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Localized surface plasmon resonance based optical biosensor using surface modified nanoparticle layer for label-free monitoring of antigen-antibody reaction

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Abstract

In recent years, label-free biosensors not requiring external modifications have been receiving intense attention. A label-free optical biosensor, which retains many of the desirable features of conventional surface plasmon resonance (SPR) reflectometry, namely, the ability to monitor the kinetics of biomolecular interactions in real-time without a label has been developed with several important advantages: the biosensor device is easy to fabricate, and simple to implement, requiring only an UV–Vis spectrophotometer or flatbed scanner. Importantly, the label-free optical biosensor can be easily multiplexed to enable high-throughput monitoring of biomolecular interactions in an array-based format. In this research, the development of a localized surface plasmon resonance (LSPR)-based label-free optical biosensor using a surface modified nanoparticle layer is aimed. This optical detection method promises to offer a massively parallel detection capability in a highly miniaturized package. The two-dimensional nanoparticle layer was formed by the surface modified silica nanoparticles. The optical properties and surface analysis of nanoparticle layer substrate were characterized through transmission measurements and atomic force microscopy (AFM). Simultaneously, the nanoparticle layer substrate was applied to the optical LSPR-based biosensor for label-free monitoring of the antigen–antibody reaction. The anti-fibrinogen antibody immobilized nanoparticle layer substrate surface, and the change in the absorption spectrum, caused by the antigen–antibody reaction, was observed. By using this anti-fibrinogen antibody immobilized nanoparticle layer substrate; the detection limit of this optical LSPR-based biosensor was 10 ng/ml. © 2005 Elsevier Ltd. All rights reserved.

Keywords: Biosensor; Nanoparticles; Localized surface plasmon resonance (LSPR); Label-free monitoring; Surface modification; Self-assembled monolayer (SAM)

1. Introduction

In the past decade, various kinds of biosensors were developed, and biosensor became a part of many fields in research, such as medicine [1], and environmental monitoring [2]. Biosensor experiments involve immobilizing a ligand on a solid substrate surface, and monitoring its interaction with an analyte in sample solution. In general, biosensors consist of two components: a biomolecule, which is a highly specific recognition element, and a transducer, such as an electrode [3], or an optical fiber [4], that converts the molecular recognition event into a quantifiable signal. Signal transduction has been carried out with electrochemical [5], quartz crystal microbalance (QCM) [6], optical absorption [7], fluorescence [8], surface plasmon resonance (SPR) [9], and other transducers. In these biosensors, biomolecules (ligand), such as enzymes [10], antibodies [11], oligonucleotides [12–14], microorganisms [15], peptides [16], cells [17] were immobilized on a solid substrate by numerous steps and used to detect the presence of an analyte, such as enzymatic substrates, antigens, oligonucleotides and so on. However, most conventional biosensors require a relatively long assay

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time that involves troublesome liquid-handling and labeling procedures, and many expensive reagents and apparatus. Especially, the labeling procedure draws attention as the most important point. Almost all of the commercially available biosensors make use of labeled reagents, such as enzymes or fluorescent dye. Therefore, a label-free biosensor is desired to monitor a biomolecular interaction easily with a short assay time [18].

Several groups developed different detection methods of label-free biosensors. Kerman et al. [19] reported the development of a label-free peptide nucleic acid (PNA)immobilized biosensor that detected low concentrations of target DNA using electrochemical detection method. Pei et al. [20] developed a glucose biosensor based on a microcantilever platform. Microcantilevers are also applicable to label-free detection of DNA hybridization and the detection of singlebase mismatches [21], nanomechanical motion induced by antibody-antigen recognition [22], and the detection of the prostate-specific-antigen cancer marker [23]. In addition, label-free biosensor using QCM [24] SPR [25] and metal nanoparticles [26-28] were also reported. However, the almost conventional label-free biosensors using QCM and SPR were high-cost apparatus, difficult to use. Therefore, a more convenient and high sensitive label-free biosensor is under demand. Therefore, we aimed the development of the localized surface plasmon resonance (LSPR)-based label-free optical biosensor using surface modified nanoparticles.

Label-free optical methods for detecting biological and chemical interactions have a number of advantages; such as possibility to monitor the biomolecular interaction in realtime and good reliability of results that are obtained using fewer operation procedures. Especially, SPR biosensor based on the phenomenon of SPR is a powerful optical tool for monitoring the binding event between biomoleculars in real-time without the need of intrinsic or extrinsic labels. In the past decade, SPR detection methods have made an important contribution to the quantification of biomolecular interactions [29]. Unfortunately, conventional SPR reflectometry (e.g. the Biacore sensor system) is difficult to realize in a large-scale array format, because of the optics associated with the detection system [30]. This latter limitation is significant, because high-throughput biochemical assays based on biosensors are urgently needed to measure the biomolecular interactions.

From these backgrounds, we developed a biosensor based on LSPR sensing devices using surface modified silica nanoparticles. Commercial SPR sensing devices generally operate in total internal reflection mode using the Kretschmann configuration [31]. LSPR-based biosensors that operate in transmission geometry have been established for ultrathin gold island films and gold or silver nanoparticles immobilized on glass [32]. In this research, we fabricated a layer of surface modified silica nanoparticles on gold layer can be utilized as a LSPR biosensor. This biosensor also operates in transmission mode, allowing for a simpler collinear optical arrangement and providing a smaller probing area than the typical Kretschmann configuration. Localized surface plasmons (LSPs) are charge density oscillations confined to coinage metal nanoparticles and nanoislands. Excitation of LSPs by light at an incident wavelength, where resonance occurs results in the appearance of intense surface plasmon (SP) absorption bands. The intensity and position of the SP absorption bands are characteristic of the type of material (typically, gold, silver, or platinum), the size, size distribution, and shape of the nanostructures and are highly sensitive to the changes of the surrounding environments. These methods can detect an immediate increase in thickness of a biomolecular layer on the surface of a sensitive element caused by a reaction between the solution component under study and the receptor layer immobilized on the surface. This optical biosensor can be applied to the real-time and label-free observation of biochemical reactions between various ligands including antigen-antibody reactions and nucleic acids hybridizations.

The several label-free optical biosensors based on LSPR were reported previously [26-29,32-35]. LSPR-based optical biosensors usually utilized noble metal nanoparticles such as gold, silver and platinum. However, these biosensors have a several kind of disadvantages, such as time-consuming synthesis and difficult control of the diameter of noble metal nanoparticles and poor reproducibility. Therefore, we were interested in developing a goldcapped nanoparticle layer substrate [36]. This gold-capped nanoparticle layer substrate could excite the LSPR and is readily applicable to label-free optical biosensors. However, a gold-capped nanoparticle layer substrate has a disadvantage about sensitivity. In this research, we achieved the improvement of the sensitivity using surface modified nanoparticles. The gold-capped nanoparticle layer using surface modified nanoparticles was also applied to detect the antigen-antibody recognition reactions.

2. Materials and methods

2.1. Materials

4,4'-Dithiodibutyric acid (DDA) for self-assembled monolayer (SAM) formation on the gold substrate surface was supplied by Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) for activation of the carboxyl group of DDA was purchased from Dojindo Laboratories (Kumamoto, Japan). 3-Aminopropyltriethoxysilane (γ-APTES) for surface modification of silica nanoparticles was purchased from Shin-Etsu Chemical Co., Ltd (Tokyo, Japan). Silica nanoparticles (100 nm i.d.) for preparing the nanoparticle layer were purchased from Polysciences, Inc. (Warrington, PA). Slide glass substrate (S-1111, $76 \times$ 26 mm, thickness: 0.8-1.0 mm) was purchased from Matsunami Glass Ind., Ltd (Osaka, Japan). Electronic grade of sulfonic acid and hydrogen peroxide for cleaning the slide glass substrate were purchased from Kanto Kagaku (Tokyo, Japan). N-hydroxysuccinimide (NHS) for antibody immobilization was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Protein A Fc binding grade for control the antibody conformation was purchased from ICN Biomedicals (Asse, Belgium). Anti-fibrinogen antibody, antigen and dinitrophenylated bovine serum albumin (DNP-BSA) were purchased from Sigma (St Louis, USA)

2.2. Apparatus

For the evaporation of gold and titanium on the slide glass substrate, a thermal evaporator (SVC-700TM/700-2) was purchased from Sanyu Electron Co., Ltd (Tokyo, Japan). For the monitoring of base pressure, an analog Ionization Vacuum Gauge was utilized (GI-TL3, ULVAC, Kanagawa, Japan). The growth rate in thickness was monitored by a quartz crystal microbalance (Model TM-200R, MAXTEK. Inc., CA, USA). Spectrometer (USB-2000-UV-VIS, wavelength range: 200-1100 nm), Tungsten Halogen light source (LS-1, wavelength range: 360-2000 nm), and optical fiber probe bundle (R-400-7 UV/VIS, fiber core diameter: 200 µm, wavelength range: 250-800 nm) for evaluation of the optical characteristics of nanoparticle layer substrates were purchased from Ocean Optics (Dunedin, USA). Atomic force microscopy (AFM) for surface analysis of nanoparticle layer substrate was performed using a commercial AFM unit (SPA-400, Seiko Instruments Inc., Chiba, Japan) with a calibrated 20 µm xy-scan and 10 µm z-scan range PZT-scanner.

2.3. Surface modification of the silica nanoparticles

Silica nanoparticles were dried over 24 h at 55 °C, and surface modified silica nanoparticles were prepared by a

reaction with 1% (v/v) γ -APTES solution in ethanol for 24 h at room temperature (RT) by stirring continuously. After the surface modification, the γ -APTES solution was removed in the centrifugal operation for 1 h at 3500 rpm and the centrifuged surface modified silica nanoparticles were washed with ethanol. Both of the washing and centrifugal operations were repeated for three times. After these operations, surface modified nanoparticles were dried up for 12 h at 55 °C. Silica nanoparticles modified with amino groups were thus obtained. Finally, surface modified nanoparticles were stored in the desiccator before use. Then, the surface modified silica nanoparticles colloid solution was prepared by dispersing in ultra pure water for the formation of the nanoparticle layer (Fig. 1).

2.4. Thermal evaporation of titanium and gold onto the slide glass substrates

Slide glass substrate, after the pretreatment of surfaces, were used as substrates for the formation of the nanoparticle layer. Slide glass substrates were cut to either 1.25×1.25 cm. All substrates were cleaned via a three-step process of ultrasonic cleaning in acetone for 30 min, soaking in sulfuric acid and hydrogen peroxide solution at a ratio of 5:1 (v/v) for 30 min, followed by thorough rinsing with ultra pure water (18.3 M Ω -cm), and drying at RT. Finally, it was stored in the desiccator before use.

For evaporation, the thermal evaporator was used at a base pressure of 4×10^{-6} Torr; the growth rate was monitored by the QCM, and manually adjusted to 1.0 Å/s. gold and titanium of 99.99% purity was obtained



Fig. 1. Surface modification of the silica nanoparticles using 3-aminopropyltriethoxysilane.



Fig. 2. Fabrication of the LSPR-based biosensor using surface modified nanoparticles.

from Furuya Metal (Tokyo, Japan). A titanium layer of 10 nm and a gold layer of 30 nm were evaporated onto the glass substrates. The same amount of gold was also evaporated onto the formed nanoparticle layer using surface modified nanoparticles for the final gold nanoparticle layer formation.

2.5. Fabrication of the LSPR-based biosensor using surface modified nanoparticles

The fabrication procedure of LSPR-based biosensor using surface modified nanoparticles is shown in Fig. 2. DDA solution concentrations (1 mM-1 nM) were changed, and added to the gold substrate surface produced by the thermal evaporation, and the self-assembled monolayer (SAM) was formed in 1 h. The SAM-functionalization was carried out with 400 mM EDC solution for 1 h, EDC activated the carboxyl groups of the DDA, and therefore, the amino groups of silica nanoparticles could form esters with the activated carboxyl groups. The surface modified silica nanoparticles witch modified with amino groups (1% w/v) by silane coupling reagent in ultra pure water were added to the activated SAM modified gold substrate surface for 1 h. The nanoparticle layer modified substrates were rinsed thoroughly with ethanol, and ultra pure water for remove the excess surface modified nanoparticles, and dried at RT. Finally, gold layer (30 nm) was deposited on the nanoparticle layer modified substrates using thermal evaporator.

2.6. Evaluation of the optical characteristics

The experimental setup was shown in Fig. 3. All absorbance spectra were taken from 400 to 800 nm on the UV–Vis spectrometer at RT. White light (tungsten–halogen light

source) emerging from the optical fiber bundle was incident onto the nanoparticle layer substrate from the vertical direction. The reflected light was coupled into the detection fiber probe of the optical fiber bundle and analyzed by the UV–Vis spectrometer. Under these experimental conditions, the optical characteristics of the nanoparticle layer substrates were investigated.

2.7. Surface analysis of the nanoparticle layer substrate surface using AFM

To analyze the surface of nanoparticle layer substrate quality in terms of particle density, periodicity, and



Fig. 3. Experimental setup of the optical characteristics evaluation.



Fig. 4. Immobilization procedure of the antibody on the gold-capped nanoparticle.layer substrate surface.

monolayer formation, AFM was carried out in tapping mode using silicon tips and cantilevers with a nominal spring constant of 18 N/m for scanning in air. All reported images were acquired at scan rates in the range 0.25–0.50 Hz. The surface coverage of the nanoparticles was calculated using calculation software including AFM operating software.

2.8. Optical characteristics evaluation by the avidin and anti-fibrinogen adsorption onto the nanoparticle layer substrate surface

Different concentrations of avidin $(1 \text{ ng/ml} \sim 100 \text{ µg/ml})$ solutions in 20 mM phosphate buffered saline (PBS, pH 7.4) were introduced onto the nanoparticle layer substrate surface directory for 1 h. After the avidin adsorption, the nanoparticle layer substrate surface was rinsed by PBS, then the optical characteristics evaluation of the avidin adsorption was carried out. Simultaneously, the optical characteristics evaluation by the anti-fibrinogen adsorption was carried out.

2.9. Immobilization of antibody on the surface of the nanoparticle layer substrate

The experimental procedure for the immobilization of the anti-fibrinogen antibody is shown in Fig. 4. The antibody immobilization was carried out with an almost similar procedure to the formation of nanoparticle layer using surface modified silica nanoparticles. DDA solution of 1 mM was added to the nanoparticle layer surface, and the SAM was formed in 1 h. The SAM-functionalization was carried out with 400 mM EDC solution for 1 h, and then 100 mM NHS solution added to the SAMfunctionalized nanoparticle layer surface for 1 h. Protein A solution of 100 µg/ml was introduced to the nanoparticle layer substrate surface for 1 h, which combined the antibody Fc region of the IgG antibody, therefore, the conformation of the antibody became possible to control. Anti-fibrinogen antibody of 100 µg/ ml was introduced to the protein A modified nanoparticle layer surface for 1 h. The immobilization of the antibody was achieved after these steps. Finally, the antibody

immobilized nanoparticle layer was rinsed thoroughly with 20 mM phosphate buffered saline (PBS, pH 7.4) and dried at RT.

2.10. Label-free measurement of the antigen–antibody reaction

The anti-fibrinogen antibody was immobilized on the nanoparticle layer substrate surface, which could recognize only fibrinogen molecules. Different concentrations of fibrinogen $(1 \text{ ng/ml} \sim 100 \mu \text{g/ml})$ were introduced to the nanoparticle layer substrate surface, and the change in the absorption spectrum caused by the antibody-antigen reaction was observed. Simultaneously, DNP-BSA was introduced to the anti-fibrinogen antibody immobilized nanoparticle layer substrate surface as the negative control.

3. Results and discussion

3.1. Characteristics evaluation of the nanoparticle layer substrate

The formation method of the nanoparticle layer by covalent attachment was observed to be more successful than the physical adsorption and electrophoretic deposition



Fig. 5. Optical characteristics of the nanoparticle layer using different concentration of DDA solutions.

at large peak and with high reproducibility (data not shown). Therefore, the covalent attachment was carried out for the nanoparticle layer formation. The changes in the absorbance strength of nanoparticle layer substrates are shown in Fig. 5. The absorbance strength dependency of the nanoparticle layer substrates formed by surface modified silica nanoparticles on DDA concentration was monitored at 552 nm. It is known that nanoparticles such as gold [37–39], silver [40], and copper [41] possess strong absorption in the visible region, often coined as localized surface plasmon absorption. Such LSPR occurs when the incident photon frequencies match the collective oscillations of the - conduction electrons of metal nanoparticles or metal islands. The particles in the nanoscale exhibit unique

optical responses within the UV–Vis region, where the absorbance shows an exponential decay with decreasing photon energy (the so-called Mie scattering) onto which a localized surface plasmon resonance band, specific for the particle material, is superimposed. The localized surface plasmon energy and intensity have been found to be sensitive to a number of factors, including particle size, shape, immediate surrounding media, etc. The absorbance strength in the visible region of the electromagnetic spectrum occurs due to the excitation of localized surface plasmon oscillations, and is responsible for the beautiful variety in the colors of the nanoparticle layer. Thus, chemically assembled nanoparticle layer of surface modified silica nanoparticles on slide glass substrate are ideal



Fig. 6. AFM images and absorbance strength for the nanoparticle layer coverage dependence on DDA concentration.

candidates for study using UV–Vis spectroscopy. To examine the optimum time scale for formation of a monolayer of surface modified silica nanoparticles on the slide glass substrate surface, UV–Vis spectroscopy measurements were carried out as a function of time for the formation of the nanoparticle layer. As mentioned earlier, the nanoparticle layer substrates, which were formed at different formation times, were washed with significant volumes of ultra pure water, and dried prior to the evaluation of the optical characteristics. A clear change in the localized surface plasmon resonance intensity in time was observed (data not shown). The time scale for the formation of a monolayer, of the nanoparticle layer substrates was set as 1 h.

AFM was used to observe the nanoparticle layer formation on the surface of the slide glass substrate. AFM images and absorbance strength of nanoparticle layer substrates with different concentrations of DDA (1 mM-1 nM) are shown in Fig. 6. Absorbance strength of the silica nanoparticles depended on the ranging DDA concentration. The AFM images clearly illustrated that the surface modified silica nanoparticles were one particle in depth, forming a nanoparticle layer. As a result of the surface analysis using AFM, different periodicity and coverage in connection with DDA concentration were obtained. From the AFM images, the surface plasmon intensity was found to be depending on the coverage and periodicity of the surface modified nanoparticles. When the formation of a nanoparticle layer substrate was performed without using DDA, the absorption peak was very weak. In addition, the adsorption of the surface modified nanoparticles was sparse in the surface analysis using AFM. Thus, this formation technique was utilized to achieve the nanoparticle layer formation. This was due to slight variations in surface modified nanoparticle density on the Au substrate surface, and the relatively small sample area. The coverage of the



Fig. 7. Particle coverage dependence of the nanoparticle layer on DDA concentration.



Fig. 8. Calibration curve of the nanoparticle layer substrate on avidin and anti-fibrinogen antibody concentration.

nanoparticles with DDA concentration dependent is shown in Fig. 7.

DDA concentration of 1 mM was observed to be the optimum condition. The surface coverage of nanoparticles was found to improve almost linearly with DDA concentration. Thus, the control of nanoparticle coverage was accomplished.

3.2. Optical characteristics evaluation by the avidin and anti-fibrinogen adsorption onto the nanoparticle layer surface

The optical characteristics evaluation was carried out using nanoparticle layer substrate. The concentration dependence of avidin adsorption strength was observed. Using our nanoparticle layer substrate, it was possible to determine the adsorption range of avidin adsorption was $1.0 \text{ ng/ml}-100 \mu \text{g/ml}$ (Fig. 8). These absorbance strength changes were due to the adsorption of avidin on the



Fig. 9. Calibration curve of the anti-fibrinogen immobilized nanoparticle layer substrate on fibrinogen concentration.



Fig. 10. Absorbance strength change by the antigen-antibody reaction.

nanoparticle layer substrate surface. As the thickness of the biomolecular layer increased, the absorbance strength also increased. Simultaneously, the similar optical characteristics were observed by anti-fibrinogen antibody adsorption onto nanoparticle layer substrate surface.

Normally, the optical characteristics changes of the LSPRbased biosensors were commonly known as peak shift of peak wavelength. However, the peak shift was not observed using this nanoparticle layer substrate. Previously, the similar optical characteristics using LSPR-based biosensor was reported [40,42]. From these results, the nanoparticle layer was thought to be similar to previous report.

3.3. Label-free measurement of the antigen–antibody interaction

The determination of fibrinogen was performed with an anti-fibrinogen antibody immobilized nanoparticle layer substrate. The antigen-antibody reaction was performed by the injection of different concentrations of fibrinogen solution for 30 min to the anti-fibrinogen antibody immobilized nanoparticle layer substrate surface. Followed by washing with blank PBS to remove the nonspecific adsorption after antigen-antibody reaction. Subsequently, optical characteristics evaluation was carried out. Using the anti-fibrinogen antibody immobilized nanoparticle layer substrate, the detection limit of this antibody immobilized optical biosensor was 10 ng/ml (Fig. 9). In addition, 100 µg/ml of DNP-BSA solution was introduced to the anti-fibrinogen antibody immobilized nanoparticle layer substrate as a negative control using different nanoparticle layer substrate. However, the adsorption strength change was not observed (Fig. 10). In this time, the difference of absorbance strength and peak wavelength was observed when the washing procedure after the formation of the nanoparticle layer. The reason of this

difference was caused by the physical adsorption of the surface modified nanoparticle on the nanoparticle modified gold substrate surface by an incomplete washing procedure. The antibody immobilized nanoparticle layer substrate reported here was found to be applicable to the immunoassays. This detection method using nanoparticle layer substrate is possible to detect the analyte conveniently with high sensitivity, because there is no necessity of labeling such as enzymes and fluorescent dye on this detection method. In addition, this nanoparticle layer is easy to form, and the apparatus cost for the optical characteristics evaluation is lower than that of conventional SPR apparatus, and the operation procedure is more convenient.

4. Conclusions

In this research, surface modification of silica nanoparticles and SAM formation on substrates was carried out to realize a two-dimensional arrangement of silica nanoparticles. A unique nanoparticle layer of surface modified silica nanoparticles was attached to the DDA modified surface of gold substrate. The surface modified nanoparticles were prepared with γ -APTES, which provided amino groups on the silica nanoparticle surface. The surface of the gold substrate was modified with DDA, which, after activation, was reacted with the amino groups on the surface modified silica nanoparticles. AFM showed a dense monolayer of 100 nm surface modified silica nanoparticles on the gold substrate surface. The surface coverage of nanoparticles dependent on DDA concentration was also observed. Simultaneously, control of the coverage of nanoparticle density was achieved.

In addition, the nanoparticle layer substrate was applied for novel label-free optical biosensor, and then the calibration characteristics evaluation were carried out. The formation technique of nanoparticle layer reported here, enables a high periodicity, and is easy to form. Therefore, a LSPR biosensor based on nanoparticle layer developed here is considered to be promising for its potential applications to detect the biomolecular interactions.

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References

- B.D. Malhotra, A. Chaubey, Biosensors for clinical diagnostics industry, Sens. Actuators B91 (2003) 117–127.
- [2] T. Endo, A. Okuyama, Y. Matsubara, K. Nishi, M. Kobayashi, Y. Morita, Y. Takamura, H. Mizukami, E. Tamiya, Fluorescencebased assay with enzyme amplification on a microflow immunosensor chip for monitoring coplanar polychlorinated biphenyls, Anal. Chim. Acta 531 (2005) 7–13.
- [3] K. Mitsubayashi, Y. Wakabayashi, S. Tanimoto, D. Murotomi, T. Endo, Optical-transparent and flexible glucose sensor with ITO electrode, Biosens. Bioelectron. 19 (2003) 67–71.
- [4] W.L. Xing, L.R. Ma, Z.H. Jiang, F.H. Cao, M.H. Jia, Portable fiberoptic immunosensor for detection of methsulfuron methyl, Talanta 52 (2000) 879–883.
- [5] K. Kerman, Y. Morita, Y. Takamura, M. Ozsoz, E. Tamiya, Modification of *Escherichia coli* single-stranded DNA binding protein with gold nanoparticles for electrochemical detection of DNA hybridization, Anal. Chim. Acta 510 (2004) 169–174.
- [6] Y. Liu, W. Zhang, X. Yu, H. Zhang, R. Zhao, D. Shangguan, Y. Li, B. Shen, G. Liu, Quartz crystal biosensor for real-time kinetic analysis of interaction between human TNF-α and monoclonal antibodies, Sens. Actuators B99 (2004) 416–424.
- [7] F. Baldini, C. Domenici, A. Giannetti, D. Masci, A. Mencaglia, Timeresolved absorption as optical method for herbicide detection, Sens. Actuators B90 (2003) 198–203.
- [8] C.J. Taylor, L.A. Bain, D.J. Richardson, S. Spiro, D.A. Russell, Construction of a whole-cell gene reporter for the fluorescent bioassay of nitrate, Anal. Biochem. 328 (2004) 60–66.
- [9] Y. Tian, L. Zhao, D. Song, X. Liu, Y. Cao, Z. Peng, Z. Liu, H. Zhang, Acousto-optic tunable filter-based surface plasmon resonance biosensor for determination of human factor B, Anal. Chim. Acta 511 (2004) 97–104.
- [10] T. Santoni, D. Santianni, A. Manzoni, S. Zanardi, M. Mascini, Enzyme electrode for glucose determination in whole blood, Talanta 44 (1997) 1573–1580.
- [11] S. Grant, F. Davis, J.A. Pritchard, K.A. Law, S.P.J. Higson, T.D. Gibson, Labeless and reversible immunosensor assay based upon an electrochemical current-transient protocol, Anal. Chim. Acta 495 (2003) 21–32.

- [12] E. Giakoumaki, M. Minunni, S. Tombelli, I.E. Tothill, M. Mascini, P. Bogani, M. Buiatti, Combination of amplification and postamplification strategies to improve optical DNA sensing, Biosens. Bioelectron. 19 (2003) 337–344.
- [13] E. Mariotti, M. Minunni, M. Mascini, Surface plasmon resonance biosensor for genetically modified organisms detection, Anal. Chim. Acta 453 (2002) 165–172.
- [14] S. Tombelli, M. Mascini, A.P.F. Turner, Improved procedures for immobilisation of oligonucleotides on gold-coated piezoelectric quartz crystals, Biosens. Bioelectron. 17 (2002) 929–936.
- [15] G.J. Chee, Y. Nomura, K. Ikebukuro, I. Karube, Biosensor for the evaluation of biochemical oxygen demand using photocatalytic pretreatment, Sens. Actuators B80 (2001) 15–20.
- [16] Y. Katayama, Y. Ohuchi, H. Higashi, Y. Kudo, M. Maeda, The design of cyclic AMP-recognizing oligopeptides and evaluation of its capability for cyclic AMP recognition using an electrochemical system, Anal. Chem. 72 (2000) 4671–4674.
- [17] Y. Matsubara, Y. Murakami, M. Kobayashi, Y. Morita, E. Tamiya, Application of on-chip cell cultures for the detection of allergic response, Biosens. Bioelectron. 19 (2004) 741–747.
- [18] A.V. Dotsenko, A.L. Diikov, T.A. Vartanyan, Label-free biosensor using an optical waveguide with induced Bragg grating of variable strength, Sens. Actuators B94 (2003) 116–121.
- [19] K. Kerman, Y. Morita, Y. Takamura, E. Tamiya, Label-free electrochemical detection of DNA hybridization on gold electrode, Electrochem. Commun. 5 (2004) 887–891.
- [20] J. Pei, F. Tian, T. Thundat, Glucose biosensor based on the microcantilever, Anal. Chem. 76 (2004) 292–297.
- [21] K.M. Hansen, H.F. Ji, G. Wu, R. Datar, R. Cote, A. Majumdar, T. Thundat, Cantilever-based optical deflection assay for discrimination of DNA single-nucleotide mismatches, Anal. Chem. 73 (2001) 1567–1571.
- [22] C. Grogan, R. Raiteri, G.M. O'Connor, T.J. Glynn, V. Cunningham, M. Kane, M. Charlton, D. Leech, Characterisation of an antibody coated microcantilever as a potential immuno-based biosensor, Biosens. Bioelectron. 17 (2002) 201–207.
- [23] G. Wu, R.H. Datar, K.M. Hansen, T. Thundat, R.J. Cote, A. Majumdar, Bioassay of prostate-specific antigen (PSA) using microcantilevers, Nat. Biotech. 19 (2001) 856–860.
- [24] X. Su, J. Zhang, Comparison of surface plasmon resonance spectroscopy and quartz crystal microbalance for human IgE quantification, Sens. Actuators B100 (2004) 311–316.
- [25] R.I.W. Osmond, W.C. Kett, S.E. Skett, E.R. Coombe, Protein heparin interactions measured by BIAcore 2000 are affected by the method of heparin immobilization, Anal. Biochem. 310 (2002) 199–207.
- [26] N. Nath, A. Chilkoti, Label-free biosensing by surface plasmon resonance of nanoparticles on glass: optimization of nanoparticle size, Anal. Chem. 76 (2004) 5370–5378.
- [27] N. Nath, A. Chilkoti, A colorimetric gold nanoparticle sensor to interrogate biomolecular interactions in real time on a surface, Anal. Chem. 74 (2002) 504–509.
- [28] N. Nath, A. Chilkoti, Interfacial phase transition of an environmentally responsive elastin biopolymer adsorbed on functionalized gold nanoparticles studied by colloidal surface plasmon resonance, J. Am. Chem. Soc. 123 (2001) 8197–8202.
- [29] H.M. Haake, A. Schütz, G. Gauglitz, Label-free detection of biomolecular interaction by optical sensors, Fresen. J. Anal. Chem. 366 (2000) 576–585.
- [30] Y.N. Abdiche, D.G. Myszka, Probing the mechanism of drug/lipid membrane interactions using Biacore, Anal. Biochem. 328 (2004) 233–243.
- [31] A.K. Sharma, B.D. Gupta, Absorption-based fiber optic surface plasmon resonance sensor: a theoretical evaluation, Sens. Actuators B100 (2004) 431–439.
- [32] M.D. Musick, C.D. Keating, L.A. Lyon, S.L. Botsko, D.J. Pena, W.D. Holliway, T.M. McEvoy, J.N. Richardson, M.J. Natan, Metal films prepared by stepwise assembly. 2. Construction and

characterization of colloidal Au and Ag multilayers, Chem. Mater. 12 (2000) 2869–2881.

- [33] A.J. Haes, R.P.V. Duyne, A nanoscale optical biosensor: sensitivity and selectivity of an approach based on the localized surface plasmon resonance spectroscopy of triangular silver nanoparticles, J. Am. Chem. Soc. 124 (2002) 10596–10604.
- [34] G. Kalyuzhny, A. Vaskevich, M.A. Schneeweiss, I. Rubinstein, Transmission surface-plasmon resonance (T-SPR) measurements for monitoring adsorption on ultrathin gold island films, Chem. A Eur. J. 8 (2002) 3849–3857.
- [35] D. Eck, C.A. Helm, N.J. Wagner, K.A. Vaynberg, Plasmon resonance measurements of the adsorption and adsorption kinetics of a biopolymer onto gold nanocolloids, Langmuir 17 (2001) 957–960.
- [36] M. Himmelhaus, H. Takei, Cap-shaped gold nanoparticles for an optical biosensor, Sens. Actuators B63 (2000) 24–30.
- [37] S.J. Chen, F.C. Chien, G.Y. Lin, K.C. Lee, Enhancement of the resolution of surface plasmon resonance biosensors by control of the size and distribution of nanoparticles, Opt. Lett. 29 (2004) 1390–1392.

- [38] M. Zayats, A.B. Kharitonov, S.P. Pogorelova, O. Lioubashevski, E. Katz, I. Willner, Probing photoelectrochemical processes in Au– CdS nanoparticle arrays by surface plasmon resonance: application for the detection of acetylcholine esterase inhibitors, J. Am. Chem. Soc. 125 (2003) 16006–16014.
- [39] R.A. Reynolds III, C.A. Mirkin, R.L. Letsinger, Homogeneous, nanoparticle-based quantitative colorimetric detection of oligonucleotides, J. Am. Chem. Soc. 122 (2000) 3795–3796.
- [40] F. Frederix, J.-M. Friedt, K.H. Choi, W. Laureyn, A. Campitelli, D. Mondelaers, G. Maes, G. Borghs, Biosensing based on light absorption of nanoscaled gold and silver particles, Anal. Chem. 75 (2003) 6894–6900.
- [41] S. Chen, J.M. Sommers, Alkanethiolate-protected copper nanoparticles: spectroscopy, electrochemistry, and solid-state morphological evolution, J. Phys. Chem. B105 (2001) 8816–8820.
- [42] S. Hrapovic, Y. Liu, G. Enright, F. Bensebaa, J.H.T. Luong, New strategy for preparing thin gold films on modified glass surfaces by electroless deposition, Langmuir 19 (2003) 3958–3965.