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ARTICLE TYPE

Development of a smart dynamic surface chemistry for surface plasmon resonance-based sensors for the detection of DNA molecules

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We report the design of a polyrotaxane surface for biosensor applications. The results show that a three-fold improvement in immobilization and hybridization efficiency has been achieved compared to PEG SAM. The results confirm that polyrotaxane is a very promising platform candidate for biosensor applications.

The selective and sensitive biomolecule interactions detection is critical in basic research as well as clinical diagnosis. There are many types of sensors and methods developed to meet this need such as surface plasma resonance (SPR)[1,2], ellipsometry[3], optical waveguide light spectroscopy[4], quartz crystal microbalance (QCM)[5], immunosorbent assays[6], and nano-particle-based methods[7,8]. Although these techniques involve different physical principles, they share one common procedure: attaching an interesting biomolecule to the surface of a sensor chip, and then monitoring the interaction between the ligand and analyte in the sample. Therefore, the substrate surface chemistry and the method used for the biological recognition element immobilization are key challenges for the construction of biosensors. Recently, Whitesides and coworkers surveyed a large number of surface functional groups and concluded that PEG with its unique molecular composition and conformation retains the most non-fouling features regarding protein adsorption[9]. Although this kind of matrix is easy to prepare and use, it suffers from: (i) the two-dimension nature limits its capacity for ligand immobilzation; and (ii) the performance of PEG-SAM depends on the Au surface quality. We found PEG-SAM formed on thermo-evaporated Au film was prone to nonspecific protein adsorption. To overcome the above limitations, researchers have developed many kinds of oligo(ethylene glycol) (OEG)-based matrices[10-12]. In particular, poly(oligo(ethylene glycol) methyl ether methacrylate) (POEGA) has been extensively investigated as a material with significant reduction of protein non-specific adsorption and a high capacity for ligand immobilization[13-16]. However, we found that the recognition capacity decreased monotonically as the polymer chain density increased[5]. Several factors might cause this phenomenon: (i) When the polymer chain density increased the analyte diffusion into and out of the 3-D matix became more difficult[17]; (ii) compared with the solution state, probes immobilized on a solid surface easily denature and lose their structures under high chain density conditions. One solution to these problems is to create a dynamic surface, which can provide probes with mobility, in near-solution conditions. In the last few decades, Yui's group has stressed the importance of molecular mobility in the design of biomaterials [18,19]. In this study, we present a two-step strategy for producing a dynamic surface based on the molecular assembly between cyclodextrins and PEG for DNA biosensor applications. In the first step, cyclodextrins were threaded onto a linear PEG chain, and followed by capping with a bulky function group. Possibly cyclodextrins can freely slide and rotate along the chain in the polyrotaxane architecture. The combination of cyclodextrins and PEG exhibits a number of fascinating features and tuneable properties such as non-fouling ability [20] and a large increase in the surface capacity for ligand immobilization in comparison with a two-dimensional structure surface. High ligand density is required to enhance mass transfer limitation as well as to bind as much as possible of analyte to gain a proper signal in concentration detection experiments. In order to improve this detection limit and enhance the SPR signal, novel matrix with higher concentrations of receptor molecules must be developed [21,22]. Furthermore, the cyclodextrin hydroxyl group can be utilized to introduce any reactive group. In addition, the resulting polyrotaxane structural characteristics involve sliding and rotational motion and thus facilitate access to binding sites on the targets which enhance the interaction efficiencies between the ligand and the analyte [23]. In the second step, we introduced a unique non-selective photo-cross-linking functional group on the cyclodextrin molecule for immobilizing the DNA probe in functional group independent manner. The fundamental mechanism is that the diazirine groups can be transformed into highly reactive carbenes upon irradiation with UV light, which can bind to or insert irreversibly into a DNA molecule. This method has been proven useful in the construction of small-molecule microarrays[24,25]. The performance of this matrix was investigated with surface plasmon resonance. The results were encouraging and suggest that the matrix based on polyrotaxane is a very promising platform candidate for future biosensor applications. Our strategy provides a new concept for designing a novel matrix for biosensor application.

Preparation of a dynamic matrix: A large numbers of methods for preparation of polyrotaxanes as a design of dynamic biomaterial have been reported elsewhere[23,26,27]. One of the most promising approaches to construct polyrotaxanes on a substrate surface is self-assembly and a subsequent capping interaction.

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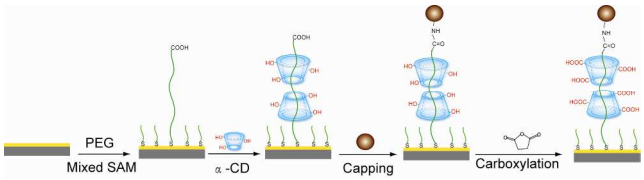
The preparation of polyrotaxanes used in this study is illustrated in scheme 1. The first step is to construct mixed SAMs of PEG thiol on a bare chip where the carboxyl-terminated PEG thiol was diluted with methyl-terminated PEG thiol to vary the carboxyl-terminated PEG chain density. α-cyclodextrins (α- CDs) were threaded onto a PEG chain by immersing the PEG modified chip into α-CDs solution (100 mg mL^{-1}) for 2 hours at room temperature followed by a subsequent capping reaction with amino acid derivatives such as L-phenylalanine. To activate the hydroxyl groups in α-CDs molecules, the chips were immersed in a dry DMF solution containing succinic anhydride and DMAP for 14 h at room temperature under an argon atmosphere. Finally, the hydroxyl groups of α-CDs were translated to carboxyl group in a controlled and functional manner. In the IR spectrum, we observed characteristic peaks at 1160 cm^{-1} which corresponds to C-O-C stretches in the PEG chain and in α-CDs. The increase in intensity of the peak at 1160 cm^{-1} (Fig. 1b,c) after the PEG-SAM immersing into α-CDs solution and capping reaction confirmed that α-CDs were threaded into the PEG chain. The new peak at 1735 cm^{-1} in Fig.1-c corresponds to the carboxyl group C=O vibration of α-CD. The threading of α-CDs into the PEG chain is also verified by the result from SPR real-time monitoring (Fig. 2). After injection of α-CD solution (100 mg mL^{-1}) to the surface, the response of SPR for short PEG (contains 6 repeat uints) is similar with bare chip, while a significant inclusion interaction take place between the long PEG (contains 44 repeat units) and α-CD. Thus, it can be concluded that α-CD can be threaded into the long PEG chain freely, while polyrotaxanes between α- CDs can PEG_6 cannot be formatted, because the PEG spacer is too short. A detail of evaluation of the stoichiometry between α-CDs and PEG was provided in electronic supplementary information.



Scheme 1 Schematic illustration of the gold surface modified with the dynamic surface based on polyrotaxanes. Functional polyrotaxane is constructed in four steps: (i) The formation of SAMs of PEG on bare chip, (ii) α- cyclodextrins (α- CDs) are threaded onto a PEG chain by immersing the PEG modified chip into a α-CDs solution, (iii) a terminal capping reaction, (iv) modification of α-CDs hydroxyl groups in polyrotaxane with succinic anhydride, making it possible to immobilize ligands in a controlled and functional manner.

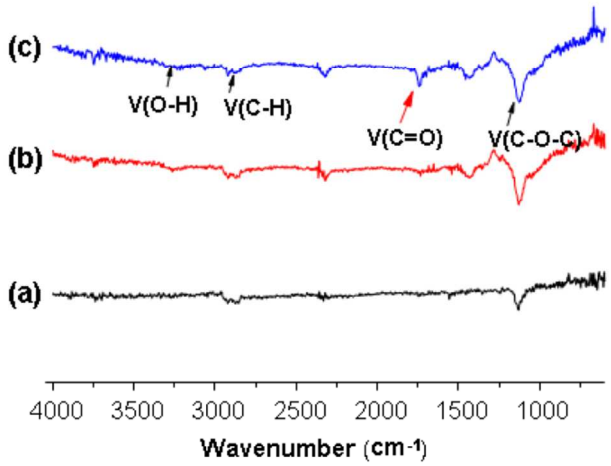


Fig.1 The FT-IR spectra of (a) PEG SAM; (b) intact polyrotaxanes surface; (c) carboxylated polyrotaxane surface.

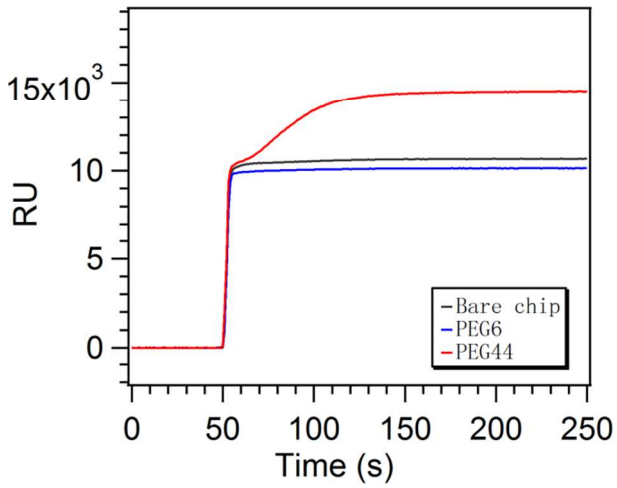


Fig.2 SPR used to real-time monitor the co-assembly reactions between α- CDs with (a) bare chip surface; (b) short PEG SAM (contains 6 repeat units); (c) long PEG SAM (contains 44 repeat units).

Immobilization of DNA probe on a dynamic matrix through a photo-cross reaction. The immobilization of DNA probe is a crucial step in the DNA biosensor application because it is directly related to the resulting biosensor performance. For decades, several methods have been developed to attach a DNA probe onto a surface through covalent and affinity binding, such as amine coupling chemistry[28], biotin/streptavidin interaction[29] and thiol coupling chemistry[30,31]. However, drawbacks regarding these selective coupling approaches are: (i) DNA probes have to possess certain functional groups (-SH, -NH2, -biotin) to be attached to matrix; and (ii) one DNA chain contains only one functional group which reduces the immobilization effectiveness. To overcome these drawbacks, we describe herein a convenient universal immobilization method through a photo-cross reaction which has been proven to introduce a variety of small molecules to a solid surface[24,32]. The procedure used to immobilize DNA probes on polyrotaxane matrix is summarized in support

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information (Scheme s1). The chip modified with carboxyl polyrotaxane was first treated with a photo-cross linker to yield photoreactive groups on α-CDs molecules. DNA probe solutions were then printed on a photo-cross linker-coated chip. Immobilization should occur in a functional group independent manner via UV irradiation at a 365 nm wavelength treatment for 30 minutes. To test this concept, we chose biotin labeled DNA as the probe, because its immobilization effectiveness can conveniently be demonstrated by a biotin and streptavidin interaction. The biotin-labeled DNA probe was immobilized on the polyrotaxane matrix via a photo-cross interaction. The chip was then probed with streptavidin (5μg mL^{-1}) using SPR technology (Fig. 3) which strongly suggests that the biotin-labeled DNA probe was successfully immobilized by a photo-cross reaction. The immobilized biotin molecule was shown to retain specific binding activity for its binding targets. Importantly, this immobilization procedure does not require a specific functional group.

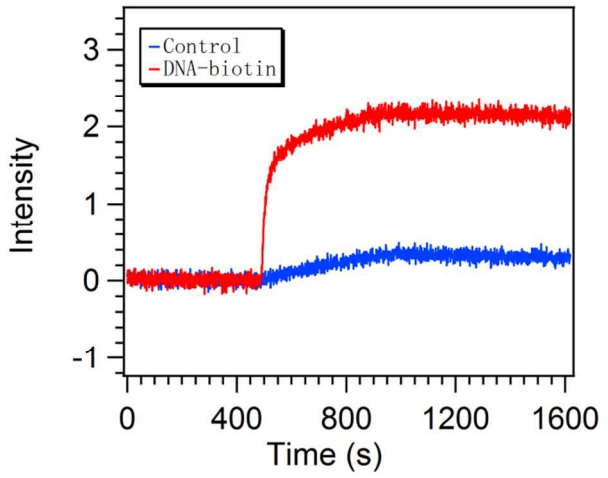

Fig.3 DNA probes immobilization on a polyrotaxane surface with a photo-cross interaction and the detection of a biotin and streptavidin interaction by using SPR technology.

Detection of DNA targets of foodborne pathogenic microorganisms. To examine the performance of DNA arrays spotted on the dynamic matrix through a photo-cross reaction, we designed 11 kinds of DNA probes to directly detect the target DNA solutions of foodborne pathogenic microorganisms (see table s1 in support information). Target probes for foodborne pathogenic microorganisms were dissolved in milli-Q water at a final concentration of 5 μM and were then printed on the chip with an automated Genetix QArray mini printer. The pattern of the spotted probes is shown in Fig.4. The chip was irradiated with 365 nm UV for 30 minutes. This strategy enables the determination of 11 kinds of food-borne pathogenic microorganisms.

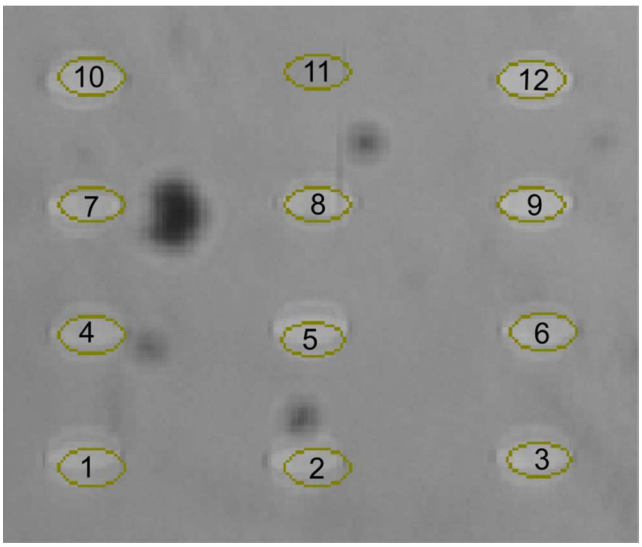

Fig.4 Fabrication of DNA array on a polyrotaxane surface. Probes (5μM) specific to the targets of food-borne pathogenic microorganism detection were immobilized. (1) Salmonella spp; (2) Shigella spp; (3) C.perfringens; (4) Y. enterocolitica; (5) L.monocytogenes; (6) B. cereus; (7) V. cholera; (8) V. parahaemolyticus; (9) S. aureus; (10) C. jejuni; (11) Control (milli-Q water); and (12) E.coli O157:H7.

After immobilizing DNA probes, the corresponding solution of synthesis target DNA (2 μM) related to food-borne pathogenic microorganisms were injected into the SPR monitoring system with a flow rate of 2 μL min^{-1}. The hybridization interaction was monitored for 800 seconds, and then the surface was washed by PBS buffer solution to remove the unbound target DNA. Any change in refraction intensity due to DNA hybridization was recorded in a real-time manner. For the detection of *E.coli* O157:H7, SPR results showed that only the probes complementary to the injected target DNA of *E.coli* O157:H7 produced recordable signals (see Fig. 5) which indicates that food-borne pathogenic microorganisms were unambiguously identified by this method. Cross reaction and unspecific hybridization were almost negligible. In all experiments, probes were regenerated with 10 mM NaOH for 1000 seconds. Typical SPR response curves for regeneration shown in Fig. 5. It was observed that the SPR response base line returned to the original value after regeneration which indicates that the target DNA can be completely removed by a NaOH solution treatment.

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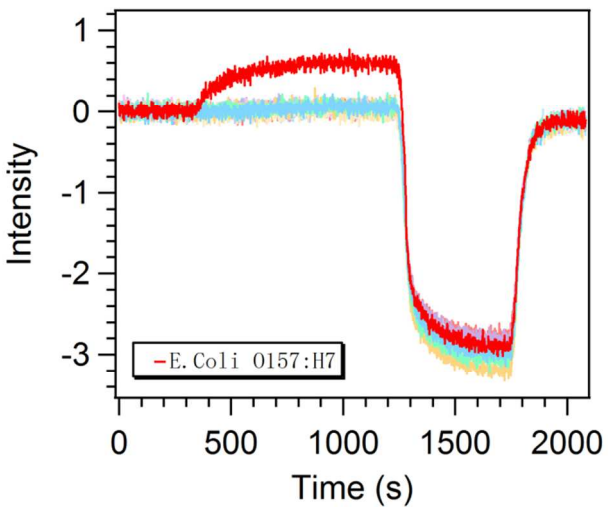

Fig.5 Typical SPR response curves for target DNA of E.coli O157:H7 detection. Note: The red curve corresponds to *E.coli* O157:H7 probe while the other curves correspond to other ten food-borne pathogenic microorganism probes.

We used SPR technology to investigate the immobilization and binding capacity of a polyrotaxane surface and compared the results with that of the PEG SAM surface (as a reference). In this study, we used biotin-labeled DNA as the probe to assess the immobilization capacity of the polyrotaxane surface. The biotin-labeled DNA was immobilized on the polyrotaxane surface with a photo-cross reaction while the probe was immobilized on the PEG-SAM was achieved with a traditional strategy such as amine coupling chemistry. SPR response signals for streptavidin binding to DNA probes were measured to be 2.14 units (430 ng cm^{-2}) and 0.76 units (152 ng cm^{-2}) for a polyrotaxane surface with a photo-cross reaction and PEG-SAM with amine coupling chemistry, respectively (see Fig. 6(a)). This result indicates that the probe immobilization capacity of a polyrotaxane surface with a photo-cross reaction was about three times stronger than that of the PEG-SAM. For the case of detection of synthesis DNA related to *E.coli* O157:H7, the binding response of polyrotaxane surface was 0.60 units (120 ng cm^{-2}), which is about three times of that of PEG SAM (0.21 unit) which corresponds to the mass change of 42 ng cm^{-2}, and demonstrates that the polyrotaxane surface is a promising alternative to the traditional PEG SAM.

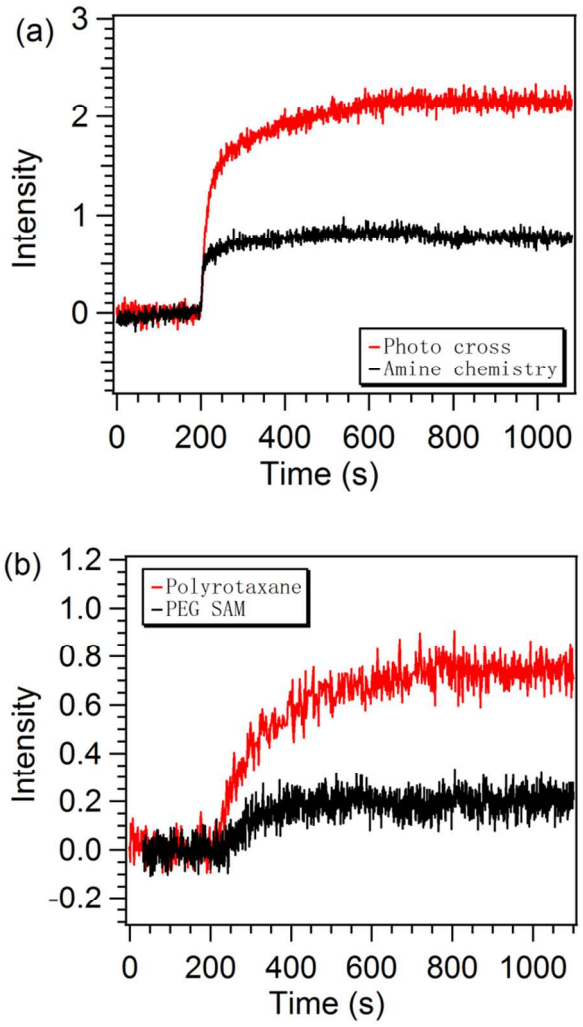

Fig.6 SPR response for (a) the specific binding of streptavidin (20 μg mL^{-1}, 0.3 μM); and (b) hybridization with target DNA of *E.coli* O157:H7 (2 μM) on the polyrotaxane surface and PEG SAM.

Conclusions

In this paper, we present a new concept of constructing biomaterial for designing dynamic matrix for biosensor application. Compared to previous reports on biosensor matrices, our approach offers the potential of higher density of immobilization sites and thereby a larger response during analyte binding. Furthermore, the moveable nature of α-CD along the PEG chain facilitates enhanced binding with the analyte. As mentioned above, this matrix exhibits good performance in DNA immobilization and DNA hybridization. It has been clarified that the supramolecular mobility of ligands introduced into a polyrotaxane significantly increases the specific affinity. The photo-cross immobilization strategy is very versatile and compatible with a broad range of biological recognition elements, and thus it is suitable for biosensor applications. This research provides a novel chemical strategy for designing an ideal matrix for biosensor applications.

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Notes and references

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‡ **Preparation of SAM:** After treating with plasma for 5 minutes, the bare chips were immersed into a 1 mM mixture of carboxyl-terminated PEG thiol and methoxyl-terminated PEG thiol at the ratio of 1:10 for 16 hours at room temperature.

Preparation of a polyrotaxanes matrix: PEG modified chips were immersed in α-CD water solutions (100 mg mL^{-1}) for 2 hours at room temperature. After the α-CDs were threaded onto the PEG chains, the carboxyl groups were activated with a freshly mixed solution of EDC (0.2 M) and NHS (0.4 M) for 30 minutes, which was followed by a subsequent capping reaction with amino acid derivatives such as L-phenylalanine. To obtain terminal carboxyl groups in the polyrotaxane matrix, the resulting polyrotaxane matrices were incubated with a DMF solution containing succinic anhydride (10 mg mL^{-1}) and DMAP (15 mg mL^{-1}) for 16 hours at room temperature with shaking.

Preparation of DNA chip: The chip was activated with a freshly prepared mixture of EDC (0.2 M) and NHS (0.4 M), and a 15 μL photo-cross linker solution (100 mM) was pipetted onto the chip, and the chip was covered with a cover slip and placed in the dark for 2 hours at room temperature. The chip was then blocked with 1 M of ethanolamine for one hour at room temperature. The probe solution(5 μM) was printed onto the chip by using an automated Genetix QArray mini printer. Subsequently, the chip was irradiated with a 365 nm wavelength of 2.8 J/cm^2 for 30 minutes by using a UV instrument (Amersham Life Science)

Surface plasmon resonance measurement: The chip was set in a Plexera Kx5 (Plexera, USA) and the phosphate buffer solution (PBS, 10 mM) was introduced at a rate of 2 μL s-1 as a running buffer until a stable baseline was achieved. The synthetic target DNA was diluted to the desired concentration with a PBS buffer. Prior to testing, the target DNA was denatured by heating for 5 min at 95℃ and then was immediately chilled in ice for 5 min to obtain denatured ssDNA. The denatured target DNA was injected into the SPR flow-cell at a rate of 2 μL s^{-1}. The hybridization reaction was monitored for 400 s; PBS was finally passed through to remove the unbound DNA and establish a stable baseline. The analytical signal is derived from the difference between the baseline value before and after the hybridization. In all experiments, the SPR chip was regenerated with 10 mM NaOH.

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