Fluctuations of the number of adsorbed molecules in biosensors due to stochastic adsorption–desorption processes coupled with mass transfer

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We derived a simple theory of fluctuations of the equilibrium number of adsorbed molecules in biosensors, caused by the stochastic nature of adsorption–desorption (AD) processes coupled with mass transfer. The two-compartment model is used for approximation of the spatial dependence of analyte concentration in the reaction chamber, which is justified when a thin layer depleted of the analyte exists close to the surface on which the binding reaction occurs. By using the obtained analytical expression for the power spectral density of fluctuations we perform for the first time the quantitative analysis of the influence of the mass transfer on the fluctuations spectrum. For realistic parameter values, the influence of mass transfer proved to be significant, causing the increase in the fluctuations level of up to two orders of magnitude compared to the rapid mixing case. The dependences of the mass transfer influenced fluctuations spectrum on various parameters of the analyte–receptor binding process are also systematically investigated. The presented theoretical model of fluctuations enables good estimation of the AD noise, which affects the total noise and the minimal detectable signal of biosensors. It provides the guidelines for improvement of the limits of detection, and for optimization of detection methods. The theory is also proposed as a basis for development of highly sensitive methods for analyte detection and characterization of biomolecular binding processes, based on the measured fluctuations spectrum. It is applicable for various types of sensors whose operation principle relies on the adsorption process of analyte molecules.

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1. Introduction

Detection and identification of biomolecules in solutions are very important tasks in medicine, environmental protection, agriculture, and homeland security. Investigation of biomolecular interactions, being the basis of various vital biochemical processes is of great significance, especially for the fundamental understanding of biology, as well as for pharmaceutical research. There is a variety of different platforms for detection of biological entities and characterization of biomolecular interactions. However, there is a need to explore novel techniques which improve the efficiency and sensitivity of detection of analyte molecules in liquids and provide additional information about biomolecular processes and their kinetics.

The principle of operation of one group of biological sensors relies on the process of affinity-based binding of analyte molecules from the solution to the specific probe molecules used for functionalization of the surface of a transducer. Binding of analyte molecules to the probe molecules results in a measurable change of one or more of the transducer’s physical (mechanical, electrical, and optical) parameters [1–6]. In the steady state, established when binding and unbinding processes of interacting molecules become balanced under given conditions, the coverage of the surface by bound molecules depends on the concentration of analyte molecules in the solution. The analyte concentration and the rate constants of the interaction processes of biomolecules are usually obtained from the steady state data and from the change of the measured parameter in time during the period of the steady state establishment [4–8]. Mass transfer (transport of the analyte molecules existing in a solution to and from the sensing surface) is an important effect that has to be considered when biomolecular binding-unbinding processes are characterized and also when decisions are made during sensor design [9–12].

Fluctuations of the number of adsorbed molecules in the steady state also contain information about the analyte, the adsorption–desorption (AD) process and its kinetics [13] (the terms binding–unbinding, adsorption–desorption and association–dissociation will be used as synonyms in this paper).

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This implies that methods based on frequency domain analysis of measured fluctuations can be developed for detection of analytes and for investigation of biomolecular interactions, as a complementary approach to conventional time domain measurements [14].

Fluctuations of the measured parameter, induced by the stochastic nature of AD processes, have traditionally been regarded as noise. Fluctuations of the number of adsorbed molecules, caused by the stochastic nature of AD processes, result in fluctuations of the sensor’s output signal, which have traditionally been regarded as noise. They are fundamental by their origin and therefore unavoidable. In devices that contain micro/nanosized functioning parts, fluctuations generated by fundamental mechanisms put a hard limit on their performance [15]. As the dimensions of the structures decrease, the influence of AD induced fluctuations increases and there is a possibility for the AD noise to start to dominate over other fundamental noises [13,16,17]. Compared to other micro/nanodevices, scaled down biological sensors are especially affected by fluctuations caused by AD processes of analyte particles (large polymer molecules, proteins, nucleic acids, cells, etc.). Therefore, with the current trend towards small-scale micro/nanofabricated biosensors, the analysis of this kind of fluctuations becomes increasingly important. Comprehensive understanding and theoretical modeling of AD induced fluctuations in biological micro/nanodevices is essential for determination of their limiting performance and for improvement of detection methods.

In general, the phenomenon of fluctuations in biosensors response belongs to the category of processes occurring in systems in which physical quantities are both space and time dependent. Fluctuations arise due to both the stochastic transition events (binding or unbinding of the interacting molecules) and the mass transfer. Therefore, the combined influence of these effects has to be considered.

The theory of fluctuations of the number of adsorbed particles for the cases where mass transfer is of negligible influence is derived in [13,18–20]. Such case is, for example, adsorption from a gaseous environment, where mass transfer is much faster than in liquids. A complex analysis of the stochastic processes in biosensors operating in liquids can be found in [21–23]. A stochastic analysis which includes mass transfer effects is considered in literature from the field of chromatographic separation-based assays [24]. However, to the best of our knowledge, a quantitative analysis of the influence of mass transfer on fluctuations spectrum of the number of adsorbed particles in the affinity-based sensors is still missing in the literature.

In this paper we analyze the fluctuations of the number of molecules adsorbed on a biosensor surface from the liquid phase. The objective of our work is the development of a simple theory which is useful for the quantitative estimation of the effects of mass transfer on the equilibrium fluctuations of the measured signal and also for investigation of biosensor performance limits. The additional motivation for the development of this theory is its application in designing of new methods for detection of biomolecules in solutions, and also for characterization of biomolecular binding processes coupled with mass transfer, using the analysis of the measured spectrum of fluctuations.

In the following sections, we derive a simple mathematical model of stochastic adsorption of analyte particles, taking into account the mass transfer process. We introduce the two-compartment model approximations, in order to obtain the equation describing the change of the number of adsorbed particles in time due to the combined effect of the intrinsic adsorption–desorption process and the mass transfer. Then, by applying the approach used for generation–recombination (GR) fluctuations of the number of charge carriers in semiconductors, which are described by an analogous stochastic equation, we obtain the analytical expression for the power spectral density of fluctuations of the number of adsorbed molecules. The numerical calculations are performed using the parameter values corresponding to realistic experimental and practical conditions in biosensors. Finally, the analysis of the results demonstrates the significance of taking into account the effects of mass transfer in biosensors operating in liquids; when fluctuations of their output signal are considered.

It is important to realize that, due to the complexity of the phenomena on the sensor surface and in the reaction chamber, it would be very difficult to establish a complete mathematical model that incorporates all the relevant physical processes. Like all approximate approaches, the theory we present in this paper is devised by introducing assumptions which limit the scope of its applicability. First, the reaction is assumed to be a simple bimolecular reversible binding reaction according to the Langmuir adsorption model. It is assumed that the functionalization of the surface is homogeneous, that there is no non-specific binding, as well as that neither analyte nor receptor particles undergo changes that can affect statistical properties of the binding process in time or over the ensemble. Also, the two-compartment model is valid for the cases of mass transfer influenced binding which leads to formation of a thin layer depleted of the analyte adjacent to the surface where adsorption process occurs.

We would like to emphasize that the fluctuations of the number of adsorbed molecules, determined by the presented theory, can be easily converted into fluctuations of a quantity (e.g. mass, resonant frequency, refraction index, etc.) that characterizes a given sensor (quartz crystal microbalance (QCM), thin film bulk acoustic resonator (FBAR), plasmonic, nanoplasmonic, etc.) whose operating principle relies on the adsorption process of analyte molecules.

2. Theoretical analysis

In the surface based biosensing techniques one type of reacting molecules (called probe molecules, capturing molecules or receptors) is immobilized on a surface of a sensing element, where they serve as the sites for binding of molecules of another reactant (i.e. the analyte). A sensing element functionalized in this way is located in a reaction chamber. When a solution containing analyte molecules is introduced in a reaction chamber, the binding reaction may occur when the analyte molecules come in the vicinity of the binding sites. We assume a simple one-to-one reaction model: the association process, whereby one analyte molecule and one receptor molecule bind to each other and create the immobilized complex, and the dissociation process, whereby this complex dissociates. The sensor response corresponds to the total number of formed analyte–receptor complexes. We also assume that the analyte particles do not interact with each other, and that all the binding sites on the sensor surface are equivalent. These assumptions are in accordance with the Langmuir adsorption model [10]. The equation describing the time evolution of the reversible binding process between analyte and receptor molecules is then

\[
\frac{d\eta_i(t,x,y)}{dt} = k_f C_i(t,x,y) (\eta_m - \eta_i(t,x,y)) - k_i \eta_i(t,x,y)
\]

(1)

where \(\eta_i(t,x,y)\) is the surface density of bound analyte molecules, \(\eta_m\) is the surface density of receptor sites (constant in time and assumed to be uniform on the surface), \(C_i(t,x,y)\) is the free analyte concentration in the immediate vicinity of the functionalized sensor surface, \(k_f\) is the association rate constant, and \(k_i\) is the dissociation rate constant (these are the intrinsic rate constants for the analyte–receptor binding–unbinding reaction). All the variables in Eq. (1) depend on an exact position on the functionalized sensor surface (i.e. on coordinates in that plane, x and y).
Besides the association–dissociation reaction described by Eq. (1), transport processes of diffusion and flow also occur in the sensor’s reaction chamber. They bring analyte molecules to, and take them away from the binding sites. While the direction of the analyte flow is along the reaction chamber (x axis direction), i.e. it is parallel with the functionalized sensor surface, the direction of the diffusion depends on the spatial distribution of concentration of the free analyte molecules. The continuity equation describes the rate of change of the spatially dependent analyte concentration \( C(t,x,y,z) \) influenced by transport processes as

\[
\frac{\partial C(t,x,y,z)}{\partial t} = -\text{div}(j(t,x,y,z))
\]

(2)

where \( j(t,x,y,z) \) is the flux vector (z-axis is perpendicular to the functionalized sensor surface). The components of \( j \) depend on the spatial distribution of the free analyte concentration, the spatial distribution of flow velocity of the solution in the reaction chamber, and the analyte diffusion coefficient. Therefore, Eq. (2) is a partial differential equation, which with appropriate initial and boundary conditions describes the change of the free analyte concentration in the reaction chamber, due to transport and AD processes. Eqs. (1) and (2) can be simultaneously solved by using numerical finite-element methods. However, since we are interested in an analytical solution for \( C(t,x,y) = C(t,x,0) \) which we can use in further analysis of the fluctuations of the number of adsorbed molecules, a simpler mathematical model is required.

There are many possible sets of transport and binding conditions in the reaction chamber, which differ depending on the geometry and dimensions of the system (reaction chamber, sensing surface), the solution flow rate, the receptor surface density, and also on the diffusivity and affinity-based binding properties of the analyte molecules. Therefore, a general analytic solution of Eqs. (1) and (2) does not exist. Instead, analytical solutions can be found with certain approximations only for some of the physically relevant cases. In microfluidic systems, two transport regimes are considered depending on the ratio of the time scale required for diffusion of analyte particles across the chamber height and the time scale for convection in the axial direction [9]. When the diffusion time scale is shorter than the convection time scale [9,10], molecules entering the chamber at an arbitrary height \( z \) are able to reach by diffusion the surface on which the binding reaction occurs. Such transport regime (which we will call type 1 transport regime) is common, for example, in shallow microfluidic channels. In other cases (the diffusion time scale greater than the convection time scale), the analyte molecules in the flow chamber that are not able to reach the reactive surface exit the sensing region, carried by the flow. Thus, only the molecules from the layer adjacent to the sensing surface can participate in the binding reaction. Higher flow rates, thicker channels and slower-diffusing analytes are in favor of such transport regime. This situation (type 2 transport regime) is typically encountered in SPR systems. It is also common in flow-through devices, such as QCM and some other surface-based sensors [9–12,25]. The following consideration and the analysis that we perform in this paper refer to type 2 transport regime.

When introducing the approximations which significantly simplify the equations to be solved, we first consider the case of the so-called rapid mixing [11]. In that case, the transport of analyte particles is assumed to be so fast compared to the binding reaction, that it leads to a rapid establishment of the uniform analyte concentration \( C_0 \) in the entire reaction chamber, which is also constant in time (\( C_0 \) is the analyte concentration in the solution entering the chamber). Then the approximation \( \frac{C_0(t,x,y)}{C_0} = C_0 \) can be used in Eq. (1). It acquires the same form as the equation used for description of reversible adsorption of particles from the gas phase [13], which can be solved analytically for \( n_0(t) \), yielding the number of bound molecules that approaches equilibrium exponentially. This corresponds to the case of reaction-limited kinetics. In other cases, in systems with type 2 transport regime, the analyte concentration near the sensing surface is spatially dependent. When the diffusion flux of analyte molecules on the reactive surface is smaller than the adsorption flux (diffusion-limited kinetics), the zone in the vicinity of the functionalized surface is depleted of the analyte due to the binding reaction, i.e. a thin mass transfer boundary layer is formed. In that case, the simplified system of equations describing the processes of analyte binding and transport can be obtained by introducing the assumptions of the two-compartment model regarding the analyte concentration dependence on spatial coordinates in the sensor’s reaction chamber.

2.1 Two-compartment model equations

The two-compartment model provides a good approximation of the mass transfer influenced binding kinetics (i.e. of the change of the number of adsorbed molecules on the sensor surface in time) in cases when a thin zone depleted of the analyte is formed close to the surface on which the binding reaction occurs. Its accuracy is experimentally confirmed [11,12,25,26]. This model uses the simplified dependence of the analyte concentration on the spatial coordinates. The reaction chamber is divided into two compartments: the inner compartment, in the immediate contact with the sensor surface, and the outer compartment, which occupies the remaining part of the chamber (Fig. 1).

The model assumes that the analyte concentration in the outer compartment is uniform in space and constant in time, and is equal to the concentration in the bulk solution, \( C_0 \) (the short time interval at the beginning of analyte injection, when the concentration increases from 0 to \( C_0 \), is neglected). The concentration of analyte molecules in the inner compartment, \( C_i \), is also uniform within the compartment (thus \( C_i = C_0 \)), but it changes in time due to both the transport of analyte molecules between compartments and the association–dissociation process taking place on the sensor’s surface. After a short period of time from the beginning of the AD process, this change becomes slow, so that the quasi-steady state approximation becomes valid (i.e. \( \frac{dC_i}{dt} \approx 0 \)) [11], but the time dependence of the concentration in the inner compartment cannot be neglected. The important benefit of the two-compartment model is the elimination of the dependence of the analyte concentration on spatial coordinates in each of the two compartments, so all the variables depend only on time and their values used in the equations are averaged over the sensor’s surface.

Based on the assumptions of the two-compartment model and the quasi-steady state approximation, the rate of change of the number of adsorbed molecules per unit area equals the net
number of molecules that enter the inner compartment in unit time per unit boundary area between the compartments, so the following equation is valid

\[
\frac{dN_b(t)}{dt} = k_m(C_0 - C_i(t))
\]  

(3)

Here \( k_m \) is the mass transfer coefficient, which characterizes the transport between the inner and the outer compartment (physically, it is the volumetric flux). The model assumes that \( k_m \) is constant.

Spatial uniformity of the variable \( N_b(t) \) over the entire functionalized sensor surface of the area \( A \) implies that the total number of bound analyte molecules on the sensor surface, \( N_b(t) \), is obtained as \( AN_b(t) \). The total number of immobilized receptor sites, \( N_m \), equals \( AN_m \). Accordingly, the system of equations describing the time change of the number of molecules adsorbed on the sensor surface is simply obtained from Eqs. (1) and (3) as

\[
k_m(C_0 - C_i(t))A = k_f C_i(t)(N_m - N_b(t)) - k_r N_b(t)
\]  

(4)

\[
\frac{dN_b(t)}{dt} = k_f C_i(t)(N_m - N_b(t)) - k_r N_b(t)
\]  

(5)

Eqs. (4) and (5) constitute the system of equations of the two-compartment model. It enables us to turn to the analysis of the nondimensional quantity \( N_b(t) \) which does not refer to a particular position on the sensing surface, but rather to the whole surface.

By elimination of the variable \( C_i(t) \) from Eqs. (4) and (5), the equation is derived which can be written in the form

\[
\frac{dN_b(t)}{dt} = g(N_b(t)) - r(N_b(t))
\]  

(6)

where the functions \( g(N_b(t)) \) and \( r(N_b(t)) \) are given as analytical expressions depending only on time. In this way, the two-compartment model enables us to describe the change in the number of adsorbed molecules by one equation which takes into account the mass transfer effects and is independent of spatial coordinates, instead of the complex system of Eqs. (1) and (2).

Eq. (6) has the same form as the one used to express the rate of change of the number of charge carriers in semiconductors due to generation–recombination processes [13,27]. The following equivalence of the quantities can be established: the rate of increase of the number of adsorbed particles \( (g) \) is equivalent to the carrier generation rate, and the rate of decrease of the adsorbed particles number \( (r) \) is equivalent to the rate of carrier recombination. In equilibrium, fluctuations of the number of adsorbed particles originate from the difference between the instantaneous values of the stochastic rates corresponding to \( g \) and \( r \), as will be shown in the next section.

2.2. Fluctuations of the number of adsorbed analyte molecules in equilibrium

Eq. (6) describes the deterministic behavior of the number of adsorbed particles in time. It enables us to obtain the number of bound molecules in the steady state \( (N_{be}) \), established when all the transient processes are finished. The condition \( dN_b/dt = 0 \) applied to Eq. (6), i.e. \( g(N_{be}) = r(N_{be}) \), yields the equilibrium number of adsorbed molecules

\[
N_{be} = \frac{k_f C_0}{k_r + k_f C_0} \cdot N_m
\]  

(7)

This expression corresponds to the Langmuir isotherm equation.

Upon reaching the equilibrium, the number of adsorbed particles still fluctuates due to the stochastic nature of the AD process coupled with mass transfer. The instantaneous value of the number of adsorbed particles in equilibrium is \( N_b(t) = N_{be} + \Delta N_b(t) \), where \( \Delta N_b(t) \) denotes small fluctuation around the equilibrium value at the moment \( t (\Delta N_b(t) \ll N_{be}) \).

In order to perform the analysis of fluctuation phenomena it is generally possible to classify the fluctuation processes in two categories. The first category includes the processes that are independent of spatial coordinates. This is the case in spatially homogeneous systems whose total number of constituents fluctuates. Carrier fluctuations in semiconductors, arising from the generation and recombination processes, belong to this class [27,28]. The resulting noise is called generation–recombination noise.

Fluctuations in spatially continuous systems, in which both spatial and time dependences of variables exist, belong to the second category. Here, fluctuations arise due to combined effects of the stochastic transitions between states (e.g. free/bound) and the transport of the system constituents.

The phenomenon of fluctuations in chemical and biological sensors generally belongs to the second category. However, in the case of rapid mixing, where there is no spatial dependence of variables due to the fast mass transfer, the analogy of AD and GR processes can be directly applied to model the fluctuations, as it is done for AD processes in gases [13,20]. In other cases belonging to type 2 transport regime, the two-compartment model leads to simplifications that permit the fluctuations of the total number of adsorbed particles to be also analyzed by applying the approach used for analysis of GR fluctuations. This is possible since the two-compartment model provides the analytical expressions for the effective rates of transition of the analyte molecules from the free state to the adsorbed state, \( g(N_b) \), and from the adsorbed to the free state, \( r(N_b) \) (equivalent to generation and recombination rates, respectively). These effective rates incorporate the influences of both the AD process and the mass transfer. It is important to note that the effective rates we obtained are spatially independent, explicitly independent of time, but dependent on \( N_b(t) \), which is the situation analogous to GR processes. Therefore, for the analysis of the random process \( N_b(t) \) (the fluctuating number of bound particles on the surface) we use the theory [29] that yields the probability distribution, mean, variance, correlations and other statistical properties of GR processes characterized by generation and recombination rates which are functions of the number of charge carriers \( n \). These processes are known in the probability theory as the birth–death processes, a class of the discrete-state, continuous time Markov processes. Ergodicity of the process \( N_b(t) \) is assumed (there is no disturbing factors in the system that would compromise stationarity and ergodicity). Starting from the master equation (in which the probabilities of transition from the state \( N_b \) to \( N_b + 1 \) and from \( N_b \) to \( N_b - 1 \) during the time interval \( \Delta t = 0 \) are related to the transition rates \( g(N_b) \) and \( r(N_b) \), respectively), it can be shown that the random variable \( N_b \) has the steady state probability distribution [27,29]

\[
P(N_b) = P(0) \prod_{i=0}^{N_b} \frac{g(v)}{v(v-1)}
\]  

(8)

where \( P(0) \) is obtained from the condition that the sum of probabilities over all possible values of \( N_b \) equals 1. The variance of the random variable \( N_b \) is [27–29]

\[
\langle (\Delta N_b)^2 \rangle = \frac{g(N_{be})}{g(N_{be}) - r(N_{be})} \cdot N_b \cdot N_{be}
\]  

(9)

After substitution of \( N_b(t) = N_{be} + \Delta N_b(t) \) in Eq. (6), followed by expansion of the expression on the right side in a Taylor series in the vicinity of \( N_{be} \), the following equation is obtained

\[
\frac{d\Delta N_b}{dt} = \left( \frac{dg}{dN_b} - \frac{dr}{dN_b} \right) \cdot \Delta N_b = -\frac{1}{r} \cdot \Delta N_b
\]  

(10)
It defines the exponential decay in time of the excess number of bound molecules $\Delta N_b(t)$ towards the equilibrium value $N_{be}$.

The corresponding stochastic equation used for the fluctuations analysis has the form

$$\frac{d\Delta N_b}{dt} = -\frac{1}{\tau} \cdot \Delta N_b + \Delta g(t) - \Delta r(t)$$

(11)

i.e. the same as for GR fluctuations. The terms $\Delta g(t)$ and $\Delta r(t)$ are the stochastic components of the effective transition rates, which account for the randomness of all the involved processes. These stochastic terms give the previous equation the form of the Langevin equation, where $\xi(t) = \Delta g(t) - \Delta r(t)$ is the Langevin stochastic source function. $\Delta g(t)$ and $\Delta r(t)$ are statistically independent processes. The Langevin equation yields the relationship between the power spectral density (PSD) of the random variable $N_b(t)$ and the uniform spectrum of $\xi(t)$ [28]

$$S_{N_b}^2(f) = \frac{S_{\xi}^2 \tau^2}{1 + (2\pi f)^2 \tau^2}$$

(12)

By integrating the expression (12) over the frequencies $f$ from 0 to $\infty$, and by equating the result with Eq. (9) which equals $g(N_{be}) \tau$ (based on Eq. (10)), the spectrum of the Langevin stochastic source is obtained as $S_{\xi}^2 = S_{N_b}^2 + S_{\Delta r}^2 = 4g(N_{be})$. Thus, the PSD of the fluctuations of the number of adsorbed analyte molecules is obtained in the form

$$S_{N_b}^2(f) = \frac{4g(N_{be}) \tau^2}{1 + 4\pi^2 f^2 \tau^2}$$

(13)

The quantity $S_{N_b}(f)$ is the rms value of fluctuations of the number of adsorbed molecules per square root Hertz, and it is also called the spectral density of fluctuations, or simply, the spectrum of fluctuations.

Eq. (10) implies that the time constant of the fluctuation process is

$$\tau = \frac{1}{k_r + k_f \eta_m} + \frac{k_r k_f \eta_m}{(k_r + k_f \eta_m)^2 k_m}$$

(14)

The first term on the right side of Eq. (14) equals the time constant in the case when the rapid-mixing model of the analyte concentration establishment is valid. It will be denoted by $\tau_R$. The second term, which we will denote by $\tau_m$, shows that the time constant is influenced by the value of the mass transfer coefficient. It is obvious that $\tau_m$ is the minimal possible value of $\tau$. It depends only on the time constants of adsorption ($\tau_a = 1/k_f \eta_m$) and desorption ($\tau_d = 1/k_r$). As the mass transfer flux decreases, $\tau$ increases due to the increase of $\tau_m$. It is interesting to note that the time constant of establishment of equilibrium of the binding process in the case of exponential binding kinetics is also given by Eq. (14) [12]. Then the ratio $\tau/\tau_R$ is a measure of the influence of mass transfer on the binding process kinetics, because it shows how much the mass transfer decelerates the establishment of the equilibrium, compared to the case of rapid-mixing. In the cases when kinetics deviates from the exponential behavior the same expression (Eq. (14)) is valid for the time parameter which influences the rate of increase of the number of bound molecules [12].

By introducing the expressions for both $\tau$ and $g(N_{be})$, Eq. (13) can be written in the explicit form

$$S_{N_b}^2(f) = \frac{4k_r k_f \eta_m A k_C + k_f \eta_m + (k_f k_r / k_m) \eta_m}{(k_f + k_r \eta_m)^2} - \frac{(k_r + k_f \eta_m)^2}{k_r + k_f \eta_m}$$

(15)

This expression enables the analysis of the shape of fluctuations when the influence of mass transfer is taken into account. In the case when the mass transfer effects are neglected the fluctuations power spectrum has the same form as in the case of rapid mixing, which can be obtained from Eq. (13) by replacing $\tau$ with $\tau_R$. By using Eq. (13) it is easy to determine the power spectrum of fluctuations of the coverage of the receptor sites by analyte molecules (the coverage is defined as $\theta(t) = N_b(t)/N_m$)

$$S_{\theta}^2(f) = \frac{4k_r k_f \eta_m A k_C + k_f \eta_m + (k_f k_r / k_m) \eta_m}{(k_f + k_r \eta_m)^2} - \frac{(k_r + k_f \eta_m)^2}{k_r + k_f \eta_m}$$

(16)

Since $S_{\theta}^2(f)$ is proportional to $1/\Lambda$, a smaller sensing area leads to increased fluctuations of the surface coverage. Eq. (13) also enables obtaining of the PSD of fluctuations of a physical quantity by means of which the detection of an analyte is performed. For example, such a quantity can be the adsorbed mass

$$S_{M}^2(f) = \frac{M^2}{N_m^2}$$

(17)

where $M_0$ is the mass of an analyte particle. However, this can also be some other quantity (resonant frequency, refraction index, etc.), depending on the type of the sensor.

3. Numerical calculations: results and discussion

In this section we report the results of the analysis of the spectrum of fluctuations of the number of adsorbed particles in biosensors, based on numerical calculations performed using the derived expressions. The parameter values ($k_r, k_f, k_m, C_o, \eta_m$) used in the analysis are within the range that corresponds to the realistic experimental and practical conditions [7,11,12,25]. They are chosen so to include the cases when the mass transfer influences the spectrum of fluctuations, as well as the cases when the spectrum is not affected by the transport of analyte molecules.

Fig. 2a and b show the spectrum of fluctuations of the number of adsorbed molecules, as a function of both the frequency $f$ and the analyte bulk concentration $C_o$. The parameters $k_r, k_f, k_m, C_o, \eta_m$ equal $8 \times 10^{-7} \text{/(M s)}, 0.08 \text{ls}^{-1}$ and $5 \times 10^{-12} \text{Mm}$, respectively, and they are common for both diagrams. A sensing area $A = 100 \times 10^{-6} \text{m}^2$ is assumed. For the case shown in Fig. 2a, $k_m = 2 \times 10^{-5} \text{m/s}$, while $k_m = 2 \times 10^{-2} \text{m/s}$ for the diagram presented in Fig. 2b. Fig. 2c shows the ratio $R_{PS}$ of the fluctuations spectra which are given in Fig. 2a and b. In Fig. 2a–c, cross vertical planes are used to make the dependences of the shown quantity on $f$ (for $C_o = \text{const}$) or on $C_o$ (for $f = \text{const}$) more obvious.

Fig. 3 shows the same quantity as Fig. 2a and b, but only as a function of frequency, with the mass transfer coefficient as a parameter ($C_o = 1 \text{mM}$, the order of magnitude of $k_m$ changes from $10^{-6} \text{m/s}$ to $10^{-2} \text{m/s}$, while the other parameters are the same as above).

The spectrum of fluctuations is of Lorentzian type, so the function $S_{\theta}(f)$ for a constant $C_0$ has a flat part in the low frequency range ($\omega \ll 1/\tau$, which is called plateau. At higher frequencies the value of the same function monotonically decreases (Figs. 2a, 2b and 3). The position of the characteristic “knee” is determined by the frequency $\omega = 1/\tau$ at which $S_{\theta}(f)$ has $2^{1/2}$ times lower value compared to the value at the plateau.

By comparing the diagrams in Fig. 2a and b it becomes obvious that the fluctuation spectrum significantly differs for different values of $k_m$. The mass transfer affects both the magnitude of the plateau of the spectrum and the position on the frequency axis of the knee of the curve $S_{\theta}(f)$ for $C_o = \text{const}$. In the case of lower mass transfer coefficient, shown in Fig. 2a, the level of the spectral density of fluctuations is higher compared to the case of higher mass transfer flux (Fig. 2b), for the same analyte bulk concentration. The increase of the level of fluctuations due to the influence of mass transfer can be clearly seen in Fig. 2c. For the parameter values given above, the level of fluctuations spectrum in the region of the plateau is about 20 times higher in the case of the lower transport flux. As $k_m$ decreases, the increase of the level of fluctuations spectrum in the plateau zone becomes more prominent (Fig. 3). This is logical, because in this frequency range the magnitude of the
spectrum is proportional to \( \tau \), which increases with the decrease of \( k_m \).

It can be seen in Fig. 2a and b that the frequency corresponding to the knee of the curve \( S_{bh}(f) \) for \( C_r = \) const differs for two different values of \( k_m \), as it is lower in the case of slower mass transfer. The knee continuously moves towards higher frequencies with the increase of the mass transfer coefficient (Fig. 3). The maximal value of the knee frequency is \( 1/\tau_g \). The measure of the influence of mass transport on the decrease of the knee frequency is the ratio \( \tau/\tau_g \) which equals \( 1 + k_f k_m [/(k_m k_r + k_f C_r)] = 1 + k_f k_m (1 - \theta_g)/k_m \). This ratio also equals the ratio of the plateau magnitude of the fluctuations spectrum for a given \( k_m \) and the same quantity in the case of rapid mixing, for the same set of parameter values (\( C_o, \eta_m, k_r \) and \( k_f \)). Based on these facts a criterion whether or not the influence of mass transfer on the fluctuations spectrum is significant can be established. When \( k_m \) is comparable to or lesser than \((1 - \theta_g)k_f \eta_m \) (the intrinsic adsorption volumetric flux in equilibrium) the influence of the mass transfer on the fluctuations spectrum of the number of adsorbed particles is significant (transport influenced fluctuations spectrum). When the condition \( k_m \gg (1 - \theta_g)k_f \eta_m \) is valid, the effects of mass transfer are practically negligible, and the fluctuations spectrum is the same as in the case of rapid mixing (reaction-determined fluctuations spectrum). In the example illustrated by Fig. 2, the mass transfer whose coefficient obeys \( k_m \gg 3.64 \times 10^{-4} \) m/s is of negligible influence on the fluctuations spectrum in the whole shown range of analyte concentration. Therefore, the spectrum shown in Fig. 2b is practically the same as the spectrum which would be obtained without taking into account the mass transfer effects.

The ratio \( \tau/\tau_g \) is also an indication of the discrepancy between the characteristic parameters (the plateau magnitude and the knee frequency) of the actual fluctuation spectrum and the one obtained by neglecting the mass transfer effects in the cases when they actually exist. Fig. 2c illustrates this discrepancy for \( k_m = 2 \times 10^{-5} \) m/s and the values of other parameters used in the presented example.

Now we will examine how the influence of the mass transfer, characterized by a certain value of \( k_m \), depends on the analyte concentration in the solution, on the receptor surface density, and also on the intrinsic association and dissociation rate constants. The analysis given above has shown that these dependences can be investigated by observing the ratio \( \tau/\tau_g \) as a function of the chosen parameter (\( C_o, \eta_m, k_r \) or \( k_f \)). The greater the ratio \( \tau/\tau_g \) at fixed \( k_m \), the stronger the influence of the mass transfer on the fluctuations
spectrum (the greater is the deviation of both the plateau magnitude and the knee frequency of the spectrum curve from the case of rapid mixing). Both qualitative and quantitative analysis of the mass transfer influence as a function of each of the parameters will be given in the following paragraphs.

The spectrum of fluctuations differs for different values of $C_0$ at a given $k_m$ (see the curves $S_{NH}(f)$ for different fixed values of $C_0$ in Fig. 2a). Fig. 2c shows that the ratio of the spectrum for a certain mass transfer flux and the spectrum when the mass transfer is neglected also depends on $C_0$. How much the influence of mass transfer is affected by $C_0$ can be seen more clearly in Fig. 4a which shows the ratio $R_r = \tau/\tau_R$ as a function of both the analyte concentration and the mass transfer coefficient, for $k_f = 5 \times 10^{-7}$ 1/(Ms), $k_i = 0.08$ 1/s, and $\eta_m = 1 \times 10^{-11}$ Mm. The purpose of the shown crossed vertical planes is to make the dependence of $\tau/\tau_R$ on each parameter more obvious (valid for Figs. 4 and 5). By observing the dependence $R_r(C_0)$ for $k_m = \text{const}$ (Fig. 4a), it can be noticed that the influence of the mass transfer on the fluctuations spectrum decreases with the increase of $C_0$. For example, for $k_m = 10^{-3}$ m/s and $C_0 = 10^{-10}$ M the plateau magnitude of the fluctuations spectrum will be 50 times greater, and the knee frequency 50 times lower compared to the case of rapid mixing. However, for the same mass transfer flux and $C_0 = 10^{-8}$ M the deviation will be lower (about 8 times). Such behavior can be explained by the decrease in the adsorption flux $(1 - \theta_c)k_f\eta_m$ due to the increase of the equilibrium surface coverage as $C_0$ increases. Therefore, at higher values of $C_0$ the factor by which $k_m$ exceeds $(1 - \theta_c)k_f\eta_m$ is greater, so $\tau/\tau_R$ is lower. The shown example also indicates that it depends on $C_0$ whether or not the influence of mass transfer characterized by a certain value of $k_m$ is negligible. The mass transfer is of negligible influence for $k_m \geq 4.7 \times 10^{-3}$ m/s when $C_0 = 10^{-10}$ M, while at $C_0 = 10^{-8}$ M the minimal value of $k_m$ for which the mass transfer influence is negligible is lower for an order of magnitude. At some higher values of $C_0$, the fluctuations spectrum can be unaffected by mass transfer at the same value of $k_m$ that made the fluctuations spectrum transport-influenced at lower $C_0$. As $C_0$ decreases, the fraction of free binding sites $(1 - \theta_c)$ is closer to 1, so $\tau/\tau_R$ increases. At $C_0 \ll k_i/k_f$ (in the shown example $k_i/k_f = 1.6 \times 10^{-9}$ M), the ratio $\tau/\tau_R$ at fixed $k_m$ asymptotically approaches the maximal value $1 + k_f\eta_m/k_m$, and the influence of mass transfer is strongest for the given $k_m$. However, if $k_m \gg k_f\eta_m$ (here $k_f\eta_m = 5 \times 10^{-4}$ m/s), $k_m \gg (1 - \theta_c)k_f\eta_m$ will be valid for an arbitrary value of $C_0$, so the influence of mass transfer will be negligible regardless of $C_0$.

The dependence of the ratio $\tau/\tau_R$ on both the receptor surface density and the mass transfer coefficient is shown in Fig. 4b, which is obtained for the following values of parameters: $k_f = 8 \times 10^{7}$ 1/(Ms), $k_i = 0.08$ 1/s, $C_0 = 5 \times 10^{-10}$ M. It can be seen from the diagram that the increase of the time constant $\tau$ relative to $\tau_R$, caused...
by the influence of mass transfer, is higher at greater adsorption sites density. Therefore, as \( \eta_m \) increases, the influence of mass transfer on AD fluctuations spectral characteristics becomes stronger. In the given example (Fig. 4b) for high receptor surface densities, the level of the plateau of the fluctuations spectrum is up to two orders of magnitude higher compared to rapid mixing systems, and the frequency of the knee of the spectral curve is lower for the same order of magnitude. The value of \( k_m \) at which the mass transfer influence becomes negligible (i.e. \( \tau \) approaches \( \tau_k \)) decreases with the decrease of the receptor surface density.

The influence of mass transfer is also more prominent for higher values of \( k_f \), as seen in Fig. 5a which shows the dependence \( R_c(k_f, k_m) \) for \( C_0 = 1 \times 10^{-9} \text{ M} \), \( \eta_m = 2.5 \times 10^{-11} \text{ Mm} \), \( k_f = 0.02 \text{ 1/s} \). For \( k_m = 10^{-5} \text{ m/s} \), \( \tau \) is 50 times greater than \( \tau_k \) at \( k_f = 10^8 \text{ 1/(M s)} \), while for the same \( k_m \) the ratio \( \tau/\tau_k \) is only slightly greater than 1 at \( k_f = 10^6 \text{ 1/(M s)} \). This can be explained by the fact that the adsorption flux increases with the increase of \( k_f \). Thus at higher values of \( k_f \) the spectrum of fluctuations will be influenced by the analyte transport of such values of \( k_m \) that result in a negligible influence at lower values of \( k_f \) (e.g. see the curve \( R_c(k_f) \) for \( k_m = 10^{-5} \text{ m/s} \) in Fig. 5a). This means that at higher intrinsic adsorption rate constants the mass transfer must be faster in order for its influence to be negligible. Fig. 5a shows that with the increase of \( k_f \), for \( k_m = \text{const} \), the ratio \( \tau/\tau_k \) asymptotically approaches the maximal value that equals \( 1 + k_f\eta_m/(C_0 k_m) \). This strongest influence is reached when \( k_f \gg k_m/C_0 \). The stronger \( k_f \) the stronger the mass transfer influence on the spectrum of fluctuations of the number of adsorbed particles. At the same mass transfer rate, the ratio \( \tau/\tau_k \) can vary for an order of magnitude, depending on the value of \( k_m \) as shown by our calculations. The dependence of the ratio \( \tau/\tau_k \) on \( k_f \) also has a maximum which equals \( 1 + k_f\eta_m/(C_0 k_m) \) when \( k_f \gg k_m/C_0 \). The mass transfer is negligible for any value of \( k_f \) that satisfies the expression \( k_f < k_m k_f (C_0/\eta_m k_m) \).

A similar conclusion arises from the analysis of the dependence of the ratio \( R_c \) on both the intrinsic dissociation rate constant and the mass transfer coefficient (Fig. 5b), obtained for \( C_0 = 5 \times 10^{-10} \text{ M} \), \( \eta_m = 1 \times 10^{-11} \text{ Mm} \), \( k_f = 5 \times 10^{-7} \text{ 1/(M s)} \): the greater \( k_f \) the stronger the mass transfer influence on the spectrum of fluctuations of the number of adsorbed particles. At the same mass transfer rate, the ratio \( \tau/\tau_k \) can vary for an order of magnitude, depending on the value of \( k_m \) as shown by our calculations. The dependence of the ratio \( \tau/\tau_k \) on \( k_f \) also has a maximum which equals \( 1 + k_f\eta_m/(C_0 k_m) \) when \( k_f < k_m/C_0 \). The mass transfer is negligible for any value of \( k_f \) that satisfies the expression \( k_f < k_m k_f (C_0/\eta_m k_m) \).

The strong dependence of the fluctuations spectrum on different parameters of a binding–unbinding molecular process, as we obtained, imply that new methods can be developed for analyte detection and for characterization of mass transfer influenced biomolecular interactions, based on the frequency domain analysis of the measured fluctuations in the sensor’s output signal. The binding kinetics and intrinsic rate constants can be determined by adjusting the parameters of the derived analytical expression to fit the experimentally obtained spectrum. Since AD intrinsic rate constants are related to the particular analyte–receptor binding pair, such methods could provide additional information about the adsorbed analyte, for example, those necessary for identification of the analyte if several substances have the affinity for binding to the same capturing probes. If the influence of mass transfer actually exists, but is neglected during the analysis of the measured fluctuations spectra, interpretation of the results will be erroneous, leading to wrong determination of analyte concentration and binding kinetics and possibly to wrong identification of the analyte.

Because it causes deceleration of the binding kinetics and the increase in signal fluctuations, mass transfer can be considered as one of the physical limitations of biosensor performance. AD fluctuations, which are already considerable in small-scale micro/nanofabricated sensors, become even greater when the influence of mass transfer exists, so the possibility for them to dominate over the fluctuations generated by other fundamental mechanisms is higher. Therefore, the limiting performance (the total noise and the minimal detectable signal) of biosensors are especially affected by AD fluctuations when the binding of analyte to receptor molecules is influenced by mass transfer. By neglecting the mass transfer effect when it actually exists, the noise and the minimal detectable signal would be underestimated. Obviously, the effects of mass transfer must be taken into account when the noise and the minimal detectable signal of biosensors used in liquids has to be determined, and also when the analysis of measured spectrum of fluctuations is performed in order to obtain additional information about the analyte or about the biomolecular interaction process.

4. Conclusion

In this paper we presented a simple theory of fluctuations of the number of adsorbed particles in the affinity-based biological sensors, which takes into account the mass transfer effects. We obtained the analytical expression for the power spectral density of the fluctuations and formulated the criterion intended for determination whether or not the influence of mass transfer of a given flux on the fluctuations spectrum is significant.

The theory relies on assumptions that limit the scope of its applicability to the cases of simple biomolecular reversible binding reactions and sensors in which analyte concentration can be well described by the two-compartment model.

The derived mathematical model is a convenient tool for estimation of the quantitative influence of the mass transfer on the spectrum of fluctuations of the measured signal. In this paper we presented for the first time the quantitative data about the influence of the fluctuations spectrum influenced by mass transfer and the spectrum not affected by it. Our numerical calculations showed that the mass transfer process can significantly influence the fluctuations spectrum. Slower mass transfer results in both the higher fluctuations magnitude and the lower knee frequency of the spectrum curve. This implies that the performance of biosensors operating in liquids, where the transfer of analyte molecules is much slower than in gases, is especially affected by the mass transfer process.
The obtained analytical expression for the PSD of fluctuations is useful for quantitative estimation of the sensor’s AD noise in the case of analyte to receptor binding influenced by mass transfer. Thus, the derived theory enables realistic estimation of the limiting performance of biosensors operating in liquids. It can also be utilized for evaluation of the dependence of sensor performance characteristics (e.g., AD noise, detectable signal) on different parameters, which is a prerequisite for minimization of mass transfer adverse influence. Therefore, the significance of this theory is in its applicability for optimization of biomolecular binding experiments and detection methods. It provides the guidelines for improvement of the limits of detection, and it is useful for development of low-noise biosensors based on miniaturized transducer structures.

Both the theory and the analysis presented in this paper can be applied for development of novel highly sensitive methods for extraction of information about the analyte and the biomolecular binding process from the measured spectrum of fluctuations in the sensor’s output signal. The presented mathematical model of AD fluctuations, which includes mass transfer effects, is a significant step towards a comprehensive theory of fluctuations, which enables proper interpretation of the experimentally obtained fluctuations spectra.

In our future work we will investigate the influence of non-specific binding, surface heterogeneity and surface diffusion of molecules on the fluctuations spectrum of the measured signal in biosensors.

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References


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