

Lactose-Functionalized Gold Nanorods for Sensitive and Rapid Serological Diagnosis of Cancer

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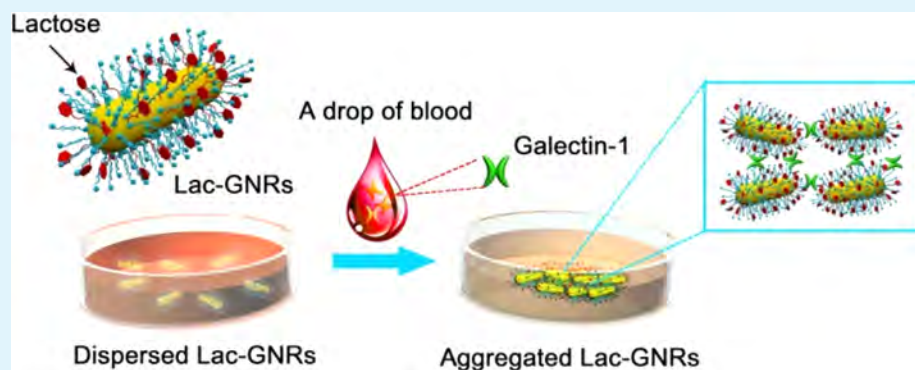
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Supporting Information



ABSTRACT: Timely and accurate diagnosis of cancer is crucial to cancer treatment. However, serological diagnosis of cancer still faces great challenge because the conventional methodology based on the enzyme-linked immune sorbent assay (ELISA) is costly, time-consuming, and complicated, involving multiple steps. Herein, lactose-functionalized gold nanorods (Lac-GNRs) are fabricated as efficient biosensors to detect cancerous conditions based on the unique surface plasmon resonance properties of GNRs and high specificity of lactose to the galectin-1 cancer biomarker. A trace concentration of galectin-1 as small as 10^{-13} M can be detected by Lac-GNRs. The comparative study among BSA, galectin-3, and galectin-1 demonstrates the good specificity of Lac-GNRs to galectin-1 either in aqueous solutions or in the complex and heterogeneous serum specimens. Clinical tests show that the Lac-GNRs biosensors can readily distinguish the serums of cancer patients from those of healthy persons simply by using a microplate reader or even direct visual observation. The Lac-GNRs biosensing platform is highly efficient and easy to use and have great potential in rapid screening of cancer patients.

KEYWORDS: gold nanorods, lactose-surface-functionalization, surface plasmon resonance, galectin-1, serological diagnosis

1. INTRODUCTION

Cancer is caused by abnormal cell growth, and according to the statistics provided by the World Health Organization (WHO) in 2014, cancer is a major public health problem with 14.1 million new cases and 8.2 million deaths.¹ It is generally accepted that early diagnosis and treatment increase the chance of recovery greatly, and timely and accurate diagnostic techniques are urgently needed. Cancerous tissues are different from normal ones in that they produce some specific biomarkers during tumor generation, development, and metastasis.² Because some of these cancer biomarkers are released into the blood of patients,³ serological detection of cancer biomarkers with ultralow sensitivity can be a rapid,

efficient, and noninvasive cancer screening technique.^{4,5} Although the enzyme-linked immune sorbent assay (ELISA) is commonly used in serological detection of cancer biomarkers, it suffers from shortcomings such as multiple steps, high cost, and time-consuming.⁶

On the heels of the rapid development of nanotechnology, various nanoparticles with unique properties have aroused interests in diagnosis.^{7–13} For instance, noble metal nanoparticles especially gold nanorods (GNRs) are excellent

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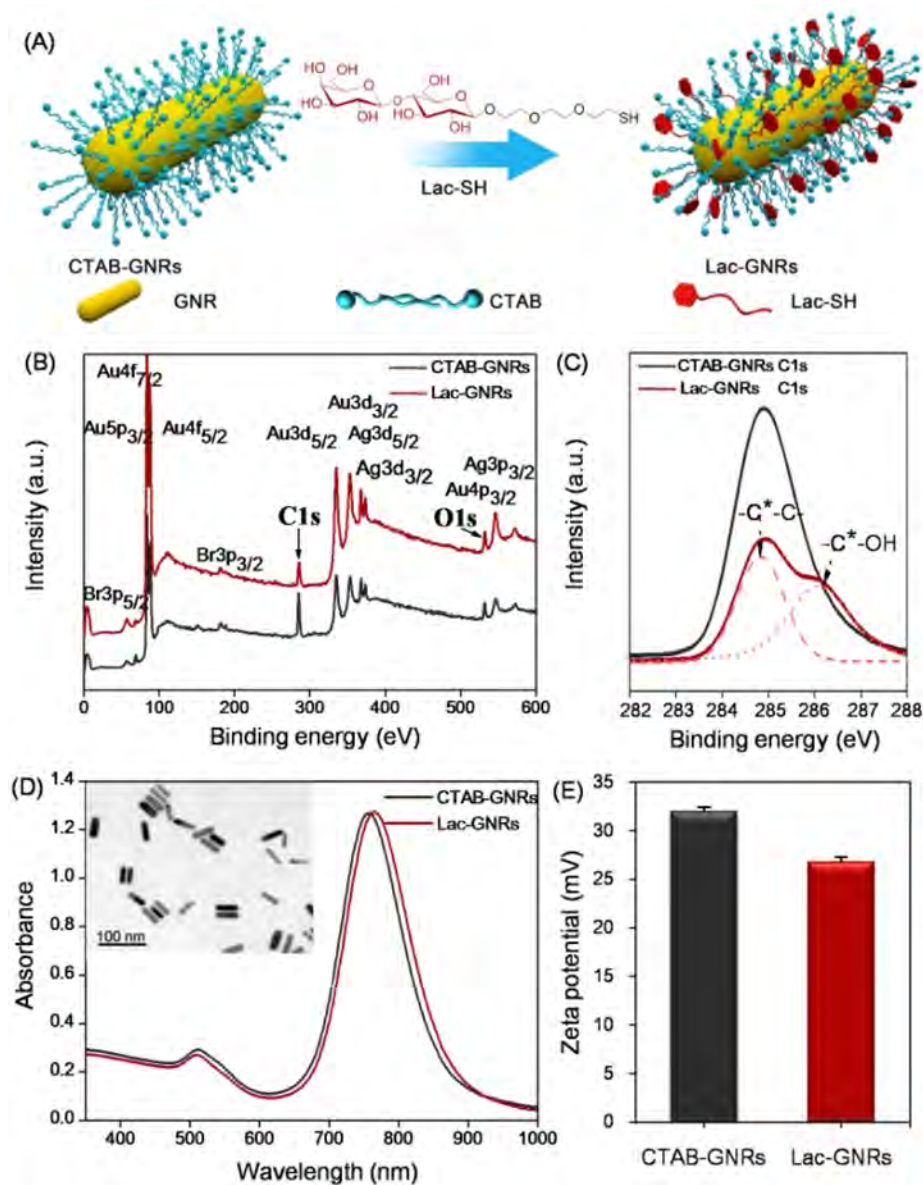


Figure 1. (A) Schematic illustration of the synthesis of Lac-GNRs. Characterization of CTAB-GNRs and Lac-GNRs: (B) Survey XPS spectra, (C) high-resolution C 1s spectra, (D) absorption spectra with an inset TEM image of Lac-GNRs, and (E) Zeta potential.

building blocks for biosensors due to their surface plasmon resonance (SPR) shifts in biorecognition events.¹⁴ GNRs have a unique longitudinal SPR (LSPR) band in the near-infrared (NIR) region,^{15–20} and their extinction coefficient is much larger than that of spherical gold nanoparticles and several orders of magnitude larger than those of traditional organic chromophores.²¹ Moreover, slight environmental variations around the GNRs can induce significant changes in the LSPR peak which has been demonstrated to be more sensitive than the transverse SPR (TSPR) peak of spherical gold nanoparticles.²² Therefore, GNRs have been suggested as efficient biosensors to detect specific targets and events including cancer biomarkers.^{23–33} Despite recent progress, there have been few in-depth studies about GNR-based biosensors in the clinical detection of cancer biomarkers³⁴ probably due to the uncontrollable SPR response in the physiological environment and rigorous instrumental requirement. As a result, a reliable SPR-based platform for practical cancer diagnosis is still not available.

As a cancer biomarker, galectin-1 plays a pivotal role in the multiple stages of tumor development including tumor invasion, metastasis, immune-escaping, secondary growth, and angiogenesis.^{35,36} Fundamental and clinical studies have indicated that galectin-1 is overexpressed in different stages of various types of cancer^{37–42} and even released into the patient's blood.^{37,42} Consequently, galectin-1 is a desirable biomarker for cancer screening and the development of a convenient and sensitive method based on rapid serological detection of galectin-1 is very attractive albeit challenging. Galectin-1 is a member of the lectin family proteins, which can selectively recognize the carbohydrate structure of glyco-proteins and glyco-lipids by the carbohydrate recognizing domain (CRD).³⁵ The unique CRD of galectin-1 can be probed by lactose (a simple disaccharide) via the special β -galactoside bond. In this study, a surface-functionalized GNRs-based biosensor is designed and produced to detect galectin-1. A sulfhydryl lactose ligand is bonded to the GNRs surface by the Au–S interaction to fabricate the lactose-functionalized GNRs (Lac-

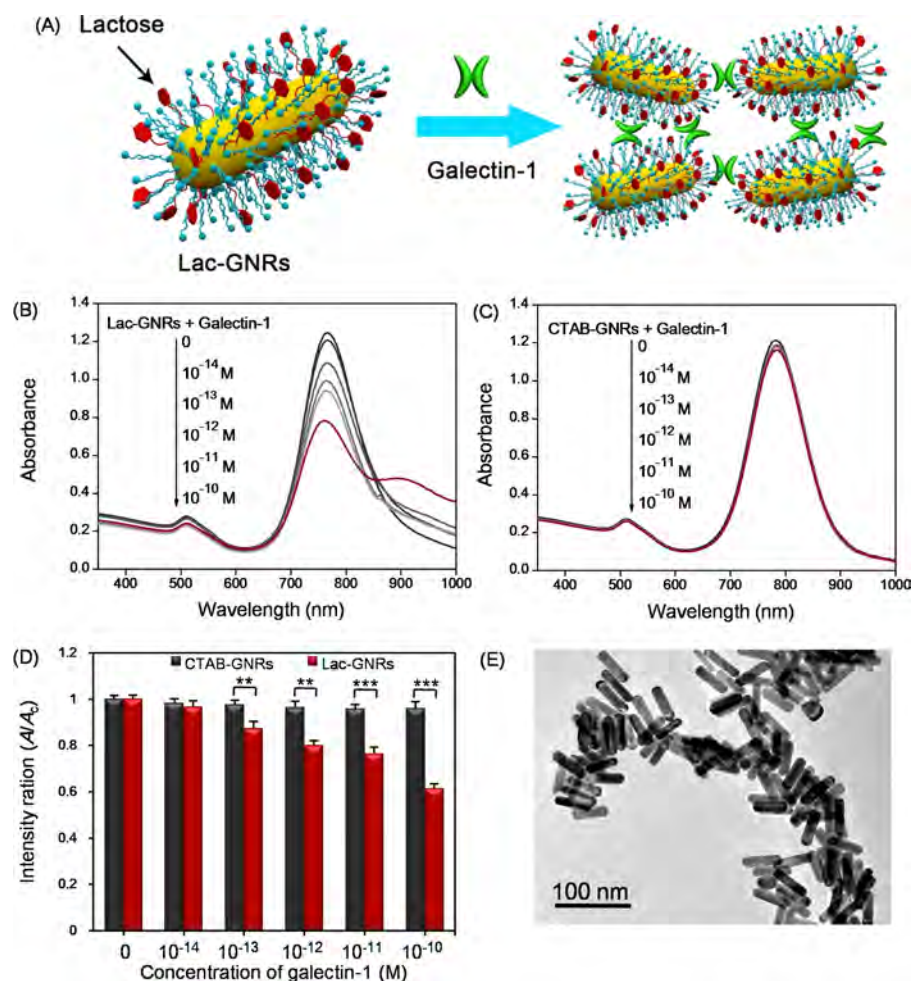


Figure 2. (A) Schematic illustration of galectin-1 induced aggregation of Lac-GNRs. Sensitivity of Lac-GNRs to detect galectin-1 in an aqueous solution: Absorption spectra of (B) Lac-GNRs and (C) CTAB-GNRs after addition of galectin-1 with concentrations from 0 to 10^{-10} M; (D) corresponding LSPR peak intensity ratio (A/A_c) versus galectin-1 concentration, where A and A_c are the LSPR peak intensity of the Lac-GNRs or CTAB-GNRs solution added with galectin-1 (A) and the same volume of distilled water (A_c), respectively; (E) TEM image of Lac-GNRs after interaction with 10^{-10} M galectin-1. One-way ANOVA is utilized to determine the level of significance, $**p < 0.01$, $***p < 0.001$.

GNRs) which exhibit good stability in the biological environment and high sensitivity to the specific galectin-1. This choreographed biosensor enables rapid and convenient screening of cancer patients.

2. RESULTS AND DISCUSSION

2.1. Synthesis and Characterization of Lac-GNRs. The Lac-GNRs are prepared by a simple surfactant exchange process illustrated in Figure 1A employing a lactose ligand with terminal sulfhydryl groups (designated as Lac-SH, see Figure S1 for the synthesis details). Lac-SH is designed as lactose with the anomeric hydroxyl group replaced by a medium-chain containing -SH group, in which the appropriate length of the medium chain facilitates the Au-S interactions between the GNRs and Lac-SH. The GNRs with the cetyltrimethylammonium bromide (CTAB) coating (designated as CTAB-GNRs, prepared by a seed-mediated growth method published previously⁴³) are incubated with Lac-SH in the aqueous solution and kept at room temperature overnight. The Lac-GNRs are obtained by partial substitution of CTAB with Lac-SH on GNRs.

The Lac-GNRs are characterized by X-ray photoelectron spectroscopy (XPS), UV-vis-NIR spectrophotometry, zeta-

potential examination, and transmission electron microscopy (TEM) with CTAB-GNRs serving as the control. As shown in the survey XPS spectra in Figure 1B, the C 1s signal decrease and O 1s peak increase are both remarkable after lactose functionalization of GNRs. The high-resolution C 1s (Figure 1C) and O 1s spectra (Figure S2) further demonstrate the abundant C-O bonds (originating from Lac-SH ligand) on Lac-GNRs, implying that the CTAB on the surface of GNRs is partly displaced by Lac-SH. Compared to CTAB-GNRs, the Lac-GNRs show a slight red-shift in the SPR band (Figure 1D) and the red-shift increases in a dose-dependent manner (Figure S3) as more CTAB molecules on the CTAB-GNRs are replaced by Lac-SH when the concentration of Lac-SH addition goes up. TEM (inset in Figure 1D) further indicates good dispersion of Lac-GNRs in the aqueous solution. Figure 1E shows that the zeta potential of Lac-GNRs (26.8 mV) is smaller than that of CTAB-GNRs (32.0 mV), confirming successful fabrication of Lac-GNRs since lactose is neutral while the CTAB bilayers make the GNRs positively charged. A colorimetric assay with anthrone/sulfuric acid⁴⁴ is performed to quantitatively determine lactose bonding on GNRs and the percentage of lactose on Lac-GNRs is calculated to be 5.5% (Figure S4).

2.2. Trace Detection of Galectin-1 by Lac-GNRs. The SPR bands of the GNRs originate from the collective oscillation of surface electrons and hence, the resonance frequency of the SPR bands relies on the aggregation/dispersion condition of the GNRs. Because galectin-1 exists as a noncovalent homodimer with one CRD per subunit, it is supposed to recognize the lactose segments on Lac-GNRs to induce final GNRs aggregation (Figure 2A) with the SPR shift. Accordingly, the Lac-GNR platform is utilized to detect galectin-1 on the molecular level.

The assay is label-free with a simple experimental protocol. In brief, a certain amount of galectin-1 is added to the Lac-GNRs solution and the mixture is incubated at room temperature for 1 h prior to the acquisition of absorption spectra on a UV-vis-NIR spectrophotometer. As shown in Figure 2B, the Lac-GNRs solution exhibits an obvious decrease in the LSPR peak intensity (at 770 nm) after galectin-1 addition. In the meantime, a new SPR band at about 900 nm emerges. In contrast, the response of CTAB-GNRs is negligible (Figure 2C) implying that the interaction between CTAB-GNRs and galectin-1 is very weak. The histogram of the LSPR peak intensity ratio (A/A_c) versus galectin-1 concentration is plotted in Figure 2D, in which A and A_c are the LSPR peak intensity of the Lac-GNRs or CTAB-GNRs solution added with galectin-1 (A) and the same volume of distilled water (A_c), respectively. The A/A_c ratio of Lac-GNRs decreases from 1.0 to around 0.6 with exponential increase in the galectin-1 concentration from 0 to 10^{-10} M. In contrary, the corresponding A/A_c value of CTAB-GNRs is consistently above 0.98. Figure 2D shows that the detection limit of galectin-1 is as low as 10^{-13} M (** $p < 0.01$) indicating the ultrahigh sensitivity. The TEM image in Figure 2E depicts aggregation of Lac-GNRs induced by galectin-1 corresponding to the alteration of their LSPR band.

Comparative experiments are further performed to assess the efficiency of Lac-GNRs in the detection of galectin-1 in the presence of abundant lactose. Even in an aqueous solution with a large concentration of lactose, the “cluster glycoside effect”^{45,46} enables the Lac-GNRs to detect a trace amount of galectin-1 as binding to galectin-1 is much stronger than the over effect of individual lactose events (Figure S5 in Supporting Information).

In addition to the high sensitivity, good specificity is essential to biosensors. The specificity of Lac-GNRs to galectin-1 is examined by comparing galectin-1 with bovine serum albumin (BSA) and galectin-3. As shown in Figure 3, by adding 10^{-10} M galectin-1 to the Lac-GNRs solution, the Lac-GNRs solution changes from red to gray (Figure 3A) and a large change in the LSPR band is observed from the absorption spectra (Figure 3B). BSA, which is a serum albumin protein derived from cows, lacks CRD for lactose recognition. Correspondingly, addition of BSA into the Lac-GNRs solution induces neither color change nor spectra alteration, even when the concentration of BSA (10^{-8} M) is a hundred times larger than that of galectin-1. Galectin-3 is another member of the lectin family which also contains a CRD enabling specific binding of β -galactosides. It has varying effects under cancerous conditions and is used as a diagnostic marker for different types of cancer.⁴⁷ When galectin-3 is added to the Lac-GNRs solution with a concentration of 10^{-9} M (10 times larger than that of galectin-1), there is no color change in the Lac-GNRs solution and only slight variation can be observed from the LSPR band. Galectin-3 is monomeric and likely to inhibit adhesion at a low concentration. It is

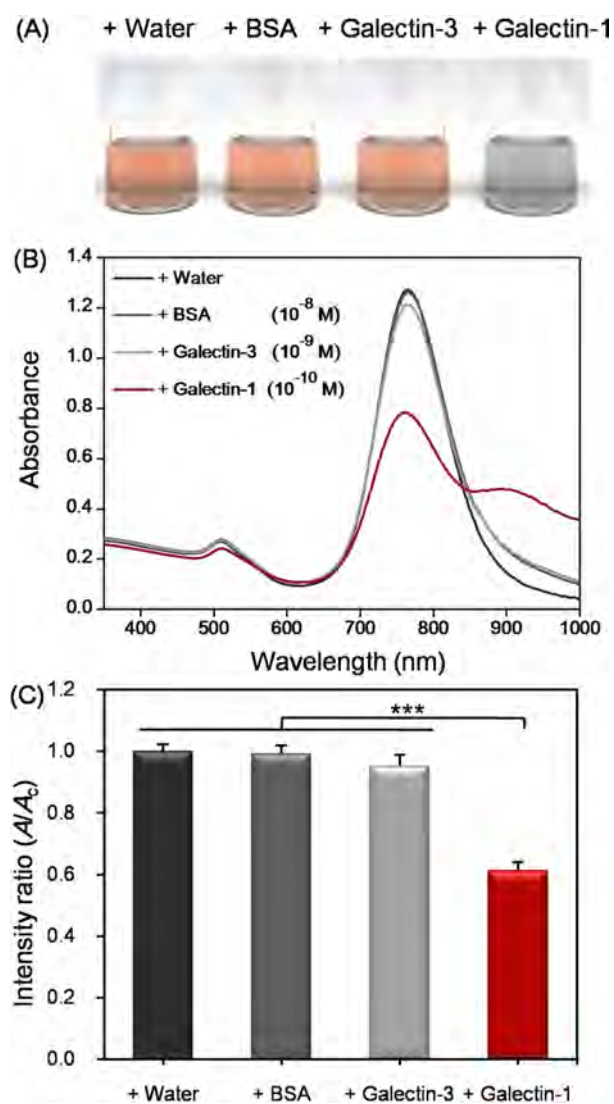


Figure 3. Specificity of Lac-GNRs in an aqueous solution: (A) photographs, (B) absorption spectra, and (C) LSPR peak intensity ratio (A/A_c) of the Lac-GNRs solution after the addition of distilled water (control), BSA (10^{-8} M), galectin-3 (10^{-9} M), and galectin-1 (10^{-10} M). One-way ANOVA is utilized to determine the level of significance, *** $p < 0.001$.

different from the existing form of galectin-1 as a homodimer.³⁵ Although galectin-3 can probe Lac-GNRs, its existence as a monomer suggests that such recognition will not induce final aggregation of Lac-GNRs (Figure S6). The results presented in Figure 3C also indicate the difference between galectin-1 and BSA/galectin-3. The LSPR peak intensity ratio A/A_c of galectin-1 is around 0.6 and those of BSA and galectin-3 are close to 1. The difference between them is quite significant (** $p < 0.001$) and good specificity to galectin-1 is thus demonstrated.

2.3. Assays of Lac-GNRs with Human Serum. Biofluids such as human serum have more complicated compositions than a single solute solution possibly leading to uncontrollable response by the nanosensors. To evaluate the reliability of Lac-GNRs in clinical applications, they are employed in the serological assays of 20 typical human serum specimens collected from 5 healthy donors and 15 cancer patients (Table S1) at the Shenzhen People's Hospital with CTAB-

GNRs serving as the control. The serum samples are separately added to the Lac-GNRs or CTAB-GNRs solutions 1 h prior to acquisition of the absorption spectra. The absorption bands observed from the serum samples incubated with Lac-GNRs or CTAB-GNRs are summarized in Figures S7 and S8, respectively. Figure S7 shows that the addition of cancer serum specimens decreases the LSPR peak intensity of Lac-GNRs accompanied by a red-shift in the LSPR band. In contrast, the red-shifted LSPR peak declines slightly when the Lac-GNRs solution is introduced with healthy serum. Figure S8 reveals that both serum specimens only decrease the LSPR peak intensity of CTAB-GNRs slightly and induce an inconspicuous red-shift in the LSPR band. There is no significant difference between the healthy and cancer serum groups by the CTAB-GNRs assays. For ease of comparison, the LSPR peak intensity ratios (A/A_c) of Lac-GNRs and CTAB-GNRs are illustrated as histograms in Figure 4, where A and A_c

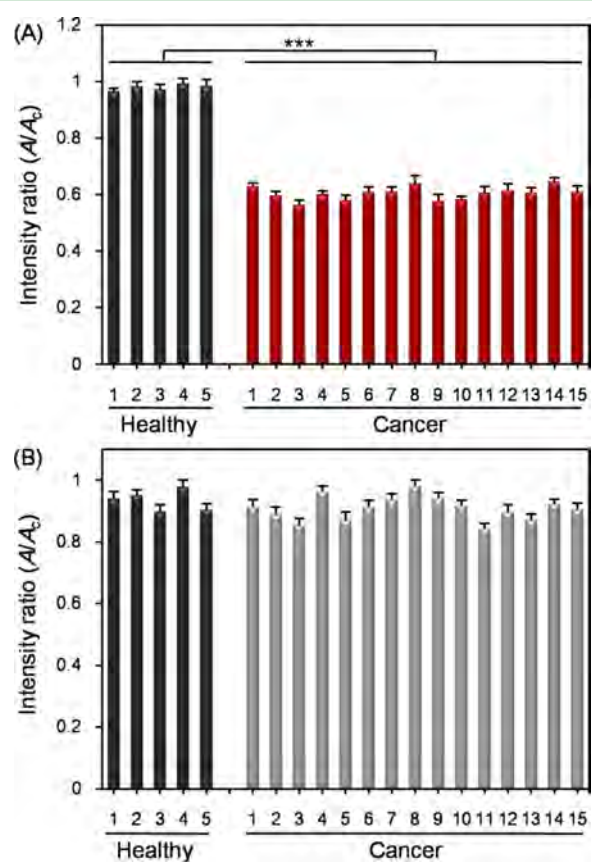


Figure 4. Serological assays by Lac-GNRs and CTAB-GNRs: LSPR peak intensity ratio (A/A_c) of (A) Lac-GNRs and (B) CTAB-GNRs after addition of the serum specimens obtained from 5 healthy donors and 15 cancer patients, where A and A_c are the LSPR peak intensity of Lac-GNRs or CTAB-GNRs solution added with serum (A) and the same volume of distilled water (A_c), respectively. One-way ANOVA is utilized to determine the level of significance, $***p < 0.001$.

are the LSPR peak intensity of Lac-GNRs or CTAB-GNRs solution added with serum specimens (A) and the same volume of distilled water (A_c), respectively. As shown in Figure 4A, the A/A_c values for the serum samples collected from 15 cancer patients range from 0.58 to 0.64 regardless of the type and stage of cancer, whereas those from 5 healthy donors vary from 0.96 to 0.99 after incubation with Lac-GNRs. The Lac-GNRs assays indicate that there is a significant difference ($***p < 0.001$)

between the healthy and cancer serum specimens, and it is quite different from the inappreciable difference determined by CTAB-GNRs assays (Figure 4B).

To further verify the specificity of Lac-GNRs to galectin-1 in the serological assays, we subjected the Lac-GNRs solution to a series of tests involving healthy human serums (from 5 donors), healthy human serums+galectin-3, and healthy human serums+galectin-1. These three groups of serum samples are incubated with Lac-GNRs separately for 1 h to determine the absorption spectra and further evaluate the corresponding LSPR peak intensity ratios (A/A_c). According to the absorption spectra in Figure S9, existence of galectin-3 in healthy serums only decreases the LSPR peak intensity of Lac-GNRs slightly, whereas the LSPR peak declines remarkably after introducing Lac-GNRs into healthy serums +1 ng/mL galectin-1. The A/A_c values in Figure 5 indicate significant differences ($***p <$

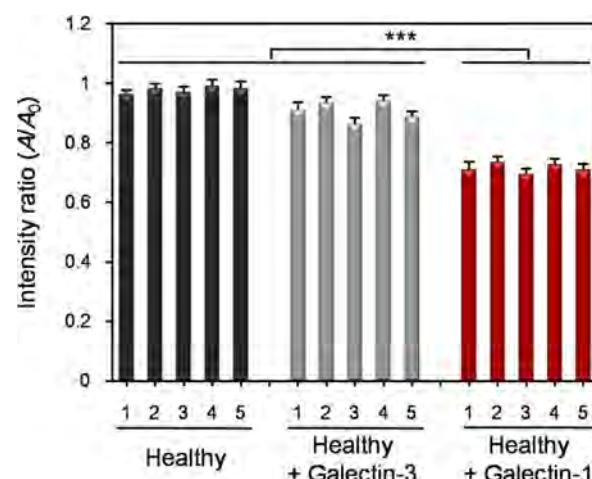


Figure 5. Specificity of Lac-GNRs in the serological tests: LSPR peak intensity ratio (A/A_c) of the Lac-GNRs solution after addition of healthy human serums, healthy human serums+galectin-3, and healthy human serums+galectin-1, where A and A_c are the LSPR peak intensity of Lac-GNRs added with various serum specimens (A) and the same volume of distilled water (A_c), respectively. One-way ANOVA is utilized to determine the level of significance, $***p < 0.001$.

0.001) between the healthy human serums+galectin-1 group and the other 2 groups statistically. In conjunction with the good specificity of Lac-GNRs nanosensors to galectin-1, the A/A_c values of healthy serums +1 ng/mL galectin-1 (0.69 to 0.73) are larger than those determined from 15 cancer serums (0.58 to 0.64). In other words, the galectin-1 concentrations in the 15 cancer serums are larger than those in healthy serums after addition of 1 ng/mL galectin-1. This is consistent with previous reports that the galectin-1 level of cancer serum is 3.07 ± 0.78 ng/mL for lung carcinoma³⁷ and ranges from 4 to 18 ng/mL for high-grade glioma.⁴² Consequently, the Lac-GNRs has adequate sensitivity in detecting trace amounts of galectin-1 in cancer patients.

Serological tests are used clinically to screen cancer patients due to the noninvasive nature and convenience. However, traditional serological detection methods for cancer diagnosis tend to be expensive and complex and reports about serological diagnosis of cancer via nanobiosensors have been scarce so far.⁵ The following issues have hampered clinical use of nanobiosensors: (1) unreliable operation and low signal-to-noise ratio in heterogeneous clinical specimens, (2) response time

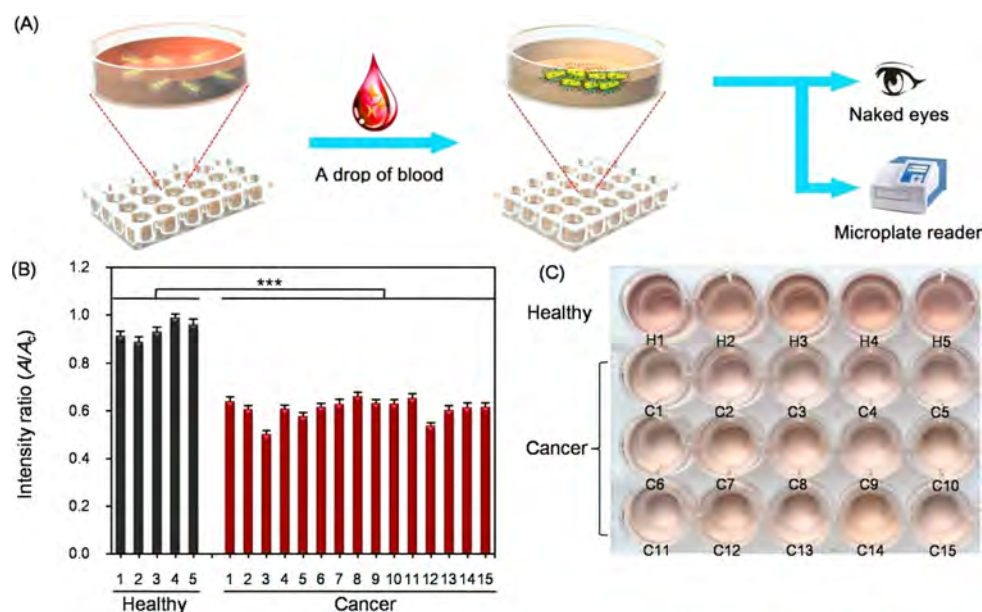


Figure 6. (A) Schematic illustration of the serological screening of suspected cancer patients by the Lac-GNRs biosensors. (B) Microplate assays reading and (C) Direct observation of the Lac-GNRs solution after addition of the serum specimens obtained from 5 healthy donors (from H1 to H5) and 15 cancer patients (from C1 to C15). Absorption intensity ratios (A/A_c) were involved, where A and A_c are the 810 nm absorption of the Lac-GNRs solution added with serum specimens (A) and the same volume of distilled water (A_c), respectively. One-way ANOVA is utilized to determine the level of significance, *** $p < 0.001$.

not compatible with diagnosis that requires fast delivery, and (3) rigorous requirement of specialized instruments for diagnosis. In response to these challenges, the Lac-GNRs biosensors described here offer reliable and fast (1 h or less) serological diagnosis of cancerous conditions. The Lac-GNRs are versatile enough to diagnose cancer in the early stage (Table S1). As a demonstration, the Lac-GNRs are applied in conjunction with a microplate reader, a common and inexpensive instrument used in clinical diagnosis, to determine dozens of serum specimens in batches (Figure 6B, microplate assays indicating a significant difference between the healthy and cancer serum specimens, *** $p < 0.001$). The obvious color change induced by Lac-GNRs can even be differentiated with the naked eye (Figure 6C, red color observed from the healthy groups and light pink observed from the cancer groups). As a kind of economical biosensors with easy-to-use properties, Lac-GNRs can even be employed by nonspecialists, making it possible to perform diagnosis at home or at a remote location.

3. CONCLUSION

Lac-GNRs biosensors are designed and fabricated for the detection of galectin-1 cancer marker based on the high specificity of lactose to galectin-1 and unique SPR properties of GNRs. The Lac-GNRs exhibit not only high sensitivity in the detection of trace amount of galectin-1 (10^{-13} M), but also good specificity to galectin-1 compared with BSA and galectin-3. The results acquired from clinical specimens indicate that the Lac-GNRs biosensors can be applied to the serological diagnosis of cancer patients. The Lac-GNRs biosensing platform is simple, sensitive, and convenient, requiring only a microplate reader, and in some cases, visual examination will suffice.

4. MATERIALS AND METHODS

4.1. Materials. All the chemicals were used as-received without further purification if not specified otherwise. Chloroauric acid

($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$), silver nitrate (AgNO_3), L-ascorbic acid, and hydrochloric acid (HCl) were obtained from Sinopharm Chemical Reagent Co., Ltd., and CTAB and sodium borohydride (NaBH_4) were purchased from Sigma-Aldrich. Galectin-1, galectin-3, and BSA were obtained from PeproTech.

4.2. Synthesis of CTAB-GNRs. The CTAB-GNRs were synthesized by the seed-mediated growth method as described previously.⁴³ The gold seed particles were prepared by adding 5 mL of 0.5 mM HAuCl_4 to 5 mL of 0.2 M CTAB and stirred vigorously followed by addition of 600 μL of freshly prepared 10 mM NaBH_4 in an ice-bath. The gold seed solution was left for more than 2 h before use. In the subsequent synthesis of CTAB-GNRs, 6 mL of 5 mM HAuCl_4 and 75 μL of 0.1 M AgNO_3 were added to 30 mL of 0.2 M CTAB, followed by the addition of 75 μL of 1.2 M HCl and 3.7 mL of 10 mM ascorbic acid. After gentle swirling, the solution changed from dark orange to colorless. Then, 50 μL of the gold seed solution was injected rapidly. The resulting solution was gently mixed for 10 s and left overnight. Finally, the CTAB-GNRs were purified by centrifugation at 10 000 rpm for 10 min, and the precipitate was resuspended in distilled water.

4.3. Synthesis of Lac-GNRs. The Lac-GNRs were prepared by adding 100 μL of 20 mM sulfhydryl lactose ligand to 5 mL of the GNRs solution and kept at room temperature for 15 h. The synthesis procedure of the sulfhydryl lactose ligand was described in more details in the Supporting Information. The as-obtained Lac-GNRs were used in subsequent experiments without further purification.

4.4. Characterization. XPS was conducted on a Physical Electronics PHI 5802 equipped with a monochromatic Al $K\alpha$ source. A constant pass energy (11.75 eV) was employed and all the data were collected at a step size of 0.1 eV. The UV-vis-NIR absorption spectra were acquired at room temperature on a PerkinElmer Lambda 25 spectrophotometer. The TEM images were acquired on a Tecnai G2 F20 S-Twin transmission electron microscope and the zeta potentials were measured on a ZetasizerNano ZS zeta analyzer.

4.5. Assays in Aqueous Solutions. First, 100 μL of the analytes (BSA, galectin-3 or galectin-1) in aqueous solution was added to 1 mL of Lac-GNRs or CTAB-GNRs solution separately. After gentle swirling, the mixtures were kept at room temperature for 1 h and diluted with 2 mL of distilled water to obtain the absorption spectra from 350 to 1000 nm on the spectrophotometer. Detection of

galactin-1 by Lac-GNRs was based on the LSPR band in the absorption spectra.

4.6. Serological Experiments. The serum specimens were obtained from 15 cancer patients and 5 healthy donors admitted to the Institute of Oncology, Shenzhen People's Hospital, Shenzhen, China. All the cancer patients had undergone pathological examination (detailed information in Table S1), and the initial serological experiment was performed in a blind test in which 20 serum samples were labeled from 1 to 20 randomly. The serum specimens were warmed to 4 °C and room temperature gradually after short storage at -20 °C. Then, 100 μ L of each serum specimen was added to 1 mL of the Lac-GNRs or CTAB-GNRs solution separately and kept at room temperature for 1 h. Afterward, each solution was diluted with 2 mL of distilled water for spectrophotometric assays according to the aforementioned protocol.

Three types of serum specimens involving healthy human serums (from 5 donors), healthy human serums + galectin-3, and healthy human serums + galectin-1 were employed in the determination of the Lac-GNRs specificity in the serological tests. After the addition of 10 μ L of 10 ng/mL galectin-3 or galectin-1 to 90 μ L of healthy human serum, the prepared serum specimens were added to 1 mL of the Lac-GNRs solution separately. Correspondingly, the added concentration of galectin-3 or galectin-1 in the serum samples is 1 ng/mL. The serum samples were kept at room temperature for 1 h and diluted with 2 mL of distilled water for spectrophotometric assays according to the aforementioned protocol.

In the microplate assays, the human serum specimens were obtained from 5 healthy donors and 15 cancer patients, and 100 μ L of each serum specimen were pipetted onto a 24-well plate containing 1 mL of the Lac-GNRs solution in each well. After remaining still at room temperature for 1 h, the mixtures on the 24-well plate were analyzed at 810 nm on a Thermo Scientific Multiskan GO microplate reader.

4.7. Statistical Analysis. All the assays for galectin-1 were performed in triplicate and the A/A_c values were expressed as means and standard deviations. Each experiment was repeated three times with data from a typical experiment shown. The one-way ANOVA test was performed to determine the level of significance.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b11192.

Experimental procedures for the synthesis of sulfhydryl lactose ligand, Figures S1–S9, and Table S1. (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting Information for
Lactose-Functionalized Gold Nanorods for Sensitive and Rapid
Serological Diagnosis of Cancer

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Procedures for the synthesis of sulfhydryl lactose ligand:

The 4Å molecular sieves (MS, 500 mg) were added to a solution of peracetylated lactose (**1**, 2.5 g, 1 equiv., 5.12 mmol) and 2-[2-(2-chloroethoxy)ethoxy]ethanol (**2**, 1.3 g, 1.5 equiv., 7.8 mmol) in anhydrous dichloromethane (CH₂Cl₂, 5 ml) and the mixture was stirred at room temperature for 10 min. 7.8 ml of BF₃·Et₂O (3.6 g, 5 equiv., 25.6 mmol) were subsequently added and the reaction mixture was stirred for another 72 h. The 4Å MS was filtered and the filtrate was extracted with CH₂Cl₂, triply washed with NaHCO₃, and dried over Na₂SO₄. The products were purified by flash column chromatography using PE/acetone as the eluent (2:1 to 1:1) to obtain the glycosylated product **3** (870 mg, 32%).

Product **3** (770 mg, 1 equiv., 1.5 mmol) was dissolved in N,N-Dimethylformamide (DMF, 3 ml) with addition of potassium thioacetate (KSAc, 550 mg, 1.5 equiv., 2.3mmol). The mixture was stirred at room temperature for 4 h. After addition of ethyl acetate (40 ml), the solution was sequentially rinsed with H₂O, NaHCO₃, and saturated NaCl solution. The organic phase was evaporated to obtain product **4** (646 mg, 80%).

Product **4** (600mg, 0.7 mmol) was dissolved in anhydrous methanol (CH₃OH, 2 ml) with addition of a certain amount of sodium. The mixture was stirred at room temperature for 12 h. Acidic ion exchange resins were added for neutralization followed by filtering and rinsing with CH₃OH. The solvent was evaporated to obtain product **5** (sulfhydryl lactose ligand, 338 mg, 95%).

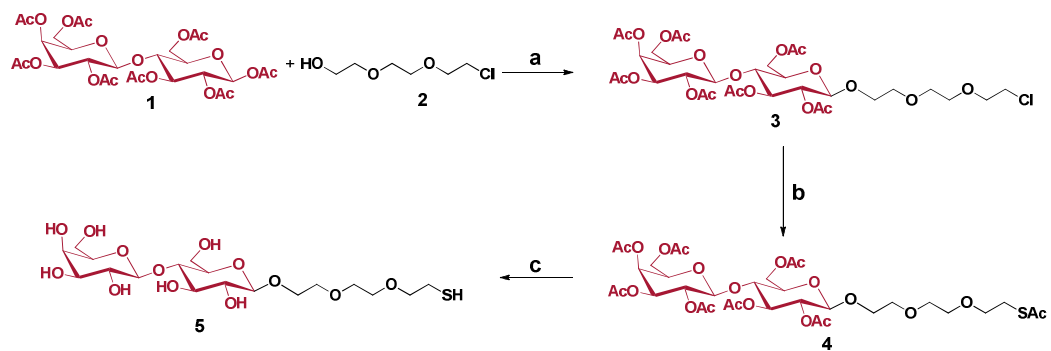


Figure S1. Synthesis of sulfhydryl lactose ligand: (a) $\text{BF}_3\text{-Et}_2\text{O}$, anhydrous CH_2Cl_2 , 4Å MS, r.t., 72h, 32%; (b) KSAc, DMF, r.t., 4h, 80%; (c) CH_3ONa , CH_3OH , r.t., 12h, 95%.

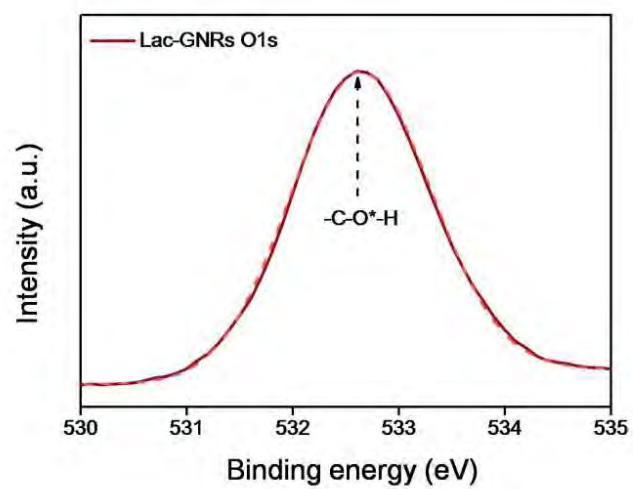


Figure S2. High-resolution O1s spectrum of Lac-GNRs with the peak corresponding to C-O*-H on lactose.

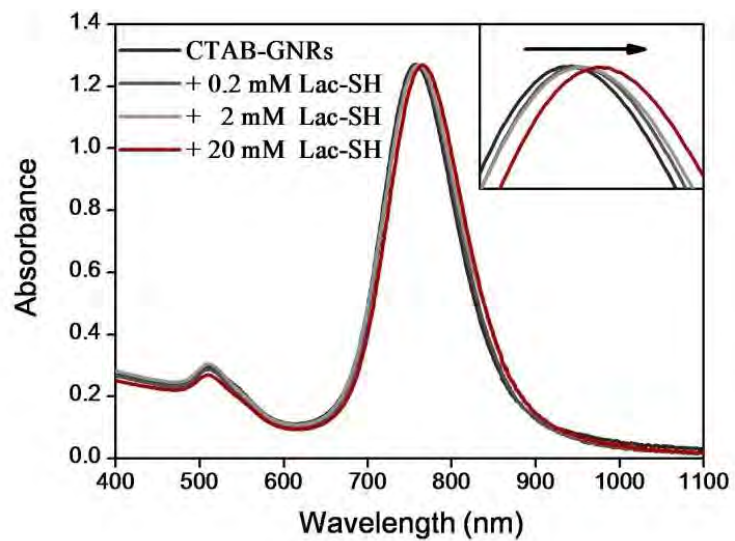


Figure S3. Absorption spectra of Lac-GNRs modified with Lac-SH at different concentrations. Inset: the magnified LSPR bands.

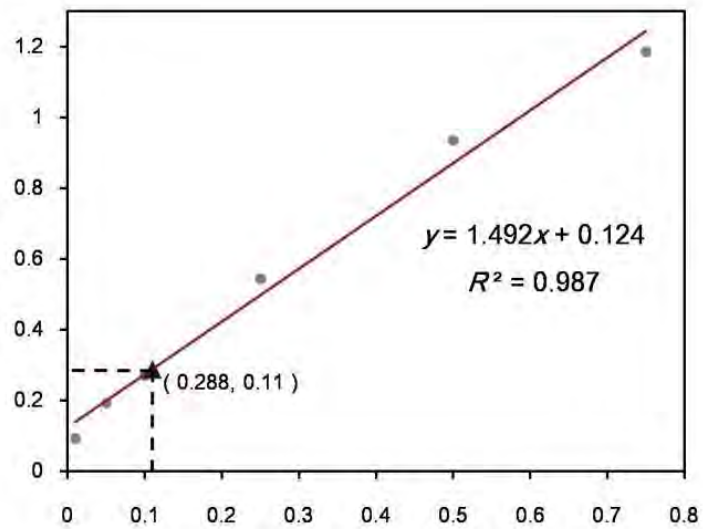


Figure S4. Quantitative measurement of lactose on Lac-GNRs by colorimetric assay with anthrone/sulfuric acid. The red line refers to the fitted curve of the standard lactose solutions (0, 0.1, 0.05, 0.25, 0.5, 0.75 mg/ml). 0.11 mg/ml of lactose (blue triangle) are determined on 2 mg/ml of Lac-GNRs according to the fitting calibration curve.

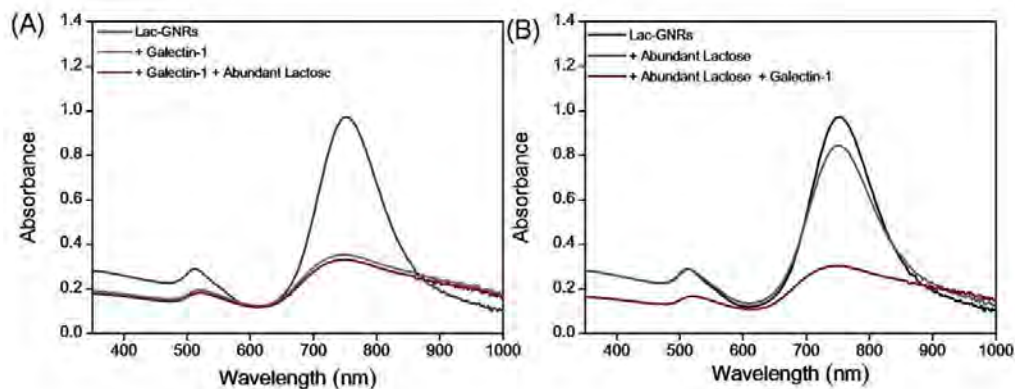


Figure S5. (A) Absorption spectra of Lac-GNRs + galectin-1 system before and after further addition of abundant lactose. In this experiment, after the addition of 100 μ l of galectin-1 (20 ng/ml) to 1 ml of Lac-GNRs, 100 μ l of lactose (100 mM) solution was added to the system for 3 h to achieve the equilibrium. No disaggregation of Lac-GNRs can be observed, suggesting that the binding between Lac-GNRs and galectin-1 is strong enough to refuse the competition from abundant lactose (lactose in the final solution is approximately 25-fold of Lac-SH). (B) Absorption spectra of Lac-GNRs + abundant lactose system before and after further addition of galectin-1. In this experiment, 100 μ l of lactose (100 mM) was firstly added to 1 ml of Lac-GNRs. Afterwards, 100 μ l of galectin-1 (20 ng/ml) was added. It can be observed that Lac-GNRs can sensitive response to a trace amount of galectin-1 even in the presence of abundant lactose which is approximately 25-fold of Lac-SH. Notably, our Lac-GNRs are capable of tracing galectin-1 in a lactose rich environment, which probably originates from the "cluster-glycoside effect" of Lac-GNRs.

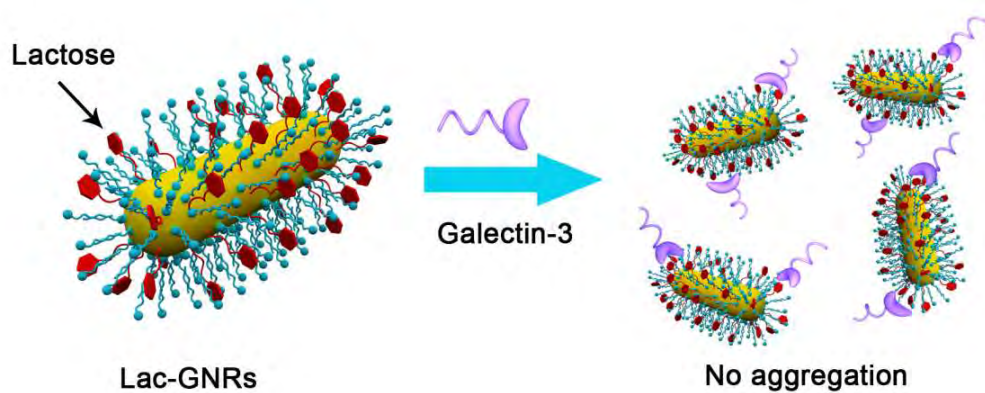


Figure S6. Schematic illustration of Lac-GNRs recognized by galectin-3 (without aggregation).

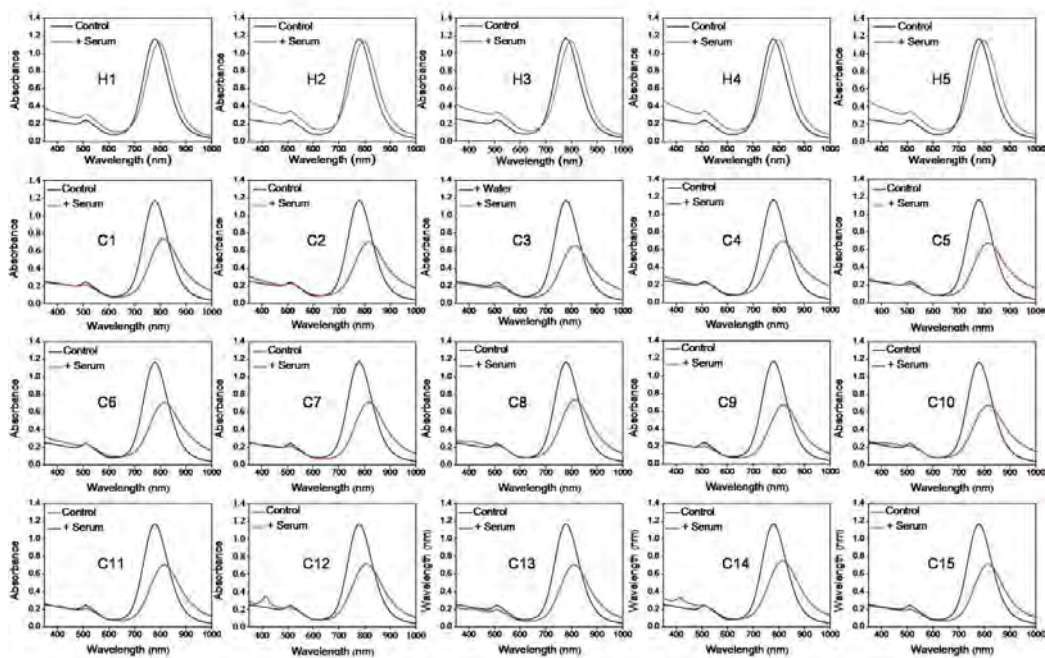


Figure S7. Absorption spectra of Lac-GNRs with addition of serum specimens obtained from healthy donors (from H1 to H5) and cancer patients (from C1 to C15). The same volume of distilled water is employed as the control.

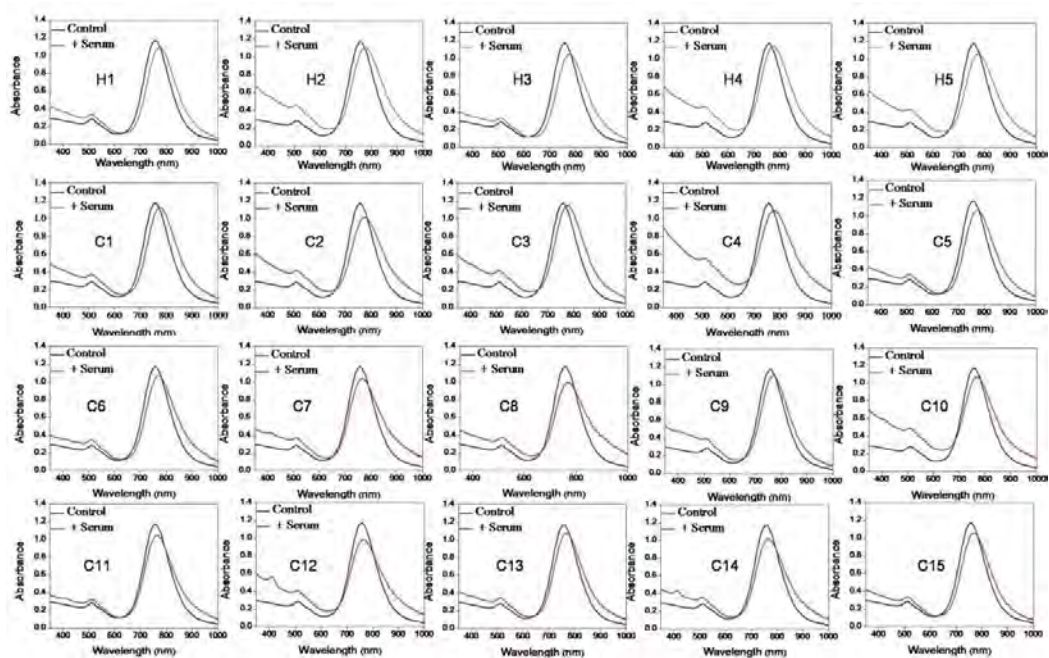


Figure S8. Absorption spectra of CTAB-GNRs with addition of serum specimens obtained from healthy donors (from H1 to H5) and cancer patients (from C1 to C15). The same volume of distilled water is employed as the control.

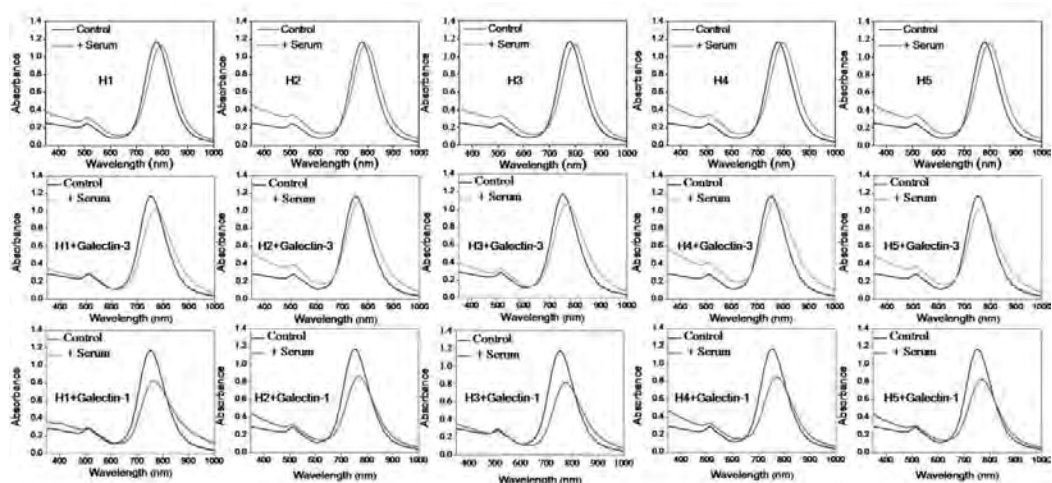


Figure S9. Absorption spectra of Lac-GNRs with addition of serum specimens obtained from healthy donors (from H1 to H5), healthy human serums+galectin-3 (from H1+galectin-3 to H5+galectin-3) and healthy human serums+galectin-1 (from H1+galectin-1 to H5+galectin-1). The added concentration of galectin-3 or galectin-1 in the healthy serum samples is 1 ng/ml. The same volume of distilled water is employed as the control.

Table S1. Detailed information about the healthy donors and cancer patients at Shenzhen People’s Hospital.

Sample	Age	Gender	Diagnosis
H1	53	Male	Healthy
H2	62	Female	Healthy
H3	48	Female	Healthy
H4	45	Female	Healthy
H5	59	Male	Healthy
C1	51	Female	Cervical carcinoma (stage IIb)
C2	31	Female	Colon carcinoma (stage IIIc)
C3	50	Male	Colon carcinoma (stage IIb)
C4	47	Female	Ovarian mucinous cystadenocarcinoma (stage IIIc)
C5	52	Female	Ovarian carcinoma (stage IV)
C6	60	Male	Left lung carcinoma (stage IV)
C7	46	Female	Right lung carcinoma (stage II)
C8	63	Male	Gastric carcinoma (stage IV)
C9	45	Male	Ascend colon carcinoma (stage IV)
C10	78	Male	Colon carcinoma (stage IIIb)
C11	64	Male	Poorly differentiated lung adenocarcinoma (stage III)
C12	50	Male	Colorectal carcinoma (stage III)
C13	64	Female	Breast carcinoma (stage IIa)
C14	43	Female	Breast carcinoma (stage IV)
C15	71	Female	Lung carcinoma (stage IV)