Electronic Supplementary Information (ESI) for Chemical Communications



# In situ tracing of cell surface sialic acid by chemoselective recognition to unload gold nanocluster probe from density tunable dendrimeric array

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## Experimental

**Materials and reagents.** The solution of generation 5.0 poly(amidoamine) (PAMAM) dendrimer with ethylenediamine core, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), L-glutathione reduced (GSH), bovine serum albumin (BSA), 3-aminophenylboronic acid (APBA), sialidase and N-Acetyl-2,3-dehydro-2-deoxyneuraminic acid (sialidase inhibitor) were purchased from Sigma-Aldrich Inc. (USA). Chloroauric acid ($HAuCl_4 \bullet 4H_2O$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Aldehyde-coated glass slide was obtained from Shanghai BaiO Technology Co., Ltd. (Shanghai, China). 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).

**Apparatus and characterization.** The microarray was spotted by a Biodot AD1500 biochip production system (Biodot, USA). The UV-vis absorption spectra were obtained with a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Japan). The fluorescence spectra were obtained with a RF-5301PC fluorescence spectrometer (Shimadzu, Japan). The transmission electron microscopic (TEM) images were observed under a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). The Zeta potential

measurements were performed with a Nano-Z zeta potential instrument (Malvern, UK). X-ray photoelectron spectroscopic (XPS) measurements were performed with an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with an ultrahigh vacuum generator. The amount of Au element for calculating the concentration of gold nanoclusters (AuNCs) was obtained by Optima 5300DV inductively coupled plasma mass spectrometer (PE, USA). The morphologies of microarray construction at different steps were observed under an Agilent 5500 atomic force microscope (AFM, USA). The cell images were observed by TCS SP5 confocal laser scanning microscope (Leica, Germany). The microarray images were recorded under a TE2000 fluorescence microscope (Nikon, Japan). The scanning parameters of the CCD camera were listed as follows: exposure time, 0.25 s; gain, 8x. The MTT assay was performed using 14 Hitachi/Roche System Cobas 6000 (Tokyo, Japan) at 550 nm.

**Cell culture and treatment.** BGC-823 cells and MTT assay kit were purchasd from KeyGEN Biotech Co., Ltd. (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 $^{o}C$ 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 re-suspended 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 1 UN sialidase for different times. Sialidase inhibitor was used to stop the sialidase reaction by adding into cell medium at 1 mg $mL^{-1}$.

**Preparation of AuNCs.** The AuNCs were prepared by a modified GSH-reduction method:[S1] GSH solution (50 mL, 25 mM) was mixed with $HAuCl_4$ solution (50 mL, 25 mM) at 1:1 molar ratios. The polymeric GS-Au(I) was formed immediately at room temperature. The color of the solution changed from colorless to stable pale yellowish after 2 weeks. The solution was first centrifuged at 21 000 g for 5 min to remove the large granule. The luminescent AuNCs were precipitated out of the solution by adding

ethanol with equal volume into the supernatant. The precipitates were then resuspended in aqueous solution and further purified using centrifugal filter units (10 kD) at 10 000 rpm for 10 min thrice. The final product was then dispersed in PBS buffer.

**Preparation of APBA-AuNC probe.** The solution of AuNCs (0.05 mM) was mixed with APBA (5 mg $mL^{-1}$) and EDC (5 mg $mL^{-1}$) at room tempreture under stirring. After reaction for 4 h, the mixture was purified using centrifugal filter units (10 kD) at 10000 rpm for 10 min thrice. The final product was then dispersed in PBS buffer. The probe concentration was calculated from the Au concentration in the probe obtained by the inductively coupled plasma mass spectrometry and the diameter of probe by TEM.

**Fabrication of density tunable dendrimeric array.** Dendrimer dissolved in ethanol with different concentrations was spotted to an aldehyde coated glass slide by the biochip production system. After reaction at 37 $^{o}$C for 4 h, the slide was washed by ethanol thrice and dried with nitrogen stream. To block the unreacted aldehyde, the slide was subjected to incubate in 10 mg $mL^{-1}$ of BSA solution at 37 $^{o}$C for 30 min. The slide was then washed by water thrice and dried with nitrogen stream.

**Unloading assay.** The dendrimeric array was incubated in 0.1 mM APBA-AuNCs for 10 min, and then washed by water thrice. The obtained probe-adsorbed dendrimeric microarray was incubated with BGC-823 cell suspensions at different concentrations (10 μL for each panel). After shaking at 37 $^{o}$C in a thermostatic shaker for 60 h, the slide was took out and washed with water to remove the cells and probe-conjugated cells. After dried with nitrogen, the slide was placed under a fluorescence microscope to acquire images using CCD camera under excitation of purple light.

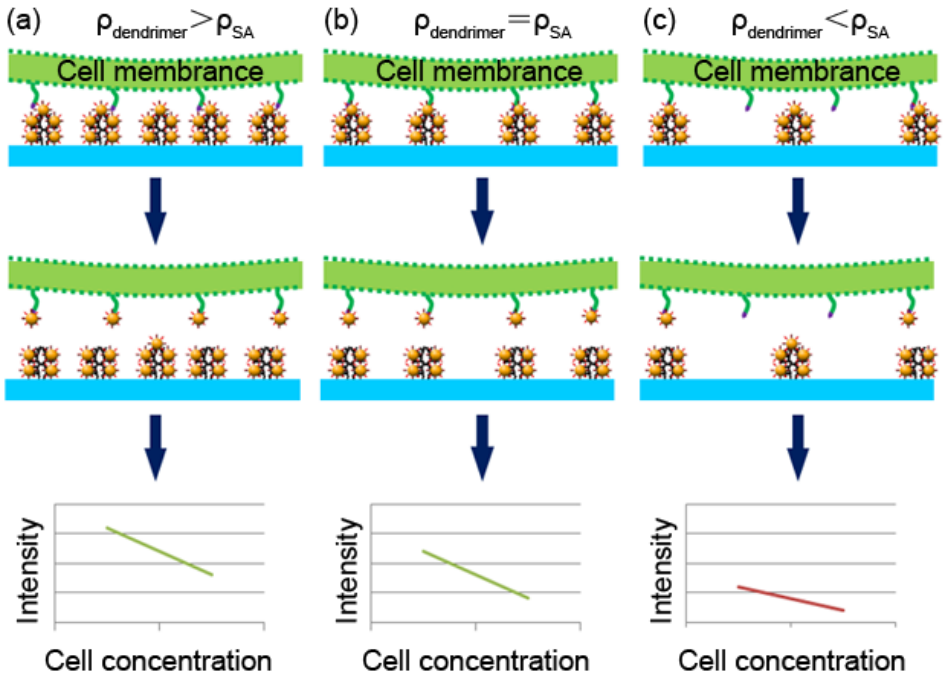
**Monitoring of cell surface SA density.** The BGC-823 cells were incubated in 4 mL PBS containing 50 mUN sialidase for different times under shaking at 37 $^{o}$C in a thermostatic shaker. The cells was then centrifuged at 1000 rpm for 3 min. Afterward, the BGC cells were incubated in 50 μg $mL^{-1}$ sialidase inhibitor for 10 min under shaking at 37 $^{o}$C in a thermostatic shaker to stop the sialidase reaction. Finally,

the cells were washed by centrifugation at 1000 rpm for 3 min thrice and subjected to the same unloading assay.

**Specific binding of APBA-AuNCs on cell surface.**

After 0.1 mg $mL^{-1}$ dendrimer was mixed with 0.01 mM AuNCs or APBA-AuNC probe for 10 min, the mixture was centrifuged at 8000 g for 5 min, and the precipitate was re-dispersed in PBS with the same volume. BGC-823 cells were seeded and cultured in the petri dish for 24 h, which were then washed with PBS twice and subjected to incubation with 1 mL AuNC or APBA-AuNC probe adsorbed dendrimer solution for 1 h at 37 °C. After washing the cells with PBS twice, the petri dish was put under the confocal laser scanning microscope under excitation at 400 nm to acquire the cell fluorescent and bright-field images.

## Mechanism for evaluation of cell surface SA density.




***Scheme S1*** Signal change upon the increasing cell concentration at three kinds of arrays with dendrimer density of (a) greater than, (b) just equal to and (c) smaller than cell surface SA density within the same unloading time.

## Characterization of AuNC and APBA-AuNC.

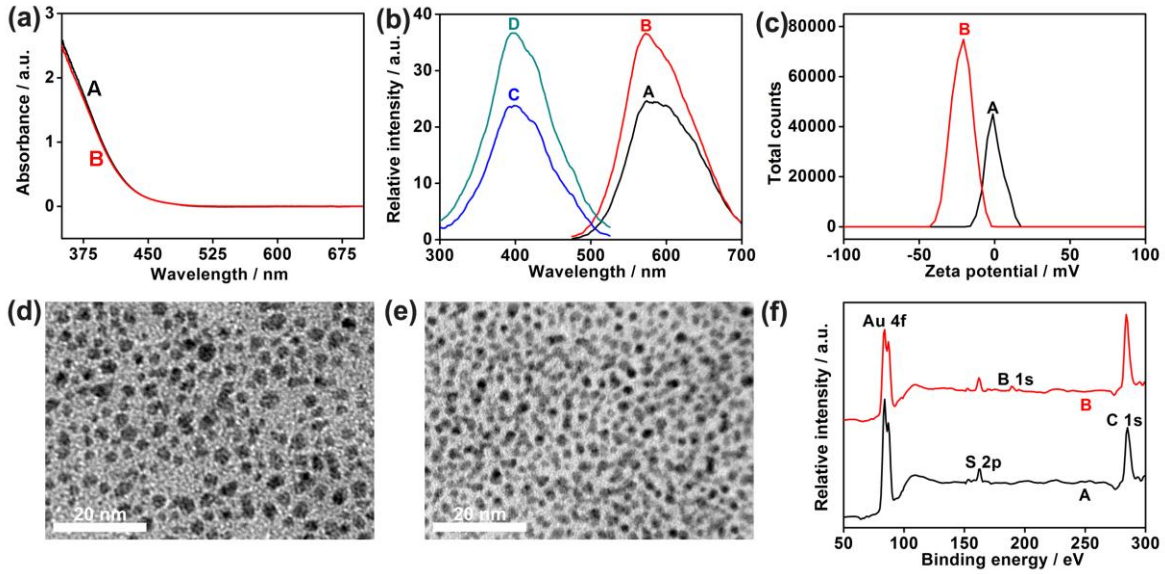



***Fig. S1*** (a) UV-vis spectra, (b) emission (A, B) and excitation (C, D) spectra and (c) zeta potential distribution of AuNCs (A,C) and APBA-AuNC probe (B,D). TEM images of (d) AuNCs and (e) APBA-AuNC probe. (f) XPS spectra of AuNCs (A) and APBA-AuNC probe (B).

## Step-by-step characterization of the probe-adsorbed dendrimer immobilized slides.

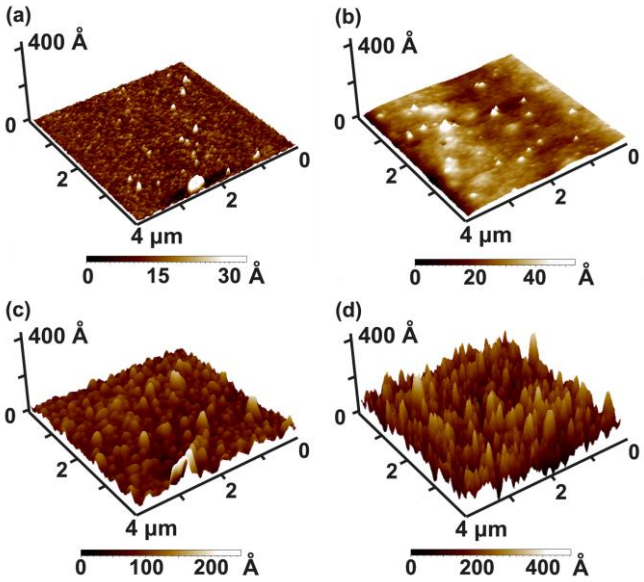



***Fig. S2*** AFM images of (a) aldehyde-coated slide, (b) dendrimer/aldehyde-coated slide, (c) dendrimer/aldehyde-coated slide blocked with BSA, and (d) APBA-AuNC incubated slide (c).

## Specific recognition of gold nanocluster probe to cell surface SAs

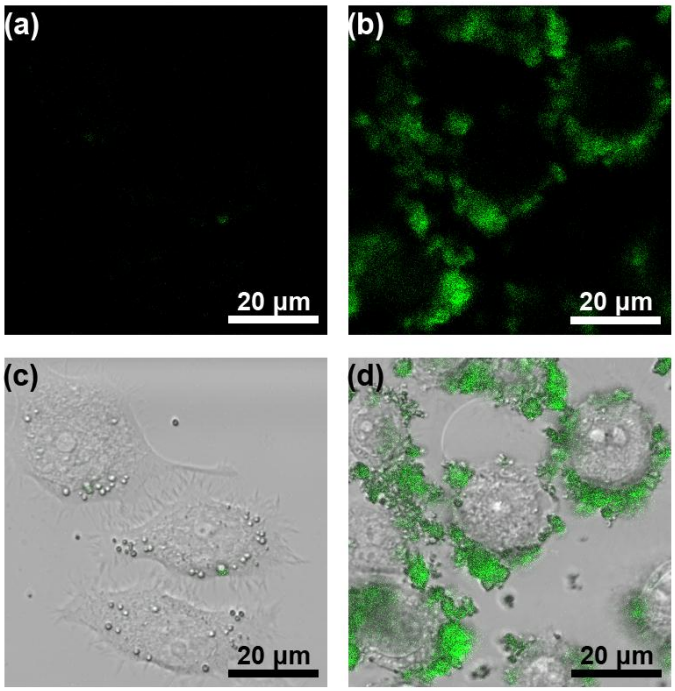



***Fig. S3*** Confocal fluorescence (a and b), and fluorescence and bright-field overlapped images (c and d) of BGC cells after treatment with AuNC adsorbed dendrimer (a and c) and APBA-AuNC probe adsorbed dendrimer (b and d).

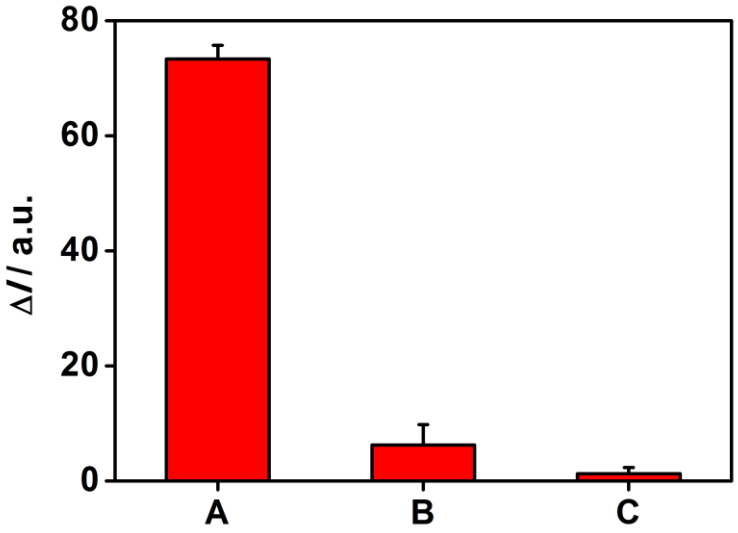



***Fig. S4*** Decreases of signal ($\Delta I$) after unloading with (A) $10^7$ cells $mL^{-1}$ BGC cells, (B) $10^7$ cells $mL^{-1}$ BGC cells after sialidase treatment for 4 h, and (C) PBS.

## Optimization of detection parameters.

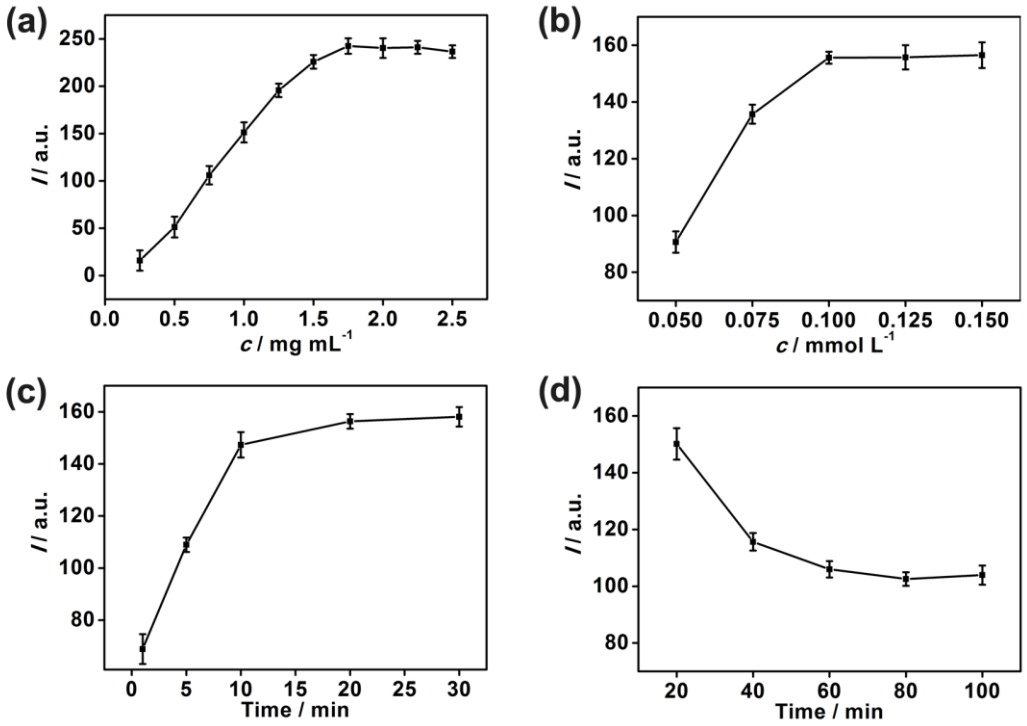



***Fig. S5*** Dependence of signal *I* on (a) dendrimer concentration for array preparation, (b) probe concentration and (c) adsorption time on dendrimer slide before unloading, and (d) unloading time by $10^6$ cells $mL^{-1}$ cells at array prepared with 1.25 mg $mL^{-1}$ dendrimer.

## Viability of BGC cells during unloading assay

The viability of BGC cells during the assay was tested by MTT assay. Briefly, after the BGC cells (100 μL, $1.0\times10^5$ cells $mL^{-1}$) were seeded in the wells of 96-well plate for 12 h, they were washed with PBS twice and subjected to incubation with different components used in unloading assay, respectively. Meanwhile, the BGC cells without treatment were incubated in culture medium as control. After these cells were incubated with MTT (50 μL, 1 mg $mL^{-1}$) for 4 h at 37 °C, 100 μL of dimethyl sulphoxide was added to each well with vibration 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 550 nm. The relative cell viability (%) was calculated by $(A_{test}/A_{control})\times100$. As shown in Fig. S6, these components did not affect the cell viability significantly.

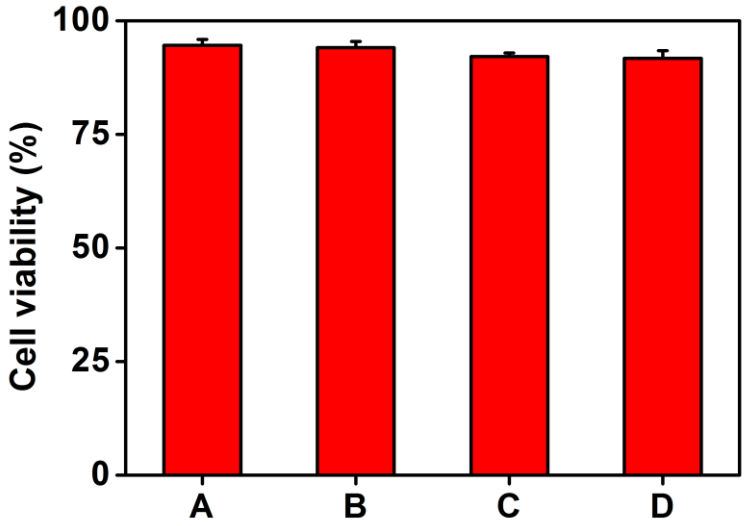



***Fig. S6*** Viability of BGC cells after incubation with (A) 0.01 mM AuNCs, (B) 0.01 mM APBA-AuNC probe, (C) 0.1 mg $mL^{-1}$ dendrimer, and (D) probe (0.01 mM) adsorbed dendrimer (0.1 mg $mL^{-1}$) for 1 h.

## References

(S1) C. Zhou, C. Sun, M. X. Yu, Y. P. Qin, J. G. Wang, M. Kim and J. Zheng, *J. Phys. Chem. C*, 2010, **114**, 7727–7732.