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Chapter · September 2020

DOI: 10.1007/978-94-024-2030-2_14

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MODEL OF INTERACTION BETWEEN TiO_2 NANOSTRUCTURES AND BOVINE LEUCOSIS PROTEINS IN PHOTOLUMINESCENCE BASED IMMUNOSENSOR

Short title: Interaction of TiO_2 and BLV proteins

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Abstract

A model of interaction between photoluminescent TiO_2 nanoparticles and Bovine Leucosis proteins *gp*51 during the formation of optical immunosensor for the determination of *gp*51 antibodies has been proposed. The main reason of the changes in the photoluminescence (PL) spectra (i.e. PL maxima shifts and PL intensity variations) as a result of Bovine Leucosis proteins adsorption on the surface TiO_2 thin film is an electrostatic interaction between the TiO_2 surface charge and partial uncompensated charges of *gp*51 proteins.

Keywords: TiO_2 nanoparticles, photoluminescence, Bovine Leucosis, immunosensor

1. Introduction

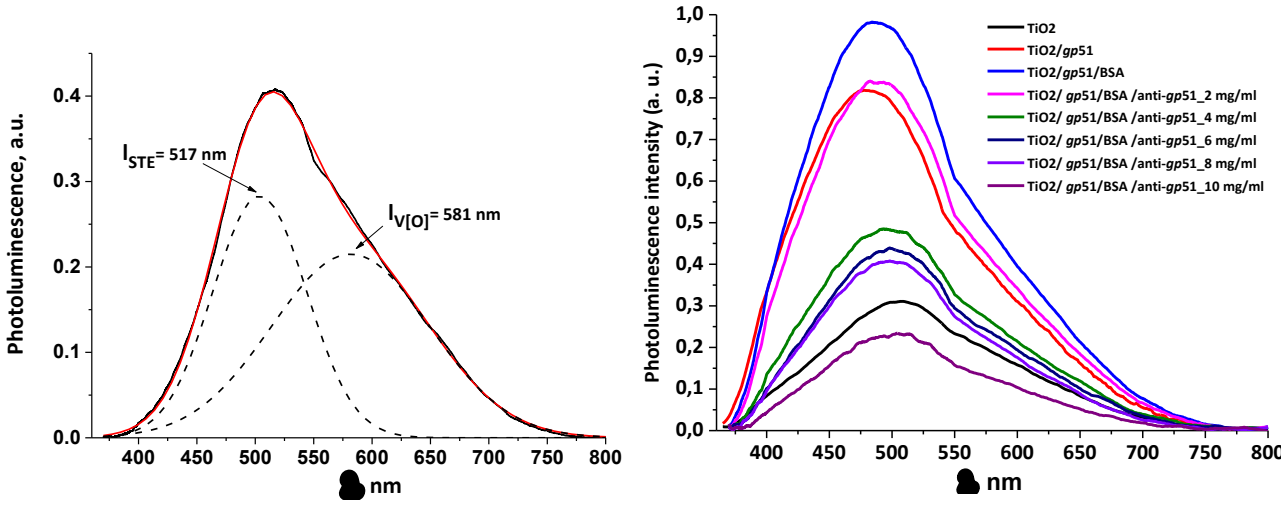
Nanostructured Titanium dioxide (TiO_2) is a popular material for biosensors application due to its good bio-compatibility and high chemical stability. Being a wide bandgap semiconductor that has an intense photoluminescence (PL) at room temperature, TiO_2 is broadly applied in optical bio- and immune sensors [1,2]. Immunosensors belong to the class of biosensors that based on the reaction between antibody and antigen by formation of an immune complex [3], where interaction between antigen-antibody couple is highly specific and selective one. Recently, immunosensors based on optical transducers that use photoluminescence, absorbance, reflectance or fluorescence signal are of great interest because they demonstrate simple, fast and accurate determination of the target analytes [4]. The main advantage of the optical systems is that optical signal can detect the bio-molecular interaction contactless, *i.e.* without contamination or significant damage of the bio-samples [1,3,4]. Besides, no additional labels of the target analytes (such as dyes or quantum dots) and no contacts for the electrical measurements are required.

In this research an optical immunosensor based on TiO_2 thin film which consisted of TiO_2 nanoparticles (anatase crystal phase) for the determination of Bovine Leucosis antibodies has been developed. In our case, the changes in the photoluminescent properties of TiO_2 nanoparticles as a result of the adsorption of Bovine leucosis proteins have been determined. Biosensors based on the changes in the photoluminescence spectra from nanostructured semiconductors like the shift of PL-maximum and variation of PL-signal intensity have been developed in the range of works [4,5,6,7]. However, the interaction of proteins and semiconductors and the reasons of the changes in the photoluminescence spectra were poorly discussed. Although the mechanism of the interaction between semiconductor and proteins is the key in solving many of problems, which are still arising during the development of TiO_2-based immunosensors, such as an improvement of sensitivity and selectivity [4]. This research is aiming to explain the origin of the changes in the photoluminescence spectra of TiO_2 resulted after the protein

adsorption on its surface during the formation of biosensitive layer, and after its interaction with the target analyte.

2. Experimental

The TiO_2 thin film consisted of TiO_2 nanoparticles (purchased from Sigma Aldrich) was formed by sol-gel synthesis on the glass substrates. The details of the deposition procedure and structural characterization of TiO_2 thin film are described in some previous authors' works [5,8]. The PL spectrum of TiO_2 nanoparticles, shown in figure 1a, is characterized by broad non-symmetric maximum around 500 nm, which can be splinted (using Origin 8.0 Pro) in two peaks, related to self-trapped exciton (STE) emission and luminescence caused by oxygen vacancies ($V_{[O]}$) [9].



a) b)

Figure 1. PL spectrum of TiO_2 thin film on glass substrate (a); PL spectra of TiO_2/*gp*51 immunosensor after its interaction with anti-*gp*51 antibodies.

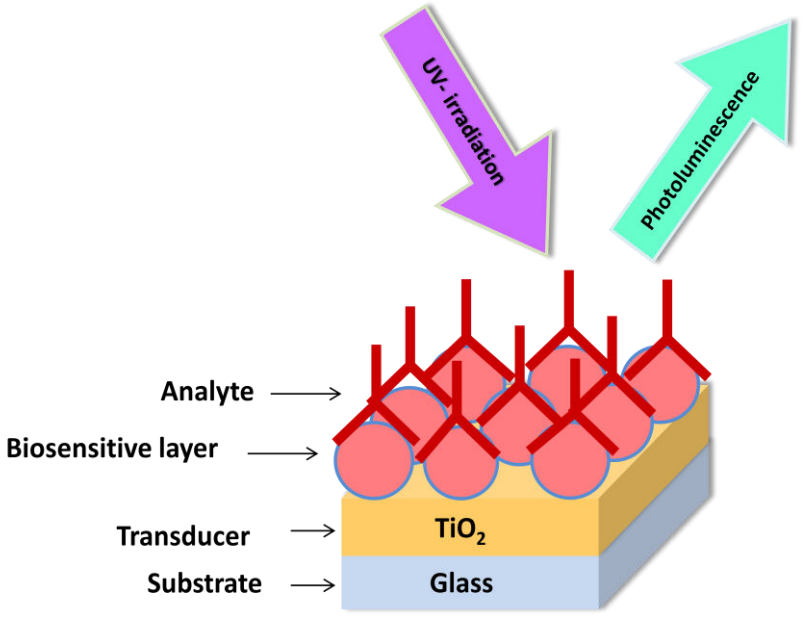


Figure 2. Photoluminescence based immunosensor scheme.

Bovine leucosis antigens *gp*51 were adsorbed on the surface of a nanostructured TiO_2 thin film by direct adsorption similarly using the process described in [5,8]. The scheme of such optical immunosensor is shown in figure 2. It was found that immobilization of *gp*51 leukemia antigens on the surface of TiO_2 is accompanied by an increase of photoluminescence signal of the sample as well as the shift of the photoluminescence peak from 517 nm to 499 nm was observed after modification of the TiO_2 by adsorbed *gp*51 antigens (Fig. 1b). Further interaction of immobilized *gp*51 antigens with *gp*51 antibodies resulted in reversed changes in TiO_2 photoluminescence spectra, i.e. a decrease in PL intensity and the backward PL peak shift from 499 nm to 516 nm. The sensitivity of the obtained immunosensor was in the range of 2-8 mg/ml [5,8].

3. Results and Discussion

Considering the interaction of proteins and semiconductor nanostructures, a few main possible mechanisms of interaction can take place: charge transfer, electrostatic interaction, resonance energy transfer, etc. Bovine Leucosis protein *gp*51 is not a redox protein, i.e. it cannot be involved in reduction-oxidation reactions therefore the charge transfer between *gp*51 antigens and TiO_2 nanoparticles is not possible [10].

TiO_2 (anatase) is known as a semiconductor of n-type conductivity, usually with an 'upward' band bending of the energy levels when closing the surface of TiO_2, which indicates the accumulation of a negative charge (bound at surface levels) on its surface [11]. The adsorption of the most of molecules is known to introduce an additional charge on the solid state surface and it can change the existing surface energy levels or form the additional ones that are involved in the exchange of charges with the volume of a solid material [12].

The proteins consist of amino acids that might contain positively and/or negatively charged radicals that are determining the charge of the different protein domains [13]. A large quantity of negatively charged groups such as aldehyde (-CHO), hydroxyl (-OH), carboxyl (-COOH) and primary amine ($-NH_2$) and some other groups, which are involved into the structure of amino acids, are responsible for the partial (δ+ and δ-) charges of particular protein domains. Therefore the proteins are characterized by electrostatic properties, and sometimes even significant electrostatic 'asymmetry of protein molecule' because the atoms and functional groups forming the protein molecules are charged differently both in their sign and in absolute charge value. Naturally, the charges at least partly are compensating each other, but since the ternary structure of proteins is relatively rigid and the charged groups have only limited degree of freedom to move within the protein globule, therefore in some parts of the protein some uncompensated charge on the surface and inside of the protein still remains. It should be taken into account that even if the structure of the most proteins is at some extent 'rigid' there is some degree of flexibility because both secondary and tertiary structures of the protein are supported by a large number of hydrogen bonds but many of them are not very strong [10]. The electrostatic bonds, which are based on Coulomb forces, between the opposite charges, van der Waals forces and disulfide bonds also play an important role in the formation of both secondary and tertiary structures of protein.

3.1. Interaction between TiO_2 and *gp*51 proteins

A *gp*51 protein molecule has a molecular mass of 51 KDa and its characteristic geometric size is about 6 nm in diameter. The authors [14], which have published a research on the formation of *gp*51 virus based capsid of BLV, have constructed an image of *gp*51 virus structure from the X-ray crystallography data

and they have reported that this protein is extra-flexible, which provides very high functionality and the ability to associate and/or dissociate of BLV capsid from the membrane of BLV infected cell. Therefore, it is expected that on the surface of TiO_2 *gp*51 forms a well-ordered monolayer. The formation of such layer was confirmed in other our researches by spectroscopic ellipsometry [15,16,17]. Although the *gp*51 protein is not a redox-protein, however, like many others, it contains a number of partially charged groups and domains, represented as partial charges "δ-" and "δ +", which mostly are lower in value than the total electron charge (1.6×10^{-19} coulombs) per charged atom or group. The presence of these partial charges suggests that the electrostatic influence on the surface charge of TiO_2 from the side of partially uncompensated charges in those parts of the *gp*51 protein that located on the surface of TiO_2 is responsible for the adsorption of this protein on the TiO_2 surface. The Coulomb interaction takes place between charged groups in the *gp*51 protein and the negatively charged surface of the TiO_2 because such electrostatic interactions are very strong at a distances ranging from several Angstroms to few nanometers. Therefore, among the others interactions such as hydrogen bonds, disulfide bonds, Van der Waals interaction, etc, which also have significant role during the adsorption of proteins, the electrostatic interaction plays one of the most important role during the adsorption of proteins to electrically charged surfaces, such as TiO_2. In addition, the local electric fields of charged domains of adsorbed proteins are affecting the PL-centers of TiO_2 and it causes the shift in the photoluminescence spectra of TiO_2 nanoparticles. Therefore, the photoluminescence maximum caused by STE shifts from 517 to 499 nm (i.e., to 18 nm), which corresponds to ~ 0.086 eV that is less than 0.1 eV, and it is one of the proofs of electrostatic interaction based physical adsorption of *gp*51 [12,18].

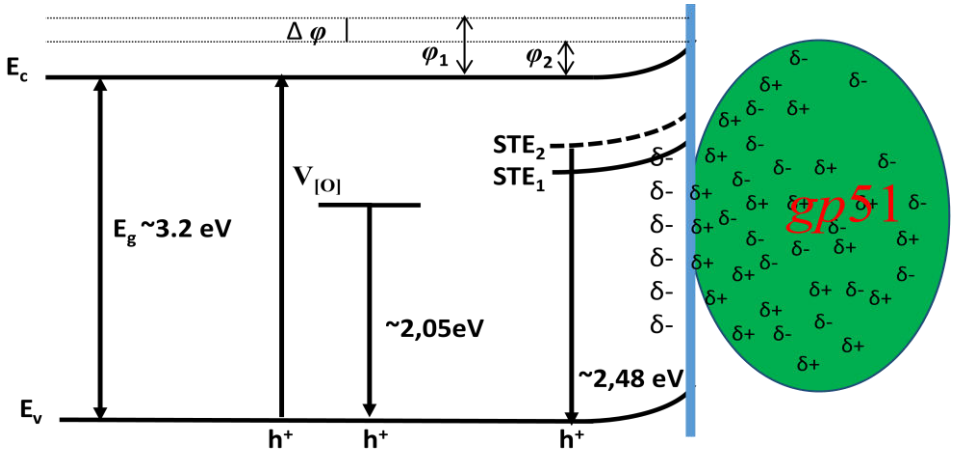


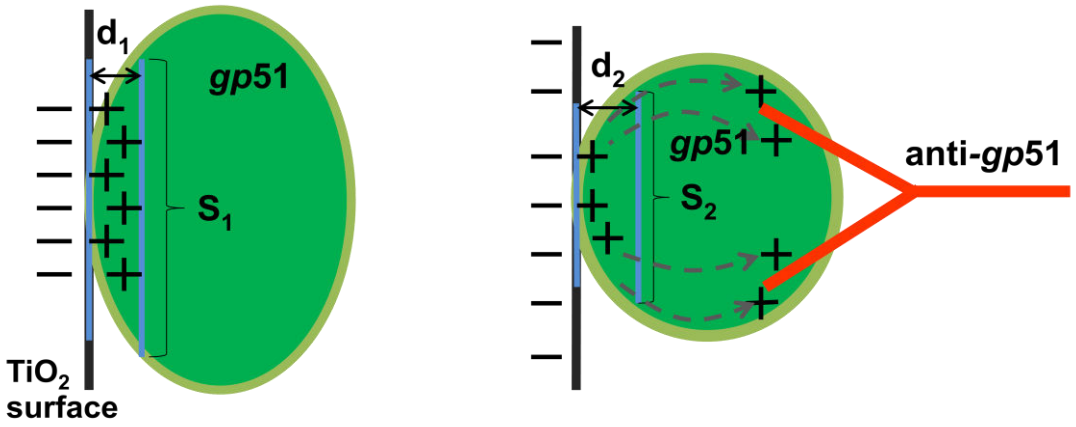
Figure 3. Energetic levels of TiO_2/*gp*51.

The splitting of the photoluminescence spectra into Gaussian curves at each stage of the experiment shows that after the adsorption of *gp*51 protein molecules on the TiO_2 surface the energy value of excitation levels, which are responsible for the luminescence and are associated with oxygen vacancies $I_{V[O]}$, almost does not change remaining at a value of 605±2 nm. At the same time, the photoluminescence maximum caused by recombination of self-trapped excitons (STE) shifts to short wavelengths, changing its position from 517 (STE_1 = 2.39 eV) nm to 499 (STE_2 = 2.48 eV) nm (Fig. 3). Since the involvement of the STE level in the process of radiative recombination is regulated by the surface, this indicates that STE level is located either on the surface plane or not very deeply within the surface layer of the TiO_2. The displacement of the light emitting recombination peak indicates that the energy level of STE is complex and has its basic and excited states. The appearance of luminescence in the region of 499 nm indicates a radiative transition from the excited STE level. The blue-shift of the photoluminescence maximum by 18 nm as a result of adsorption of the *gp*51 protein, which corresponds

to ΔE_{STE} = STE_2 - STE_1 = 0.086 eV, also indicates that the initial value of the potential barrier φ_1 on the TiO_2 surface has decreased by a value of 0.086 eV *(Δφ)* (Fig. 3). Variation of the potential barrier means that the value of negative charge localized on the TiO_2 surface has also changed, due to the charge-charge-based interaction with adsorbed protein *gp*51. Positively charged atoms and groups, which are provided by the *gp*51 protein, partially compensates the surface charge of TiO_2 and therefore reduces the energy of electrons localized at the surface levels, which are the most responsible for the generation of PL-signal (Fig. 4a). Taking into account the fact that the total negative charge predominates on the TiO_2 surface, the positively charged parts of the *gp*51 protein electrostatically interact with the negatively charged TiO_2 surface. As a result, a partial decrease of the surface charge reduces the electric field in the TiO_2 surface region (Fig. 4a).

3.2. Interaction of TiO_2/*gp*51 immunosensing structure with anti-*gp*51 proteins

Further interaction of TiO_2/*gp*51 structure with anti-*gp*51, which is also a protein, leads to the inverse changes in the photoluminescence spectra, i.e., to UV-shift of the spectrum (Fig. 1b) and decrease the photoluminescence intensity to the value that corresponds to the pure TiO_2. The latter effect is based on the formation an immune complex between immobilized antigens *gp*51 and anti-*gp*51 antibodies, which were present in aliquot. Formation of this immune complex, besides of the van der Waals interaction and other interactions, at a very high extent is based on the interaction between oppositely charged domains, functional groups and atoms in *gp*51 and anti-*gp*51 molecules (including the formation of number of hydrogen bounds, which can be estimated as specific kind of electrostatic interaction). It can be assumed that uncompensated charges (δ+ and δ-) of both proteins are involved in electrostatic interactions during the formation of immune complex. As a result, some of the charged groups that were originally involved in the interaction between *gp*51 and TiO_2 are at least partially compensated by the opposite charge of the anti-*gp*51 protein groups, thereby reducing the direct electrostatic effect from immobilized *gp*51 proteins to the charged surface of TiO_2 and to light emitting centers (Fig. 4b). The effects described above have an effect on the shift of PL-maximum and on the decrease in the potential barrier on TiO_2/*gp*51 interface due to the charge-charge interaction between TiO_2 and *gp*51. The potential barrier at the interface between TiO_2 and *gp*51 has greater value in TiO_2/*gp*51 structure in comparison with that in TiO_2/*gp*51/anti-*gp*51 due to partial compensation (decrease in value) and/or delocalization of charges, which were initially involved into interaction between TiO_2 and *gp*51 after formation of TiO_2/*gp*51 structure [18].



a) b)

Figure 4. Flat capacitor based model of the charges interaction between TiO_2 surface and *gp*51 proteins: a) electrostatic interaction of uncompensated charges of immobilized protein *gp*51 with charges located

on the surface of TiO_2; b) model of interaction that takes into account the electrostatic interaction of charges within *gp*51 antigens and anti-*gp*51 antibodies.

The distribution of charges in TiO_2/*gp*51 structure can also be interpreted as a model based on an 'imaginary flat capacitor' (Fig. 4), formed as a result of the electrostatic interaction between oppositely charged protein *gp*51 layer and the TiO_2 surface [18]. The capacitor is formed as a result of *gp*51 protein adsorption on TiO_2 surface, after which the charges are distributed in the most favorable way (energetically), partially compensating each other. Thus, the positive 'imaginary capacitor plate' is based on the positive charges, which are predominant in the protein *gp*51 area that after adsorption appears in close proximity to TiO_2/*gp*51 interface and/or due to the negative electrostatic effect of TiO_2 are induced/attracted closer to negatively charged surface. These charged atoms/groups/domains of *gp*51, localized in the close proximity to the TiO_2 surface, electrostatically affect the TiO_2 emission centers and the energy value of the surface potential barrier. Therefore, the position of the energy levels of the TiO_2 emission maximum depends on TiO_2 surface modification stage (TiO_2 or TiO_2/*gp*51) and shifts from/backwards the initial position of the demarcation level. Figure 4a represents an imaginary flat capacitor consisting of a negatively charged plate on the surface of TiO_2 and an 'imaginary positively charged plate' formed in *gp*51 protein in close proximity to TiO_2/*gp*51 interphase. Hence, the interaction of TiO_2/*gp*51 with anti-*gp*51 antibodies and the formation of *gp*51/anti-*gp*51-based immune complex leads to a 'deformation' and the reduction of charge 'stored' on 'the positive imaginary capacitor plate' (Fig. 4b). This is mainly due to the redistribution and partial compensation of charges during the formation of the *gp*51/anti-*gp*51 immune complex, which in turn reduces the charge of 'the imaginary capacitor plate' based on *gp*51 ($q_2 < q_1$). Due to this reduced charge it can be interpreted as the reduction of the area of the same plate (S_2) and/or the increase of the distance (d_2) between the two imaginary capacitor plates based on *gp*51 and TiO_2 which leads to the decrease of capacitance according to equation (1):

$$C = \frac{\varepsilon \varepsilon_0 S}{d} \qquad (1)$$

This effect is observed because some of the *gp*51 protein charges move from the TiO_2/*gp*51 interface towards interacting anti-*gp*51 protein and are partially compensated by the charge present in anti-*gp*51, whereby an imaginary positive *gp*51-based capacitor plate of the capacitor is reduced in imaginary surface area and/or correspondingly moving apart from the negative TiO_2 plate. This effect leads to a decrease in the capacitance of this imaginary capacitor and the electric field induced by *gp*51 becomes reduced. Therefore, after the interaction of TiO_2/*gp*51 with anti-*gp*51 antibodies and the formation of *gp*51/anti-*gp*51 complex, which is involved into TiO_2/*gp*51/anti-*gp*51 structure, the electrostatic effect of *gp*51 initially adsorbed on TiO_2 towards the TiO_2 surface significantly decreases. The PL shifts are attributed to the variations in the self-trapped exciton energy level, which were induced by the changes of electrostatic interaction between positively charged atoms and groups, provided by the adsorbed *gp*51 protein and negatively charged surface of TiO_2.

4. Conclusions

A model of interaction mechanism between nanostructured TiO_2 layer and Bovine Leukemia virus proteins *gp*51, during the formation of PL-based immunosensor, have been developed. The main reason of changes in the photoluminescence spectra of TiO_2 as a result of adsorption of *gp*51 antigens is the electrostatic interaction between the TiO_2 surface charge and the partial uncompensated charges of *gp*51 proteins. Subsequent interaction of the immunosensing structure of TiO_2/*gp*51 with the target analyte

anti-*gp*51 leads to inverse changes in the photoluminescence spectra due to the charge distribution as a result of the formation of an immune complex. The charge–charge-based interaction in the double charged layers *gp*51/TiO_2 can also be interpreted as a model based on 'imaginary capacitor', formed as a result of the electrostatic interaction between oppositely charged protein *gp*51 layer and the TiO_2 surface. The proposed interaction mechanism provides the general understanding of the interaction between TiO_2 and proteins, what is a key in the development of new PL-immunosensors and solving of many issues related to an improvement of performance of PL-based immunosensors, first of all, related to the sensors' sensitivity and selectivity.

Acknowledgment This work was supported by joint Ukrainian-Lithuanian research project "Application of hybrid nanostructures which are based on TiO_2 or ZnO and modified by biomolecules, in optoelectronic sensors", funded by Lithuanian Research Council (project No. P-LU-18-53).

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