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Fluorous-based carbohydrate Quartz Crystal Microbalance

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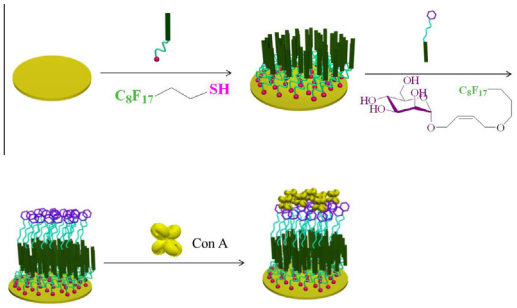
ABSTRACT

Fluorous chemistry has brought many applications from catalysis to separation science, from supramolecular materials to analytical chemistry. However, fluorous-based Quartz Crystal Microbalance (QCM) has not been reported so far. In the current paper, fluorous interaction has been firstly utilized in QCM, and carbohydrate–protein interaction and carbohydrate–carbohydrate interaction have been detected afterward.



1. Introduction

Gladysz, Horváth and Curran introduced fluorous chemistry at the end of last century.[1,2] As a kind of non-covalent interaction, fluorous interaction is based on the specific affinity between fluorous compounds (organic compounds contain C_nF_{2n+1}-group (n = 6–8), fluorous tail). The interaction is quite unique and independent to the well-known hydrophobic and hydrophilic ones, which brings many applications in different fields. For example, separation of fluorous and non-fluorous compounds can be easily achieved via fluorous solid phase extraction (F-SPE)[3], in which separation was achieved via the fluorous-modified silica gel. In 2005, the interaction was first introduced to small molecular microarray by Pohl et al.[4] Fluorous-modified carbohydrates were immobilized to glass surface via fluorous interaction and their binding ability to lectins was measured. Later on, various small molecules were introduced to glass surface via fluorous interaction aiming at microarray measurements.[5]

Considering the success of fluorous microarray, expanding the fluorous interaction to other analytical instruments is of great importance. Quartz Crystal Microbalance (QCM) is a mass sensor based on the piezoelectric properties of quartz crystals with many applications in both scientific research and industry.[6] Recently, QCM with dissipation monitoring (QCM-D) technique was developed as an extension, which simultaneously measures changes in the induced energy dissipation (ΔD) and the frequency (ΔF), and enables a label-free detection and analysis of bio-recognition events in real-time.[7] Comparing to microarray, QCM has its own pros and cons. Currently high throughput screening could not be easily achieved, which might be the major drawback of QCM. However, by using QCM, interactions between protein and ligand can be easily measured without any fluorescent labeling and the whole binding process can be monitored in situ. These two

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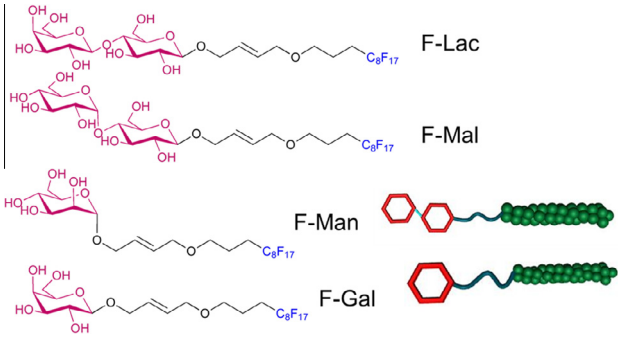
advantages make QCM more suitable to measure some complicated interactions with the analytes of high molecular weight. Furthermore, in a typical QCM-D measurement, energy dissipation (ΔD) could provide a viscoelastic parameter related to the properties of the propagation material.[8]

Thus it is quite valuable to combine fluorous interaction with QCM-D, which in fact has never been reported before. In this paper, the first example of QCM based on fluorous interaction will be demonstrated. Carbohydrate–protein interaction and carbohydrate–carbohydrate interaction (CCI) will be detected on fluorous surface. Under the help of ΔD, non-specific interaction can be differentiated from the specific ones. Thus the method reported in this paper has a promising future to be developed into a general detection method for various interactions.



Scheme 1. Chemical structures of fluorous sugars used in this study.

2. Results and discussion

Typically, pre-cleaned gold chip was first immersed in an isopropanol solution of 1*H*,1*H*,2*H*,2*H*-perfluorodecyl thiol (F-SH, 17% v:v). After 24 h, the chip was washed with pure isopropanol twice then water, and dried under nitrogen. The successful immobilization of fluorodecanethiol was proved by contact angle measurement. As shown in Figure 1a and b, the contact angles of gold surface before and after immobilization of fluorodecanethiol are 63° ± 2° and 105° ± 1°, respectively. This transformation from hydrophilic to hydrophobic supports the successful modification of F-SH on gold surface.

The next step is to coat fluorous-modified sugars (F-sugars) to the fluorous surface of gold chips. Four different F-sugars, including **F-Lac**, **F-Mal**, **F-Gal**, and **F-Man** (Scheme 1) are prepared according to the reported synthetic procedures.[9] The coating step looks straightforward at first, because F-sugars were successfully loaded to fluorous-coated glass slide in microarray assays. In microarray experiments, F-sugars were first dissolved in the mixture of DMF/MeOH/water, then the sample was loaded to the fluorous glass surface via automated pin in microliter scale. The spots finally obtained on glass surface were in the size of ca 100 μm, which was very easy to be dried and immobilized. No further washing is necessary because the amount of F-sugars on surface was very limited. However, at first, a similar strategy could not be directly transformed to the gold chip surface, because QCM measurement requires a single layer of F-sugars on the fluorous chip in cm scale with their fluorous tag interacting with the surface and their sugars toward solution, which is crucial to the success of QCM measurement. Inspired by the fluophilic condition for F-SPE, the coating procedure was designed as follows and proved successful. Typically, the gold chip was immersed in the solution of F-sugars (0.2 mg/mL) in MeOH/water = 3:2 (v:v) overnight. Then the chip was washed gently with the mixed solvent and dried. The successful immobilization of F-sugars was characterized by contact angle (Fig. 1c and d), which was measured as 38° ± 3° showing hydrophilicity from the previous hydrophobic fluorous surface.

After F-sugar has been immobilized on the gold chip, QCM measurement was then performed. As shown in Figure 2, after a short wash of HEPES buffer, peanut agglutinin (PNA) solution (0.2 mg/mL, in HEPES buffer) was loaded to the chip immobilized with **F-Lac**. Immediately, a dramatic frequency ($-\Delta F$) increase of the chip was observed within several seconds, indicating the successful absorption of proteins on surface. When $-\Delta F$ reached around 37 Hz, this quick increase reached equilibrium for a short time. Then more proteins were absorbed onto the surface in a slower rate with the total ΔF change less than 5 Hz within 700 s. At the moment, the surface turned to equilibrium again without any increase of $-\Delta F$. Subsequently, PNA in HEPES buffer at a higher concentration (0.5 mg/mL) was flowed to the chip instead of the previous one. After this switch, another 6 Hz increase of $-\Delta F$ was observed within 800 s, which was even much slower to reach

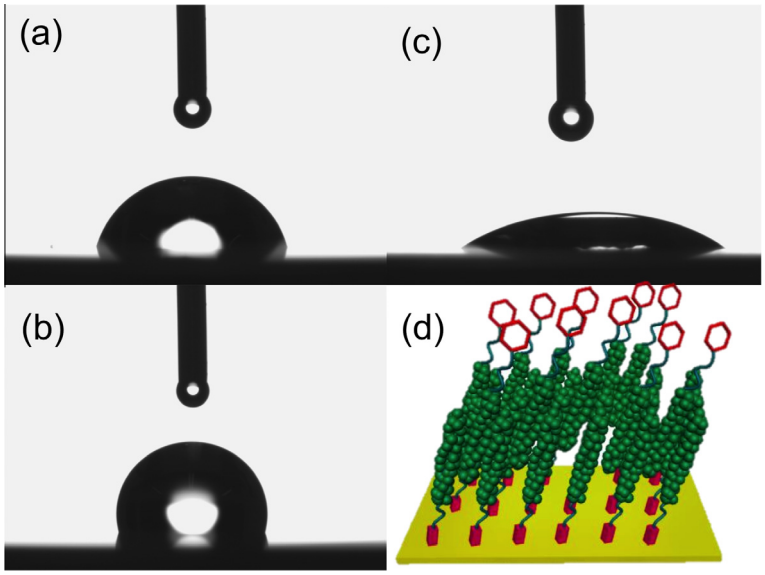


Figure 1. Contact angles of (a) bare gold, (b) fluorous gold surface, (c) after F-sugar coating. (d) Cartoon representation of the gold surface after coating with F-SH and F-sugar.