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In Situ Scanometric Assay of Cell Surface Carbohydrate by Glyconanoparticle-Aggregation-Regulated Silver Enhancement

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A convenient and label-free scanometric approach for in situ cell surface carbohydrate assay was designed by integrating the bioconjugation and aggregation of glyconanoparticles, silver signal amplification, and spot test. The novel glyconanoparticles were prepared by a one-pot procedure. In the presence of lectin, using concanavalin A and mannose as a couple of model, the glyconanoparticles exhibited fast aggregation. The aggregation process could be inhibited by the specific recognition of lectin by the carbohydrate on the cell surface. Combining the gold nanoparticle catalyzed silver enhancement and scanometric detection, the number of cell surface carbohydrate groups could be conveniently read out. The average number of mannose units on a single living intact BGC cell was detected to be $(4.5 \pm 0.4) \times 10^7$. This largely noninstrumental method took the advantages of a nanoparticle-based recognition and an aggregation-regulated signal amplification and avoided cell pretreatment and labeling processes. It could determine cell surface densities of different carbohydrates in parallel and thus would contribute considerably to meeting the challenges in decipherment of the glycomic codes.

Glycans are a large group of biomolecules with diverse structures that exist on all eukaryotic cell surfaces. Virtually every class of biomolecules, such as proteins, lipids, tRNA, and many secondary metabolites, can be found in glycosylated forms.[1] Glycans play key roles in a myriad of biological events, including protein folding, trafficking, and stability, organ development, cellular adhesion, cell signaling, immune response, and pathological processes.[2−4] Importantly, variations in the sugar signatures of glycoconjugates, such as overproduction of mucin and an increase of global sialylation, indicate certain disease states such as cancer and inflammation.[5] Therefore, analysis of carbohydrates on living cells in various events is keenly desirable for basic science advancement, clinical diagnostics, and therapeutics.[6]

Current efforts for decoding cell surface glycosylation have focused on mass spectrometric methods.[7] Although this technique can provide molecular details, it is time-consuming and not amenable to living cell interrogation due to the destructive nature. The use of lectins and antibodies with defined glycan specificities[8] offers an alternative nondestructive tool to profile cell surface glycans and correlate global changes in their expression with developmental stages and disease.[1] These methods, including lectin-array-based microscopic approaches[9−11] and probe-tagged lectin-based electrochemical[12−15] and flow cytometric[16] strategies, usually use fluorescent probes, nanoparticles, or enzymes to label cells or lectins. However, all these existing methods require complicated instruments, and the labeling processes affect the biological activity of cells and proteins. Thus, the development of a facile, cost-effective, and label-free method is of significant advantage in the study of glycobiology.

Great progress has been made in the development of bioassay strategies owing to the immense application of biofunctionalized nanoparticles.[17] Among them, carbohydrate-modified metal and semiconductor nanoparticles, such as gold nanoparticles (AuNPs)[18−24] and quantum dots,[25] have attracted attention as novel probes,

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(1) Agard, N. J.; Bertozzi, C. R. *Acc. Chem. Res.* **2009**, *42*, 788–797.
(2) Varki, A.; Cummings, R. D.; Esko, J. D.; Freeze, H. H.; Stanley, P.; Bertozzi, C. R.; Hart, G. W.; Etxler, M. E. *Essentials of Glycobiology*, 2nd ed.; Cold Spring Harbor Laboratory Press: New York, 2008.
(3) Ohtsubo, K.; Marth, J. D. *Cell* **2006**, *126*, 855–867.
(4) Marth, J. D.; Grewal, P. K. *Nat. Rev. Immunol.* **2008**, *8*, 874–887.
(5) Dube, D. H.; Bertozzi, C. R. *Nat. Rev. Drug Discovery* **2005**, *4*, 477–488.
(6) Krishnamoorthy, L.; Mahal, L. K. *ACS Chem. Biol.* **2009**, *4*, 715–732.
(7) Pilobello, K. T.; Mahal, L. K. *Curr. Opin. Chem. Biol.* **2007**, *11*, 300–305.
(8) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
(9) Hsu, K.; Pilobello, K. T.; Mahal, L. K. *Nat. Chem. Biol.* **2006**, *2*, 153–157.
(10) Pilobello, K. T.; Slawek, D. E.; Mahal, L. K. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11534–11539.
(11) Zheng, T.; Peelen, D.; Smith, L. M. *J. Am. Chem. Soc.* **2005**, *127*, 9982–9983.
(12) Ding, L.; Cheng, W.; Wang, X. J.; Ding, S. J.; Ju, H. X. *J. Am. Chem. Soc.* **2008**, *130*, 7224–7225.
(13) Cheng, W.; Ding, L.; Ding, S. J.; Yin, Y. B.; Ju, H. X. *Angew. Chem., Int. Ed.* **2009**, *48*, 6465–6468.
(14) Cheng, W.; Ding, L.; Lei, J. P.; Ding, S. J.; Ju, H. X. *Anal. Chem.* **2008**, *80*, 3867–3872.
(15) Ding, L.; Ji, Q. J.; Qian, R. C.; Cheng, W.; Ju, H. X. *Anal. Chem.* **2010**, *82*, 1292–1298.
(16) Zhelev, Z.; Ohba, H.; Bakalova, R.; Jose, R.; Fukuoka, S.; Nagase, T.; Ishikawa, M.; Baba, Y. *Chem. Commun.* **2005**, 1980–1982.
(17) Katz, E.; Willner, I. *Angew. Chem., Int. Ed.* **2004**, *43*, 6042–6108.
(18) Kikkeri, R.; Lepenies, B.; Adibekian, A.; Laurino, P.; Seeberger, P. H. *J. Am. Chem. Soc.* **2009**, *131*, 2110–2112.
(19) Lin, C.-C.; Yeh, Y.-C.; Yang, C.-Y.; Chen, C.-L.; Chen, G.-F.; Chen, C.-C.; Wu, Y.-C. *J. Am. Chem. Soc.* **2002**, *124*, 3508–3509.

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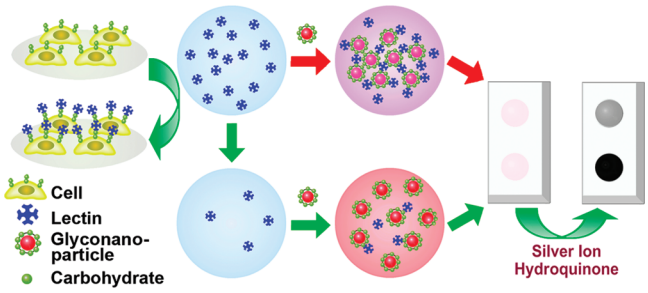

Figure 1. Scheme of the scanometric strategy for in situ detection of mannose groups on living cells.

especially for the study of carbohydrate−lectin and carbohydrate−carbohydrate interactions. These so-called glyconanoparticles integrate the clustered multivalent presentation of the biologically relevant carbohydrates on the surface and the excellent biocompatibility and substantial signal amplification effect of nanomaterials. For example, the AuNP-based glyconanoparticles have provided sensitive platforms for colorimetric assay of the lectin−carbohydrate interaction.[20−22]

In this work, a convenient and label-free scanometric approach for in situ cell surface carbohydrate assay was designed by combining the bioconjugation and aggregation of glyconanoparticles with the aggregation-regulated silver signal amplification and a spot test. Here, the scanometric technology implied that the information-recording and acquirement procedure is performed with a flatbed scanner.[26,27] The glyconanoparticle was obtained with a one-pot synthetic process by conjugation of gold nanoparticles with mannan (a polysaccharide comprised exclusively of mannose). As shown in Figure 1, concanavalin A (Con A), a mannose-specific lectin with four binding sites for sugar epitopes,[8] could induce the aggregation of mannose unit exposed glyconanoparticles.[20,21,24] In the presence of mannose motif abundant cells, the lectin-induced aggregation could be inhibited by the specific binding of lectin to the cell surface carbohydrate to decrease the concentration of free lectin in the glyconanoparticle recognition system. Followed by a AuNP-catalyzed silver enhancement process, the lectin-induced aggregation could be directly and quickly visualized due to the fact that the aggregated AuNPs had lower silver enhancement capability than dispersed AuNPs.[28] Thus, the extent of silver signal enhancement depended proportionally on the expression level of the corresponding cell surface carbohydrate. The visual observation or scanometric detection provided an easy-to-use method for in situ monitoring of cell surface carbohydrates. It avoided the pretreatment of cells, the labeling process of the recognition element, and the use of sophisticated instruments and thus possessed potential applications in clinical diagnosis and elucidation of carbohydrate functions on living cells.

(20) Schofield, C. L.; Haines, A. H.; Field, R. A.; Russell, D. A. *Langmuir* **2006**, *22*, 6707–6711.
(21) Nath, S.; Kaittanis, C.; Tinkham, A.; Perez, J. M. *Anal. Chem.* **2008**, *80*, 1033–1038.
(22) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226–8230.
(23) Reynolds, A. J.; Haines, A. H.; Russell, D. A. *Langmuir* **2006**, *22*, 1156–1163.
(24) Tsai, C.-S.; Yu, T.-B.; Chen, C.-T. *Chem. Commun.* **2005**, 4273–4275.
(25) van Kasteren, S. I.; Campbell, S. J.; Serres, S.; Anthony, D. C.; Sibson, N. R.; Davis, B. G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 18–23.
(26) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757–1760.
(27) Lee, J. S.; Mirkin, C. A. *Anal. Chem.* **2008**, *80*, 6805–6808.
(28) Zhang, Z.; Chen, C. L.; Zhao, X. S. *Electroanalysis* **2009**, *21*, 1316–1320.

EXPERIMENTAL SECTION

Materials and Reagents. Con A, wheat germ agglutinin (WGA), bovine serum albumin (BSA), mannan (M7504-250MG, from *Saccharomyces cerevisiae*), and a silver enhancer kit including enhancement solutions A and B were purchased from Sigma-Aldrich Inc. [3-[(2-Aminoethyl)amino]propyl]trimethoxysilane (AEPTS) was from Alfa Aesar China Ltd. $AuCl_3HCl\cdot 4H_2O$ (>48% Au) and sodium citrate were obtained from Shanghai Chemical Reagent Co., Ltd. (China). Phosphate-buffered saline (PBS; pH 7.2) contained $NaNO_3$ (100 mM) and 10 mM Na_2HPO_4/NaH_2PO_4. All other reagents were of analytical grade. All aqueous solutions were prepared using ultrapure water (≥18 MΩ, Milli-Q, Millipore).

Cell Culture. BGC-823 cells were cultured in RPMI 1640 medium (GIBCO) supplemented with fetal calf serum (10%), penicillin (100 μg/mL), and streptomycin (100 μg/mL) at 37 °C in a humidified atmosphere containing 5% CO_2. The cells in the exponential growth phase were trypsinized and resuspended in culture medium to obtain a homogeneous cell suspension for cell surface carbohydrate assay.

Apparatus and Characterization. The real-time observation of Con A-induced aggregation of glyconanoparticles was performed by taking photos using a digital camera (DSC-W30, Sony, Japan). Glyconanoparticle-spotted glass slides were scanned using an HP Scanjet 2400 scanner. The scanning parameters were as follows: highlights, 255; shadows, 6; midtones, 1.85; output level white, 159; output level black, 0. The UV−vis absorption spectra were obtained with a UV-3600 UV−vis−NIR spectrophotometer (Shimadzu, Kyoto, Japan). The observation of glyconanoparticles was carried out using a JEOL JEM-2100 transmission electron microscope (Japan). The static water contact angles were measured with a contact angle system (OCA30, Dataphysics Instruments GmbH, Germany) using droplets of 3 μL of ultrapure water at 25 °C.

Preparation of Glyconanoparticles. The glyconanoparticles were prepared using mannan as the stabilizing reagent and citrate as the reducing agent. The solution (39.66 mL) for preparation of glyconanoparticles contained 0.01% $HAuCl_4$ and 0.081 mg/mL mannan. After the mixture was heated to 60 °C with a water bath and continuously stirred for 20 min, 348 μL of a 10 mg/mL sodium citrate solution was rapidly added to the mixture. The resulting mixture was then allowed to stir for 1 h at 60 °C. Afterward, a clear red glyconanoparticle solution could be obtained.

To obviate the nonspecific interaction between Con A and glyconanoparticles, BSA was used to block the glyconanoparticles by adding 1 mL of a 1% BSA solution to 9 mL of the freshly prepared glyconaoparticle solution under stirring. After 30 min, the resultant solution was centrifuged (Eppendorf, 5417R) at 16000*g* for 60 min. The precipitate was then washed in PBS three times by centrifugation to remove the excessive mannan and BSA and resuspended with PBS to 333 μL. The as-prepared solution of glyconanoparticles (~75 nM) was kept at 4 °C and was stable for at least 8 months.

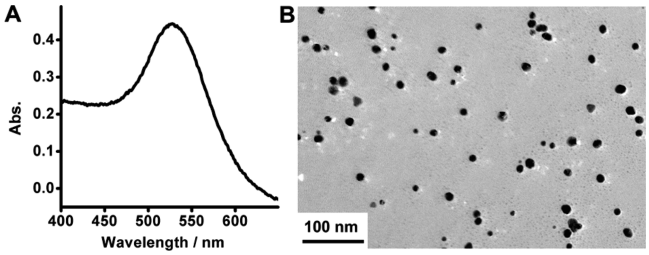

Figure 2. (A) UV−vis spectrum of the 1.9 nM glyconanoparticles and (B) TEM image of the glyconanoparticles obtained on a 400 mesh carbon-coated copper grid.

Preparation of AEPTS-Modified Glass Slides.[29] Glass slides were first dipped in a piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 12 h. After being washed thoroughly with water, they were dried under a stream of nitrogen and silanized by immersion in a solution of 1 mM HAc containing 1% AEPTS for 30 min at room temperature. After being rinsed with water and dried under a stream of nitrogen, the slides were baked for 30 min at 120 °C.

Monitoring of Lectin-Induced Aggregation of Glyconanoparticles by a Spot Test. 5.7 μL of 36 nM glyconanoparticles was mixed with 30.3 μL of Con A solutions at different concentrations (0 ∼ 1.0 μM) and 4 μL of 2 mM Ca^{2+}/Mn^{2+}, and then the mixture was allowed to react for 5 min at room temperature. Ca^{2+} and Mn^{2+} were used for retaining the binding activity of Con A to the mannose groups. A 3 μL volume of the mixture was transferred onto the AEPTS-modified glass slide and allowed to dry. Then silver enhancement was performed by immersing the slides in a 1:1 mixture of silver enhancement solutions A and B for 3 min. After the slides were rinsed with water and dried, this process was performed again. For quantification of the aggregation extent of glyconanoparticles, the slides were scanned using a scanner, and the resultant images were quantified by reading the gray scale intensity using Adobe Photoshop software.[30] The gray scale here represents the mean shades of gray of the chosen field, varying from black at the weakest intensity (0) to white at the strongest (256). Considering that a stronger silver signal showed a darker spot, which corresponded to a lower value of gray scale intensity, in this work we used the relative gray scale intensity (I) to quantify the silver signal of the spots by subtracting the gray scale intensity value of the spot from that of the background. After this data processing, a stronger silver signal corresponded to a higher I.

Cell Surface Carbohydrate Assay. After cell counting, the BGC-823 cells at different concentrations were seeded in culture dishes (35 mm × 10 mm) and incubated under the same conditions as those for cell culture for 4 h. After the culture solution was removed, the cells were washed with sterile PBS five times and incubated with 400 μL 0.5 μM Con A solution containing 0.2 mM Ca^{2+}/Mn^{2+} for 30 min. Then the solution in the culture dish was taken out and centrifuged at 200g for 5 min to obtain the supernatant, in which the Con A concentration was in inverse proportion to the amount of cell surface mannose groups. Thus, the cell surface mannose groups could be detected by analyzing the Con A level in the supernatant.

For the spot test, 30.3 μL of PBS, 0.5 μM Con A, and the supernatant were added to a tube containing 5.7 μL of 36 nM glyconanoparticles and 4 μL of 2 mM Ca^{2+}/Mn^{2+}. After the mixture was allowed to stand for 5 min at room temperature, the spotting, silver enhancement, scanning, and reading of the gray scale intensities were performed by the same procedures mentioned above. For each set of cell samples, four replicates of the spot test were carried out to obtain I_{PBS}, $I_{Con\ A}$, and $I_{supernatant}$. The amount of Con A bound to the cell surface carbohydrate ($n_{cell\text{-}bound\ Con\ A}$) was calculated using the following equation:

$$n_{\text{cell-bound Con A}} = \frac{I_{\text{supernatant}} - I_{\text{Con A}}}{I_{\text{PBS}} - I_{\text{Con A}}} n_{\text{Con A}} \qquad (1)$$

Here, $n_{Con\ A}$ represents the total amount of Con A added to the cell culture dish. The average amount of mannose groups on each cell could be considered as the quotient obtained by dividing the $n_{cell\text{-}bound\ Con\ A}$ by the cell number used for the culture process.

RESULTS AND DISCUSSION

Preparation and Characterization of Glyconanoparticles. For the initial proof of this concept, mannose and Con A, a mannose-specific lectin with four subunits, were selected as the recognition pair.[8] Mannose is one of the six primary carbohydrate units on the cell surface. The novel glyconanoparticles, mannan-conjugated AuNPs, were prepared through the direct reduction of $HAuCl_4$ by citrate in the presence of mannan. In contrast to the previously reported polysaccharide-stabilized AuNPs, the preparation of glyconanoparticles involved the conjugation of biologically relevant mannose groups on the AuNP surface, where the sugar conferred biological functionality to the inorganic core of the nanomaterial rather than playing a simple stabilizing role. The red-colored solution of glyconanoparticles exhibited a UV−vis absorption peak centered at 528 nm (Figure 2A), a typical surface plasmon resonance band for AuNPs, indicating the formation of AuNPs. The transmission electron microscopy (TEM) photo of the glyconanoparticles showed a uniform spherical shape and monodispersity with a diameter of 14 nm (Figure 2B). The conjugation between mannan and the gold nanoparticle surface was based on the interaction of the oxygen-to-gold dative bond, which could be formed between the hydroxyl groups of mannan and the gold surface.[31] Although the interaction between a single functionality and the gold surface was not strong, a high binding affinity could be obtained through the collective sum of weak interactions, which ensured the stability of the formed glyconanoparticles. The good dispersion of the glyconanoparticles was ensured by the steric protection originated from the surface-anchored polysaccharide.[32] After blockage of the surface-active sites of glyconanoparticles with BSA for preventing the nonspecific interaction, the obtained glyconanoparticles could be stable in PBS for more than 8 months.

Fabrication of the Spot Substrate. The visualization and scanometric detection of the lectin-induced aggregation of glyco-

(29) Chrisey, L. A.; Lee, G. U.; O'Ferrall, C. E. *Nucleic Acids Res.* **1996**, *24*, 3031–3039.
(30) Nam, J.-M.; Jang, K.-J.; Groves, J. T. *Nat. Protoc.* **2007**, *2*, 1438–1444.
(31) Calzolari, A.; Cicero, G.; Cavazzoni, C.; Felice, R. D.; Catellani, A.; Corni, S. *J. Am. Chem. Soc.* **2010**, *132*, 4790–4795.
(32) de la Fuente, J. M.; Penadés, S. *Biochim. Biophys. Acta* **2006**, *1760*, 636–651.

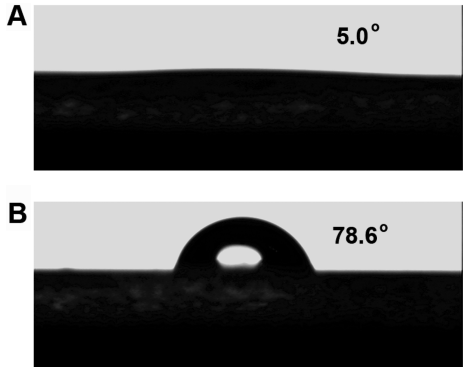

Figure 3. Contact angles of a piranha solution treated glass slide (A) before and (B) after silanization with AEPTS.

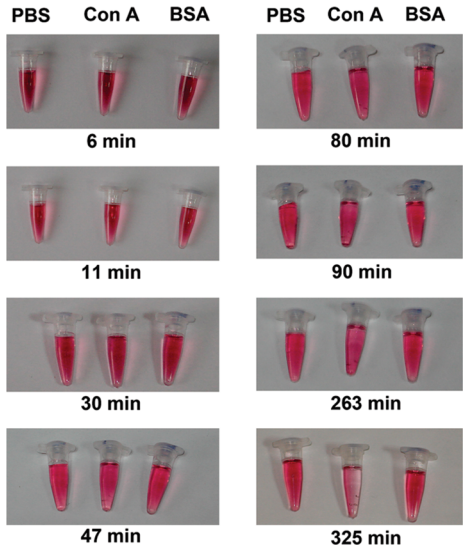

Figure 4. Photos of tubes after mixing 28.5 μL of 36 nM glyconanoparticles and 20 μL of 2 mM Ca^{2+}/Mn^{2+} with 151.5 μL of 0.05 mg/mL Con A, PBS, or 0.05 mg/mL BSA for different times.

nanoparticles after aggregation-regulated silver enhancement were achieved using a spot test. To get a well-defined glyconanoparticle spot, an amine-modified surface was fabricated. AEPTS was used to modify the glass slides for the preparation of the substrates for the spot test, which led to a contact angle of 78.6° (Figure 3). After 3 μL of the glyconanoparticle solution was spotted on the substrate and dried after being allowed to stand at 25 °C for 20 min, the spot was immensely stable against the washing procedure in silver enhancement, owing to the interaction between the primary amine of AEPTS and the gold nanoparticle surface.[33] Moreover, the substrate without spotting did not exhibit any background during the silver enhancement process.

Visualization of Lectin-Induced Aggregation. The prepared glyconanoparticle solution showed a stable red color, which was the characteristic color of dispersed AuNPs (Figure 4). When Con A was added to the glyconanoparticle solution containing Ca^{2+} and Mn^{2+}, a purple color of the solution could be observed after 90 min, owing to the red-shifted plasmon band of the

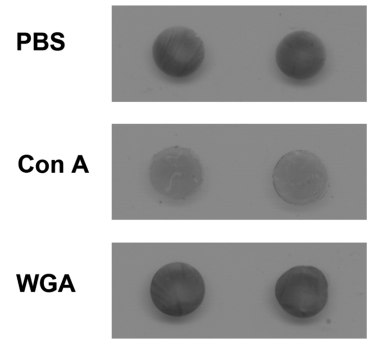

Figure 5. Duplicated scanometric images of the glyconanoparticles incubated with PBS, 2 μM Con A, and 2 μM WGA.

AuNPs. This was a well-understood diagnostic feature of the aggregation process.[34] The aggregate could be observed after incubation for 325 min (Figure 4). However, the glyconanoparticles incubated in PBS or BSA solution in the presence of Ca^{2+} and Mn^{2+} did not show any distinguishable change (Figure 4). The formation of the aggregate was therefore attributed to the cross-linking of mannan-conjugated AuNPs by Con A with four binding sites.

This aggregation process could be easily and quickly visualized by combining an aggregation-regulated silver signal amplification with the spot test. The glyconanoparticle solutions incubated with Con A, WGA (an *N*-acetyl-D-glucosamine-specific lectin), and PBS were spotted on an AEPTS-modified glass slide after mixing for 5 min. After two 3 min silver enhancements, the color of the spots from the sample with Con A was obviously lighter than those of the spots from samples with WGA and PBS (Figure 5). This demonstrated both the efficacy of the silver signal intensity as a signaling tool for the identification of Con A and the recognition specificity of the mannose groups on glyconanoparticles for Con A. Furthermore, the silver-enhanced spot test greatly improved the sensitivity and shortened the assay time. The decrease of the silver signal of the aggregated sample was due to the fact that only the exposed AuNPs could catalyze the reduction of silver ion by hydroquinone to produce the silver staining spot, which led to a much weaker catalytic ability of the aggregate compared to dispersed AuNPs.[28] Now that the silver signal was closely related to the aggregation extent of AuNPs, which depended on the amount of Con A, a facile way to monitor the Con A concentration could be developed.

The silver signal generated on glyconanoparticles during the spot test could be further quantitatively detected by a flatbed scanner associated with Adobe Photoshop.[30] The relative gray scale intensity (I), calculated by subtracting the gray scale intensity value of the spot from that of the background, was used to quantity the silver signal. A higher I corresponded to a darker spot or stronger silver signal.

Optimization of the Silver Enhancement Time, Glyconanoparticle Concentration, and Incubation Time. The intensity of the silver signal was dictated by a variety of experimental parameters, including the silver enhancement time, the concentration of glyconanoparticles, the incubation time of the glyconanoparticles with Con A, and the concentration of Con A. To improve

(33) Lévy, R.; Thanh, N. T. K.; Doty, R. C.; Hussain, I.; Nichols, R. J.; Schiffrin, D. J.; Brust, M.; Fernig, D. G. *J. Am. Chem. Soc.* **2004**, *126*, 10076–10084.

(34) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078–1081.

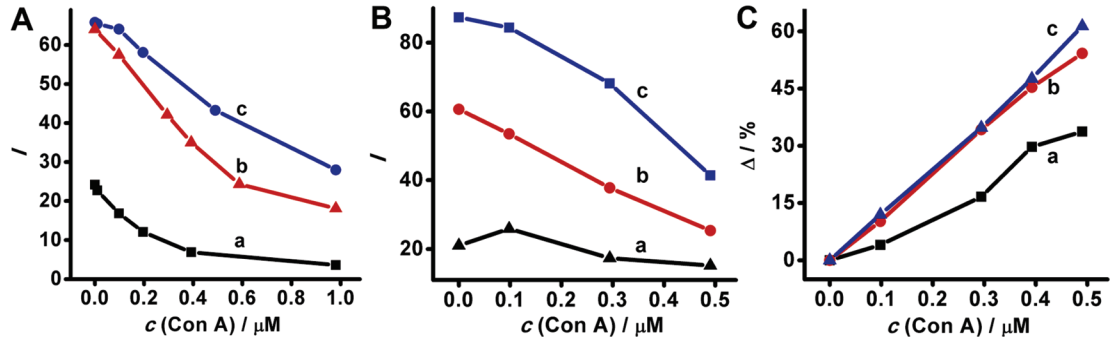

Figure 6. (A) Influence of the Con A concentration on *I* after incubation with 5.7 μL of 12 (a), 36 (b), and 59 (c) nM glyconanoparticles for 5 min and two silver enhancements, (B) dependence of *I* on the Con A concentration after one (a), two (b), and three (c) silver enhancements, and (C) variation percentage of *I* (Δ) vs the Con A concentration after incubation with 36 nM glyconanoparticles for 3 (a), 5 (b), and 7 (c) min.

the detection sensitivity of Con A, the first three parameters were optimized. With an increase of the glyconanoparticle concentration, *I* increased, and a wider linear response range of *I* with the Con A concentration could be obtained at a higher glyconanoparticle concentration (Figure 6A). In comparison with curve b, however, the sensitivity decreased in a certain Con A concentration range (0−0.6 μM) when the glyconanoparticle concentration was higher than 36 nM (Figure 6A, curve c). In the following experiments, a glyconanoparticle concentration of 36 nM was selected to maximize the percentage of variation upon the addition of Con A in a certain Con A concentration range (0−0.6 μM).

In the same range of Con A concentration, *I* increased with increasing enhancement time (Figure 6B). However, three 3 min silver enhancements led to a smaller change with an increase of Con A in a low concentration range (curve c, Figure 6B), which was unfavorable for the subsequent assay of the cell surface carbohydrate. Thus, two 3 min enhancements were selected to provide a linear correlation without sacrificing sensitivity.

The silver enhancement process was regulated by glyconanoparticle aggregation upon addition of lectin. With the longer aggregation time, the silver signal weakened and *I* decreased. However, the percentage change of *I* showed the variation tendency as shown in Figure 6C. At an incubation time of 5 min, the plot of the change percentage vs the Con A concentration showed the maximum slope and was therefore selected for the following assay.

Monitoring of Lectin-Induced Aggregation of Glyconanoparticles. The images of spots from glyconanoparticle solutions with various concentrations of Con A on AEPTS-modified glass slides showed a clear correlation of the darkness of the silver signal with the level of Con A (Figure 7A). Under the optimal conditions, *I* of the silver signal decreased with increasing concentration of Con A with a linear fit and reached a plateau at ~0.6 μM. The linear correlation range was determined to be between 0.1 and 0.6 μM with a correlation coefficient of 0.997 and a detection limit of 39 nM (Figure 7B). The variation of *I* upon addition of lectin for inducing the aggregation of glyconanoparticles was sensitive enough for the subsequent cell surface carbohydrate assay.

Cell Surface Carbohydrate Assay. With these experimental parameters in hand, the applicability of the designed protocol in the assay of the cell surface carbohydrate was examined. Here cell surface mannose motifs acted as the binding partner of Con A for its removal from the incubation mixture. An adhesive human gastric cell line (BGC-823) was chosen as the initial test target. The cells in the exponential growth phase at a certain concentration were first seeded in a culture dish. After incubation for 4 h, all cells were adhered to the dish surface. The cells were washed with PBS and then incubated with 0.5 μM Con A solution for 30 min. A 30.3 μL volume of the supernatant collected after the incubation solution was subjected to centrifugation was mixed with 5.7 μL of 36 nM glyconanoparticles to initialize the aggregation process in the presence of 4 μL of 2 mM Ca^{2+}/Mn^{2+}. Afterward, the spot test was performed.

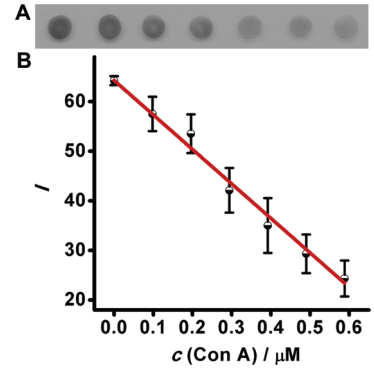

Figure 7. (A) Scanometric images of the glyconanoparticles incubated with 0, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 μM Con A (from left to right) and (B) *I* vs Con A concentration. The error bar represents the SD of six independent experiments.

The silver signals of the spots from the supernatants of four cell samples incubated with 0.5 μM Con A were obviously greater than that from 0.5 μM Con A without incubation in the cell suspension, but lower than that from PBS (Figure 8A). With increasing cell number, the silver signal increased, indicating that more cells caused less Con A to be left; thus, the aggregation extent decreased. The difference of *I* values obtained from the mixtures with and without incubation with cells corresponded to the portion of Con A specifically bound to cell surface mannose units. The amount of cell-bound Con A (*n*) calculated with eq 1 showed a linear relationship with the cell number (Figure 8B). The slope of the plot of *n* vs the cell number represented the average number of Con A molecules bound to each cell, which was equal to the average number of mannose units on a single BGC-823 cell owing to the specific binding of Con A to mannose groups on the cells. Four independent batches of experiments

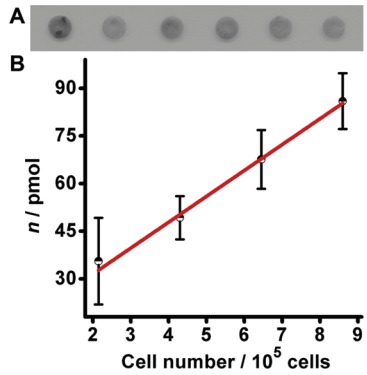

Figure 8. (A) Scanometric images for PBS, 0.5 μM Con A, and supernatants obtained after incubation of 0.5 μM Con A with 8.2 $\times$ 10^5, 6.15 $\times$ 10^5, 4.1 $\times$ 10^5, and 2.05 $\times$ 10^5 cells (from left to right) and (B) amount of cell-bound Con A (*n*) vs the cell number.

indicated that the surface of each cell had (4.5 $\pm$ 0.4) $\times$ 10^7 mannose motifs. This value was in good agreement with that of 5.3 $\times$ 10^7 mannose motifs based on the electrochemical detection of a catalytic product from a lectin-linked enzyme.[14]

Compared with the existing methods for monitoring glycan expression on the cell surface, this strategy offered the following advantages: (1) the proposed method could give the quantitative information of carbohydrate expression on the cell surface, which could not be obtained by currently used lectin-array-based optical methods; (2) the procedure obviated the need for cell pretreatment, such as cell lysis and cell labeling, thus ensuring the cells would be studied in their natural state; (3) the recognition element, lectin, was not labeled to any support such as nanoparticles and signal molecules such as enzymes; thus, the biological activity of the protein could be maintained to the largest extent;[35] (4) the measurement process was convenient and fast, particularly with much less demand on the apparatus and technique; (5) the glyconanoparticle-aggregation-regulated silver enhancement could be seamlessly used to monitor the formation of the lectin-cross-linked glyconanoparticle network, thus providing a novel analytical scheme for lectins.

(35) Jonkheijm, P.; Weinrich, D.; Schröder, H.; Niemeyer, C. M.; Waldmann, H. *Angew. Chem., Int. Ed.* **2008**, *47*, 9618–9647.

CONCLUSIONS

A facile and label-free scanometric approach for in situ cell surface carbohydrate assay has been developed. This approach integrates the bioconjugation and aggregation of glyconanoparticles, the specific recognition of lectin by the carbohydrate on the cell surface and glyconanoparticles, the aggregation-regulated silver signal amplification, and the spot test. The novel glyconanoparticles, mannan-conjugated AuNPs, can be prepared by a one-pot procedure. The specific binding of lectin to a carbohydrate group can be developed for monitoring both lectin and carbohydrate on the cell surface by scanometric detection. The glyconanoparticle-aggregation-regulated silver signal amplification provides the largely noninstrumental method with good sensitivity. The practicality of the proposed method has been demonstrated by quantifying the surface carbohydrate expression on living intact cells. The proposed strategy provides a significant analytical tool to meet the challenges of elucidation of the complex mechanisms underlying carbohydrate-related biological processes.

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