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# Tunable, dynamic and electrically stimulated lectin–carbohydrate recognition on a glycan-grafted conjugated polymer†

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**An electroactive platform for multivalent, reversible and electrically stimulated lectin–carbohydrate recognition based on mannose-functionalized conjugated polymer is reported and tuned by electro-copolymerization of mixture of tri(ethylene glycol)-functionalized EDOT and its α-mannose conjugate.**

Multivalent and oligomeric carbohydrates (glycans) found in cell surface glycoproteins and glycolipids play an important role in numerous normal and pathological biological events.[1–3] Lectins are multimeric glycan-binding proteins that show a significant increase of binding affinity per carbohydrate unit with increase of glycan size (cluster glycoside effect).[4] The glycan–lectin recognition regulates such biological processes as cell proliferation, adhesion, immune responses, cell–cell interaction and communication, among others.[5–8] Various glycan arrays and glyconanostructures utilizing fluorescence,[9,10] surface plasmon resonance (SPR)[11–13] and quartz crystal microbalance (QCM),[14,15] among others, have been developed to measure the lectin–glycan binding affinity, analyze recognition mechanism[13] and perform pathogen sensing.[14,16]

Electrically active platforms based on conjugated polymers have been utilized to understand and evaluate biomolecular recognition events, including complementary DNA binding and aptamer/protein recognition due to the great current interest for the development of low cost, portable lab-on-a-chip bioanalytical and diagnostic devices. The connection between glycans and pathological events allows this pair to be a promising target. In addition, there has been a growing interest to study the electrical stimuli on physiological characteristics of cells, such as neurons, for potential application in tissue engineering.[17,18] Integration of the glycan-mediated cellular information with electrical stimulation would be important to decode the cellular–cellular communication within such supplications. With the ultimate aim of developing such a platform, herein we describe the specific, tunable, dynamic and electrically stimulated recognition of a biomolecule (exemplified by a plant lectin, Concanavalin A) and a conjugated polymer, poly-(3,4-ethylenedioxythiophene) (PEDOT), the surface of which is modified by the complementary ligand, α-mannoside. PEDOT[19] is a widely used organic electronics material and it also demonstrates a great potential for biological applications because of its superior biocompatibility and long-term stability of its materials characteristics in aqueous solutions.[20,21] So far, only a few glycan-conjugated conjugated system have been reported for biosensing application.[22,23]

Starting from hydroxymethyl-functionalized 3,4-ethylene-dioxythiophene (EDOT-OH, Chart 1), triethylene glycol substitute monomer (EDOT-EG3) was synthesized (ESI†). This moiety was chosen because oligoethylene glycol could increase the hydrophilicity of the surface and prevent unwanted non-specific binding of proteins and cells.[24,25] After the compound was synthesized, α-mannoside can be easily conjugated to yield the desired monomer, EDOT-Man. Conjugated polymers poly(EDOT-Man) obtained from electropolymerization of EDOT-Man showed considerably stronger binding to Concanavalin A (Con A) than poly(EDOT-OH) and Au substrates (ESI†). Moreover, poly(EDOT-EG3) displayed reduced non-specific binding of bovine serum albumin (BSA) compared to poly(EDOT-OH) because of the greater hydrophilicity.

The surface functional group density for functionalized PEDOTs prepared from microemulsion electropolymerization is usually within the range of $10^{15}$ cm$^{-2}$.[26] At this relatively high surface density of mannose functional groups, multivalent carbohydrate–lectin recognition with a high apparent binding constant is expected.[11,13,15] This inhomogeneous binding event

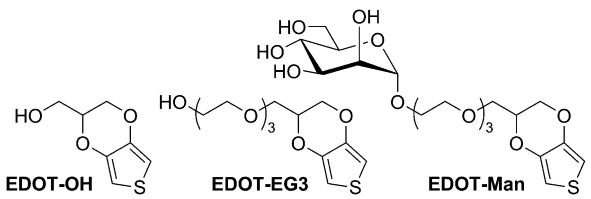


**Chart 1**

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may lead to complex and unpredictable surface properties that are inferior for diagnostic or bioanalytical devices.

For example, treatment of poly(EDOT-Man) with a competitive inhibitor, methyl-α-D-mannoside (100 mM) in a quartz crystal microbalance (QCM) chamber showed a strong retention of Con A on the chip surface. The mannosylated PEDOT surface was irreversibly blocked by Con A due to the strong multivalent recognition. In that case, surface-bioconjugated poly(EDOT-Man) was limited to a single use in biosensing and other bioengineering applications. From a practical point of view, a recyclable and reusable device is highly desirable. To that end, the apparent binding affinity needs to be optimized to a range where it is sufficiently strong to induce a signal, yet sufficiently weak to ensure reversibility of the binding. On this platform, this goal can be achieved by electropolymerization of a mixture of conjugated and non-conjugated monomers to adjust the surface density of the mannose functional group, thereby optimizing the surface affinity for Con A.

Non-specific binding of bovine serum albumin (BSA) and specific binding of Con A were evaluated for two copolymers, poly(EDOT-OH)-*co*-(EDOT-Man) and poly(EDOT-EG3)-*co*-(EDOT-Man) with EDOT-Man molar composition in monomer solution mixture ranging 0–100%, as shown in Fig. 1. In general, the binding of Con A increased with a higher EDOT-Man composition percentage. At the same time, the non-specific binding of BSA was greatly reduced with increasing EDOT-Man monomer percent in poly(EDOT-OH)-*co*-(EDOT-Man). This was mainly due to the greater hydrophilicity provided by mannose functional groups to prevent non-specific binding. On the other hand, non-specific binding of BSA with poly(EDOT-EG3)-*co*-(EDOT-Man) was further reduced compared to poly(EDOT-OH)-*co*-(EDOT-Man). As shown in Fig. 1b, the binding of Con A on poly(EDOT-EG3)-*co*-(EDOT-Man) experienced a dramatic and non-linear increase up to about 50% EDOT-Man monomer content and levelled off thereafter. It was also intriguing to observe that the Con A binding was greater on poly(EDOT-EG3)-*co*-(EDOT-Man) compared to it on poly(EDOT-OH)-*co*-(EDOT-Man). This indicated that the specific binding was enhanced by the more hydrophilic surface.

Poly(EDOT-EG3)-*co*-(EDOT-Man) prepared at the optimal 50% EDOT-Man monomer content proved to be capable of repeated Con A recognition (Fig. 2a). Recovery of the active biointerface was achieved by "washing off" with methyl-α-D-mannoside solution. The release of Con A from the surface was mainly due to the competitive inhibition binding. However, the real-time monitoring by the quartz crystal microbalance (QCM) indicated that a certain number of biomolecules were still bound to the surface (step (v) in Fig. 2(b)). The residual biomolecules could be washed off upon further rinsing with Tris buffer, hence it was suspected that these molecules were methyl-α-D-mannoside physically adsorbed on or hydrogen-bonded to the surface. Adsorption of methyl-α-D-mannoside was also detected on freshly prepared poly(EDOT-EG3)-*co*-(EDOT-Man) surfaces (Fig. 2c). The binding and releasing event can be repeated consistently, indicating a well-controlled surface for lectin–carbohydrate recognition.

The advantageous feature of a recognition event on a conjugated polymer is that it provides an electroactive platform to study both the recognition event with an electrical signal and the electrical stimulation effect on the biological interface. When a potential of −200 mV (*vs.* Ag/AgCl) was applied to the substrates, the quantity of Con A bound to the surface almost doubled compared to the substrate without applying any electrical stimulation, as shown in Fig. 3a.

Evaluation of the electrical stimulation during the Con A binding *in situ* by applying alternating potentials of −200 mV and 0 V for 20 s, respectively, clearly displayed that the frequency dropped immediately after the electrical pulse was applied. This indicated more adsorption of Con A (Fig. 3b and c). Con A binding when a positive potential from 0 to 500 mV (*vs.* Ag/AgCl) was applied did not show a difference from the non-electrically-stimulated sample. A control experiment showed only little frequency decrease when an identical electrical pulse was applied

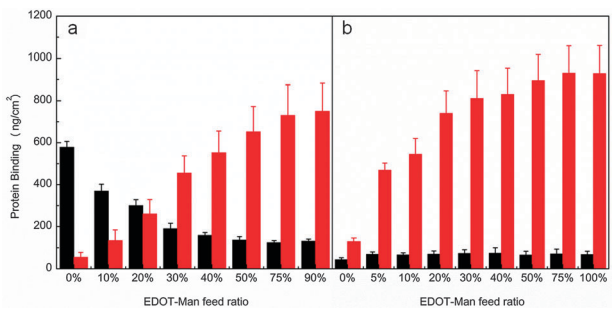


**Fig. 1** Protein binding of non-specifically targeted BSA (■) and specifically targeted Con A (■) on (a) poly(EDOT-OH)-*co*-(EDOT-Man) and (b) poly(EDOT-EG3)-*co*-(EDOT-Man). The mass of the protein binding layer was estimated by Sauerbrey relationship[27] based on 3rd overtone data obtained from the quartz crystal microbalance (QCM).

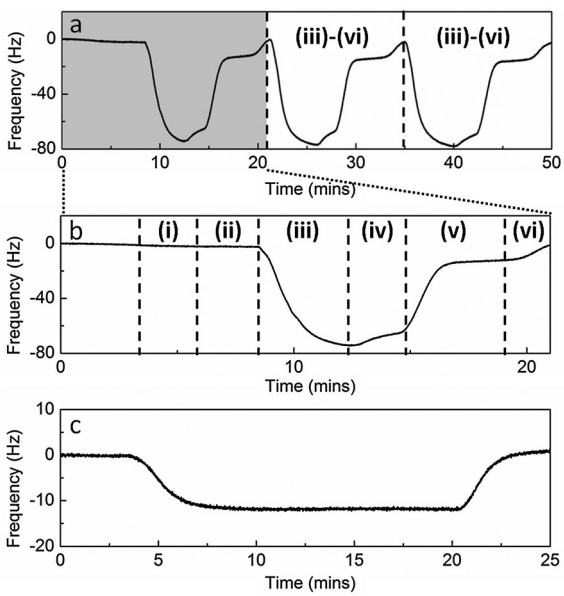


**Fig. 2** Real-time monitoring of repeatable Con A recognition by QCM: (a) and (b) cycles of Con A binding and releasing on poly(EDOT-EG3)-*co*-(EDOT-Man); (c) adsorption of methyl-α-D-mannoside on poly(EDOT-EG3)-*co*-(EDOT-Man). The real-time experiment steps are as following: (i) addition of BSA (1 mg $mL^{-1}$), (ii) rinsing with Tris-buffer, (iii) binding of Con A (1 mg $mL^{-1}$), (iv) rinsing with Tris-buffer, (v) releasing Con A in the presence of methyl-α-D-mannoside (100 mM) and (vi) rinsing with Tris-buffer.

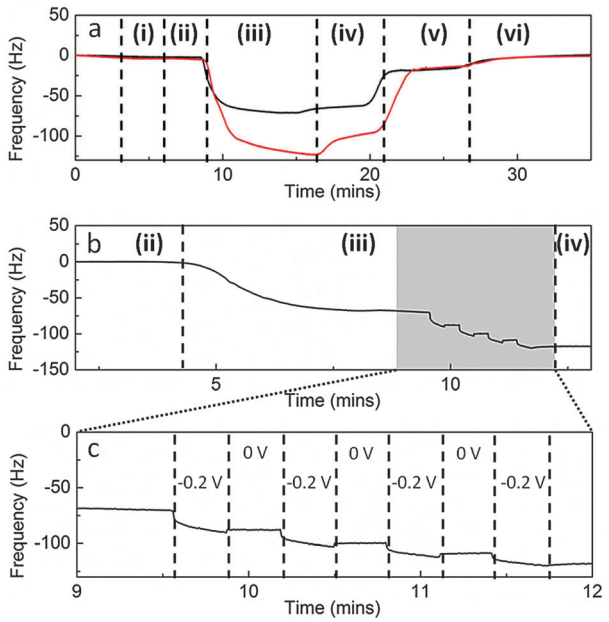


**Fig. 3** Real-time monitoring of Con A recognition on poly(EDOT-EG3)-*co*-(EDOT-Man) films upon applying electrical stimulation by QCM: (a) Con A binding and releasing with (——) and without (—) applying a potential (−200 mV *vs.* Ag/AgCl); (b) controlled Con A recognition by an alternating electrical pulse (−200 mV and 0 V *vs.* Ag/AgCl for 20 s) during the Con A binding; (c) enlarged figure of the real time monitoring of the frequent change by applying alternating electrical pulse. The real-time experiment steps are as following: (i) addition of BSA (1 mg $mL^{-1}$), (ii) rinsing with Tris-buffer, (iii) binding of Con A (1 mg $mL^{-1}$), (iv) rinsing with Tris-buffer, (v) releasing Con A in the presence of methyl-α-D-mannoside (100 mM) and (vi) rinsing with Tris-buffer.

to the same substrate without Con A (ESI†). In this control experiment, the drop in frequency was mainly due to the adsorption of cations present in the buffer through electrostatic attractions. When −200 mV was applied to the conjugated polymer, the polymer would exhibit less positive charges through de-doping procedure. On the other hand, Con A is negatively charged at pH = 7.4. Therefore the enhanced binding was not due to electrostatic interactions. A plausible explanation for the observed increase was that the local concentration of $Mn^{2+}$ and $Ca^{2+}$ cations present in the proximity to the surface was increased upon applying a negative potential. These cations are necessary in the mannose–Con A binding and the high local concentration increased the apparent binding affinity of Con A to the mannosylated substrate.

In summary, we have prepared an electrically active platform with glycan-grafted conjugated polymers. The surface density of the mannose ligand that is utilized for this mimic can be easily adjusted by controlling the composition of the mannosylated monomer, EDOT-Man. The lectin–carbohydrate interaction is affected by the surface composition of both the ligand density and surface hydrophilicity. The binding event can be further controlled by using electrical stimulation. By applying a potential of −200 mV (*vs.* Ag/AgCl), the binding of Con A was enhanced dramatically. Importantly, Con A can be removed by methyl-α-D-mannoside solution, allowing for the substrate to be recycled for multiple binding events.

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