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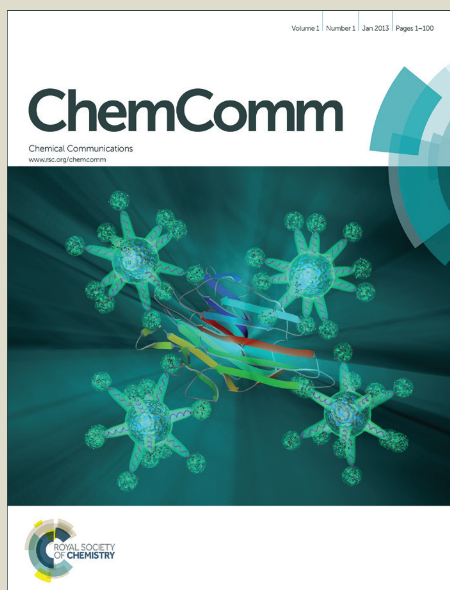

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ARTICLE TYPE

Surface plasmon resonance assay coupled with hybridization chain reaction for amplified detection of DNA and small molecule

Xuemei Li, *[a] Yan Wang, [b] Linlin Wang, [b] and Qingli Wei [b]

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A surface plasmon resonance (SPR) detection system based on hybridization chain reaction (HCR) was developed for amplified detection of DNA and small molecule with high sensitivity.

Surface plasmon resonance (SPR) is a leading technique for the fast, real-time and in situ bioaffinity detection of a wide range of unlabeled biomolecular targets.[1] SPR techniques measure the change in refractive index of the solvent near the surface that occurs during complex formation or dissociation.[2] SPR has been employed for the detection of protein,[3] nucleic acid[4] and small molecules.[5] However, it is important to point out that SPR assays generally suffer from low signal intensity and nonspecific binding, which has impeded further application of SPR in proteomics and disease diagnostics, especially for detection of trace targets in complex biological samples.[6] To overcome this drawback, a variety of amplification approaches have been explored.[7] Recent technological developments mostly focus on the application of Au and magnetic nanoparticles (NPs) conjugated to secondary antibodies as a part of a surface sandwich assay to enhance SPR response.[8] For example, SPR sensor based on aggregation of a network of AuNPs,[9] DNA modified AuNPs,[3b] superparamagnetic particles[10] and magnetic molecularly imprinted polymers[8b] have been applied to enhance SPR signal. Unfortunately, a key limitation relevant to all NP-based enhanced SPR detection studies is that there is often a relatively narrow dynamic range over which the measurement can be performed.

The sensitive and selective detection of nucleic acids is important in biological studies, molecular diagnostics and biomedical development since they are routinely used as biomarkers to help diagnose pathogenic infections and genetic disorders. The amplified detection of DNA has attracted substantial research efforts, and a number of amplification strategies have also been reported in our previous studies including strand-displacement amplification (SDA), RNA ribozyme cyclic amplification, bio-bar-code technique.[11] There is quite few reports on the combination of the novel and powerful signal enhancement methods with SPR detection.[12]

Hybridization chain reaction (HCR) is another type of nucleic acid amplification reactions and this class of mechanisms suggests the possibility of constructing biosensors solely from unmodified single-stranded DNA.[13] In the present work, a SPR detection system based on HCR was developed for amplified detection of DNA and ATP. The target DNA was partly hybridized with the immobilized capture sequence on the Au chip and the unpaired fragment of target DNA worked as triggers to open the hairpin DNA structures in sequence and propagate a chain reaction of hybridization events to self-assemble into complex structures driven by the free energy of base pair formation without enzyme.[14] Amplifying SPR response was observed by the introduction of the long nicked duplex sequence. Through specific design of the trigger sequence, the HCR based SPR sensor can be further applied for detection of ATP.

The scheme of continuous SPR screening of trigger DNA at a single chip is illustrated in Scheme 1B. After magnetic separation, the solution containing $\mathbf{S_2}$ was introduced into the Au chip, on which the thiolated capture DNA was anchored via sulfur-gold affinity. The key factor to a HCR system is the storage of potential energy in short loops protected by long stems.[15] Each hairpin has a stem of 16 base pairs and also has an additional sticky end at the 5' end of H1 (complementary to the loop of **H2**) and at the 3' end of **H2** (complementary to the loop of **H1**). In the presence of ATP, the released $\mathbf{S_2}$ was worked as trigger DNA to interactes with the sticky end of **H1**, which undergoes an unbiased strand-displacement interaction to open the hairpin. The newly exposed sticky end of **H1** linked at the sticky end of **H2** and opens the hairpin to expose a sticky end on **H2**. This sticky end is identical in sequence to the initiator strands. In this way, nanodevices were self-assembled in a cascading manner from **H1** and **H2** through HCR, as confirmed by agarose gel electrophoresis and AFM (Fig. S1 in ESI†). Thus, a large amount of Fc-modified DNA sequences are linearly and periodically assembled to the Au chip and the enhancement of the SPR signal is achieved by increasing the refractive index of the surface.

[a] *School of Chemistry and Chemical Engineering, Linyi University, Linyi 276005, P. R. China*
[b] *State Key Laboratory Base of Eco-chemical Engineering, College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, P.R. China.*
E-mail:xuemei_li@yeah.net; Fax: 86-539-8766867; Tel: 86-539-8766867.
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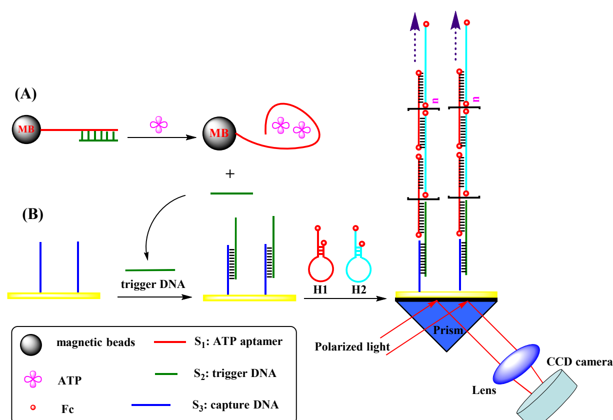

Scheme 1 Schematic representation of the SPR assay for ATP detection with the DNA-based hybridization chain reaction.

We used SPR to study the HCR process from the planar substrate, a typical SPR response curve of this process is shown in Fig. 1. Compared with the bare Au substrate (Fig. 1A, curve a), the modification of $\mathbf{S_3}$ induced the increase of SPR angle due to the coverage of $\mathbf{S_3}$ onto Au Chip (Fig. 1A, curve b). The SPR angle value of the $\mathbf{S_3}$-modified Au chip was set as zero for clarity. After $\mathbf{S_3}$ functionalized substrate was incubated with $\mathbf{S_2}$ for 2 h, the SPR angle increased from 71 degree to 71.025 degree as a result of the hybridization process (Fig. 1B). Then **H1** and **H2** were introduced into the flow cell, and the SPR angle further increased 0.205 degree, which corresponded to the formation of the HCR products. In order to subtract background signal such as bulk refractive index change or environmental noise, two fluidic channels were used by serially flowing the **H1** and **H2** mixture solution into a reference channel (no capture probe preimmobilized) and the analytical channel. In Figure 1B, no binding signal in curve a was expected, indicating that the nonspecific adsorption of **H1** and **H2** is absent at the Au film modified with $\mathbf{S_3}$. This suggests that $\mathbf{S_3}$ would not react with **H1** and **H2** without the trigger DNA sequence. Interestingly, upon mixing $\mathbf{S_3}$ with $\mathbf{S_2}$, we found that the SPR angle increased greatly, indicating the attachment of the HCR products.

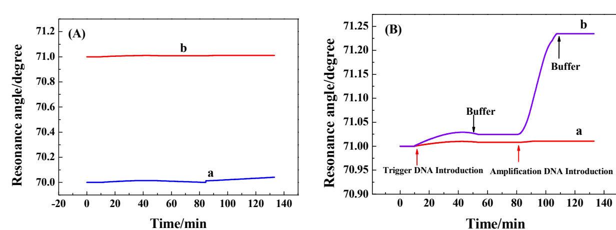

Fig. 1 (A) The SPR curve of the bare Au chip (a) and modified with the capture DNA $\mathbf{S_3}$ (b). (B) SPR sensorgrams corresponding to injections of 20 nM **H1** and **H2** mixture at 10 μL/min into fluidic channels wherein the substrate had been exposed to (a) $\mathbf{S_3}$ modified Au chip, (b) $\mathbf{S_3}$ modified Au chip and hybridized with $\mathbf{S_2}$. The arrows indicate the beginnings of injections.

We determined the optimal concentrations of capture probe and **H1**/**H2** and reaction time for the most extensive HCR system (see ESI†). Under the optimal conditions, the dynamic range of the designed method for detection of trigger DNA was examined. The introduction of $\mathbf{S_2}$ at different concentrations to the sensing interface induced different increases in SPR signal associated with the formation of longer DNA double helix. This allows one to construct standardized curves that can be used to determine the concentration of ligand in an unknown sample. The SPR signal was proportional to the $\mathbf{S_2}$ concentration over from 0.5 to 500 fM as shown in Fig. 2. A detection limit of 0.3 fM can be estimated using 3σ, which is 6 orders lower than a localized surface plasmon resonance (LSPR) amplified with gold nanoparticles.[16] The relative standard deviation (RSD) for eleven replicate determinations of $\mathbf{S_2}$ with different chips at 50 fM was 7.9%, indicating a good reproducibility of the sensing strategy.

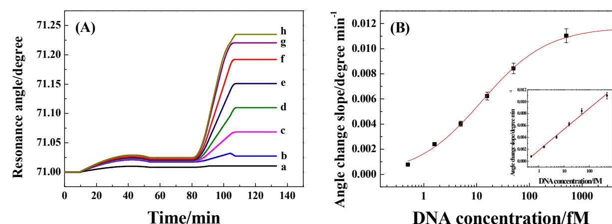

Fig. 2 (A) Real-time resonance angle responses of HCR biosensor for trigger DNA detection. The concentration of trigger DNA from a to g: 0, 0.5, 1.5, 5, 15, 50, 500, 5000 fM. (B) Calibration curve of the determination of trigger DNA. Inset is the linear relationship between the resonance angle shifts and the trigger DNA concentrations. The error bars are standard deviations of three repetitive measurements.

For DNA sensor, it is desirable that assays be performed rapidly and continuously.[3a] Furthermore, accuracy in quantitively determining the trigger would be significantly improved if comparison of two concentrations of the trigger were made with results obtained from the same SPR chip. The biosensor surface could be regenerated by injecting 1 M HCl into the fluidic channels to remove the hybridized double-strand DNA (Fig. 3). After 5 regenerations, the sensor retained 94.2% of the original sensor signal, implying the reusability of the Au chip. The decrease in sensor performance might be attributed to the structure degradation of the capture probe during the denaturation and renaturation processes.

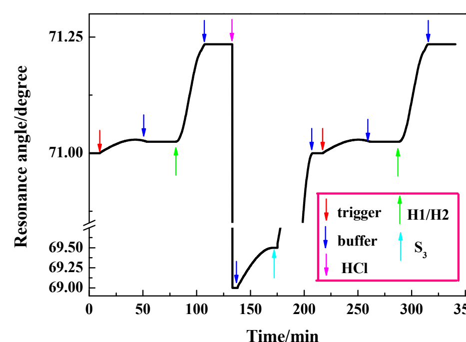

Fig. 3 Continuous screening of trigger DNA at a single SPR chip. 500 fM $\mathbf{S_2}$, 2.5 pM **H1**/**H2** complex, buffer were injected into an SPR channel and the sensor surface was regenerated using 1 M HCl.

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To explore the universality of our design, the HCR-based method was applied to detect small molecules. It has been suggested that ATP plays fundamental roles in the regulation and integration of cellular processes and also been used as substrate for cell viability and cell injury.[17] Therefore, we employed ATP as the model target analyte. The determination of ATP was achieved based on switching structures of aptamers from DNA/DNA duplex to DNA/ATP complex.[18] In this work, the **S_1**-MBs were prepared by direct immobilization of amino-modified ATP aptamer on the carboxyl magnetic beads through the amide bond in the presence of EDC and NHS, and hybridized with its complementary sequence **S_2** (Scheme 1A). These complexes were used as sensing interface to recognize ATP and separation tool. When ATP is introduced into the hybrid-modified MBs, the aptamer part bound to ATP and folded to the complex structure. As a result, the complementary **S_2** was released into the solution. After magnetic separation, the solution containing **S_2** was introduced into the Au chip, on which the thiolated capture DNA was anchored via sulfur-gold affinity. The HCR reaction was triggered as a result.

The introduction of ATP in different concentrations to the sensing interface induced different released trigger DNA. The SPR signal is proportional to the ATP in the range of 1.0 to 5000 nM (shown in Fig. S5). A detection limit of 0.48 nM can be estimated using 3σ. A series of eleven repetitive measurements of 500 nM ATP were used for estimating the precision, with the value of RSD being 8.7%. This sensitivity is about 3 orders of magnitude higher than that of colorimetric method based on cyclic enzymatic signal amplification,[19] fluorescent method by employing graphene oxide (GO) as a mass amplifier[20] and chemiluminescence resonance energy transfer (CRET) method.[21] The result was also comparable with our previously reported chemical assays.[11a,b]

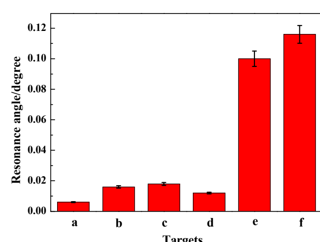

Fig. 4 Specificity for the detection of ATP. The resonance angle responses to (a) buffer, (b) CTP, (c) GTP, (d) UTP, (e) ATP, (f) ATP spiked in serum sample. The concentrations of all targets are 500 nM. The error bars are standard deviations of three repetitive measurements.

To examine the specificity of the method for the detection of ATP, experiments were conducted on ATP analogues such as CTP, GTP and UTP. As shown in Fig. 4, no apparent SPR angular shift was observed for the CTP, GTP and UTP detection. However, the introduction of ATP resulted in the dramatic increase in the SPR signal. In addition, comparable responses were obtained for ATP spiked in buffer and human serum. Inspired by the high sensitivity and selectivity, we applied the proposed method to analyze ATP in complicated biological samples. In this case, analysis of cellular ATP from lysates of HeLa cell and K562 leukemia cell were carried out.[22] The concentration of ATP is 3.9 μM for HeLa cell and 3.5 μM for K562 cell lysate. The results of the present work were validated with HPLC analysis and were listed in Table S2. The results of the present method are in good agreement with those of HPLC method.

In conclusion, we have developed a simple and highly sensitive SPR biosensor for amplified detection of DNA and small molecules by coupling the DNA-based hybridization chain reaction with the SPR strategy. The SPR signal could be amplified by the introduction of the long duplex sequence in the presence of the initiator strands. The proposed label free SPR strategy showed high sensitivity and selectivity without the participation of enzyme molecules, thereby avoiding the limitation of a thermostable enzyme for protein enzymes and the requirement of specific recognition site for nicking endonuclease. Significantly, due to the universality of aptamers, the methodology can be generalized to a wide variety of targets such as proteins, cells and other small molecules that are relevant to medical diagnostics and environmental monitoring.

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