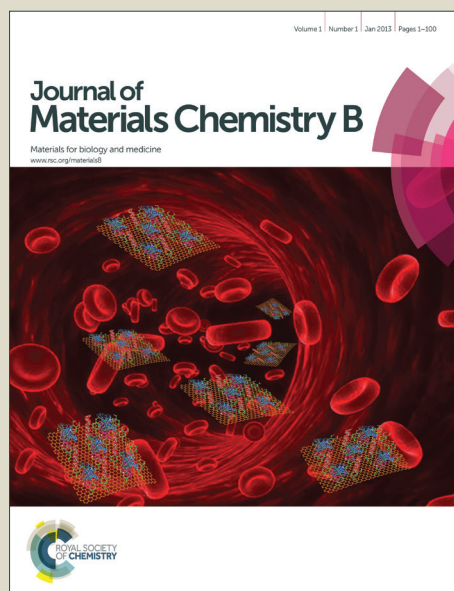


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ARTICLE

Signal amplified two-dimensional photonic crystal biosensor immobilized with glyco-nanoparticles

Cite this: DOI: 10.1039/x0xx00000x

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Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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A two-dimensional, glycopolymer-immobilized, photonic crystal (PhC) biosensor was developed for the detection of proteins. Glycopolymers with different conformations, homopolymers and sugar-incorporating nanoparticles were immobilized on the PhC using intermediate succinimide-containing polymers and proteins. The surface modification was analyzed in detail, and the sugar-protein interaction was detected by monitoring changes in the reflection intensity that was expressed by the two-dimensional PhC. The surface modifications were performed successfully, and specific interactions were detected between the glycopolymers and the proteins. Stronger bonds were present between the glycopolymers and the target proteins than between the glycopolymers and the monovalent sugar, because of a clustering effect. The sugar-incorporating nanoparticles showed a larger binding capacity compared with the homopolymers, and low protein concentrations (with a detection limit of 6.0 ng mL⁻¹) were detected using the sugar-incorporating nanoparticle-immobilized PhC. The detection limit of the developed biosensor was lower than that of surface plasmon resonance sensor (1.43 μg mL⁻¹). The results of this study indicated the potential of the developed biosensor for the detection of a variety of biomolecules.

1. Introduction

There is an increasing need for accurate biosensors that can detect the infection disease, cancer and pathogens, and the biosensors are also useful for the basic biological science. Biosensors typically consist of two parts; the detection part and the molecular recognition part. Recently, various detection methods are utilized for the biosensor such as optical analyses (e.g. fluorescence¹, surface plasmon², structural color³ etc), electrochemistry⁴, quartz crystal microbalance⁵ and field emission transistor.⁶ Among them, optical analyses are promising due to the high sensitivity and the recent development of the laser technology. Structural colors are one of the optical analysing methods that enable the simple and rapid detection of the target molecules.³ Because of dye-free color creation, the production of durable colors with long-term retention properties is possible. There have been several ambitious reports for biosensor using structural colors of photonic crystals (PhCs).⁷⁻¹⁷ Asher et al. reported PhC biosensors using crystalline colloidal arrays,¹⁸ and Takeoka et al. reported colorimetric glucose sensors using inverse opal hydrogels.¹⁹ In the former reports, they prepared the PhCs, which was successful in the biosensing, however, at the same time, preparing the PhCs is tedious for preparation of versatile sensing devices.

On the other hand, our group previously reported PhC biosensors based on the structural color of nano-imprinted two-dimensional PhCs, where the two-dimensional PhCs could be produced easily and utilized by a simple modification step.²⁰ We have also reported the detection of antigen-antibody reactions using two-dimensional PhCs.²¹ Therefore, the nano-imprinted two-dimensional PhCs is a novel and convenient materials for biosensing based on the structural color. One important thing in PhCs is the appropriate surface modification for immobilization of biomacromolecules. So far we have also reported the surface modification using polymers, where we have achieved the protein detection by glycopolymers.²² In the reports, the modification of various materials with glycopolymers such as gold (substrates²³ and nanoparticles²²) and siliceous materials (glass,²⁴ silica,^{25,26} and silicon wafers²⁷) was achieved.

In the view of molecular recognition for biosensing, the glycopolymers are attractive, because sugars play important roles *in vivo*. It is known that sugars bind specifically to proteins.^{28,29} It has been discovered that sugar-protein interactions are relevant to various vital phenomena, including pathogenic infections, the metastasis of cancer, and cell adherence.³⁰ In human bodies, sugars exist in domains such as glycoproteins and glycolipids. These compact, dense sugar

structures result in stronger interactions between the sugars and proteins; this is known as the cluster effect.³¹ The cluster effect can be enhanced via the use of synthesized glycopolymers. Because glycopolymers are capable of binding with proteins strongly and specifically, they have the potential as highly effective molecular recognition components in biosensors. We have previously reported glycopolymers and sugar-incorporating polymer nanoparticles that interact with target molecules strongly and specifically.²²⁻²⁷

In this study, we developed the two-dimensional PhCs for biosensing. In our previous work, we immobilized succinimide-containing polymers on two-dimensional PhCs.^{20,21} In this study, the surface modification of two-dimensional PhCs was analyzed in detail. Concanavalin A (ConA) was used as a target protein. Mannose was used to achieve the specific recognition of ConA. It is known that ConA binds specifically to mannose.^{32,33} Two different types of polymer—specifically, mannose homopolymers (**ManHP**), and mannose-incorporating nanogel particles (**ManNP**)—were synthesized. These mannose-incorporating polymers were successfully immobilized on the nano-imprinted cyclo-olefin polymer film (PhCs film) using an intermediary consisting of the succinimide-containing polymer and the ConA. The strong and specific interactions between the mannose-incorporating polymers and the ConA were detected by monitoring the changes in the reflection intensity of the PhCs film. Because sugars act as ligands for various proteins, bacteria, and viruses, sugar-incorporating polymers can be used for the recognition of various kinds of biomolecules.³⁴⁻³⁶ The results of this study suggested that the developed biosensor has great potential, and could be applied for the development of PhC biosensors.

2. Experiments

2.1 Reagents

The following chemicals were obtained from commercial sources, and were used as received: *N*-acryloxysuccinimide (NAS), *N,N'*-methylenebisacrylamide (BIS), and 3,3'-dithiodipropionic acid were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan); (trimethoxysilyl) propyl methacrylate (TMSMA), poly ethylene glycol ethyl ether methacrylate (PEGMA), and bovine serum albumin (BSA) were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA); azobisisobutyronitrile (AIBN), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMAc), *N,N*-dimethylsulfoxide (DMSO), sodium methoxide, acrylic acid (AAc), *N-tert*-butylacrylamide (TBAm), sodium dodecyl sulfate (SDS), and 4,4'-azobis(4-cyanovaleric acid) (V-501) were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); ConA was obtained from J-Oil Mills, Inc. (Tokyo, Japan); and *N*-hydroxysuccinimide was obtained from Kishida Chemical Co., Ltd. (Osaka, Japan). *N*-Isopropylacrylamide (NIPAm) (Wako Pure Chemical Industries, Inc., Osaka, Japan) was recrystallized from hexane.

The water used in this study was purified using a Direct-Q Ultrapure Water System (Merck, Ltd., Darmstadt, Germany).

2.2 Apparatus

The hydrodynamic diameter of the **ManNP** was determined using dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments Ltd, Worcestershire, UK.). The polymer molecular weight was determined using gel permeation chromatography (GPC), with the chromatograph connected to a Shodex LF804 column (Showa Denko KK, Kanagawa, Japan). Nano-imprinted cyclo-olefin polymer films were purchased from Scivax Corp., Kanagawa, Japan. An atomic force microscope (AFM) (Bruker Corp., Phoenix, AZ, USA) (AFM1) with a cantilever (NCHV-10V; Veeco Instruments Inc., NY, USA) was used to evaluate the morphology of the PhCs film. To evaluate the holes on the PhC film surface, a commercial AFM unit (SPA-400; Seiko Instruments, Inc., Chiba, Japan) (AFM2) with a calibrated PZT scanner (20nm xy-scan and 10-nm z-scan ranges) and a cantilever (SI-DF20; Hitachi High-Tech Science Corp., Tokyo, Japan) were used. The existence of functional groups and polymers on the PhCs films was confirmed using C(1s) and N(1s) X-ray photoelectron spectroscopy (XPS) (AXIS-ultra; Shimadzu/Kratos, Kyoto, Japan). A 27-MHz quartz crystal microbalance (QCM) system (Affinix Q4; Initium Inc., Kanagawa, Japan) was used to quantify the interactions between the mannose-incorporating polymer and the ConA. For the evaluation of the optical characteristics, a UV-VIS spectrophotometer (USB-4000-UV-VIS, wavelength range: 200–1100 nm), a tungsten halogen light source (LS-1, wavelength range: 360–2000 nm), and an optical fiber probe bundle (R-200-7-UV-VIS, fiber core diameter: 200 nm, wavelength range: 250–800 nm) were purchased from Ocean Optics (Dunedin, FL, USA).

2.3 Synthesis of poly(NAS-*r*-PEGMA-*r*-TMSMA)

To achieve the synthesis of poly(NAS-*r*-PEGMA-*r*-TMSMA), first, a Schlenk flask was heated and subjected to negative pressure to remove any water. The air in the Schlenk flask was then nitrogen-substituted twice. After the Schlenk flask was cooled to room temperature, 3 mmol of NAS, 3 mmol of PEGMA, 3 mmol of TMSMA, and 10 mL of DMF were added to the Schlenk flask. Nitrogen was bubbled through the reaction mixture for 30 min. Following the addition of 0.1 mmol of AIBN, the polymerization was carried out at 70°C for 20 h (Scheme 1).

2.4 Synthesis of ManNP

p-Acrylamidophenyl- α -D-mannopyranoside (**Man**) was synthesized using an existing method (Scheme 2).^{25,37} **Man** (1.5 mol%), NIPAm (63.5 mol%), TBAm (20 mol%), BIS (5 mol%), AAc (5 mol%), and 2.07 μ M of SDS were dissolved in 30 mL of water, resulting in a total monomer concentration of 52 mM. Nitrogen was bubbled through the reaction mixture for

30 min. Following the addition of V-501, the polymerization was carried out at 70°C and for 3 h under a nitrogen atmosphere. The polymerized solution was purified using dialysis (molecular weight cut off: 100,000) against an excess of water. The yield of **ManNP** was determined by measuring the weight of nanoparticles obtained via the lyophilization of 10 mL of the dialyzed solution (160 mg, 85%). The diameter of the **ManNP** was determined to be in the range of 80–200 nm, using DLS.

2.5 Synthesis of ManHP

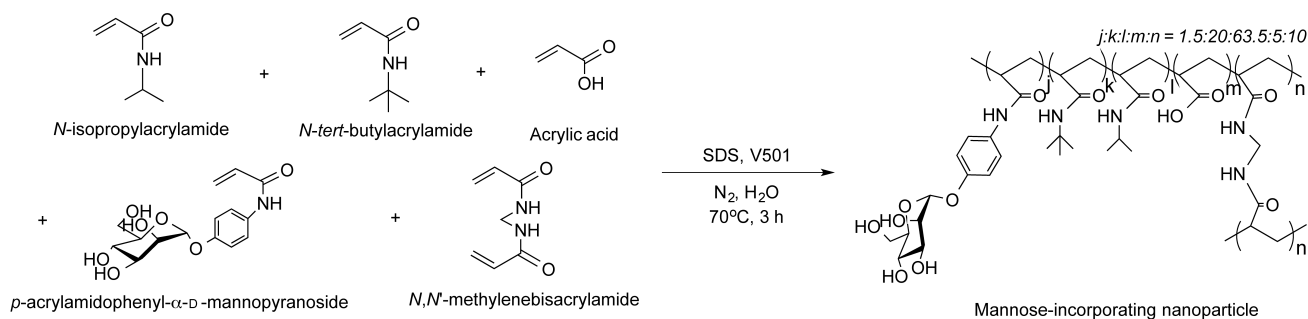
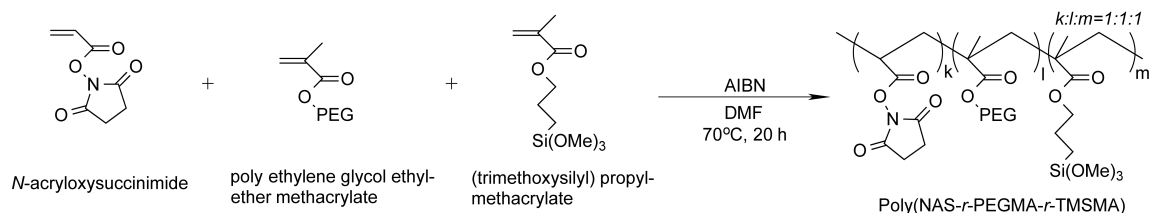
p-Acrylamidophenyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside (**AcMan**) was synthesized using an existing method.²³ 0.75 mmol of **AcMan** monomer and 15 μ mol of AIBN were dissolved in 400 μ L of DMAc. The polymerization was carried out at 70°C and for 3 h under a nitrogen atmosphere. The solution was vacuum dried to remove any DMAc. The remaining solute was then dissolved into DMSO, and the solution was purified using dialysis (molecular weight cut off: 1,000) against an excess of DMSO. The solution was vacuum dried to remove any DMSO. The remaining solute (**AcManHP**) was then dissolved into MeOH, and sodium methoxide was added until the pH of the solution increased to 10. After the solution was stirred for 1 h, the solution was vacuum dried to remove any MeOH. The molecular weight of the **AcManHP** was obtained using GPC (M_w : 2.01×10^5 , M_n : 1.29×10^5 , $M_w/M_n = 1.57$), and the molecular weight of the **ManHP** was calculated from the molecular weight of the

2.7 Immobilization of mannose-incorporating polymers on the PhCs films

The mannose-incorporating polymers were immobilized on the PhCs films as shown in Fig. 1. Ultraviolet/ozone treatment was performed on the films for 30 min, to form hydroxyl groups. The films were immersed in a poly(NAS-*r*-TMS-*r*-PEGMA) solution (10 g L⁻¹ in DMF) at room temperature for 1 h, and the solution was then heated to 38°C and maintained at this temperature for 1 h. The films were washed with DMF, and then cured at 120°C for 5 min. The resulting films were immersed in a 600 nM ConA solution for 1.5 h. After the films were washed with water, they were dried by air blow, and then immersed in a 30 μ g mL⁻¹ **ManHP** or **ManNP** solution for 1.5 h. The films immobilized with the mannose-incorporating polymers were then washed with water to remove any non-immobilized polymers, and then dried by air blow. After each step, the morphology was observed using AFM and XPS. Each AFM observation was replicated using two different AFM instruments. AFM1 was used to evaluate the morphology of the PhCs films, and AFM2 was used to evaluate the detailed size of holes in the PhCs films.

2.8 Analysis of the binding between the mannose-incorporating polymer and the proteins, using QCM

3,3'-Dithiopropionic acid (2 μ L, 100 mM in ethanol) was loaded into the QCM cells and incubated for more than 30 min. The cells were washed with pure water, and the carboxylic acid



AcManHP (M_w : 1.33×10^5 , M_n : 0.85×10^5 , $M_w/M_n = 1.57$).

groups on the gold electrode surfaces were activated for 30 min by loading 0.1 mL of a 1:1 v/v aqueous solution of 1-ethyl-3-

(3-dimethylaminopropyl)carbodiimide (100 mg mL⁻¹) and *N*-hydroxysuccinimide (100 mg mL⁻¹) to form *N*-hydroxysuccinimidyl esters. Ten millimoles of HEPES buffer (pH 7.4, 137 mM NaCl, 2.68 mM KCl, 1.8 mM CaCl₂, and 0.49 mM MgCl₂, 500 μL) was added to the cells, and the cells were set on the QCM system. After the oscillation frequency was stabilized, the ConA solution was injected into the cell with the activated electrodes (to give a concentration in the cell after injection of 600 nM). After rinsing the ConA-immobilized cells using the 10 mM HEPES buffer (to remove the non-immobilized Con A), the cells were filled with the buffer (500 μL) and set on the QCM system. After the stabilization of the oscillation frequency, the **ManHP** or **ManNP** solutions were sequentially injected into the cells (concentration in the cells: 1–50 μg mL⁻¹). After rinsing the mannose-incorporating, polymer-immobilized cells to remove the non-immobilized polymers, the cells were filled with the HEPES buffer, and then set on the QCM system. The ConA solution was sequentially injected into the cells, and the QCM frequency change was monitored for more than 45 min (concentration in the cells: 5–360 nM).

2.9 Detection of protein adsorption on the mannose-incorporating polymers via monitoring of the changes in the reflection intensity of the PhCs film.

To evaluate the reflection intensity of the PhCs films, all reflection spectra were obtained in the wavelength range of 350–800 nm, using a UV/VIS spectrophotometer at room temperature under dry conditions. The PhCs films were irradiated vertically with white light from the optical fiber bundle. The reflected light was coupled to the detection fiber probe of the optical fiber bundle, and analyzed using UV/VIS spectrophotometry, using OOIBase32 operating software. The changes in the reflection intensity were directly related to the concentration of protein added to the mannose-incorporating polymer-immobilized PhCs films.

Protein adsorption on the mannose-incorporating polymer-immobilized PhCs films was detected using this detection setup. In the blank measurements, the surfaces of the PhCs films were immobilized with mannose-incorporating polymers. The films were then immersed in solutions with various

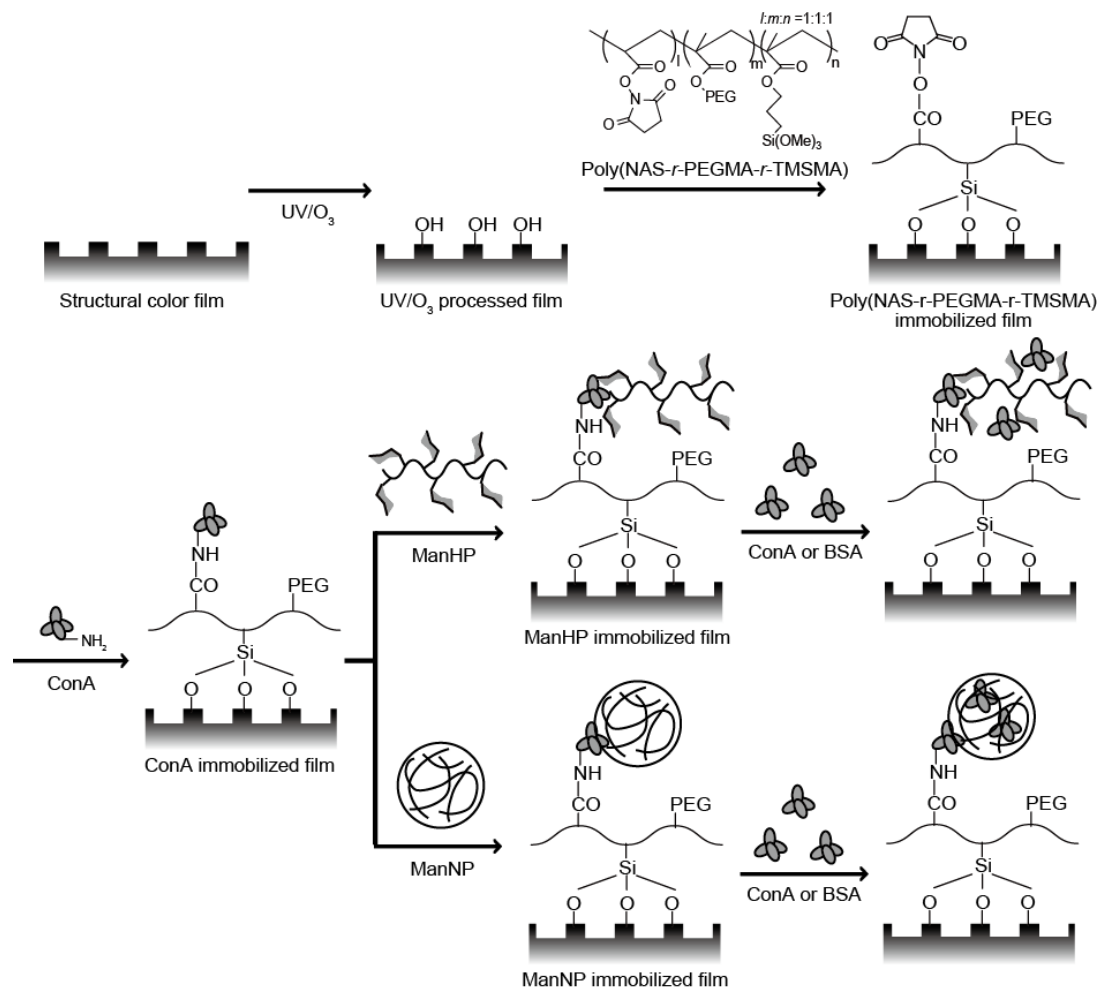


Fig. 1 Immobilization of mannose-incorporating polymer on the PhCs films, and the adsorption of proteins.

concentrations of ConA or BSA for 2 h, at room temperature (protein concentrations of 10^{-8} – 10^0 g L⁻¹). After the films were washed with water, they were dried by air blow, and the reflection intensity of the films was determined

3. Results and discussion

3.1 Surface analysis of the PhCs films using XPS

The surfaces of the PhCs films were measured using XPS (Fig. 2 and 3).^{23–26,38–40} In the C(1s) spectra of the UV/O₃-treated film, peaks corresponding to C-H and C-C bonds (285.0 eV), and C-O bonds (286.0 eV) were observed.³⁸ In the C(1s) spectra of the poly(NAS-*r*-PEGMA-*r*-TMSMA)-immobilized film, peaks corresponding to C-H, C-C and C-Si bonds (285.0 eV), C-O bonds (286.0 eV), and C=O bonds (288.0 eV) were observed. In the C(1s) spectra of the ConA-immobilized film, peaks corresponding to C-H and C-C bonds (285.0 eV), C-O bonds (286.0 eV), C-N bonds (287.0 eV), and C=O bonds (289.0 eV) were observed. In the C(1s) spectra of the **ManHP**-immobilized film, peaks corresponding to C-H and C-C bonds (285.0 eV), C-O bonds (286.0 eV), C-N bonds (287.0 eV), and C=O bonds (288.0 eV) were observed.^{25,26,39,40} In the C(1s) spectra of the **ManNP**-immobilized film, peaks corresponding to C-H and C-C bonds (285.0 eV), C-O bonds (286.0 eV), C-N bonds (287.0 eV), and C=O bonds (288.0 eV) were observed.

In the N(1s) spectra of the poly(NAS-*r*-PEGMA-*r*-TMSMA)-, ConA-, and **ManNP**-immobilized films, the intensity of the peak corresponding to N-H and N-C bonds (400.5 eV) increased as the immobilization step proceeded. In the C(1s) spectra of the UV/O₃-treated PhC film, the presence of C-H and C-C bonds, which were attributed to the cyclo-olefin polymers, and C-O bonds, which were attributed to hydroxyl groups, was confirmed. In the poly(NAS-*r*-PEGMA-*r*-TMSMA) immobilized film, C=O bonds were newly observed. This suggested that the poly(NAS-*r*-PEGMA-*r*-TMSMA) was immobilized on the film. Moreover, all the peaks corresponding to bonds included in poly(NAS-*r*-PEGMA-*r*-TMSMA) except N-C bonds were confirmed. The peak corresponding to N-C bonds was not confirmed because the succinimide groups were hydrolyzed before the XPS measurements. Peaks corresponding to N-C and N-H bonds were observed in the ConA-immobilized-film; these peaks were not observed for the poly(NAS-*r*-PEGMA-*r*-TMSMA)-immobilized film. The peaks corresponding to N-C and N-H bonds were observed because of the amine groups in the ConA. In fact, these results suggested that the immobilization of ConA on the film had been achieved. In the **ManHP**- and **ManNP**-immobilized films, the intensity of the peaks corresponding to the N-C and N-H bonds increased by approximately three times compared with the ConA-immobilized film. This was due to the presence of the Man monomers, NIPAm, TBAm, and BIS. Furthermore, all the peaks corresponding to bonds included in **ManHP** and **ManNP** were confirmed. This indicated that the immobilization of **ManHP** and **ManNP** on the films had been

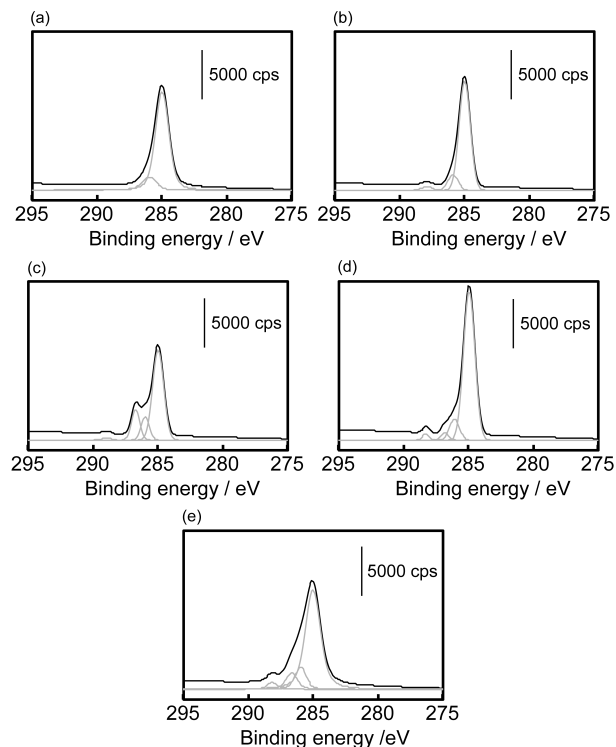


Fig. 2 C(1s) spectra for (a) UV/O₃-treated (b) poly(NAS-*r*-PEGMA-*r*-TMSMA)- (c) ConA-, (d) ManHP-, and (e) ManNP-immobilized PhCs films.

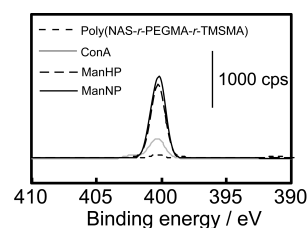


Fig. 3 N(1s) spectra for poly(NAS-*r*-PEGMA-*r*-TMSMA)-, ConA-, ManHP-, and ManNP-immobilized PhCs films.

achieved. These results indicated that each immobilization step was successfully achieved.

3.2 Surface analysis of PhCs films using AFM

The morphology of the PhCs film surfaces was determined using AFM (Fig. 4). The unmodified structural-color film showed a flat surface with hole patterns (Fig. 4(a)). The average diameter of the holes was approximately 230 nm. The holes were arranged regularly at intervals of 150 nm. The poly(NAS-*r*-PEGMA-*r*-TMSMA)-immobilized PhC film showed a rounded surface with hole patterns (Fig. 4(b)). The ConA-immobilized film showed a rounded and rough surface with hole patterns (Fig. 4(c)). The **ManNP**-immobilized film showed a rough surface with hole patterns (Fig. 4(d)).

The regularly arranged hole patterns on the surface of the unmodified PhCs film were observed in our previous work.¹⁵ The surface of the poly(NAS-*r*-PEGMA-*r*-TMSMA)-

immobilized film was more rounded than the surface of the unmodified PhCs film. This drastic change in the surface morphology suggested that the poly(NAS-*r*-PEGMA-*r*-TMSMA) was immobilized on the PhCs film. The ConA-immobilized PhCs film also showed a rounded surface, but wrinkles were observed. The appearance of the wrinkles suggested that the ConA had been immobilized on the film. The **ManNP**-immobilized film showed an extremely rough surface. Compared with the ConA-immobilized PhCs film, the morphology of the surface showed dramatic changes; this suggested that the immobilization of **ManNP** had been achieved. Furthermore, holes with a diameter of 230 nm and a depth of 200 nm were clearly observed in the unmodified, **ManHP**-immobilized and **ManNP**-immobilized PhCs films using the stronger tapping mode of AFM (Fig. S1 and S2 in Supporting Information). The continued presence of the holes suggest that the exhibition of structural color was possible even after the immobilization of the mannose-incorporating polymers. When combined, the AFM and XPS results confirmed the immobilization of each material on the structural color-PhCs films, and the exhibition of structural color after the immobilization of the mannose-incorporating polymers.

3.3 Analysis of the interaction between the mannose-incorporating polymers and proteins using QCM

The interactions between the mannose-incorporating polymers and proteins were confirmed by measuring the frequency change of a QCM (Fig. 5 and Fig. S3 in Supporting information). After the addition of ConA, a concentration dependency in the frequency change was observed for both the **ManHP**- and **ManNP**-immobilized QCM electrodes. For both the **ManHP**- and the **ManNP**-immobilized electrodes, the frequency change increased until a concentration of 240 nM was reached; no further frequency changes were observed as the ConA concentration was increased further. The frequency change was 1.5 times larger for the **ManNP** electrode than for the **ManHP** electrode. The K_a values between the mannose-incorporating polymers and the ConA were estimated using Langmuir's equation. The K_a values for **ManHP** and **ManNP** were calculated as $1.08 \times 10^7 \text{ M}^{-1}$ and $1.87 \times 10^7 \text{ M}^{-1}$, respectively. No frequency change was observed with the addition of BSA, for both the **ManHP**- and the **ManNP**-immobilized QCM electrodes.

The K_a values for the **ManHP** and **ManNP** electrodes were larger than that for methyl-**Man**, which is on the order of $10^3 - 10^4 \text{ M}^{-1}$.⁴¹ It is known that sugars can enhance the strength of the interactions with proteins via the cluster effect. We believe that the K_a value was higher in the mannose-incorporating polymers than in monovalent mannose because of the cluster effect. Comparing **ManHP** and **ManNP**, no large difference in the K_a values was observed. The number of mannose molecules per gram was 25 times higher in **ManHP** compared with **ManNP**, despite that fact that **ManNP** had fewer binding sites than **ManHP**. Because **ManHP** had a higher density of

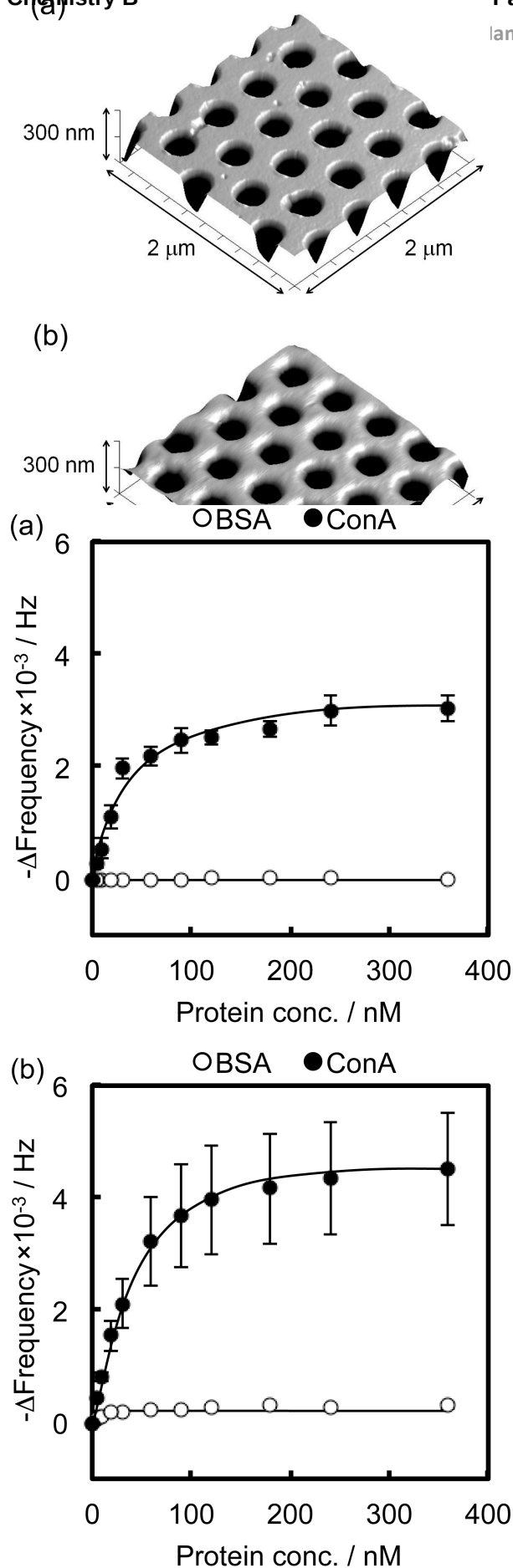


Fig. 5 QCM frequency changes produced by the adsorption of proteins on (a) **ManHP**- and (b) **ManNP**-immobilized Au electrodes.

mannose molecules compared with **ManNP**, the cluster effect could be observed much easier in **ManHP**; however, there was no difference between the K_a values for **ManHP** and **ManNP**. It is believed that **ManNP** interacted with ConA three-dimensionally. The diameter of **ManNP** was in the range of 80–200 nm. ConA has a tetrahedral structure with four mannose-binding sites on each corner, and the length of each side is 10 nm.⁴² **ManNP** brought out better binding ability compared to **ManHP**, because **ManNP** trapped ConA in the 3D network of polymer chains and bound to ConA more efficiently than **ManHP**, at multiple sites. For both polymers, no frequency change was observed with the addition of BSA. No non-specific interactions were observed between the BSA and the mannose-incorporating polymers. This was attributed to the hydrophilization of the PhCs film surface. Sugar-immobilized surfaces are typically hydrophilized, and thus prevent non-specific interactions.^{43–45} These results suggested that there were strong and specific interactions between the mannose-incorporating polymers and the ConA. On the other hand, **ManNP** showed the larger error bars than **ManHP**, which is discussed in the later section.

3.4 Detection of protein adsorption on mannose-incorporating polymers via monitoring of the changes in the reflection intensity of the PhCs film.

To achieve the detection of proteins using the mannose-incorporating polymer-immobilized PhCs films, the changes in the reflection intensity of the PhCs films were measured (Fig. 6). After the addition of ConA, concentration-dependent changes in the reflection intensity were observed for both the **ManHP**- and **ManNP**-immobilized PhCs films. In the **ManHP**-immobilized film, the reflection-intensity increased linearly, from approximately 10^{-4} g L⁻¹ to 10^{-1} g L⁻¹. In the **ManNP**-immobilized film, the reflection intensity increased linearly, from approximately 10^{-5} g L⁻¹ to 10^{-1} g L⁻¹. The change in the reflection intensity was approximately three times larger in the **ManNP**-immobilized film than in the **ManHP**-immobilized film. No change in the reflection intensity was observed with the addition of BSA, for either of the **ManHP**- or **ManNP**-immobilized PhCs films.

The change in the reflection intensity was approximately three times larger for the **ManNP**-immobilized PhCs film than for the **ManHP** immobilized film. The change in the reflection intensity depended on the amount of protein adsorbed on the surface of the films. This suggested that **ManNP** could adsorb three times as much ConA, compared with **ManHP**. A similar trend was observed in the QCM measurement. The binding capacity of **ManNP** was higher than that of **ManHP**. An approximate line of best fit was applied in the part of the plot where the reflection intensity increased. For **ManHP** and **ManNP**, the approximate line of best fit was applied in the range of 10^{-4} – 10^{-1} g L⁻¹, and 10^{-5} – 10^{-2} g L⁻¹, respectively. The ConA detection limit was calculated from the intersection point of the approximate line of best fit and $\Delta\text{Intensity} = 0$. The detection limits of the **ManHP**- and **ManNP**-immobilized

PhCs films for the detection of ConA were 16.7 ng mL⁻¹ and 6.0 ng mL⁻¹, respectively. The ConA detection limit of the Surface plasmon resonance sensor is 1.43 $\mu\text{g mL}^{-1}$.⁴⁶ Low concentrations of ConA were detected using the two-dimensional biosensor with structural color. Lower concentrations of ConA were detected with **ManNP** than with **ManHP**, because the binding capacity of **ManNP** was three times higher than that of **ManHP**. Neither **ManHP** nor **ManNP** showed any change in the reflection-intensity with the addition of BSA. The specific interaction between the mannose-incorporating polymers and the ConA was confirmed by the changes in the reflection intensity.

The development of a structural-color biosensor using sugar-incorporating nanogel particles was achieved. The surface modification of the biosensing film was confirmed using XPS and AFM. By immobilizing various materials on the PhCs films, the films could be applied in other types of biosensors, such as biosensors for the detection of antigen-antibody interactions. The results from the QCM analysis showed that

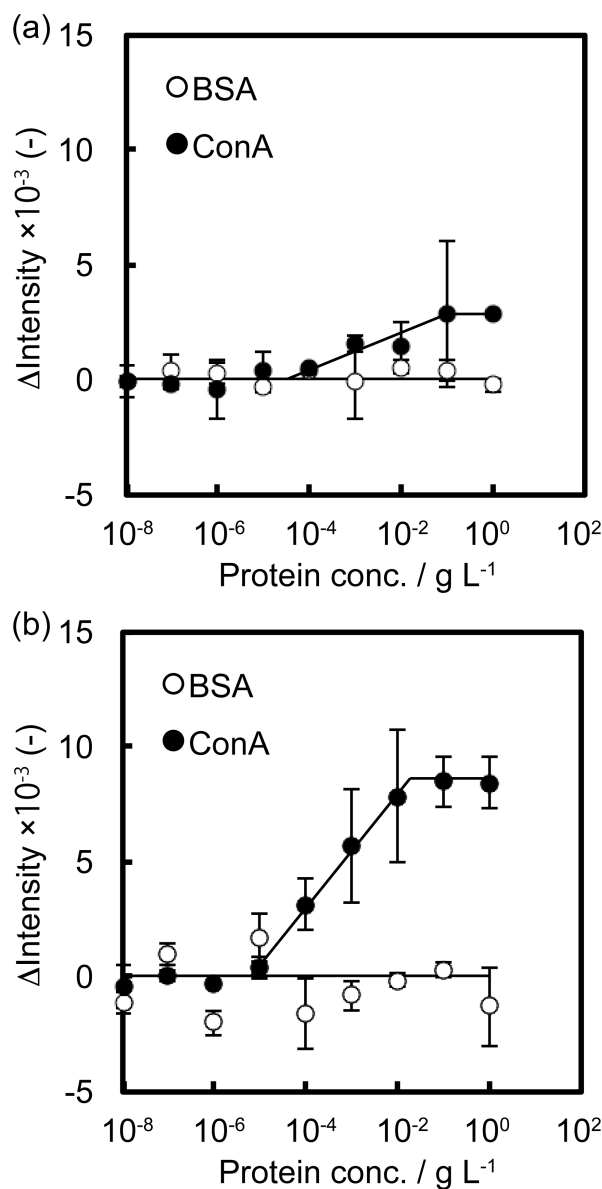


Fig. 6 Changes in the reflection intensity of the PhCs film upon the adsorption of protein on (a) **ManHP**- and (b) **ManNP**-immobilized PhCs films.

the K_a values for **ManHP** and **ManNP** were very similar. **ManNP** bound ConA as strongly as **ManHP**, and had a higher binding capacity compared with **ManHP**. These results suggested that **ManNP** had a better ability to bind ConA, compared with **ManHP**. Furthermore, the ConA detection limit of the **ManNP**-immobilized PhCs film was superior to that of the **ManHP**-immobilized PhCs film. The three-dimensional structure of **ManNP** enhanced the sugar-protein interaction. Meanwhile, the **ManNP**-immobilized PhCs films showed the larger error bars than **ManHP**-immobilized films. Since the **ManNP** is spherical nanogel with diameters of 80-200nm, the immobilization of **ManNP** caused a nanoscale heterogeneity to the film. Furthermore, the size of the **ManNP** had a dispersity. Though **ManNP** was advantageous on the molecular recognition ability, the roughness of the surface reflects the large error bar, which will be improved by the formation of uniform interface.⁴⁷

Sugars are known to bind with various biomolecules such as proteins, viruses, and pathogens. By incorporating other kinds of sugars into the nanogel particles, this biosensing system could be applied in virus biosensors and pathogen biosensors. **ManNP** has the potential to act as a highly effective molecular recognition component in biosensors.

Conclusions

The surface modification of two-dimensional PhCs was successfully achieved using succinimide polymers and glycopolymers for biosensing. The polymer modification didn't damage the optical properties of PhCs, and are an advantage on the biosensing. The modification of PhCs enables the molecular recognition interface, and inhibits the non-specific adsorption of proteins. Our investigation showed the possibility of polymer modification by not only glycopolymer but also by various biopolymers including polyethylene glycol, which extend the application of the PhCs sensor. We investigated the glycopolymer-interface, which showed the specific biosensing of a lectin, and inhibition of the non-specific adsorption. Low-concentrated proteins were detected using the glycopolymer immobilized two-dimensional PhC biosensor with structural color. The results of this study indicated that this biosensor has the potential to be applied for the detection of various biomolecules such as bacteria, viruses, and pathogens.

Acknowledgements

This work was supported by a Grant-in-Aid for Young Scientists A (23685027), a Grant-in-Aid for Young Scientists B (24750073), Grant-in-Aid for Exploratory Research (23685027) and Asahi Glass Foundation.

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† Electronic Supplementary Information (ESI) available: [AFM images of structural-color films, and detection of proteins using a structural-color film without glycopolymers]. See DOI: 10.1039/b000000x/

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