Electronic Supplementary Information (ESI) for Chemical Communications

In situ electrochemical assay of cell surface sialic acids featuring high-efficient chemoselective recognition and a dual-functionalized nanohorn probe

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Experimental

Materials and reagents. Avidin, avidin-fluorescein isothiocyanate (avidin-FITC), biotin hydrazide (BH), mannan, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*-hydroxysuccinimide (NHS) and sialidase were purchased from Sigma-Aldrich Inc. (USA). 3-aminophenylboronic acid (ABPA) and sodium periodate ($NaIO_4$) were from Alfa Aesar China Ltd. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Sodium borohydride ($NaBH_4$) and D-fructose were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Single-walled carbon nanohorns (SWNHs) were kindly provided by Prof. Iijima's group (Japan Science and Technology Agency). Phosphate buffer saline (PBS, pH 7.4) contained 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na_2HPO_4 and 1.41 mM KH_2PO_4. All other reagents were of analytical grade. All aqueous solutions were prepared using ultra pure water (≥ 18 MΩ, Milli-Q, Millipore).

The mannan-conjugated AuNPs were prepared by mixing a cooled solution (49.4 mL) containing 0.01% $HAuCl_4$ and 3.2 mg mannan as the stabilizing reagent[1] with 0.6 mL ice-cold solution of $NaBH_4$

(0.1 M) as the reducing agent[2] under continuously stirring, which was then stirred for 1 h and stored at 4 °C.

Apparatus and characterization. Scanning electron microscopic (SEM) images were obtained using a Hitachi S-4800 scanning electron microscope (Japan). Transmission electron microscopic (TEM) image was observed on a JEM-1010 transmission electron microscope (JEOL Ltd., Japan). The infrared spectra were recorded on a Nicolet 400 Fourier transform infrared (FT-IR) spectrometer (Madison, WI). The luminescent image of BGC cells after chemoselective labeling and avidin-FITC recognition was obtained at room temperature using a cooled low-light charge-coupled device (CCD) camera with high resolution (BioImaging Systems Chemi HR 410 camera, UVP, USA) at an excitation wavelength of 365 nm. The UVP parameters were as follows: exposure, 45 s; focus, 4.7; light mode, high; gain, 75 and green 365 nm. The UV-vis absorption spectrum was obtained with a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Japan). Differential pulse voltammetric (DPV) measurements were performed on a CHI 630D electrochemical analyzer (CH Instruments, Inc., USA). The MTT assay was performed using Hitachi/Roche System Cobas 6000 (Tokyo, Japan) at 490 nm.

Cell culture and treatment. BGC-823 cells were kindly provided by Affiliated Zhongda Hospital of Southeast University, Nanjing, China, and cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}), and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO_2. The cell cycle period and the interphase of BGC-823 cells are 41 and 40 h, respectively. At the logarithmic growth phase, the cells were trypsinized and washed twice with culture medium by centrifugation at 1000 rpm for 6 min. The sediment was resuspended in the culture medium to obtain a homogeneous cell suspension. Cell number was determined using a Petroff-Hausser cell counter (USA). Sialidase-treated BGC-823 cells were obtained by incubating the cells in culture medium in the presence of 10 μg mL^{-1} sialidase for 12 h.

Preparation of dual-functionalized nanohorn probe. SWNHs were dispersed in 30% HNO_3 and then refluxed for 24 h at 140 °C to obtain carboxylic group-abundant SWNHs. After centrifugation, the sediment was washed with water until the pH reached 7.0 and then dispersed in ultrapure water (1 mg

mL^{-1}). After adding NHS (2.5 mg) to 0.25 mL of SWNH solution, EDC (1 mg, 150 μL) was quickly added and the mixture was stirred for 1 h at room temperature. Avidin (1 mg mL^{-1}, 500 μL) and ABPA (0.5 mg, 100 μL) were then added. After the mixture was stirred overnight at room temperature, the avidin and ABPA co-conjugated SWNHs were obtained, which were then washed and resuspended in PBS to a concentration of 1 mg mL^{-1}. The solution of avidin and ABPA dual-functionalized nanohorn probe could stably be stored at 4 ^{o}C for at least 6 months.

Chemoselective labeling of cell surface sialic acids (SAs) and electrochemical detection. BGC cells at different concentrations (50 μL) were seeded in the wells of 96-well plates and incubated for 1 h under the same conditions as those for cell culture. After removing the culture solution and washing the adhered cells twice, 50 μL $NaIO_4$ (1 mM) was added to each well at 4 ^{o}C for 30 min, which introduced an aldehyde group at the C-7 position of the SAs on cell surface.[3] After the wells were washed twice with PBS, the cells were incubated with 50 μL BH (0.1 mM, pH 6.7) containing 10 mM aniline at 4 ^{o}C for 1.5 h to produce a biotin tag linked on cell surface SA sites.

After the chemoselectively labeled cells were washed twice with PBS, 30 μL of nanohorn probe (1 mg mL^{-1}) was added in each well and incubated at room temperature for 1 h. The wells were washed twice with PBS, and then 50 μL of mannan-conjugated AuNPs was added to each well. The boronic groups on nanohorn probe bound to cell surface allowed the multiple binding of mannan-conjugated AuNPs. After reaction for 1 h at room temperature and washing the wells twice to remove the free AuNPs, the cells were incubated with 50 μL of fructose solution (3.6 mg mL^{-1}) at room temperature for 40 min to release the mannan-conjugated AuNPs bound by ABPA. The supernatant solution containing the released AuNPs and fructose was pipetted out and mixed with 50 μL 0.1 M HCl. The mixture was then dropped on a screen printed carbon electrodes (SPCE) to perform the electrochemical measurement, which was carried out by electrooxidation of AuNPs at +1.3 V for 40 s, and then cathodic DPV scanning from +0.6 V to 0 V with a step potential of 4 mV, a pulse amplitude of 50 mV and a pulse period of 0.2 s.[4]

Evaluation of average amount of SAs on BGC cell surface. The DPV peak current (i_p), corresponding to the total amount of nanohorn probe (n) or AuNPs captured by cells, was directly related to both the cell

number (a) and the amount of SA sites on cell surface. Thus, the plot of i_p *vs.* the logarithm of BGC cell concentration or cell numbers (a) can be described as follows:

$$i_p = \text{mlog}\,(a) + b_1 \quad (1)$$

In order to obtain the relationship between the i_p and the amount of nanohorn probe, different amounts of the nanohorn probe in 500 μL solution were mixed with excessive amount of mannan-conjugated AuNPs, and reacted at room temperature under continuous stirring for 1 h. The resultant AuNPs-conjugated nanohorn probe was washed with PBS to remove the free AuNPs, and resuspended with 50 μL of fructose solution (3.6 mg mL^{-1}). After reaction at room temperature for 40 min, the released AuNPs from the nanohorn probe by competition with fructose was collected to perform the DPV detection. The i_p showed a linear relationship with the logarithm of the amount of nanohorn probe (n):

$$i_p = \text{mlog}\,(n) + b_2 \quad (2)$$

At the same i_p value, the amount of bound or used nanohorn probe (n) or released AuNPs should be the same. Thus the average number (k) of SAs on each cell surface ($k = n/a$) could be calculated from eqs 1 and 2:

$$k = 10^{\frac{b_1 - b_2}{m}} \quad (3)$$

Dynamic monitoring of SA expression on cell surface. 50 μL BGC cell suspensions (10^6 cells mL^{-1}) were seeded in the wells of 96-well plates and incubated with sialidase (10 μg mL^{-1}) for different times. After carefully washing with PBS twice, the sialidase treated cells were subjected to the chemoselective labeling and electrochemical detection. Considering that the same cell number (a) was used, i_p was directly related to the average number of SAs on sialidase-treated cell (k'), which could be calculated using the i_p value obtained from treated cells and eq. 2:

$$k' = \frac{10^{\frac{i_p - b_2}{m}}}{a} \quad (4)$$

Demonstration of specific binding of BH to aldehyde group by aniline catalysis:

50 μL BGC cell suspensions (10^6 cells mL^{-1}) were seeded at defined locations on a glass slide (the diameter of each spot was 4 mm) and incubated for 1 h under the same conditions as those for cell culture. For panel (a) in Fig S1, the cells were then subjected to the $NaIO_4$ oxidation and BH linkage in the presence of aniline. The panels (b) and (c) in Fig S1 correspond to the absence of $NaIO_4$ oxidation or aniline, respectively. After washing twice with PBS, all the cell spots at the slide were incubated with 50 μL avidin-FITC (0.2 mg mL^{-1}) at room temperature for 1 h and carefully washed twice with PBS to remove the non-tagged avidin-FITC. Finally the slide was taken a photograph with a CCD camera at the excitation wavelength of 365 nm at room temperature and the fluorescent intensity (FI) was obtained by the BioImaging System.

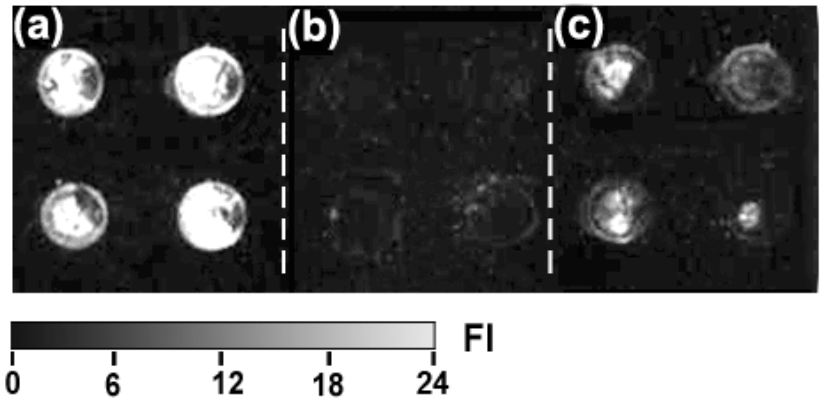

Fig. S1 Fluorescent images of avidin-FITC incubated BGC cells pretreated with (a) $NaIO_4$ and BH (in presence of aniline) successively, (b) BH (in presence of aniline), and (c) $NaIO_4$ and BH (in absence of aniline) successively.

Characterization of the nanohorn probe

In comparison with the SEM image of the oxidized SWNHs (A in Fig. S2a), the SEM image of the APBA and avidin dual-functionalized nanohorn probe (B in Fig. S2a) showed an apparent larger diameter. Compared with the FT-IR spectrum of the oxidized SWNHs (Fig. S2b, curve A), the FT-IR spectrum of the nanohorn probe (Fig. S2b, curve B) displayed the absorption peaks from the vibration of amide I and amide II at 1648 and 1563 cm^{-1}, and the peak from asymmetric B-O stretching at 1409 cm^{-1}. These tests confirmed the successful preparation of the nanoprobe. The image of the nanohorn probe after AuNP attachment was obtained by TEM (Fig. S2c), which showed the homogeneously dispersed AuNPs on the surface of the SWNHs with diameter of 3.5 nm.

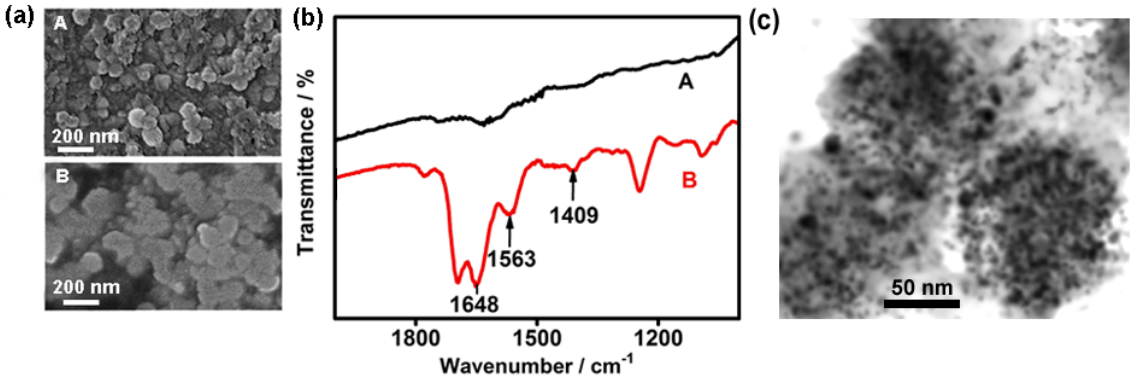

Fig. S2 (a) SEM images of (A) oxidized SWNHs, (B) nanohorn probe, (b) FT-IR spectra of (A) oxidized SWNHs, (B) nanohorn probe, and (c) TEM image of the SWNH probe with attached AuNPs

Characterization of mannan-conjugated AuNPs

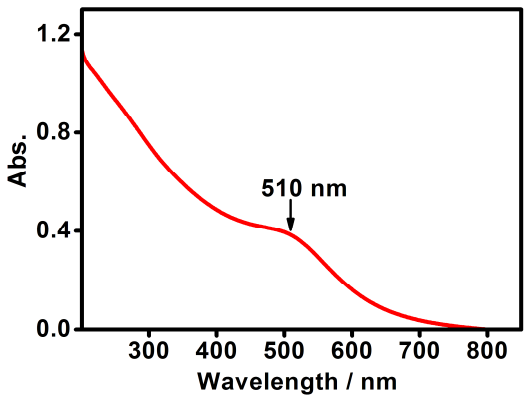

Fig. S3 UV-vis spectrum of the mannan-conjugated AuNPs.

Demonstration of specific binding of nanohorn probe and mannan conjugated AuNPs

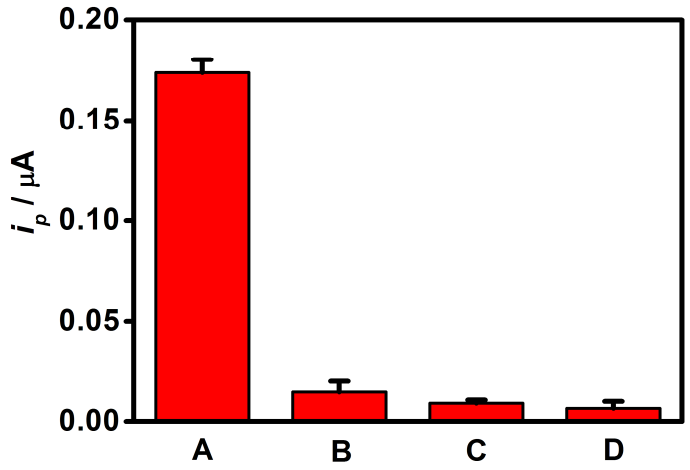

Fig. S4 DPV responses corresponding to (A) BGC cells at 10^6 cells mL^{-1}, (B) sialidase-treated BGC cells at10^6 cells mL^{-1}, (C) no BGC cells and (D) BGC cells at10^6 cells mL^{-1} without the binding of nanohorn probe.

Optimization of nanohorn probe preparation and detection parameters

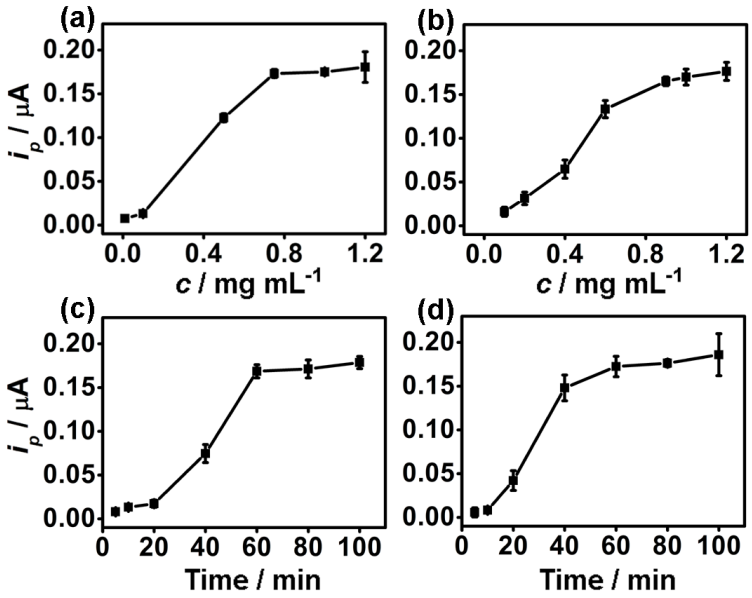

Fig. S5 Dependence of DPV peak current on (a) avidin concentration for preparation of nanohorn probe, (b) nanohorn probe concentration, (c) incubation time with nanohorn probe, and (d) reaction time with mannan-conjugated AuNPs. When one parameter changes, the others are at their optimal conditions.

Viability of BGC cells during the sequential detection procedure

The viability of BGC cells during the sequential detection procedure was analyzed by MTT assay. Briefly, after the BGC cells（100 μL, 1.0×10^4 cells mL^{-1}) were seeded in the wells of 96-well plate for 4 h, the medium was discarded. The cells were then washed by PBS twice and subjected to the sequential processes of cell detection, respectively. Meanwhile, the BGC cells without labeling were incubated with equal culture medium as control. MTT (50 μL, 1 mg mL^{-1}) was then added to each well. After incubation for 4 h at 37 °C, the medium was removed and 100 μL of dimethyl sulphoxide was added to each well and the cell plate was vibrated for 15 min at room temperature to dissolve the crystals formed by the living cells. Finally, the absorbance of each well was measured using Hitachi/Roche System Cobas 6000 (Tokyo, Japan) at 490 nm. The relative cell viability (%) was calculated by $(A_{test}/A_{control})\times100$. As shown in Fig. S6, after treatment of BGC cells with 1 mM $NaIO_4$ for 30 min, the cells exhibited very high viability (column A). The subsequent steps of the treatment by BH, nanohorn probes, AuNPs and fructose did not affect the cell viability significantly (column B, C, D and E). These results demonstrated that the designed method did not affect the viability of living cells.

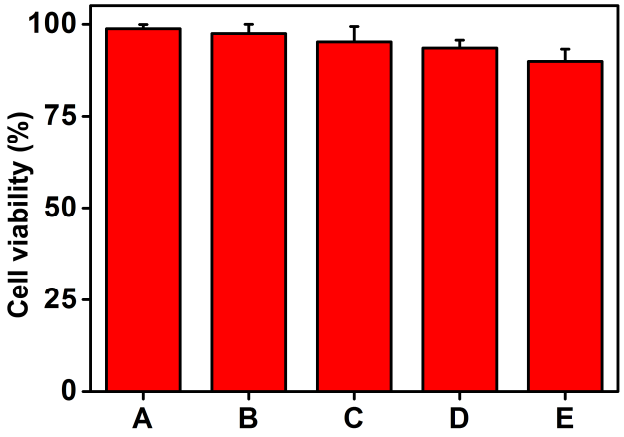

Fig. S6 Viability of BGC cells during the sequential experimental procedure: (A) BGC cells treated with $NaIO_4$, (B) BGC cells treated with $NaIO_4$ and BH, (C) BGC cells treated with $NaIO_4$, BH and nanohorn probe, (D) BGC cells treated with $NaIO_4$, BH, nanohorn probe and AuNPs, and (E) BGC cells treated with $NaIO_4$, BH, nanohorn probe, AuNPs and fructose.

References

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