Bioimaging of targeting cancers by aptamer-conjugated carbon nanodot

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Electronic Suporting Information(ESI)

Experimental sections

Materials

Glycerol, NaH_2PO_4 and 2- beta mercaptanol were procured from sigma Aldrich (St. Louise, MO,USA). TTA1(5'-Maleimide-CCTGCACTTGGCTTGGATTTCAGAGAAGGGAG-3', mal-Tnc) aptamer and a random DNA sequence (5'-Maleimide-TTCCTCCTCCTCCTTCTC CTCCTCCTCC-3', mal-Mt) was purchased from Bioneer (Bioneer, Seoul, Korea). And all the chemicals were analytical reagent grade and were used without further purification.

Synthesis of the SH-gC-Dots

The SH-gC-Dots were synthesized by a microwave assisted pyrolysis method. In the typical processor, 35 ml glycerol and 15 ml 7.1 mM phosphate solution were mixed with 10 ml of 2- beta mercaptanol under vigorous stirring to form a homogeneous solution in 250 ml beaker. Then the beaker was treated with microwave for 13 minutes using domestic microwave oven (700 W). Finally, solution was cooled down to room temperature, the yellow solution was then diluted and dialyzed against pure water for 2 days using 25kDa cellulose dialysis back. Purified yellow color solution was stored in glass tube for further analysis. The samples were dried by lyophilization technique for overnight and concentartions were measured as 4µg/mL. The various concentarion of the SH-gC-Dots **(**0.8, 4, 8 and 16 µg/mL) were conjugated with 10 pmole of mal-Tnc in PBS buffer at room temeperature by incubation for one hour.

Instrumentation and characterizations

TEM measurements were performed on JEOL JEM-2010 at operating voltage of 200 kV. Drops of a dilute aqueous solution of the gC-Dots, SH-gC-Dots and TTA1-C-Dots were deposited on a carbon-coated copper grid at dried in air for overnight. The size was presented as the mean ± standard deviation. Emission and absorbance spectra were recorded 200 µl of gC-Dots and SH-gC-Dots using by Synergy Mx Monochromator-Based Multi-Mode Microplate Reader. The emission spectra were recorded for different excitation wavelength ranging from 360 nm to 560 nm. The absorbance spectra were recorded in the range between 300 nm to 800 nm. XPS measurements were performed with a Mg Kα source (1253.6 eV) from Escalab 220i-XL (Thermo VG, U.K.). Spectra were acquired with 10 eV pass energy and a Shirley type background was subtracted to each region. The gC-Dots and SH-gC-Dots solutions were mounted on sample holder and the spectra were measured by a Perkin-Elmer

Spectrum 2000 FTIR spectrophotometer. A baseline correction was applied after measurement.

Cell culture

CHO, HeLa and C6 cells were purchased from the American Type Culture Collection (ATCC). The culture medium for cells consisted of Dulbecco's modified Eagle's medium (DMEM, life technology Seoul, Korea) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Grand Island, NY) and 1% Antibiotics (Invitrogen, Grand Island, NY) in a standard incubator (5% CO_2 atmosphere at 30°C). The cells were split at regular intervals.

In vitro cell viability assay

CHO, HeLa and C6 cells were cultured using established procedures. In the 96-well plates, each cells were plated at 1×10^5 cells per well over night. The various concentrations of the SH-gC-Dots (8, 16, 32, 80, or 160 µg/mL) were treated with CHO, HeLa and C6 cell for 24 hours. Cells cultured in the free medium were taken as the control. The cell viability was evaluated by the 3-(4, 5-dime-thythazolz-yl)-2, 5-diphenyletrazolium bromide (MTT) assay. The optical density was read at 490 nm on a multi well spectrometer (Synergy MX, Biotek Ltd, VT, USA). All assay performed in triplicate. The results were presented as reduction of metabolic activity in percentage when compared to control cells cultured in growth medium only (100%).

In Vitro fluorescence analysis

CHO, HeLa and C6 cells (1×10^5) were transected with 16 µg/ml of the SH-gC-Dots or 16

μg/ml of TTA1-C-Dots within the PBS buffer at 37˚C and transferred into dark 96-well microplates. The fluorescent intensities were measured using by Synergy Mx Monochromator-Based Multi-Mode Microplate Reader.

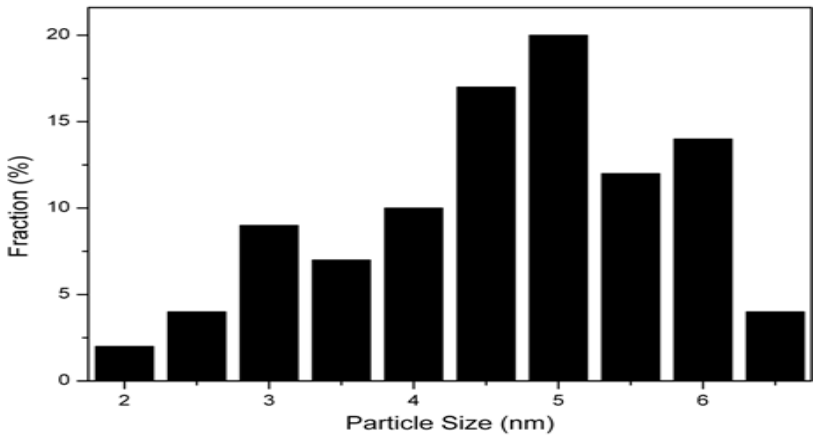
Confocal Analysis

CHO, HeLa and C6 cells (1×10^4) were seeded into 4-well plates containing 25-mm-diameter cover glass and cells were grown for 24 hours at room temperature. Before tratment with SH-gC-Dots (16 μg/ml), the cells were incubated for 30 minutes at 4°C. After 2 hours incubation with SH-gC-Dots, Mt-C-Dots and TTA1-C-Dots, the cells were washed with Dulbecco's phosphate-buffered saline (D-PBS) twice for 5 mina and then fixed to 4% paraformaldehyde. The cover slips from the four-well plates were placed into a glass slide solution was added (Vector Laboratories, Inc., CA, USA). The confocal images were obtained at excitation wavelength of 488 nm using by LSM 510 (Carl Zeiss Inc., Berlin, Germany).

Agarose electrophoresis

The conjugation efficiency of mal-Tnc aptamer with SH-gC-Dots was evaluated by agarose gel electrophoresis. The concentration of the 0.8, 4, 8 and 16 μg/mL of SH-gC-Dots were prepared with 10 pmol of mal-Tnc aptamer by incubation for 30 min at room temperature, 8 μL of complex-containing solution was mixed with 2 μL loading buffer, and loaded into a 2% agarose gel containing ethidium bromide (0.5 μg/mL). The electrophoresis experiment was carried out for 40 min in 1 x TAE buffer at a constant voltage of 100 V. The mean intensity of the mal-Tnc aptamer band was measured by imageJ software (NIH, USA).

(a)



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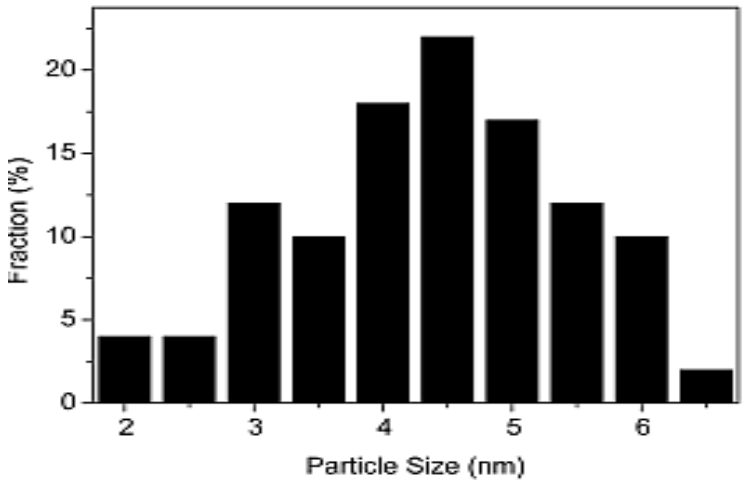
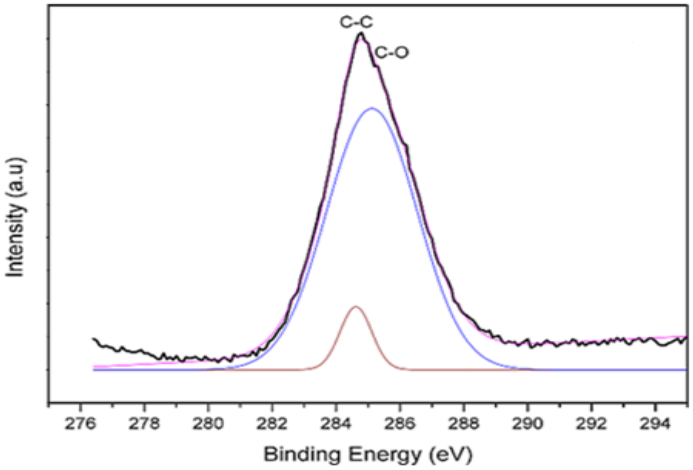


Figure. S1. Particle size histogram of (a) the gC-Dots and (b) the SH-gC-Dots . The particle size histograms were measured from TEM images.

(a)



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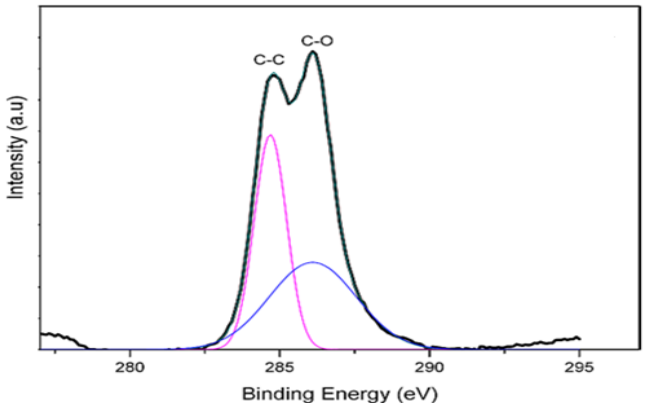

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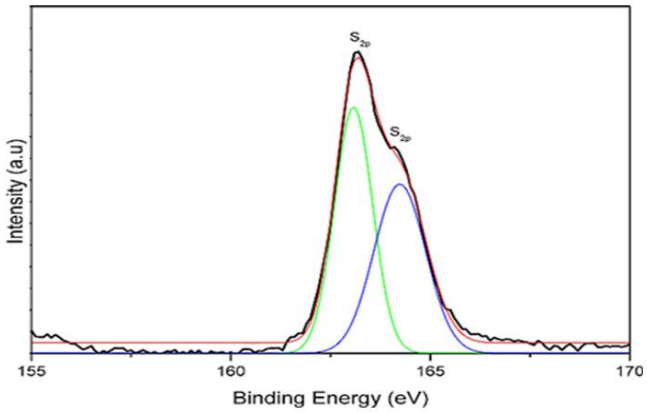
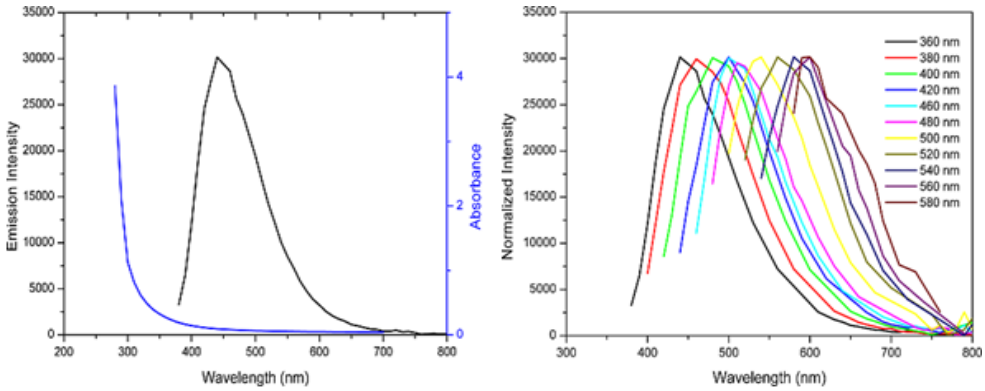


Figure. S2. (a) High-resolution XPS C1s spectrum of gC-Dots, (b) High-resolution XPS C1s spectrum and (c) XPS S_{2p} spectrum of the SH-gC-Dots .

(a)



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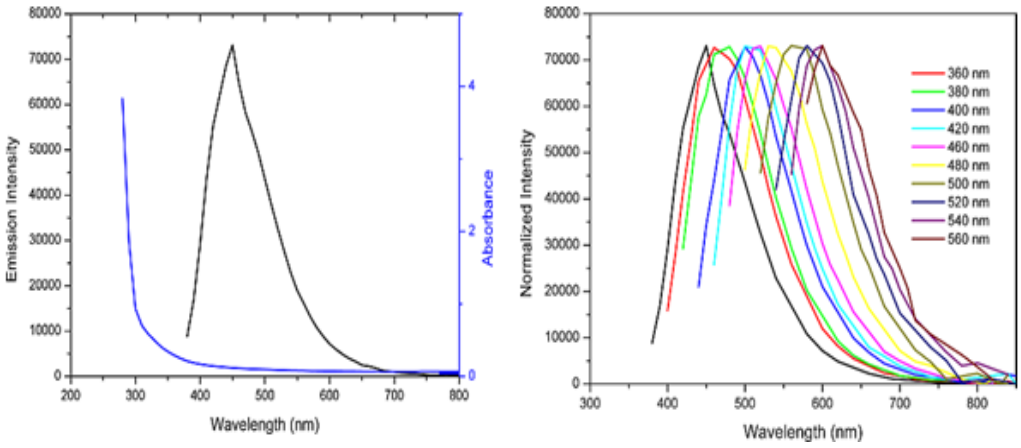
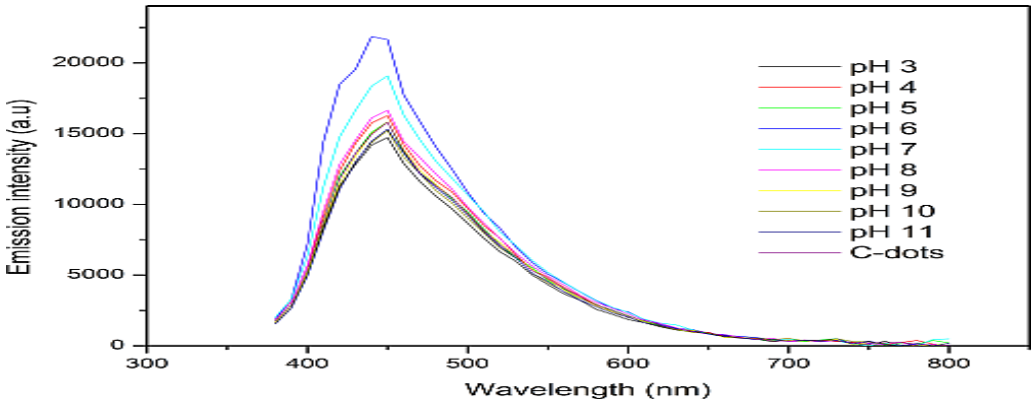


Figure. S3. (a) UV/Vis absorption, emission spectra (excition at 360 nm) and normalized emission spectra of the gC-Dots (b) UV/Vis absorption, emission spectra (excitation at 360 nm) and normalized emission spectra of the SH-gC-Dots.

(a)



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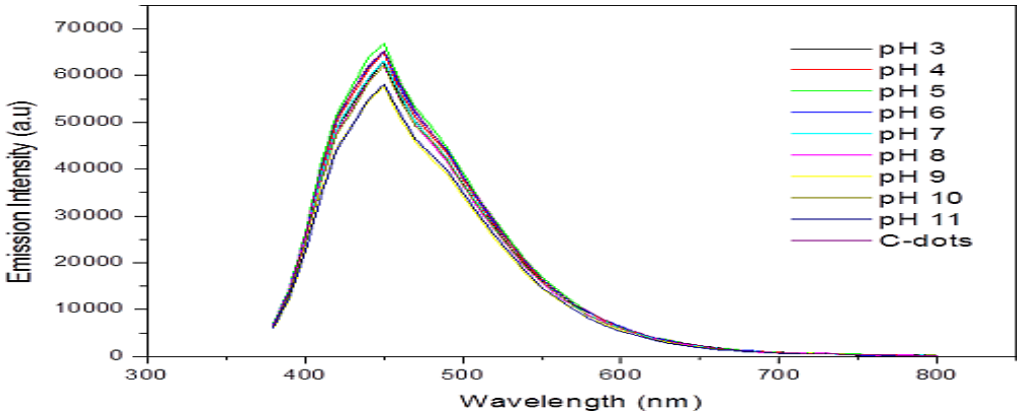


Figure. S4. pH-dependent emission spectra of (a) the gC-Dots and (b) the SH-gC-Dots. Different type of buffer from pH 3 to pH 11 were prepared by mixing of sodium phosphate monobasic dehydrate and sodium phosphate dibasic. The 100 µL of each puffer were placed into the dark 96-well microplates and 100 µL of the gC-Dots and the SH-gC-Dots were mixed. The emission spectra were recorded for the excitation wavelength of 360 nm.

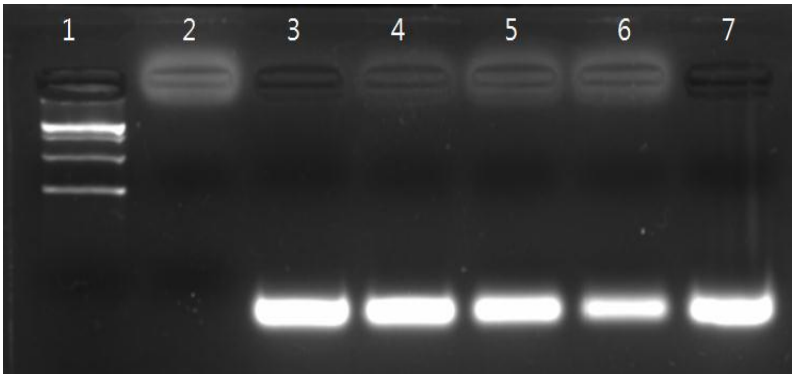
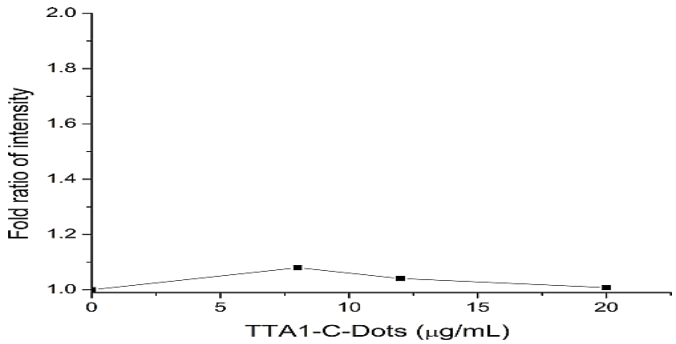
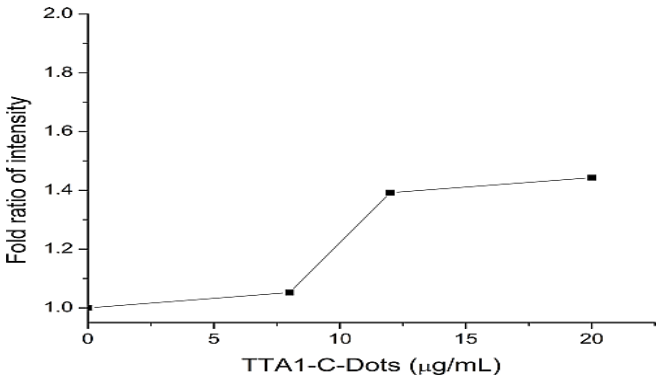


Figure. S5. Gel electrophoresis of molecular ladder of 1kb (lane 1), the SH-gC-Dots alone (lane 2), 0.4 µg/mL of the SH-gC-Dots with TTA1 (lane 3), 0.8 µg/mL of the SH-gC-Dots with TTA1 (lane 4), 4 µg/mL of the SH-gC-Dots with TTA1 (lane 5), 8 µg/mL of the SH-gC-Dots with TTA1, 16 µg/mL of the SH-gC-Dots with TTA1 (lane 6) and TTA1 alone (lane 7) (TTA1 concentration =10 pmole).

(a)



(b)



(c)

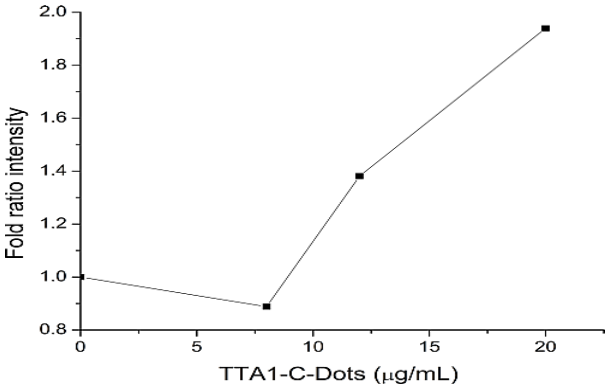


Figure. S6. Fold ratio of fluorescence intensity for figure 3a. Fluorescence intensities of the SH-gC-Dots (20 μg/mL) and TTA1-C-Dots (8, 12, 20 μg/mL) at 360nm of excitation and 450 nm of emisstion were obtained from CHO (a), HeLa (b) and C6 (c) cells in figure 3a. Fold ratio was normalized against the SH-gC-Dots (20 μg/mL).

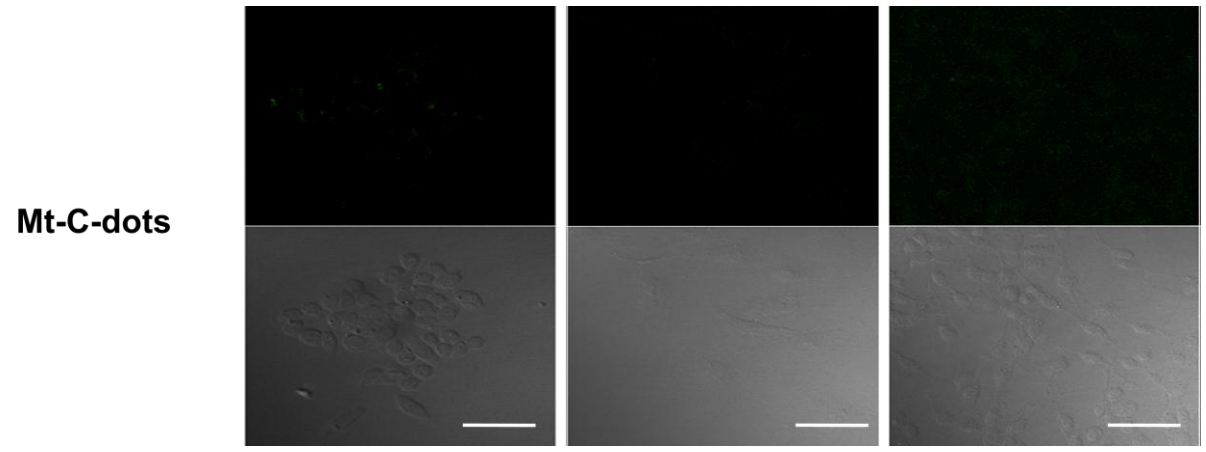


Figure. S7. Confocal microscopy image of CHO (1st column), HeLa (2nd column) and C6 (3rd column) cells treated with 16μg/mL of the Mt-C-Dots. Cellular morphology of each cell line was displayed with 20 μm of scale bar

Table S1: Comparison of emission spectra for different excitation wavelength of gC-Dots and SH-gC-Dots.

gC-Dots		SH-gC-Dots	
Excitation(nm)	**Emission(nm)**	**Excitation(nm)**	**Emission(nm)**
360	460	360	450
380	490	380	470
400	500	400	490
420	520	420	511
440	520	440	511
460	530	460	528
480	541	480	538
500	560	500	560
520	580	520	580
540	590	540	590
560	610	560	600