analytical chemistry

Letter

pubs.acs.org/ac

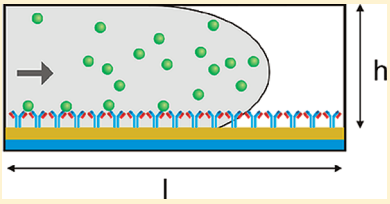
Analytical Value of Detecting an Individual Molecular Binding Event: The Case of the Surface Plasmon Resonance Biosensor

Hana Šípová, David Vrba, and Jiří Homola*

Institute of Photonics and Electronics, Academy of Sciences of the Czech Republic, Chaberská 57, 182 51 Prague, Czech Republic

Supporting Information

ABSTRACT: We explore the ultimate limits of the performance of bioanalytical approaches based on the detection of individual molecular binding events taking place at the sensor surface interfaced with a microfluidic flow-through cell. As a case study, we investigate and compare the bioanalytical performance of flow-through surface plasmon resonance (SPR) sensors based on (1) localized surface plasmons (LSP) which detect a single binding event and (2) propagating surface plasmons (PSP) which integrate a great number of simultaneously occurring binding events. We demonstrate that for the biomolecular interactions most relevant to biosensing the single-binding-event LSP approach is inferior to the integrating PSP approach. We estimate that the number of biorecognition elements available to interact with the analyte molecules would need to be, depending on the size of the analyte and parameters of the molecular interaction, in the order of 10 to 10^3 to increase the probability of the positive response of the LSP-based sensor to that of the PSP-based sensor.



The need for technologies for rapid and sensitive detection of chemical and biological species exists in numerous fields (e.g., medical diagnostics, environment monitoring, food safety, security) and has been the major driving force of biosensor research and development. In the past decade, advances in nanotechnologies have given rise to highly miniaturized biosensors (down to individual nanoparticles) capable of probing molecular events at the micro/nanoscale.[1,2] Several works have reported detection of individual binding/dissociation events,[3,4] thus opening avenues to new bioanalytical modalities. However, as the miniaturization of biosensors reaches the submicrometer scale, not only the technological boundaries but also intrinsic factors, such as the nature of the molecular interaction and the transport of the analyte to the sensing area, may become factors limiting analytical performance of the biosensors. In affinity biosensors, the interaction between the biorecognition elements immobilized on the surface of the sensor and target analytes in a liquid sample can take place in a variety of formats. The most common formats for biosensor-based analysis of macroscopic samples incorporate flow-through (micro)fluidic cells or cuvettes interfaced with a biosensor.[5] The transport of the analyte to the sensor plays an important role and has been investigated with respect to the geometry and flow conditions in micro and nanoscale sensors.[6,7]

In this work we explore the potential value of detecting single-molecule binding events for bioanalytical applications. We analyze and compare the bioanalytical capabilities of flow-through surface plasmon resonance (SPR) sensors based on (1) localized surface plasmon (LSP) registering a single binding event and (2) propagating surface plasmon (PSP) producing the sensor response by integrating the signal from a great number of molecular interactions distributed over an extended sensing surface. These configurations are investigated by simulating molecular interactions between the analyte molecules contained in a solution flowing in a microfluidic channel of a flow-through cell and biorecognition elements immobilized on the sensing surface at the bottom of the microfluidic channel. The effects of the size of the analyte and the interaction properties of the molecules involved are also studied.

APPROACH

We investigate molecular interactions occurring between analyte molecules contained in a solution flowing in a microfluidic channel and biorecognition elements on one (sensing) wall of the channel in two different formats: (a) a single biorecognition element placed at the input of the fluidic channel (Figure 1A) and (b) biorecognition elements distributed along the sensing surface (Figure 1B).

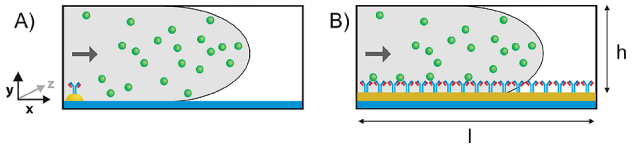


Figure 1. Molecular interactions in microfluidic channels incorporating (A) an individual biorecognition element at the input of the fluidic channel and (B) biorecognition elements distributed homogeneously along the sensing channel.

The flow-channel is assumed to have a rectangular cross section (length l, height h, and width w); as $w \gg h$, variations in concentrations across the width of the microfluidic channel are

Received: October 19, 2011
Accepted: December 6, 2011
Published: December 6, 2011

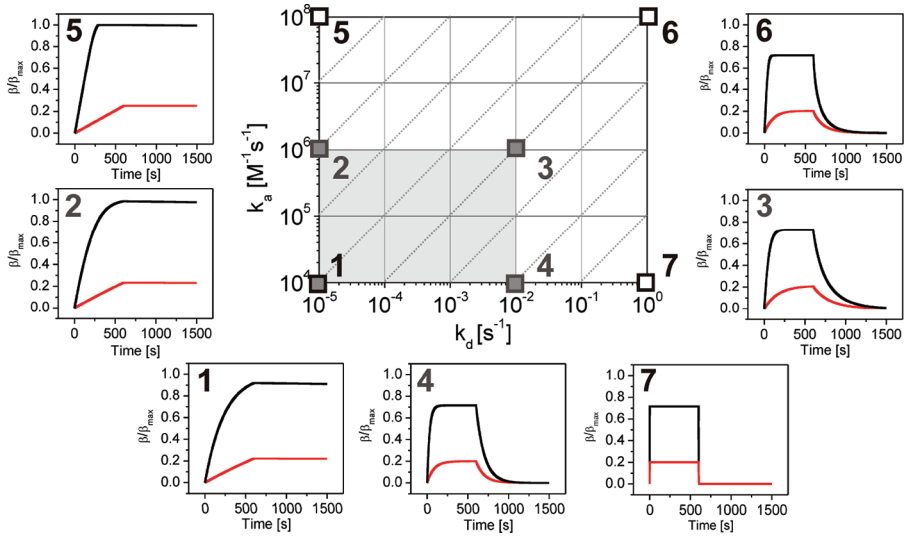


Figure 2. Center: The k_a−k_d space of biomolecular interactions; the biomolecular interactions used in SPR bioanalytical sensors are typically within the shaded area. Left, down, and right: Model temporal sensor responses calculated for the seven selected model interactions and two different concentrations of analyte equal to LOQ_{PSP} (Table 1) times 100 (black curve) and 1000 (red curve), respectively. Microfluidic channel dimensions: h = 0.05 mm, l = 4 mm. Flow velocity: v_{max} = 10 mm s^{-1}; analyte was flowed along the sensing surface from t = 0 s to t = 600 s.

neglected. Furthermore, the flow is assumed to be laminar along the whole length of the fluidic channel and have the maximal flow velocity v_{max}.

The interaction between analyte and biorecognition elements distributed along the length of the microfluidic channel is described by the kinetic equation characterizing the molecular interaction itself and the diffusion equation describing the transport of analyte molecules to the sensing surface. For the considered geometry and the simple 1:1 interaction model, the diffusion equation and kinetic equation can be written as follows:

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2}\right) - 4v_{max}\frac{y}{h}\left(1 - \frac{y}{h}\right)\frac{\partial C}{\partial x} \quad (1)$$

$$\frac{d\beta}{dt} = k_a C_{y=0}(\beta_{max} - \beta) - k_d\beta \quad (2)$$

where C is the concentration of analyte, D is the diffusion coefficient, k_a and k_d are the association and dissociation rates, β is the surface concentration of the complexes, β_{max} is the surface concentration of the biorecognition elements, and t is time.[8]

In order to estimate the ultimate analytical potential of the detection approaches based on registering individual binding events in a flow-through format, we will assume an LSP-sensor carrying only a single biorecognition element located at the beginning of the microfluidic channel (Figure 1A), which is capable of registering a binding event regardless of how long the complex has existed. The probability $p(t)$ that the LSP-sensor has registered a binding event can be expressed as follows

$$\frac{dp}{dt} = k_a C_{x,y=0}(1 - p) \quad (3)$$

(for the derivation of the equation, see the Supporting Information). To solve the system of differential eqs 1 and 2 or eqs 1 and 3 with Neumann and Dirichklet boundary conditions, we used the method of finite elements on a two-dimensional space. The time evolution of both the diffusion and the kinetic equations was modeled in parallel using the explicit Euler method.

The most important biomolecular interactions used in SPR biosensing (DNA hybridization, antibody−antigen interactions, aptamer−ligand interactions, etc.) are typically located in the $k_a - k_d$ space marked by four combinations of k_a and k_d values: (1) $k_a = 10^4$ M^{-1} s^{-1}, $k_d = 10^{-5}$ s^{-1}, (2) $k_a = 10^6$ M^{-1} s^{-1}, $k_d = 10^{-5}$ s^{-1}, (3) $k_a = 10^6$ M^{-1} s^{-1}, $k_d = 10^{-2}$ s^{-1}, and (4) $k_a = 10^4$ M^{-1} s^{-1}, $k_d = 10^{-2}$ s^{-1}. This area is highlighted in gray in Figure 2 (center), which shows its position with respect to the majority of biomolecular interactions.[9]

In order to compare the detection performance of the LSP and PSP-based biosensing approaches, we calculated the probability of detection of the analyte using the LSP-based sensor for the time required for the PSP-based sensor to detect the analyte with a probability of >99.73% (i.e., the probability of false negative <0.3%). In bioanalytical sensors, this level of probability is typically associated with the limit of quantification (LOQ), which is defined as a concentration which corresponds to the sensor response equal to 10 standard deviations of the sensor response obtained for a blank sample.[10] The LOQ of a model PSP-based sensor was determined as the concentration of analyte producing the surface coverage of 1 pg mm^{-2}. This value was calculated as 10 times 0.1 pg mm^{-2} that is the minimum detectable surface coverage achieved by several research as well as commercial high-performance PSP-based sensors.[11]

RESULTS AND DISCUSSION

Figure 2 (left, down, and right) shows the kinetic curves obtained for the PSP-based sensor and selected model interactions, assuming a surface concentration of receptors of 5×10^{12} molecules cm^{-2} and the diffusion coefficient of 5×10^{-4} mm^2 s^{-1} (which corresponds to the size of the analyte of approximately 50 kDa). For each type of interaction, the kinetic curves were calculated for two different concentrations of analyte. For molecular interactions 1−7, they were 424 nM, 9.1 nM, 27 nM, 2.56 μM, 6.0 nM, 25.4 nM, 251 mM (black curves) and concentrations 10 times lower (red curves), respectively.

dx.doi.org/10.1021/ac202774k | Anal. Chem. 2012, 84, 30−33

Table 1 shows the values of LOQ calculated assuming a detection time of 10 min. The LOQs obtained for the larger analyte are typically 10 times lower than those for the smaller molecule as expected. The main exception from this trend is the case of the interaction with $k_a = 1 \times 10^8$ M^{-1} s^{-1} and $k_d = 1 \times 10^{-5}$ s^{-1}, for which the LOQ for the larger molecule is only 2.2 times lower than that for the small molecule. This effect is caused by the transport limitation of the interaction, which becomes more pronounced for a larger analyte and high association rate.

Table 1. Parameters of the Model Interactions, The Estimated Limit of Quantification of the PSP-Based Sensor (LOQ_{PSP}), the Probability of a Positive Signal from the LSP-Based Sensor (P_{LSP}), and the Number of Biorecognition Elements Required to Achieve the Probability of Detection of 99.73% ($NBE_{0.9973}$)

interaction no.	k_a [M^{-1} s^{-1}]	k_d [s^{-1}]	LOQ_{PSP} [nM]	P_{LSP} [%]	$NBE_{0.9973}$
Small Analyte (MW ~ 5 kDa, $D = 50 \times 10^{-5}$ mm^2 s^{-1})					
1	1×10^4	1×10^{-5}	4.2	2.5	234
2	1×10^6	1×10^{-5}	0.05	3.2	185
3	1×10^6	1×10^{-2}	0.26	14.3	38
4	1×10^4	1×10^{-2}	25.7	14.3	38
5	1×10^8	1×10^{-5}	0.013	52.8	8
6	1×10^8	1	0.26	100	1
7	1×10^4	1	2560	100	1
Medium-Size Analyte (MW ~ 50 kDa, $D = 5 \times 10^{-5}$ mm^2 s^{-1})					
1	1×10^4	1×10^{-5}	0.42	0.25	2363
2	1×10^6	1×10^{-5}	0.009	0.54	1090
3	1×10^6	1×10^{-2}	0.027	1.60	369
4	1×10^4	1×10^{-2}	2.5	1.50	394
5	1×10^8	1×10^{-5}	0.006	30.1	17
6	1×10^8	1	0.025	78.2	4
7	1×10^4	1	251	77.8	4

Table 1 presents the probability of detection of the analyte using the LSP approach and the estimated number of biorecognition elements that would be needed in order to increase the probability of the LSP-based sensor to the value of 99.73%, while keeping the detection time of 10 min. The concentrations used in these calculations were equal to LOQ_{PSP} (Table 1). The results suggest that the probability of detection of the analyte using the LSP approach depends strongly on the size of the analyte and the association and dissociation rates. Clearly, for the biomolecular interactions most relevant to SPR biosensing (shaded area in Table 1), the probability of obtaining a positive signal from the LSP-based sensor within 10 mins is less than 15% and 2% for small and medium-size analytes, respectively. The number of biorecognition elements required to achieve the probability of detection equal to that of the PSP-based sensor (99.73%), $NBE_{0.9973}$, was calculated for each model interaction (Table 1, see the Supporting Information for details of the calculation). The simulations imply that $NBE_{0.9973}$ would range from several tens to several hundreds for small analytes and would be even an order of magnitude higher for medium-size analytes. However, it should be noted that these values were determined assuming that the interactions proceed independently on each biorecognition element and that the mass transport is not limiting the interaction rate at this (low) number of biorecognition elements. It should also be stressed that this study assumes the best possible scenario for the LSP approach. A typical plasmonic nanoparticle can only host a limited number of biorecognition elements and even fewer are located in the hot-spot areas in which individual binding events may be detected. The performance comparable with that provided by the PSP approach can only be achieved for interactions with an extremely high dissociation rate constant ($k_d = 1$ s^{-1}) and small analytes (~5 kDa).

This analysis suggests that in a flow-through detection format and in terms of the lowest detectable concentrations, nanoplasmonic (LSP) biosensors provide an inferior performance compared to that of PSP-based plasmonic sensors. The sensitivity of the LSP-based nanobiosensor is outweighed by the low analyte flux to the nanobiosensor, which at low analyte concentrations substantially reduces the overall probability of the binding. It should be noted that applications where small numbers of molecular targets in microscopic samples are to be analyzed under no flow (e.g., detection of the mRNA expression of a single cell) lie outside the scope of the presented analysis; however even in these formats, analyte transport limitations need to be considered.[6]

CONCLUSIONS

The reduction of the size of the biosensor down to a single nanoparticle capable of detecting individual binding events has been perceived as an important step toward new biosensor technologies with unsurpassed sensitivity and multiplexing capabilities. We compared the performance of nanoplasmonic (LSP) and macroscopic (PSP) biosensors in a flow-through format and concluded that for the molecular interactions most relevant to biosensing, localized biosensing using a single nanoparticle is strongly disfavored by the low probability of binding due to the low number of biorecognition elements available. Therefore, in order to achieve the detection performance of the biosensors based on integrating a large number of binding events across a larger surface area, the nanoparticle-based sensors would require the simultaneous employment of multiple nanoparticles or very long detection times. Although the quantitative results were obtained for the case of SPR biosensors using localized and propagating surface plasmons, the trends are expected to hold for other types of affinity biosensors which are based on recording individual binding events in flow-through detection formats.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Phone: +420 266 773 448. Fax: +420 284 681 534. E-mail: homola@ufe.cz. URL: http://www.ufe.cz.

ACKNOWLEDGMENTS

This research was supported by the Academy of Sciences of the Czech Republic by Praemium Academiae and under the Contract KAN200670701 and by the Ministry of Education, Youth and Sports under Contracts OC09058 and LH11102.

REFERENCES

(1) Cui, Y.; Wei, Q. Q.; Park, H. K.; Lieber, C. M. *Science* **2001**, *293*, 1289−1292.
(2) Fritz, J.; Baller, M. K.; Lang, H. P.; Rothuizen, H.; Vettiger, P.; Meyer, E.; Guntherodt, H. J.; Gimzewski, J. K.; Gerber, C. *Science* **2000**, *288*, 316−318.
(3) Mayer, K. M.; Hao, F.; Lee, S.; Nordlander, P.; Hafner, J. H. *Nanotechnology* **2010**, 21.
(4) Sannomiya, T.; Hafner, C.; Voros, J. *Nano Lett.* **2008**, *8*, 3450−3455.
(5) Ward, L. D.; Winzor, D. J. *Anal. Biochem.* **2000**, *285*, 179−193.
(6) Sheehan, P. E.; Whitman, L. J. *Nano Lett.* **2005**, *5*, 803−807.
(7) Squires, T. M.; Messinger, R. J.; Manalis, S. R. *Nat. Biotechnol.* **2008**, *26*, 417−426.
(8) Homola, J., Ed.; Springer: Berlin, Heidelberg, Germany, 2006; pp 69−91.
(9) Onell, A.; Andersson, K. *J. Mol. Recognit.* **2005**, *18*, 307−317.
(10) Armbruster, D. A.; Pry, T. *Clin. Biochem. Rev.* **2008**, *29* (Suppl 1), S49−52.
(11) Homola, J. *Chem. Rev.* **2008**, *108*, 462−493.