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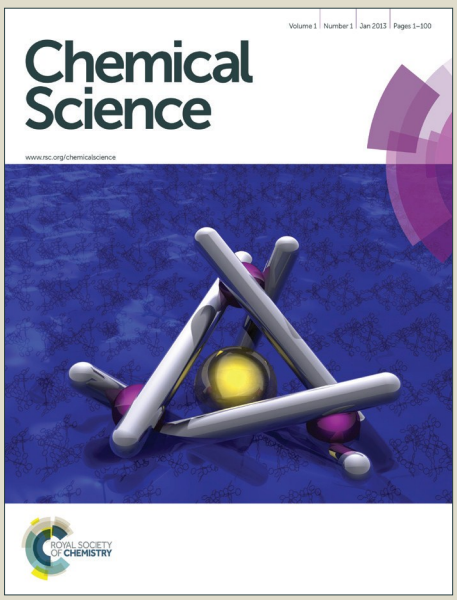

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ARTICLE

A novel probe based on phenylboronic acid functionalized carbon nanotubes for ultrasensitive carbohydrate determination in biofluids and semi-solid biotissues

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Carbohydrates are known to be involved in a wide range of biological and pathological processes. However, due to the multiple hydroxyl groups structure, carbohydrate recognition is a particular challenge. Herein, we reported an ultrasensitive solid-phase micrextraction (SPME) probe based on phenylboronic acid (PBA) functionalized carbon nanotubes (CNTs) for direct in vitro or in vivo recognition of carbohydrate in biofluids as well as semi-soild biotissues. The coating of the proposed probe possessed 3D interconnected porous architecture formed by the stacking of CNTs. As a result, the binding capacity toward carbohydrate was excellent. The proposed approach was demonstrated to be much superior to most of carbohydrate sensors, including higher sensitivity, wider linear range, and excellent qualitative ability in multi-carbohydrate coexistent system. Thus, this approach opened up new avenues to the facile and efficient recongnition of carbohydrate for important applications such as glycomics.

Introduction

Carbohydrates are known to be involved in a wide range of biological processes.[1] Simultaneously, the concentration of carbohydrates in biological system is vital to several pathological processes. For example, the diabetes mellitus, which is one of the biggest public health threats, demands continuous carbohydrates monitoring.[2] Thus, precise determination of carbohydrates is necessary for not only fundamental researches but also clinical diagnoses.

Unlike nucleic acids, amino acids and lipids, determination of carbohydrate in aqueous solution is a tough challenge for chemists and biologists.[3] Carbohydrates are hydrophilic species and therefore difficult to be extracted from water by traditional pre-treatment methods. As the nature of multiple hydroxyl groups, they are also hydromimetic, blending easily into a background of water molecules.[4,5] Synthetic receptor for specific carbohydrates recognition is a challenging yet highly impactful area of research.[6-9] The phenylboronic acid (PBA) and its derivatives, known be able to rapidly and reversibly interact with a 1,2 or 1,3-diol in aqueous media, are the hottest candidates for carbohydrate receptor design.[10-12] However, the synthesis routes of the receptor for carbohydrate sensor are usually complicated and tedious, and the efficiency and selectivity of the synthetic receptors, particularly ones that in the competitive solvents, remain a major challenge. The reasons for this are that the interactions of a receptor with the OH groups of a carbohydrate-derived substrate do not fundamentally differ from that with water molecules, which causes the cross-interference of the determination signals, and the structural similarity of many carbohydrates, d-glucose and d-mannose, for example, differ in the configuration of only a single OH group on the ring.[5] In addition, the determination principles mainly depended on the physicochemical signal changes of the receptor exposed to the sample, such as fluorescence,[13-15] swelling/shrinking degree,[16-18] diffraction[19] and conductivity[20] and so on. It inevitably caused that: 1) the limits of detection of the carbohydrate sensors were mostly ranged from hundreds of micromoles to millimoles per lite. The potential ability to application in unconventional body fluids containing low carbohydrate concentrations, such as interstitial fluid extracted by iontophoresis, tears, saliva and urine and at intracellular concentrations at the single-cell level in metabolomic studies,[21-24] were infeasible; 2) The synthetic receptors were not capable of application for carbohydrate recognition in the semi-solid or solid biological tissues, which are the main components that comprised the organism.

Carbon nanomaterials, such as carbon nanotubes (CNTs), have been explored extensively for carbohydrate-related biomolecule recognition in recent years.[25,26] Pristine carbon nanomaterials suffer from low solubility, thus the surface properties of CNTs can be tuned not just to improve their water solubility but also to enable these versatile

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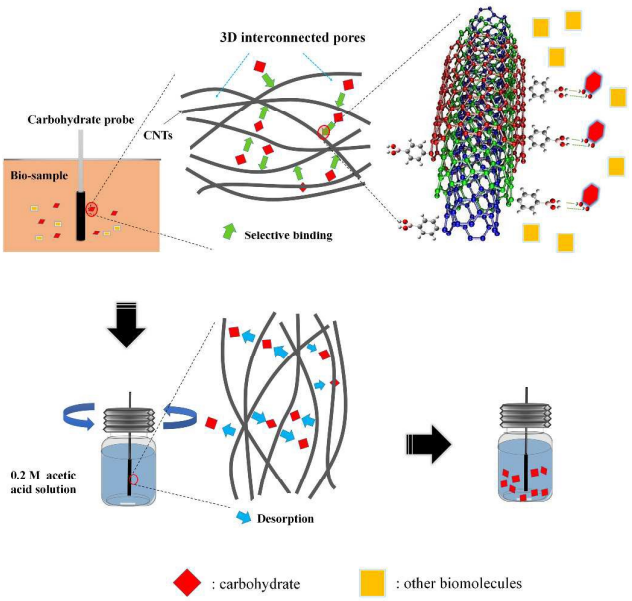
nanomaterials to interact selectively with biological systems in aqueous systems. Chen et al. recently reported a review, which highlighted the strategies for synthesis of functionalized carbon nanomaterials and their applications in biosensing and biomedicine.[27] As reported recently, functionalized CNTs could serve as an excellent one-dimensional scaffold for ligand display, which exhibited strong affinity to lectin[28], glucose[9] and glycan[29] in aqueous system. In addition, a novel nanocomposite consisting of 3-aminophenylboronic acid and CNTs was synthesized and constructed an impedance-cell sensor.[30] Herein, we fabricated an ultrasensitive SPME probe based on PBA functionalized CNTs, which enabled fast, quantitative, and direct carbohydrate analysis in biofluids or semi-solid biotissues, by coupling with gas chromatograph-mass spectrometry (GC-MS). Firstly, PBA functionalized-CNTs was synthesized and acted as the carbohydrate nano-receptor. The hybrid, containing proper ratio of nano-receptor to other biocompatible and acid resisting polyacrylonitrile (PAN), was attached to the pretreated quartz fiber through dip-coating method forming a novel pH-controlled capture/release miniature probe for selective capture of carbohydrate. Owing to the 3D interconnected architecture formed by the stacking of PBA functionalized-CNTs in the coating, the proposed probe possessed excellent binding capacity toward carbohydrate (the enrichment factors were as high as 151). Only by adjusting the pH of the eluent, this proposed probe was feasible to couple with GC-MS for carbohydrate separation and detection, which dexterously avoided the cross-interaction effect and greatly improved the sensitivity for carbohydrate recognition simultaneously (Scheme 1). Interestingly, the proposed probe was valid to identify and determinate carbohydrate in a multi-carbohydrates coexistence system. Moreover, this carbohydrate probe was successfully applied to determinate glucose in bovine serum and human urine without any expensive enzymes or tedious pretreatment procedure. Importantly, the excellent biocompatibility and mechanical strength of the probe made it possible to directly immerse the probe into the semi-solid biological tissues (plant leaf and stem) for in vivo carbohydrate recognition and continuous carbohydrate monitoring.

Results and discussion

Synthesis and Characterization of the receptor

As the ultrahigh ratio of surface area to volume of the carbon nanotube (CNTs), and the extreme sensitivity of their surface atoms to any surface reaction events. Herein, PBA functionalized-CNTs, with an external diameter about 40 nm (Figure 1A and S1), was synthesized and used as a carbohydrate receptor. The IR spectrums (Figure 1B) and XPS (Figures 1C and S2) confirmed the existence of PBA groups on CNTs. The affinity of the PBA functionalized-CNTs to diol unit was first confirmed using adenosine as a test compound, which contains a pair of cis-diol groups and it has UV absorbance at about 260 nm. The deoxyadenosine was used as an interferrant, which also has UV absorbance at about 260 nm while contains no cis-diol moiety. As shown in Figure 1D, the PBA functionalized CNTs exhibited excellent selectivity toward adenosine, and the binding amount was depended on the exposure time (Figure S3). In addition, the binding capacity toward adenosine was measured to be 50.9 ± 2.3 μmol/g. These results demonstrate that the synthesized receptor shown excellent selectivity toward cis-diol.



Scheme 1. Representation of the carbohydrate recognition with the probe based on PBA functionalized-CNTs.

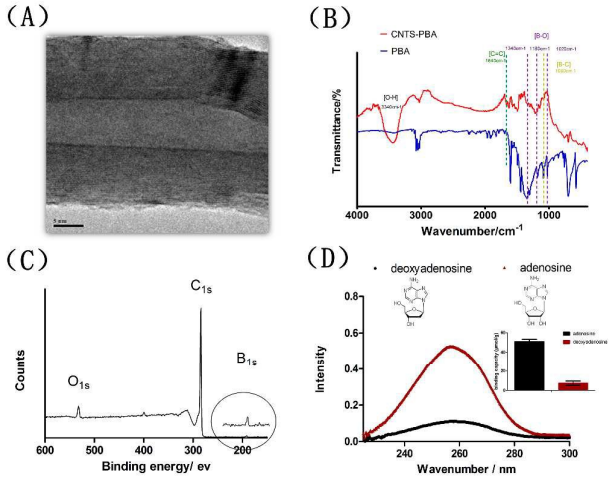


Figure 1. High TEM image (A), IR spectrums (B) and entire XPS spectrum (C) of the synthesized PBA functionalized-CNTs; (D) The selectivity of PBA functionalized-CNTs toward cis-diol.

Binding capacity toward diol of the probe

There are two probe design challenges: 1) an appropriate auxiliary for fixing the receptor on the solid substrate (quartz fiber); 2) prevent the auxiliary material from covering the receptor. Otherwise, the covering auxiliary could block binding sites of the receptor. Herein, PAN (Mw=150000, dissolved in dimethylformamide, V:V=1:10), which was regarded as a biocompatible and acid resisting polymer,[31-33] was selected as the auxiliary for attaching the receptor to the pretreated quartz fiber through dip-coating method. Meanwhile, dimethylformamide was enabled to evaporate at high temperature (120 °C, 40 min) and feasible to create a porous structure of the coating (Figure 2A). In addition, as seen in Figures 2A-B and S4, the stacking of CNTs could form 3D interconnected pores compared with the nanoparticle stacking (We fabricated another PBA functionalized-carbon dots based probe as a nanoparticle stacking probe model, which was shown in Figures 2C-D). This architecture possessed high specific surface area and facilitated the mass transfer in the coating, which greatly enhanced the availability of PBA groups (Figure 2E). Meanwhile, owing to the reversible binding of PBA groups-diol unit[10] and the high acid resisting ability of PAN, the bound diol unit was feasible to be released for further qualitative or quantitative analysis only by adjusting the pH of the eluent (Figure 2F). As shown in Figure 2G, the extraction efficiency of the proposed probe toward diol was much superior to the other probes widely used in biological analysis, including polydimethylsiloxane (PDMS) and C18.[34-37] In addition, the comparison result between the PBA functionalized probe and non-PBA functionalized probe indicated that the extraction performance of the proposed probe was contributed to the PBA groups in the coating, which demonstrated that the PBA groups in the probe were available and provided a specific scaffold for carbohydrate binding.

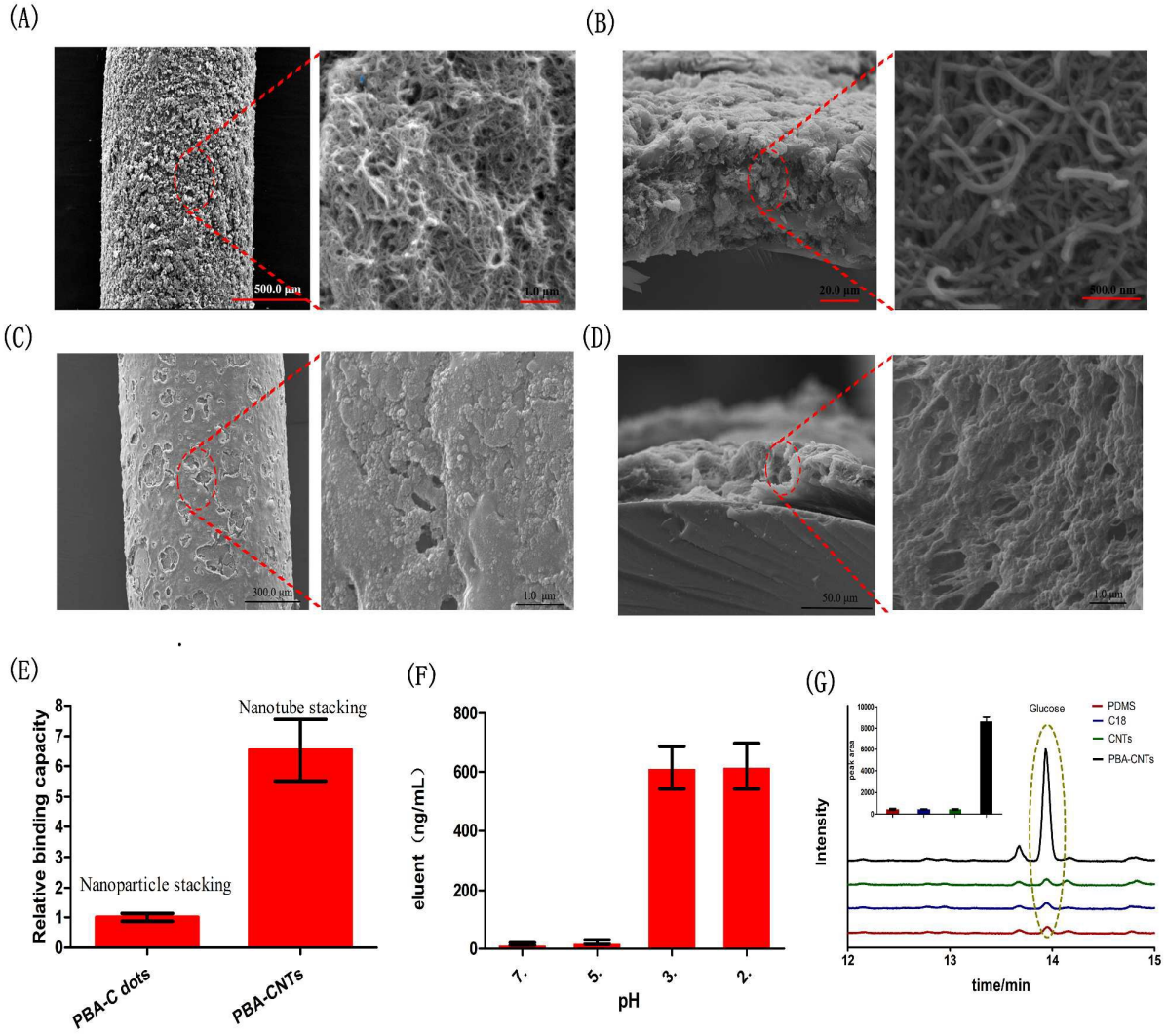


Figure 2. TEM images of the surface (A) and cross section (B) of the PBA functionalized-CNTs probe; TEM images of the surface (C) and cross section (D) of the PBA functionalized-carbon dots probe. It could be observed that no 3D interconnected pores was formed in the PBA functionalized-carbon dots probe; (E) the proposed probe with nanotube stacking possessed high binding capacities than the ones based on PBA functionalized-carbon dots (nanoparticle stack). Two kinds of probes were prepared in the same way; (F) concentrations of glucose in eluent under different pH (exposure time: 40 min); (G) compared the binding capacities with other widely used probe and non-PBA functionalized probe.

Specificity of the probe toward carbohydrate

Selectivity is a critical parameter to evaluate the performance of the probe. We firstly studied the response of the proposed probe toward the potential interfering substances coexisting in biofluids, including various amino acids, aliphatic acid, glutathione, and uric acid. As shown in Figure 3, the extraction capacity of the probe toward these substances was negligible. The exclusive extraction of carbohydrate was resulted from the following reasons. Firstly, the carbohydrate possessed specific multiple hydroxyl groups structure while these coexisting substances have no such special structure. Secondly, as demonstrated in Figures 1D and 2, the probe provided a specific scaffold for diol unit, especially for the cis-diol. Thus, the carbohydrate, possessed various 1,3 or 1,2 cis-diol units, could be easily captured by the scaffolds.

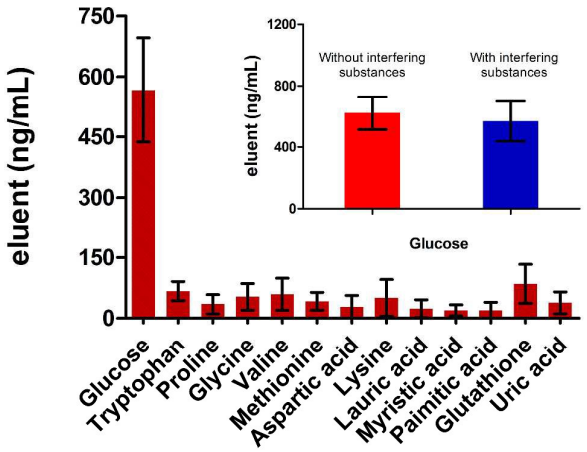


Figure 3. The response of the probe toward various potential interfering substances. The mixture contained glucose, various amino acids, aliphatic acid, glutathione, and uric acid (the concentrations were both of 10 μM) was extracted by the probe for 40 min. It showed that the probe presented specific selectivity toward carbohydrate. The inserted figure was the response of the probe toward glucose in the solution without and with interfering substances.

Glucose recognition in PBS

The performance of the prepared probe for carbohydrate assay was firstly evaluated in the phosphate buffer solution (PBS). Meanwhile, glucose, a typical monosaccharide, was used as carbohydrate model, and 0.2 M acetic acid (pH≈2) was selected as the eluent. As shown in Figure 4A, the binding reaction was completed within 20 min. Such a beneficial feature was due to the 3D interconnected pores and fixed orientation of the PBA molecules within the probe, which facilitated the efficiencies of mass transfer and complexing simultaneously.

Under the optimized conditions, the probe was applied for glucose determination in PBS solution. Due to the enrichment effect of the probe (see below) and the high resolution detector, the linear range of the probe was ranged from 1 μM to 100 μM for glucose determination (Figure 4B), with a limit of detection of 0.12 μM (signal-to-noise ratio of 3). To our knowledge, the linear range and the detection limit of the proposed probe were much better than most of previous boronic acid based sensors (Table S1). A higher sensitivity for glucose assay is important not only in low concentration biofluids, such as tears, saliva and urine, but also in high glucose level biofluids, such as blood (several to tens of millimoles per liter). The reason is that highly sensitive probes would allow a sufficient sample dilution during assay, which can effectively reduce the interference from the complicated matrix (blood/serum).[38] It is noteworthy that the whole assay procedure, consisting of binding, elution and detection, was less than 2 h. In addition, the reusability of the probe was evaluation. It shown that the extraction efficiency had not significant change after 20 times uses (Figure S5).

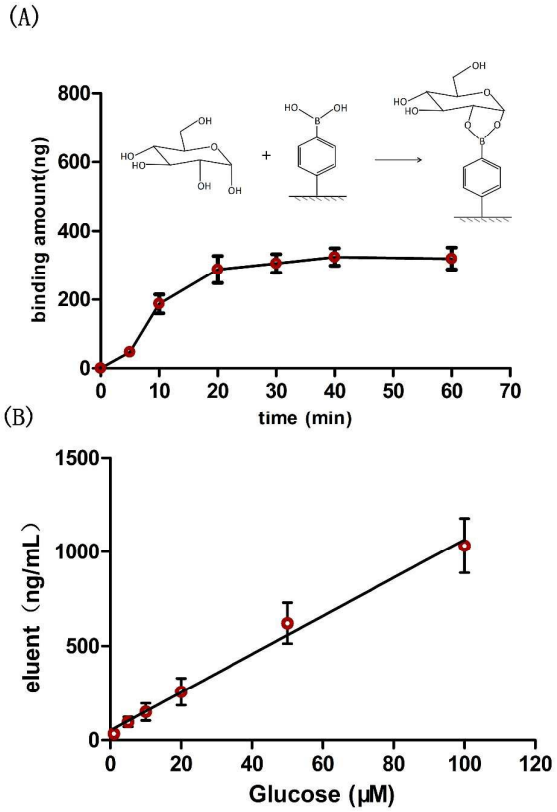


Figure 4. (A) Binding kinetic of the proposed probe toward glucose in PBS solution (100 μM glucose); (B) the wide linear range of the glucose in PBS solution.

Multi-carbohydrate recognition in PBS

Carbohydrate is one of the most abundant molecules that comprise human life, and the kinds of carbohydrate are various. The approaches based on sensors for carbohydrate recognition have some drawbacks, one is that it is not capable of qualitative or quantitative recognition of multi-carbohydrate in biofluids. In parallel with the emergence of glycomics, the novel assay for fast and accurate carbohydrate recognition in the multi-carbohydrates coexisting biofulids was required.[39-42] Here, owing to excellent separation ability of chromatography as well as the high resolution of mass spectrum, the proposed probe coupled with GC-MS was proved to be feasible to recognize carbohydrate in a multi-carbohydrates coexisting PBS solution, with the linear ranges ranged from 0.5 μM to 20 μM (Figure 5A), which could compensate for the low qualitative or quantitative ability of the previous boronic acid based sensors.

Owing to the unique 3D interconnected architecture in the coating, the enrichment factors, defined as the ratio of the carbohydrate concentrations in probe and in matrix, were measured to be ranged from 63 to 151 (Figure 5B). Generally, the probe showed higher enrichment capacity toward hexose than pentose. The reasons we explained were that: 1) PBA can bind with cis 1,2- or 1,3-diols to form a diol–phenylboronate complex with either five- or six-membered ring systems, respectively,[43,44] 2) The binding amount is largely depended on the probability of directional collision. From this point, the hexose, which has additional hydroxy unit compared with the pentose, should easy to complex with PBA. It was noteworthy that no selectivity against structural similar carbohydrate, such as mannose, glucose and galactose, was required in this approach. Since the probe was easy to couple with GC-MS.

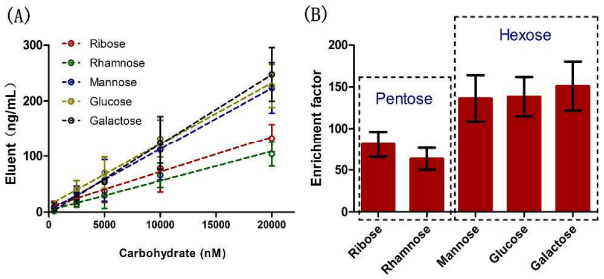


Figure 5. The linear ranges (A) and enrichment factors of multi-carbohydrates (B) in PBS solution using the proposed probe.

Assay in serum and urine

Given the simplicity and ultra-sensitivity of carbohydrate determination with the proposed probe, the approach can be said to be an ideal device for monitoring carbohydrate in real biofluids. As a proof of concept, bovine serum was firstly used as a biofluid model. Considering the high glucose level in serum, 15 μL bovine serum was diluted 100 times with the PBS solution, and then was transferred into a 2 mL vial. The probe was directly immersed into the serum for glucose determination without any expensive enzymes or tedious pretreatment procedure. As shown in Table 1, the glucose obtained by the proposed probe was well agreement with the values measured by a commercial blood glucose monitor. Benefited by the ultrasensitive property of the proposed approach, the probe was also successfully applied for glucose determination in the trace glucose contained sample, urine (Table 1).

Table 1. Glucose assay in bovine serum and human urine.

Sample[a]	S-1	S-2	S-3	Average
Serum	4.6 mM	5.7 mM	6.9 mM	5.7 mM
Urine	16.0 μM	16.2 μM	17.7 μM	16.6 μM

[a] the average detected concentration of glucose in serum was 5.7 mM with the proposed probe, which was well agreement with the values measured by the commercial blood glucose monitor (5.9 mM). Notably, the glucose (16.6 μM) was detectable using the proposed probe.

In vivo carbohydrate recognition

Previous fully-implantable sensor, embedded in the body for carbohydrate recognition, are suffering challenges mainly due to the insufficient physicochemical signal intensity required for transdermal detection.[45] Fortunately, the proposed probe could dexterously avoid this tough challenge due to the different detection principle. Thus, besides the above in vitro analysis, other major novel application of the proposed probe was the in vivo carbohydrate recognition in semi-solid biotissues, such as plant stem and leaf. Figure 6A was briefly presented the in vivo sampling procedure in plant tissues. To achieve this goal, the probe should be capable of resisting the adhesion of biological macromolecules on the surfaces. Otherwise, the adhered biological macromolecules would block the binding sites of the probe and alter the ionization efficiencies when the macromolecules were desorbed in the desorption solvents. It was demonstrated through MALDI-TOF MS that no macromolecules were existed in the eluent of probe exposed in aloe leaf or Malabar spinach stem for 30min (Figures 6B and S6). Moreover, the excellent mechanical strength of the probe, which could be observed in Supplementary movie 1, make it suitable for direct immersing into semi-solid biotissues. Regarding to the carbohydrate selected in this study, glucose was the main monosaccharide detected in Malabar spinach while rhamnose, mannose, glucose and galactose were detected in aloe without any plant sacrifice (Figure 6C). The abundant species of monosaccharide detected in aloe leaf with the proposed assay were well agreement with the previous analysis consisted of several tedious pretreatment,[46,47] which demonstrated the feasibility for in vivo analysis. Finally, the probe was used for noninvasive and long-term in vivo continuous carbohydrate monitoring in aloe leaf (Figure 6D).

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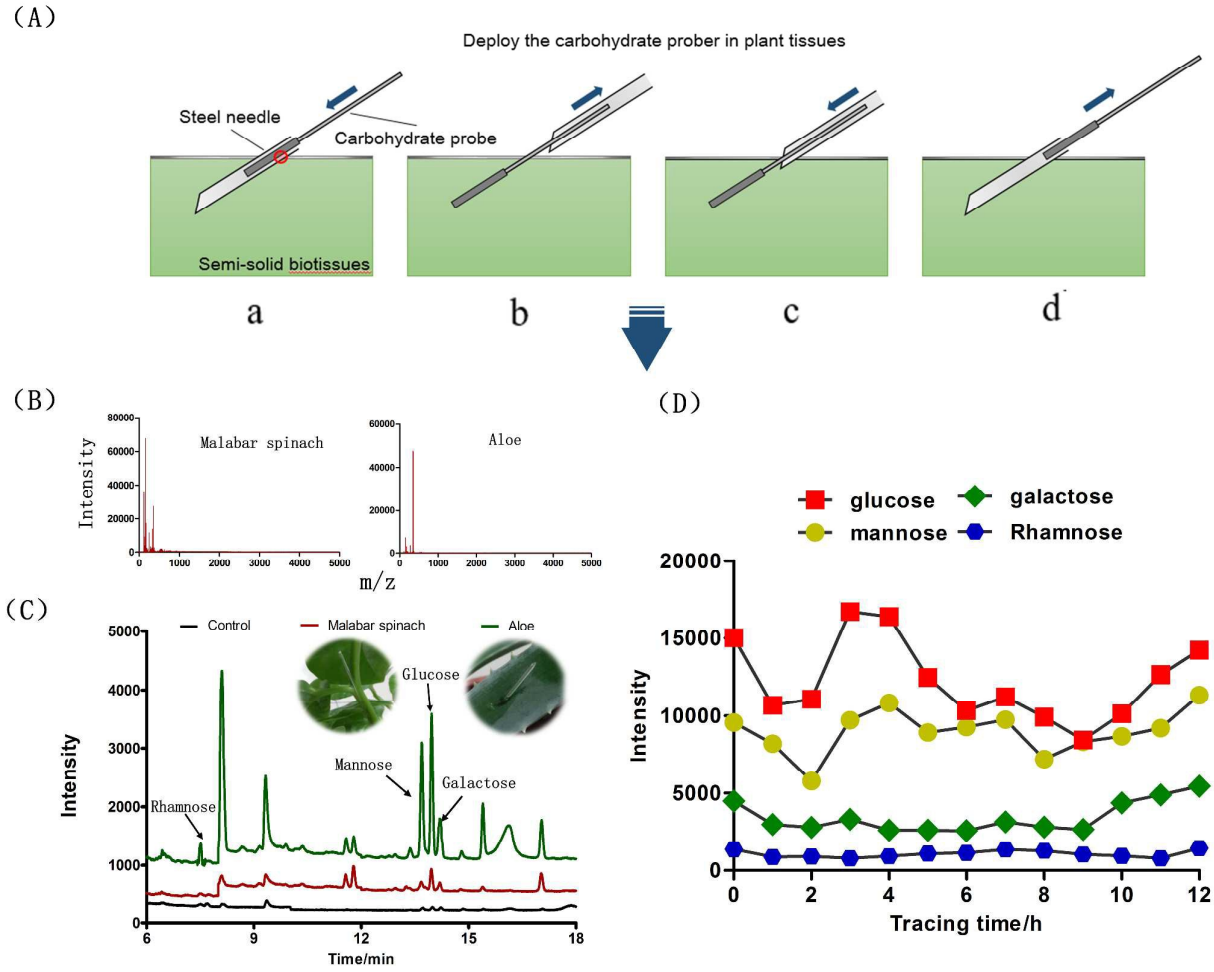

Figure 6. (A) The in vivo sampling procedure in plant tissues. Deploy the carbohydrate probe under the guidance of a steel needle (a), remove the steel needle and expose the carbohydrate in plant tissues (b), carefully put back the steel needle in the plant tisues at the end of sampling (C), and remove the carbohydrate probe (d). The probe was easy to be inserted into or removed from plant tissues; (B) biological macromolecules analysis in the eluent with MALDI-TOF MS; (C) carbohydrate assay in the stem of Malabar spinach and leaf of aloe. The signals of the corresponding carbohydrate were apparent; (D) in vivo continuous carbohydrate monitoring in aloe leaf using the proposed probe.

Conclusions

In summary, a novel SPME probe based on PBA functionalized-CNTs was proposed for fast and ultrasensitive determination of carbohydrate in biofluids and semi-solid biotissues. The proposed approach was demonstrated to be much superior to the carbohydrate sensors. It exhibited several significant advantages, including higher sensitive, wider linear range, and excellent qualitative ability. Unlike the method based on carbohydrate sensors, no more complicate and tedious synthesized route of the receptor was need in the proposed approach. Moreover, the probe was capable of direct in vitro or in vivo determination of multi-carbohydrates in complex real sample matrix, and the total analysis procedure was time-saving (less than 2 h). Notably,

the preparation approach of the probe can be applicable to other nanomaterials. In conclusion, this approach opened up new avenues for facile and efficient tracing and recognizing carbohydrate in bio-sample and could be a promising approach for important applications such as glycomics.

Experimental

Reagents and materials

Multi-walled carbon nanotube (MWCNTs), diamine, N,N'-diisopropylcarbodiimide (DIC), N,N-Diisopropylethylamine (DIEA), N-hydroxysuccinimide (NHS), dichloromethane (DCM), dimethylformamide (DMF), anhydrous tetrahydrofuran (THF) and all analytes for saccharides detection experiments were purchased from Aladdin Reagent (Shanghai, China); thionyl chloride (SOCl2) was obtained from Thermo Fisher Scientific (Guangzhou, China); 4-carboxyphenylboronic acid (4-CPBA), polyacrylonitrile (PAN), sodium borohydride and methylimidazole were purchased from J&K Scientific (Beijing, China); Nitric acid, ethanol, diethyl ether, acetic acid; acetic anhydride were from Guangzhou Reagent Company (Guangzhou, China). Quartz fiber (420 μM O. D.) was obtain from Scitlion Technology Co., Ltd (Beijing China); Bovine serum was purchased from Sigma Aldrich (Shanghai, China) and human urine was obtain form Centre for Disease Prevention and Control of Guangdong Province (Guangzhou, China). All the plants used in this study were cultivated from seeds in the plant growth chamber (Conviron A1000, Cannada).

Synthesis of the PBA-functionalized CNTs

Firstly, the raw sample of CNTs was refluxed in nitric acid for 11 h, and the carboxylated CNTs were filtered and washed with deionized water until pH was 7 and dried in vacuum oven. Then 300 mg of carboxylated CNTs were stirred in 60 ml of a 20:1 mixture of SOCl2 and DMF at 70 ◦C for 24 h. After the acyl chlorination, the CNTs were centrifuged and washed with anhydrous THF for six times followed by dry under vacuum. Then the acyl-chlorinated CNTs were reacted with 100 ml diamine solution at 100 ◦C for 48 h. After cooling to room temperature, the amino functionalized-CNTs (A-CNTs) were obtain and washed with ethanol for five times to remove excess diamine. Lastly, 100 mg A-CNTs, 0.4 mmol 4-CPBA, 0.5 mmol DIC, 0.5 mmol DIEA, and 0.5 mmol NHS were dissolved in the mixture solution of dichloromethane and DMF. The reaction solution was kept stirring for 24 hours at room temperature. Then, diethyl ether was added to stop the reaction followed by filtration and washing with diethyl ether, water and methanol. The product was dried under vacuum to get the PBA functionalized-CNTs.

The selectivity of PBA-functionalized CNTs

For demonstration the selectivity of the PBA-CNTs toward cis 1,2-diol compound, adenosine and deoxyadenosine were used as the model compound. 2 mg PBA functionalized-CNTs was added to 1 mL solution of 1 mg/mL adenosine or deoxyadenosine. The tubes were shaken on a rotator (400 rpm) for 1 h at room temperature. Then the suspension was centrifuged and the collected PBA functionalized-CNTs was rinsed with 1.5 mL of the PBS solution (pH 8.5) for 6 times each. Afterwards, the PBA functionalized-CNTs was resuspended in 1 mL of 0.2 M acetic acid solution and eluted for 1 h on a rotator with 600 rpm speed. Finally, the PBA functionalized-CNTs was collected by centrifuge again and the eluates were collected by pipetting carefully. The eluates were used for UV analysis.

The binding capacity of the receptor

For measurement of the binding capacity, a series of adenosine solution (1.00, 0.50, 0.10, 0.050, 0.0010, 0.00050 mg/mL) was prepared. The absorbance of the six solutions was detected to draw the standard curve. 2 mg PBA functionalized-CNTs were added to 1.5 mL of 1.0 mg/mL adenosine solution in a 2 mL plastic tube. The solution was shaken on a rotator for 12 h at room temperature (in order to measure the binding site was entirely complexed with adenosine). The following centrifuge and elution procedures were consistent with the ones mentioned above. According to the measured absorbance of the eluent and the standard curve, the concentration of the eluate was obtained, from which the binding capacity was calculated. The binding capacity of PBA functionalized-CNTs was measured to be 50.9±2.3 μmol/g.

Preparation of the probe based on PBA functionalized-CNTs

The quartz fibers (QFs) were cut into 4-5 cm segments followed by sonication in water, menthol and acetone. After sonication, the QFs were then soaked in 0.1 M sodium hydroxide for 30 min to activate the surface, the excess sodium hydroxide was neutralized with hydrochloric acid. Finally, the OFs were dried at room temperature.

100 mg PAN was fully dissolved with 1 g anhydrous DMF in a 1.5 mL plastic tube through 1 h sonication. 40 mg PBA functionalized-CNTs was then added to the plastic tube. Another 30 min sonication was conducted to form the dispersive slurry. The pretreated QFs were dipped into the slurry and removing them slowly, a uniform coating of slurry of PAN and PBA functionalized-CNTs with 1.5 cm length was prepared on the surface of the QFs. The QFs were dried under flowing nitrogen, and finally curing them for 40 min at 120 ◦C, which facilitated DMF to evaporate and ensure better adherence of the coating to the QFs.

Binding capacity of the probe

To study the advantage of the nanotube stacking of the coating, another probe based on PBA functionalized-carbon dots, which possessed nanoparticle stacking (Figure 2C and D), was used as a reference. The synthesized method of PBA functionalized-carbon dots was referenced to Shen. P. and Xia. X..[48] Briefly, 0.2 g of phenylboronic acid was dissolved in 20 mL of ultrapure water, followed by adjusting the pH to 9.0 by adding 0.1 M NaOH under stirring, then bubbling nitrogen gas for 1 h to remove dissolved O_2. Finally, the solution was transferred to the Teflon-lined autoclave chamber and heat

to 160 °C for 8 h. TEM images and fluorescence spectra of the PBA-carbon dots were provide in Figure S7. The preparation of the probe based on PBA functionalized-carbon dots, including material dosage and preparation process, was consistent with the method mentioned in the section of "Preparation of the probe based on PBA functionalized-CNTs." The characterizations of micro-morphologies were shown in Figure 2C and D. For evaluation of the binding capacity, each kinds of probe was immersed into the glucose aqueous solution (10 μM, 1.5 mL) and then shocked on a rotator. After 60 min, the probe was removed and rinsed with deionized water for 30 s followed dried with a Kimwipe tissue. Finally, the bound glucose on the probe was then eluted in 1.5 mL 0.2 M acetic acid solution, the concentration of glucose was detected by GC-MS.

Compared the extraction performance with other commonly used biological probe

PDMS and C18 commercial probes (both 45 μm in thickness) were obtain from Supelco Inc (Shanghai, China), and the other CNTs probe without PBA modified was prepared throng the same method mentioned above in our lab. The concentration of glucose used was 50 μM. All the experimental parameters and relative operating process was consistent with the ones mentioned in the section of "Evaluation of the performance in PBS solution".

Evaluation of the performance in PBS solution

For glucose assay in PBS solution, a series of glucose solution (dissolved in PBS, 1.0, 5.0, 10.0, 20.0, 50.0, 100.0 μM) was prepared in 2 mL brown vial with cap gasket (polytetrafluoroethylene). The procedure of introduction and fixation of the probe in PBS solution was as follow: a steel needle of a hypodermic syringe head was pierced into the cap gasket to create a hold. The end of the QFs, which is the opposite end of the coating, was then inserted into the cap gasket. Afterwards, the cap with QFs was screwed (Figure S8). The extraction was performed on a rotator with 400 rpm speed for 20 min. After extraction, the cap was removed and the probe was then rinsed with deionized water for 30 s and dried with a Kimwipe tissue. Subsequently, the probe was immersed into the glass vial (250 μL 0.2 M acetic acid) and eluted for 30 min (optimized in Figure S9) on a rotator with 600 rpm speed. The eluent was processed with a simple derivatization prior to introduce into GC-MS for glucose determination. For multi-carbohydrate assay in PBS solution, a series of carbohydrate solution, contained ribose, rhamnose, mannose, glucose and galactose (dissolved in PBS, 0.50, 2.5, 5.0, 10.0, 20.0 μM), was prepared. The assay procedure was consistent with the ones in glucose solution.

Ex-vivo glucose assay in bovine serum and human urine

The bovine and human urine was stored at -80 ◦C prior to analysis. For assay in bovine serum sample, 15 μL thawed bovine serum was diluted to 1.5 mL PBS solution in a 2 mL brown vial. For assay in human urine sample, due to the trace glucose contained in urine, 1.5 mL thawed human urine was directly transferred into 2 mL brown vial by pipetting without further dilution. The probe was then directly immersing into the serum or urine sample for glucose assay without any expensive enzymes and tedious pretreatment procedure, and the assay procedure was consistent with the ones mentioned in the section of "Evaluation of the performance in PBS solution".

In vivo carbohydrate assay in plant

The customized hollow steel needle was stabbed into the leaf or stem of the plant to a depth of about 2 cm. The probe was inserted into the needle and reached to the end of the needle. Subsequently, the needle was carefully withdrawn back to let the probe be exposed in the plant tissues. After a certain duration, the needle was put back to plant under the guidance of the probe to a depth of about 1.5 cm. Then, the probe was withdrawn from the needle, and the needle was removed. The total sampling duration was controlled to be 30 min. The probe were then rinsed with deionized water for 3 times (60 s for each time) and dried with a Kimwipe tissue. Subsequently, the probe was immersed into the glass vial (250 μL 0.2 M acetic acid) and eluted for 30 min on a rotator with 600 rpm speed. The eluent was processed with derivatization and then introduced into GC-MS for carbohydrate determination.

GC-MS analysis

The eluent for carbohydrate analysis needs a simple derivatization prior to GC-MS analysis. Briefly, 2% 0.2 mL sodium borohydride solution (dissolved in ammonium hydroxide) was added into the eluent. After reaction for 20 min at 40 ◦C, 0.4 mL acetic acid, 0.3 mL methylimidazole and 1 mL acetic anhydride was added to the solution followed another 10 min reaction. Finally, the derivatives of carbohydrate were dissolved in 500 μL dichloromethane. The carbohydrate detection was performed on an Agilent 6890N gas chromatograph equipped with a MSD 5975 mass spectrometer The GC-MS and electron-impact ionization (EI). A split/splitless-type injector was used for sample introduction. Chromatographic separation was carried out with a HP-5MS capillary column (30 m × 250 μm × 0.25 μm, Agilent Technology, CA, USA). The inlet temperature was 240 °C, and the oven temperature programs were as follows: The initial oven temperature was 140 °C (held for 0. 5 min), ramped at 30 °C/min up to 190 °C (held for 5 min), and ramped at 2 °C/min up to 210 °C (held for 2 min). Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. The MSD was operated in the electron impact ion (EI) mode with a source temperature of 230 °C. The electron energy was 70 eV and the filament current 200 A.

Biochemical Analyzer for Glucose Assay

To evaluate the feasibility of the proposed probe for determination of glucose, the concentrations of glucose in human serum were assayed by an enzymatic (hexokinase) text using PUZS-300 automatic biochemical analyzer. The methods was referenced by Shen. P. and Xia. X..[48] Briefly, the

sample was firstly added into the sample cup, which was then placed in sample frame for the measurement after setting the parameters. Under the catalytic effects of hexokinase, glucose and adenosine triphosphate (ATP) can react and form glucose-6-phosphate and adenosine diphosphate (ADP). The former can dehydrogenize and form 6-phosphate glucose acid in the presence of glucose-6 phosphate dehydrogenase. At the same time, nicotinamide adenine dinucleotide phosphate (NADP) is reduced and form nicotinamide adenine dinucleotide phosphate (NADPH). The production rate of NADPH is proportional to the concentration of glucose, which can be monitored by the absorbance at 340 nm and measure the glucose concentration.

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Notes and references

1 M. S. Timmer, B. L. Stocker, P. H. Seeberger, *Curr Opin Chem Biol* 2007, **11**, 59-65.
2 M. B. Davidson, *Diabetes Care. Dec.* 1998, **21**, 2152-2160.
3 D. Walt, K. F. Aoki-Kinoshita, B. Bendiak, C. Bertozzi, G. Boons, A. Darvill, G. Hart, L. Kiessling, J. Lowe, R. Moon, Transforming glycoscience: A roadmap for the future, National Academies Press, Washington, DC, 2012.
4 A. P. Davis, R. S. Wareham, *Angew. Chem. Int. Ed.* 1999, **38**, 2978-2996.
5 S. Kubik, *Angew. Chem. Int. Ed.* 2009, **48**, 1722-1725.
6 T. K. Dam, C. F. Brewer, *Chem. Rev.* 2002, **102**, 387-430.
7 C. Ke, H. Destecroix, M. P. Crump, A. P. Davis, *Nat. Chem.* 2012, **4**, 718-723.
8 M. Rauschenberg, S. Bomke, U. Karst, B. J. Ravoo, *Angew. Chem. Int. Ed.* 2010, **49**, 7340-7345.
9 L. N. Cella, W. Chen, ,N. V. Myung, A. Mulchandani, *J. Am. Chem. Soc*. 2010, **132**, 5024-5026.
10 S. Jin, Y. Cheng, S. Reid, M. Li, B. Wang, *Med Res Rev.* 2010, **30**, 171-257.
11 M. Dowlut, D. G. Hall, *J. Am. Chem. Soc.* 2006, **128**, 4226-4227.
12 B. Mu. B, T. P. McNicholas, J. Zhang, A. J. Hilmer, Z. Jin, N. F. Reuel, J.-H. Kim, K. Yum, M. S. Strano, *J. Am. Chem. Soc.* 2012, **134**, 17620-17627.
13 R. Freeman, L. Bahshi, T. Finder, R. Gill, I. Willner, *Chem. Commun.* 2009, 764-766.
14 S. G. Cordes, S. Bakthan, *Angew. Chem., Int. Ed.* 2006, **45**, 3829-3832.
15 W. Wu, T. Zhou, A. Berliner, P. Banerjee, S. Zhou, *Angew. Chem., Int. Ed.* 2010, **49**, 6554-6558.
16 A. Matsumoto, K. Yamamoto, R. Yoshida, K. Kataoka, T. Aoyagi, Y. A. Miyahara, *Chem. Commun.* 2010, **46**, 2203-2205.
17 C. Ancla, V. Lapeyre, I. Gosse, B. Catargi, V. Ravaine, *Langmuir* 2011, **27**, 12693-12701.
18 N. Fortin, H.-A. Klok, *ACS Appl. Mater. Interfaces* 2015, **7**, 4631-4640.
19 S. A. Asher, V. L. Alexeev, V. Goponenko, A. C. Sharma, I. K. Lednev, C. S. Wilcox, D. N. Finegold, *J. Am. Chem. Soc.* 2003, **125**, 3322-3329.
20 F. H. Arnold, W. Zheng, A. S. Michaels, *J. Membr. Sci.* 2000, **167**, 227-239.
21 M.-C. Lee, S. Kabilan, A. Hussain, X. Yang, J. Blyth, C. R. Lowe, *Anal. Chem.* 2004, **76**, 5748-5755.
22 X. D. Ge, L. Tolosa, G. Rao, *Anal. Chem.* 2004, **76**, 1403-1410.
23 M. Miyashita, N. Ito, S. Ikeda, T. Murayama,; K. Oguma, J. Kimura, *Biosens. Bioelectron.* 2009, **24**, 1336-1340.
24 M. Fehr, H. Takanaga, D. W. Ehrhardt, W. B. Frommer, *Mol. Cell. Biol.* 2005, **25**, 11102-11112.
25 Y.N. Chen, H. Vedala, G.P. Kotchey, A. Audfray, S. Cecioni, A. Imberty, S. Vidal, A. *ACS Nano*. 2012, **6**, 760-770.
26 D. Chen, H.B. Feng, J.H. Li, *Chem. Rev.* 2012, **112**, 6027-6053.
27 Y.A. Chen, A. Star, S. Vidal, *Chem. Soc. Rev.* 2013, **42**, 4532-4542.
28 P.J.G. Luo, H.F. Wang, L.R. Gu, F.S. Lu, Y. Lin, K.A. Christensen, S.T. Yang, Y.P. Sun, *ACS Nano.* 2009, **3**, 3909-3916.
29 N.F. Reuel, J.H. Ahn, J.H. Kim, J.Q. Zhang, A.A. Boghossian, L.K. Mahal, M.S.Strano, *J. Am. Chem. Soc.* 2011, **133**, 17923-17933.
30 X. Zhong, H.J. Bai, J.J. Xu, H.Y. Chen, Y.H. Zhu, *Adv. Funct. Mater.* 2010, **20**, 992-999.
31 D. Vuckovic, J. Pawliszyn, *Anal. Chem.* 2011, **83**, 1944-1954.
32 D. Vuckovic, S. Risticevic, J. Pawliszyn, *Angew. Chem., Int. Ed.* 2011, **50**, 5618-5628.
33 E. Cudjoe, B. Bojko, I. Lannoy, V. Saldivia, J. Pawliszyn, *Angew. Chem., Int. Ed*. 2013, **52**, 12124-12126.
34 G. Ouyang, K. D. Oakes, L. Bragg, S. Wang, H. Liu, S. Cui, M. R. Servos, D. G. Dixon, J. Pawliszyn, *Environ. Sci. Technol.* 2011, **45**, 7792-7798.
35 O. P. Togunde, K. D. Oakes, M. R. Servos, J. Pawliszyn, *Environ. Sci. Technol.* 2012, **46**, 5302-5309.
36 M. L. Musteata, F. M. Musteata, J. Pawliszyn, *Anal. Chem.* 2007, **79**, 6903-6911.
37 F. S. Mirnaghi, J. Pawliszyn, *Anal. Chem.* 2012, **84**, 8301−8309
38 Y. Xia, J. Ye, K. Tan, J. Wang, G. Yang, *Anal. Chem.* 2013, **85**, 6241-6247.
39 E. Palecek, J. Tkac, M. Bartosik, T. Bertok, V. Ostatna, J. Palecek, *Chem. Rev.* 2015, **115**, 2045-2108.
40 P. N. Spahn, N. E. Lewis, *Curr Opin Biotech* 2015, **30**, 218-224.
41 R. Raman, S. Raguram, G. Venkatataraman, J. C. Paulson, R. Sasisekharan, *Nat methods*, 2005, **2**, 817-824.
42 P. H. Seeberger, D. B. Werz, *Nature*, 2007, **446**, 1046-1051.
43 T. D. James, K. R. A. Sandanayake, S. Shinkai, *Angew. Chem. Int. Ed.* 1996, **35**, 1910 – 1922.
44 J. Yan, H. Fang, B. Wang, *Med. Res. Rev.* 2005, **25**, 490-520.
45 R. Ballerstadt, C. Evans, R. McNichols, A. Gowda, *Biosens Bioelectron.* 2006, **22**, 275-284.
46 R. H. Davis, Aloe vera: A Scientific Approach, Vantage Press, New York, 1997.
47 A. Femenia, E. S. Sanchez, S. Simal, C. Rosselló, *Carbohydr. Polym.* 1999, **39**, 109 -117
48 P. Shen, Y. Xia, *Anal. Chem.* 2014, **86**, 5323−5329.