Available online at www.sciencedirect.com
SCIENCE DIRECT®
Sensors and Actuators B 95 (2003) 336–343
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Self-assembled multilayer superstructures as immobilization support for bioreceptors

B.A. Snopok[a,*], Yu.G. Goltsov[b], E.V. Kostyukevich[a], L.A. Matkovskaja[b], Yu.M. Shirshov[a], E.F. Venger[a]

[a] *Institute of Semiconductor Physics, National Academy of Sciences of Ukraine, Kiev, Ukraine*
[b] *L.V. Pisarzhevskii Institute of Physical Chemistry, NAS of Ukraine, Kiev, Ukraine*

Abstract

A new strategy for the preparation of layer-by-layer inorganic assemblies are discussed which allows simple fabrication of multifunctional thin film on various substrates. The procedure involves alternating layers of the cyanoferrate derivatives with layers of copper ions. A film growth was investigated in situ by surface plasmon resonance (SPR) and characterized by atomic force microscopy (AFM). It was shown that the topography of the surface strongly depends on a number of layers. In particular, the change-over to the formation of 3D aggregates after 12–15 cycles of deposition was observed. It was demonstrated that biological captors, immunoglobulins, can easily be immobilized onto these films in closely packed oriented monolayer, and stable theoretically predicted response was obtained in SPR geometry. This suggests that the functional multilayer built using this approach will have useful application owing to their controllable chemical reactivity.

Keywords: Layer-by-layer assembly; Cyanoferrate; Immunoglobulins immobilization; Surface plasmon resonance

1. Introduction

Biosensor control systems is one of the fastest developing branches of modern science [1,2]. The highly specific interactions that are characteristic of the molecular biosystems involved in the immune response of living organisms (antigen–antibody reaction) are well known and widely used to form sensing layers in various biosensor systems. Indeed, immunological processes based on the antigen–antibody reaction and underlying most of the affinic biosensors continue to play a leading role in control systems of both different proteins and their complexes [3,4], as well as high-weight (e.g., viruses/cells [5,6]) and low-weight (e.g., hormones, drugs, etc. [7–9]) molecular biosystems. Despite a variety of physical transducers of different types [10] used for creation of bioanalytical control systems, the basic principles of biosensor system function remain unchangeable and are determined, first of all, by their capacity to keep optimal conditions for function of receptor centers of biological origin in artificial systems. Taking into account prime-turn requirements of long-term stability both from the viewpoint of their sensitivity and selectivity, which are imposed upon instrumental means of environment monitoring, interface sensitive architectures should satisfy the following main requirements [11]:

(1) immobilization milieu similar to the natural state: conditions at the surface should maintain, or even increase, receptor stability and functioning;
(2) strong receptor-membrane binding, preferably via covalent coupling;
(3) directional immobilization: to increase active fraction of receptors, their active sites should face outwards from the electrode surface;
(4) arrangement of receptor centers: optimal packing density of receptor centers at the interface should be specific for high-binding activity;
(5) negligible nonspecific binding: to achieve high sensitivity and specificity, nonspecific binding at both receptor and membrane should be suppressed;
(6) controlled immobilization: a mechanism of film formation should preferably be based on self-limiting/self-assembled approaches;
(7) "physically" inert support: a film should permit the transducing mechanism to function satisfactorily;
(8) low-cost technology.

* Corresponding author. Tel.: +380-44-265-5626; fax: +380-44-265-1827.
E-mail address: snopok@isp.kiev.ua (B.A. Snopok).
URL: http://www.isp.kiev.ua.

0925-4005/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0925-4005(03)00536-7

Unfortunately, attempts to design sensing elements that meet these standards have not been successful to date, although various procedures of immobilization (e.g., adsorption on insoluble matrices [12], entrapment in polymers [13], crosslinking by a multifunctional reagents [14], covalent binding onto a membrane [15], etc.) and types of support (such as dextran matrix [8,9], lipid monolayers [16], biotin-streptavidin systems [17], etc.) have been proposed. In this case, sensors based on physical transducers that are sensitive to the parameter changes near the transducer surface (caused by the nature, concentration, or spatial arrangement of guest molecules [18]) should meet strict requirements, such as small thickness, possibility of being fabricated at the surface of small electrodes, stability in liquid phase, "plane" surface, etc.

The general requirements for formation of biocompatible immobilization support with desired receptor orientation at the interface can be attained if nanochemical approaches are used. The results mentioned above are foregone conclusion that immobilization layers will be organized in a rigid framework in a nanometer scale and have active surface groups suitable for direct coupling of bioreceptors in an oriented manner. Thus, both immobilization process and support formation for biorecognition interface must be nano-organized and will be based on a self-assembling methodology. In this work, we suggest a new method for forming an immobilizing structure for affinic sensors of the immune response based on the ideology of self-assembling the whole sensitive biorecognizing architecture.

2. Optical biosensors

A number of standard approaches have been advocated for the analysis of protein adsorption. At the same time, many successes achieved during investigations of biochemical processes at the molecular level have become possible largely owing to the sensor instrument engineering that is actively developed [19]. Various (electrical, thermal, mechanical, optical, etc.) physico-chemical transducers are used in sensor systems. The most promising of them seem to be those based on optoelectronic sensing elements, which can be used to monitor the binding process of unlabeled proteins in real time and represent the biosensor approach in biochemical and analytical sciences [20,21].

Biosensor is the system that transforms information about the process of biochemical interaction of the analyte and the system of receipting centers immobilized on the surface of the physical transducer into the signal that is convenient for further treatment [22]. Usually, it is supposed that receptor centers have biological origin or are the synthesized analogues of the biological ones. Methods of fabrication and purification of receptor centers that have biological origin are widely described in biochemical papers as well as the measurement techniques and the necessary equipment. At the same time, optimization of the immobilization support specific both to the registration method and bioreceptor remains a challenge in many respects. There are two directions of the interfacial optimization, namely

- the aim of the "physical" direction is to provide the most efficient transformation of the changes in the sensitive layer into the output signal; the efficiency is determined by both functional, and structural (topographical and morphological) properties of the immobilization support;
- the aim of the "chemical" direction is to create the conditions for the oriented and nondestructive immobilization of the receptor centers. The "chemical" optimization has two principal aspects: (i) the topographical one that controls the possibility of organized structure formation on the rough surface and (ii) the protecting aspect that prevents the denaturation of biomolecules on the transducer surface.

Application of the optoelectronic effects for the registration of specific intermolecular interaction provides both optimal conditions for biological molecules (in view of the minimization of external influence) and high sensitivity. To illustrate, the systems using SPR offer a number of undeniable advantages that are inherent to optical sensor systems [23]. Indeed, using this direct, nondestructive and quantitative technique, one can work in situ without additional fluorescent or other tracers. In reality, conditions of surface plasmon excitation at the interface between thin gold film and solution under investigation (the effect of the surface plasmon resonance, SPR) depend on small variations of the optical constants of the solution in the vicinity of the surface. Besides, it is possible to monitor the molecular recognition processes in real time and spend only small-volume samples per single analysis procedure. Processes of the intermolecular interaction cause the shift of the minimum of the excitation angle of the polariton states; the changes take place due to the bounding (or releasing) of the molecules from the solutions with the receptor centers that are immobilized on the surface of the optoelectronic transducer—an essential part of biosensing instruments. All these enable one to obtain additional reliable information on kinetics of processes involving molecular biosystems of high sensitivity.

Although optical transducers where SPR is used possess the above advantages, the following two main requirements substantially retard wide use of such systems when analyzing various biochemical processes occurring at the molecular level. First, to provide the highest responsivity, the immobilization layer thickness has to be substantially less than the penetration depth for an evanescent wave (i.e., below 100–200 nm for radiation from the visible spectral region). Second, adsorption of biomolecules onto the transducer surface should not lead to loss in their functional capabilities. The restrictions discussed above dictate a need for quest of transition layers that should reduce a destructive effect of metal surface. Besides, the sensing surface of a transducer has to provide optimum conditions for receptor centers to function, as well as retention of high stability

and manufacturing compatibility with techniques used to fabricate sensing elements.

3. Experimental

3.1. Probe molecules and supports

Goat Anti-Mouse IgG, Mouse IgG, Goat Anti-Rabbit IgG, Rabbit IgG, Goat Anti-Human Fibrinogen, Rabbit Anti-Bovine Serum Albumin and Bovine Serum Albumin (BSA) were obtained from Sigma Chemicals and were used as supplied without further purification.

Human fibrinogen was prepared from human plasma by sodium sulfate precipitation [24]. All other chemicals were reagent grade or better. Water was deionized and then distilled in a silica and Teflon apparatus prior to use.

Protein reactions were made in phosphate buffer saline PbS solution (pH 7.2) at the ambient temperature (20±2 °C).

3.2. Film deposition

The chip support is a trapeziform glass (K-8) prism of dimensions 18 mm × 12 mm × 6 mm and inclination angle of 68°. The refractive index of the prism is 1.515. For the sensor formation, the upper side of the support prism was sputtered with a ca. 1 nm adhesive layer of Cr, followed by 45 nm of Au. During deposition the prism was kept at the 22 °C with subsequent holding (3 h) in high vacuum (5×10^{-4} Pa) before removing from the evaporation equipment (VUP-5M). Sensor chips were used for measurements without any additional cleaning procedures prior to an experiment. Untreated chips were mounted in the instrument and flushed with buffer until the baseline drift was less than ±30 angle second.

3.3. Immobilization procedure and reagents

The formation scheme of gold surface multilayer modification system for site-oriented immobilization of antibodies (4) (Fig. 1) includes two steps [25]. At first, self-assembled carboxylic-acid-terminated alkanethiol monolayers was deposited (2). The surface of gold was modified by exposing the newly produced films to the ethanol solution of dodecanthiol ($HS(CH_2)_{11}COOH$, concentration of 5×10^{-4} mol/l) for 13 h at a constant temperature of 297 K. The samples were then washed in the flow of pure ethanol for 1 h to remove residue of physically sorbed dodecanthiol and were dried in a flow of dry and dust-free air. We used these highly ordered organic films as the protective and stabilizing ones for gold surface (1), as well as for chemical attachment of layered cyanoferrate nanoarchitectures (3). After that, fabrication of the copper aminopentacyanoferrate(II) $Cu_3[Fe(CN)_5NH_3]_2$ film was carried out using alternating adsorption of metal (Cu^{2+}) and complex ($[Fe(CN)_5NH_4]^{3-}$) ions from water solution $CuSO_4$ and $Na_3[Fe(CN)_5NH_3]$.

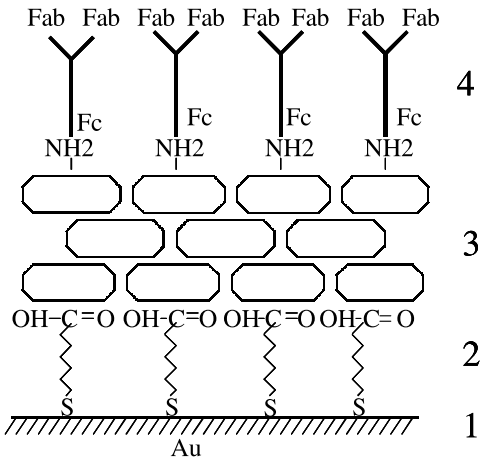

Fig. 1. A copper aminopentacyanoferrate hybrid superstructure onto a suitably functionalized substrate shown for site-oriented immobilization of antibodies (4): 1—gold film; 2—self-assembled carboxylic-acid-terminated alkanethiol monolayers $HS(CH_2)_{15}COOH$; 3—layered copper aminopentacyanoferrate(II) film $Cu_3[Fe(CN)_5NH_3]_2$.

Si_3N_4 surface activation was performed using $Br–(CH_2)_2–Br$ bifunctional compound in accordance with [26]. After this procedure C–Br groups were created at the surface, these groups being capable of bonding with ethylendiamine (en, $NH_2–(CH_2)_2–NH_2$). The support with modified surface was used for layer-by-layer deposition described above.

Formation of copper aminopentacyanoferrate multilayer was performed using freshly prepared sodium aminopentacyanoferrate(III) that was synthesized by standard principles [27] on the base of $K_4[Fe(CN)_6]\cdot 3H_2O$ compound and analyzed with visible and infra-red spectra (“Specord-M40”, “Specord-75IR”).

3.4. Surface analysis

The AFM imaging was undertaken using a commercial Nanoscope IIIa (Digital Instrument, Santa-Barbara) equipped with a 80 μm scanner. For the AFM analysis we used Si_3N_4 plates. The scans were performed in the tapping force mode using commercially available AFM tips made of silicone nitride with a scan frequency of approximately 1 Hz.

3.5. Instrumentation

The detailed description of the Surface Plasmon Resonance Sensor Instrument *BioHelper* (PLASMON-002) built by the Institute of Semiconductor Physics (ISP NASU, Kiev, Ukraine) was discussed early [21,28]. Briefly (Fig. 2), the light beam from the He–Ne laser ($\lambda = 632.8$ nm) is deflected by a mirror to the prism with sensitive layer mounted on the revolving table, which can be rotated by a stepper motor by means of a micrometer screw with a lever arm. The position

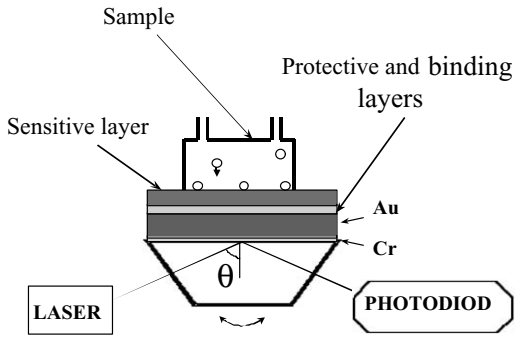

Fig. 2. Schematic arrangement of the components in the surface plasmon resonance analytical instrument *BioHelper* (Plasmon-002).

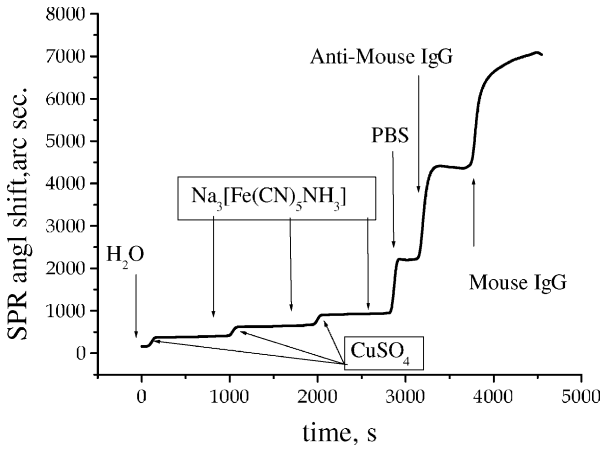

Fig. 3. Procedure of layer-by-layer formation of inorganic architecture (alternating injection $CuSO_4$ and $Na_3[Fe(CN)_5NH_3]$ water solution)—three steps and one-step immobilization of Anti-Mouse IgG and its interaction with Mouse IgG (100 μg/ml concentration).

of the stepper motor is monitored by software means with respect to the hardware reference point. A photodiode detector is positioned at the opposite side of the prism and it monitors the laser light undergoing total internal reflection on the sensor chip. For a complete scan (ca. 15°), the table must move from the left-most to the right-most position and back again, which takes about 15 s. A "track minimum" scan which is used to analyze binding and dissociation kinetics lasts ca. 2 s. The peristaltic pump provides flow of solutions through the experimental cell, which is formed of silicone rubber covered by the Teflon holder and is mounted directly onto the sensor chip surface. The cell has a round cross-section of ca. 25 mm^2 in the direction perpendicular to the prism surface, with a height of 1 mm and a volume of ca. 25 μl.

4. Results and discussion

At first we will analyze the formation of self-assembled structures monitored by SPR in order to find out the influence of the number of layers on effective optical parameters of thin cyanoferrate films. Then we will discuss the results of the topographic (AFM) investigations in view of their correspondence to the SPR results. In the next section, the in situ investigations of both antibodies immobilization adsorption and subsequent interactions with corresponding antigens are described in detail. The final section is devoted to the discussion of possible immobilization mechanisms and peculiarities of the interfacial bioreceptor structure.

4.1. Layer-by-layer formation of immobilization support: in situ SPR monitoring

It is essentially to note that optoelectronic transducers like those based on SPR phenomenon can be also used to investigate processes of formation of spatially organized structures within tens and hundreds angstroms scale that are important for both fundamental researches and applications because they are the base for the development of new technologies in microelectronics, heterogenic catalysis, and sensor production. It is obvious that the possibility of forming the above self-assembled monolayers with given chemical functionality, which are responsible for the specificity of the sensor, depends mainly on the possibility of creating the conditions for self-organizing the molecular ensembles on the surface of solids.

In order to illustrate the procedure of layer-by-layer formation of inorganic layered architecture and investigation of biomolecular interaction, a typical experiment was monitored by SPR system (Fig. 3). The water was allowed to flow through the cell with carboxylic-acid-terminated alkanethiol monolayer covered SPR chip and then sharply substituted with the $CuSO_4$ water solution. After the sensor response reached an equilibrium state without flow, the $Na_3[Fe(CN)_5NH_3]$ water solution was added and after it achieved equilibrium, was again replaced by water. The procedure was repeated in accordance with a given number of layers. The dependence of an SPR angle shift on the layer number is shown in Fig. 4. As Fig. 4 testifies, the SPR angle increases with the number of layers (if it is more than 3), with the mean value of an increment of about 300–400. It is necessary to note that the response increase has a linear character with an increasing number of layers (at least, if this number is more than 3 and less than 10), which can testify to the formation of an ordered structure on the surface. In this case, layer parameters are identical in the considered thickness range. Some features of first-layer formation (less than the response values) are conditioned by the presence of support nonuniformities (and, consequently, of the thiol layer, as shown, e.g., in [29]), which was repeatedly observed when forming the self-assembled films [30]. It is necessary to stress that no additional loosely bound molecules from the sensor surface was removed during washing with pure water. Such a result is in excellent agreement with the previous assumption that those surface transformations include self-organization of

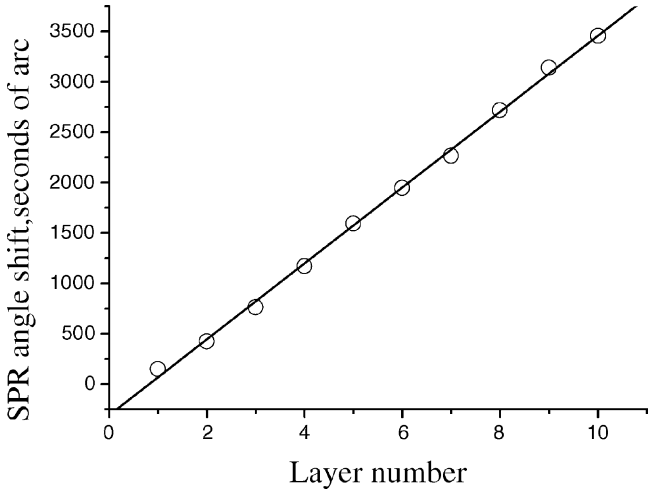

Fig. 4. Angle shift of the SPR minimum position (second of arc) vs. layer number of copper aminopentacyanoferrate(II) film $Cu_3[Fe(CN)_5NH_3]_2$.

copper aminopentacyanoferrate ($Cu_3[Fe(CN)_5NH_3]_2$) layers fastened by Cu^{2+} bridges with different arrangement of primer nucleator molecules on the surface.

4.2. AFM imaging

The same procedure to form the immobilization support on the gold-coated SPR prisms was used for fabrication of the samples for AFM investigations. In this case, as substrates we used silicon plates with an external layer of silicon nitride. Low values of roughness intrinsic to these samples, even after processing them with dibromethane and ethylendiamine, enabled us to check for the proper growth of the ordered cyanoferrate structure neglecting a contribution of large-scale relief nonuniformities inherent to gold films. Indeed, the AFM image in Fig. 5 shows that the gold surface is not atomically smooth with the grain diameter of the gold coating ranging from 20 to 30 nm. The surface roughness shows a maximum height variation of 2.5 nm for a 500 nm scan, with the area root-mean-square (RMS) surface roughness ca. 0.84 nm. The situation with Si_3N_4 samples is quite different,—the RMS is less than 0.4 nm and without long-range fluctuations.

In order to fabricate the cyanoferrate films on the Si_3N_4 surface, previous treatment was done in accordance with the procedure described in [27] and described briefly above. The AFM results depicted in Fig. 5 clearly show that under the adsorption conditions employed in this study, some first layers repeat the substrate structure, at least in a statistical sense, while on increasing the layer number over 10 we can clearly observe formation of three-dimensional aggregates. Thus, with the increasing layer number, defects condensing in the course of this layer-by-layer growth cause destruction of two-dimensional ordering and create conditions for the following preferable formation of a bulk phase. It is obvious that for purposes of forming ordered layers of bioreceptors this regime is less preferable. It is especially important to note that the SPR method implies the use of plane-parallel boundaries of surface architectures to calculate layer parameters using a value of registered signals when solving the reverse problem [31]. But really the thickness dispersion of the immobilization layer that reaches tens of nanometers can essentially reduce adequacy of this method. Moreover, it was also shown that as the number of layers increased, the number of defects and holes in the layer also increased. Taking into consideration relatively large dimensions of this object, it is possible to suggest that this observation may have originated possibly due to some local self-organization in molecular layers with participation of metal (Cu^{2+}) and complex ($[Fe(CN)_5NH_4]^{3-}$) ions. To go inside the internal structure of such aggregates, additional information about the possible packed structures is required.

4.3. Immobilization performance

Our analysis of the immobilized antibody quantity (for anti-BSA) on the layer number (resonance angle shift amount to 4750 ± 250 arc s. for 3, 5 and 10 layers) testifies to the fact that this quantity has only weak dependence on the layer number in the range between 3 and 10 where one can observe the most ordered structure of the interface layer using data both AFM and SPR. The presence of small-scale defects has no essential role in this case as the immunoglobulin molecule size considerably exceeds the lattice constant of the structure that enables us eventually to provide effective immobilization of antibodies. At the same time, immobilization efficiency strongly depends on the antibody source (see Table 1). But this effect is not unique, e.g., protein A affinity also considerably differs when using various antibody sources [32]. While in the last case this selectivity is related to some features of protein A spatial structure, it is not acceptable for cyanoferrate layers. It enables us to assume that the immobilization efficiency is determined by chemical activity of Fc fragments in immunoglobulin molecules, which have a various compositions in different immunoglobulins.

Although the immobilized receptor quantity is an important parameter, however, it is valid only in the case when the immobilized centers can combine with complementary

Table 1

Antibody/antigen couple	Maximal sensor response for immobilized antibody (arc second)	Antigen/antibody ratio
Anti-Mouse IgG/Mouse IgG	2400	0.91
Anti-Rabbit IgG/Rabbit IgG	4540	0.47
Anti-Bovine Serum Albumin/Bovine Serum Albumin	5150	0.08
Anti-Human Fibrinogen/ Fibrinogen	6560	0.74

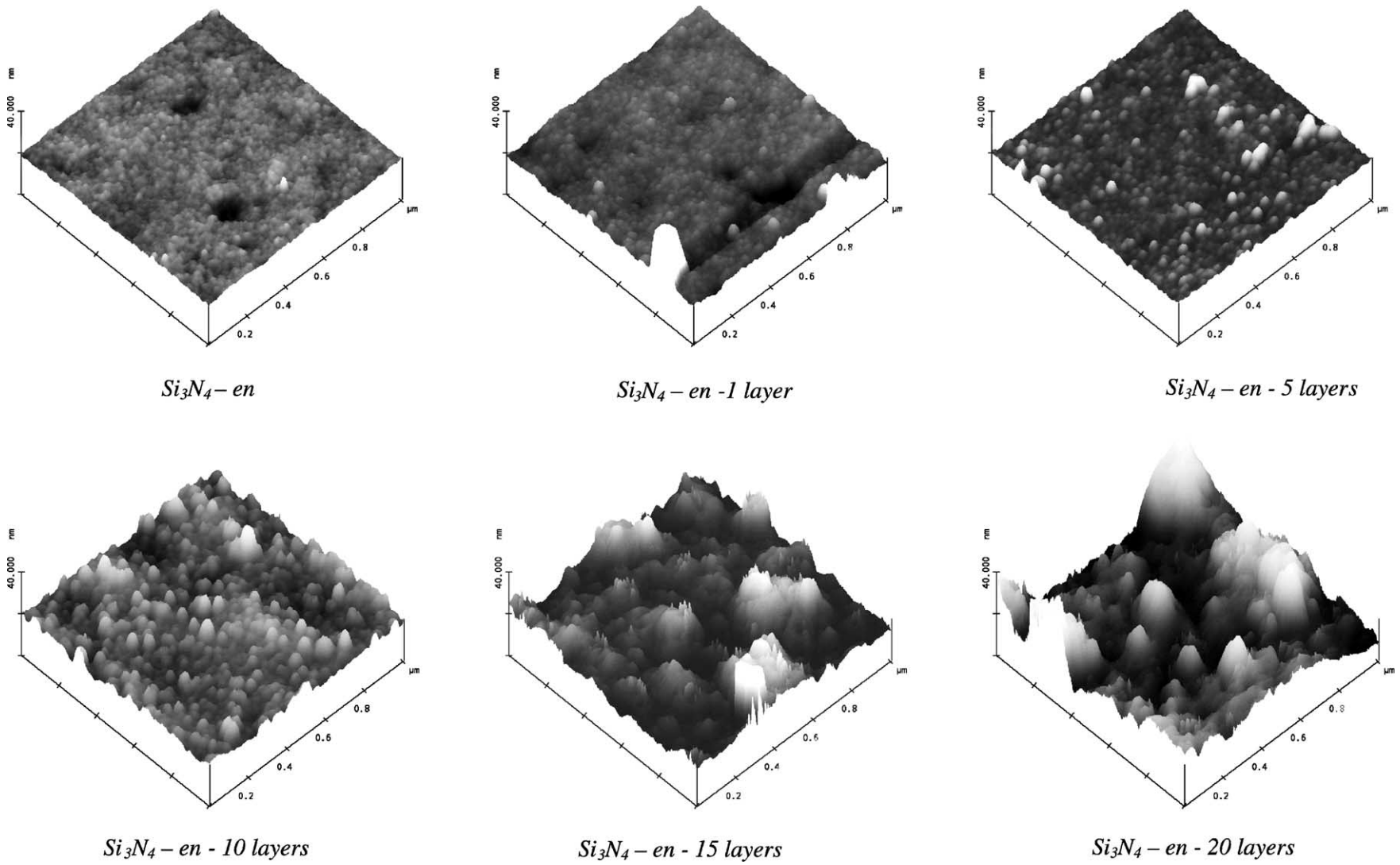

Fig. 5. Atomic force microscope images (1000 nm square scan) of self-assembled multilayers obtained by alternate deposition of $CuSO_4$ and $Na_3[Fe(CN)_5NH_3]$ water solutions.

antigens at respective selectivity. Data given in Table 1 show that, as noticed above, not only the immobilization efficiency but also the capability to bind antigen essentially depends on the antibody nature. Indeed, despite the large absolute responses to Anti-Bovine Serum Albumin, the response to complementary antigen (BSA) is absent. At the same time, for the couple of mouse immunoglobulins (Goat anti-Mouse IgG/Mouse IgG) at a minor increase of an absolute signal value as compared to nonmodified gold surface, the reaction has a character of monomolecular interaction. This enables us to use this immobilization procedure for these immunoglobulins. Peculiarities of interaction of Goat anti-Rabbit IgG/Rabbit IgG when using a cyanoferrate immobilization structure results in the fact that no more than a half of antibodies can interact with complementary antigens. The most interesting result is observed in the case of the couple Goat anti-Human Fibrinogen/Human Fibrinogen. In this case, the density of immobilized antibodies approaches a theoretically possible limit of the antibody density on a plane surface calculated from the conditions of a dense package. Antigen/antibody signal ratio approaches to 0.7 that exceeds, to some extent, that of a dense-packed fibrinogen layer on such plane surface. Indeed, taking the density of protein layers to be the same and taking into account the immunoglobulin thickness (ca. 10 nm) and that of the fibrinogen D-fragment (ca. 4–5 nm), the fibrinogen/antibody signal ratio should not exceed 0.5. The observed increase may be explained by a fibrinogen bend in the fragment E range that increases an effective thickness of the fibrinogen layer. All these enable us to conclude that the fibrinogen molecule is disposed as lying on the surface of polycnonal antibodies as it was observed by us earlier in the case of nonmodified gold surface, too [21].

5. Concluding remarks

The most commonly used convention for the formation of sensitive layers employs either biomimetic methodology with simplified models of natural products or biotechnology approaches based on the utilization of native biomolecules. However, the formation of novel sensor systems requires the integration of major achievements in both fields to take advantage of the high sensitivity and specificity peculiar to bio- or synthetic receptors—from the one hand, and reproducibility and stability of artificial membrane (support system)—from the other. The main efforts in this way must be devoted to the control of status of bioreceptor at the interface.

Self-assembled superstructures, including layer-by-layer assembly of multicomposite films, have generated great interest during the last few years for various sensor applications because of the possibility of controlling the composition, orientation, thickness, and chemical functionality of interfacial captors. Several approaches have been developed for fabrication of the layered architectures with wide spectrum of driving forces controlling supramolecular organization at the interface [33–37]. These include multilayer self-assembly based not only on ionic interactions, but also on charge transfer, hydrogen bonding, and more recently, using coordination chemistry. The “hybrid” metal system described here related to the coordination complexes, which combine the different chemistries of substituted cyanoferrate complexes and copper ions. The immobilization mechanism is assumed to be based on hydrolysis of the substituted cyanoferrate groups. The combination of these two self-assembly chemistries allows for tractable growth by simple consecutive adsorption from solution of hybrid superstructures with interesting properties, such as one-step immobilization of immunoglobulins. This suggests that superstructure multilayers built using this chemistry will have use not only as materials but also as effective immobilization support.

References

[1] P. Bongrand, Ligand-receptor interactions, Rep. Prog. Phys. 62 (1999) 921–968.
[2] A.F. Collings, F. Caruso, Biosensors: recent advances, Rep. Prog. Phys. 60 (1997) 1397–1445.
[3] G.V. Beketov, Yu.M. Shirshov, O.V. Shynkarenko, V.I. Chegel, Surface plasmon resonance spectroscopy: prospects of superstrate refractive index variation for separate extraction of molecular layer parameters, Sens. Actuat. B 48 (1998) 432–438.
[4] K.V. Kostyukevych, B.A. Snopok, S.A. Zinio, Y. Shirshov, I. Kolesnikova, E.V. Lugovskoi, New opto-electronic system based on the surface plasmon resonance phenomenon: application to the concentration determination of DD-fragment of fibrinogen, in: Proceedings of the SPIE, Start-up Opportunities, and Strategic Alliances, Quebec, vol. 3414, 1998, pp. 290–301.
[5] P. Boltovets, V. Boyko, I. Kostikov, N. Dyachenko, B. Snopok, Yu. Shirshov, Simple method for plant virus detection: effect of antibody immobilization technique, J. Virol. Meth. 105 (1) (2002) 141.
[6] M.H.V. Regenmortel, The antigenicity of tobacco mosaic virus, Phil. Trans. Roy. Soc. Lond. B 354 (1999) 559–568.
[7] U. Schlecht, Y. Nomura, T. Bachmann, I. Karube, Reversible surface thiol immobilization of carboxyl group containing haptens to a biacore biosensor chip enabling repeated usage of a single sensor surface, Bioconjugate Chem. 13 (2002) 188–193.
[8] P. Gomes, D. Andreu, Direct kinetic assay of interactions between small peptides and immobilized antibodies using a surface plasmon resonance biosensor, J. Immunol. Meth. 259 (2002) 217–230.
[9] S. Tombelli, M. Minunni, M. Mascini, A surface plasmon resonance biosensor for the determination of the affinity of drugs for nucleic acids, Anal. Lett. 35 (4) (2002) 599–613.
[10] B.A. Snopok, I.V. Kruglenko, Multisensor systems for chemical analysis: state-of-the-art in electronic nose technology and new trends in machine olfaction, Thin Solid Films 418 (2002) 21–41.
[11] B.A. Snopok, K.V. Kostyukevych, G.V. Beketov, S.A. Zinio, Yu.M. Shirshov, E.F. Venger, Biochemical passivation of metal surfaces for sensor application: reactive annealing of polycrystalline gold films in hydrogen sulfide atmosphere, Semicond. Phys. Quant. Electron. Optoelectr. 3 (1) (2000) 59–68.
[12] M. Knichel, P. Heiduschka, W. Beck, G. Jung, W. Goepel, Utilization of a self-assembled peptide monolayer for an impedimetric immunosensor, Sens. Actuat. B 28 (1995) 85–94.

[13] K. Arisumi, F. Feng, T. Miyashita, H. Ninomiya, Functionalization of polymer Langmuir–Blodgett films using active ester groups, Langmuir 14 (1998) 5555–5558.
[14] W.H. Scouten, J.H.T. Luong, R.S. Brown, Enzyme or protein immobilization techniques for applications in biosensor design, Tibtech 13 (1995) 178–185.
[15] B. Raguse, V. Braach-Maksvytis, B.A. Cornell, L.G. King, P.D.J. Osman, L. Wieczorek, Tethered lipid bilayer membranes: formation and ionic reservoir characterization, Langmuir 14 (3) (1998) 648–659.
[16] B. Raguse, V. Braach-Maksvytis, B.A. Cornell, L.G. King, P.D.J. Osman, R.J. Pace, L. Wieczorek, Tethered lipid bilayer membranes: formation and ionic reservoir characterization, Langmuir 14 (1998) 648–659.
[17] F.-J. Schmitt, L. Haussling, H. Ringsdorf, W. Knoll, Surface plasmon studies of specific recognition reactions at self-assembled monolayers on gold, Thin Solid Films 210–211 (1992) 815–817.
[18] S. Spinke, M. Liley, F.J. Schmitt, H.S. Guder, L. Angermaier, W. Knoll, Molecular recognition at self-assembled monolayers: optimization of surface functionalization, J. Chem. Phys. 99 (1993) 7012–7019.
[19] J.J. Ramsden, Optical biosensors, J. Mol. Recogn. 10 (1997) 109–120.
[20] W. Knoll, Guided wave optics for the characterization of polymeric thin films and interfaces, in: R.E. Hummel, K.H. Guenther (Eds.), Handbook of Optical Properties, Thin Films for Optical Coatings, vol. 1, CRC Press, Boca Raton, 1997, Chapter 3.
[21] B.A. Snopok, K.V. Kostyukevich, S.I. Lysenko, P.M. Lytvyn, O.S. Lytvyn, S.V. Mamykin, S.A. Zynyo, P.E. Shepelyavyj, E.F. Venger, S.A. Kostyukevich, Yu.M. Shirshov, Optical biosensors based on the surface plasmon resonance phenomenon: optimization of the metal layer parameters, Semicond. Phys. Quant. Electron. Optoelectr. 4 (1) (2001) 56–69.
[22] D. Thevenot, K. Toth, R. Durst, G. Wilson, Pure Appl. Chem. 71 (12) (1999) 2333.
[23] P.M. Boltovets, B.A. Snopok, Yu.M. Shirshov, N.S. Dyachenko, Arranged protein layers for optoelectronic biosensor systems: oriented protein immobilization on modified gold surface, Rep. Nat. Acad. Sci. Ukraine 11 (2001) 136–143.
[24] T.V. Varetskaya, Microheterogeneity of fibrinogen. Cryofibrinogen, Ukrainskii Biokhimichnii Zhurnal 32 (1960) 13–24 (in Ukrainian).
[25] Yu.G. Goltsov, L.O. Matkovskaya, B.A. Snopok, E.V. Kostyukevich, Yu.M. Shirshov, Bio-optoelectronic transducer for stereo chemical control of active molecules, Ukrainian Patent UA 37078 (2001).
[26] M.A. Karymov, A.A. Kruchinin, Yu.A. Tarantov, I.A. Balova, L.A. Remisova, Yu.G. Vlasov, Fixation of DNA directly on optical waveguide surfaces for molecular probe biosensor development, Sens. Actuat. B 29 (1995) 324–327.
[27] G. Brauer (Ed.), Handbook on Inorganic Synthesis, vol. 5, Mir, Moskow, 1985, pp. 1761–1762 (in Russia).
[28] B.A. Snopok, K.V. Kostyukevych, O.V. Rengevych, Yu.M. Shirshov, E.F. Venger, I.N. Kolesnikova, E.V. Lugovskoi, A biosensor approach to probe the structure and function of the adsorbed proteins: fibrinogen at the gold surface, Semicond. Phys. Quant. Electron. Optoelectr. 1 (1) (1998) 121–134.
[29] S.I. Lysenko, B.A. Snopok, V.A. Sterligov, E.V. Kostyukevich, Yu.M. Shirshov, Light scattering by molecular-organized films on the surface of polycrystalline gold, Opt. Spectrosc. 90 (4) (2001) 606–616.
[30] B.A. Snopok, P.E. Strizhak, E.V. Kostyukevich, V. Serebriy, S.I. Lysenko, P.E. Shepeliavii, S.L. Priatkin, S.A. Kostyukevich, Yu.M. Shirshov, E.F. Venger, Interfacial architecture on the fractal support: polycrystalline gold films as support for self-assembling monolayers, Semicond. Phys. Quant. Electron. Optoelectr. 2 (3) (1999) 86–97.
[31] O.V. Rengevich, Yu.M. Shirshov, Yu.V. Ushenin, A.G. Beketov, Separate determination of thickness and optical parameters by surface plasmon resonance: accuracy consideration, Semicond. Phys. Quant. Electron. Optoelectr. 2 (2) (1999) 8–35.
[32] N.L. Brown, S.P. Bottomley, M.G. Gore, Affinity purification of human IgG using immunobilised, immunoglobulin-binding domains from protein A of Staphylococcus aureus, Biochem. Soc. Trans. 26 (3) (1998) S249.
[33] G. Decher, Layered nanoarchitectures via directed assembly of anionic and cationic molecules, in: G. Abberti, T. Bein (Eds.), Comprehensive Supramolecular Chemistry, Elsevier, Netherlands, 1996, Chapter 14, pp. 507–530.
[34] C. Mingotaud, C. Lafuente, J. Amiell, P. Delhaes, Ferromagnetic Langmuir–Blodgett film based on Prussian blue, Langmuir 15 (2) (1999) 289–292.
[35] M.A. Ansell, A.C. Zeppenfeld, K. Yoshimoto, E.B. Cogan, C.J. Page, Self-assembled cobalt-diisocyanobenzene multiplayer thin films, Chem. Mater. 8 (3) (1996) 591–594.
[36] M.A. Rodrigues, D.F.S. Petri, M.J. Politi, S. Brochsztain, Novel self-assembled films of zirconium phosphonate 1,4,5,8-naphthalenediimides, Thin Solid Films 371 (2000) 109–113.
[37] M.A. Ansell, E.B. Cogan, G.A. Neff, R. Roeschlaub, C.J. Page, Self-assembly of thin film superstructures based on alternating metal-bisphosphonate and cobalt-disocyanide layers, Supramol. Sci. 4 (1–2) (1997) 21–26.

Biographies

B.A. Snopok received his MSc degree in Physics, Chemistry and Biology from Moscow Institute of Physics and Technology in 1988, PhD from Institute of Physics, Kiev in 1995, and is currently Senior Scientific Officer at the Institute of Semiconductor Physics, Kiev. Current research interests include interfacial phenomena in (bio)chemical sensor application, chemical imaging, monolayers and Langmuir–Blodgett films, organic semiconductors, self-organization and growth processes of thin organic films, intermolecular interactions and electron/phonon processes in organic materials.

E.V. Kostyukevich was born in Kiev (Ukraine) on May 26, 1955. She received her engineering degree in semiconductor electronics (1977) from Kiev University, Radiophysics Department, and PhD in Physics and Mathematics from the Institute of Semiconductor Physics (National Academy of Sciences of Ukraine) in 2001. Research interests are focused on technological aspects of development of the optical sensor sensitive elements and methods of investigation of the molecular interactions.

Yu.M. Shirshov was born in Orenburg, Russia, on January 25, 1940. He received the MS degree from Chernovitz University in 1963. Since 1963 he has done postgraduation at the Institute of Semiconductor Physics, National Academy of Sciences of Ukraine (Kiev). Since 1966 he has been senior researcher (since 1970), leading researcher (since 1975), head of the Laboratory of Molecular Electronics (since 1984), head of department of functional optoelectronics (since 1995). Candidate of Phys.-Math. Sci. (1970), Doctor of Phys.-Math. Sci. (1991). Areas of research activity: molecular phenomena on the semiconductor and insulator surface, optoelectonic and microelectronic chemical and biosensors. He has authored and coauthored over 100 research papers and a book.

E.F. Venger was born on April 19, 1947. He received the MS degree from Vinnitsa Educational College in 1970. Since 1978 he has been with the Institute of Semiconductor Physics, National Academy of Sciences of Ukraine (Kiev) as junior researcher, senior researcher (since 1988), leading researcher (since 1990), head of the Department of Semiconductor Heterostructures (since 1992). Doctor of Phys.-Math. Sci. (1990), Professor (1992). Areas of research activity: spectroscopy of surface vibrational and plasma polaritons in semiconductor structures; computer simulation of relaxation processes in ion-implanted semiconductors; optical properties of the ultra-dispersed systems.