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3D-quantification of biomolecular covers using surface plasmon-polariton resonance experiment

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ABSTRACT

The concentration of surface molecules N_s and components of molecular susceptibility $\chi_{ji}(\omega)$ can both be determined from surface plasmon-polariton resonance (SPPR) experiments, instead of effective layer thickness and index of refraction, which are usually determined. The theoretical consideration of a molecular layer as monolayer of separated 3D-oscillators provides a new perspective for investigating molecules during SPPR experiment. It is shown that SPPR response and the form of the reflective curve depend on the form of a biomolecule and its orientation relative to the surface of the metal-carrier of plasmon oscillations. The experimental data for immunological reaction for the calculation of surface molecular concentration and mass of biomolecular covering are presented.

1. Introduction

The surface plasmon resonance (SPR) effect is widely used in modern sensors as a sensitive method for the study of physical properties of molecular layers or coverings at the surface of solids [1]. The main principle of the method is a measurement of the shifts of resonant angle if the molecular covering is present at the sensitive surface of the sensor. The angle shift is, of course, dependent on molecular concentration as well as the type of the molecules. The physical models usually used for the description of this shift are based mainly on the concept of an additional layer on the surface of SPR-converter, which is characterized by the effective thickness h and refractive index N, analogous to the similar idea of ellipsometry of thin films [1–4]. Another approach is based on the idea of ultra-thin film representation [5]. The main point of this approach is the representation of the molecular layer as effective ultra-thin homogeneous film characterized by any susceptibility, which was calculated with self-consistent equations for local field using molecular polarization and effective film thickness. The calculations do not utilize boundary conditions and molecules are represented as point-like objects. It is clear that similar approaches do not allow one to obtain information about the concentration or individual dielectric properties of molecules at the surface. To describe the optical properties of molecular coverings at the surfaces, one needs to take the individual properties of the adsorbed molecules, their interaction with the surface and intermolecular (lateral) interactions into account. Bobbert and Vlieger have shown [6] that one solution of the problem of light reflection from a substrate covered with spherical particles can be obtained by definition of the reflected electromagnetic wave as a sum of Fresnel's plane wave and number of the spherical waves, which are raised at the scattering on the spherical particles in accordance to Mie theory. Another method of calculation of reflection coefficient for the surface covered by molecular layer is based on the Green function concept [7]. Here we develop an approach based on the linear response concept for the non-point-like protein molecules, which have the shape of oblate or prolate ellipsoids.

2. Theory

2.1. Susceptibility of molecular layer at the surface

To calculate the reflection coefficient one needs to know the effective susceptibility of the molecular layer. We consider the

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dilute thin layer of organic molecules homogeneously distributed on the surface. The molecular susceptibility $\chi_{jl}(\omega)$ is considered as known. The molecules are assumed as prolate or oblate ellipsoids. The field at an arbitrary point in the system obeys the equation [8]:

$$E_i(\vec{R},\omega) = E_i^{(0)}(\vec{R},\omega) - a\sum_{\alpha=1}^{Q}\int_{V_\alpha} d\vec{R}' G_{ij}(\vec{R},\vec{R}',\omega)\chi_{jl}(\omega)E_l(\vec{R}',\omega), \quad (1)$$

where $E_i^{(0)}(\vec{R},\omega)$ is the external long-range field, a is the coefficient defined by the system of units (for SI $a = \omega^2/c^2\varepsilon_0$), Q is the number of molecules on the surface, V_α is the molecular volume, $G_{ij}(\vec{R},\vec{R}',\omega)$ is a photon propagator that describes propagation of light of frequency ω from point $\vec{R}'$ to point $\vec{R}$ [9]. Summation is made over all positions which are occupied by the molecules. Because molecular linear dimensions are much less than light wavelength and average distances between the molecules are considered to be larger than molecular linear dimension (submonolayer cover), one can make the next approximation:

$$\sum_\alpha \int_{V_\alpha} d\vec{R}' G_{ij}(\vec{R},\vec{R}',\omega)\chi_{jl}(\omega)E_l(\vec{R}')$$

$$\approx \sum_\alpha G_{ij}(\vec{r}-\vec{r}_\alpha, z, z_\alpha, \omega)\tilde{\chi}_{jl}(\omega)E_l(\vec{r}_\alpha, z_\alpha), \quad (2)$$

where $\tilde{\chi}_{jl}(\omega) = V_\alpha\chi_{jl}(\omega)$, V_α is the molecular volume. Here $\tilde{\chi}_{jl}(\omega)$ is the response on the local (total) field which connects the polarization of the molecule and the local field via

$$P_j(\vec{r}_\alpha, z_\alpha, \omega) = \tilde{\chi}_{jl}(\omega)E_l(\vec{r}_\alpha, z_\alpha, \omega). \quad (3)$$

The averaging over molecular coordinates if molecules are homogeneously distributed along the surface is performed using the equation:

$$\overline{\sum_{\alpha=1}^{Q} G_{ij}(\vec{r}-\vec{r}_\alpha, z, z_\alpha, \omega)\tilde{\chi}_{jl}(\omega)E_l(\vec{r}_\alpha, z_\alpha, \omega)}$$

$$= \frac{1}{S^{Q-1}}\int d\vec{r}_1 d\vec{r}_2 \ldots d\vec{r}_N \sum_{\alpha=1}^{Q}\int \frac{d\vec{k}}{(2\pi)^2} e^{-i\vec{k}(\vec{r}-\vec{r}_\alpha)}$$

$$G_{ij}(\vec{k}, z, z_\alpha, \omega)\tilde{\chi}_{jl}(\omega)\int \frac{d\vec{k}'}{(2\pi)^2} e^{-i\vec{k}'\vec{r}_\alpha}E_l(\vec{k}', z_\alpha, \omega)$$

$$= N_s\int \frac{d\vec{k}}{(2\pi)^2} e^{-i\vec{k}\vec{r}}G_{ij}(\vec{k}, z, z_\alpha, \omega)\tilde{\chi}_{jl}(\omega)E_l(\vec{k}, z_\alpha, \omega), \quad (4)$$

where S is the area of the surface at which the Q molecules are situated, $N_s = Q/S$ is molecular concentration. Then, an equation of self-consistent field in the Wail-representation can be written as

$$E_i(\vec{k}, z_\alpha, \omega) = E_i^{(0)}(\vec{k}, z_\alpha, \omega) - N_s a G_{ij}(\vec{k}, z_\alpha, z_\alpha, \omega)\tilde{\chi}_{jl}(\omega)$$

$$E_l(\vec{k}, z_\alpha, \omega). \quad (5)$$

Making Fourier transformation in the plane of the surface, one obtains from Eq. (3):

$$E_l(\vec{k}, z_\alpha, \omega) = (\tilde{\chi}_{jl}(\omega))^{-1}P_j(\vec{k}, z_\alpha, \omega). \quad (6)$$

Then, Eq. (5) can be rewritten in the form:

$$(\tilde{\chi}_{ij}(\omega))^{-1}P_j(\vec{k}, z_\alpha, \omega) = E_i^{(0)}(\vec{k}, z_\alpha, \omega) - N_s a G_{ij}(\vec{k}, z_\alpha, z_\alpha, \omega)$$

$$P_j(\vec{k}, z_\alpha, \omega). \quad (7)$$

The solution of this equation is

$$P_j(\vec{k}, z_\alpha, \omega) = [(\tilde{\chi}_{ji}(\omega))^{-1} + N_s a G_{ij}(\vec{k}, z_\alpha, z_\alpha, \omega)]^{-1}E_i^{(0)}(\vec{k}, z_\alpha, \omega). \quad (8)$$

Then, the effective susceptibility of sub-monolayer of the molecules at the surface which connects the Fourier-transformants of layer polarization and external field has a form:

$$\tilde{X}_{ij}(\vec{k}, z_\alpha, \omega) = [(\tilde{\chi}_{ij}(\omega))^{-1} + N_s a G_{ji}(\vec{k}, z_\alpha, z_\alpha, \omega)]^{-1}. \quad (9)$$

2.2. Reflection coefficient

For SPPR simulation one needs to know the reflection coefficient of the molecular layer (see Fig. 1a). For calculation of the reflection coefficient, let us consider the planar layered medium, the electrodynamical properties of which are characterized by photon propagator $G_{ji}(\vec{k}, z, z', \omega)$. Let the light propagation from semispace $z>0$ to the same semispace be described by photon propagator $G_{ij}^{(+,+)}(\vec{k}, z, z', \omega)$, the light propagation from semispace $z<0$ to semispace $z>0$ – by photon propagator $G_{ij}^{(+,-)}(\vec{k}, z, z', \omega)$, and the light propagation from semispace $z>0$ to semispace $z<0$ – by photon propagator $G_{ij}^{(-,+)}(\vec{k}, z, z', \omega)$. Then, an effective susceptibility of the molecular layer situated at the surface of semispace $z<0$ is defined by equation (9) with photon propagator $G_{ij}^{(-,-)}(\vec{k}, z, z', \omega)$. If the field $E_i^{(0)}(\vec{k}, z, \omega)$ acts at the molecular layer, the field reflected by the layer will be written as

$$E_i^{(R)}(\vec{k}, z, \omega) = N_s G_{ij}^{(+,-)}(\vec{k}, z, z_\alpha, \omega)X_{jl}(\vec{k}, \omega)E_l^{(0)}(\vec{k}, z_\alpha, \omega), \quad (10)$$

where z_α is z-coordinate of the centre of molecule. Then, the reflection coefficient of the molecular layer, which connects the amplitudes of reflected by the layer and incident p-polarized fields $E_p^{(R)} = R_p E_p^{(0)}$, can be written in the form:

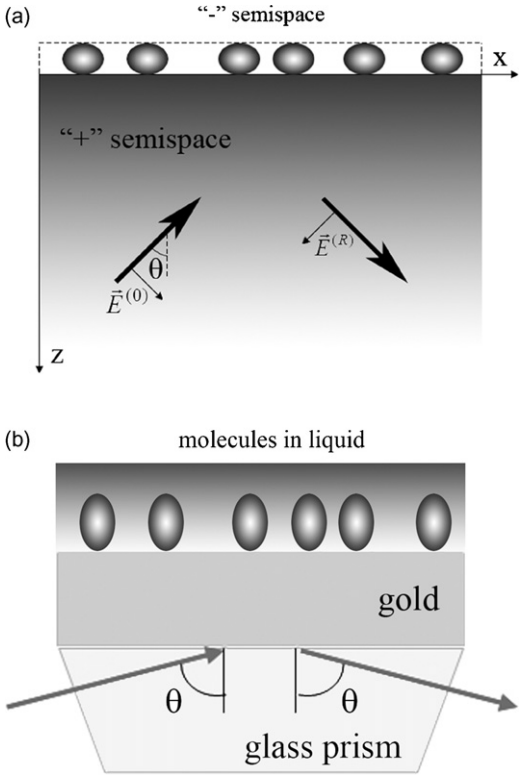

Fig. 1. (a) Reflection of the light by molecular layer situated at a surface. (b) Schematic presentation of the system under investigation.

$$R_p^{(M)}(\theta,\omega) = G_{xj}^{(+,-)}(\vec{k},z,z_\alpha,\omega)N_s X_{jx}(\vec{k},\omega) + G_{zj}^{(+,-)}(\vec{k},z,z_\alpha,\omega)$$
$$N_s X_{jz}(\vec{k},\omega) + [G_{xj}^{(+,-)}(\vec{k},z,z_\alpha,\omega)N_s X_{jz}(\vec{k},\omega)$$
$$+G_{zj}^{(+,-)}(\vec{k},z,z_\alpha,\omega)N_s X_{jx}(\vec{k},\omega)]\cos\theta\sin\theta \quad (11)$$

where θ is incident angle. Because light reflection occurs both by molecular layer and by the surface, total reflection coefficient should be written as the sum:

$$R_p^{(T)}(\theta,\omega) = R_p^{(0)}(\theta,\omega) + R_p^{(M)}(\theta,\omega), \quad (12)$$

where $R_p^{(0)}$ is the Fresnel reflection coefficient of the surface.

2.3. Modeling of SPPR curves

For modeling the SPPR experiment as shown schematically in Fig. 1b one needs to calculate the reflection coefficient of the system consisting of a glass attenuated total reflection (ATR) prism, a thin gold film and the liquid with an adsorbed molecular layer (see Fig. 1b). If one is going to use the approach described above, it is evident that the molecular layer is situated at a surface of metal film placed on glass ATR prism. Using Eq. (12) one can calculate the SPR curves relating to the different shapes of the molecules. It is clear from Eqs. (9) and (11) that the reflection coefficient defining the SPPR curve is dependent on molecular concentration and on molecular shape. We calculated the reflection coefficient using specially developed software, according to Eq. (12) for different values of parameter $\zeta = h_{||}/h_\perp$ (where $h_{||}$ and $h_\perp$ are ellipsoid semiaxes parallel (||) or perpendicular ($\perp$) to the substrate surface plane) which defines the shape of the molecules having the same mass. It turned out that the SPPR curve corresponding to molecules having prolate ellipsoid shape are characterized by a rather strong shift when parameter ζ is changed (see Fig. 2a). Molecules characterized by oblate ellipsoid shape demonstrate very small shifts for various values of parameter ζ. For example, changing the ζ value from 1.1 to 10 leads to a change in value of the minimum angle from $\theta_{min} = 64.121^\circ$ to 64.262°.

Calculations show that the shift of the minimum of SPR curve with increasing molecular concentration is rather evident. Namely, increasing the molecular concentration (for molecules characterized by $\zeta = 0.15$) by 5% leads to angle shift of $\Delta\theta = +0.1^\circ$. This implies that the proposed approach could be useful for the development of experimental results using SPR measurements for evaluating biomolecular coatings or layers, because the concentration of molecules that have individual molecular characteristics such as polarization of a single molecule or its shape at the surface can be determined using this approach.

One should note that developing this approach allows us to envisage the possibility of a two-component covering of the SPR sensor surface into account. Namely, one may calculate the SPPR curves for molecular layers consisting of molecules having both prolate and oblate shapes. The dependences of SPPR curves on the composition of the molecular film consisting of prolate ($\zeta = 0.12$) and oblate ($\zeta = 2.0$) molecules are shown in Fig. 2b. One can see that changing part f of prolate molecules from $f = 1$ (which corresponds to molecular layer consisting only of prolate molecules) to $f = 0.5$ leads to a shift of the curve minimum of about 0.505°. This result is evidence of a rather strong dependence of dispersion properties of the molecular layer on the molecular shape.

3. Materials and methods

The surface plasmon-polariton resonance Kretschmann type spectrometer "NanoSPR 321" (Chicago, USA) with a light-emitting diode light source ($\lambda = 650$ nm) and 45 nm Au-covered glass slides ($n = 1.61$, 1.5-cm^2 area exposed to the solution) was used in this work. The proteins were purchased from Aldrich. The biospecific reaction (see Fig. 3a) between the immunoglobulines pair IgG–anti-IgG in flowing (10 μl/min) regime using PBS (pH 7.3) as running buffer was performed. The concentration of both IgG and anti-IgG solutions was 200 μg/ml. Glycine buffer (pH 2.2) for estimation of specificity of reaction was used.

4. Results and discussion

The experimental kinetic dependences of SPPR angular position for specific reaction IgG–anti-IgG and protein G–IgG presented in Fig. 3a and b could prove the theoretical consideration. The immunoglobulines are characterized as biomolecules with strong eccentricity (size 3 nm × 4 nm × 25 nm) [10], so our theoretical approach can be applied for this biostructure. As mentioned above, the presented theoretical approximation relates to monolayers. From this point of view the part of immunological reaction A–B and part A–C both can be theoretically considered as monolayers with different shape parameters ζ for IgG molecules and for IgG–anti-IgG complex. The angular position of SPPR after washing with glycine buffer (pH 2.2) indicates that influence of nonspecific adsorption of anti-IgG on the SPPR response in this case is small and the gradient between B and C levels reflects mainly the binding of anti-IgG molecules with specific sites of IgG molecules and the creation of a complex. The value ζ for complex IgG–anti-IgG can be taken as noticeably smaller in comparison with a single IgG molecule due to increased eccentricity of the complex relative to a single molecule

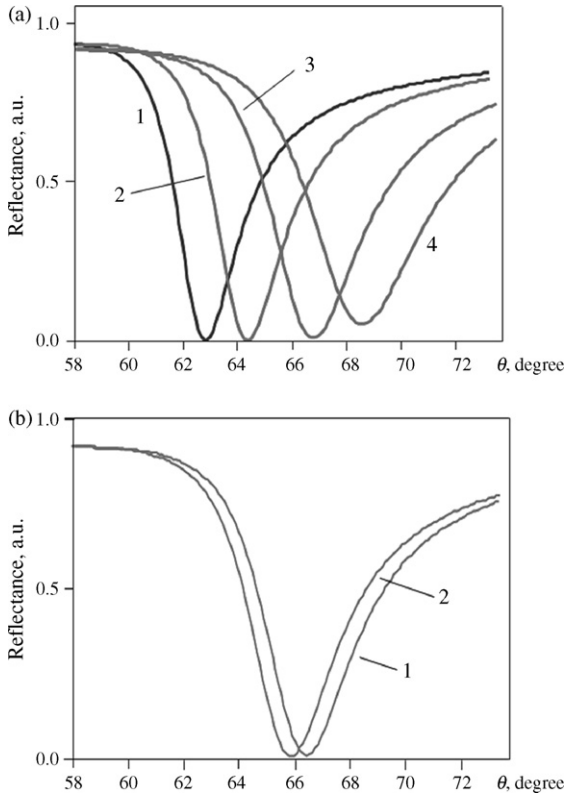

Fig. 2. (a) Calculated SPPR curves dependent on the shape of molecules. (1) Free surface, $\theta_{min} = 62.747^\circ$; (2) oblate molecules $\zeta = 2.0$, $\theta_{min} = 64.262^\circ$; (3) prolate molecules $\zeta = 0.12$, $\theta_{min} = 66.585^\circ$; (4) prolate molecules $\zeta = 0.11$, $\theta_{min} = 68.302^\circ$. (b) Calculated dependences of SPPR curves on the composition of the molecular film consisting of prolate ($\zeta = 0.12$) and oblate ($\zeta = 2.0$) molecules. Part of prolate molecules: $f = 1$ (curve 1, $\theta_{min} = 66.282^\circ$) and $f = 0.5$ (curve 2, $\theta_{min} = 65.777^\circ$).

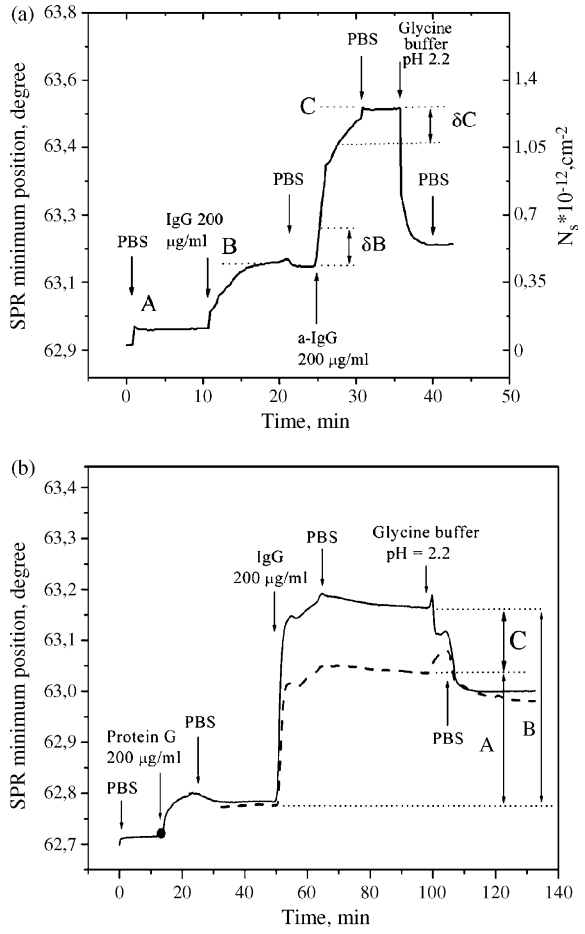

Fig. 3. (a) The kinetic dependence of SPPR angular position for reaction IgG–anti-IgG. The right axis represents surface concentration for IgG monolayer (up to level B) or for complex IgG–anti-IgG (up to level C); δB and δC: deviations of SPPR minimum angular position (arrows show direction of deviation). (b) The typical comparative kinetic dependence of SPPR angular position at the adsorption of IgG molecules onto bare gold surface (dash line) and surface covered by protein G molecules (solid line). (A) SPPR response for IgG adsorption onto the bare gold surface after PBS washing; (B) the same for the surface, modified with protein G; (C) difference, related to modification of sensors surface.

and, respectively, the shift of SPPR angle position after IgG–anti-IgG binding is expected to be greater. This supposition correlates with calculated SPPR shift values (see Fig. 2a) and with those observed in Fig. 3a for kinetic dependence where shift of SPPR angle position for anti-IgG binding is noticeably larger, than for IgG monolayer adsorption despite of the equal concentration of IgG and anti-IgG solutions (200 μg/ml) and approximately the same molecular mass both for IgG and anti-IgG. It should be noted that the result shown

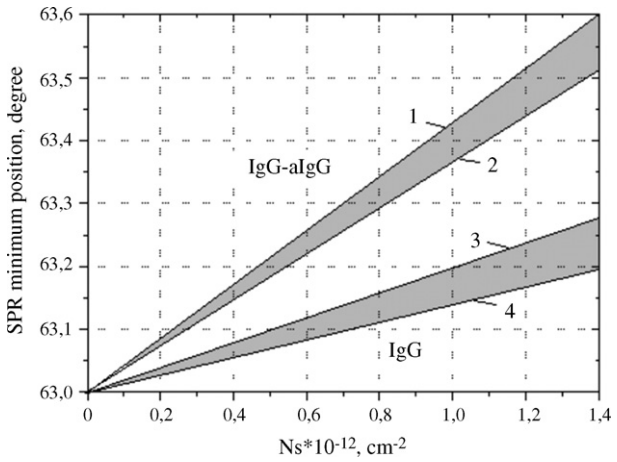

Fig. 5. The calculated dependences of the surface concentration for IgG molecules and IgG–anti-IgG complexes via SPPR angular response. For positions 1 and 3: $\zeta = 0.15$; for positions 2 and 4: $\zeta = 2$.

in Fig. 3a represents the maximum registered response on anti-IgG binding, whereas when experiments were repeated, it was observed that the SPPR-response deviated within certain limits, denoted as δB and δC. It is often observed in SPPR experiments and emphasized here that a strong deviation can readily be explained by changing of parameter ζ for considered monolayers during adsorption of IgG onto Au surface or for IgG–anti-IgG binding process. This supposition is proved by SPPR experiments with oriented immobilization of IgG molecules using protein G (see Fig. 3b). It is known that molecules of protein G, immobilized as the first layer, promote the position of immunoglobulin normally oriented relative to the surface of sensor [11] (see Fig. 4a). The set of two-channel comparative SPPR experiments indicates a clear difference in SPPR response for IgG immobilization onto the gold surface that was modified initially with protein G. A rational explanation for the observation of this difference lies in the presence of the initial protein G layer that would affect the normal orientation of IgG molecules. This explanation is reasonable, because the SPPR response after the removal of specifically immobilized IgG molecules (using glycine buffer, pH 2.2) appears to be the same as the response on the randomly adsorbed IgG monolayer onto the bare gold surface, so the value of the SPPR response in this case depends on part of normally (or not horizontally) oriented IgG molecules for both of the comparative experiments.

Following the theoretical approach for the results presented here, the SPPR response in this study depends on the spatial position of separate biomolecule or biomolecular complex on the surface of Au, if the shape of these objects is elongated, which is a peculiarity of immunoglobulines. Certainly, we are aware that a difference in orientation for similar biomolecules is not the same

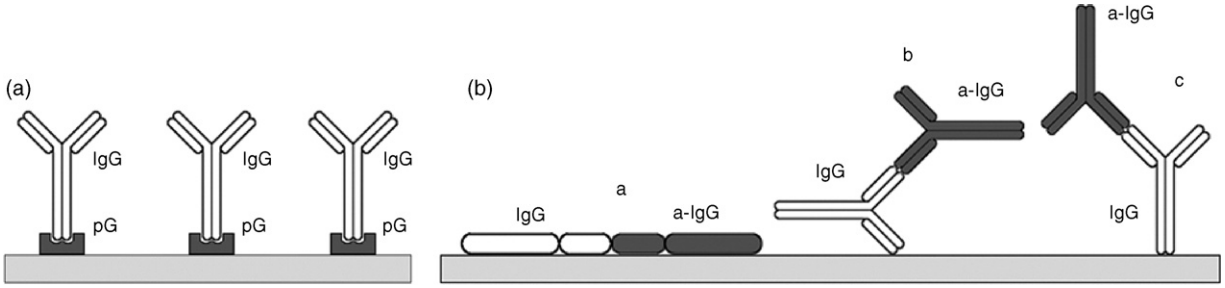

Fig. 4. (a) Scheme of possible orientation of IgG relatively to the surface of Au, modified with protein G. (b) Scheme of possible orientation of IgG–anti-IgG complex relatively to the surface of Au. Here $\zeta_a > \zeta_b > \zeta_c$.

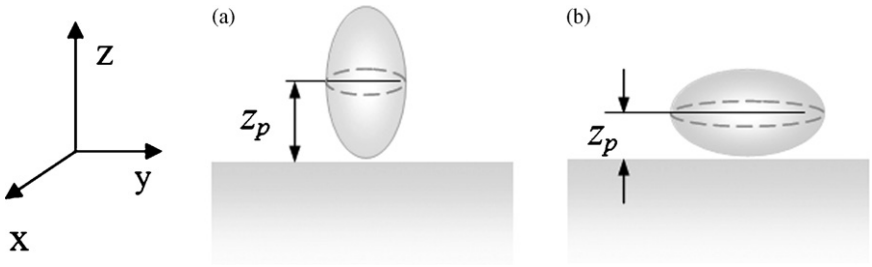

Fig. 6. Albumen molecule at the surface: (a) as prolate ellipsoid; (b) as oblate ellipsoid.

as the difference between prolate and oblate ellipsoids; rather, it is a strong approximation. Concerning this, we could approximate the molecular complexes differently oriented relative to the surface by ellipsoidal particles, characterized by different shape from oblate to prolate. In this case, the maximum SPPR response would be observed when single molecules or complexes are orientated normally to Au surface when immobilized (position c in Fig. 4b). For the intermediate position b a response is decreased and becomes minimal when the complex or molecule is orientated horizontally to the surface (position a). Moreover, one can suppose that the presence of molecular complexes of type c at rather high concentration provides for evident angle shift because for positions a and b, which are modeled by oblate ellipsoids, the angle shift is very weakly dependent on the particle shape.

The roughness of the Au surface plays a part in the disordered character of IgG adsorption and increases the δB and δC values. The influence of biomolecular shape on the deviation of SPPR response becomes smaller with decreasing eccentricity of biomolecule form and is minimal for spherical objects.

The above consideration concerning the 3D-shape of biomolecules makes it possible to calculate nomograms for monolayers of specified type of biomolecule or biomolecular complex, accounting for their geometrical form (or position). Making the assumption that monolayers of biomolecules in the described experiments are packed with the average distances between molecules about their linear dimension, the nomograms for the definition of surface concentration of IgG molecules or IgG–anti-IgG complexes were calculated (see Fig. 5).

The observed deviation of surface concentration N_s (shaded areas) relates to the value of parameter ζ, which characterizes the spatial position of IgG molecule or IgG–anti-IgG complex. Note, nomograms account only for specifically built complexes, without accounting of nonspecific adsorption of anti-IgG at the Au surface. Using the SPPR method it is thus possible to make an approximate estimation of the molecular adsorption directly in units of surface concentration N_s, which for our experiments was about of $(1.1 \pm 0.1) \times 10^{12}\,\mathrm{cm}^{-2}$ for saturated monolayers. Furthermore, the mass of biomolecular layer M_s can be calculated from Eq. (13) directly in mg/m^2 [7]:

$$M_s = \frac{N_s M}{A_m}, \tag{13}$$

where M is molecular mass of biomolecule or biomolecular complex and $A_m = (\tilde{\chi}_\perp + \tilde{\chi}_{||})/2$ (where $\perp$ and $||$ subscripts show that corresponding molecules are situated perpendicularly or parallel to the surface) when the numbers of prolate and oblate particles are equal. The mass of IgG layer was found to be $3.66 \pm 0.5\,\mathrm{mg/m}^2$, whereas the mass of complex was $6.5 \pm 1.0\,\mathrm{mg/m}^2$, about twice the value, accounting for the observed weak nonspecific adsorption.

5. Conclusion

The theoretical background and experimental approach for 3D-quantification of the surface concentration of biological molecules from SPPR data is proposed. In addition to a previously developed method [7] based on the physical model of molecular layer which uses two parameters—polarizability and surface concentration of molecules, the novel approach presented here allows an estimation of the influence of the spatial form of a biomolecule on the SPPR angle position. The SPPR measurements of biospecific reaction between IgG and anti-IgG molecules on the Au surface are performed and the surface concentration of biomolecular layer is estimated. This approach may be useful to determine or estimate the orientation of biomolecules relative to the surface of SPPR-sensors for a more comprehensive understanding and quantitative estimation of biomolecular layers.

Acknowledgements

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Appendix A. Susceptibility of the single molecule at the surface

To calculate the reflection coefficient of the molecular layer one needs to know the initial polarizabilities of the molecule $\chi_{ij}(\omega)$ which describe the linear response of a single molecule situated at the surface on the local field. Because the SPPR simulations here will be performed for the molecules of immunoglobulin, one can make the approximation that the molecule at the surface has a form close to ellipsoid. The linear response of the ellipsoidal particle at the surface was calculated earlier [12]. If semiaxes of the molecule are h_x, h_y, h_z, molecular volume is V_p, the planar component of molecular susceptibility is

$$\tilde{\chi}_{ii} = \varepsilon_r V_p \frac{\varepsilon_p - \varepsilon_r}{\varepsilon_r + (\varepsilon_p - \varepsilon_r) m_i} L_{||}, \quad i = x, y, \tag{A.1}$$

where planar local-field factor is

$$L_{||} = \left[1 + \frac{(\varepsilon_r - \varepsilon_m)(\varepsilon_p - \varepsilon_r)}{3(\varepsilon_r + \varepsilon_m)(\varepsilon_r + (\varepsilon_p - \varepsilon_r) m_i} \vartheta\right]^{-1}, \tag{A.2}$$

where ε_p is a dielectric constant of the particle, ε_m and ε_r are dielectric constants of the substrate and ambient, respectively, m_i is the depolarizing factor and $\vartheta = h_x h_y h_z/(2z_p)^3$ is the local-field factor.

The normal component of molecular susceptibility is

$$\tilde{\chi}_{zz} = \varepsilon_r V_p \frac{\varepsilon_p - \varepsilon_r}{\varepsilon_r + (\varepsilon_p - \varepsilon_r) m_i} L_\perp, \tag{A.3}$$

with

$$L_{\perp} = \left[1 + \frac{(\varepsilon_r - \varepsilon_m)(\varepsilon_p - \varepsilon_r)}{3(\varepsilon_r + \varepsilon_m)(\varepsilon_r + (\varepsilon_p - \varepsilon_r)m_i} 2\vartheta\right]^{-1}. \quad (A.4)$$

The depolarizing factors for molecule as prolate ellipsoid for which $h_z > h_x = h_y$ (see Fig. 6a):

$$m_z = \frac{1-\eta^2}{\eta^3}\left(\frac{1}{2}\ln\frac{1+\eta}{1-\eta} - \eta\right), \quad m_x = m_y = \frac{1}{2}(1 - m_z), \quad (A.5)$$

where $\eta = (1-\zeta^2)^{1/2}$, $\zeta = h_x/h_z$.

For molecule as oblate ellipsoid for which $h_x = h_y > h_z$ (see Fig. 6b) the depolarizing factors are:

$$m_z = \frac{1+\nu^2}{\nu^3}(\nu - \arctan\nu), \qquad m_x = m_y = \frac{1}{2}(1 - m_z), \quad (A.6)$$

where $\nu = (\zeta^2 - 1)^{1/2}$.

One should note that interaction between the molecule and the surface can lead to local field enhancement effect which causes strong enhancement of molecular polarizability [13].

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