Lithuanian Journal of Physics, Vol. **54**, No. 4, pp. 256–265 (2014)

EFFECT OF LIGHT ON STABILITY OF THIOL-CAPPED CdSe/ZnS QUANTUM DOTS IN THE PRESENCE OF ALBUMIN

A. Kalnaitytė [a], S. Bagdonas [a], and R. Rotomskis [a, b]

[a] *Department of Quantum Electronics, Faculty of Physics, Vilnius University, Saulėtekio 9, LT-10222 Vilnius, Lithuania*

[b] *Laboratory of Biomedical Physics, National Cancer Institute, Santariškių 1, LT-08406 Vilnius, Lithuania*

E-mail: agne.kalnaityte@gmail.com

Received 14 May 2014; revised 17 July 2014; accepted 10 December 2014

The effect of exposure to the green laser light on the photoluminescence (PL) stability of CdSe/ZnS quantum dots (QDs) capped with either mercaptopropionic (MPA) or thioglycolic (TGA) acid was studied in aqueous suspensions and in the presence of bovine serum albumin (BSA). The results of absorbance and luminescence measurements suggest that the capacity of protein to change the coating structure of thiol-capped QDs and the stability of photoluminescence depends on the nature of stabilizing surface ligands. The interaction of BSA molecules with TGA-capped quantum dots increases their PL quantum yield (QY) and makes PL more stable, however, the effect is opposite for MPA-capped QDs. The light exposure instantly increases the PL intensity and the quantum yield of TGA-capped QDs but does not change those of MPA-capped QDs. In the medium with BSA, however, the occurrence of light-induced PL enhancement does not depend on stabilizing thiol ligands of QDs and it lasts for a relatively long period of time.

Keywords: quantum dots, photostability, protein, photoluminescence

PACS: 81.07.Ta, 78.67.Hc

1. Introduction

Semiconductor quantum dots (QDs) – nanometre-size particles – have been widely used in the fields of medicine, biology, and material science. In comparison with organic dyes and fluorescent proteins, QDs have a narrow and tuneable emission band, a broad absorption spectrum, a high photoluminescence quantum yield and enhanced photostability [1]. Besides semiconductor core and shell, which determine the photophysical properties [2], QDs usually have their surface capped with different ligands to prevent the aggregation and precipitation. High quality QDs (possessing a coating with good resistance to degradation) are necessary to minimize the release of Cd element in a living organism [3]. Because QDs are used in a living organism, the potential negative effects of these nanoparticles on biological systems could not be ignored [4, 5]. The QDs capped with hydrophobic organic ligands usually have a high fluorescence quantum yield [6] but are not suspendable in aqueous solutions. In order to be biocompatible nanoparticles with good colloidal stability, the QDs must be made hydrophilic and non-toxic. To make them suitable for the application in medicine, the surface of quantum dots has to be appropriately modified using various hydrophilic ligands [7]. The thiol acids that include mercaptopropionic acid and thioglycolic acid are chosen most often to improve the photochemical stability of quantum dots in the aqueous solution [8]. However, not only the coating of the surface can change the spectroscopic properties of QDs [2], but also the surrounding environment [9] and exposure to light [10, 11] can affect the QDs photoluminescence. Thus, optical spectroscopy becomes a useful technique to assess the quality and biocompatibility of QDs on the basis of their spectroscopic properties (the quantum yield of photoluminescence, the features of emission spectra) being measured under various model conditions.

In this study, we applied spectroscopic methods to compare the stability and photostability of negatively charged CdSe/ZnS quantum dots that were capped with several thiol-based stabilizing ligands and suspended in an aqueous medium. The effects of two factors, the interaction between QDs and bovine serum albumin (BSA) as well as the exposure of QDs to the green laser light, on the photoluminescence properties of QDs have been investigated.

2. Materials and methods

Absorption and steady state fluorescence spectroscopy measurements were performed on CdSe/ZnS

quantum dots (Invitrogen, USA), the surface of which had been modified with thioglycolic (TGA) or mercaptopropionic acids (MPA) (PlasmaChem GmbH, Germany) in the Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, to make them water-soluble (Fig. 1). Studies involved two batches of TGA-capped QDs (batches No. 1 and No. 2), which were independently modified in accordance with the same protocol, and one batch of MPA-capped QDs.

Quantum dots were suspended in a phosphate buffer solution (0.05 M, pH 7) and in a solution with bovine serum albumin (BSA) (Albumin, V fraction, M = 69,000 g/mol, Carl Roth GmbH, Germany). The final concentration of QDs ($c = 3.6 \cdot 10^{-7}$ M) was prepared from the stock solution at a dilution ratio of 200. The final concentration of BSA in samples with QDs was 10^{-5} M. The continuous-wave semiconductor laser (532 nm, 38 mW/cm^2) was used to irradiate the samples. The samples were exposed to the laser light in a 10 × 4 mm quartz cuvette (Hellma, Germany) and the spectral measurements were performed immediately after the light exposure. Different irradiation times were chosen for different experimental sessions, as shown in the figures. The volume of each sample was 2 ml. Between the measurements the samples were kept in the dark at +4 °C.

The initial absorbance spectra were measured immediately after the modification of QDs surface with a Varian Cary Win UV absorption spectrometer (Varian Inc., Australia). The initial photoluminescence (PL) spectra were measured with a Varian Cary Eclipse spectrofluorimeter (Varian Inc., Australia). An excitation wavelength for photoluminescence was set at 405 nm, the excitation and emission slits were 5 nm. For the rest of experiments an Avantes AvaSpec-3648 fibre optic spectrometer (Avantes, Netherlands) was used to register absorption spectra, and a PerkinElmer LS55 spectrofluorimeter (PerkinElmer, USA) was used to register PL spectra. An excitation wavelength for PL was set at 450 nm, the excitation slit was 4 nm, and the emission slit was 3 nm.

Quantum yield (QY) of the photoluminescence of quantum dots was calculated according to the Formula [12]:

$$\Phi = \Phi_s \times \frac{1-10^{-As}}{1-10^{-A}} \times \frac{Int}{Int_s} \times \frac{n^2}{n_s^2},$$

where the s subscript refers to the standard specimen and other symbols have the following meanings: Φ is the quantum yield, A is the absorbance at the excitation wavelength, Int is the integrated emission area across the band (in inverse centimetre scale), n is an index of refraction. The rhodamine B ($c = 4.7 \cdot 10^{-6}$ M) in 64% w/w aqueous ethanol was used as the standard solution. The fluorescence quantum yield Φ_s of rhodamine B is 0.7 for excitation at 510 nm and 0.65 for 610 nm [13]. According to another study, the rhodamine B quantum yield is 71% and depends on the temperature [14, 15]. Thus, for excitation at 450 nm being applied in this study, $\Phi_s = 0.7$ was used (Formula). Since the samples were measured in the aqueous media and the standard solution was measured in 64% ethanol, the corresponding refractive indexes of water ($n = 1.34$ [16]) and of 64% ethanol solution ($n_s = 1.36$ [17]) at 20 °C were included in QY calculations. The errors of PL QY of QDs were evaluated taking into account the systematic errors of absorption measurements (±0.001 o. d. u.). The calculated values of PL QY are summarised in the tables.

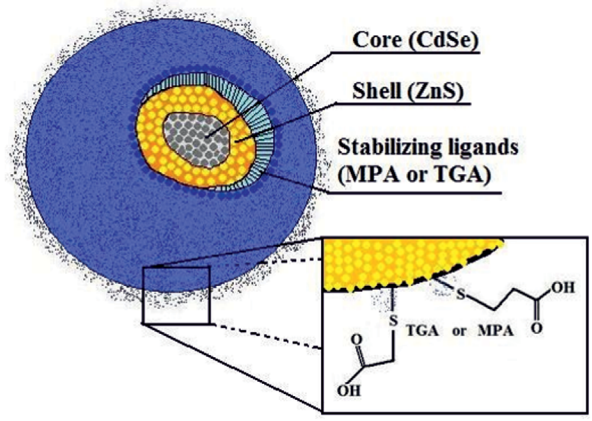

Fig. 1. The structure of quantum dots. The TGA and MPA molecules possess a thiol group, SH, and links to the QDs via an S–S bond. The size of ligands is not in scale with QDs.

3. Results

The initial absorption and photoluminescence spectra of CdSe/ZnS quantum dots, which were registered in aqueous suspensions of the same QDs concentration, immediately after the modification with a stabilizing ligand (TGA or MPA), are presented in Fig. 2. The absorption of MPA-capped QDs was less intense than that of TGA-capped QDs (prepared from batch No. 2). However, the photoluminescence intensity was greater for MPA-capped QDs. It is evident that the quantum yield of photoluminescence of MPA-capped QDs was greater in comparison with that of TGA-capped QDs. In addition, the spectral position of the PL band was different: 546 nm – for quantum dots capped with TGA and 548 nm – for QDs capped with MPA. No changes were observed in the PL spectra of the samples that were prepared from the stock solutions of

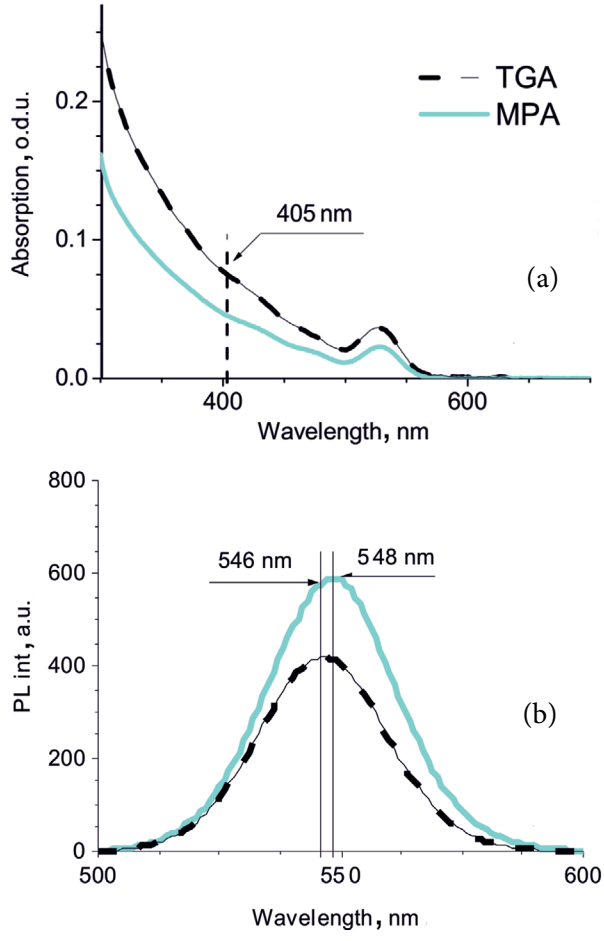

Fig. 2. Absorption (a) and photoluminescence (b) spectra of CdSe/ZnS quantum dots capped with TGA or MPA in a phosphate buffer, pH 7. Excitation wavelength was 405 nm.

TGA-capped and MPA-capped QDs after a month of storage, when the experiments with light and BSA were started. However, the changes occurred in the PL spectra of TGA-capped QDs during subsequent experiments as well as in those samples, which were prepared from the stock solution after longer periods of storage.

3.1. TGA-capped quantum dots

The measurements of absorption and PL spectra of TGA-capped QDs modified in accordance with the same protocol, showed some differences in spectral properties between the samples of QDs prepared from batches No. 1 (TGA-QDs-1) and No. 2 (TGA-QDs-2) (Fig. 3). The spectral position of the PL band (at 548 nm) of TGA-capped QDs from batch No. 2 was red-shifted. The absorbance of TGA-QDs-1 was slightly lower and the last absorption band was broader at the blue side in comparison with TGA-QDs-2. The spectral properties of TGA-QDs-1 samples did not change in the aqueous solution and no precipitation was observed for two weeks. On the other hand, although no spectral changes

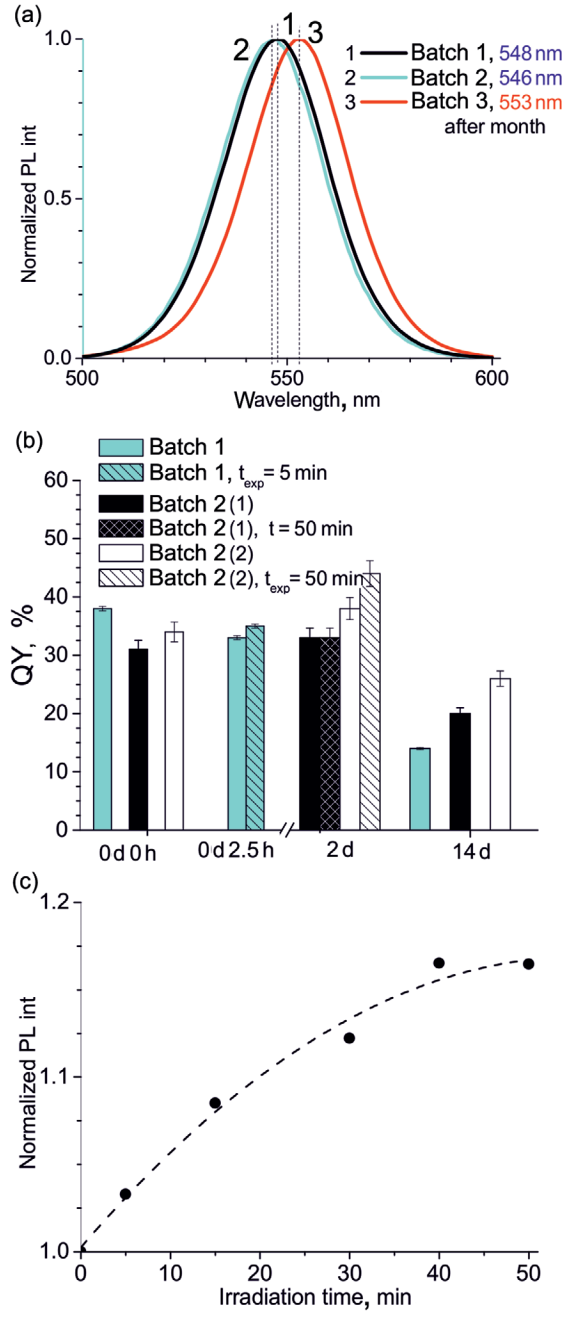

Fig. 3. (a) Photoluminescence spectra of TGA-capped QDs from batches No. 1 and No. 2; bands' spectral positions are shown. (b) The dependence of PL QY of TGA-capped QDs (control and irradiated) on time; t_{exp} – time of irradiation, the samples were exposed for 5, 50 minutes or kept non-exposed. (c) The dependence of the normalized PL intensity (at 549 nm) of TGA-capped quantum dots (batch No. 2) on the irradiation time. PL excitation wavelength was 450 nm.

were detected in the absorption spectra of TGA-capped QDs from batch No. 2, the PL band shifted from initial 546 nm to 549 nm, and the precipitation has occurred.

Different exposure doses were applied to investigate the light-induced short-term effects on TGA-capped QDs in the solutions without BSA. The histogram in Fig. 3(b) shows the increase of the photoluminescence QY of irradiated QDs compared with non-irradiated QDs after five minutes (batch No. 1) and fifty minutes (batch No. 2) of exposure. The initial quantum yield of TGA-QDs-1 was a little higher than QYs of two TGA-QDs-2 samples at a zero day of the experiment (Table 1). It has to be noted that the PL intensity of each TGA-QDs-2 sample prone to precipitation was repeatedly measured three times immediately after mixing. Therefore, the errors of PL QY for TGA-QDs-2 included the calculated standard deviation of the measured PL intensity (±10 a. u.). The PL quantum yield of TGA-QDs-1 slightly increased after exposure to light for 5 minutes at the zero day. When TGA-QDs-2 samples were exposed after two days, their PL intensity increased slightly after the exposure for 5 minutes and the saturation was reached at about 50 minutes (Fig. 3(c)), when the quantum yield increased by a few percents. After two weeks the PL QY diminished for QDs samples from both batches, but while the QY of non-exposed TGA-QDs-2 was higher than the QY of exposed TGA-QDs-1, it remained lower than the QY of their exposed counterparts.

The presence of BSA in the suspension also affected the spectroscopic properties of TGA-capped CdSe/ZnS quantum dots ($c = 3.6 \cdot 10^{-7}$ M). The intensity of absorbance of TGA-QDs-1 has increased becoming almost the same as that of TGA-QDs-2. No further spectral changes in QDs absorbance were observed during the period of experiments. It is notable that while the PL intensity of TGA-QDs-1 decreased in the suspension without BSA within the first 2.5 hours after the sample preparation, the PL intensity stayed stable in the suspension with BSA during these hours (Fig. 4(a)). However, the increased PL intensity of QDs was observed after the exposure of samples to the laser light for five minutes irrespective of the absence or the presence of protein (Fig. 4(b)). The calculated value of the initial PL quantum yield of TGA-QDs-1 was about 38% (Table 1, Fig. 5). Similarly as the PL intensity, the PL QY of samples also decreased without BSA but remained stable with BSA during the first 2.5 hours. The PL QY of the exposed quantum dots increased by a few percents, but in the suspension with BSA the QY increased slightly more than in the suspension without BSA. Moreover, after a day the PL QY of QDs in the suspension with BSA increased by some percents regardless of whether the samples were exposed to light or not. At the same time the PL QY of QDs without BSA decreased by a few percents. After five days the decrease of QY was observed for all samples of TGA-QDs-1. The PL QY of exposed QDs in the suspension without protein was almost half as high as the QY of non-exposed QDs in the medium with protein. The value of PL QY for QDs, which underwent a combined treatment (were exposed to light in the presence of BSA), remained the highest. At the 5th day the samples were exposed for the second time to a doubled light dose (10 min), but in this case the exposure had no effect on the PL QY for QDs in the sample without BSA or the PL QY increased only a little (for QDs in the sample with BSA). After two weeks, in the suspension with BSA the PL quantum yield of non-exposed QDs further decreased for about one third of the initial

Table 1*. The values of the photoluminescence quantum yield of TGA-capped QDs in solutions with BSA or without BSA.

Experimental days	QY of photoluminescence, %				
	Batch 1			Batch 2	
	Light	Dark + BSA	Light + BSA	Dark	Light
0 d, 0 h	38.1 ± 0.4	38.1 ± 0.4	38.1 ± 0.4	31.2 ± 1.6	33.6 ± 1.7
0 d, 2.5 h	33.2 ± 0.3	38.1 ± 0.4	38.1 ± 0.4	–	–
0 d, 2.5 h t_{exp} = 5 min	35.2 ± 0.4	38.1 ± 0.4	41.2 ± 0.4	–	–
1 d	31.8 ± 0.3	41.6 ± 0.4	45.1 ± 0.5	–	–
2 d	–	–	–	32.8 ± 1.7	37.5 ± 1.9
2 d t_{exp} = 5 min	–	–	–	–	38.5 ± 2.0
2 d t_{exp} = 50 min	–	–	–	32.8 ± 1.7	43.6 ± 2.2
5 d	21.8 ± 0.2	37.9 ± 0.4	42.7 ± 0.4	–	–
5 d t_{exp} = 10 min	22.2 ± 0.2	35.5 ± 0.4	44.3 ± 0.4	–	–
14 d	14.1 ± 0.1	26.7 ± 0.3	35.3 ± 0.4	20.4 ± 1.0	26.3 ± 1.3

* Dark means non-exposed; light means exposed to laser light; t_{exp} is time of irradiation, values of PL QY of exposed QDs after exposure. PL excitation wavelength was 450 nm.

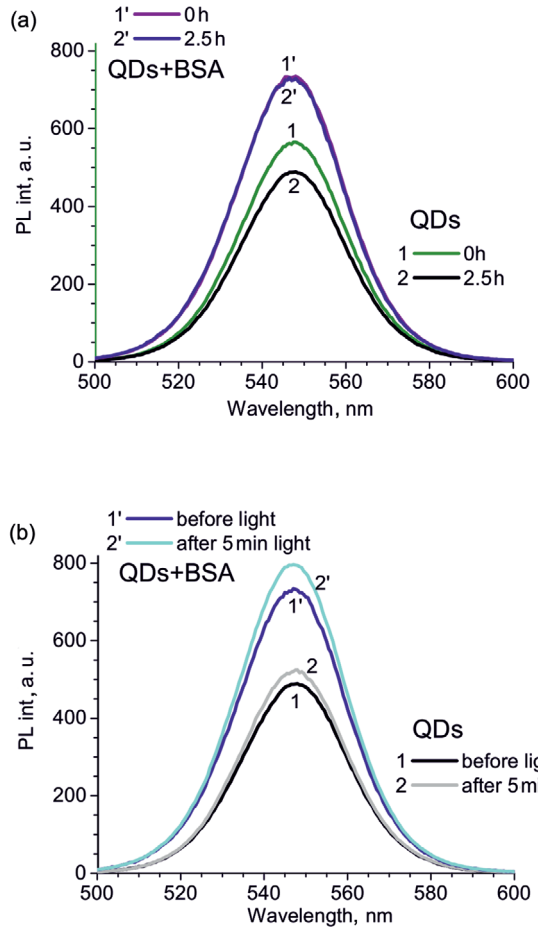

Fig. 4. Photoluminescence spectra of TGA-capped quantum dots (batch No. 1) in solutions with or without BSA: (a) non-exposed, freshly-made (0 h) and after 2.5 hours; (b) before and after exposure to the laser light at 532 nm for 5 min. PL excitation wavelength was 450 nm.

value. However, the relative decrease in the PL QY of exposed QDs was slight. Within two weeks the spectral PL band shifted 3 nm towards shorter wavelengths in all QD samples with protein, while no spectral changes were observed in samples without protein.

The combined effect of light and protein has also been measured on photoluminescence properties of

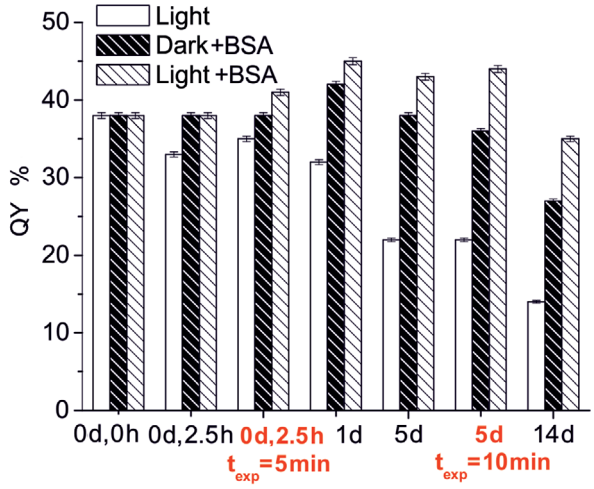

Fig. 5. Time-dependent changes in the photoluminescence quantum yield of TGA-capped QDs (batch No. 1) in solution with BSA (dark means non-exposed, light means exposed to laser light) or without BSA (exposed to laser light); t_{exp} is time of irradiation, values of PL QY of exposed QDs after exposure. PL excitation wavelength was 450 nm.

TGA-capped QDs from batch No. 2 prepared from the stock solution after storage for two months. Such storage period had no effect on the initial absorbance intensity and spectral features of the last absorption band. However, the spectral position of the PL band of these QDs samples was shifted to a red side at 553 nm (Fig. 3(a)), and the precipitation was observed. The presence of BSA induced no changes in intensity and the spectral position of the last band of TGA-QDs-2 absorbance during the period of experiments. The initial values of the PL QY of TGA-QDs-2 were lower than in the previous experiments reaching about 20% and increased by a few percents after the day (Table 2, Fig. 6). In the suspensions with BSA the initial values of the PL QY of TGA-QDs-2 were similar as in pure suspensions and increased in about the same few percents after the day. After exposure to light for 15 min on the next day (at about 24 hours

Table 2*. The values of the photoluminescence quantum yield of TGA-capped QDs (batch No. 2) in suspensions with and without BSA.

Experimental days	QY of photoluminescence, %			
	Dark	Light	Dark + BSA	Light + BSA
0 d	21.4 ± 1.1	20.1 ± 1.0	23.2 ± 1.2	20.1 ± 1.2
1 d	25.6 ± 1.3	23.5 ± 1.2	29.8 ± 1.5	29.8 ± 1.5
1 d t_{exp} = 15 min	–	29.7 ± 1.5	–	37.0 ± 1.9
3 d	24.4 ± 1.2	27.1 ± 1.4	29.7 ± 1.5	36.4 ± 1.8

* Dark means non-exposed; light means exposed to laser light; t_{exp} is time of irradiation, values of PL QY of exposed QDs after exposure. PL excitation wavelength was 450 nm.

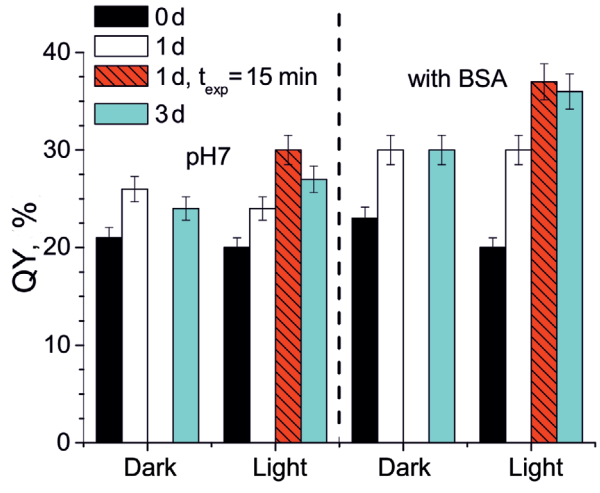

Fig. 6. Changes of the photoluminescence quantum yield of TGA-capped QDs (batch No. 2) on time in suspensions with and without BSA, both non-exposed (dark) and exposed to the laser radiation (light); t_{exp} is time of irradiation. PL excitation wavelength was 450 nm.

after preparation of samples), the further increase of the photoluminescence QY was observed regardless of the presence of protein in QDs samples. After three days the PL QY of quantum dots slightly decreased in the medium without protein, regardless of the exposure of samples, and stayed stable in the suspensions with protein. The PL QY of exposed QDs in both pairs of samples remained higher in comparison with non-exposed QDs, while the combined treatment, like in the case of TGA-QDs-1, resulted in the highest PL QY value (Table 2, Fig. 6).

3.2. MPA-capped quantum dots

In order to compare how the spectroscopic properties of modified QDs depended on the surface ligands, the effects of light and protein have been measured on the photoluminescence of MPA-capped CdSe/ZnS quantum dots (Table 3, Fig. 7). As mentioned earlier, the initial value of the PL quantum yield of MPA-capped quantum dots was higher than that of TGA-capped QDs. The PL intensity and the quantum yield of MPA-capped QDs did not change in the suspension without BSA within three days varying about the mean value of 58%. There were no notable changes in the spectroscopic properties of MPA-capped QDs even after their exposure to light. However, after three days the PL QY of exposed QD was slightly higher. The addition of BSA into the samples caused a rapid decrease in the PL quantum yield of MPA-capped QDs, but not in the PL QY of TGA-capped QDs. Also, the PL band of QDs broadened slightly for one nanometre towards shorter wavelengths. Exposure to light for 15 minutes had no additional effect on the PL QY of QDs. Further decrease of the PL QY was observed after four and six days, but then, like in the pure suspensions, the QY of exposed MPA-capped QDs was higher.

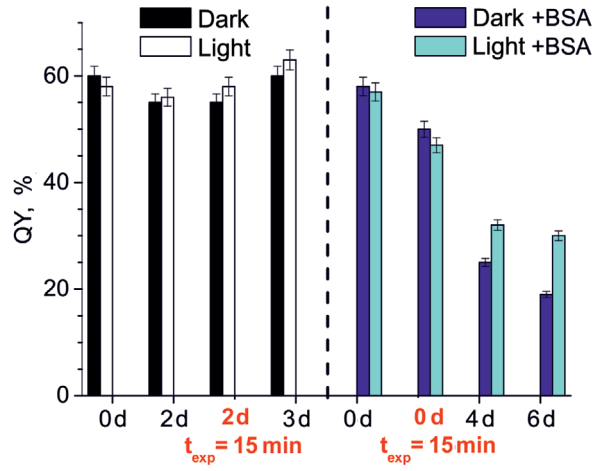

Fig. 7. The changes of PL quantum yields of MPA-capped QDs on time in solutions with and without BSA; non-exposed (dark) and exposed to laser radiation (light); t_{exp} is time of irradiation. PL excitation wavelength was 450 nm.

Table 3*. The values of the photoluminescence quantum yield of MPA-capped QDs in solutions with and without BSA.

Experimental days	QY of photoluminescence, %			
	Dark	Light	Dark + BSA	Light + BSA
0 d	59.8 ± 1.8	58.3 ± 1.7	58.4 ± 1.7	56.6 ± 1.7
0 d t_{exp} = 15 min	–	–	49.5 ± 1.5	47.0 ± 1.4
2 d	54.8 ± 1.7	56.4 ± 1.7	–	–
2 d t_{exp} = 15 min	54.8 ± 1.7	58.4 ± 1.7	–	–
3 d	59.4 ± 1.8	64.6 ± 1.9	–	–
4 d	–	–	24.6 ± 0.8	31.9 ± 1.0
6 d	–	–	18.8 ± 0.6	29.5 ± 0.9

* Dark means non-exposed; light means exposed to laser light; t_{exp} is time of irradiation, values of PL QY of exposed QDs after exposure. PL excitation wavelength was 450 nm.

4. Discussion

In this study we compared the effects of light and protein (BSA) on the photoluminescence stability of thiol-capped (TGA and MPA) QDs after the surface modification that made them water-suspendable. Despite the fact that the surface of TGA-capped QDs in both examined batches was modified according to the same protocol, some differences between the samples were observed spectroscopically. Firstly, the last absorption band of TGA-QDs-1 was broader at the blue side in comparison with the absorption band of TGA-QDs-2. Secondly, the spectral position of the PL band of TGA-QDs-1 did not change in pure aqueous solutions (548 nm), but shifted towards a blue side at 545 nm in suspensions with BSA after two weeks of the experiment, while the PL spectral band of TGA-QDs-2 shifted towards longer wavelengths (Fig. 3(a)) and the precipitation was observed regardless of the presence of protein. The initial values of PL QY were higher for TGA-QDs-1 than for TGA-QDs-2 (Table 1, Fig. 3(b)). However, the later comparison between TGA-QDs-2 and TGA-QDs-1 showed the opposite situation after two weeks (Table 1, Fig. 3(b)). The relatively reduced stability of TGA-QDs-1 photoluminescence could be a result of accidentally formed structural variations during the modification procedure, presumably leading to increased numbers of defects in the coating layer and diminished insulation of the QDs surface.

Aggregation of suspended QDs takes place when the surface stabilizing ligands are removed from the nanocrystal surface via protonation [18] or, possibly, photooxidation [8]. The bathochromic shift of the PL band of TGA-QDs-2 in suspensions, which were prepared from the stock solution after two months of storage, was even bigger than that in earlier prepared QDs samples – after two experimental weeks. Thus, the observed differences in spectral properties (such as a decrease of PL QY and a shift of the PL spectral band towards longer wavelengths) together with precipitation reflected the higher aggregation state of TGA-QDs-2. The dilution of the stock solution with an aqueous medium kept the PL intensity and QY of TGA-QDs-1 reduced not only within the first 2.5 hours but also after a day (Table 1, Fig. 5), however, the PL intensity and QY of the TGA-QDs-2 increased (Table 2, Fig. 6). Such variation can be explained by the change in the dynamic equilibrium of an aggregation state of TGA-QDs-2 diluted in the aqueous medium when the rising numbers of detached QDs increased the PL intensity and the QY. Thus, we suppose that time-dependent spectral differences observed between TGA-capped QDs from two batches can be resulted by lower stability of the capping layer formed from the stabilizing ligands in the case of TGA-QDs-2, which reduces its capacity to prevent aggregation in comparison with TGA-QDs-1.

Despite the differences between TGA-capped QDs from two batches, the addition of BSA in the medium enhanced the PL intensity and QY of both TGA-QDs-1 (Table 1, Fig. 5) and TGA-QDs-2 (Table 2, Fig. 6) within a day after the preparation of samples. It was reported that the denatured BSA can be conjugated to the surface of QDs and thereby improve the chemical stability and the PL QY of the water-soluble CdTe QDs [19]. As it was noted earlier, after two weeks the PL spectra of the TGA-QDs-1 suspended with BSA became blue-shifted and the PL QY decreased. Nevertheless, it was still higher than the PL QY of QDs in pure suspensions. These observations could be explained by the gradual removal of TGA molecules from the QDs surface in the presence of BSA. It has been found that the interaction with BSA improved the PL of TGA-capped QDs, but not at the initial phase [20]. The interaction of water suspended QDs with protein could lead to the formation of an additional coating layer that stabilizes quantum dots [20, 21]. Thus, the BSA-induced increase of the PL QY of TGA-capped QDs is sustained, but the enhancement of QDs stability is a short-term effect (Table 1, Fig. 5).

Comparing the two types of QDs capped with stabilizing ligands having a thiol group (TGA or MPA), we observed that the PL QY of MPA-capped QDs (Table 3, Fig. 7) in the aqueous suspension without protein was higher and remained more stable than the QY of TGA-capped QDs (Table 1, Fig. 5). It was shown in another study that the chain length of the stabilizing ligand is an important factor controlling the stability of QDs [22]. Indeed, the MPA ligands with longer chains enhanced the photoluminescence stability more than TGA. Moreover, while the addition of BSA in the medium increased the PL intensity and QY of TGA-capped CdSe/ZnS quantum dots (Table 1, Fig. 5), it decreased the PL QY of MPA-capped QDs (Table 3, Fig. 7) [23]. These observations imply that BSA cannot properly attach to the surface of QDs when longer chains of MPA molecules are present in the surrounding medium, and their interaction with BSA molecules might impair the formation of the protein coating layer. Thus, the capacity of protein to modify and stabilize/insulate the QDs surface can be strongly influenced by the nature of stabilizing ligands.

The exposure to green light induced the instant increase of the PL intensity and QY of the studied thiol-capped QDs in suspensions without BSA (Fig. 4(b)). Comparison between non-exposed and exposed samples did not show any light-induced shifts of QDs spectral bands. The PL intensity and QY of the exposed TGA-QDs-1 increased by a few percents but no more

than up to initial values (Table 1, Fig. 5). At this point (Table 2, Fig. 6), the PL intensity and QY of the exposed TGA-QDs-2 also increased by the same few percents above the values before irradiation but below the initial values (Table 1, Fig. 3(b)). Thus, the light-induced instant increase of the PL intensity and the QY is the effect independent on the initial stability of the capping layer on QDs surface. However, no general consensus on the reasons of the short-term enhancement of PL has been reached yet. Some suggested mechanisms include the photoneutralization of the local charged centres inside and outside the QDs [24], the elimination of the topological surface defects [25–27], the passivation of the surface trap states by photoadsorbed molecules [10, 11, 28], for example, water molecules [29, 30] and the photoinduced rearrangement of surface stabilizing agents [2, 31–33]. The reason why the MPA-capped QDs possessed higher values of the PL QY and enhanced stability than the TGA-capped QDs might be an unequal effect that these thiol ligands had on PL self-quenching. The MPA ligands with longer chains can hold nanoparticles farther apart from each other and form a more stable surface capping layer than TGA ligands with shorter chains. Light did not affect the PL QY of MPA-capped QDs (Table 3, Fig. 7) but increased the QY of TGA-capped QDs (Tables 1–2, Figs. 5–6). For the latter case, the light-induced rearrangement of the stabilizing agents or photoadsorbed molecules in the surface layer might diminish self-quenching of thiol-capped QDs causing the increase of photoluminescence.

In the absence of light the decrease of the PL QY of thiol-capped QDs exposed in the suspension with BSA was observed during several days (Tables 1–3, Figs. 5–7). However, the PL QY of TGA-capped QDs was still higher than before irradiation and also higher than the QY values of non-exposed control samples (Tables 1–2, Figs. 5–6). While in the case of MPA-capped QD the decrease of the PL intensity and the PL QY was observed independently of light exposure within 15 minutes after addition of BSA, after a week the PL QY of exposed MPA-capped QDs was greater than the QY of non-exposed QDs (Table 3, Fig. 7). Despite the obvious effect of stabilizing ligand's nature on the interaction with protein, the enhancing effect of light on photoluminescence of QDs in the suspension with BSA takes place independently on the surface ligands. The light-induced PL enhancement of thiol-capped QDs is instant and also relatively long-lasting in the presence of BSA in comparison with the samples being exposed without protein. Such combined effect of light and protein might be caused by the reduction of PL self-quenching in the exposed samples and the steric interactions between suspended QDs with adsorbed BSA molecules keeping nanoparticles farther apart from each other.

5. Conclusions

This study aimed to investigate the changes of the photoluminescence intensity and the quantum yield of thiol-capped (TGA or MPA) quantum dots influenced by external factors: the exposure to the green light and the presence of protein (BSA). The capacity of the protein to modify and stabilize the surface layer of thiol-capped QDs can be strongly influenced by the nature of stabilizing ligands. The BSA molecules cannot improve the stability and the PL QY of MPA-capped QDs, while it can improve the PL intensity and the PL QY of TGA-capped QDs. The increase of the PL QY of TGA-capped QDs is presumably caused by the formation of an additional coating layer of BSA that provides additional stabilization and insulation for the surface of quantum dots. The light can increase the PL intensity and QY of thiol-capped QDs and this instant increase might be caused by the photoadsorption of water molecules on the QDs surface or the rearrangement of stabilizing ligands leading to decreased PL self-quenching. The light-induced PL enhancement of thiol-capped QDs occurs independently on the nature of stabilizing ligands and is relatively long-lasting in the medium with BSA, presumably, due to the stabilisation of decreased PL self-quenching by steric interactions between BSA molecules on the surface of QDs. Since both light exposure and BSA act as independent factors, their mutual effect on the PL intensity and the QY of thiol-capped QDs might be suppressive or enhancing with respect to untreated samples depending on the nature of stabilizing ligands. However, the green light never reduces the PL of thiol-capped QDs and the presence of BSA enhances its positive effect.

Acknowledgements

This work was partly supported by the project "Promotion of Student Scientific Activities" (VP1-3.1-ŠMM-01-V-02-003) from the Research Council of Lithuania (A. K.). This project is funded by the Republic of Lithuania and the European Social Fund under the Operational Programme for the Development of Human Resources 2007–2013, Priority 3.

References

[1] T. Jamieson, R. Bakhshi, D. Petrova, R. Pocock, M. Imanib, and A.M. Seifalian, Biological applications of quantum dots, Biomaterials **28**, 4717–

4732 (2007), http://dx.doi.org/10.1016/j.biomaterials.2007.07.014

[2] J.A. Kloefper, S.E. Bradforth, and J.L. Nadeau, Photophysical properties of biologically compatible CdSe quantum dot structures, J. Phys. Chem. B **109**, 9996–10003 (2005), http://pubs.acs.org/doi/abs/10.1021/jp044581g

[3] A.M. Derfus, W.C.W. Chan, and S.N. Bhatia, Probing the cytotoxicity of semiconductor quantum dots, Nano Lett. **4**(1), 11–18 (2004), http://pubs.acs.org/doi/abs/10.1021/nl0347334

[4] J. Ma, J.Y. Chen, J. Guo, C. Wang, W. Yang, L. Xu, and P. Wang, Photostability of thiol-capped CdTe quantum dots in living cells: the effect of photooxidation, Nanotechnology **17**, 2083 (2006), http://dx.doi.org/10.1088/0957-4484/17/9/002

[5] Y. Qu, W. Li, Y. Zhou, X. Liu, L. Zhang, L. Wang, Y-f Li, A. Iida, Z. Tang, and Y. Zhao, Full assessment of fate and physiological behavior of quantum dots utilizing *Caenorhabditis elegans* as a model organism, Nano Lett. **11**, 3174–83 (2011), http://pubs.acs.org/doi/abs/10.1021/nl201391e

[6] K. Susumu, H. Tetsuo Uyeda, I.L. Medintz, T. Pons, J.B. Delehanty, and H. Mattoussi, Enhancing the stability and biological functionalities of quantum dots via compact multifunctional ligands, J. Am. Chem. Soc. **129**, 13987–13996 (2007), http://pubs.acs.org/doi/abs/10.1021/ja0749744

[7] W.W. Yu, E. Chang, R. Drezek, and V.L. Colvin, Water-soluble quantum dots for biomedical applications, Biochem. Biophys. Res. Commun. **348**, 781–786 (2006), http://dx.doi.org/10.1016/j.bbrc.2006.07.160

[8] J. Aldana, Y.A. Wang, and X.G. Peng, Photochemical instability of CdSe nanocrystals coated by hydrophilic thiols. J. Am. Chem. Soc. **123**, 8844–8850 (2001), http://pubs.acs.org/doi/abs/10.1021/ja016424q

[9] F. Koberling, A. Mews, and T. Basché, Oxygen-induced blinking of single CdSe nanocrystals, Adv. Mater. **13**(9), 672–676 (2001), http://dx.doi.org/10.1002/1521-4095(200105)13:9<672::AID-ADMA672>3.0.CO;2-W

[10] V. Biju, R. Kanemoto, Y. Matsumoto, S. Ishii, S. Nakanishi, T. Itoh, Y. Baba, and M. Ishikawa, Photoinduced photoluminescence variations of CdSe quantum dots in polymer solutions, J. Phys. Chem. C **111**, 7924–7932 (2007), http://pubs.acs.org/doi/abs/10.1021/jp0713514

[11] S.R. Cordero, P.J. Carson, R.A. Estabrook, G.F. Strouse, and S.K. Buratto, Photo-activated luminescence of CdSe quantum dot monolayers, J. Phys. Chem. B **104**, 12137–12142 (2000), http://pubs.acs.org/doi/abs/10.1021/jp001771s

[12] D.F. Eaton, Reference materials for fluorescence measurement, Pure Appl. Chem. **60**(7), 1107–1114 (1988), http://list.iupac.org/publications/pac/1988/pdf/6007x1107.pdf

[13] F. López Arbeloa, P. Ruiz Ojeda, and I. López Arbeloa, Flourescence self-quenching of the molecular forms of Rhodamine B in aqueous and ethanolic solutions, J. Lumin. **44**(1–2), 105–112 (1989), http://dx.doi.org/10.1016/0022-2313(89)90027-6

[14] D. Magde, G.E. Rojas, and P.G. Seybold, Solvent dependence of the fluorescence lifetimes of xanthene dyes, Photochem. Photobiol. **70**(5), 737–744 (1999), http://dx.doi.org/10.1111/j.1751-1097.1999.tb08277.x

[15] T. Karstens and K. Kobs, Rhodamine B and rhodamine 101 as reference substances for fluorescence quantum yield measurements, J. Phys. Chem. **84**(14), 1871–1872 (1980), http://pubs.acs.org/doi/abs/10.1021/j100451a030

[16] M. Daimon and A. Masumura, Measurement of the refractive index of distilled water from the near-infrared region to the ultraviolet region, Appl. Opt. **46**(18), 3811–3820 (2007), http://dx.doi.org/10.1364/AO.46.003811

[17] J. Nowakowska, *The Refractive Indices of Ethyl Alcohol and Water Mixtures*, Master's Theses, Paper 668 (Loyola University, Chicago, 1939), http://ecommons.luc.edu/luc_theses/668

[18] J. Aldana, N. Lavelle, Y.J. Wang, and X.G. Peng, Size dependent dissociation pH of thiolate ligands from cadmium chalcogenide nanocrystals, J. Am. Chem. Soc. **127**, 2496–2504 (2005), http://pubs.acs.org/doi/abs/10.1021/ja047000%2B

[19] Q. Wang, Y. Kuo, Y. Wang, G. Shin, Ch. Ruengruglikit, and Q. Huang, Luminescent properties of water-soluble denatured bovine serum albumin-coated CdTe quantum dots, J. Phys. Chem. B **110**, 16860–16866 (2006), http://pubs.acs.org/doi/abs/10.1021/jp062279x

[20] V. Poderys, M. Matulionyte, A. Selskis, and R. Rotomskis, Interaction of water-soluble CdTe quantum dots with bovine serum albumin, Nanoscale Res. Lett. **6**, 9 (2011), http://www.nanoscalereslett.com/content/6/1/9

[21] B. Sahoo, M. Goswami, S. Nag, and S. Maiti, Spontaneous formation of a protein corona prevents the loss of quantum dot fluorescence in physiological buffers, Chem. Phys. Lett. **445**, 217–220 (2007), http://dx.doi.org/10.1016/j.cplett.2007.07.075

[22] J. Lovr, S. Ju Cho, F.M. Winnik, and D. Maysinger, Unmodified cadmium telluride quantum dots induce reactive oxygen species formation leading to multiple organelle damage and cell death, Chem. Biol. **12**, 1227–1234 (2005), http://dx.doi.org/10.1016/j.chembiol.2005.09.008

[23] M. Matulionyte, V. Poderys, and R. Rotomskis, Influence of surface coating on CdTe quantum dots stability and interaction with bovine serum albumin, in: *Proceedings of 8th International Conference on Medical Physics in the Baltic States* (Kaunas, Lithuania, 2010), http://alephfiles.rtu.lv/TUA01/000035052_e.pdf

[24] M. Oda, A. Hasegawab, N. Iwamib, K. Nishiurab, N. Andob, A. Nishiyamab, H. Horiuchib, and T. Tania, Reversible photobluing of CdSe/ZnS/TOPO nanocrystals, Colloids Surf. B **56**, 241–245 (2007), http://dx.doi.org/10.1016/j.colsurfb.2006.12.012

[25] J. Jasieniak and P. Mulvaney, From Cd-rich to Se-rich – the manipulation of CdSe nanocrystal surface stoichiometry, J. Am. Chem. Soc. **129**, 2841–2848 (2007), http://pubs.acs.org/doi/abs/10.1021/ja066205a

[26] Y. Wang, Z. Tang, M.A. Correa-Duarte, I. Pastoriza-Santos, M. Giersig, N.A. Kotov, and L.M. Liz-Marzan, Mechanism of strong luminescence photoactivation of citrate-stabilized water-soluble nanoparticles with CdSe cores, J. Phys. Chem. B **108**, 15461–15469 (2004), http://pubs.acs.org/doi/abs/10.1021/jp048948t

[27] Y. Wang, Z. Tang, M.A. Correa-Duarte, L.M. Liz-Marzán, and N.A. Kotov, Multicolor luminescence patterning by photoactivation of semiconductor nanoparticle films, J. Am. Chem. Soc. **125**, 2830–2831 (2003), http://pubs.acs.org/doi/abs/10.1021/ja029231r

[28] K. Pechstedt, T. Whittle, J. Baumberg, and T. Melvin, Photoluminescence of colloidal CdSe/ZnS quantum dots: the critical effect of water, J. Phys. Chem. C **114**, 12069–12077 (2010), http://pubs.acs.org/doi/abs/10.1021/jp100415k

[29] J. Silver and W. Ou, Photoactivation of quantum dot fluorescence following endocytosis, Nano Lett. **5**(7), 1445–1449 (2005), http://pubs.acs.org/doi/abs/10.1021/nl050808n

[30] S. Patra and A. Samanta, A fluorescence correlation spectroscopy, steady-state, and time-resolved fluorescence study of the modulation of photophysical properties of mercaptopropionic acid capped CdTe quantum dots upon exposure to light, J. Phys. Chem. C **117**, 23313–23321 (2013), http://pubs.acs.org/doi/abs/10.1021/jp407130e

[31] H. Bao, Y. Gong, Z. Li, and M. Gao, Enhancement effect of illumination on the photoluminescence of water-soluble CdTe nanocrystals: toward highly fluorescent CdTe/CdS core-shell structure, Chem. Mater. **16**, 3853–3869 (2004), http://pubs.acs.org/doi/abs/10.1021/cm049172b

[32] M. Jones, J. Nedeljkovic, R.J. Ellingson, A.J. Nozik, and G. Rumbles, Photoenhancement of luminescence in colloidal CdSe quantum dot solutions, J. Phys. Chem. B **107**, 11346–11352 (2003), http://pubs.acs.org/doi/abs/10.1021/jp035598m

[33] K. Sato, S. Kojima, S. Hattori, T. Chiba, K. Ueda-Sarson, T. Torimoto, Y. Tachibana, and S. Kuwabata, Controlling surface reactions of CdS nanocrystals: photoluminescence activation, photoetching and photostability under light irradiation, Nanotechnology **18**, 465702 (2007), http://dx.doi.org/10.1088/0957-4484/18/46/465702

ŠVIESOS POVEIKIS TIOLIAIS DENGTŲ CdSe/ZnS KVANTINIŲ TAŠKŲ STABILUMUI TERPĖJE SU ALBUMINU

A. Kalnaitytė [a], S. Bagdonas [a], R. Rotomskis [a, b]

[a] *Vilniaus universiteto Fizikos fakulteto Kvantinės elektronikos katedra, Vilnius, Lietuva*

[b] *Nacionalinio vėžio instituto Biomedicininės fizikos laboratorija, Vilnius, Lietuva*

Santrauka

Veikiant CdSe/ZnS kvantinius taškus, dengtus merkaptopropionine (MPR) ar tioglikoline (TGR) rūgštimis, žalia lazerio spinduliuote, ištirtas jų stabilumas vandeninėje terpėje ir terpėje su jaučio serumo albuminu (JSA). Iš sugerties bei liuminescencijos matavimų rezultatų nustatyta, jog baltymo gebėjimas pakeisti tioliais dengtų kvantinių taškų dangalo struktūrą ir fotoliuminescencijos (FL) intensyvumą priklauso nuo stabilizuojančių paviršiaus ligandų prigimties. Kvantiniams taškams, dengtiems TGR, sąveikaujant su JSA molekulėmis padidėdavo jų FL kvantinis našumas, o fotoliuminescencija tapdavo stabilesnė, priešingas efektas gautas su MPR degtais kvantiniais taškais. Šviesa iš karto po švitinimo padidindavo TGR dengtų kvantinių taškų fotoliuminescencijos intensyvumą ir kvantinį našumą, bet kvantinių taškų, dengtų MPR, spektroskopinių savybių nepakeisdavo. Terpėje su JSA fotoliuminescencijos padidėjimas stebimas nepriklausomai nuo kvantinių taškų dengiamojo sluoksnio prigimties bei stabilumo, o fotoliuminescencija išlieka santykinai stabilesnė.