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Design of a "Turn-Off/Turn-On" Biosensor: Understanding Carbohydrate-Lectin Interactions for Use in Noncovalent Drug Delivery

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Supporting Information

ABSTRACT: A low-cost and highly sensitive biosensor system is designed to investigate carbohydrate-lectin interactions. This combination of glyco-gold nanoparticles and boronic acid biosensor system opens a way to study noncovalent drug delivery.

Understanding the binding specificity for carbohydrate-lectin recognition events[1] encompasses the intricate details associated with a cascade of biological events[2] that occur during molecular recognition as well as lectin-prompted drug delivery.[3] Carbohydrate-lectin interactions display extremely low affinity (dissociation constants 10^{-6}–10^{-7} M). To achieve maximum affinity in biological systems, carbohydrates usually display multivalency, allowing appropriate orientation and spacing with respect to corresponding lectin ligand.[4] Artificial multivalent model systems mimic the natural biomolecular systems and allow us to efficiently evaluate carbohydrate-protein interactions. Myriad multivalent model systems have been proposed for the investigation of carbohydrate-lectin interactions.[5] Among them, carbohydrate-functionalized hybrid gold nanoparticles (GNPs) have interested scientists from various disciplines in developing new glyco nanotechnology strategies, which were effectively utilized for biomimetic purposes at the molecular level.[6] Various biosensors based on transduction processes such as electrochemical, electrical, thermal, magnetic, piezoelectric, and optical methods have been employed to address the weak protein-carbohydrate complex interactions.[7] Biosensor techniques are limited due to their low limit of detection (LOD), low sensitivity, and complicated instrumental setup which in turn requires high technical capability.[8] Out of many well-established and explained techniques, calorimetric/optical biosensors are particularly impressive because they offer real-time detection of biological events.[9] Calorimetric bioassay-based experiments rely on aggregation and dispersion of glyco-GNPs.[10] The development of technologically innovative, efficient, highly sensitive, and clinically applicable diagnostic probes is a challenge.[11] A "smart" approach for designing novel diagnostic tools should enable simultaneously the investigation of biomolecular recognition events and efficient drug delivery. In this context, to address the difficulties associated with regular biosensor techniques, herein we design and describe a cost-effective, highly sensitive, robust, and operationally simple optical biosensor that relies on the interactions between multivalent glyco-GNPs and fluorescent boronic acid molecules. This molecular recognition system not only provides details about carbohydrate-lectin binding affinities but also represents an efficient noncovalent glycoconjugate-GNP drug carrier system.

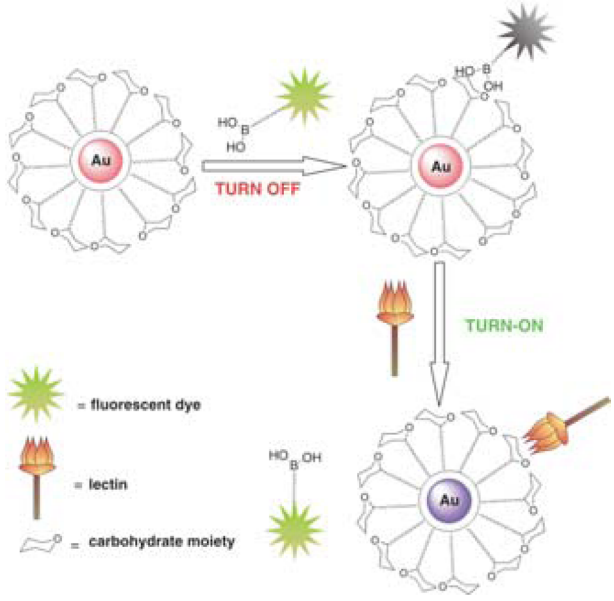

Figure 1. A "turn-off" and "turn-on" system based on glyco-GNP-FB complex to probe carbohydrate-lectin interactions.

Boronic acid forms reversible cyclic esters with diols of monosaccharides, and this unique ability can be exploited in the design of biosensors.[12,13] Several carbohydrate sensors[14] and nucleotide and carbohydrate transporters[15] have been constructed that use boronic acid as their recognition moiety. The majority of reported "turn-off/turn-on" type boronic acid-based biosensors operate on photoelectron transfer (PET), fluorescence resonance energy transfer (FRET), and twisted internal charge transfer (TICT) processes.[16] However, in our system, interaction between fluorescent-dye-tagged boronic acid and carbohydrate-encapsulated GNPs quenches fluorescence through a phase-induced radiative rate suppression phenomenon ("turn-off" process).[17,18] When carbohydrate-specific lectin is subsequently added to the GNP-fluorescent boronic acid complex, the incoming lectin molecules replace the fluorescent

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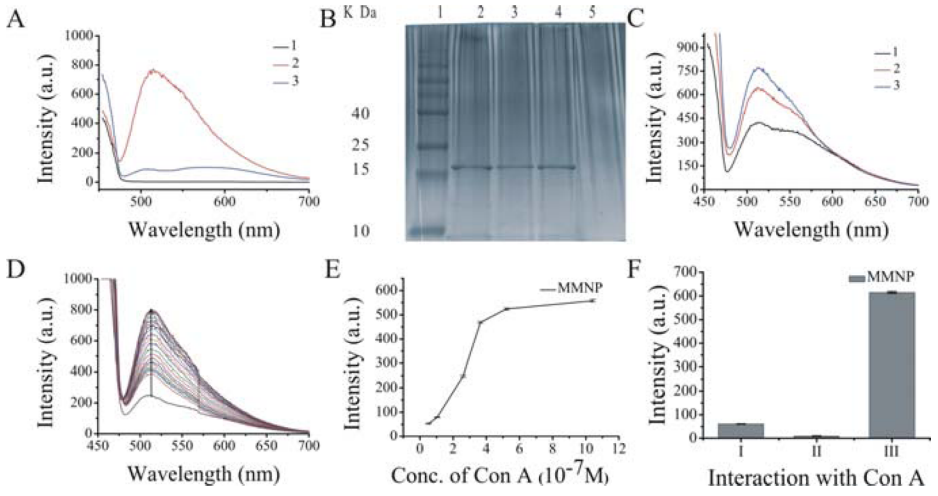

Figure 2. (A) Fluorescence spectra of (1) MMNP solution in 100 μL PBS buffer, (2) 100 μL PBS buffer with 1 μL FB ligand solution (1.04×10^{-4} M), and (3) 100 μL MMNP incubated with 1 μL FB solution (1.04×10^{-4} M). (B) Gel electrophoresis: lane 1, standard fermantas marker; lane 2, 2 μL ConA (1.04×10^{-4} M) in PBS buffer and 2 μL loading buffer; lane 3, 2 μL ConA (5.2×10^{-6} M) in PBS buffer and 2 μL loading buffer; lane 4, MMNP with ConA, 2 μL PBS buffer, 2 μL loading buffer; lane 5, FB-MMNP in 2 μL PBS buffer and 2 μL loading buffer. (C) Fluorescence measurements showing ion effect on the FB-MMNP-ConA system (1) with only Ca^{2+} ions, (2) with only Mn^{2+} ions, and (3) with both Ca^{2+} and Mn^{2+} ions. (D) Fluorescence measurement of ConA-MMNP binding for 2 mg/mL (2.08×10^{-5} M) ConA concentration; no further increment in intensity was observed after 120 min. (E) Fluorescence intensity pattern with ConA concentration showing gradual increase in intensity with increasing ConA concentration. (F) Interaction of different carbohydrate ligands with ConA: (I) β-Glc-Am-MNP, (II) β-Glc-MNP, and (III) MMNP.

boronic acid ligand in the chromophore-GNP complex due to the high interaction affinity between carbohydrate-specific lectin and corresponding carbohydrates. Removal of the fluorescent boronic acid ligand would regenerate the fluorescence of the system ("turn-on" process), vindicating the carbohydrate-protein reciprocal recognition. Besides these interaction studies, the boronic acid-glyco-GNP system was also deployed in Jurkat cell line, where it was found to act as a potent delivery system (Figure 1).

In our initial study, α-mannopyranoside was glycosylated with a thiol-terminated ethylene glycol linkage, and GNPs were functionalized with mannosides with the aid of thiol appendages. MALDI-TOF was used to characterize mannose mono-modified gold nanoparticles (MMNPs). Similarly, fluorescein boronic acid (FB) ligand was synthesized from *p*-tolueneboronic acid. This was followed by incubating FB with MMNPs to obtain FB-MMNPs. Extensive fluorescence studies were carried out to corroborate the significance of boronic acid in FB-MMNP complex (see Supporting Information for detailed experiments). As predicted, fluorescence of FB was quenched when it was incubated with MMNPs (Figure 2A, curve 3). In our study of MMNP-lectin interactions, we used *Concanavalin ensiformis* (ConA), a plant lectin that is widely exploited for studying carbohydrate-lectin interactions. ConA exists as a tetramer at neutral pH and binds selectively with α-mannopyranoside and α-glucopyranoside.[19,20] To confirm that ConA would replace the FB ligand and bind with MMNP, we conducted gel electrophoresis studies, which showed a strong binding affinity between ConA and MMNP (Figure 2B, lane 4). Finally, sensing studies were done to establish the affinity of ConA toward MMNP in replacing the boronic acid from FB-MMNP complex. A 5 μL aliquot of ConA (1 mg/mL devoid of Ca^{2+} and Mn^{2+} ions) was added to the FB-MMNP complex solution. The emission profile showed nominal or negligible growth in the intensity (192 nm) upon excitation of the resultant nanoparticle solution with 514 nm. To circumvent this negligible growth in intensity, the experiment was repeated, supplementing Ca^{2+} and Mn^{2+} ions to the ConA solution. There was a tremendous increase in the intensity (from 192 to 764 au) of FB-MMNP solution, substantiating the significance of Ca^{2+} and Mn^{2+} ions for lectin-carbohydrate binding[21] (Figure 2C, curve 3). It is also worth noting that the immense increase in fluorescence intensity (764 au) in fact competes with that of FB alone (Figure 2A, curve 2), indicating a greater exchange rate between ConA and FB-MMNP, which also results in the discharge of a colossal amount of FB. This clearly establishes the reliable performance of this biosensor system. We further discerned that Mn^{2+} ions augment the lectin-carbohydrate interaction better than Ca^{2+} (Figure 2C, curves 2 and 3). Intricate details associated with ConA-MMNP binding were estimated to assess the LOD of the system, efficiency of the biosensor, and also the performance compared with the earlier reported biosensors. This was done by estimating the minimal ConA concentration required for optimal MMNP-ConA binding.

A series of fluorescence experiments with varying ConA concentrations and time were done to determine the performance efficiency of this biosensor. Initially, 2 mg/mL of ConA was employed to investigate the ConA-MMNP interactions while fluorescence was measured at 5 min intervals. Steady increase in fluorescence intensity was observed until 120 min, at which time the maximum intensity was recorded (802 au, Figure 2D). This indicates that, for a specific concentration of ConA, after a specified time, the binding interactions become saturated. Similarly, 1, 0.7, 0.5, 0.1, and 0.01 mg/mL concentrations of ConA were employed to measure the fluorescence intensity (see the Supporting Information). Similar trends in fluorescence intensity were observed for each specific concentration of ConA. A gradual decrease in the intensity pattern (802 to 30 au) was also observed with decreasing ConA concentration (2 to 0.01 mg/mL, Figure 2E). Based on our meticulous experiments, it was deduced that 0.01 mg/mL of ConA can successfully swap the FB from FB-MMNP complex, suggesting that this biosensor system could detect a 4.9 nM ConA concentration, its maximum LOD. A detailed LOD calculation is provided in the Supporting

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Information. This value competes fairly with other techniques generally employed for carbohydrate-lectin interactions,[22] and our biosensor is highly sensitive when compared with earlier reported calorimetric-based biosensor technologies. Additionally, we investigated the interaction between MMNPs and other plant lectins, such as peanut agglutinin and lectins from *Triticum vulgaris*. These lectins did not show any response to MMNP (Figure 2F), in agreement with already established studies.

The imperative presence of Mn^{2+} and Ca^{2+} ions for proper protein-carbohydrate interactions was validated using regeneration tests. Experimental details and discussion are provided in the Supporting Information.

This successful technique to establish binding interactions between MMNPs and ConA was also used to study other carbohydrate-lectin interactions. Other saccharide interactions, like β-glucose-MNP and β-glucosamine-MNP, were studied. However, no such interactions were observed between β-glucose-modified gold nanoparticles (Gl-MNP) or β-glucosamine-modified gold nanoparticles (GlAm-MNP) and ConA.

We also extended this biosensor system to hybrid nanoparticle systems or mixed monolayer protected clusters (MMPCs). MMPCs are GNPs grafted with more than one kind of carbohydrates. The advantages associated with these dimodified GNPs for studying carbohydrate-lectin interactions are the following: (a) Difunctionalized GNPs mimic natural cell systems (cells contain more than one kind of carbohydrate on their surface); hence, results on the lectin-sugar interaction would be more accurate. (b) Concentration of the lectin helps in estimating the amount of different carbohydrates adsorbed on each dimodified gold nanoparticle (DNP), which could in turn be useful for estimating the amount of carbohydrate present in a particular cell system. To study these MMPCs, we investigated three kinds of mannose-glucose DNPs: 1:1 mannose-glucose-DNP (1:1 MG-DNP), 1:5 mannose-glucose-DNP (1:5 MG-DNP), and 1:10 mannose-glucose-DNP (1:10 MG-DNP). DNPs were synthesized in such a way that the total amount of carbohydrates was kept constant while incubating with gold nanoparticles, and in each type of DNP, the percentage of mannose decreased from 1:1 to 1:10 MG-DNPs.

MG-DNPs were incubated with FB as described earlier to obtain a FB-mannose-glucose-dimodified gold nanoparticle complex (FB-MG-DNP). Each type of FB-MG-DNP complex (1:1, 1:5, and 1:10) was tested with different concentrations of ConA. We conjectured that there should be a steady decrease in fluorescence as the mannose concentration is decreased from 1:1 to 1:10 MG-DNPs. However, the fluorescence intensity behavior from 1:1 to 1:10 MG-DNPs revealed was interesting. Fluorescence intensity from 1:1 to 1:5 decreased as expected because of the decrease in mannose concentration on MG-DNP. Surprisingly, fluorescence increased from 1:5 to 1:10. It was anticipated that, due to the extremely low concentration of mannose on 1:10 MG-DNP, the fluorescence intensity would be lower than that of 1:5 MG-DNP; however, in this case fluorescence intensity was increased. For instance, intensity increased from 295 to 311 au at a ConA concentration of 3.12×10^{-5} M (Figure 3A). A similar increasing tendency was observed with two other ConA concentrations, 2.08×10^{-5} and 1.04×10^{-5} M. This observation is quite unusual, and intense molecular level experiments are needed to understand the complex interactions between mannose and ConA. To understand this phenomenon more precisely, we synthesized 1:1, 1:5, and 1:10 mannose-glucosamine-dimodified GNPs (MGlAm-DNPs) and repeated the sensing experiments (experiment details are

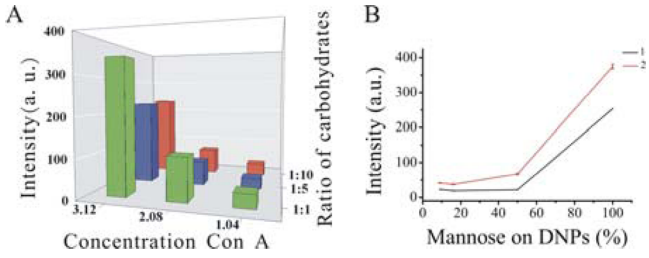

Figure 3. (A) Fluorescence intensity patterns of 1:1, 1:5, and 1:10 MG-DNPs with different ConA concentrations. (B) Fluorescence intensity response curves (each curve is obtained by plotting different concentrations of 1:0, 1:1, 1:5, and 1:10) of MG-DNPs and MGlAm-DNPs in (1) 1.04×10^{-5} and (2) 2.08×10^{-5} M ConA.

provided in the Supporting Information). These experiments showed a similar fluorescence tendency from 1:5 to 1:10 MGlAm-DNPs. For 3.12×10^{-5} M ConA, the intensity increased from 283 to 291 au. The combined fluorescence intensity results of MG-DNP and MGlAm-DNP were plotted against percentage of mannose linkers, which ultimately furnished the concentration of mannose present on a particular DNP system (Figure 3B). This technique is in turn highly beneficial in measuring the amount of a particular carbohydrate on a cell system or artificial cell system such as carbohydrate-encapsulated nanoparticles/quantum dots. These carbohydrate-encapsulated NPs mimic natural cell systems for our understanding of molecular-level cellular communication and interactions.

Their inertness, nontoxic nature, and ease of synthesis over a wide range of diameters (1−150 nm) make GNPs potential drug carrier candidates to unload various payloads into the target.[23] The large surface volume of smaller GNPs enables them to functionalize with small drugs, large biomolecules such as nucleic acids, genes, and proteins, which in turn would result in high payload/carrier ratios and site-specific delivery.[24] Synthesis of mixed-monolayer protected clusters (MMPCs) and release of payload by the "place-exchange reaction" mechanism proved to be an efficient delivery system.[25] However, the presence of cationic ligands over MMPCs is mandatory, as it would facilitate the passage through the cell-membrane barrier.[26] Recently, Raines et al. showed that pendant boronic acids enable the cytosolic delivery of protein into the cells, and these boronates represent a new class of noncationic carrier systems.[27] We anticipated that interactions between FB-MMNP complex and the glycocalyx of the cell surface could facilitate the entry of model hydrophobic drug. Jurkat human T lymphoma cells were incubated with FB-MMNP solution for 30 min to understand the function of the MMNP biosensor. Confocal images showed the absorbance of fluorescein boronic acid by the cells. Control experiments were also performed in order to understand the bioassay analysis and to confirm the presence of fluorescence in the cells is due to the FB-MMNP complex. In this control experiment, cells were incubated with molecular fluorescein boronic acid only, and no fluorescence was detected in these control cells, indicating that the payload dye (fluorescein boronic acid) has not been released into the cells. Thus, we infer that the combination of FB-MMNP could only facilitate the FB to transmit into the cell culture (Figure 4). We reasoned that, when cells were incubated with FB-MMNP system, ConA present on the cell surface interacts with FB-MMNP, and there will be binding between cell surface ConA and MMNP. This would sequentially eliminate FB from the FB-MMNP complex. Subsequent boronate ester formation between glycocalyx

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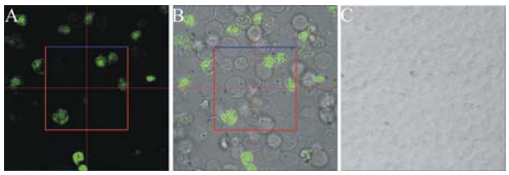

Figure 4. Multiplexed confocal analysis of Jurkat cells incubated with FB-MMNP: (A) Jurkat cells after incubating with FB-MMNP at 454 nm; (B) cell culture; and (C) Jurkat cells after incubating with FB at 454 nm (control experiment).

carbohydrate 1,2-diols and the released FB molecules would further trigger the cellular uptake of hydrophobic dye. However, the delivery of the dye largely depends on the affinity of the boronic acid and the relevant carbohydrates prevalent on the cell-surface glycocalyx.

In conclusion, we have demonstrated a novel "turn-off/turn-on" biosensor detection system employing fluorescein boronic acid and carbohydrate-modified gold nanoparticles. Salient features of this biosensor include that it (a) operates on simple fluorescence spectroscopy and hence is cost-effective and operationally simple; (b) is highly sensitive, with a LOD found to be 4.9 nM; (c) is rapid and robust, allowing observation of fluorescence intensities within a few seconds; (d) provides visible evidence for carbohydrate-lectin interaction; (e) employs dicarbohydrate-modified gold nanoparticles, which enables it to calculate the amount of specific carbohydrate adsorbed on a unknown cell system; and (f) could be employed as a noncovalent drug carrier.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and additional data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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