Supporting Information for

Real-time tracking the intracellular delivery of 2D nanosystem by progressively activatable fluorescence platform for cancer diagnosis

Materials and Instrumentation: 1-Ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC), deoxycholic acid, N-hydroxysuccinimide (NHS) were purchased from Sigma Aldrich (Saint Louis, Missouri, United States). Rhodamine B (RhB) was obtained from Alfa Aesar. Sodium hyaluronate (MW≈47 KDa) and adipicdihydrazide were purchased from Aladdin. Trypsin, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and hyaluronidase (Hyal) from bovine vitreous humor were purchased from Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). All chemical agents were of analytical grade. Nanopure water (18.2 M Ω; Millpore Co., USA) was used in all experiments and to prepare all buffers. FAM-labeled aptamer was synthesized by Sangon Biotechnology Co. (Shanghai, China)

DNA sequences:

FAM-5'-TTT TTT TTT TgC AgT TgA TCC TTT ggA TAC CCT gg-3'

FT-IR analysis was carried out on a Bruker Vertex 70 FT-IR Spectrometer. AFM measurements were performed using Nanoscope V multimode atomic force microscope (Veeco Instruments, USA). Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer NanoS apparatus equipped with a 4.0 mW laser operating at λ = 633 nm. UV–Vis spectroscopy was carried out with a JASCO V-550 UV–Vis spectrometer. Fluorescence measurements were carried out on a JASCO FP-6500 spectrofluorometer.

Synthesis of RhB based probe: Rhodamine B based probe was synthesized according to literature procedures. Rhodamine B (958 mg, 2 mmol) was dissolved in 20 mL of methanol, followed by addition of ethylenediamine (0.67 mL, 10 mmol). The reaction mixture was refluxed for 6 hours till the fluorescence of the solution was disappeared. The reaction was cooled to room temperature, and the precipitate was collected and washed with 10 mL of cold ethanol. Crude product was purified by recrystallization from acetonitrile as a colorless solid.

Synthesis of HA-DA and HA-R Conjugates: Amphiphilic HA-DA conjugates were

prepared by chemical grafting deoxycholic acid (DA) to adipic dihydrazide modified HA (HA-ADH) through amide formation as previously reported [17]. Successful introduction of DA into HA polymers was confirmed using ^{1}H NMR in D_2O. The amount of DA in the conjugate was quantitatively characterized from the integration ratio between the characteristic peak of the *N*-acetyl group in HA (δ =∼1.81 ppm [3H, -COCH_3-]) and that of the methyl group in DA (δ= ∼0.51 ppm [3H, -CH_3]) The degree of substitution (defined as the number of DA per 100 sugar residues of HA polymer) value was around 15% for HA-DA conjugates.

For cellular imaging, RhB based probe was used to label HA-DA (HA-R). HA-DA (100 mg) was reacted with EDC (28.8 mg, 0.15 mmol) and NHS (17.25 mg, 0.15 mmol) in 5 mL 50% DMSO/H_2O, stirring at room temperature for 30 min before adding RhB based probe (24.2 mg, 0.05 mmol). The mixture was stirred at room temperature for another 24 h. HA-R was purified by dialysis against 50% methanol/H_2O. The amount of conjugated probe onto HA backbone was determined using the UV-Vis spectrometer by measuring absorbance at 560 nm and about 8 RhB molecules were conjugated to each HA chain (molecular weight of HA = 47 kDa).

Quantitative analysis of the content of RhB molecules in HA-R: To quantify the content of RhB molecules in the conjugate by UV−vis spectroscopy, variable concentrations of Rhodamine B were added to a solution containing 1% w/v HA-DA and the absorption spectra of the different solutions were recorded. The increase in the absorbance at λ=560 nm corresponded to the amount of Rhodamine B. A calibration curve relating the absorbance features of the systems as a function of Rhodamine B concentration was generated. Then, 1% w/v of HA-R (HA-DA-Rhodamine B) was prepared and the absorbance at λ=560 nm was recorded. Based on the calibration curve, the Rhodamine B concentration in 1% w/v HA-R solution was evaluated spectroscopically. Based on this quantitative analysis, about 8 RhB were conjugated to one HA-DA polymer chain..

Synthesis of GO-HA-R: GO was synthesized from graphite by modified Hummers method. An amount of GO was dispersed by sonicating with 2 mg/mL HA-R solution for 1 h. To avoid heating, the solution was immersed in an ice-bath during sonication. The resulting solution was filtered through a 0.1 μm pore-sized microporous membrane to remove free HA-R and the obtained product was dispersed in PBS buffer and then stored at 4 °C until further use.

Enzyme and pH Activated Fluorescence Recovery Experiments: A series of standard pH buffers were prepared by mixing 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 at varied volume ratios. The pH value was measured by pH-meter. Activated fluorescence recovery was monitored under different conditions by spectrofluorometer (JASCO FP-6500). Fluorescent quantification data of GO-HA-R solutions were obtained after the GO-HA-R solutions were incubated with different concentrations (0–400 unit/mL) of Hyal in a PBS buffer (pH =6.0, 37 °C) for different time. The excitation wavelength was 488 nm and the emission wavelengths were in the range from 500 to 650 nm.

Cell Experiments: MCF-7 and NIH3T3 cells were cultured in 25 cm^2 flasks in Isceve's Modifi ed Dulbecco's medium (IMDM) (Gibco) containing 10% (v/v) fetal bovine serum (Gibco) at 37 °C in an atmosphere of 5% (v/v) CO_2 in air. The media was changed every three days, and the cells were passaged by trypsinization before confluence.

Typically, the cells were positioned in an inverted configuration and incubated with 5.0 mL of the culture medium containing nanoprobes at 37 °C in a 6-well culture plate. After indicated time, we removed the medium from each well and added 0.8 mL of pristine medium (containing no nanoprobes) to wash off the loosely-bound nanoparticles on the cell surface. Fluorescence imaging experiments were performed on a Zeiss LSM700 confocal laser scanning microscope. Flow cytometric analysis was performed on BD FACS Aria.

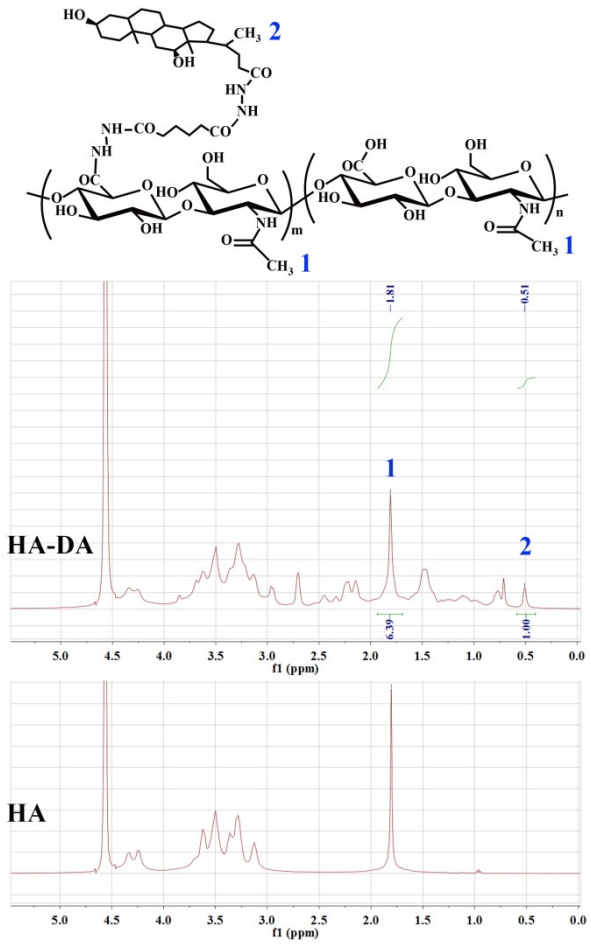


Figure S1. The structure and ^{1}H NMR spectra of HA-DA conjugates.

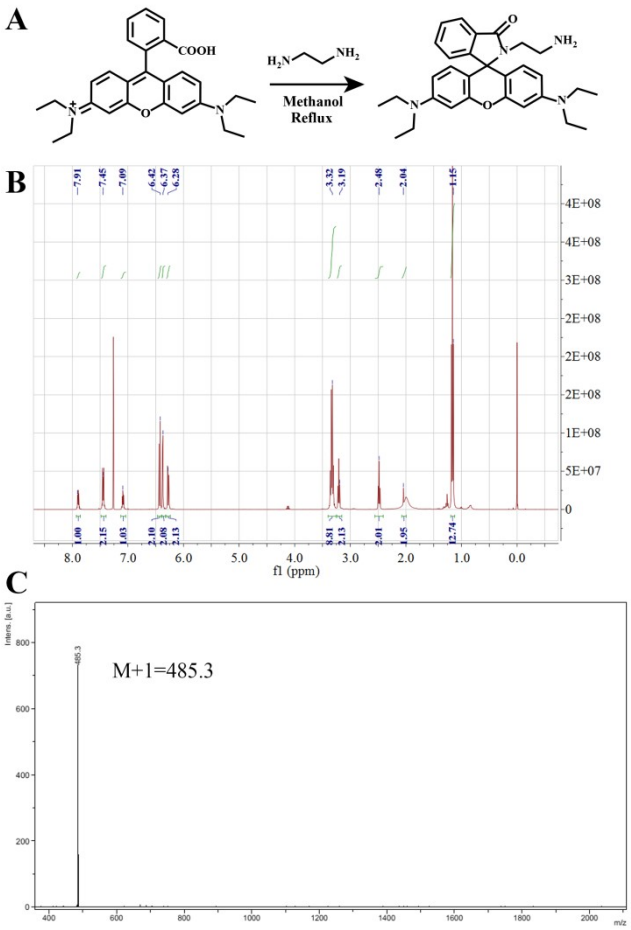


Figure S2. (A) Synthetic route of RhB based probe. (B) ^{1}H NMR spectrum recorded in $CDCl_3$ for RhB based probe. (C) Mass Spectrum of RhB based probe.

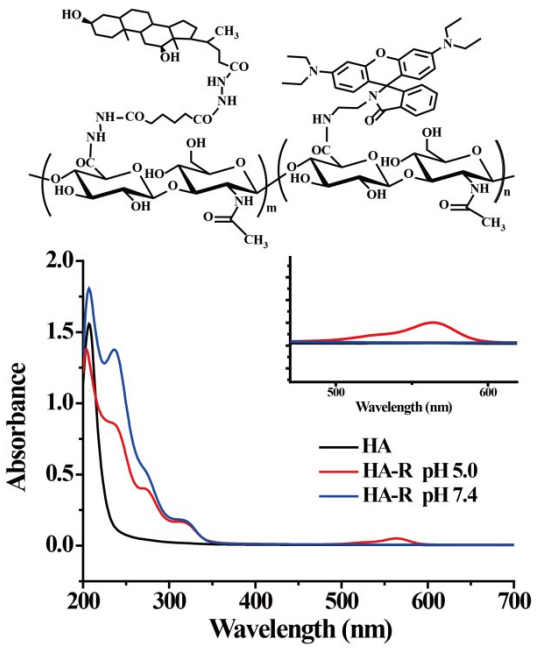
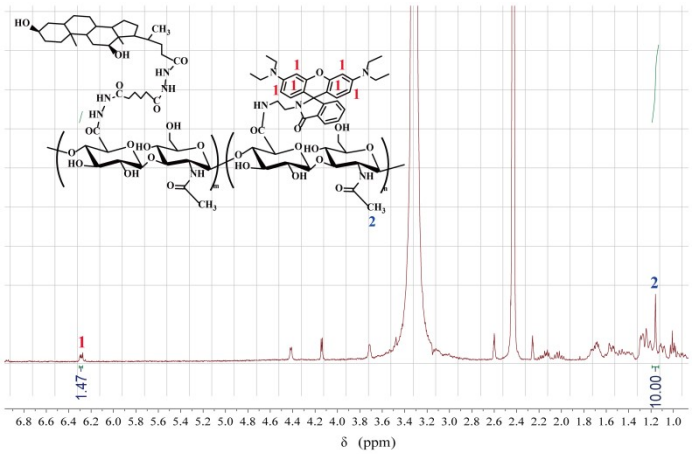


Figure S3. The structure and UV-Vis spectra of HA-R conjugates.



$$\frac{N_H(HA)}{N_H(RhB)} = \frac{10/3}{1.47/6} = 13.6$$

$$N_H(RhB) = \frac{N_H(HA)}{13.6} = \frac{4.7\times10^4/403.31}{13.6} \approx 8$$

$N_H(HA)$ represents the number of repeating units of HA

$N_H(RhB)$ represents the number of Rhodamine

Figure S4. ^{1}H NMR spectra of HA-R conjugates and the number of RhB molecules per polymer chain is calculated according to NMR spectrometer.

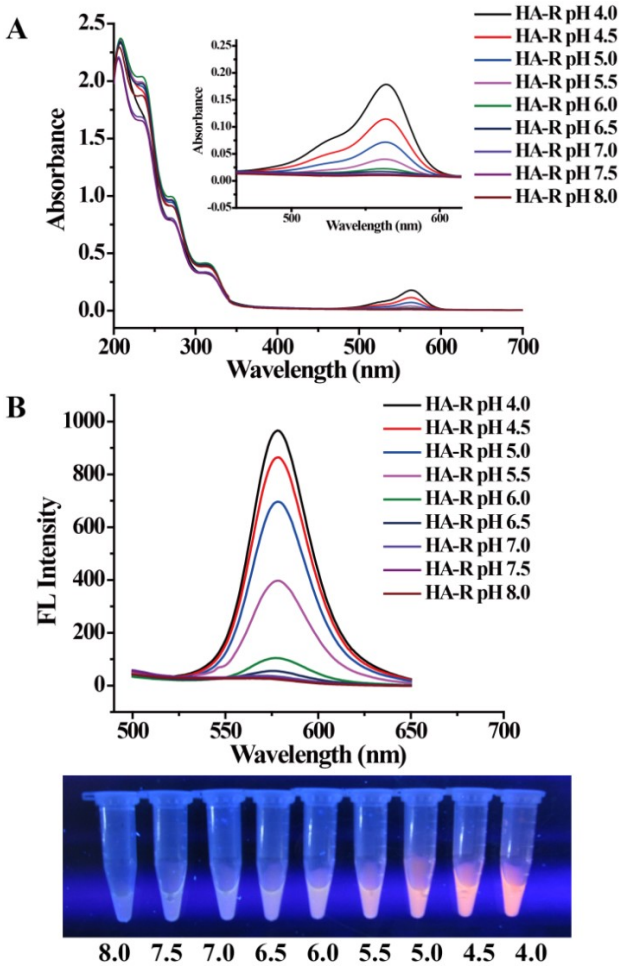


Figure S5. pH-switchable UV-Vis absorption spectra (A) and fluorescence emission spectra (B) of HA-R conjugates.

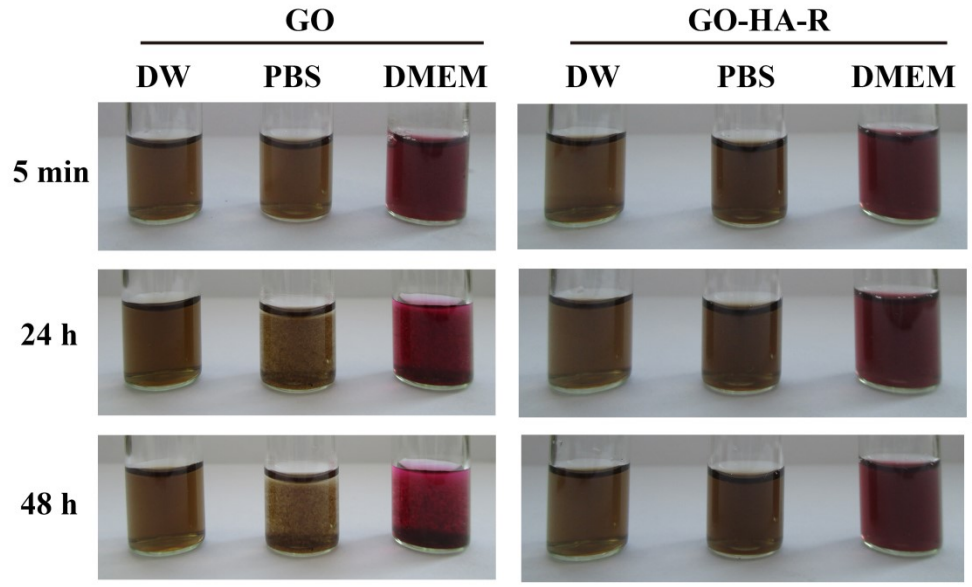


Figure S6. The photographs of GO and GO-HA-R dispersed in deionized water (DW), PBS buffer solution, cell culture medium (DMEM). All these photos are taken at different periods after dispersion by sonication

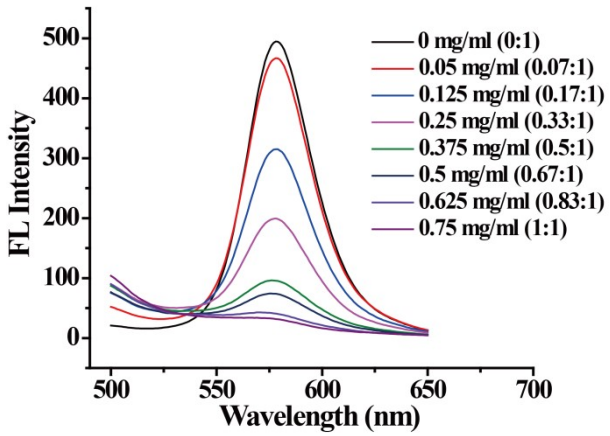


Figure S7. Fluorescence quenching of the HA-R in the absence (black) and presence of GO with a series of concentrations.

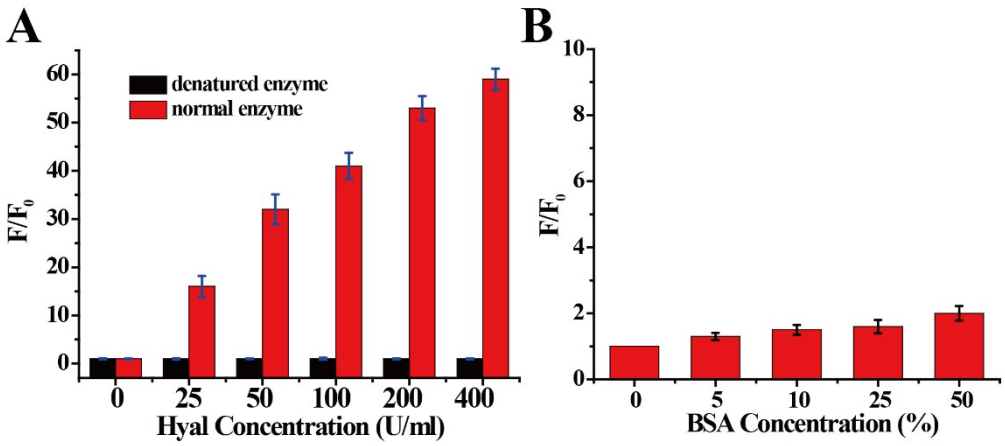


Figure S8. In experiment A), the enzyme was denatured by heating enzyme solutions at 90 °C for 60 min before adding to the nanoassemblies. As expected, no fluorescence recovery was observed. In experiments B), we found that the GO-HA-R composite showed only slight fluorescence enhancement upon incubation with bovine serum albumin (BSA) contained cell culture medium.

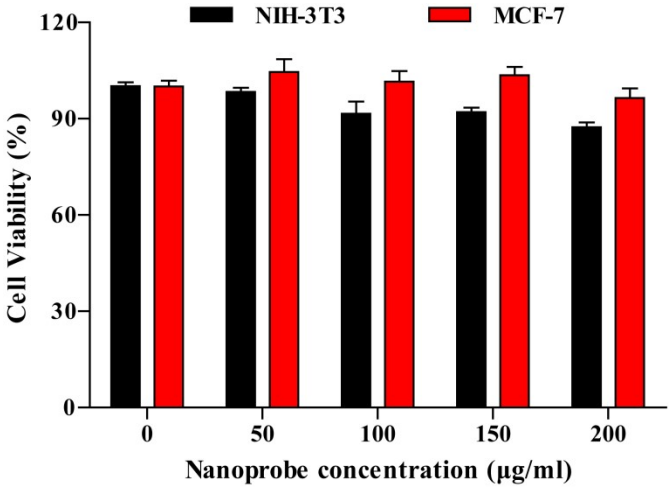


Figure S9. Relative viabilities of NIH-3T3 and MCF-7 cells after treatment with different concentrations of GO-HA-R-Apt-F nanoprobe for 24 h.

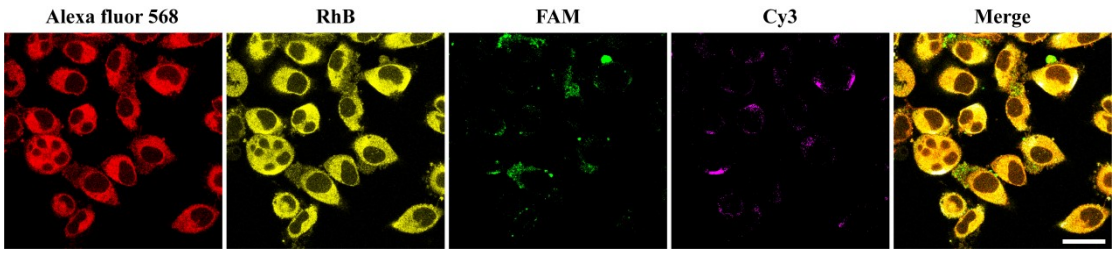


Figure S10. CLSM images of MCF-7 after sequential incubation with GO-HA-R-Apt-F nanoprobe, CellTrace™ Red CMTPX and Cy3-labelled MUC1 antibody. Alexa fluor 568: CellTrace™ Red CMTPX; RhB: HA-R; FAM: Apt-F; Cy3: Cy3-labelled MUC1 antibody. Scar bar = 30 μm.