Electronic Supplementary Information

Rituximab-Gold Nanoprobes for Simultaneous Dark-field Imaging and DAB Staining of CD20 Over Expression on Raji Cell

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Experimental Details:

Materials

Reagents used in the synthesis are all standard procedures and commercially available. Potassium carbonate, disodium hydrogen phosphate, potassium chloride, Tween-20 and paraformalclehyde (Lingfeng, Shanghai). Phosphotungstic acid. sodium chloride and ethanol (Sinopharm, Shanghai). Hydrogen peroxide (Aladdin, Shanghai). PEG-20000 (Rongyuan, Taiyuan). Potassium dihydrogen phosphate (Xilong, Shantou). Bovine serum albumin (Biosharp, Korea). Human IgG (Boster, Wuhan). Rituximab Injection (Roche, Switzerland). DAB enzyme substrate color development kit (DAB-0031)(20*) (Maxim, Fuzhou). All the chemicals mentioned above were used as-received without any further purification.

Synthesis and characterization of Rituximab-Au nanoprobes

Synthesis of Rituximab-Au nanoprobes

Tri-sodium citrate—tannic acid protocol was used for preparing gold nanoparticles. The concentration of the gold nanoparticles was adjusted to 289.5μg/ml by centrifugation and resuspension, with OD value of 4.3. The pH of 1ml gold nanoparticle colloidal solution was adjusted to approximately 9.5 with 40μl of 0.1M K_2CO_3 aqueous solution. 78μg Rituximab was added into the gold nanoparticle colloidal solution and the obtained mixture was incubated for 5min. The mixture was then centrifuged to remove unbound antibodies at 12000 rpm for 40 min at 4℃. Deposition was resuspended with 1% BSA aqueous solution (pH has been adjusted to 9.5) to obtain purified Rituximab-Au nanoprobes. The Rituximab-Au nanoprobes were stored at 4℃.

TEM, UV-vis spectroscopy and DLS measurements

Gold nanoparticles and the corresponding Rituximab-Au nanoprobes were detected by transmission electron microscopy (TEM, JEOL JEM-2100, Japan)，UV-vis spectroscopy (UV-3600, Shimadzu, Japan) and dynamic light scattering (DLS) device (Brookhaven-Zetaplus, Brookhaven, UK).

According to the TEM images, the average diameter of gold nanoparticles was about 10.0 nm and remained unchanged almost when coated with antibody and BSA (Fig. S1). In the UV-vis absorption spectra, the maximum absorbance peak of the Rituximab-Au nanoprobes red-shifts, and the peak intensity reduces compared with gold nanoparticles, attributed to the alteration of dielectric environment around gold nanoparticles (Fig. S2). The average hydrodynamic size of gold nanoparticles was about 16.7 nm and for the Rituximab-Au nanoprobes it was about 63.6 nm (Fig. S3). The coating of antibodies and BSA led to the increase of hydration layer thickness.

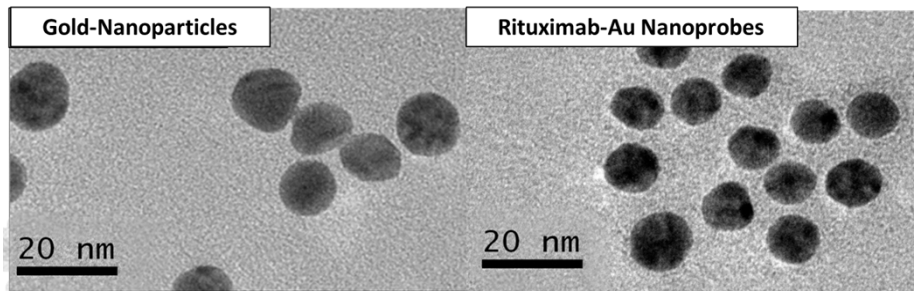


Fig. S1 TEM images of gold nanoparticles and the corresponding Rituximab-Au nanoprobes.

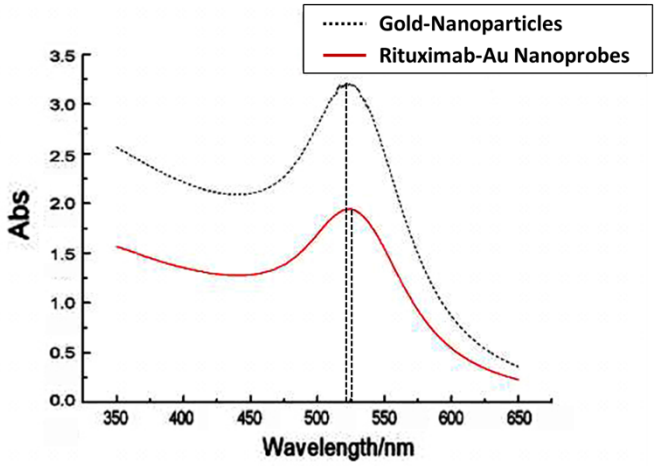


Fig. S2 The UV-vis spectra of gold nanoparticles and the corresponding Rituximab-Au nanoprobes.

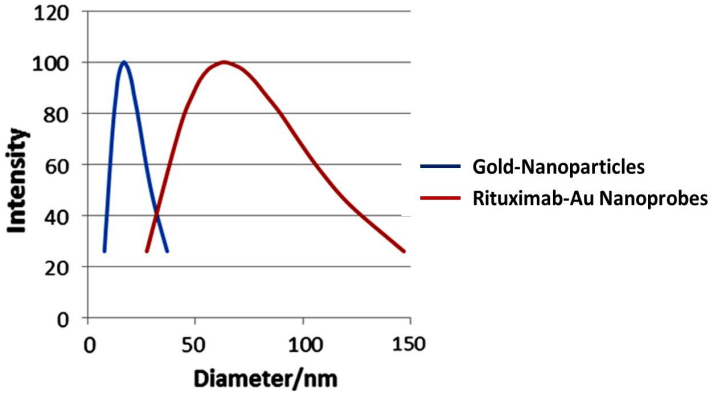


Fig. S3 The hydrodynamic size of gold nanoparticles and the corresponding Rituximab-Au nanoprobes.

BCA Protein Quantitation Kit tests

After purified with centrifuge, Rituximab-Au nanoprobes without BSA blocking were deposited at the bottom of centrifuge tubes while unbound antibodies were left in the supernatant. BCA kit was used to figure out the percent of unbound antibodies.

Percent of Antibodies Bound to Gold Nanoparticles = [1－Amount of Antibodies in Supernatant / (Antibodies in Supernatant ＋ Antibodies bound to Gold Nanoparticles)]×100%

Number of Antibodies on a Single Gold Nanoparticle = Number of Antibodies conjugated to gold nanoparticles / Number of Gold Nanoparticles

According to the calculation results, the percent of antibodies bound to gold nanoparticles was 57.6% and the average number of antibodies on a single gold nanoparticle was 7.5.

Processing Method of Light Scattering Images

In order to reasonably compare the dark-field images of different control groups, light scattering images of cells were adjusted to a uniform background color by using an image manipulation software of Photoshop 8.0.1. Images were first desaturated to black and white and then set to a uniform color background without changing the contrast. As a result, the scattering intensity of the nanoprobes on the surfaces of cells becomes easy to compare (Fig. S4).

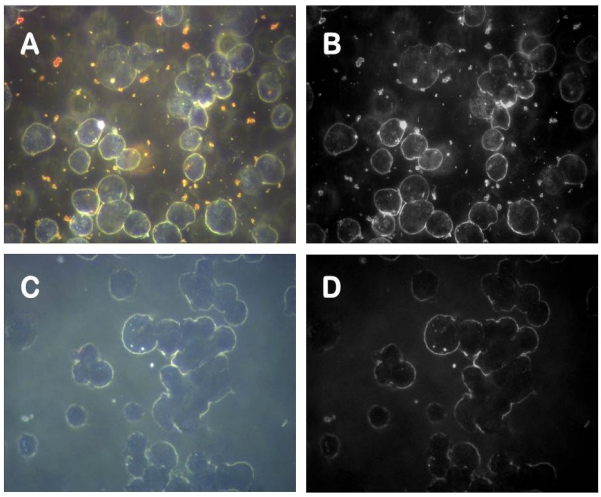


Fig. S4 (A, C) The original dark-field images obtained directly from a libratory microscope. (B, D) The processed image relative to a and c respectively. As the original dark-field images obtained from a libratory microscope were affected heavily by the environment and hard to be compared directly, a uniform color background for each light scattering image was set by using an image manipulation software of Photoshop 8.0.1.

CD20 labeling with gold nanoprobes, dark-field imaging and DAB staining

Every Raji cell smear of the experimental group was incubated with 100 μl Rituximab-Au nanoprobes (OD=4.3) at 37℃ for 1 hour and then washed with 1% PBS-T for three times, 5min per time. Pictures of the smears were taken using an optical microscope with light and dark-field mode (ECLIPSE E-200, Nikon, Japan). Generally, the light scattering images were firstly observed under the dark-field mode. After that, a 200μl chromogenic agent containing 3, 3'-diaminobenzidin (DAB) and hydrogen dioxide (H_2O_2) was incubated with the smear for 3min and then the smears were washed and detected under the bright-field mode. Here gold nanoparticles were used as mimetic peroxidase to catalyze color action of DAB in the presence of H_2O_2.

Immunochemically stained Raji cell smears were observed under both the bright-filed and dark-field mode. The bright-field image showed the similar state of Raji cells on different slides under bright-field (Fig. S5). The gold nanoparticles group displayed red because of the non-specific deposition of gold nanoparticles in great quantities while cells of other groups (The experimental group, the competitive inhibition group, the unrelated cells group, the unrelated nanoprobes group and the blank group) were intact, clear and transparent, which proves that the bright light-scattering cell circles observed in the dark-field was unrelated to the cell status, but due to the dark-field light scattering from gold nanoparticles bound to the surface of cells.

Cells of the competitive inhibition group were blocked with 100 times amount of Rituximab. Cells of the unrelated cells group were replaced with K562. Antibodies of the unrelated nanoprobes group were replaced with human IgG. Cells of these three groups were not expected to be labeled with nanoprobes.

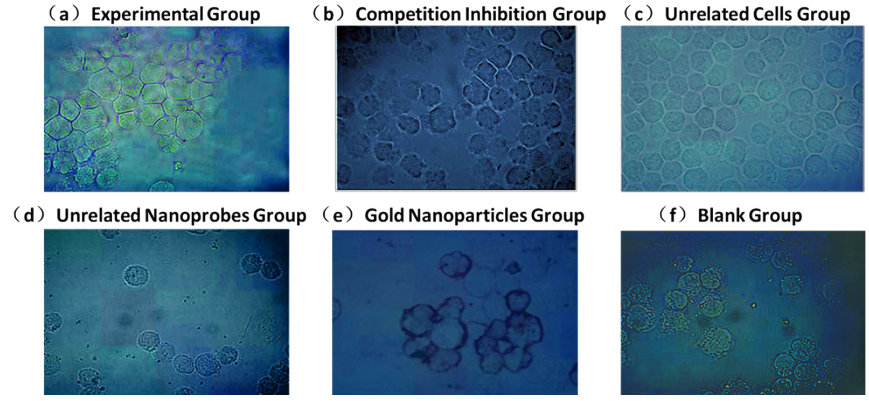
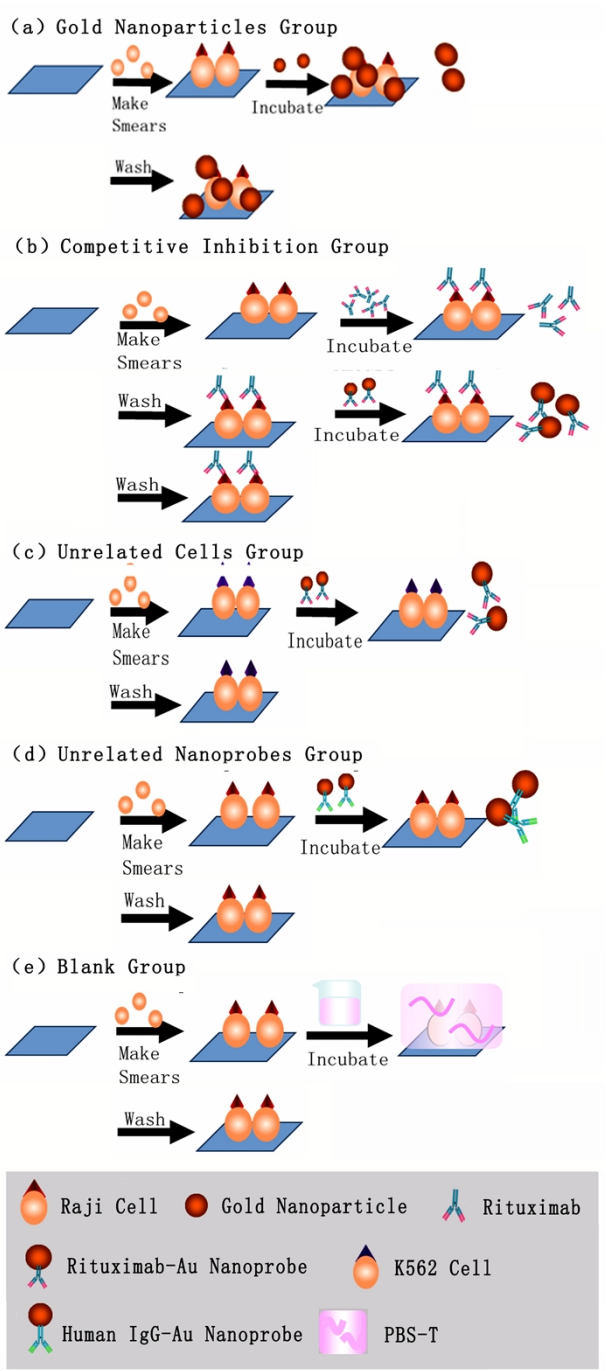


Fig. S5 The bright-field image of Raji cells after labeled by the Rituximab-Au nanoprobes for the experimental group (a). The bright-field images for other five control groups (b-f).



Scheme S1 The principle of other control groups：(a) the gold nanoparticles group; (b) the competitive inhibition group; (c) the unrelated cells group; (d) the unrelated nanoprobes group; (e) the blank group.

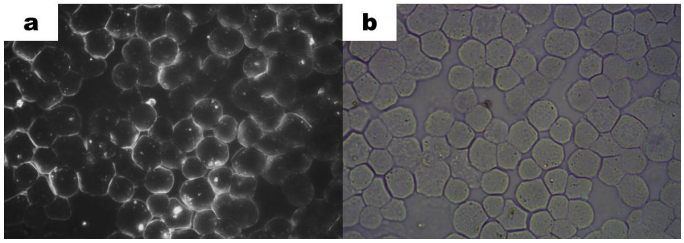


Fig. S6 Dark-field (a) and bright field (b) image of Raji cells after labelled by the Rituximab-Au nanoprobes.

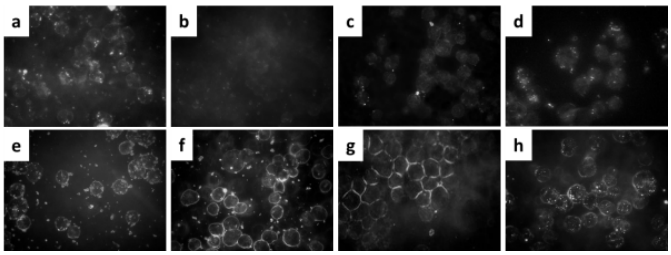


Fig. S7 Dark-field images of different methods of decreasing non-specific binding. a. Au=289.5 μg/ml, incubated 60 min, without BSA&PEG; b. Au=289.5 μg/ml, incubated 15 min, with BSA&PEG; c. Au=289.5 μg/ml，incubated 30 min, with BSA&PEG; d. Au=57.9 μg/ml, incubated 60 min, with BSA&PEG; e. Au=115.8 μg/ml，incubated 60 min，BSA&PEG; f. Au=289.5 μg/ml, incubated 60 min, with BSA&PEG; g. Au=289.5 μg/ml, incubated 60min, with BSA&PEG, short-time low-speed centrifugation; h. Au=289.5 μg/ml，incubated 60min, with BSA&PEG, dipped with PBS-T.

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