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Effect of Nucleic Acid and Albumin on Luminescence Properties of Deposited TiO_2 Quantum Dots

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Luminescent quantum dots (QDs) have gained a great attention as promising candidates in optical detection of various biomolecules such as DNA, proteins, etc. In this paper, we focused on fabrication of QDs in the deposited form, which still belongs to less clarified and used approach in biosensing compared to colloidal one. The deposited QDs from non-toxic titanium dioxide (TiO_2) were prepared by template based technique, namely anodic oxidation of titanium film through nanoporous alumina template. This method allows to creation of QDs strongly fixed on silicon substrate without addition of any other fixation matrix (e.g. polymer). Highly ordered TiO_2 QDs with broad luminescence peak in visible range were achieved. The QDs array was characterized by SEM confirming the QDs size being below 10 nm, which is necessary to reach the quantum size effect. Preliminary results showed that subsequent modification of QDs surface with chicken DNA and bovine serum albumin (BSA) resulted in partial or complete quenching of fluorescence depending on the biomolecules concentration.

Keywords: quantum dots; luminescence; biosensors; TiO_2; template method; DNA; bovine serum albumin

1. INTRODUCTION

Excellent optical properties of QDs (namely high quantum yield, broad absorption spectra and narrow, symmetric fluorescence spectra from UV to NIR, large effective excitation and emission

Stokes shifts), long life-time (high resistance to photobleaching) compared to ordinary fluorophores and stability (resistance to photo- and chemical degradation) predestinate them in usage for imaging and as optical probes for detection of peptides, proteins, nucleic acids and other biomolecules [1-5]. Even the systems with colloidal QDs are more frequently used for biosensing application, the systems with QDs grew on various solid substrates is no less attractive but markedly less studied. Nowadays, new approaches in biosensing with QDs consist in usage of QDs fixed on some solid substrate. However, this is generally provided by colloidal QDs incorporation in some polymer matrix or formation of colloidal QDs thin films via self-assembly [6-8]. Moreover, most of these QDs are toxic hence some biocompatible layer should cover the QDs core [9].

Therefore, there is a demand for direct, easy, cheap and non-toxic QDs growth with no requirement of other fixation agent addition, for example through nanoporous template [10]. This nanoporous template can be easily fabricated by anodic oxidation of aluminium layer. The nanopore size is influenced by anodization parameters, namely temperature, time, electrolyte and mainly anodization voltage. By setting these conditions, the required nanostructure size is reached to ensure the quantum size effect. The method is well reproducible and cost-effective [11]. In this paper, we proposed a new approach for biomolecules (protein, peptides, nucleic acids) detection using non-toxic TiO_2 QDs fluorescence array prepared by template based technique – see Figure 1.

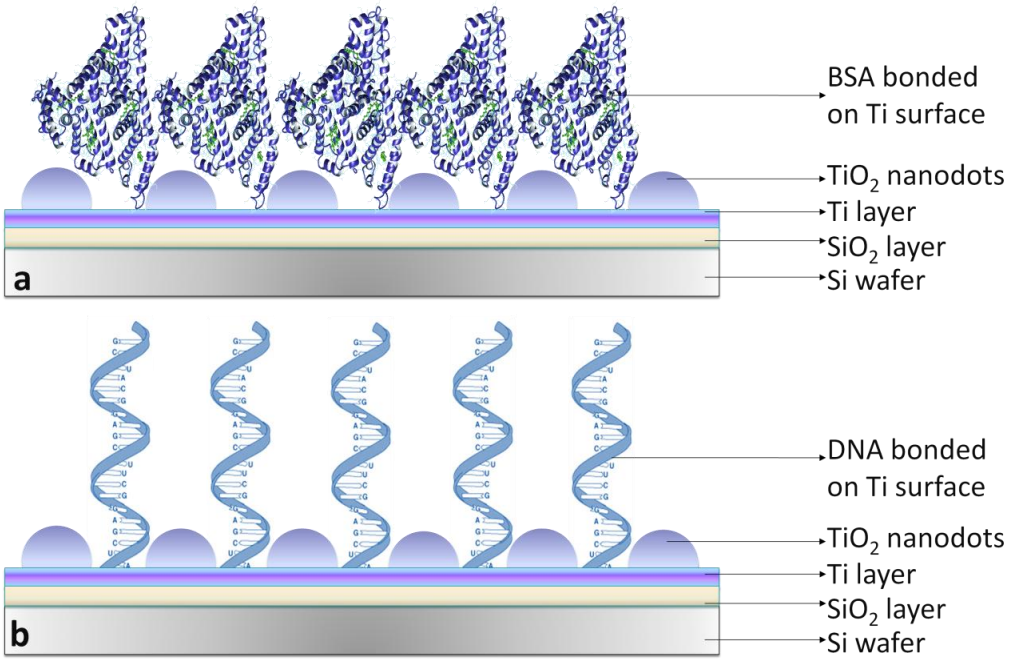

Figure 1. Schematic illustration of biomolecules sensing with TiO_2 QDs fluorescence array: **(a)** sensor with BSA (bovine serum albumin), **(b)** sensor with DNA.

In our probes, the QDs represent a central component of biosensors and serve as substrate for biomolecules immobilization on their surface. The sensor photoluminescence signal is changed depending on biomolecules concentration. The supposed change of QDs emission is based on

quenching effect instead of luminescence peak shift. The excitation of QDs can be from wide range of wavelengths in ultraviolet region. Nevertheless some items still remain in the optimization of these sensing systems. One of these issues lies in interfacial chemistry, which influences among others the sensor regeneration and reuse in further assays.

2. EXPERIMENTAL PART

2.1 Chemicals

Sulphuric acid (97%, p.a.), phosphoric acid (84%, p.a.) and chromium trioxide (99%, p.) were purchased from Penta (Czech Republic). Deionised water was obtained from Millipore RG system MiliQ (Millipore Corp., USA, 18.2 MΩ). Lyophilised highly polymerized DNA (Reanal, Hungary) was isolated from chicken erythrocytes (Mw = 400 000 g/mol). Bovine serum albumin and other used chemicals were purchased from Sigma-Aldrich (USA).

2.2 Titanium and aluminium layers preparation

Prior to titanium deposition, the wafers were thermally treated (wet CVD) in order to create silicon dioxide layer with thickness of 1.1 μm. A high-purity titanium layer (99.5%, Safina, Czech Republic) with thickness of 100 nm was prepared on 4 inch silicon wafer by magnetron sputtering (Leybold - Heraeus Z 550) with PK150 target under Ar (purity of 99.999 %) pressure of 0.85 Pa. Subsequently, high purity aluminium layer (99.99%) with thickness of 1 μm was deposited by thermal evaporation (U311B Tesla Opočno, Czech Republic) from Al target 5N (Sigma-Aldrich) under pressure of 2.10^{-4} Pa.

2.3. Anodization process

Ordered arrays of titanium nanodots were achieved by successive anodization of aluminium and titanium layers using the utility model equipment for electrochemical post-processing deposition fabricated in our laboratory (a detailed description of the tool is reported in [12]). Briefly, the system consists of 3 moving plates driven by step-motors forming 3D position system, a flow-system controlled by membrane pump, power source, thermostat and personal computer providing automatic driving of all processes. The pump assures that the flow of the liquid (electrolyte, etching or washing solution) circulates from vessel through thermostat to the deposition cell which is situated above the sample with Ti and Al layers. Thanks to different anodizing behaviour of these layers, the same electrolyte can be applied during the whole process. The tested potential values were following: 4, 5, 7, 9, 11, 15, 20 and 24 V. The temperature changed from 9 to 11 °C, only in the case of 24 V the temperature of 2 °C was tested. Sulphuric acid in concentration scale 1, 2 and 3 M was chosen as electrolyte in constant potential mode. This acid provides smaller pore diameter in alumina template compared to other commonly used electrolytes (oxalic or phosphoric acids). An aqueous solution of

H_3PO_4 (50 ml L^{-1}) and CrO_3 (30 g L^{-1}) was used for chemical etching of alumina template (5 min at 60 °C). Finally the samples were heated in vacuum oven at 400 °C for 1 h in order to reach the transformation of amorphous TiO_2 into crystallographic phase of anatase.

2.4. Scanning electron microscopy

The size of QDs and pores in alumina template were estimated using scanning electron microscopy (FE Tescan Mira II LMU) under following conditions: work distance of 2.5 nm, high vacuum mode (10^{-3} Pa), voltage of 15–20 kV, and spot size of 2.4 nm.

2.5. Photoluminescence measurement

The photoluminescence properties of annealed as well as non-annealed TiO_2 QDs array were characterized by fluorescence spectroscopy (Horiba, Jobin-Yvon) with laser diode excitation at 350 nm, photomultiplier (S2-A PMT) detector and 400 nm cut-off filter. For fluorescence quenching test, two biomolecules aqueous solution (DNA and BSA) were prepared in following concentration scale: 1, 10 and 100 μg mL^{-1}. 6 μL of each solution was dropped on TiO_2 annealed QDs surface and dried in oven (BMT, Czech Republic) for 10 min at 35 °C.

3. RESULTS AND DISCUSSION

3.1 TiO_2 QDs array topography

We prepared the array of TiO_2 QDs for biosensing purposes with strong adhesion to SiO_2/Si substrate using one step anodization technique. The nanostructures grew through nanoporous alumina template, which was fabricated in the same time by anodic oxidation of aluminium layer deposited on titanium. Concerning the quantum size effect of TiO_2 QDs (in anatase phase), Monticone et al. observed almost no variation of the band gap energy with particle size 2R down to 15 nm [13]. Therefore, the most important step in preparation process was to determine the appropriate anodizing potential and deposition time to ensure the required nanostructure dimensions.

In our work, we changed several parameters to observe their influence on final QDs size, namely anodization voltage from 4 to 24 V, electrolyte temperature from 2 to 11 °C and sulphuric acid concentration from 1 to 3 M. We found, that the size of TiO_2 QDs decreased with decreasing value of anodization potential: from 25–30 nm at 20 V to the lowest value of 8–10 nm reached at 5 V (see Table 1). Simultaneously with decrease in potential we needed to increase the electrolyte concentration to ensure the sufficient current value during the process. The electrolyte temperature growth enhanced the rate of anodization and facilitates the nanostructure ordering. The influence of anodization conditions on final QDs size confirmed by SEM can be seen on Figure 2.

The SEM characterization of Al_2O_3 template and TiO_2 QDs provided important information about QDs ordering and homogeneity. Whatever anodization conditions were applied, the created nanostructures

(see Figure 2a) as well as pores in alumina template were hexagonally arranged as expected. We revealed that optimal anodization conditions for TiO_2 QDs formation are 5 V, 11 °C and 3 M sulphuric acid as electrolyte, which resulted in template pore size in the range of 5–8 nm. This dimensions of pores allowed the creation of titania QDs with size of about 8–10 nm (see Figure 2c). The interpore spacing in template prepared under these conditions was estimated at approx. 15 nm.

Table 1. Anodization conditions influence on TiO_2 QDs size.

Anodization voltage (V)	Electrolyte temperature (°C)	Electrolyte concentration (M)	Size of TiO_2 nanodots (nm)
20	9	1	25–30
15	9	1	20–25
11	9	2	15–20
9	9	2	14–18
5	9	2	10–13
5	11	3	8–10

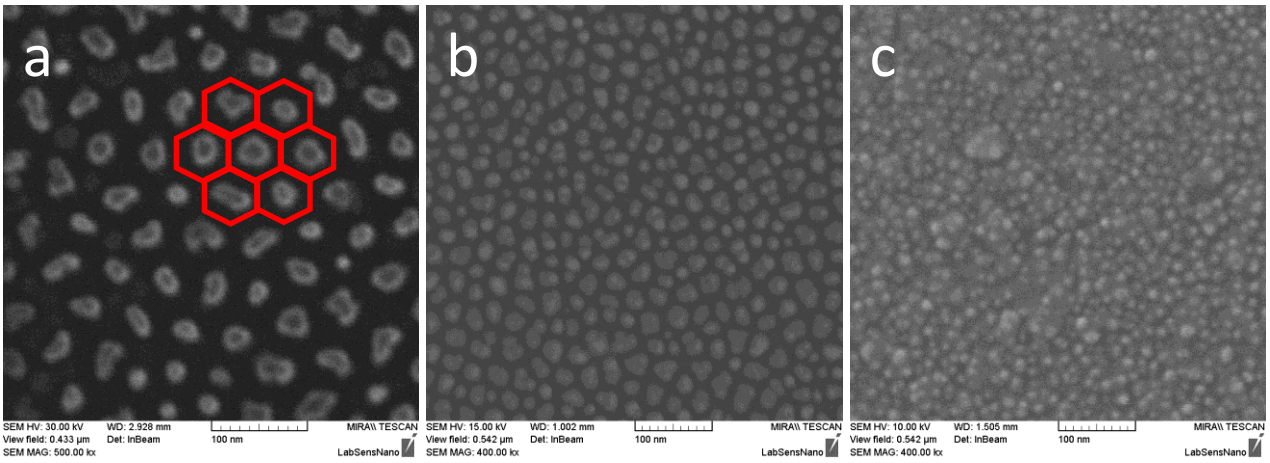

Figure 2. SEM characterization of TiO_2 QDs under different anodization conditions with bar of 100 nm: **(a)** 20 V, 9 °C, 1 M sulphuric acid (magnification of 500,000, work distance of 2.9 mm), **(b)** 11 V, 9 °C, 2 M sulphuric acid (magnification of 400,000, work distance of 1 mm), **(c)** optimal conditions: 5 V, 11 °C, 3 M sulphuric acid (magnification of 400,000, work distance of 1.5 mm).

3.2. TiO_2 QDs photoluminescence properties

The heat-treatment of TiO_2 QDs prepared under optimal anodization conditions resulted in transformation of amorphous titanium into crystallographic form, which is necessary in order to observe QDs luminescence properties. Therefore, the emission spectra were firstly collected for annealed and non-annealed TiO_2 QDs sample (excitation at 350 nm), which revealed that only heat treated titanium QDs show remarkable luminescence peak in visible range with maximum position in

the range of 514–521 nm (Figure 3). The small peak around 400 nm presented in all spectra is supposed to be some unspecific scattering.

3.3. Monitoring of biomolecules influence on TiO_2 QDs photoluminescence quenching

To verify the functionality of prepared TiO_2 QDs as biomolecules sensing system, we chose two biomolecules aqueous solutions, namely BSA and chicken DNA, to test their influence on TiO_2 QDs luminescence quenching. Three different concentrations of each biomolecule were tested: 1, 10 and 100 μg mL^{-1}. The collected emission spectra of TiO_2 QDs modified with particular biomolecule (DNA in the Figures 4A and B and BSA in the Figures 5A and B) were compared with spectrum of annealed TiO_2 QDs. The luminescence intensity of biomolecules solution (in concentration of 100 μg mL^{-1}) deposited on pure microscopic glass was also analysed: in both cases, the intensity (after glass spectrum substraction) was similar and about 6 times lower than the intensity of TiO_2 QDs (see Figure 6).

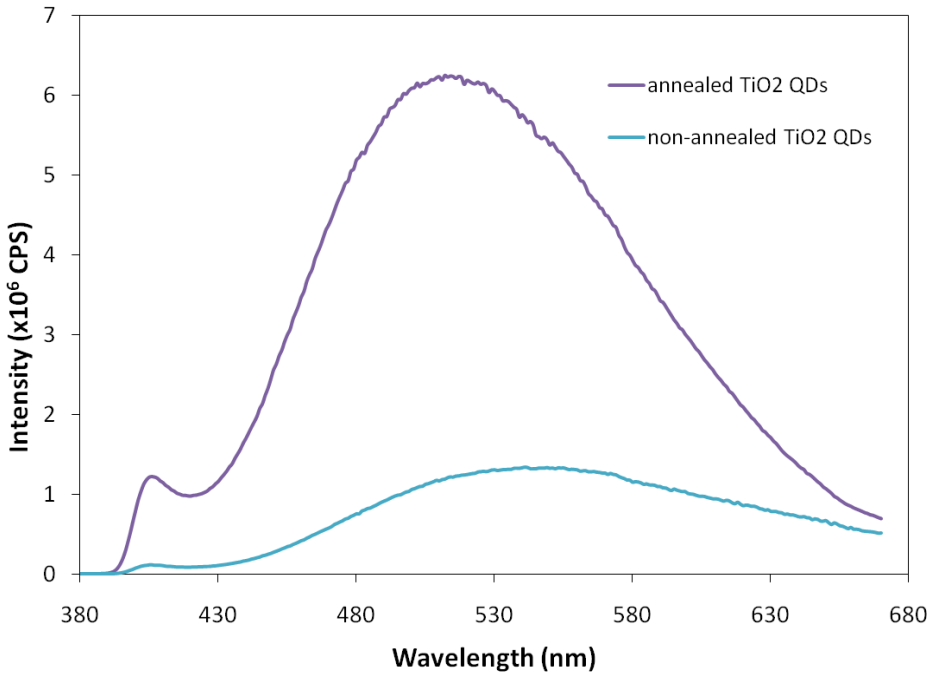

Figure 3. Emission spectra comparison of annealed and non-annealed TiO_2 QDs.

As supposed, we observed only luminescence intensity decrease with the increasing biomolecules concentration regarding to origin TiO_2 QDs luminescence maximum and no peak shift (the maximum position is the same for all tested samples). The same small peak around 400 nm appeared in all spectra (unspecific scattering). We found that concentration of 100 μg mL^{-1} resulted in complete quenching whatever biomolecule was used (spectra not shown). In the case of 10 μg mL^{-1} biomolecule solutions, we detected the partial quenching with intensity decrease of 48 % for DNA and

intensity decrease of 57 % for BSA. In the case of the lowest concentrated solutions (1 μg mL^{-1}), the intensity decrease of 33 % and 24 % were measured for BSA and DNA, respectively.

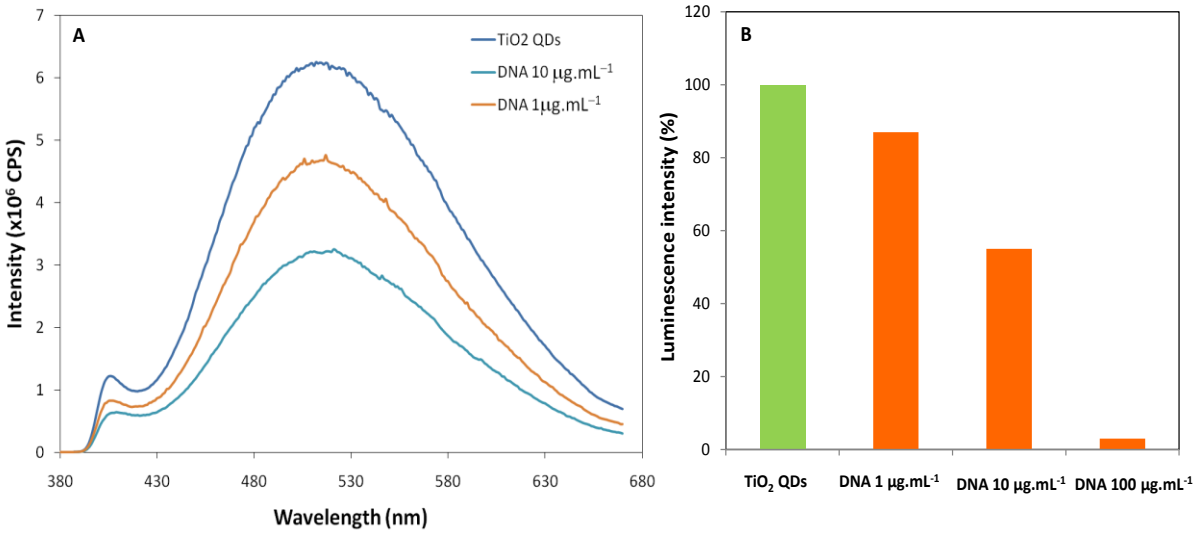

Figure 4. (A) Emission spectra of TiO_2 QDs and TiO_2 QDs modified with DNA. **(B)** Decrease in luminescence intensity with the increasing concentration of DNA.

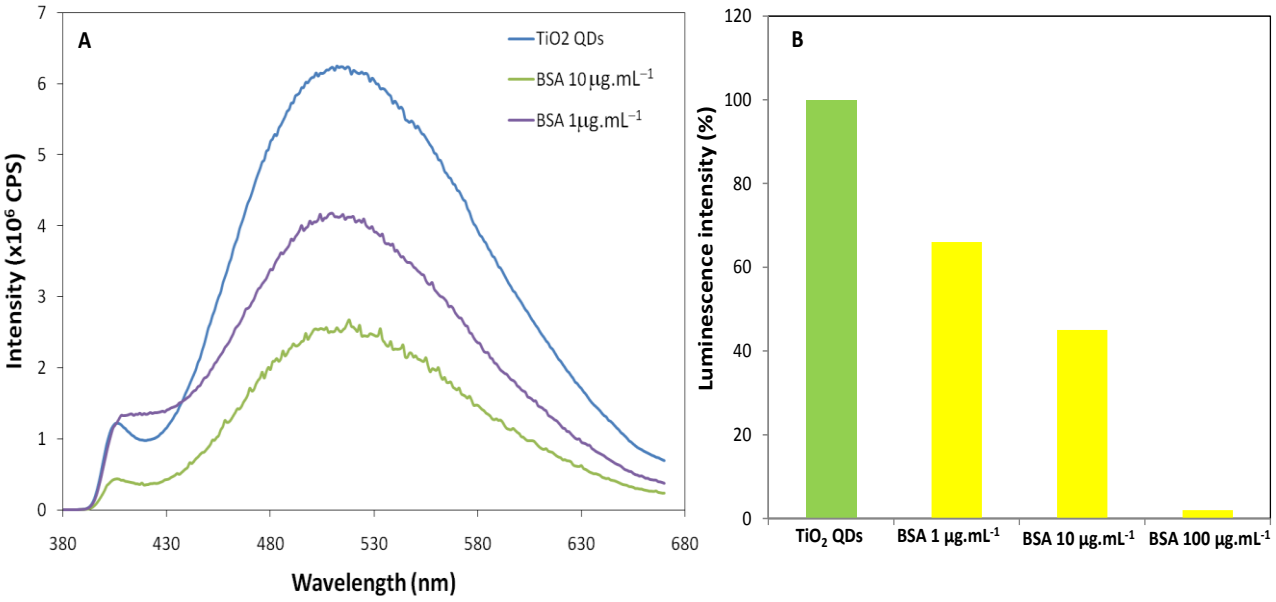

Figure 5. (A) Emission spectra of TiO_2 QDs and TiO_2 QDs modified with BSA. **(B)** Decrease in luminescence intensity with the increasing concentration of BSA.

From these preliminary results, we can conclude that BSA has higher influence in TiO_2 QDS luminescence quenching than DNA. This could be a consequence of biomolecules shape and size: even

the DNA is bigger molecule, it has rather tubular shape, while BSA is spatially larger due to complex 3D protein structure. However, more precise study with various concentration level of each biomolecule should be done in this field in order to observe the quenching mechanism in more details.

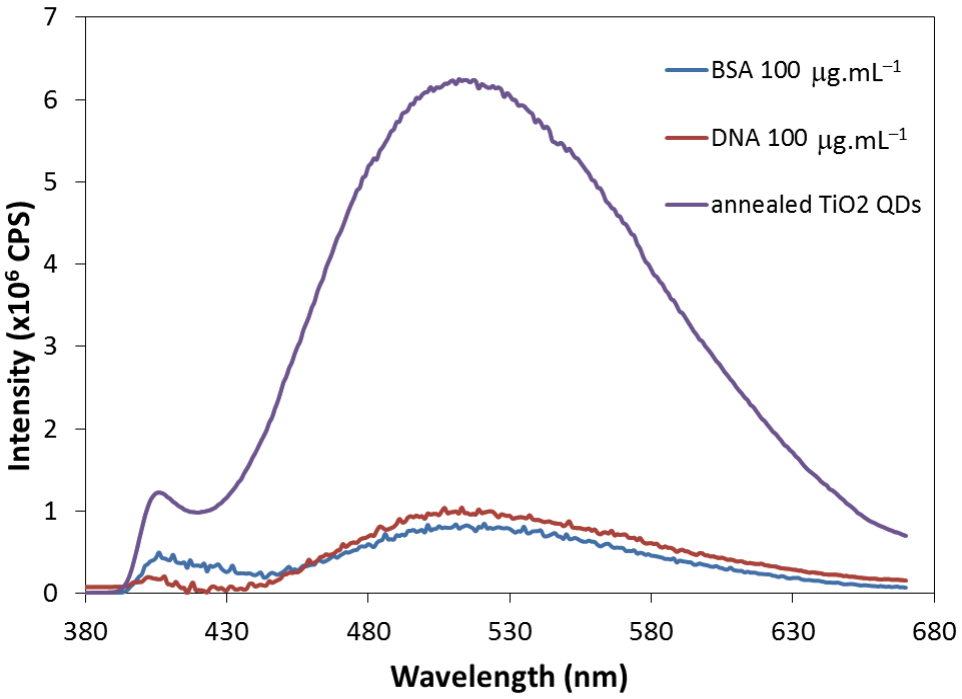

Figure 6. Emission spectra of annealed TiO_2 QDs and biomolecules solution in concentration of 100 μg.mL^{-1} deposited on glass (after glass spectrum subtraction).

4. CONCLUSIONS

New exploitation of cheap and rapid template based technique with good reproducibility was used for preparation of TiO_2 QDs array. The application of optimal anodization conditions enabled to create QDs with size below 10 nm and densely covering the titanium surface. The prepared titanium QDs showed strong photoluminescence peak in visible range after annealing at 400 °C, which resulted in transformation of amorphous TiO_2 in crystallographic phase (the most probably anatase). The luminescence quenching test with two biomolecules, BSA and DNA, was performed showing that biomolecules concentration has strong influence on luminescence intensity decrease. From these preliminary results, we suppose that our non-toxic TiO_2 QDs are suitable for various biomolecules (DNA, proteins) sensing *in vitro*. The usage of this fluorescence array detector may also be of great importance for clinical diagnostics and *in vivo* imaging [14,15]. The big advantage of these deposited QDs compared to colloidal ones is their infinite life-time, which predestines those to many times repeatable tests. Based on these promising results, continuing in the testing of various substances, conditions and concentrations will be necessary to find the potential influence on biomolecules

solution spreading on TiO_2 QDs sensor array and hence avoiding the oven-drying before luminescence properties measurement.

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