Electronic Supplementary Information

Rolling “Wool-Balls” : Rapid Live-Cell Mapping of Membrane Sialic Acids via Poly $p$-Benzoquinone/Ethylenediamine Nanoclusters

Bin-Bin Chen, Xiao-Yuan Wang and Ruo-Can Qian*

East China University of Science and Technology
E-mail: ruocanqian@ecust.edu.cn
Supplementary Methods
Materials and reagents, Apparatus, Preparation of nanoclusters, Cell culture, Imaging of sialic acids (SAs) in living cells, Imaging of sialic acids (SAs) in HeLa cells treated with sialidase, Information extraction from the images.

Supplementary Figure S1
HRTEM image of nanoclusters.

Supplementary Figure S2
FL spectra of the wool-ball solution under different excitation wavelengths.

Supplementary Figure S3
FT-IR spectrum of nanoclusters.

Supplementary Figure S4
XPS spectra of nanoclusters. The (a) XPS spectrum and corresponding (b) C 1s, (c) N 1s and (d) O 1s spectra of nanoclusters.

Supplementary Figure S5
Plot of integrated FL intensity vs. absorbance of quinine sulfate and nanoclusters.

Supplementary Figure S6
The stability investigation in different PBS buffer solutions.

Supplementary Figure S7
The treatment of 2D pseudo-color and 3D images by Matlab software.

Supplementary Figure S8
The estimation of curvature radius of HeLa cells by Matlab.
Bright field and 3D images of HeLa cells treated with nanoclusters. (a) Bright field and (b) 3D images of HeLa cells treated with nanoclusters (scale bar: 20 μm).

**Supplementary Figure S10**

Bright field and FL images of untreated HeLa cells. (a) Bright field, (b) FL, (c) pseudo-color, and (d) 3D images of untreated HeLa cells (scale bar: 20 μm).
Supplementary Methods

Materials and reagents. Hydroquinone and hydrogen peroxide (H₂O₂) were commercially available from Shanghai Lingfeng Chemical Reagent Co., Ltd. Ethylenediamine (EDA) was purchased from Aladdin Reagent Co., Ltd (Shanghai, China). All solutions were prepared using 18 MΩ.cm ultrapure water (EMD Millipore, TONDINO, Shanghai). HeLa cervical cancer cells, MCF-7 breast cancer cells and RAW264.7 macrophage cells were obtained from Shanghai Moxi Boil Co., Ltd.

Apparatus. A JEM-2100 high-resolution transmission electron microscope (JEOL, Japan) was used for the morphology characterization of nanoclusters. A USB 2000+ spectrometer (Ocean Optics Inc., USA) was used for UV-Vis characterization. The fluorescence (FL) images were obtained by the same microscope using a mercury lamp as the light source (100 W Epi illuminator). The element composition of nanoclusters was measured with an ESCALAB 250 X-ray photoelectron spectrometer. The FT-IR spectrum of nanoclusters was collected on a Hitachi FTIR-8400S Fourier Transform Infrared spectrometer (Tokyo, Japan).

Preparation of nanoclusters. Nanoclusters were prepared with a major revision as previously reported. Briefly, 100 mg hydroquinone was dissolved in 1.75 mL water, and then 2.5 mL H₂O₂ (20 %) was added into the solution. The nanoclusters can be formed within 30 min after the addition of 0.75 mL EDA at room temperature. Through a cellulose ester dialysis membrane (100-500 MWCO) over night, the large carbon quantum particles were retained in dialysis bag, while the ultra-small nanoclusters were exuded from the dialysis bag. Then, we collected the nanoclusters solution outside dialysis membrane and obtained nanoclusters powder by lyophilization.

Cell culture. HeLa cells were cultured in DMEM (Gibco) with 10 % fetal bovine serum (FBS, Sigma), streptomycin (100 μg/mL), and penicillin (100 μg/mL). MCF-7 and RAW264.7 cells were cultured by RPMI-1640 (Gibco) with FBS (10 %, Sigma), streptomycin (100 μg/mL), and penicillin (100 μg/mL). The culture dishes were placed in a humid atmosphere at 37 ºC with 5 % CO₂.

Imaging of sialic acids (SAs) in living cells. HeLa cells in DEME supplemented with 10 % FBS were added to culture dishes. Then cells were cultured for 24 h in an incubator (37 ºC, 5 % CO₂). After 24 h incubated, the culture medium was replaced with 5 mL DEME containing 2 mM NaIO₄ at 37 ºC for 30 min, then rinsed with PBS buffer three times. Next, 2.5 mL DEME containing 40 μg/mL nanoclusters were added to culture dishes. The cells were cultured for 30 min in an incubator (37 ºC, 5 % CO₂), and then rinsed with PBS buffer three times, and transferred for FL imaging.

Imaging of sialic acids (SAs) in HeLa cells treated with sialidase. HeLa cells in DEME supplemented with 10 % FBS were added to culture dishes. Then cells were cultured for 24 h in an
incubator (37 °C, 5 % CO₂). After 24 h incubated, the culture medium was replaced with 3 mL DEME containing different concentrations of sialidase (1 μg/mL, 10 μg/mL and 100 μg/mL) at 37 °C for 4 h, then rinsed with PBS buffer three times. Next, the culture medium was replaced with 5 mL DEME containing 2 mM NaIO₄ at 37 °C for 30 min, then rinsed with PBS buffer three times. Then, 2.5 mL DEME containing 40 μg/mL nanoclusters were added to culture dishes. The cells were cultured for 30 min in an incubator (37 °C, 5 % CO₂), and then rinsed with PBS buffer three times, and transferred for FL imaging.

**Information extraction from the images.** From the FL images, the green channel intensity of the nanoclusters was analyzed using Adobe Photoshop software. Matlab was used for the extraction of 2D pseudo-color of green channel intensity and 3D color-coded green channel intensity corresponding to HeLa cells. Moreover, Matlab was also applied for the estimation of curvature radius of HeLa cells.
Supplementary Note 1. HRTEM image of nanoclusters.

Figure S1. HRTEM image of nanoclusters.
Supplementary Note 2. FL spectra of nanoclusters.

Figure S2. FL spectra of the wool-ball solution under different excitation wavelengths.
Supplementary Note 3. FT-IR spectrum of nanoclusters

FT-IR spectrum of nanoclusters was collected through Fourier Transform Infrared spectrometer. The FT-IR spectrum showed characteristic absorption bands of N-H stretching vibration at 3437 cm\(^{-1}\), C-H stretching vibrations at 2924 cm\(^{-1}\) and 2863 cm\(^{-1}\), C=N stretching vibration at 1631 cm\(^{-1}\), N-H bending vibration at 1554 cm\(^{-1}\), C-N stretching vibration at 1377 cm\(^{-1}\) and C-O stretching vibration at 1106 cm\(^{-1}\), respectively.\(^1\),\(^2\)

Figure S3. FT-IR spectrum of nanoclusters.
Supplementary Note 4. XPS spectra of nanoclusters

The XPS spectra of nanoclusters were measured through an X-ray photoelectron spectroscopy. XPS analysis showed that the nanoclusters were composed of carbon, nitrogen, and oxygen elements and the C 1s, N 1s and O 1s peaks were located about at 284.8, 399 and 531.8 eV, respectively (Figure S4a). Moreover, the atomic percentages of carbon, nitrogen and oxygen were 64.77 / 13.68 / 21.55. The C 1s spectrum (Figure S4b) showed three peaks at 284.5, 285.5 and 287.2 eV, which was ascribed to the C-C, C-N, and C=N/C=O bonds, respectively.\(^1,3\) The N 1s spectrum (Figure S4c) showed one peak at 399.1 eV, which was due to the C-N group.\(^1\) The two peaks at 531.4 and 532.1 eV in the O 1s spectrum (Figure S4d) could be attributed to the C=O and C-OH/C-O-C groups, respectively.\(^1,3\)

Figure S4. XPS spectra of nanoclusters. (a) XPS spectrum and corresponding (b) C 1s, (c) N 1s and (d) O 1s spectra of nanoclusters.
Supplementary Note 5. Plot of integrated FL intensity vs. absorbance of the Quinine sulfate and nanoclusters.

The quantum yield ($\Phi$) of nanoclusters was calculated using quinine sulfate as a reference. Quinine sulfate ($\Phi = 0.55$) was dissolved in 0.1 M H$_2$SO$_4$ (refractive index ($\eta$) = 1.33) while the nanoclusters were dissolved in ultra-pure water ($\eta = 1.33$). Then the quantum yield of nanoclusters was calculated by comparing the integrated FL intensities (excited at 370 nm) and the absorbance values (at 400 nm) of nanoclusters with quinine sulfate. As shown in Figure S5, the FL intensity vs. absorbance of nanoclusters showed good linearity. The quantum yield was calculated using the following equation:

$$\Phi_X = \Phi_{ST} \left( \frac{m_X}{m_{ST}} \right) \left( \frac{\eta_X^2}{\eta_{ST}^2} \right)$$

Where $\Phi$ is the quantum yield, $m$ is slope, $\eta$ is the refractive index of the solvent, ST is the standard and $X$ is the sample. The quantum yield for nanoclusters is determined to be 12%.

Figure S5. Plot of integrated FL intensity vs. absorbance of quinine sulfate and nanoclusters.
Supplementary Note 6. The stability investigation in different PBS buffer solutions

The stability of synthesized nanoclusters was tested under various pH values. The concentrations of PBS and nanoclusters were 10 mM and 1 µg/mL, respectively. Result showed that the FL of nanoclusters hardly change in the pH range from 5.0 to 8.0.

**Figure S6.** The stability investigation in different PBS buffer solutions.

![Graph showing FL intensity vs pH](image_url)
Supplementary Note 7. Treatment of 2D pseudo-color and 3D images using Matlab software

The 2D pseudo-color and 3D color-coded green channel intensity corresponding to HeLa cells could be obtained by Matlab software. The codes (left column) and corresponding comments (right column) were as follows:

```
I = imread('D:\files name');        %read image
J = imresize(I,[100 100]);         %change the image size to 100*100 pixels
g = J(:,:,2);                       %extract green channel
contour(g)                          %contour map
colormap(jet)                       %fill the contour map with color
colorbar                            %add color bar
figure(2),surf(g)                   %3D surface map
shading interp
```

**Figure S7.** The treatment of 2D pseudo-color and 3D images by Matlab software.
**Supplementary Note 8. The estimation of curvature radius of HeLa cells by Matlab**

The curvature radius of HeLa cells could be estimated by Matlab software. Briefly, we firstly selected a fluorescent photograph (**Figure S8a**) of single HeLa cell as a sample for estimating curvature radius. Then, we draw a circle roughly (**Figure S8b**) matching the outline of the cell in **Figure S8a**, and read the grayscale matrix of the image by Matlab. Extracted points greater than 0, and draw a scatter plot with the indexes of the chosen points. Fitted data points with ellipses, so its curvature formula was obtained (**Figure S8c**). And the elliptic equation:

\[-0.001738x^2 - 0.001619xy + 1.404x - 0.001083y^2 + y - 309.0 = 0\]

Next, the four vertices (**Figure S8d**) of the ellipse were estimated on the fitted curve, and the corresponding values were obtained using the curvature formula. The equation for calculating curvature:

\[k = \frac{|((0.001619(0.003477x + 0.001619y-1.404))/((0.001619x+0.002166y-1.0)^2 - 0.003477/(0.001619x + 0.002166y - 1.0) - (1.0 ((0.002166 (0.003477x + 0.001619y - 1.404))/(0.001619x + 0.002166y - 1.0)^2 - 0.001619/(0.001619x + 0.002166y-1.0)) (0.003477x+0.001619y - 1.404))/(0.001619x + 0.002166y - 1.0))/((1.0 (0.003477x + 0.001619y - 1.404) ^2)/((0.001619x + 0.002166y - 1.0) ^2 + 1.0) ^{(3/2)})|}

Finally, the equation for curvature radius: \(\rho = 1/k\)

The codes of curvature and curvature radius were shown in **Figure S8e** and **Figure S8f**, respectively.

![Images](image-url)
Figure S8. The estimation of curvature radius of HeLa cells by Matlab.
Supplementary Note 9. Bright field and 3D images of HeLa cells treated with nanoclusters

Figure S9. Bright field and 3D images of HeLa cells treated with nanoclusters. (a) Bright field and (b) 3D images of HeLa cells treated with nanoclusters (scale bar: 20 μm).
Supplementary Note 10. Bright field and FL images of untreated HeLa cells

Figure S10. Bright field and FL images of untreated HeLa cells. (a) Bright field, (b) FL, (c) pseudo-color, and (d) 3D images of untreated HeLa cells (scale bar: 20 μm).
References

