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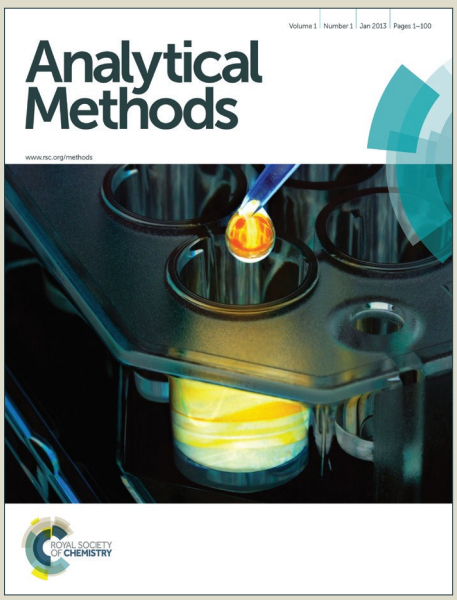

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ARTICLE

Calix[4]arene crownether as an oriented linker for highly sensitive detection of zinc ions using peptide probe

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Abstract: Peptides, as the simplest biological recognition elements have been developed as probes to capture multitudinous targets. One important factor in fabricating peptide biochip is to immobilize peptide without losing their activity on a solid phase. To keep them functional, it is necessary to immobilize peptide in an oriented way. Calix[4]arene crownether (CC) have been used as a proteins linker molecule on the solid surface. In this study, calix[4]arene crownether was self-assembled modified on the gold surface. The calix[4]arene crownether monolayer was characterized by EIS and SPR. An elaborately designed peptide probe Ac-CCPGCAAAARRRR-NH_2 is employed here for assay of Zn^{2+}. This tridecapeptide consists of binding part (CCPGC), spacer (AAAA) and immobilization part (RRRR) to interact with CC SAM orientedly. We compared the sensitivity and the specificity of the linker molecules with that of common attachment agent using a zinc ions binding peptide. The fabricated chip showed a superior sensitivity and a much lower detection limit than those chips prepared by other methods. Thus, the calix[4]arene crownether chip can be used as a powerful peptide linker with a wide range of applications, including peptide-drug interaction, peptide-cell interaction, and an enzyme activity assay.

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ARTICLE

1. Introduction

The calix[n]arenes are among the most widely studied organic macrocyclic host systems in supramolecular chemistry [1]. Calix[4]aren derivatives can be used as an artificial linker system for the immobilization of protein, enzyme and antibody. Calix[4]arene crownether (CC) is a well-known calixarene derivative which has been utilized for guanidinium, antibody, enzyme and membrane receptor's immobilization in former works [2-5]. It was found that CC may have a unique surface chemistry allowing the formation of a self-assembled monolayer (SAM) on gold surface and tight binding of the captured proteins to the crown moiety of the linker molecules. The major binding force can be attributed to the ionized amine groups of capture analytes that bind to the crown moiety of the linker molecule via host-guest interactions [6, 7]. CC as an artificial linker molecules have been widely used to antibody and membrane proteins adsorption attracted lots of researchers' attention. It was verified that CC as a protein linker can preserve the novel activity of immobilized proteins. Thus, to our best of knowledge, there has been no report for the bioactive peptide immobilization methods based on artificial linkers.

As the second most abundant transition metal in living organisms and an essential trace element in biological system, zinc ions (Zn^{2+}) play a vital role in cellular metabolism, gene expression, neurotransmission, apoptosis, enzyme regulation and physiological processes including the binding with metalloproteins as cofactor, the central nervous system[8-11]. Then, Zn^{2+} also can produce adverse effects on human health and environment. For instance, inadequate zinc (II) absorption, increased zinc (II) losses from the body or increased requirements for zinc (II) will result in zinc (II) deficiency in human organisms, which brings about several disorders, for example, growth retardation, the decrease of the immunological defense, eye lesion and some skin disease [12-14]. It also has been shown that several neurological disease are closely correlated with the disorder of Zn^{2+} metabolism, such as Alzheimer's disease (AD), epilepsy, Parkinson and cerebral ischemia [15, 16]. Hence, developing robust environment-friendly method detect trace Zn^{2+} with high sensitivity and selectively has become crucial important.

In recent studies, lots of methods had been developed for zinc ions detection including electrochemistry [17], surface enhanced Raman scattering (SERS) [18], absorption and fluorescence spectrometry [19-21], label-assisted laser desorption ionization mass spectrometry (LA-LDI MS) [22]. However, these traditional methods are laborious, time-consuming and considerable expertise. Therefore, it is still essential to develop highly sensitive, selective and directly approaches to monitoring Zn^{2+}.

The current methods for zinc ion recognition probe most focus on luminescent lanthanide [23], organic compounds [24, 25] and fluorescent substance [26]. The focus on peptide as a probe for zinc ions detection [27] is very few. Peptides, which have a good affinity for metal ions, are particularly attractive for development of electrochemical sensors in view of the following merits. Peptides, as the simplest biological recognition elements able to capture multitudinous targets [28], not only allow for cost-efficient large scale synthesis, but also can make facile modification to facilitate its accommodation into a sensing element and optimization of amino acid sequence or local structure to produce designer peptides with enhanced target recognition ability [29]. Additionally, peptides own multidentate binding sites beneficial to exhibiting a strong binding affinity to metal ions and permitting the redox active center to remain in close proximity to the transducer for maximum signal output [29]. Taking advantages of these merits, peptides have been used as probes for metal ions detection, such as the measuring of Pb^{2+}, Cu^{2+}, Cd^{2+}, Ni^{2+} and Co^{2+} and Zn^{2+} [29-32].

The sequence of CCPGCC is known as has a high affinity for Zn^{2+} with a binding constant similar to the native zinc-containing proteins, then only the first three cysteine residues participated in coordination of Zn^{2+} [27, 33]. The interaction between the three cysteine residues and Zn^{2+} induce a conformational change of the peptide. In this study, we designed a label-free SPR sensor for the monitoring of zinc ions based on a specific peptide probe immobilized onto the surface of CC SAM. Three different peptide immobilization methods were compared in an aspect of peptide surface coverage and Zn^{2+} sensing capability (Fig. 1). CC can be immobilized on the gold surface through self-assembled binding thiol group on CC's upper ring onto Au chip. Then, an elaborately designed peptide probe Ac-CCPGCAAAAARRRR-NH2 is employed here for assay of Zn^{2+}. This tridecapeptide consists of three important parts: one is the binding part with the first five residues containing tricysteine (CCPGC); the middle part includes four residues (AAAA) here as a spacer; the last four residues (RRRR) is designed here contributes to high-density immobilization on CC monolayer via host-guest interactions [34, 35]. The spacer avoids perturbation of peptides' orientation when immobilized to the surface of CC SAM. In the presence of zinc (II) ions, the peptides can capture the Zn^{2+} and along with the conformation change. This zinc (II) ions SPR sensor shows excellent selectivity and sensitivity comparing with other metal cations (Mg^{2+}, Ca^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Ba^{2+}, Ni^{2+} and Co^{2+}). Comparing with three different immobilization methods, the strategy based on CC as peptide linker has highest surface coverage and good Zn^{2+} sensing capability.

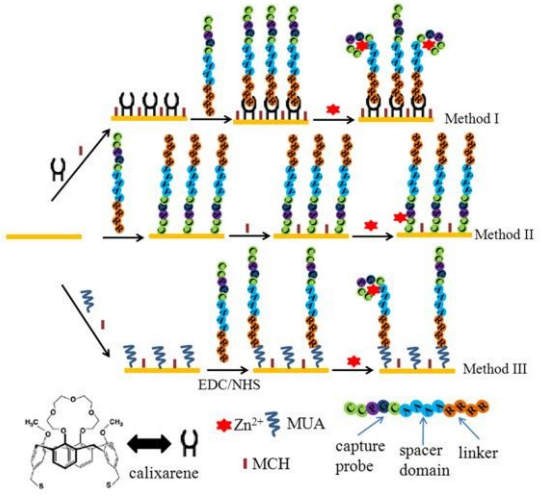

Figure 1 Experimental scheme for the Zinc ion detection on the SPR chip and three immobilization methods compare for evaluation of the sensor chip's activity. Peptide immobilization via the covalent bond between Au and the thiol group (method II) and induce a linker between the peptide and gold surface, which are CC (Method I) and MUA (Method III).

2. Experimental

2.1 Chemicals and reagents

Calix[4]arene crownether (Fig. 1) was obtained from Proteogen Co. (Seoul, Korea, www.proteogen.co.kr). Dimethyl sulfoxide (DMSO), tris(2-carboxyethyl) phosphine hydrochloride (TECP), 6-mercapto-1-hexanol (MCH), 11-mercaptoundecanoic acid (MUA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA, www.sigmaaldrich.com). Zn^{2+}-binding peptide with a sequence of Ac-CCPGCAAAARRRR-NH_2 was synthesized by Sangon Biotch Co. Ltd. (Shanghai, China, sangon.bioon.com.cn). Metal salts of $CuCl_2$, $MgCl_2$, $CaCl_2$, $ZnCl_2$, $PbCl_2$, $MnCl_2$, $BaCl_2$, $NiCl_2$ and $CoCl_2$ were purchased from Sigma-Aldrich (St. Louis, Mo, USA). Phosphate buffer saline (PBS) buffer solution was prepared by NaCl, $Na_2HPO_4{\cdot}2H_2O$, NaH_2PO4 (Sigma-Aldrich Co. Ltd). All chemicals and solvents were analytical reagents and used without further purification. The deionized water (resistance >18 MΩ·cm) which was purified with a Direct-8 Millipore purification system (Branstead, USA) was used to make buffer.

2.2 Fabrication of calix[4]arene crownether SAM on the gold chip

A microscope cover glass (18 mm×18 mm×0.15 mm, refractive index = 1.515; Matsunami, Japan) with 50 nm gold layer was used as a substrate for the formation of CC SAM. The gold film was deposited on the cover glass by a sputter coater (Q150Rs, Quorum Technologies, Kent, UK) under conditions of 3.0×10−2 mbar and 40 mA for 150 s. The thickness of the gold layer was evaluated through simulation of its SPR curve. The fabricated bare gold chip was rinsed with ultra-pure water, ethanol and ultra-pure water, sequentially. After gently blowing with nitrogen gas, the bare gold chip is ready to use.

The CC solution was prepared by dissolved in the mixture of chloroform and methanol (1:3, v/v). The CC SAM was fabricated by immersion of the gold chip into the 0.10 mM CC solution at room temperature. After the immobilization, the sensor chip was rinsed with chloroform–methanol mixture solutionfor 1~2 h. After this, the sensor chip was rinsed using mixture solution, ethanol and ultra-pure water, sequentially. Then the modified sensor chip was dried under nitrogen stream softly.

2.3 Characterization of calix[4]arene crownether SAM

Electrochemical impedance spectroscopy (EIS) was carried out on a Metrohm electrochemical analyzer (Metrohm Autolab B.V., the Netherlands) respectively in 5 mM $[Fe(CN)_6]^{3-/4-}$ (containing 0.1 M PBS, pH 7.4). A three-electrode system consisting of a modified gold electrode (diameter, 3 mm) as the working electrode, a saturated calomel reference electrode (SCE) and a platinum counter electrode was used for all the electrochemical measurements. The whole reaction process on the electrode was measured by EIS. In order to prevent the denaturation of biomolecules on the electrode, after each modification step, the electrode must be used immediately. In addition, the CC SAM also could be characterized by cyclic voltammetry (CV). The CV measurements were performed by using a BAS-100B electrochemical analyzer (Bioanalytical Systems Inc., USA). A three-electrode system consisted of an Ag/AgCl reference electrode with a filling solution of saturated KCl, a platinum coil as the auxiliary electrode and gold as a working electrode was used. The scan rate was 50 $mV \cdot s^{-1}$. SPR spectroscopic was also employed here to characterize the formation of the CC SAM. The SPR spectroscopic measurements for the monolayer formation of CC SAM on the gold surface were performed by a home-made SPR system.

2.4 Immobilization of peptide on the calix[4]arene crownether SAM

After formation of the CC SAM, the modified gold chip was fixed on the home-made SPR instrument. Then, the gold disk was rinsed with PBS buffer (0.1 M, pH 7.4) about 2 h for the gold chip balance and backfilled with 1 mM MCH (dissolved in 0.1 M PBS buffer, pH 7.4) for 1 h. After the gold chip was rinsed with PBS buffer about 30 min, the peptide immobilization occurs. The immobilization of Zn^{2+}-binding peptide on the gold chip was performed at 25 ℃ (±0.3) by incubation with 50 μM peptide solution (dissolved in 0.01 M PBS buffer, pH 7.4) for about 2 h. After the immobilization, the gold chip was rinsed with PBS buffer to remove the nonspecific adsorbed peptide. The process of the immobilization was monitored by SPR spectroscopy.

2.5 SPR measurements

SPR measurements were performed using a home-made SPR system based on the traditional Kretschmann configuration which was described in our previous report detailly [36, 37]. Briefly, a laser diode (LD, λmax= 675 nm) was used as the light source. The reflected intensity of light through the polarizer and the prism was measured using a photodiode detector (ANDO Electric Co. Ltd., AQ-1976, Kanagwa, Japan). The incident angle of the prism was varied with the motorized rotary stage and its controller (Suruga Seiki, D80, Shizuoka, Japan). The signal of the photodiode can be converted through a signal process board (K-MAC Co., Spectra View 200, Taejeon, Korea), then could be interfaced using a computer. The angle resolution of the SPR system was determined the resolution of the motorized rotary stage was 0.004°.

SPR measurements were performed at 25 ℃ (±0.3). A scheme diagram of sensor chip configuration is shown in Fig.1. An optimum pH for zinc sensing was observed in a range of pH 5.9-7.9. Eight different Zn^{2+} solutions with concentrations in the range of 1.0×10^{-13} to 1.0×10^{-6} M were prepared in PBS buffer solution. These solutions were injected into the channel, monitored by SPR, and rinsed with PBS buffer solution to remove unbounded Zn^{2+}. To measure the sensor's specificity, 200 μL, 1 μM other metal ions solutions were respectively injected into the chip surface using similar procedures.

2.6 Surface coverage calculation

The surface concentration of immobilization peptide was calculated according to the previously reported method [38].

Optical parameters required for calculation of surface concentration of immobilized peptide were determined by simulation using experimental SPR data. The surface concentration of immobilization peptide on the surface was calculated by the following equation:

$$\Gamma = 3d \frac{(n^2 - n_b^2)}{(n^2 + 2)(r(n_b^2 + 2)) - v(n_b^2 - 1)} \quad (1)$$

where Γ and d are surface concentration of the adsorbed molecules and the thickness of the adsorbed layer respectively. nb and n are the refractive index of the buffer solution and the adsorbed layer, respectively. r and v are the specific refractivity of global protein (0.243 mL/g) and the partical specific volume of protein deposited on the linker layer (0.729 mL/g) [39].

3. Results and discussion

3.1 Characterization and Optimization of the Sensor Scaffold.

To fabricate a sensor chip for Zn^{2+} detection, we prepared a calix[4]crown surface on a gold electrode. The EIS and CV are employed to characterize the formation of CC SAM. EIS is sensitive to the changes of interfacial properties at electrode surface [40]. Fig. 2a illustrates the EIS of different electrodes in the presence of 5 mM $[Fe(CN)6]^{3-/4-}$ (containing 0.1 M PBS, pH 7.4). The bare gold electrode exhibited a very small semicircle domain, which means that the charge-transfer process very fast. After incubation with the CC, the electron-transfer resistance (Ret) was remarkably increased to 1000 Ω, which validates the successful assembly of CC on the gold electrode surface through Au-S bonds. The properties of the electrode modified with the CC SAM can be estimated by subjecting the electrode to reductive desorption experiments. The reductive desorption peak of the CC SAM on the Au electrode was shown in our previous work [41]. The peak attributed to reductive desorption of thiolated compounds that are chemisorbed on the Au electrode, and all thiolated compounds are reduced/oxidized in the CV experiments. Moreover, the surface coverage can be determined from the CV measurements [42, 43]. After accounting for the surface roughness of the Au electrode, the surface coverage of CC SAM was calculated to be 1.19×10^{-10} mol cm^{-2}, which was consistent with the theoretical monolayer value (6.002×10^{-10} mol cm^{-2}) obtained using CS ChemdrawTM (Cambridgesoft Co., USA).

The above results suggest that the self-assembled CC monolayer, which can provide favorable conditions that increase the determination resolution of Zn^{2+}. Therefore, to sense the formation of CC SAM on the gold chip surface, SPR was measured regularly. As shown in Fig. 2b, the black scatter dot recorded the bare gold chip's incident angle and the blue scatter dot represents the chip when modified with CC; then the red and dark cyan line respectively represents the calculation curve for the bare gold chip's incident angle and the chip modified with CC. The optical parameters and peptide surface concentration were shown in Table 1. The results of EIS, CV and SPR combined clearly demonstrated the CC was immobilized as a dense monolayer. Then, the constructed artificial linker monolayer on the gold surface was ready to absorb peptide.

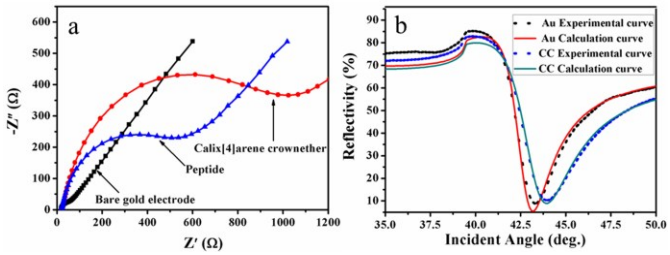

Figure 2 Characterization of the calix[4]crown SAM: (a) Nyquist plots corresponding to (black line) bare gold electrode, (red line) CC modified electrodeand and (blue line) peptide modified electrode, Bias potential: 0.224 V, Frequency range: 0.1 Hz to 100 kHz; Scan rate: 100 mV s^{-1}; (b) Experimental and Calculation SPR curve for the bare Au chip and after modified with CC.

Table 1 Surface concentration of the immobilized molecules and optical parameters determined by theoretical simulation.

Layer	Optical parameters			Surface concentration
	n	k	d	
Gold	0.289	3.3	49	
CC SAM	1.207	0.211	1.54	1.19×10^{-10} mol cm^{-2}
Peptide	1.375	0.001	5.06	283 ng cm^{-2}

n, k and *d* denote refractive index, extinction coefficient, geometrical thickness (nm) respectively.

Zn^{2+}-binding peptide was immobilized on the CC SAM via host-guest interactions between the guanidinium of arginine and

crown-ether moiety as described elsewhere [2]. EIS and SPR were employed here to characterize the immobilization. As shown in Fig. 2a, when the modified electrode immobilized with peptide, the electron-transfer resistance was decreased from 1000 Ω to 600 Ω. Considering that most of the guanidinium group of peptide exists as an ionized from in a physiological condition (pH=7.4), the resistance decrease may due to the attraction of guanidinium ions to $[Fe(CN)_6]^{3-/4-}$.

Fig. 3a shows the sensorgram of immobilization processes of peptide on CC SAM. Before incubation in the peptide solution, MCH was used to backfill the bare gold surface. SPR signal increased 0.02 degree slightly, which verifies the CC SAM on gold surface is very compact. The chip was then immersed with peptide solution. Due to adsorption of peptide onto the chip surface, SPR angle was gradually increased and saturated within 2 h. Immobilization of peptide resulted in SPR angle change of 0.396 degree, which confirms that peptide layer was constructed on the CC SAM in a dense state due to the artificial constructed linker layer.

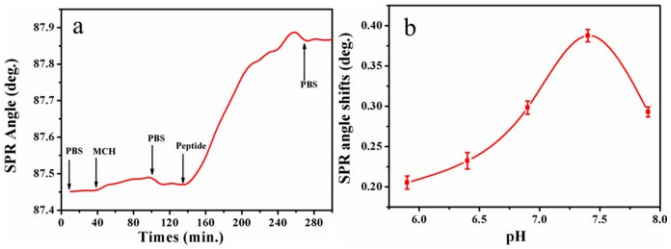

Figure 3 (a) The sensorgram of immobilization processes of peptide on CC SAM; (b) The influence of pH on the SPR signal response of the peptide immobilization on CC SAM. Error bars represent standard deviations of three independent measurements.

3.2 Optimization of detection conditions and sensor's selectivity

The influence of pH for the peptide immobilization was optimized. As shown in Fig. 3b, with increase the pH from 5.9 to 7.9, the SPR signal increased firstly and then decreased, which may due to the pH change affects the ionization of guanidinium group. At the physiological condition (pH=7.4), peptide has a maximum immobilization on the CC SAM. pH 7.4 was selected for peptide immobilization in next experiment.

In order to improve the detection sensitivity, the influence of pH on the sensors response was tested using 1μM Zn^{2+} solution in the pH value range of 5.9 - 7.9. Varying the pH would also cause the change of peptide property such as density, orientation and charge holding, resulting in the variation of interaction between peptide and Zn^{2+}. As shown in Fig. 4a, it was found the fabricated sensing system has a higher SPR response near pH 7.4. Thus the optimal value of pH 7.4 was selected in next measurements.

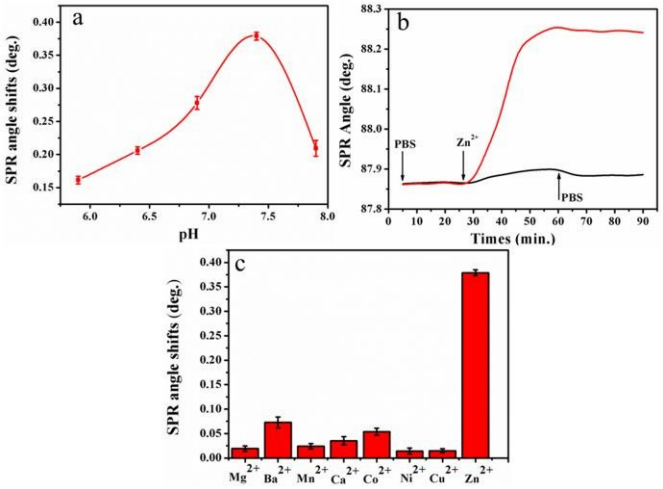

Figure 4 (a) The effect of pH for the capture of Zn^{2+}; (b) Control experiments of Zn^{2+} binding on Calix[4]arene crownether with and without the peptide media (red line: CC SAM with peptide; black line: CC SAM without peptide); (c) SPR angle shifts histogram of various ions in the same concentration of 1 μM upon injection of zinc ions. Error bars represent standard deviations of three independent measurements.

In order to confirm the specific binding between Zn^{2+} and peptide, 1μM Zn^{2+} solution was injected onto the two channel chip surface modified with CC SAM and CC SAM/peptide respectively. As shown in Fig. 4b, Zn^{2+} on the CC SAM/peptide results an evidently increase (red line) comparing with.that of CC SAM only (black line). To demonstrate the selectivity of the peptide immobilized on the CC SAM for Zn^{2+} detection, the control experiments were performed by using Ca^{2+}, Cu^{2+}, Ba^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+} and Co^{2+} (1 μM) to compare with Zn^{2+} (1 μM) in the same experimental conditions. Fig. 4c shows the histogram of the SPR angle shifts with different ions. It was found that no remarkable signal was observed in comparison with that in the presence of Zn^{2+}. Thus, the experiment results effectively confirmed a good selectivity and specificity of the peptide to Zn^{2+}.

3.3 Comparasion of different peptide immobilization methods

Three immobilization methods were compared for evaluation of the sensor chip's activity (Fig.1). In method II, peptide was immobilized on the gold chip via the covalent bond between Au and the thiol group of cysteine. The other two methods of peptide immobilization are induced a linker between the peptide and gold surface, which are CC (Method I) and MUA (Method III). As shown in Fig. 5, peptide can be immobilized with a highest surface coverage in Method I. Method I show the biggest SPR signal change when measuring same concentration of Zn^{2+}, which means Method I is the most sensitive sensing system for Zn^{2+}. Method II may damage the novel structure of peptide due to cysteine in the binding peptide bond to the gold, which may result a lowest sensing ability among three methods. In case of method III, peptide was covalently bound MUA to form amide linkages through EDC/NHS-activation. Peptide has a lowest surface coverage among three methods, which results a lower sensing ability. CC as a peptide linker through a host-guest interaction can preserve peptide's novel activity. In a summary these results demonstrated that the peptide immobilization via a CC as a linker has a best sensitivity.

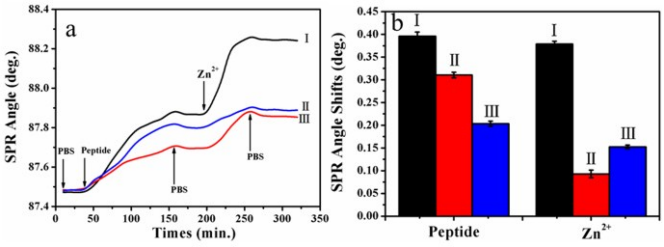

Figure 5 Different methods for peptide immobilization on the gold chip. The concentration of Zn^{2+} is 1 μM. Error bars represent standard deviations of three independent measurements.

3.4 Sensor's sensitivity and detection Zn^{2+} in real samples

The detection of different concentrations of Zn^{2+} based on SPR was implemented to evaluate the sensing performance. After rinsing with the PBS buffer, SPR angle was carried out at each concentration. As shown in Fig. 6a, SPR angle shifts according to different Zn^{2+} concentrations from 1×10^{-13} M to 1×10^{-6} M respectively. Even at the lowest concentration of Zn^{2+}, SPR angle shift 0.122 degree. However, when it reached at 1.0×10^{-7} M, even after injected the higher concentration of Zn^{2+} into the SPR chamber, the SPR angle is kept stable. Every concentration has been replicated at least three times under the same experimental conditions. As shown in Fig. 6b, a linear relationship between the SPR angle shifts and the logarithm of Zn^{2+} concentration was established from 1.0×10^{-13} M to 1.0×10^{-9} M with a correlation coefficient of 0.997 (y = 0.05322x + 0.80793, n=3). The detection limit of Zn^{2+} was caculated to be 0.094 pM (LOD=3.3×standard deviation/slope). Comparing with previous reports, method I has a broader detection window and lower detection limit (Table 2).

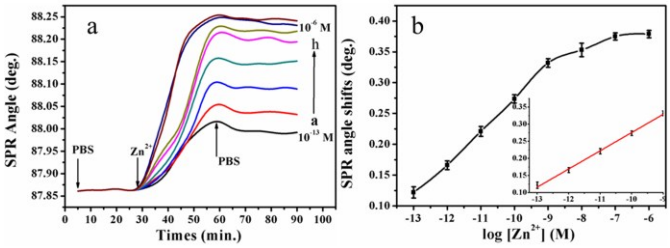

Figure 6 (a) SPR angle increase according to treatment with different concentration of Zn^{2+} (1×10^{-13}~1×10^{-6} M); (b) calibration curve for the detection of Zn^{2+} (1×10^{-13}~1×10^{-6} M). Insert shows the linear relationship in the range from 1×10^{-13}~1×10^{-9} M. Error bars represent standard deviations of three independent measurements.

Table 2 Comparison of some different Zn^{2+} sensing systems

Method	Probe	Analytical ranges (μM)	Detection limit (μM)	Ref.
Fluorescence	3-mercaptopropionic acid-capped CdTe QDs	1.6-35	1.2	8
	pyridoxal-based fluorescein derivative	0-200	0.021	13
	Schiff-base derivative	0.16-10	0.15	16
	1,8-naphthalimide derivative	0-4.0	1.03	24
	CdSe/ZnS QDs	0.9-16	0.7	26
Absorption	N,N'-Diferrocenylidene-2,7'-diaminobiphenyl	~	10	11
	peptide-AuNPS	0.024-1.2	0.01	27
LA-LDI MS	polyaromatic	~	10	22
SPR	CC-peptide	1×10^{-7}-1.0	9.4×10^{-8}	This study

To investigate feasibility of the proposed method for application to real samples, we employed urine (InnoReagents, www.innoreagents.com) and water samples (tap water and pond water) for real samples detection. The as-prepared urine and water samples were spiked with Zn^{2+} at different concentration levels (0.1μM and 1μM) separately. Every sample was injected into the channel for monitoring separately. The experimental results were summarized in Table 3. Detection recovery was found from 92%-103%, which confirmed that the fabricated Zn^{2+} sensor can be applied to complex matrix.

Table 3 Determination of Zn^{2+} in urine and water samples

	Sample	Added (μM)	Found (μM)	Recovery
Urine	1-1	0.1	0.103 ± 0.002	103%
	1-2	1.0	0.99 ± 0.01	99%
Tap water	1-1	0.1	0.092 ± 0.004	92%
	1-2	1.0	1.02 ± 0.01	102%
Pond water	1-1	0.1	0.098 ± 0.003	98%
	1-2	1.0	0.96 ± 0.05	96%

4. Conclusions

In this work, a simple strategy has been developed based on calixarene captured peptide SPR chip to sensitively and specifically detect zinc ions. This approach just needs two steps modification on the gold chip. The increase of SPR angle can characterize the different concentrations of Zn^{2+} that the peptide captured. The fabricated sensor is very sensitive as the limit of detection is 0.094 pM. Therefore, this method provides a novel platform for metal ions research and may contribute to the development of the detection of zinc ion in aqueous samples and could be useful in clinical diagnosis.

Acknowledgements

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Calix[4]arene crownether as an oriented linker for highly sensitive detection of zinc ions using peptide probe

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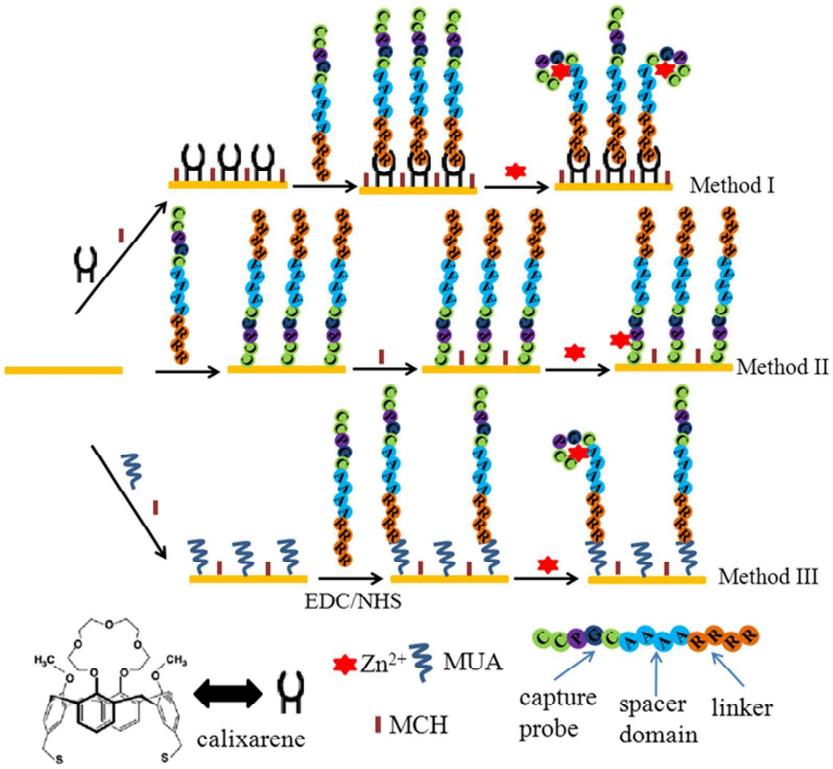

Experimental scheme for the Zinc ion detection on the SPR chip.

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