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Theoretical understanding of the SPR sensor response on the protein adsorption

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This research deals with consideration of the SPR sensor response in the framework of the scattering matrix approach with the modeling of biomolecular layer using Green's function formalism and effective medium theories. It was found out that modeling of the SPR sensor response using abovementioned approaches in the densely packed monolayer approximation gave not enough agreement with the experiment. The reason was that real molecular layers are rarely densely packed. They usually can be characterized by molecular surface concentration or monolayer filling factor. Approximation of these parameters allows obtaining their values, which correspond to real biomolecular layers, and gives better agreement with the experiment.

1. Introduction

Surface plasmon resonance (SPR) is a highly sensitive modern technique which has many applications, especially with investigation of biological objects [1-5]. For example, this technique can be used to study the proteins adsorption [6-8]. In fact, sometimes the problem of results interpretation appears. The SPR sensor response is usually modeled in the framework of the scattering matrix formalism [9-12]. However, the results of such modeling often do not conform well to the experiment, because this approach does not consider the internal structure of the biomolecular layer. So, we extended this approach by using the effective medium theories [13] and Green's functions formalism [14,15] for biomolecular layer modeling.
The advantages of the Green's functions approach were considered in detail in the work [15]. Using this method to process the SPR experimental data, the authors have calculated the surface concentration and the components of the molecular permittivity tensor of the biomolecular layer instead of refractive index and thickness of the biomolecular layer. It was shown that SPR response depends on the shape and orientation of adsorbed molecules with respect to the SPR sensor surface.
The effective medium theories are usually used for the consideration of multicomponent media such as rough surfaces [16], nanoparticles in dielectric matrices [17,18], nanoparticle thin films [19], island films [20] etc. We have applied the effective medium theory approach to biomolecular layers on solid surface, which can be also considered as a two-phase multicomponent system consisting of biomolecules and buffer solution.
Therefore, the main aim of the present research is to compare the results of SPR response modeling by abovementioned approaches with the SPR experimental data obtained for the proteins adsorption.

2. Experimental

Commercially available optoelectronic SPR biosensor NanoSPR-321 was used in the present

research. The TF glass SPR slides with thermally evaporated 45 nm gold film and 5 nm chromium adhesion sublayer were exploited as plasmon oscillations carrier. The reflected light intensity versus the angle of incidence (SPR curve) was measured. At the angle, which is called resonant, the surface plasmon resonance occurs that causes sharp decrease of reflected light intensity. The presence of the biomolecular layer on the sensor surface results in SPR minimum angular position shift towards larger values.

Phosphate buffered saline (PBS) solutions of trypsin (24 kDa, globular protein with size 5 nm) and bovine serum albumin (BSA, 66.43 kDa, ellipsoidal protein with size 4×4×14 nm) with concentrations of 250 μg/ml were exploited during the experiments.

3. Theory

SPR sensor response on biomolecular adsorption was modeled in the framework of the scattering matrix formalism to characterize the multilayer system (glass-Cr-Au-biomolecular layer-buffer solution) with additional consideration of the biomolecular layer using Green's functions approach and effective medium theories.

In the Green's functions approach [21] for spherical molecules, the biomolecular layer is characterized by molecular surface concentration N_s and molecular polarizability A_m. Then the reflectance coefficient of *p*-polarized light by the system will be [14]:

$$R_p = R_{0p} + \frac{(\beta A_m g_{xx} - A_m f_{zz})(1+R_{0p}^2) - 2(\beta A_m g_{xx} + A_m f_{zz})R_{0p}}{(1+A_m a g_{xx})(\beta + A_m a f_{zz}) - a(\beta A_m g_{xx} - A_m f_{zz})R_{0p}},$$

where R_{0p} is the reflectance coefficient of *p*-polarized light by the system without biomolecular layer,

$$A_m = \frac{3M}{4\pi N_a \rho}\frac{n^2-1}{n^2+2}$$

is the polarizability of the spherical molecule,

$$\beta = \frac{1+4\pi A_m}{n_1^2}, \quad a = 2\pi N_s,$$

$$g_{xx} = -x_1 / n_1^2, \quad f_{zz} = -\frac{k^2}{n_1^2 x_1}$$

are some combinations of the wave vector,

$$k = k_0 n_1 \sin\theta, \quad x_1 = n^2 k_0^2 - k^2, \quad k_0 = \frac{\omega}{c},$$

n is the refractive index of the protein molecule, n_1 is the refractive index of the surrounding medium, M is the molecular weight of the protein molecule, ρ is the protein density, N_a is the Avogadro's number. If the protein molecule is not spherical, then abovementioned equations are substituted with respective formulas [14].

In the effective medium approach, the biomolecular layer is characterized by the effective refractive index of the biomolecular layer n_{eff} and filling factor f. Depending on the topology of heterosystem, different effective medium theories can be used [13]. For example, for Lorentz-Lorenz effective medium theory the refractive index of the biomolecular layer can be derived from the equation [13]

$$\frac{n_{eff}^2-1}{n_{eff}^2+2} = \frac{n_1^2-1}{n_1^2+2}\cdot f + \frac{n_2^2-1}{n_2^2+2}\cdot(1-f),$$

where n_1 is the refractive index of the surrounding medium, n_2 is the refractive index of the

protein molecule. Similarly, for the Maxwell-Garnett theory the effective refractive index can be found from the formula

$$\frac{n_{eff}^2 - n_1^2}{n_{eff}^2 + 2n_1^2} = f\,\frac{n_2^2 - n_1^2}{n_2^2 + 2n_1^2};$$

for the Bruggeman nonsymmetrical and symmetrical theories the effective refractive indices are the solutions of equations

$$\left(\frac{n_1^2 - n_2^2}{n_1^2 - n_{eff}^2}\right)^3 = \frac{1}{f^3}\cdot\frac{n_2^2}{n_{eff}^2}$$

and

$$f\,\frac{n_2^2 - n_{eff}^2}{n_2^2 + 2n_{eff}^2} + (1-f)\,\frac{n_1^2 - n_{eff}^2}{n_1^2 + 2n_{eff}^2} = 0,$$

respectively.

The following values were used for the calculation of the multilayer structure parameters: the refractive index of the glass slide was n_g = 1.61, chromium complex refractive index n_{Cr} = 3.66 + 4.365i [22], refractive index of protein molecules n = 1.46 [23], the approximate thicknesses of adsorbed trypsin and BSA layers were d_{tr} = 5.0 nm and d_{BSA} = 4.0 nm (BSA long axis is parallel to the surface), respectively [24]. Gold complex refractive index was fitted for the curves corresponding to the initial PBS flow, with n_{Au} = 0.151 + 3.811i [25] chosen as a guess value for the fitting.

4. Results and discussion

The kinetic dependences of SPR curve minimum (SPR sensograms) for trypsin and BSA adsorption were experimentally obtained (Figs. 1, 2).

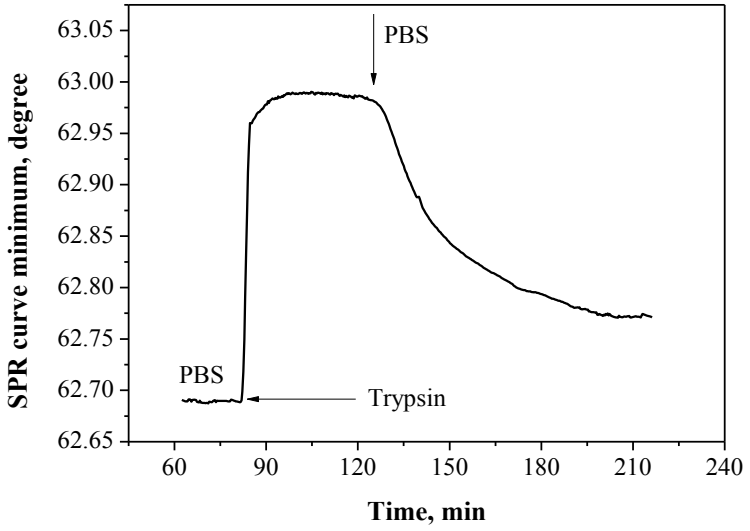

Fig. 1. The kinetic dependence of SPR curve minimum for trypsin adsorption.

SPR sensograms represent the following processes at the SPR sensor surface. The first horizontal

region corresponds to the stabilization of the gold-liquid interface after filling the measurement cell with buffer solution. The next stage with increasing SPR curve minimum position corresponds to the protein adsorption process and the last region with the decreasing of the SPR curve minimum is connected with the removal of the non-adsorbed protein due to rinsing with buffer solution.

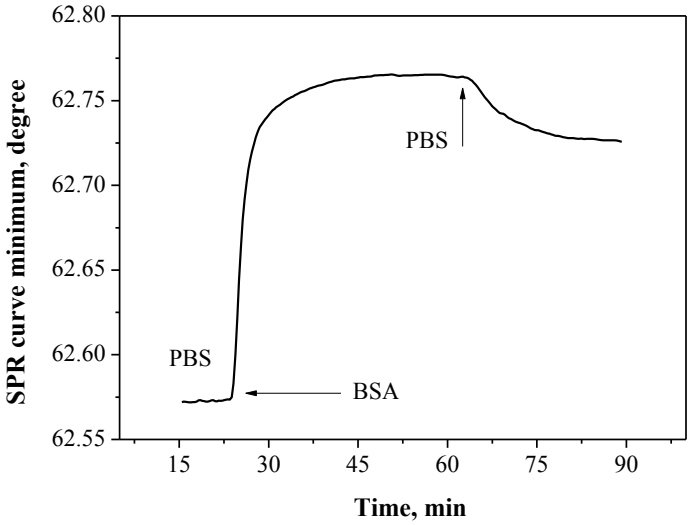

Fig. 2. The kinetic dependence of SPR curve minimum for BSA adsorption.

The final angular positions of SPR curves minimum θ_{exp}, which correspond to the formed biomolecular layer on the SPR sensor surface, were compared with the similar values, calculated using the abovementioned theoretical approach in the densely packed monolayer approximation. Biomolecules filling factor (for effective medium theories) was equal to 0.524 and surface concentrations (for Green's functions formalism) of the trypsin and BSA layers were $4 \cdot 10^{16}$ m^{-2} and $1.79 \cdot 10^{16}$ m^{-2}, respectively (Table I).

Table I. Comparison of the experimental and calculated (Green's functions and effective medium theories) SPR curve minimum values.

	θ_{exp}, degree	θ_{Green}, degree	θ_{L-L}, degree	θ_{M-G}, degree	$\theta_{Br.nonsym.}$, degree	$\theta_{Br.sym.}$, degree
Trypsin	62.77	63.0283	62.9541	62.9576	62.9584	62.9582
BSA	62.72	63.3860	62.7787	62.7814	62.7821	62.7819

As one can see, the calculated values of SPR curve minimum are close but not equal to the experimental ones. The reason is that real molecular layers can't be considered as densely packed. They are characterized by some molecular surface concentration N_s (for Green's function formalism) or filling factor f (for effective medium theories). If we change the filling factor (or surface concentration for Green's function formalism) among all possible values and calculate the respective position of the SPR curve minimum, we shall be able to choose such value of filling

factor (surface concentration) which will correspond to experimental position of the SPR curve minimum. Therefore, approximation of the abovementioned parameters allows obtaining better agreement with the experiment. It should be noted that different effective medium theories gave almost the same results which is rather unexpected. This can be explained by the fact that the filling factor for the calculations was 0.524 and this value is situated at the limit of some theories application. So, further we shall apply only one effective medium theory, namely, Bruggeman effective medium theory because it is applicable not only for small filling factors as, for example, Maxwell-Garnett theory. The results of the calculations in the framework of sparsely packed layers are presented in Table II.

Table II. Experimentally obtained values of SPR curve minimum and calculated molecular surface concentrations, filling factors and effective refractive indices of trypsin and BSA layers.

	θ_{exp}, degree	N_s, 10^{16} m^{-2}	f	$N_{eff.Br.}$
Trypsin	62.77	1.3	0.191	1.3553
BSA	62.72	0.4	0.386	1.3804

Evidently, Table II demonstrates the approximated molecular surface concentration N_s and filling factor f values that we have obtained which more correctly characterize the real biomolecular layers and give better agreement with the experiment.

5. Conclusion

The scattering matrix approach for consideration of SPR experiment results of biomolecular adsorption study was extended using theoretical models describing sparsely packed molecular layer. As a result, it was found that modeling of the SPR sensor response using Green's function formalism and effective medium theories allows more correct explanation of the experimental results taking into account sparsely packed structure of biomolecular layer. Proposed approach allows estimating the surface molecular concentration and filling factors, which are important parameters describing the biomolecular layer.

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