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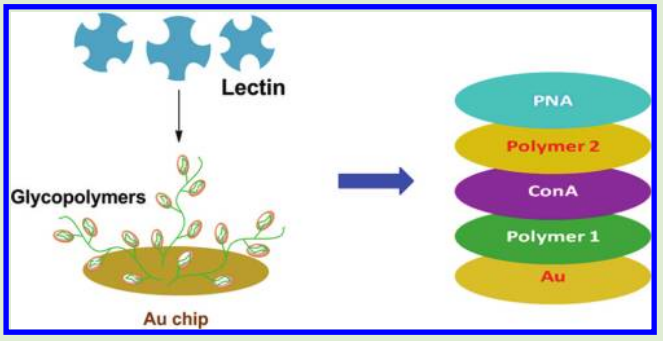
Controlled Alternate Layer-by-Layer Assembly of Lectins and Glycopolymers Using QCM-D

Yanzi Gou, Stacy Slavin, Jin Geng, Lenny Voorhaar, David M. Haddleton,* and C. Remzi Becer*

Department of Chemistry, University of Warwick, Coventry CV4 7AL, United Kingdom

Supporting Information

ABSTRACT: Layer-by-layer (LBL) assembly of concanavalin A (Con A), peanut agglutinin (PNA) plant lectins, and well-defined synthetic glycopolymers via their biological affinities have been prepared using a quartz crystal microbalance with dissipation monitoring (QCM-D). We demonstrate the use of mannose/galactose glycopolymers as lectin binders due to their selective binding to Con A/PNA, respectively. A detailed analysis of the adsorption processes and the adsorbed layer are provided and tuning the composition of multilayers using a series of well-defined glycopolymers differing only in the pendant sugar ratio is discussed.



Lectin–carbohydrate interactions[1] play key roles in a large number of important biological processes such as immune response, cell–cell communication, and pathogen infection.[2] However, the affinities between most monosaccharides and their lectin receptors are usually weak. This can be overcome and dramatically enhanced by multivalent carbohydrate ligands through the "glycoside cluster effect".[3] However, the structural diversity and complexity of carbohydrates in nature has frustrated chemists to synthesize desired carbohydrates and to analyze lectin–carbohydrate interactions.[4]

This difficulty gave rise to various carbohydrate mimics, especially glycopolymers, which are synthetic macromolecules with pendant carbohydrate moieties.[5] As they can be both antagonists and agonists of many biological processes,[6] glycopolymers have been applied in areas such as targeted drug delivery[7] and macromolecular drugs.[8] For example, because the first inhibitor of influenza hamagglutinin based on a glycopolymer was synthesized by Bovin and co-workers in 1990,[9] glycopolymeric treatments of influenza viruses have been developed.[10] Recently, various self-assembled structures of glycopolymers such as glyconanoparticles,[11] carbon nanotubes,[12] microarrays,[13] multilayer films and capsules[14] have emerged as novel carbohydrate-based biomaterials showing even more promising advantages over conventional application of glycopolymers.[15] Sulfonated glycopolymer films on a silicon oxide surface exhibited improvements for in vitro blood compatibility.[16] Hyperbranched poly(glycoacrylate) polymer films were supportive for human vein endothelial cell growth in culture.[17]

LBL self-assembly[18] has been significantly developed in recent years for the deposition of multilayer films from solutions onto solid surfaces.[19] An advantage of LBL assembly is that it allows for a choice of various substrates and has very good control over the film thickness, composition, stability and surface functionality. LBL assembled films have been prepared previously via electrostatic forces of poly(cationic) and poly(anionic) materials,[20] small molecule hydrogen bonding,[21] or biological affinities such as avidin–biotin,[22] lectin–carbohydrate,[23] and antigen–antibody[24] interactions.

In this current work, we make use of biological affinities to demonstrate in situ alternate LBL assembly of lectin and glycopolymers controlled by QCM-D, Figure 1. Well-defined

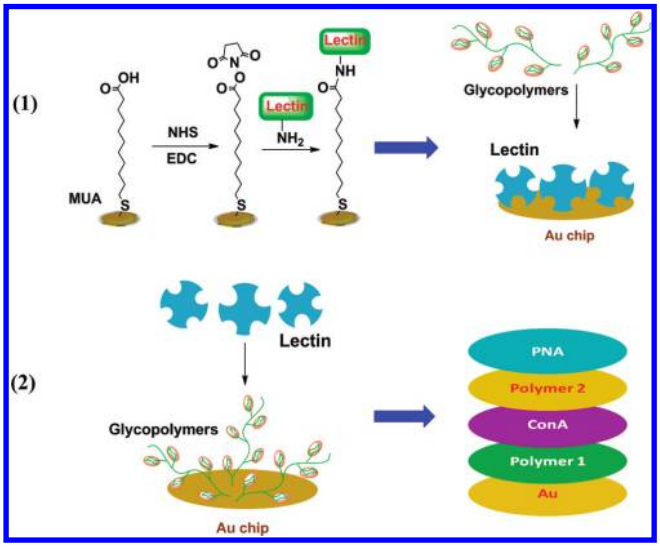


Figure 1. Schematic diagram of the LBL self-assembly by QCM-D using well-defined glycopolymers.

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glycopolymers were prepared to achieve fine-tuning of the composition of the resulting multilayer films and the deposition process was under real time control by using QCM-D. Two different ways were investigated to prepare the multilayer films in two different orders in which either lectins or glycopolymers were immobilized first onto the gold surface of the quartz crystal chip.

Both Con A and PNA were selected as the model lectins in this study. Con A, extracted from jack bean seeds, is a mannose-selective and well-studied homotetramer with four subunits (26.5 kDa each). PNA is a lectin isolated from peanuts as a 110 kDa tetramer composed of four identical subunits, which binds selectively to galactose.[25] In all QCM-D experiments, HBS buffer (10 mM HEPES, 150 mM NaCl, pH 7.4) containing 1 mM Ca^{2+}, Mg^{2+}, and Mn^{2+} was used as the presence of divalent metal ions and is essential for the activity of these lectins.[25] The flow rate was set to 50 μL min^{-1} to get efficient mass coverage over the quartz crystal surface and to reduce the time caused by the assays.

The glycopolymers (Scheme 1) were synthesized via a combination of copper(I)-mediated living radical polymerization and copper-catalyzed azide−alkyne cycloaddition (CuAAC) click reaction following a common and versatile general procedure.[26] The mannose-galactose glycocopolymers (**P1**∼**P5**) were prepared using the same polymeric backbone framework, and their compositions and molecular weights are shown in Table 1. The disulfide mannose glycopolymer (**P6**) was polymerized using a disulfide functionalized initiator (Supporting Information).

Scheme 1. Chemical Structures of Glycopolymers Used in this Study

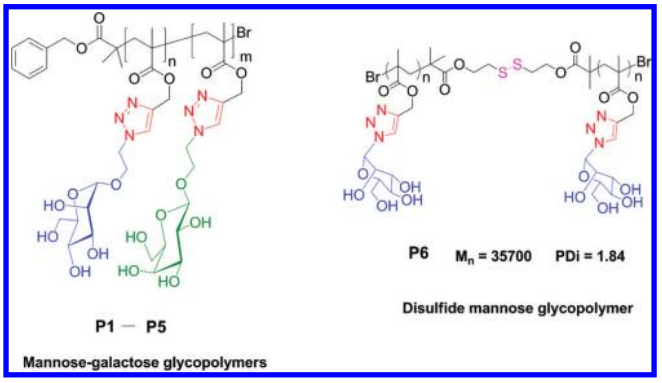

Table 1. Composition of Mannose-galactose Glycopolymers

	Man. (%)	Gal. (%)	M_n (kDa)	PDi
P1	100	0	22.1	1.31
P2	75	25	22.5	1.34
P3	50	50	22.2	1.33
P4	25	75	22.1	1.31
P5	0	100	22.1	1.29
P6	100	0	35.7	1.84

First, it is desirable to immobilize Con A onto the Au chip surface. The binding of Con A to bare gold was found not to be stable and the lectin was washed off slowly with HBS buffer. In order to circumvent this the Au chip was chemically modified with 11-mercaptoundecanoic acid (MUA), followed by 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide (EDC) hydrochloride and *N*-hydroxysuccinimide (NHS; Supporting Information). Con A was then bound to the modified surface via nucleophilic substitution with lysine, present on the surface. In this case, the QCM-D data showed that Con A had indeed attached to the modified quartz crystal surface and was not removed with extensive washing with HBS buffer, Figure 2 (left).

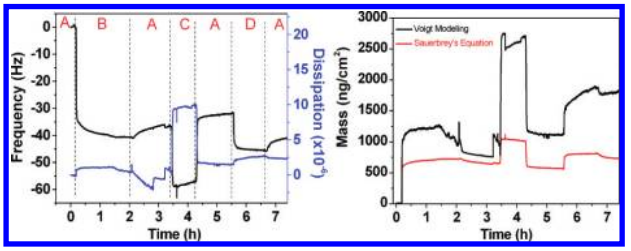

Figure 2. QCM-D plot (left) and estimated mass (right) of materials deposited on the modified Au chip surface over time: (A) HBS buffer; (B) Con A in HBS buffer (0.5 mg mL^{-1}); (C) ethanolamine HCl in HBS buffer (1 M, pH 8.5); (D) **P1** in HBS buffer (0.5 mg mL^{-1}).

The resonating frequency of the Au coated chip decreased when Con A buffer solution passed over the surface. After it had reached a plateau, it was necessary to rinse the surface with HBS buffer so as to exclude the bulk effect from changes in composition and viscosity of the injected Con A solution until a further plateau was reached. Ethanolamine hydrochloride (1M, pH = 8.5) was used to block unreacted NHS groups to prevent their reacting with the glycopolymers. Subsequently, the mannose containing glycopolymer **P1** in HBS solution was passed through the system. As the pendant mannose interacted with immobilized Con A, **P1** on the surface was stable when washed by HBS buffer. The mass of the materials deposited on the quartz crystal surface over time was estimated by both Sauerbrey's equation and by Voigt modeling, Figure 2 (right).

An alternative approach involves binding the glycopolymers to the gold coated quartz crystal. As the mannose-containing glycopolymer, **P1**, did not remain bound to the chip, it was replaced with the disulfide-containing mannose glycopolymer **P6**.[27] In this case, the disulfide bond resulted in adsorption, and **P6** remained bound and stable on the surface when washed with HBS buffer for several hours (Supporting Information). Thus, bilayer assemblies were constructed by adsorption of the disulfide mannose glycopolymer **P6** directly onto the Au chip surface and then passing Con A buffer solution over the QCM-D chip, Figure 3 (left). Con A remained on the surface as the second layer when washed with HBS buffer, indicating that Con A interacted with glycopolymer **P6**. Two concentrations of

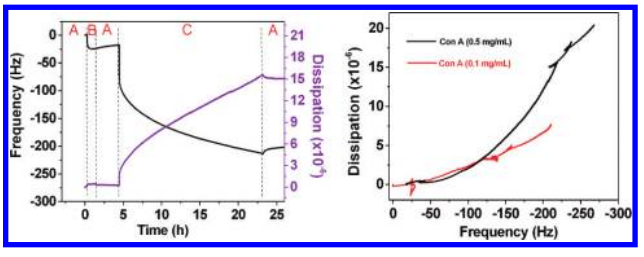

Figure 3. QCM-D plot (left) and *D*-*f* plot (right) of the self-assembly between **P6** and Con A: (A) HBS buffer; (B) **P6** in HBS buffer (0.5 mg mL^{-1}); (C) Con A in HBS buffer (0.5 mg mL^{-1}).

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Con A buffer solution were investigated. The change in frequency of the chip caused by Con A in buffer solution (0.1 mg mL^{-1}), $\Delta f = -108$ Hz, while that caused by using a higher concentration of Con A (0.5 mg mL^{-1}), $\Delta f = -185$ Hz. The mass and thickness of the two layers can be tuned by changing the concentration of **P6** and Con A. In this case, the factors that affect the lectin−carbohydrate interaction, such as concentration, pH value, temperature, ionic strength, and so on, can be investigated systematically due to the advantages of QCM-D.

The structure and dynamic viscoelastic properties of the absorbed layers were obtained by using a dissipation versus frequency plot (*D-f* plot), Figure 3 (right). For adsorption of glycopolymer **P6**, the dissipations for both concentrations were very small and the *D-f* relations were approximately linear, which indicated that the two films formed by **P6** were close to rigid. The adsorption of Con A was more complicated. The slope of the *D-f* plot for Con A increased in both cases, signaling more dissipation per added molecule during the adsorption process. This showed the binding of Con A to the **P6** monolayer film was not only kinetically controlled, but also influenced by the transport limitations, such as the conformational rearrangement of Con A, trapped liquid in the layer, or even the interfacial processes.[28] The influence on the layer of Con A with higher concentration (0.5 mg mL^{-1}) was larger than that of the lower concentration (0.1 mg mL^{-1}), and the absorbed film was less rigid.

Lectin−glycopolymer assemblies, starting with the attachment of the disulfide bonds in the polymeric backbones to quartz crystal surface provide a facile approach to multilayer bioactive films, Figure 4 (left). The glycopolymer **P6** (0.5 mg

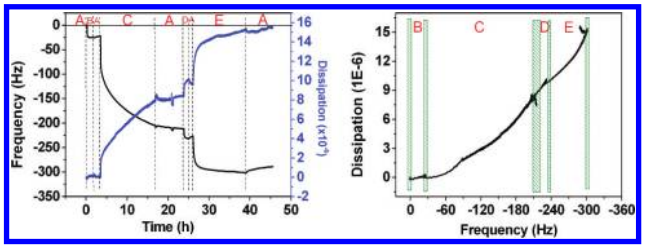

Figure 4. QCM-D plot (left) and *D-f* plot of the multilayer films assembly: (A) HBS buffer; (B) **P6** in HBS buffer (0.5 mg mL^{-1}); (C) Con A in HBS buffer (0.5 mg mL^{-1}); (D) **P3** in HBS buffer (0.5 mg mL^{-1}); (E) PNA in HBS buffer (0.5 mg mL^{-1}).

mL^{-1}, $\Delta f = -23$ Hz) was attached to the surface, followed by Con A (0.5 mg mL^{-1}, $\Delta f = -187$ Hz), mannose−galactose glycocopolymer **P3** (0.5 mg mL^{-1}, $\Delta f = -15$ Hz) and PNA (0.5 mg mL^{-1}, $\Delta f = -63$ Hz) sequentially. The four layer alternate assembly was achieved using QCM-D as the mannose groups in **P3** bound to Con A, while the galactose moieties interacted with PNA.

The slope of the *D-f* plot for **P3** adsorption was higher than that for **P6** adsorption, which was comparable to the slope for the lectin Con A and PNA adsorption, Figure 4 (right). This indicates that the absorbed **P3** layer was less rigid than the **P6** layer and the interactions between **P3** and Con A, PNA, and **P3** were viscoelastically similar. In addition, the part of the *D-f* plot for **P3** binding was nearly linear, which indicated a monolayer was formed by **P3** without any obvious conformational changes. This factor should be taken into consideration when preparing multilayer films.

The mass of the rigid, uniform film on the quartz crystal surface in air or vacuum can be calculated using the Sauerbrey's equation (see Supporting Information). However, modeling[29] of the mass will be needed for the viscoelastic films deposited on the Au chip surface from solution. The mass data obtained from both Sauerbrey's equation and Voigt modeling showed in Table 2. In these two experiments (Figures 2 and 4), the

Table 2. Estimated Mass Data (ng cm^{-2}) from Two Experiments[a]

	Figure 2			Figure 4		
	Con A	**P1**	**P6**	Con A	**P3**	PNA
Sauerbrey's eq.	658	163	396	3394	264	1109
Voigt modeling	790	699	413	3707	319	769

[a]25 °C; pH 7.4; flow rate, 50 μL min^{-1}; concentration of each material, 0.5 mg mL^{-1}.

concentrations of Con A used were both 0.5 mg mL^{-1}. However, there was a large difference in the mass of Con A deposited. The signals of interactions were amplified greatly by using glycopolymer **P6**, Table 2. It was more efficient to use disulfide glycopolymer **P6** initially to prepare the bioactive films.

The use of a series of well-defined glycopolymers differing only in the ratio of different pendant sugars to tune the composition of the self-assembled multilayer films was also achieved, Figure 5. After the formation of two layers of

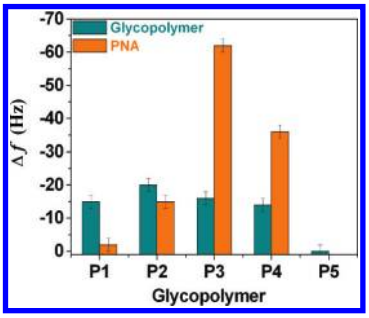

Figure 5. Frequency changes by passing HBS solution of glycopolymers (**P1**∼**P5**, respectively, with the same concentration as 0.5 mg mL^{-1}) and PNA (0.5 mg mL^{-1}), sequentially.

glycopolymer **P6** (0.5 mg mL^{-1}) and Con A (0.5 mg mL^{-1}) on the gold chip surface, glycopolymer **P1**∼**P5** of the same concentration was passed over the surface respectively, followed by a solution of PNA. The layer of glycopolymer **P5** did not form as galactose cannot bind with Con A. Although frequency changes of the binding of other glycopolymers **P1**∼**P4** to the preformed bilayer film were similar, the following adsorption of PNA onto the glycopolymer layers varied greatly. The layer of PNA did not form for glycopolymer **P1** as PNA cannot bind with mannose. The adsorption of PNA was most for glycopolymer **P3** bearing pendant mannose and galactose in 1:1 ratio.

In summary, two different ways have been demonstrated to prepare the LBL alternate self-assembled bioactive multilayer surfaces via the biological affinities of different lectins and their specific carbohydrates. The gold-coated quartz crystals need to be chemically modified by MUA, EDC, and NHS to attach the lectin Con A to form a stable layer. The disulfide glycopolymers bound directly to the chip surface to apply glycopolymers as the

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first layer, which also exhibited signal amplification. The tuning of the composition of the multilayer films was also studied using different glycopolymers. The controlled LBL multilayer self-assembly of lectins and synthetic glycopolymers through their specific interactions controlled by QCM-D showed a facile way to prepare multilayer bioactive films. The future studies will focus on the binding kinetics of lectins and glycopolymers using QCM-D.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis procedures and characterization of the glycoplymers, QCM-D experimental procedures, and complementary results. This material is available free of charge via the Internet at http://pubs.acs.org

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: c.r.becer@warwick.ac.uk; d.m.haddleton@warwick.ac.uk.

Notes

The authors declare no competing financial interest.

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