Biosensors and Bioelectronics 90 (2017) 91–95

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Self-tuning interfacial architecture for Estradiol detection by surface plasmon resonance biosensor

Praskoviya Boltovets[a], Svitlana Shinkaruk[b,c], Luc Vellutini[b,d], Borys Snopok[a,*]

[a] V. Lashkaryov Institute of Semiconductor Physics, NAS Ukraine, 41 Prospect Nauki, Kyiv 03028, Ukraine
[b] University Bordeaux, UMR 5255, ISM, F-33000 Bordeaux, France
[c] INSERM, U862, Neurocentre Magendie, F-33000 Bordeaux, France
[d] CNRS, UMR 5255, ISM, F-33405 Talence, France

ARTICLE INFO

Keywords:
Biosensor
Surface plasmon resonance
Electrostatic levitation
Estradiol
Stretched exponential function
Complex systems

ABSTRACT

This study reports the operation principles for reusable SPR biosensors utilizing nanoscale-specific electrostatic levitation phenomena in their sensitive layer design. Functional macromolecular building blocks localized near the "charged" surface by a variety of weak electrostatic interactions create a flexible and structurally variable architecture. A proof-of-concept is demonstrated by an immunospecific detection of 17β-Estradiol (E2) following the competitive inhibition format. The sensing interfacial architecture is based on the BSA-E2 conjugate within the BSA matrix immobilized on the "charged" (as a result of guanidine thiocyanate treatment) gold surface at pH 5.0. Kinetic analysis for different E2 concentrations shows that using parameter β of the stretched exponential function $\sim(1-\exp(-(t/\tau)^{\beta})$ as an analyte-specific response measure allows one to substantially decrease the low detection limit (down to 10^{-3} ng/ml) and increase the dynamic range (10^{-3}–10^{3} ng/ml) of the SPR biosensor. Finally, it's concluded that the created interfacial architecture is a typical complex system, where SPR response is formed by the stochastic interactions within the whole variety of processes in the system. The E2 addition destroys the uniformity of the reaction space (where an interaction of the antibody (Ab) and the analog of E2 in the self-tuneable matrix takes place) by the redistribution of the immunospecific complexes $Ab(E2)_x$ (x=0, 1, 2) dependent on E2 concentration. Binding dynamics changes are reflected in the values of β which summarize in compact form all "hidden" information specific for the evolving distributed interfacial system.

1. Introduction

"Fluidity" is an inherent property of biological membranes, the most important interfaces in animate nature. Therefore, the desired sensing architecture of artificial membranes must have the properties of at least flexibility and variability in order to successfully implement the natural recognition ability of biomolecules. This aspect is particularly important for transducer-based biosensors (e.g. SPR, QCM, SAW etc.) owing to the complex interplay between the affinity reactions that give rise to the signal and the association between the biological macromolecules and the solid support (Snopok, 2012; Wittliff et al., 2008; Ramsden, 1997). Indeed, apart from (i) retaining the biological activity of the molecular biosystems under conditions different from their natural environment, the interfacial design must (ii) guarantee the accessibility of its recognition sites to the target analyte and (iii) diminish the nonspecific binding of other molecules (Mitchell et al., 2006; Patching, 2014). In addition, (iv) functional units must be located as close as possible (nm range) to the surface for sensitive enough signal readout (Snopok, 2012). To achieve that interfacial architecture must ensure some freedom for functional elements aboard. Although there is an enormous interest to achieve (i)–(iv), no complete approach exists for both fabrication and practical application of complex interfacial architectures mimicking native membrane structures.

One of the possible ways to create a "soft" sensitive architecture is by using an electrostatic immobilization approach utilizing globular biological molecules as upgradable building blocks. The approach is based on the possibility to control the position and/or orientation of the macromolecules near the "charged" (via chemical treatment etc.) surface by tuning their Coulomb shell (via pH changing etc.) (Boltovets et al., 2001a, 2001b). As a result of the displacement reaction, a spatially extended poly-ionic macromolecule replaces small counterions (Hirsh et al., 2013) and finally becomes localized near the surface by a number of weak electrostatic interactions (nanoscale specific

* Corresponding author.
E-mail address: snopok@isp.kiev.ua (B. Snopok).

http://dx.doi.org/10.1016/j.bios.2016.11.017
Received 5 September 2016; Received in revised form 31 October 2016; Accepted 3 November 2016
Available online 12 November 2016

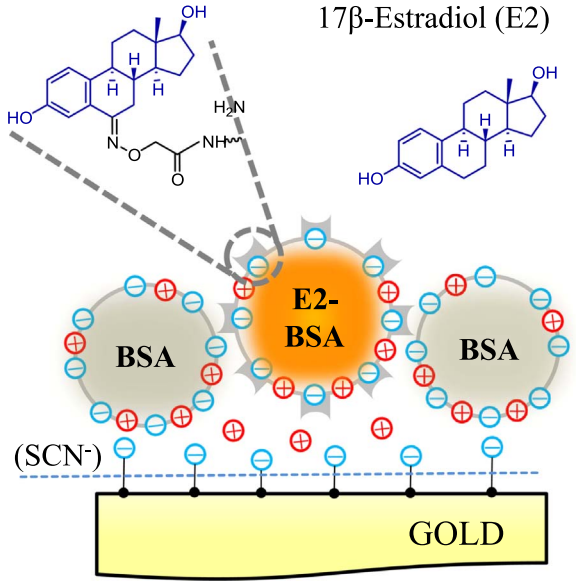

Fig. 1. Schematic representation illustrating the interfacial protein architecture based on the E2-BSA conjugate within the BSA matrix utilizing the electrostatic levitation phenomenon for forming a "fluid"-like interfacial membrane with rotation freedom and in-plane mobility of membrane components.

electrostatic levitation phenomenon, ns-ELP) (Boltovets et al., 2002, 2013a,b). However, no experiments have been reported to date in which fully miscible functional (e.g. BSA conjugate) and inert (e.g. BSA) macromolecular building blocks (exploiting the ns-ELP) introduce "fluidity" into the functional interfacial architecture (Fig. 1). Such studies may have a considerable impact on the further development of the electrostatic immobilization approach for forming a monolayer-like adaptive sensing architecture on the transducer surface. To explore this possibility, it is necessary to show how the interfacial design and peculiarities of interfacial processes are manifested in the sensor response. The present work is focused on these issues of ns-ELP based self-tuning sensing architectures.

In the present study 17β-Estradiol (E2) (Fig. 1) was chosen as a model analyte (Makin and Gower, 2010). A natural estrogenic hormone occurs at levels in the range from pg/ml to ng/ml in blood and urine depending on the phase of menstrual cycle of adult females (Fontana, 2014; Wang et al., 2001; Xin et al., 2010; Wirth et al., 2007). E2 is essential for the development and maintenance of female reproductive tissues, as well as for male reproduction (Alomary et al., 2001; Pentikäinen et al., 2000). It has neurotrophic and neuroprotective properties (Behl et al., 1995; Belelli and Lambert, 2005) as well as important functions in many other tissues including bone (Ecarani et al., 1997), liver (Tian et al., 2012) or blood vessels (Collins et al., 1995).

Because of low E2 weight and the fluctuation of external factors (temperature etc.), the analyte itself cannot generate a response with a reasonable signal to noise ratio in optical biosensors, in particular the ones based on Surface Plasmon Resonance phenomena (SPR) (Supplementary materials). Therefore, for the quantitative detection of E2 usually a competitive format with specific antibodies or Estradiol receptors as selective enhancer is used (Zhang et al., 2013; Miyashita et al., 2005; Supplementary materials). In the present work we used a competitive inhibition format with monoclonal antibodies as the macromolecular competitor-enhancer and E2-BSA conjugate as the interfacial building block with an analog of target steroid on its surface (Fig. 1). Taking into account the mechanism of SPR phenomenon (Snopok, 2012), this procedure results in the formal amplification of the signal that is proportional to the ratio of antibody and analyte molecular weights (c.a. 150 kDa/272 Da~550 in the case of monodentate binding).

2. Materials and methods

2.1. Materials

Chemical (DMSO, guanidine thiocyanate (rhodanide) $NH_2C(=NH)NH_2.HSCN$, HCl (18%), H_2O_2 (30%), acetonitrile) and biochemical (17β-Estradiol (E2), E2-6-(O-carboxymethyl)oxime-BSA conjugate (E2-BSA), BSA) reagents were purchased from Sigma-Aldrich (l'Isle d'Abeau, France), Mouse monoclonal anti-Estradiol antibody was obtained from Meridian LifeScience (Memphis, TN, USA). All buffers (PBS (pH 6.7), citrate buffer (CB, pH 5.0/ pH 4.0), acetonitrile-NaOH (ACN-NaOH regeneration solution, the 10% solution of the acetonitrile in 50 mM NaOH) were prepared according to the standard procedures using bidistilled water.

2.2. Sample preparation

Because of the low solubility of E2 in water, pure DMSO was used for stock solution preparation. To exclude the difference in DMSO concentrations, all final solutions and buffers (CB-DMSO, PBS-DMSO) contained 0.1% DMSO.

E2 stock solution was prepared with a 1 μg/ml concentration. E2-BSA conjugate was dissolved in PBS with a 1 mg/ml concentration, aliquoted and stored at −20 °C. Working concentration was 50 μg/ml. An antibody working solution (5 μg/ml) was prepared from stock solution immediately before measurements. An incubation of the mix of the antibodies and E2 was executed at the room temperature during 40 min.

2.3. Instrumentation

Scanning spectrometer "BioHelper" (ISP NASU, Kiyv, Ukraine) was used for the SPR measurements with standard chips (50 nm Au/ 1.5 nm Cr/Glass (n=1.61)) (Snopok et al., 2006a,b,c). The measurements were performed in the static mode without sample flow; the volume of an open cell was 400 μl.

2.4. Data processing algorithms

SPR kinetics were analyzed with a model that takes heterogeneous processes on the surface into account using a stretched exponential function (Snopok, 2014; Boltovets et al., 2013b; Snopok and Kruglenko, 2005):

$$\Gamma(t) = \Gamma_{max}\cdot\left(1 - \exp\left(-\left(\frac{t}{\tau}\right)^{\beta}\right)\right) \quad (1)$$

where Γ_{max} is the saturation level of the response, τ is the time constant, and β is the parameter that indicates the mechanism of surface layer evolution.

The dependencies of the SPR response Γ versus analyte concentration C were approximated using the Morgan–Mercer–Flodin equation or the logistic curve (Snopok et al., 2006b):

$$\Gamma = \frac{const\cdot[Ab]}{1 + ([C]/[M]\cdot const)^{p}} \quad (2)$$

const is the instrument specific parameter, [Ab] is the concentration of macromolecular competitor (antibody in our case), [C] and [M] are the volume concentration of the analyte (E2) in the solution and the surface concentration of its immobilized analog on the surface respectively. Power *p* represents the order of the reaction in the analyte – an effective value indicating the stechiometry of the processes occurring in the system.

To approximate the kinetic and standard curves, the nonlinear regression analysis procedures of OriginPro 7.5 (OriginLab Corporation) were used.