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ORIGINAL PAPER

Real-time monitoring of biomolecular interactions in blood plasma using a surface plasmon resonance biosensor

Tomáš Špringer · Marek Piliarik · Jiří Homola

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Abstract We report a novel approach to biosensor-based observations of biomolecular interactions which enables real-time monitoring of biomolecular interactions in complex media. This approach is demonstrated by investigating the interaction between the human chorionic gonadotropin (hCG) and its antibody in blood plasma using a surface plasmon resonance biosensor and a dispersionless microfluidics system. The real-time binding data obtained in blood plasma are compared with those obtained in buffer and blood plasma using a conventional method. It is also demonstrated that the proposed approach can enhance the capability of the biosensor to detect biomolecules in complex samples in terms of detection time and sensitivity. In the model experiment, this approach is shown to enable direct detection of hCG in blood plasma at levels which are five times lower than those detected using the conventional detection approach.

Keywords Surface plasmon resonance · Molecular interaction analysis · Microfluidics · Optical biosensor

Introduction

In the last two decades an increasing effort has been dedicated to the development of real-time label-free optical biosensors [1]. This effort resulted in a wide variety of optical biosensor technologies suitable for biomolecular interaction analysis and rapid detection of chemical and biological species with applications in sectors such as biomedical and pharmaceutical research, medical diagnostics, environmental monitoring, food safety, and security [1, 2]. In medical diagnostics, detection of a wide range of molecular markers in bodily fluids is desirable. These biomarkers are present at concentrations ranging typically from pg/mL to μg/mL. For example, levels of human chorionic gonadotropin (hCG) in the blood plasma of healthy individuals are below 1 ng/mL and can increase to μg/mL in patients suffering from trophoblastic cancer or epithelial ovarian tumors [3].

Biosensor-based studies of biomolecular interactions are typically carried out in buffers which are well-defined and rather simple environments. This approach fails to take into account potentially complex effects of other molecules which are present in the medium in which biomolecular interactions occur in reality (e.g., human blood, blood plasma) [4–6]. For instance, proteins in blood serum were demonstrated to interfere with certain drugs, affecting their pharmacological activity [4, 5]. Therefore, the monitoring of biomolecular interactions in native environments presents an important research goal. However, this goal poses numerous challenges, as the complex media influence sensor response both by affecting the molecular interaction under study [6] and by interfering with the measurements (e.g., through non-specific adsorption on the sensor surface) [7]. Non-specific adsorption from blood plasma produces a sensor response which is often comparable or larger than that associated with the specific interaction under study [7]. In order to reduce the interfering effects from complex samples, samples can be diluted [8, 9]. However, the dilution of sample decreases concentration of analyte and

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T. Špringer · M. Piliarik · J. Homola (✉)
Department of Optical Sensors,
Institute of Photonics and Electronics AS CR, v. v. i.,
Chaberská 57,
182 51 Prague, Czech Republic
e-mail: homola@ufe.cz

therefore reduces sensitivity of the method. Interfering effects are commonly reduced by means of referencing in which measurement is performed simultaneously in two channels—sensing channel that is sensitive to both specific molecular interaction and interfering effects and reference channel that is sensitive just to interfering effects. However, when applied to measurements in blood plasma, response of each channel may substantially exceed the specific response of the sensing channel. The accuracy of referencing ranges between 95% and 99% of the sensor response [1] and therefore the resulting error may still be larger than the sensor response to the specific molecular interaction. In order to improve conditions for the direct observation of molecular interactions in complex media, various sensor surface modifications suppressing the non-specific interactions have been proposed. For instance, biointerfaces based on zwitterionic polymers [7, 10] have been demonstrated to exhibit non-specific protein adsorption from undiluted blood plasma of less than 3 ng/cm^2 [7].

Various approaches have been proposed to detect protein analytes in complex samples. Sandwich detection formats with secondary or tertiary antibodies or gold nanoparticles have been widely used to enhance sensor response to target molecules [11–15]. Combination of colloidal nanoparticles and secondary antibody was used in the study of Schneider et al. [13] to detect the hCG down to 0.5 ng/mL in undiluted blood plasma. Teramura and Iwata [14] used secondary and tertiary antibody to amplify the sensor response to α-fetoprotein. This approach increased the sensor response by a factor of ~6 and allowed achieving the limit of detection in the ng/mL level. However, the sandwich detection format requires multiple steps which makes the real-time monitoring of the interaction of the target molecule with the receptors impossible. Moreover, the requirement of multiple steps increases the detection time, complexity and costs of the assay.

We report a novel approach to biosensor-based observation of biomolecular interactions which enables direct real-time monitoring of biomolecular interactions in complex media. The approach is demonstrated by investigating a model biomolecular interaction between hCG diluted in 50% blood plasma and its antibody immobilized on the surface of a surface plasmon resonance (SPR) sensor.

Materials and methods

Reagents

Sodium acetate, KH_2PO_4, Na_2HPO_4, KCl, NaCl, ethanolamine (EA), and bovine serum albumin (BSA) were purchased from Sigma Aldrich, USA, in molecular biology grade or higher. *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from GE Healthcare, USA. hCG and the respective antibody (anti-hCG) were obtained from Scripps Laboratories, USA. Carboxylic (HS-C_{11}-$(EG)_6$-OCH_2-COOH) and hydroxylic (HS-C_{11}-$(EG)_4$-OH) alkanethiols were purchased from Prochimia, Poland. Ethanol for spectroscopy (purity ≥99.9%) was purchased from Merck, USA. Blood plasma was kindly provided by the Institute of Hematology and Blood Transfusion, Prague, Czech Republic (IHBT). The blood samples were obtained at IHBT from informed consent patients in accordance with the rules of the Ethical Committee of IHBT. Plasma was centrifuged from ACD whole blood of healthy donors and stored at −80 °C until use. The composition of the phosphate buffer PB was 1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.7 mM KCl, pH 7.4 at 25 °C. The PBS and PBS_{Na} buffer had the same composition as the PB buffer with the exception of NaCl concentration which was 137 mM and 0.75 M, respectively. PBS_{BSA} and $PBS_{Na/BSA}$ buffers were prepared by adding 20 mg/mL BSA and 2 mg/mL BSA into PBS and PBS_{Na}, respectively. SA_{10} consisted of 10 mM sodium acetate, pH 5 at 25 °C. All buffers were prepared using deionized water (18 MΩ/cm resistance, Direct-Q from Millipore).

Sampling method for monitoring of biomolecular interactions

The proposed method (herein referred to as the sampling method) is based on sequential injections of complex sample (e.g., blood plasma) and a washing solution (e.g., buffer). Fig. 1

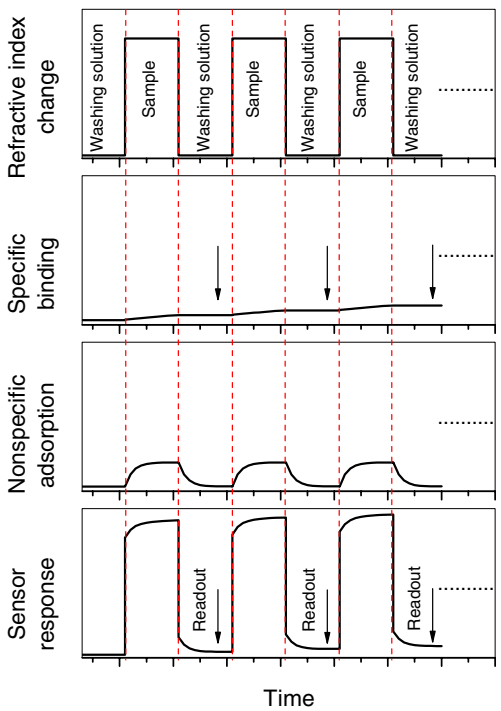

Fig. 1 Sampling method: specific and non-specific effects occurring during the sequential injection of the complex sample and washing solution

illustrates several cycles of the sampling method with the interaction taking place in the complex sample and sensor readout being performed in the washing solution. When the sensor is exposed to the sample, specific binding, non-specific adsorption, and refractive index change takes place at the sensor surface. The washing solution is designed in such a way that it removes non-specifically adsorbed proteins, while it does not disrupt binding of analyte molecule to their biospecific partner immobilized on the sensor surface. We demonstrate that almost complete removal of the non-specifically adsorbed proteins can be achieved during the washing period as short as several seconds, while only a small fraction of specifically bound analyte molecules is desorbed. Therefore the level of specifically captured analyte molecules can be assessed at the end of the washing solution cycle (Fig. 1) and thus the real-time binding data can be reconstructed.

Surface plasmon resonance sensor

A laboratory SPR sensor (PLASMON IV) developed at the Institute of Photonics and Electronics, Prague, Czech Republic [16] was used in this study. The sensor is based on the wavelength spectroscopy of surface plasmons and the attenuated total reflection method. The sensor allows for simultaneous monitoring of biomolecular interactions in four independent sensing channels. Sensor chip is made of a glass slide coated by an adhesion-promoting titanium film (thickness: 1 nm) and a gold film (thickness: 50 nm). In the SPR sensor, the chip is interfaced with a flow-cell with four separate flow chambers facing four sensing areas on the sensor chip (details of the fluidic system are given in the following section). The sensor is equipped with temperature stabilization, enabling SPR measurements with the temperature baseline stability better than 0.01 °C. The sensor response is represented by the change of the SPR wavelength and can be calibrated to the change in the surface coverage of bound molecules. The calibration coefficient is proportional to the molar weight and depends on the resonant wavelength [17]. For the SPR sensor used in this study and SPR wavelength of 750 nm, a 1-nm SPR wavelength shift represents a change in the protein surface coverage of 17 ng/cm^2.

Dispersionless microfluidics

In order to allow for fast switching between the analyzed complex sample and the washing solution, a recently developed dispersionless microfluidics [18] was used. This microfluidics system allows switching between two samples without dispersion of the liquid and intermixing before the sample reaches the sensing area. The principle of operation of the microfluidics system is illustrated in Fig. 2. The sensing area, where the sample is to be delivered, is comprised of a microfluidic channel and two input and two output ports.

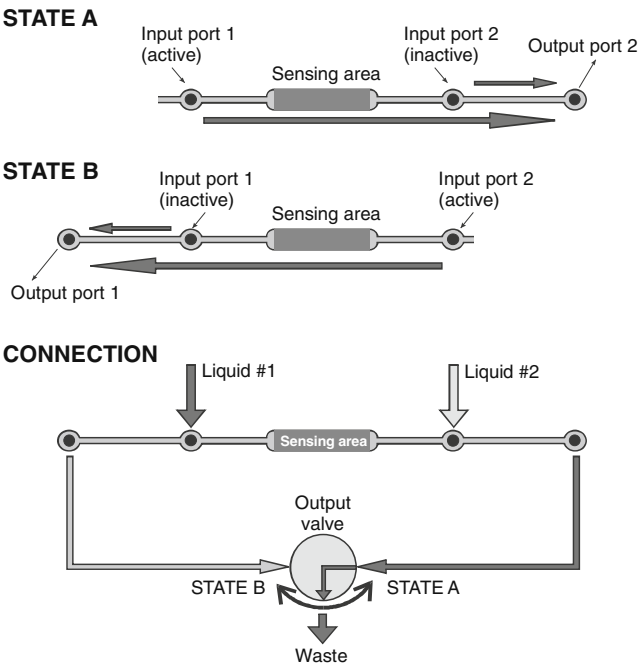

Fig. 2 Principle of operation of the dispersionless microfluidics

Two different liquids are introduced to the microfluidic channel through the two input ports. In state A, output port 2 is opened and output port 1 is closed, which makes liquid #1 flow through the sensing area and then to the waste container while liquid #2 flows directly to the waste container. In state B, the direction of flow is reversed, which makes liquid #2 flow through the sensing area. The microfluidic structure is formed from 60-μm-thick self-adhesive vinyl foil which is attached to the surface of a polished acrylic manifold. The input ports are connected to two multichannel peristaltic pumps and output ports are connected to microelectric valves (VICI Valco Instruments Co. Inc., USA).

Sensor functionalization

Sensing areas on the surface of the SPR chip were functionalized to yield sensing and reference channels with low fouling background. A self-assembled monolayer of mixed carboxy- and hydroxy-terminated alkanethiols was used as a linker layer onto which anti-hCG antibody and BSA (sensing channel) or only BSA (reference channel) were covalently anchored via amino coupling chemistry. Details of the functionalization procedure are given below. A 3:7 mixture of HS-C_{11}-$(EG)_6$-OCH_2-COOH and HS-C_{11}-$(EG)_4$-OH thiols was dissolved in ethanol in a total concentration of 0.2 M. A clean SPR chip was immersed in the thiol mixture at 40 °C for 10 min and stored at a room temperature overnight. Then the chip was rinsed with ethanol, mounted into the SPR setup and deionized water was flowed along the sensing area. Subsequently, the chip

surface was incubated for 10 min in aqueous mixture of 0.5 M NHS/0.1 M EDC to activate the carboxylic groups. After activation, a solution of 10 μg/mL anti-hCG in 10 mM SA_{10} buffer was pumped across the sensing area (sensing channel). The immobilization with anti-hCG was stopped when the SPR sensor response reached 7 nm (~119 ng/cm^2), which corresponds to one third of the saturation surface coverage of anti-hCG. The sensor surface was then incubated with 1 mg/mL BSA in SA_{10} for 30 min (both sensing and reference channels). Non-covalently bound anti-hCG and BSA was rinsed with PBS_{Na} for 5 min. At the end of the surface functionalization, 1 mM EA (pH 8 at 25 °C) was introduced for 5 min to deactivate carboxylic groups of alkanethiols. During the functionalization procedure, the flow rate and the temperature were set to 20 μL/min and 25 °C, respectively.

Monitoring interaction between hCG and anti-hCG

Real-time monitoring of the interaction between anti-hCG antibody immobilized on the sensor surface and hCG in blood plasma was performed using both the sampling method and conventional referencing method to allow for direct comparison.

The sampling method in plasma was performed as described below. After the functionalization of the SPR chip with antibodies, the input port 1 was used as the active input port. Washing solution ($PBS_{Na/BSA}$) was pumped through the active input port 1 and flowed across the sensing area until a stable baseline was achieved. Blood plasma mixed with PBS_{BSA} (1:1 ratio) was spiked with hCG (final hCG concentration: 250 ng/mL) and loaded into the input port 2 of the microfluidics system (inactive). In this configuration the blood plasma sample was directed to the waste without entering the sensing area. The sampling approach was carried out by sequentially switching the active input port between input ports 1 and 2. Ten 30-s cycles of blood plasma sample and washing solution were performed (total time: 10 min). Finally, the microfluidics system was switched to the input port 1 and the washing solution was flowed through the sensing area until equilibrium was achieved. The flow rates used in these experiments were as follows: blood plasma sample was pumped at a flow rate of 20 μL/min while a flow rate of 60 μL/min was used for the washing solution to increase the desorption rate of the non-specifically adsorbed molecules and to improve the accuracy of the readout of the specific sensor response. Reference channel functionalized with BSA was used to account for the non-specific adsorption.

The referencing method was performed in blood plasma and buffer as described below: after functionalization of the chip, PBS_{Na} was flowed through the sensor area until a stable baseline was established (flow rate of 60 μL/min). Subsequently, the sample containing hCG was injected in the flow channel (250 ng/mL hCG in PBS or in 1:1 blood plasma/PBS_{BSA} solution). The analyte solution was flowed through the sensor flow-cell at a flow rate of 20 μL/min. Then, the solution was replaced with PBS_{Na} buffer which was flowed through the sensing area until equilibrium was reached. Reference channel functionalized with BSA was used to account for the non-specific adsorption.

Detecting hCG in blood plasma

Detection of hCG in blood plasma was performed using the sampling method and the results were compared to those obtained using the conventional referencing method.

In the sampling method, a series of blood plasma samples (50% in PBS_{BSA}) spiked with different concentrations of hCG (25, 50, 100, 250, and 500 ng/mL) were analyzed. Blood plasma sample and the washing solution were introduced into the two respective input ports of a microfluidic channel. A sequence of ten 30-s injections of blood plasma sample and washing solution was performed. The total time the blood plasma sample was in contact with the sensor surface was therefore 5 min. Each concentration was measured using dedicated sensing and reference channels.

The hCG-spiked blood plasma samples were also analyzed using the conventional referencing method. In these experiments, PBS was used as the running buffer. Blood plasma samples (50% diluted in PBS) spiked with different concentrations of hCG (75, 100, 150, 250, and 500 ng/mL) were pumped through the sensing and reference channels for 5 min and then replaced with the running buffer. After flowing the running buffer through the flow-cell for 6 min, PBS_{Na} was injected and let flow for 3 min to remove the non-specifically adsorbed proteins. Finally, PBS buffer was injected again.

Data analysis

In all experiments reported herein, sensor response measured in the reference channel was subtracted from the sensor response obtained in the sensing channel to produce a reference-compensated sensor response. The reference-compensated sensor response corresponding to a given concentration of analyte was quantified using two different methods. For the conventional referencing method, the sensor response was calculated as a difference between the sensing and reference channels in the running buffer after the washing step. On the other hand, for the sampling method, the sensor output was calculated as the initial binding rate determined by applying linear regression to the initial linear portion of the hCG binding curve. In order to assess reproducibility of the sensor, data obtained from at least three independent measurements for each concentration using a new chip for each measurement were used. First, the relative error (standard

deviation of the sensor output divided by its mean value) was expressed as percentage. Then, the reproducibility was calculated by subtracting the relative error from 100%. The statistical operations were performed using the commercial data analysis software Origin 8.1 (OriginLab Corporation, USA).

Results and discussion

Monitoring interaction between hCG and anti-hCG in blood plasma

The temporal sensor response of the sensing and reference channels is shown in Fig. 3. Clearly, the injection of blood plasma sample results in high response in both the sensing and reference channels. Upon switching to the washing solution, the change in the bulk refractive index of solution and desorption of non-specifically absorbed proteins causes a quick change in the sensor response which comes to a stable value within 10 s (in both sensing and reference channels). Desorption of specifically bound hCG to anti-hCG (in the sensing channel) is much slower compared to the change in refractive index and desorption of non-specifically adsorbed proteins. The dissociation rates of the hCG/anti-hCG complex were measured in PBS_{BSA} buffer for four different concentrations of hCG (500, 250, 100, and 50 nM) and the following dissociation rates were obtained: $(1.3 \pm 0.1) \times 10^{-3} s^{-1}$, $(1.2 \pm 0.2) \times 10^{-3} s^{-1}$, $(1.0 \pm 0.2) \times 10^{-3} s^{-1}$, and $(1.4 \pm 0.8) \times 10^{-3} s^{-1}$ (see Electronic Supplementary Material). These rates are rather close and imply that the decrease in the specific sensor response during the 30-s washing period is between 3% and 4%. Therefore, the sensor response measured at the end of each washing step (denoted with red circles in Fig. 3) differs from the correct value corresponding to hCG–anti-hCG system without washing step by 4% or less. This value is comparable with the typical error in SPR-biosensor-based measurements.

Figure 4 shows the real-time sensor response to hCG binding obtained using the sampling method and the conventional referencing method in blood plasma and buffer. The real-time binding data obtained in blood plasma using the sampling method show a good agreement with the data measured in buffer both during the association and dissociation phases of the interaction. After 5 min of hCG binding, the sensor response reaches 0.33 and 0.35 nm for the sampling method in blood plasma and referencing method in buffer, respectively. The difference between the sampling method and the measurement in buffer is consistent with the

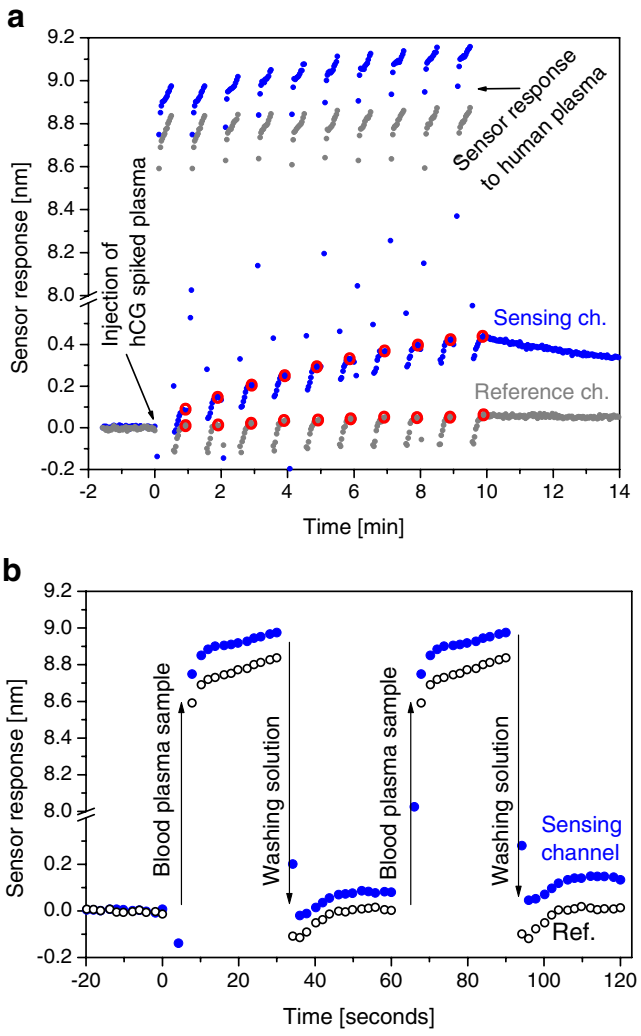

Fig. 3 Sampling method: **a** temporal sensor response to hCG in 50% blood plasma in the sensing and reference channels; hCG concentration: 250 ng/mL. **b** Detail of the first two binding/washing cycles (120 s)

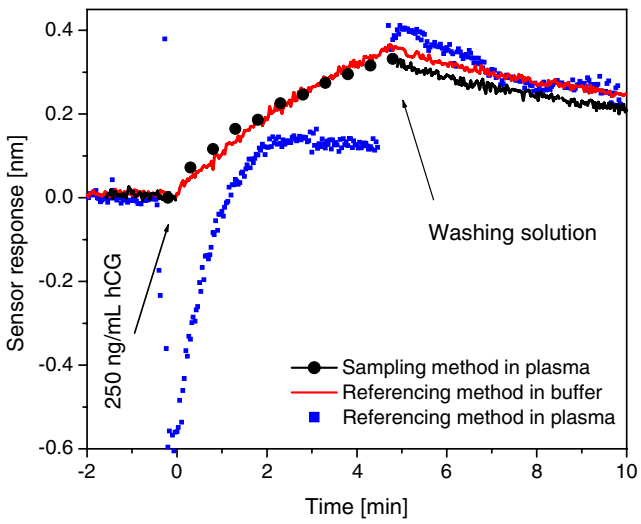

Fig. 4 Reference-compensated sensor response to hCG binding in buffer and in diluted blood plasma using the sampling method and conventional referencing method; hCG concentration: 250 ng/mL

expected 4% decrease in the sensor response due to the short dissociation steps of the sampling method. Dissociation rates determined from the dissociation phase of the sensor response - 1.4×10^{-3} s^{-1} (sampling method) and 1.6×10^{-3} s^{-1} (referencing method in buffer) - show a good agreement. The sensor response obtained using the conventional referencing method in blood plasma exhibits a more pronounced association phase (in particular, in the initial stage of the binding). We believe that this effect is in part due to differences in the non-specific binding of plasma proteins to the surfaces of sensing and reference channels. After injection of the washing solution, the sensor response returns quickly to the level of 0.41 nm, which is by 17% higher than the value obtained in buffer. The dissociation rate was determined to be 2.3×10^{-3} s^{-1}. The faster dissociation, compared to that observed in buffer, is likely to be associated with (faster) dissociation of other blood plasma protein adsorbed on the sensor surface.

Detecting hCG in blood plasma

The binding data for anti-hCG immobilized on the SPR sensor surface and hCG in blood plasma obtained using the sampling method are shown in Fig. 5. As follows from Fig. 5, the sensor response increases with the concentration of hCG. At low concentrations of hCG (50 and 100 ng/mL), the sensor response increases with time at a constant rate. At higher concentrations of hCG, the temporal sensor response departs from linear and at a concentration of hCG of 500 ng/mL, the sensor response eventually approaches an equilibrium. The equilibrium sensor response is 0.45 nm which corresponds to the surface coverage of 7.7 ng/cm^2. The sensor response observed in the reference channels during these experiments did not exceed 0.08 nm (for 5-min detection cycle), which corresponds to the surface coverage of 1.3 ng/cm^2. This level of non-specific adsorption is very low and is comparable with the amount of non-specifically adsorbed proteins on the most advanced non-fouling biointerfaces [7]. We believe that this is mainly due to the fact that the high ionic strength washing solution removes the non-specifically adsorbed molecules during each sampling cycle before they can form a solid layer enabling secondary non-specific adsorption on this layer.

Reproducibility of the sensor output increases with the concentration of hCG. For the sampling method, the reproducibility ranges from 72% (hCG concentration: 25 ng/mL) to 90% (hCG concentration: 500 ng/mL). Reproducibility of the sensor response in the conventional referencing method follows the same trend and ranges from 67% (hCG concentration: 50 ng/mL) to 79% (hCG concentration: 500 ng/mL).

Using the SPR sensor output determined for a set of different concentrations, calibration curves were established for both the sampling method and the conventional referencing method. In the sampling method, the sensor output (the initial binding rate) was determined from the initial linear portion of reference-compensated sensor response (Fig. 5). The time interval used in the regression varied from 1.5 min for 500 ng/mL to 5 min for 25 ng/mL. The sensor output in the referencing method was determined as a difference between the sensing and reference channels in the running buffer after the washing step. The resulting calibration curves are presented in Fig. 6. The binding rate obtained using the sampling method is directly proportional to the analyte concentration across the whole considered range of concentrations. Binding rates measured for the blank sample (50% plasma not spiked with hCG) in

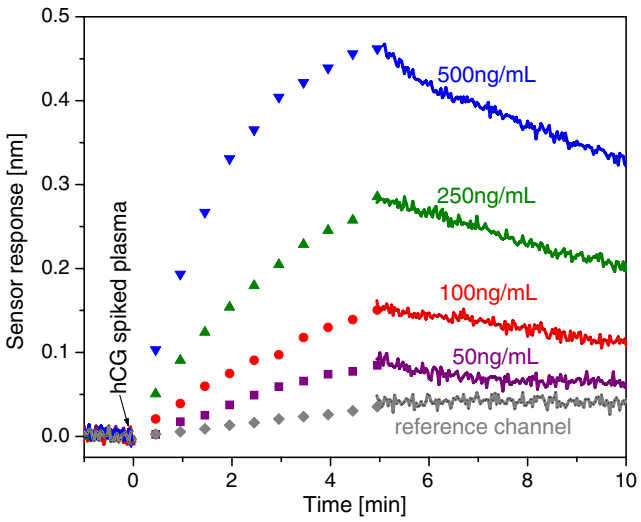

Fig. 5 The binding kinetics measured using the sampling method in spiked blood plasma samples for different hCG concentrations (50–500 ng/mL)

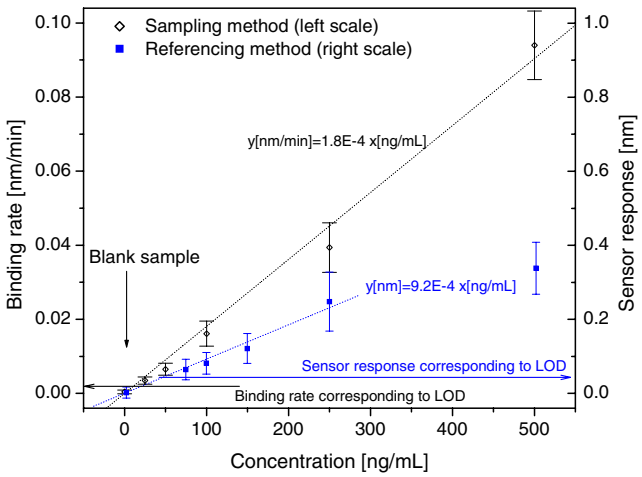

Fig. 6 Calibration curves for hCG detected in blood plasma using the sampling method (*black*, *open symbols*) and the conventional referencing method (*blue-filled squares*, *right axis*). *Dashed lines* correspond to linear fit through zero (weighted, parameter of the linear fit provided in the figure, correlation coefficients: $r_{sampling}$=0.995, $r_{referencing}$ = 0.985). The sensor outputs corresponding to LOD for the sampling and conventional referencing methods are denoted with horizontal lines

the sensing and reference channels were $(6.8 \pm 0.1) \times 10^{-3}$ and $(6.4 \pm 0.5) \times 10^{-3}$nm $\times$ min^{-1}, respectively. The sensor response obtained using the conventional referencing method is proportional to concentrations; however, at higher hCG concentrations (500 ng/mL) the sensor response levels off. Sensor responses measured for the blank sample were (0.10±0.02) and (0.10±0.01) nm for sensing and reference channel, respectively. The limit of detection (LOD) was calculated from the calibration curves as the concentration of hCG which corresponds to the sensor output equal to three standard deviations of the sensor output for a blank sample. This corresponds to LOD of 10 ng/mL for the sampling method and 50 ng/mL for the conventional referencing method. It should, however, be noted, that LOD serves here mainly for comparison of the two approaches and that the lowest concentrations at which a quantitative measurement of hCG can be done are higher (approximately three times higher than the respective LOD).

Conclusions

A novel approach to biosensor-based observation of biomolecular interactions is reported which enables real-time monitoring of biomolecular interactions in complex media. Using the interaction between hCG and the respective antibody in 50% blood plasma as a model system, we demonstrate that this approach allows reconstruction of the real-time binding data and rapid detection of biomolecular analytes in complex samples. It is demonstrated that the ability to access the real-time binding data makes it possible to improve detection performance by a factor of five. This enhancement is comparable with those achieved previously by the use of complex assays employing secondary and tertiary antibodies. The experiments demonstrate that biomolecular analytes occurring at concentrations as low as 10 ng/mL can be directly and specifically detected in blood plasma in 10 min. The speed of analysis and sensitivity may be of particular interest in point-of-care applications. Furthermore, the possibility of obtaining real-time binding data for molecules occurring in their native environments may be attractive feature in molecular biology and biomedicine. This method can be readily adapted to other types of sensors including interferometric sensors, fluorescence-based sensors, QCM, etc.

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References

1. Homola J (2008) Chem Rev 108:462
2. Fan XD, White IM, Shopoua SI, Zhu HY, Suter JD, Sun YZ (2008) Anal Chim Acta 620:8
3. Wu J, Fu ZF, Yan F, Ju HX (2007) TrAC, Trends Anal Chem 26:679
4. Cooper MA (2003) Curr Opin Pharmacol 3:557
5. Baneres-Roquet F, Gualtieri M, Villain-Guillot P, Pugniere M, Leonetti JP (2009) Antimicrob Agents Chemother 53:1528
6. Bidlingmaier M, Strasburger CJ (2007) Pituitary 10:115
7. Vaisocherova H, Yang W, Zhang Z, Cao ZQ, Cheng G, Piliarik M, Homola J, Jiang SY (2008) Anal Chem 80:7894
8. Kikuchi Y, Uno S, Nanami M, Yoshimura Y, Iida S, Fukushima N, Tsuchiya M (2005) J Biosci Bioeng 100:311
9. Nagel T, Gajovic-Eicheimann N, Tobisch S, Schulte-Spechtel U, Bier FF (2008) Clin Chim Acta 394:110
10. Vaisocherova H, Zhang Z, Yang W, Cao ZQ, Cheng G, Taylor AD, Piliarik M, Homola J, Jiang SY (2009) Biosens Bioelectron 24:1924
11. Wei JY, Mu Y, Song DQ, Fang XX, Liu X, Bu LS, Zhang HQ, Zhang GZ, Ding JH, Wang WZ, Jin QH, Luo GM (2003) Anal Biochem 321:209
12. Huang L, Reekmans G, Saerens D, Friedt JM, Frederix F, Francis L, Muyldermans S, Campitelli A, Van Hoof C (2005) Biosens Bioelectron 21:483
13. Schneider BH, Dickinson EL, Vach MD, Hoijer JV, Howard LV (2000) Biosens Bioelectron 15:597
14. Teramura Y, Iwata H (2007) Anal Biochem 365:201
15. Suwansa-ard S, Kanatharana P, Asawatreratanakul P, Wongkittisuksa B, Limsakul C, Thavarungkul P (2009) Biosens Bioelectron 24:3436
16. Vaisocherova H, Zitova A, Lachmanova M, Stepanek J, Kralikova S, Liboska R, Rejman D, Rosenberg I, Homola J (2006) Biopolymers 82:394
17. Vaisocherova H, Mrkvova K, Piliarik M, Jinoch P, Steinbachova M, Homola J (2007) Biosens Bioelectron 22:1020
18. Springer T, Piliarik M, Homola J (2010) Sens Actuators, B 145:588