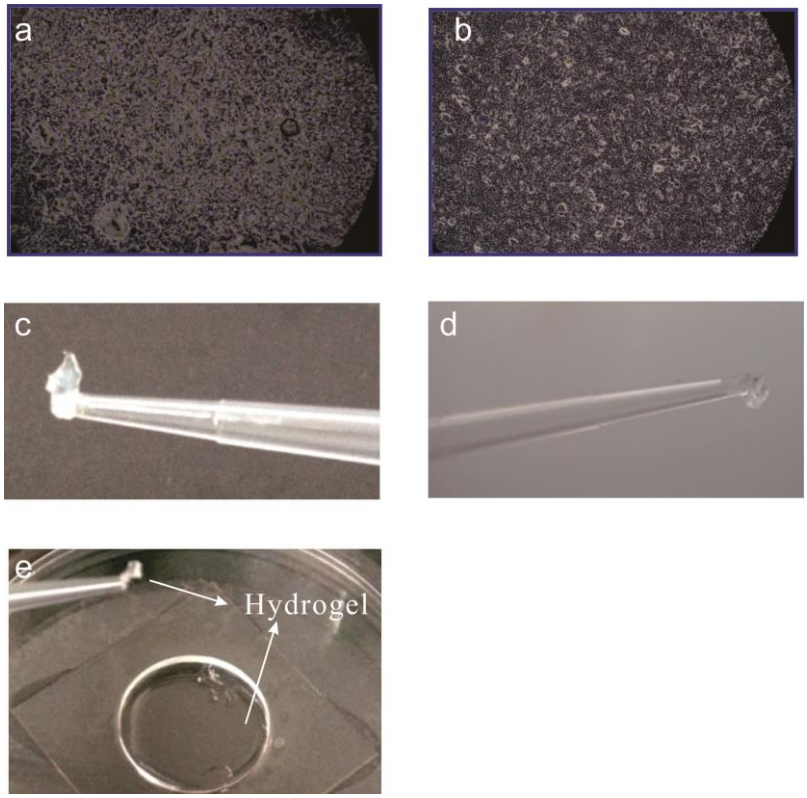

Figure S2. Fabrication of DNA hydrogel by using cancer cells dispersed in different buffer solutions (a-d) and adhered on cell culture dish (e). (a) The microscopy image for DNA hydrogel formed in 1×PBS, (b) The microscopy image for DNA hydrogel formed in 1×TAE, (c) The photograph of DNA hydrogel formed in 1×PBS, (d) The photograph of DNA hydrogel formed in 1×TAE. (e) DNA hydrogel can successful form via atcHCR triggered by cells adhered on cell culture dish.

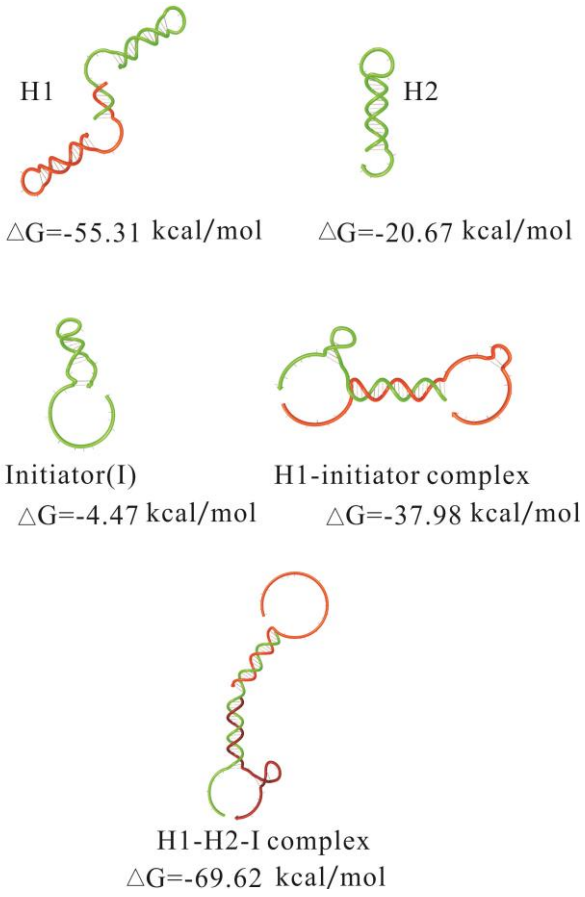

Figure S3. The free energy and secondary structure of H1, H2, Initiator, H1-initiator complex and H1-H2-Initiator complex were predicted through NUPACK.

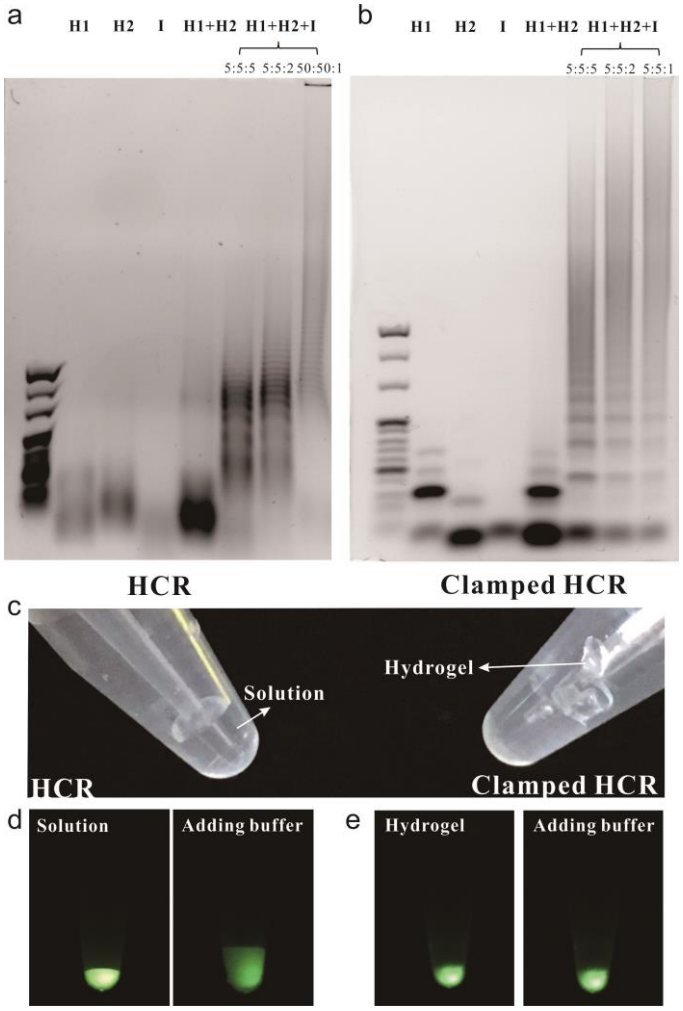

Figure S4. (a) The gel electrophoresis characterization of traditional HCR (lane 1: DNA marker; lane 2: H1; lane 3: H2; Lan4: Initiator (I); lane 5: mixture of H1 and H2; Lane 6-8: HCR products with various ratio of H1, H2 and I). (b) The gel electrophoresis characterization of atcHCR (lane 1: DNA marker; lane 2: H1; lane 3: H2; Lan 4: Initiator (I); lane 5: mixture of H1 and H2; Lane 6-8: atcHCR products with various ratio of H1, H2 and I). (c) Picture of traditional HCR demonstrated that the HCR products remained solution state, whereas, atcHCR forms the DNA hydrogel. (d) When we added buffer into the tube of traditional HCR products, we observed dispersed DNA solution under UV light (DNA was stained by SYBR Green 1). (e) When we added buffer into the tube of atcHCR products, the stained DNA kept the form of hydrogel without dispersing with added buffer.

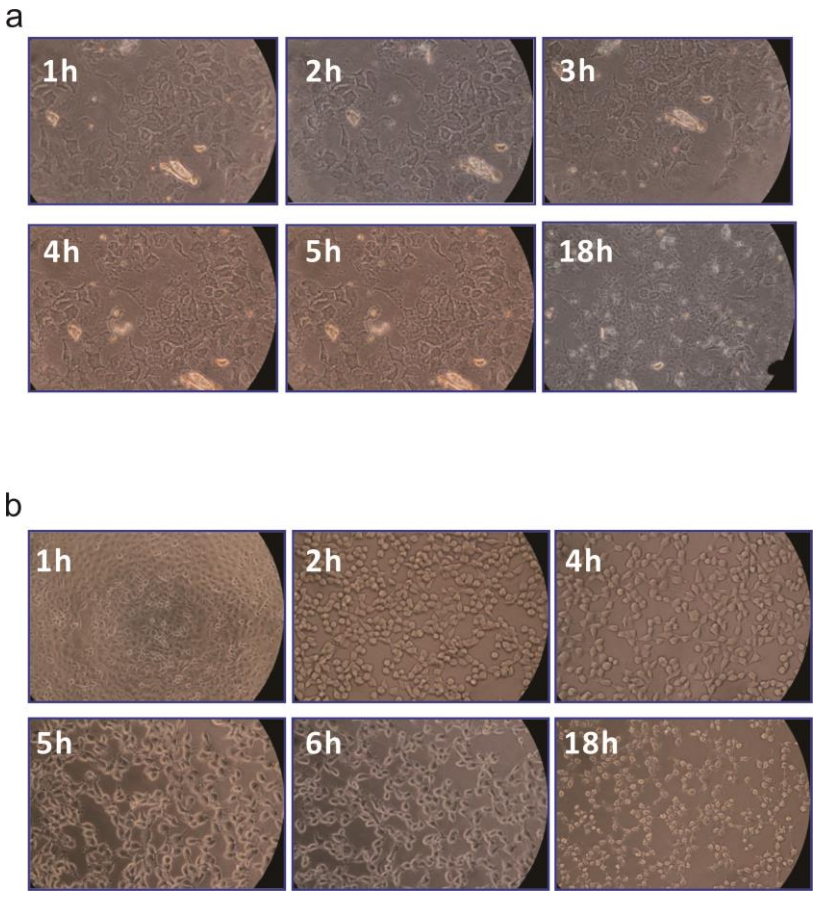

Figure S5. (a) Retention of the morphology of MCF-7 cells during the gelling process. (b) In contrast, the morphology of MCF-7 cells in buffer solution changed.

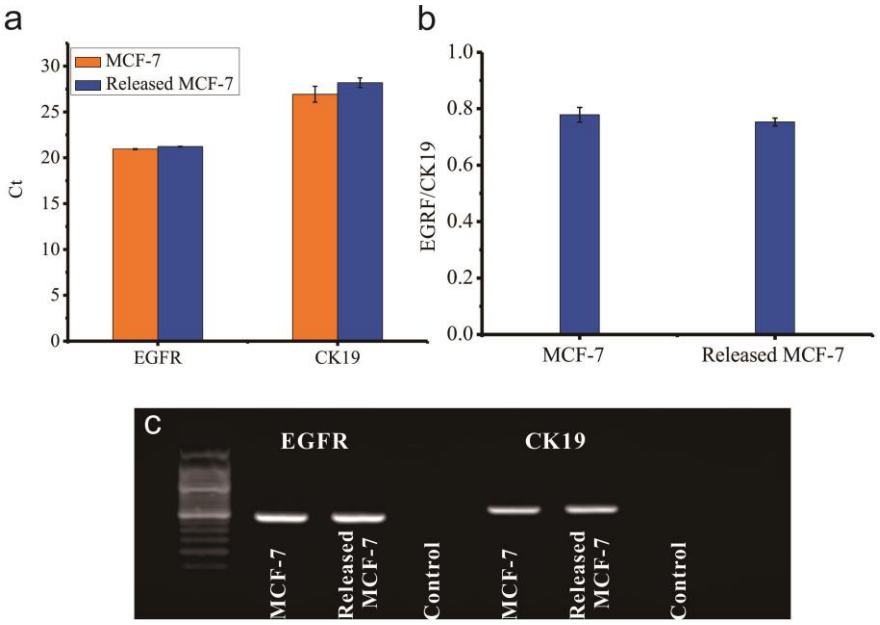

Figure S6. RT-PCR analysis of the mRNAs of MCF-7 cells and released MCF-7 cells. (a) Comparison of Ct value of EGFR and CK19 for MCF-7 and released MCF-7. (b) Comparison of Ct ratio of EGFR and CK19 for MCF-7 and released MCF-7. (c) Gel electrophoresis analysis of EGFR and CK 19 for MCF-7 and released MCF-7.

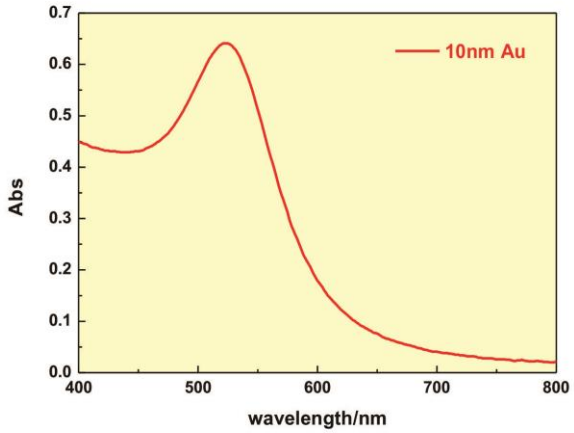

Figure S7. Representative adsorption spectrum of well dispersed AuNPs in solution.

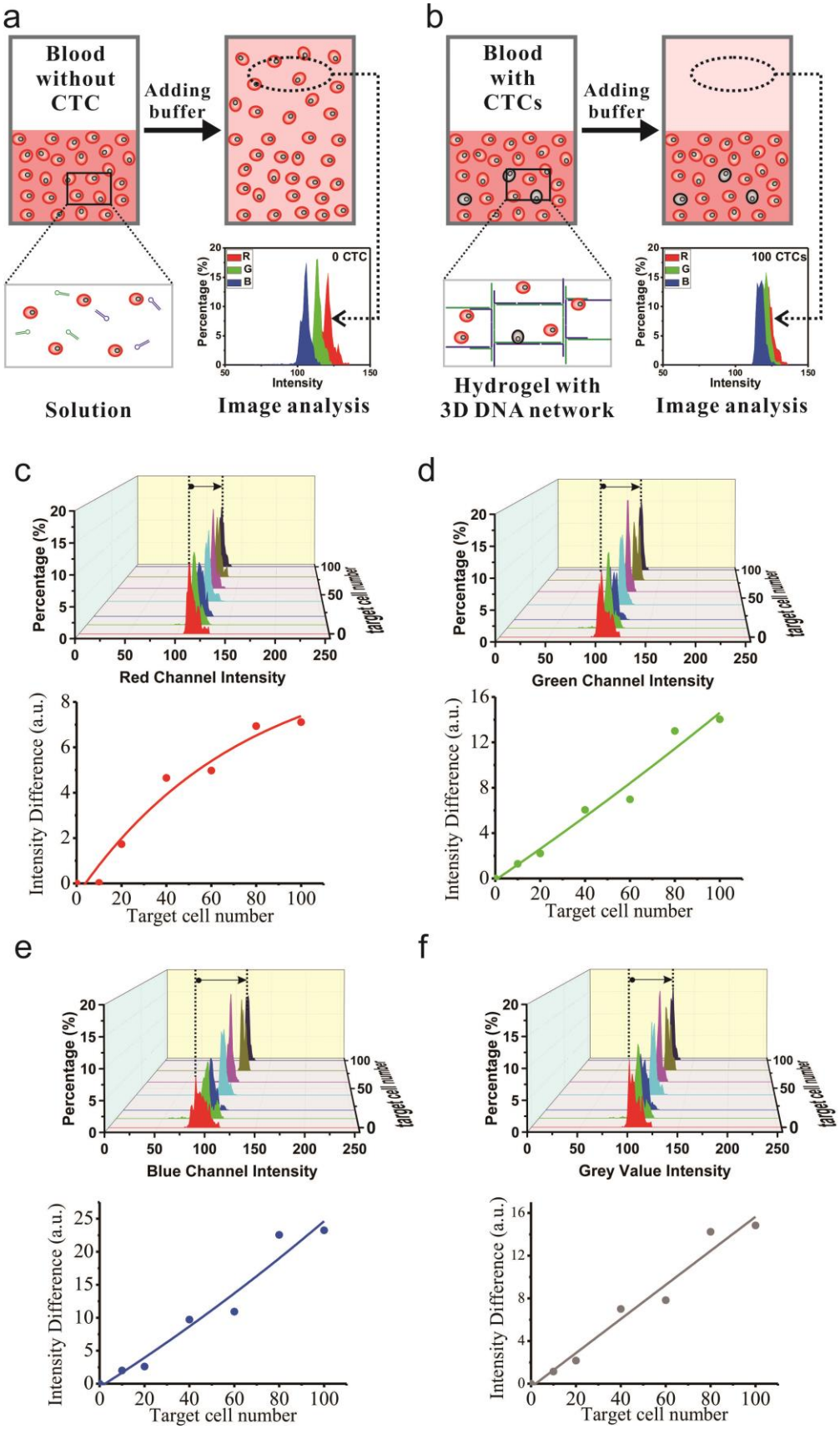

Figure S8. CTC quantification with DNA gelation-based CTC cloaking. (a) The intrinsic blood cells in the blood sample were used as indicator before the gelling process. Without

cancer cells in blood sample, the hydrogel could not form and blood cells could be dispersed into the buffer solution after adding buffer solution. (b) Upon formation of the DNA hydrogel initiated by the cancer cells, the blood cells were trapped within the hydrogel and could not be dispersed into the upper layer of buffer solution. (c-f) Quantify the CTC numbers in blood sample using the histogram function with red channel (c), green channel (d), blue channel (e), and grey channel (f) option of the Adobe Photoshop. The average value from the histogram window was used to calculate the intensity of each samples.