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Magnetic nanoparticle-enhanced SPR biosensor

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Abstract

We report a new concept of surface plasmon resonance (SPR) biosensor for detection of chemical and biological analytes that holds potential for increased sensitivity and shorten detection time. It is based on magnetic nanoparticles (MNPs) assays in which the MNPs are employed to simultaneously serve as a) 'carriers' for efficient delivery of target analyte to the sensor surface and b) as labels that can dramatically increase refractive index changes associated with molecular binding events. We implemented this approach by using a sensor chip with diffraction grating and SPR-active gold and magnetic $Co_{70}Fe_{30}$ layers. A periodically modulated polymer sensor surface was coated by a magnetic $Co_{70}Fe_{30}$ layer for a rapid (and reversible) docking of MNPs followed by a deposition of a gold layer for diffraction-based excitation of surface plasmons. In conjunction with sandwich assays, this concept is expected to provide means for detection of molecular and biological analytes that is not hindered by their slow diffusion-controlled mass transport to the sensor surface.

Keywords: Optical sensors; biosensors; surface plasmon resonance; magnetic nanoparticles; magnetic layer

1. Introduction

Surface plasmon resonance (SPR) biosensors become increasingly popular technology for label-free detection of chemical and biological analytes in important areas such as medical diagnostics, food control and environmental monitoring [1]. In this method, a capture of target analyte by recognition elements on a metallic sensor surface is probed by resonantly excited surface plasmons. The highly localized field of surface plasmon waves originates from coupled oscillation of a charge density and associated electromagnetic field on surfaces of metals and it is extremely sensitive to molecular binding-induced refractive index changes. In order to improve detection limits of SPR biosensors, numerous approaches were investigated including an increasing the yield of analyte capture by using large binding capacity biointerfaces [2], through assays with an amplification of binding events on the surface by gold nanoparticles [3] or enzymatic reactions [4] as well as by combining SPR with fluorescence spectroscopy [5]. However, all these heterogeneous assays-based schemes are inherently limited by slow diffusion-controlled mass transport of analyte from a sample to the sensor surface which impedes their sensitivity and detection time [6].

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Magnetic nanoparticles (MNPs) are often used in analytical methods for efficient extraction of analyte molecules from crude samples. Recently, they were combined with SPR biosensors and were employed as labels that enhance the sensor signal through increasing the mass change on their surface associated with molecular binding [7, 8]. In this paper, we present an approach to further advance the MNPs-enhanced SPR biosensors in which target analyte captured by MNPs are *in situ* collected on the SPR sensor surface by applied magnetic field. It is based on sensor chip with periodically modulated magnetic and metallic layers that enables a) probing the sensor surface by grating-coupled SPR and b) manipulating nanoparticles above the sensor surface through magnetic field. The structure was optimized in order to reversibly dock MNPs on the surface by a short-range interaction with magnetic domains on the surface. In order to bring the MNPs close to the surface, an external magnetic field was applied. This approach is expected to offer a new route to overcome diffusion limited-mass transport to the sensor surface in SPR biosensors.

2. Materials and methods

2.1. Chemicals

Magnetic nanoparticles modified with dextran layer (fluidMAG-CMX) with a diameter of 100 nm (Fe_3O_4 magnetic core with a diameter of 75 nm and 12.5 nm thick dextran shell) were purchased from Chemicell (Berlin, Germany). PBS buffer tablets and Tween-20 were purchased from Sigma-Aldrich. PBS-Tween buffer (PBST) was prepared by adding Tween 20 (0.05%) in PBS buffer solution. Dithiolaromatic PEG6-carboxylate (thiol-COOH) and dithiolaromatic PEG3 (thiol-PEG) were purchased from SensoPath Technologies (Bozeman, USA).

2.2. Optical setup and sensor chip

The optical configuration of used SPR biosensor and the sensor chip are depicted in Fig.1A. A light beam emitted from a stabilized He-Ne laser (wavelength of λ=633 nm) passed through a polarizer to select transversal magnetic (TM) polarization and was reflected at a surface of a diffraction grating sensor chip. The assembly of the sensor chip and flow-cell was mounted on a rotation stage (Huber AG, Germany) in order to control the angle of incidence θ. The intensity of the laser beam reflected at the sensor surface was measured by using a photodiode (PD) connected to a lock-in amplifier (Princeton Applied Research, USA). For time resolved measurements, the reflectivity changes δR were monitored at an angle of incidence θ set to the value providing the highest slope $\delta R/\delta\theta$. A sinusoidal grating structure (period of 500 nm and depth of 27 nm) was prepared on a 1 mm thick BK7 glass by using a soft lithography from a holographically prepared master followed by a deposition of magnetic (10 nm-thick $Co_{70}Fe_{30}$) and a gold (thickness between 45 and 50 nm) layers by using sputtering (UNIVEX 450C from Leybold Systems, Germany). For a comparison study, identical sensor chips without the magnetic layer were prepared. On the top of a gold surface, mixed thiol self assembled monolayer (SAM) consisting of thiol-COOH and thiol-PEG was prepared from 1 mM ethanol solution and molar ratio of 1:9, respectively. From below a sensor chip, an external magnetic field with a gradient of dB/dx= 0.17 T/mm was applied by using a permanent magnet (NdFeB). Liquid samples were flowed through a flow cell (depth of 500 μm and volume 550 μL) with the flow rate of 0.503 ml/min by using a peristaltic pump.

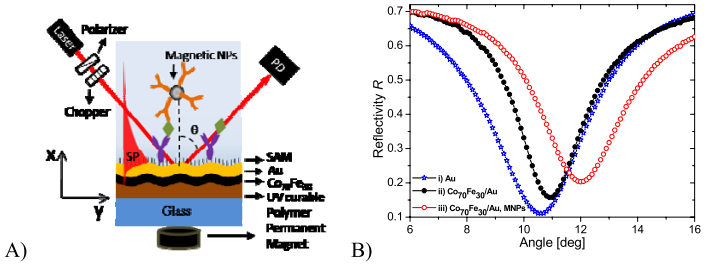

Fig. 1. A) SPR sensor with a diffraction grating sensor chip and the concept of MNPs assays. B) Reflectivity spectra measured for Au sensor chip without i) and with ii) magnetic layer $Co_{70}Fe_{30}$ and iii) reflectivity spectrum after the 30 min flow of MNPs at the concentration of 1.8 nM in PBST over the chip with the magnetic layer.

3. Results and discussion

Firstly, the angular reflectivity spectra measured for sensor chips with gold SPR-active and magnetic layers were analyzed, see Fig. 1B. Compared to the spectrum from the sensor chip without a magnetic layer (i), the spectrum measured for a chip with a magnetic layer $Co_{70}Fe_{30}$ (ii) exhibited slightly decreased surface plasmon coupling efficiency and narrower resonant dip with the full width in half minimum (FWHM) of 2.8º. The reason for this observation is probably due to the fact that the grating modulation depth was decreased by the additional $Co_{70}Fe_{30}$ layer. As shown in Fig. 1B, the resonance dip shifted by $\delta\theta_{SPR}$=1º after a flow of solution with 1.8 nM MNPs for 30 min over the sensor chip with the magnetic layer. Based on the effective medium theory [9] and measured sensitivity of SPR resonant angle ($\delta\theta_{SPR}$) to refractive index changes of $\delta\theta_{SPR}/\delta n$=92 deg/RIU (data not shown), this resonance change corresponds to the surface coverage of MNPs on the sensor chip of about 8 %. This surface coverage is comparable to the density of regions with large gradient of magnetic field at boundaries between magnetic domains of prepared $Co_{70}Fe_{30}$ with typical size few hundreds of nanometers.

Afterwards, the time kinetics of adsorption of MNPs on the SPR sensor surface was measured. Fig.2A compares the time evolution of SPR sensor reflectivity R measured upon a flow of MNPs dissolved in PBST at concentration of 180 pM MNPs. It shows that no obvious change in the reflectivity R was observed for a chip without the magnetic layer, neither with (ii) nor without (i) an external magnetic field applied. However, for a structure with a magnetic layer, the reflectivity changed rapidly upon the flow of MNPs with the slope of $\delta R_1/dt$=0.0028 min^{-1}. When an external field was applied, this slope was further enhanced by a factor of about 16 to $\delta R_2/dt$=0.0448 min^{-1}. These results indicate the amount of collected particles on the surface can be efficiently enhanced through the local magnetic field of $Co_{70}Fe_{30}$ film and external magnetic field gradient. For higher concentrations of MNPs (data not shown), we observed a strong decrease in the reflectivity signal upon a capture of particles on a metallic surface through the external magnetic field gradient. The reason for this observation is the strong absorption and scattering of Fe_3O_4 MNP core. However, for structures without the magnetic layer the SPR dip did not significantly shift, which indicate that the particles formed clusters in vicinity to the surface and they did not spread along the metallic interface. Through the local magnetic field gradient of the magnetic $Co_{70}Fe_{30}$ layer, the formation of clusters was perturbed and particles spread on the surface manifested as a strong SPR dip shift. Let us note, that the penetration depth of SP into an aqueous sample is about L_p=180 nm which makes SPR method sensitive only to one layer of MNPs.

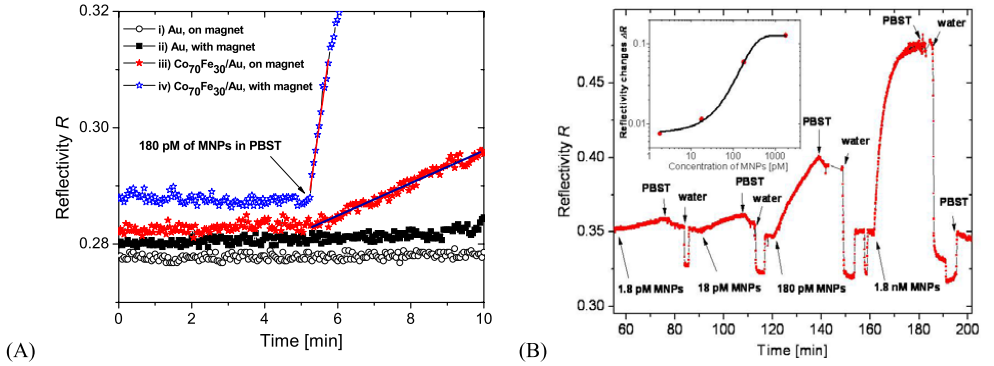

Fig. 2. (A) The time-evolution of reflectivity changes measured upon a flow of MNPs over a sensor chip without the magnetic layer (i, ii) and with the magnetic layer $Co_{70}Fe_{30}$ (iii, iv) for external magnetic field (ii, iv) applied and without an external magnetic field (i, iii). (B) Time-dependent reflectivity changes for series of injections of MNPs (with the concentration between 1.8 pM and 1.8 nM) in PBST measured for a sensor chip with 10 nm of $Co_{70}Fe_{30}$ layer and without external magnetic field. The inset shows the MNPs concentration-dependent reflectivity changes of the magnetic sensor surface after incubated with MNPs in PBST.

In biosensor applications for detection of target analytes, the trapped MNPs need to be easily removed from the

surface in order to discriminate those reacted with biomolecular recognition elements on the sensor surface (see the concept in Fig.1A). For the developed sensor platform, MNPs can be easily removed from the surface by rinsing with water. Figure 2B shows the time-dependent reflectivity changes upon the series of injections of MNPs with the concentration from 1.8 pM to 1.8 nM. After 20-minute flow of a sample, the sensor surface was rinsed with water for 3-5 min. As the figure shows, the sensor response reached the original baseline after the rinsing with water which indicates all adsorbed MNPs were removed. The reason the rapid removal of trapped MNPs is probably due to the charge interaction between MNPs and flat gold surface with thiol SAM. Both MNPs and thiol SAM carry negatively charged carboxylic moieties. In PBST buffer, the charge interaction is screened by the ions in the solution. However, the Coulomb repelling interaction become stronger than the attractive magnetic force in water with lower ionic strength which leads to a desorption of MNPs. Inset of Fig.2B indicates that the sensor responded to the MNPs at concentrations lower than 1.8 pM (corresponding to 60 ng/mL).

4. Conclusions

A new SPR biosensor platform for magnetic nanoparticles (MNPs) assay was developed in which magnetic nanoparticle can simultaneously serve as a “carrier” and a label for detection of target analyte molecules. We show that through a design of SPR sensor chip layer architecture with plasmonic and magnetic characteristics, MNPs can efficiently and reversibly dock on its surface. This approach is expected to provide a) shorten detection times through accelerated mass transport of the analyte to the surface by applied magnetic field gradient and b) higher sensitivity owing to the large mass of magnetic nanoparticle that can be used as labels. Compared to a recently reported approach on TIR-based detection with MNPs assays [10], the utilization of SPR allow employing of smaller particles and potentially offer higher sensitivity owing to the strong surface plasmon field intensity. Moreover, the usage of smaller nanoparticles is expected to offer the advantage of lower steric hindrance. The future work will include the implementation of MNPs immunoassay for detection of biomarkers.

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