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Abstract. The excitation-related problems in photodynamic therapy of cancer might be solved by combining two-photon (TP) irradiation and quantum dots (QDs) as effective energy donors for conventional photosensitizers (PS). Here, it is demonstrated for the first time that QD–chlorin e_6 (Ce_6) complex formed due to the hydrophobic interaction between Ce_6 molecules and lipid coating of QDs can be effectively excited via TP irradiation at 1030 nm, which spectrally coincides with the biological tissue optical window. TP absorption cross-section for free QDs and Ce_6 at 1030 nm was 3325 and 13 Goeppert-Mayer, respectively. Upon TP excitation of QD – Ce_6 solution, the fluorescence band of bound Ce_6 molecules was observed via energy transfer from excited QDs. Increasing concentration of Ce_6 resulted in quenching of the photoluminescence of QDs and an increase in the fluorescence intensity of bound Ce_6 molecules. These intensity changes coincided well with those observed upon single-photon excitation of QD – Ce_6 solution when QDs alone are excited. The efficiency of energy transfer in QD – Ce_6 complex upon TP excitation was about 80% (QD : Ce_6 1 : 5). These results indicate that the effective excitation of PS with a low TP absorption cross-section is possible in such type noncovalent complexes via energy transfer from TP excited QDs. [DOI: 10.1117/1.JBO.18.7.078002]

Keywords: photodynamic therapy; two-photon irradiation; quantum dots; chlorin e_6; energy transfer.

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1 Introduction

Nanostructures such as semiconductor quantum dots (QDs) emerged in the fields of biology and medicine as promising imaging and therapeutic agents.[1–6] Lately, it was proposed that QDs could be used in the photodynamic therapy (PDT) of cancer.[5,6] Unique optical properties of QDs such as size-tunable photoluminescence (PL) spectrum, high PL quantum yield and long lifetime as well as high extinction coefficient and broad absorption spectrum make them advantageous energy donors for conventional photosensitizers (PS) used in PDT.[3–5,7] Moreover, due to extremely large two-photon (TP) absorption cross-section of QDs[8–10] it becomes possible to use TP excitation in a QD (donor)–PS (acceptor) system for indirect excitation of PS, which usually have a low TP absorption cross-section.[11–14] TP excitation is usually provided within the near infrared (NIR) spectral region, which coincides with the tissue optical window. NIR irradiation can penetrate deeper into the tissue compared to visible light, allowing more profound examination and treatment of tumor. In addition, since TP excitation is confined within the focal volume of laser beam, selective activation of PS is achieved, which effectively enhances spatial resolution and produces less damage to surrounding healthy tissue.[15] To our knowledge, there are only a few studies to date focusing on TP excitation of QD–PS systems,[16–18] which show that TP excited QDs can efficiently donate energy to electrostatically[16,17] or covalently[18] attached PS. However, covalent coupling of QD–PS complexes requires additional chemical procedures which are not always feasible, while QD–PS complexes formed via electrostatic interaction might be less stable in biological media. In our study, for the first time, we have exploited the hydrophobic interaction to design QD–PS complex. Furthermore, we have used biocompatible QDs with lipid-based coating, which not only guarantees biocompatibility and stability of QDs for future applications in biological medium, but also serve as binding sites for amphiphilic PS ensuring its close localization to QD core and subsequently efficient Forster resonance energy transfer (FRET). As a PS, chlorin e_6 (Ce_6) was chosen. It is a typical light-activated drug for PDT with a high singlet oxygen generation quantum yield. However, a small TP absorption cross-section limits its application in TP PDT.

We have already shown, using single-photon (SP) excitation, that lipid-coated CdSe/ZnS QD form a stable, noncovalent complex with PS Ce_6.[19,20] Ce_6 molecules penetrate into the lipid coating of QD and localize close enough to QD shell/core for the FRET to occur. Here, we demonstrate that TP excited QD can work as efficient energy donors for Ce_6 as well.

2 Materials and Methods

Ce_6 tetrasulfonic acid was purchased from Frontier Scientific Inc. (USA). CdSe/ZnS QDs ($\lambda_{em} = 625 \pm 5$ nm, core diameter—7.1 ± 0.5 nm,[21] hydrodynamic diameter—22.8 ± 3 nm) coated by phospholipid layer with PEG and terminal carboxyl groups were obtained from eBioscience Inc. (USA). All materials were used without further purification. Ce_6 was dissolved in a small amount of 0.2 M NaOH solution and further diluted with a phosphate buffer (PB) (pH 7) to concentrations varying from 0.02 to 0.3 μM. Stock solution of QDs (10 μM) was diluted with PB to 0.02 μM working concentration. QD – Ce_6 solutions were

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made by adding 5 μL of concentrated Ce_6 solution to the already prepared 0.02 μM solution of QDs, obtaining QD:Ce_6 molar ratios from 1:1 up to 1:15. Dilution effect for QD – Ce_6 solution after addition of Ce_6 was encountered by adding 5 μL of PB to the control QD solution.

SP absorption spectra were recorded with Cary 50 UV–Vis spectrophotometer (Varian Inc., Palo Alto, California). SP excited PL spectra were measured with Cary Eclipse spectrophotometer (Varian Inc., USA). TP excitation of samples was produced by laser system PHAROS (Light Conversion, Lithuania) operating at 1030 nm wavelength with pulse duration of 280 fs and 1 kHz repetition rate. The average laser power of focused beam entering the sample was approximately 200 mW (pulse energy 200 μJ; peak pulse power 0.7 GW). TP induced PL signal was collected at a 90-deg angle and guided to spectrophotometer by an optical fiber. All experiments were performed at room temperature (RT).

TP absorption cross-sections of samples were calculated by a comparative method[22] using Rhodamine 6G TP absorption cross-section $\sigma_2^{(r)}(\lambda_2)$ as a reference [$\sigma_2^{(r)}(\lambda = 1030\ \text{nm}) = 9.8$ Goeppert-Mayer (GM) in methanol].[23] SP and TP induced PL intensities of samples were measured at their corresponding PL band maxima $F_1^{(s)}(\lambda^{(s)})$ and $F_2^{(s)}(\lambda^{(s)})$ respectively. In both cases $\lambda^{(s)} = 627$ nm. The SP extinction coefficient at the excitation wavelength of the samples $\varepsilon_1^{(s)}(\lambda_1)$, ($\lambda_1 = 515$ nm) was evaluated from the absorption spectra. The Rhodamine 6G SP and TP induced PL intensities at their corresponding band maxima $F_1^{(r)}(\lambda^{(r)})$ and $F_2^{(r)}(\lambda^{(r)})$, respectively, ($\lambda^{(r)} = 556$ nm) and SP extinction coefficient $\varepsilon_1^{(r)}(\lambda_1), \lambda_1 = 515$ nm were evaluated in the same way as for investigated samples. TP absorption cross-section can then be calculated as follows:

$$\sigma_2^{(s)}(\lambda_2) = \frac{F_2^{(s)}(\lambda^{(s)})}{F_2^{(s)}(\lambda^{(s)})}\frac{F_1^{(s)}(\lambda^{(s)})}{F_1^{(s)}(\lambda^{(s)})}\frac{\varepsilon_1^{(s)}(\lambda_1)}{\varepsilon_1^{(r)}(\lambda_1)}\sigma_2^{(r)}(\lambda_2). \quad (1)$$

By this method, neither the parameters of the excitation light (pulse energy, pulse duration, spatial, and temporal intensity distribution), nor the parameters of the detection setup (detector characteristics and refractive indexes of solvents), nor PL quantum yield need to be known.

The efficiency E of FRET between QDs and Ce_6 molecules was calculated from the decrease of QD (donors) PL in the presence of Ce_6 molecules (acceptors):

$$E = 1 - \frac{F'_D}{F_D}, \quad (2)$$

where F_D and F'_D correspond to the intensities of donor PL intensity in the absence and in the presence of acceptor, respectively.

3 Results and Discussion

SP absorption and PL spectra of pure Ce_6 and QDs aqueous solutions are shown in Fig. 1. Absorption spectrum of Ce_6 consists of the intensive Soret band at 405 nm and less intensive Q bands, with the most intensive $Q(I)$ in the red spectral region at 654 nm. In aqueous solution the maximum of Ce_6 fluorescence band is at 660 nm. Absorbance of QDs gradually decreases from UV to the red spectral region, ending with the last excitonic band at 615 nm. QDs have an intensive, narrow, and symmetrical PL band with a peak at 627 nm. It can be clearly seen that the PL band of QDs overlaps with the absorption spectrum

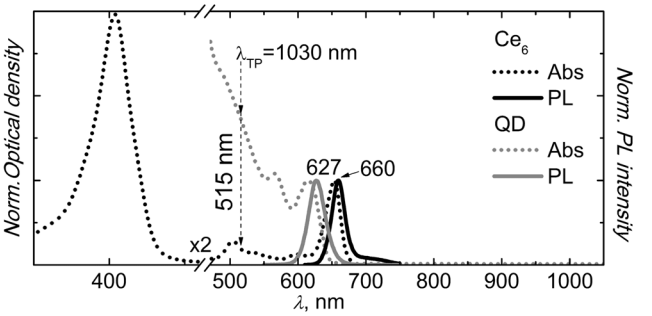

Fig. 1 Normalized SP absorption and PL spectra of pure QDs and Ce_6 aqueous solutions (PB pH 7). The arrow indicates a wavelength used for SP at 515 nm and corresponding TP at 1030 nm excitation of the samples.

of Ce_6 (Fig. 1). This satisfies the main requirement for FRET between QDs and Ce_6 to occur. Our previous study showed that addition of Ce_6 to QD aqueous solution results in stable QD – Ce_6 complex formation, driven by the hydrophobic interaction between nonpolar moiety of Ce_6 and lipids of QD coating.[19,20] Here, PL spectra after SP excitation of QDs solution mixed with different amounts of Ce_6 are shown in Fig. 2(a). Localization of Ce_6 molecules in the hydrophobic interior of the lipid part of QDs results in shift of Ce_6 fluorescence band from 660 to 670 nm. Analogous red shift of Ce_6 fluorescence maximum to 670 nm has been reported when Ce_6 molecules were incorporated into lipid bilayer.[24,25] An increasing Ce_6 concentration significantly quenches PL intensity of QDs, while the fluorescence intensity of bound Ce_6 molecules at 670 nm simultaneously increases [Figs. 2(a) and 3]. A significant increase in fluorescence intensity of Ce_6 is observed up to the QD:Ce_6 molar ratio of 1:5 however, further rise in Ce_6 concentration leads only to negligible changes in Ce_6 fluorescence intensity [Figs. 2(a) and 3(b)]. Meanwhile, the PL intensity of QDs noticeably decreases even at higher concentrations of Ce_6 (Ce_6/QD > 5) [Figs. 2(a) and 3(a)]. This might be explained by the self-quenching of bound Ce_6 molecules on the surface of QDs at relatively high concentrations of Ce_6. Moreover, the fluorescence intensity of pure Ce_6 under SP (at 515 nm) excitation is much lower than that bound to QDs [Fig. 2(a)], as can be clearly seen in inset of Fig. 3(b), where the intensity of pure Ce_6 fluorescence maximum (660 nm) is shown against the concentration. In addition, in our previous experiments we have shown that the fluorescence excitation spectra measured at 670 nm, contain a significant contribution of QDs spectrum.[19] Also, the average PL decay time of QDs decreases upon QD – Ce_6 complex formation.[19] All spectral features listed above indicate FRET occurring between QDs and bound Ce_6 molecules. The efficiency of FRET in QD – Ce_6 complex at QD:Ce_6 molar ratios of 1:1 and 1:5 was found to be 45% and 82%, respectively. Similar PL measurements for pure QDs, Ce_6, and QD – Ce_6 aqueous solutions at different QD:Ce_6 molar ratios were conducted using TP excitation at 1030 nm [Fig. 2(b)]. The intensive PL band of QDs observed at 627 nm confirms that QDs can be effectively excited using TP excitation. QDs are known to have a large TP absorption cross-section, which ranges from 75 to 47,000 GM, depending on their chemical structure, size, and excitation wavelength.[9] Here, TP absorption cross-section for QDs was calculated according to Eq. (1) $F_2^{(s)}(\lambda^{(s)}) = 1600$ a.u.; $F_2^{(r)}(\lambda^{(r)}) =$

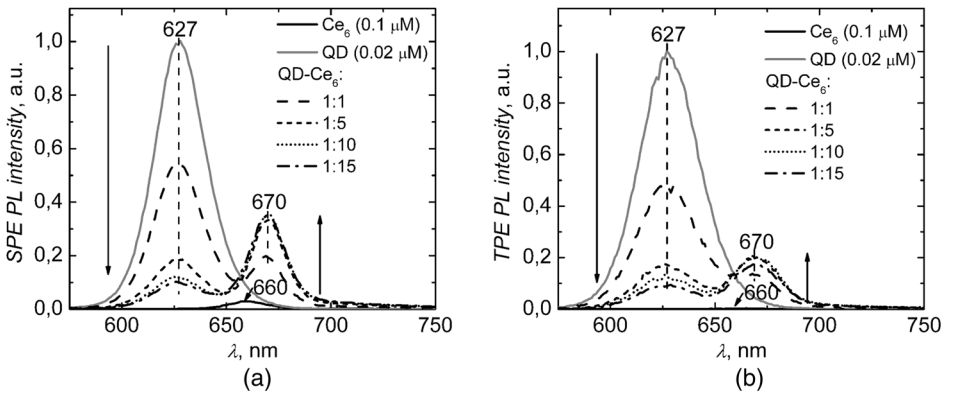

Fig. 2 PL spectra of pure QDs (0.02 μM), Ce_6 (0.1 μM), and mixed QD – Ce_6 aqueous solutions at different QD:Ce_6 molar ratios (1:1 to 1:15) under excitation of (a) SP 515 nm and (b) TP 1030 nm. Spectra were normalized to the PL peak of QDs at 627 nm.

1500 a.u.; $F_1^{(r)}(\lambda^{(r)})=9600$ a.u.; $F_1^{(s)}(\lambda^{(s)})=330$ a.u.; $\varepsilon_1^{(s)}(\lambda_1) = 724720\ \mathrm{l\cdot mol-1\cdot cm^{-1}}\varepsilon_1^{(r)}$; $(\lambda_1) = 65890\ \mathrm{l\cdot mol^{-1}\cdot cm^{-1}}$ and found to be 3325 GM, which is in agreement with the previous reports.[8–10]

Meanwhile, only a negligible emission signal can be observed for pure Ce_6 solution under TP excitation, since Ce_6 TP absorption cross-section is significantly lower (13 GM ($F_2^{(s)}(\lambda^{(s)}) = 110$ a.u.; $F_1^{(s)}(\lambda^{(s)}) = 57$ a.u.; $\varepsilon_1^{(s)}(\lambda_1) = 6830\ \mathrm{l\cdot mol^{-1}\cdot cm^{-1}}$)). For organic dyes and porphyrin type molecules TP absorption cross-section are usually in the order of tens of GM units or less.[11–14,22,23] Thus, effective application of such organic molecules in TP PDT is limited. Higher TP absorption cross-section for QDs is obtained at shorter wavelengths as 800 nm.[10] However, 1030 nm wavelength light is more suitable for FRET investigation in QD – Ce_6 complex, since only QD is excited via TP excitation.

Upon TP excitation similar spectral changes in mixed QD – Ce_6 solutions are observed as in the case of SP excitation [Figs. 2(b) and 3]. In mixed QD – Ce_6 solution PL intensity of QDs significantly decreases compared to pure QDs and an intensive fluorescence peak at 670 nm appears. No significant differences in PL changes of QDs between SP and TP excitation were observed suggesting that energy transfer pathway to bound Ce_6 molecules is independent on the excitation way [Fig. 3(a)].

Fluorescence intensity of Ce_6 at 670 nm, under TP excitation at 1030 nm of QD – Ce_6 solution increases until QD:Ce_6 molar ratio of 1:5, and begins to decrease for higher concentrations of Ce_6 [Fig. 3(b)]. This suggests that up to five Ce_6 molecules per single QD can localize within the lipid part of QDs coating without loss in fluorescence intensity. Further increase in concentration negatively acts on PL of both the QDs and Ce_6, without any enhancements on energy transfer efficiency or PL intensity. Such decrease was not observed under SP excitation at 515 nm most probably because it is compensated for by the fluorescence signal from direct excitation of free Ce_6. However, when SP excitation at 465 nm is used, where absorption of Ce_6 is minimal, a similar decrease can be observed (data not shown). As mentioned previously, it can be explained by fluorescence self-quenching of bound Ce_6 molecules due to their possible aggregation on the surface of QDs at higher Ce_6 concentrations. Under TP excitation at 1030 nm, the fluorescence of Ce_6 can be selectively observed only due to FRET from TP excited QDs to bound Ce_6 molecules. FRET efficiency under TP excitation from QD to Ce_6 in QD – Ce_6 complex at QD:Ce_6 molar ratio of 1:1 was found to be 49% and increased to the maximum 82% at QD:Ce_6 molar ratio 1:5. FRET efficiencies under TP excitation coincide with those obtained under SP excitation. It can be assumed that TP excitation can also be used to effectively excite other amphiphilic PS that can hydrophobically interact with the lipid coating of QDs.[20] It is noteworthy to mention that FRET efficiency values were obtained at RT; however, during the biomedical applications (experiments in cell cultures and

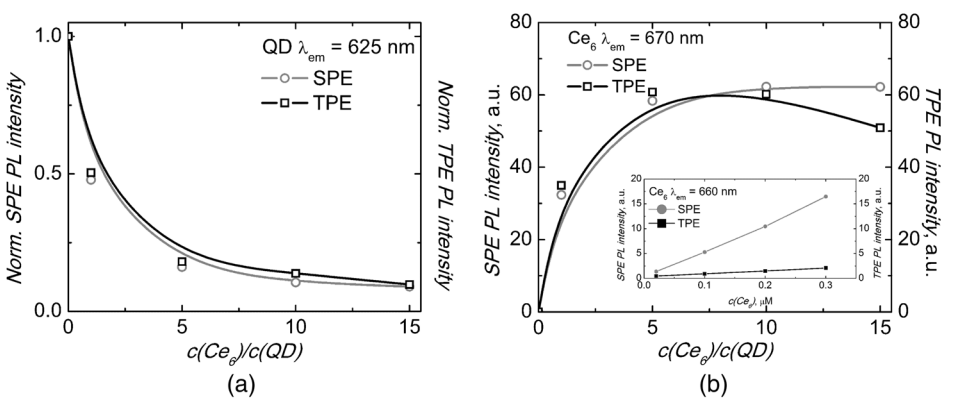

Fig. 3 Changes in the PL intensities of QD – Ce_6 solutions upon increasing QD:Ce_6 molar ratio from 1:1 to 1:15 under SP and TP excitation (a) at QD maximum at 627 nm (values are normalized to the initial intensity of QDs without Ce_6) and (b) at bound Ce_6 maximum at 670 nm. Inset shows the intensity changes of pure Ce_6 solution at fluorescence maximum at 660 nm by increasing the concentration of Ce_6 from 0.02 to 0.3 μM under SP and TP excitation wavelength.

experimental animals) QD – Ce_6 complexes will be exposed to higher temperatures. The increase of the temperature to 50°C causes decrease in FRET efficiency approximately by 10% (data not shown). It can be considered as negligible and QD – Ce_6 complex can be used for biomedical applications.

4 Conclusions

For the first time, we demonstrated that QD – Ce_6 complex self-assembled due to the hydrophobic interaction between nonpolar moiety of Ce_6 molecules and lipids of QDs coating can be effectively excited via TP irradiation at 1030 nm, which spectrally coincides with the transparency window of biological tissue. Indirect excitation of Ce_6 by energy transfer from TP excited QDs, overcomes difficulties in direct TP excitation of Ce_6, thus allowing one to utilize therapeutic action of Ce_6 under TP irradiation. The highest efficiency of FRET within QD – Ce_6 complex under TP excitation reached up to 82% when multiple Ce_6 molecules are bound to QD, which is significantly higher than that obtained by other similar QD–PS systems to date.[16–18] High FRET efficiency within QD – Ce_6 complex exploits full PDT potential of Ce_6 at relatively small concentrations of PS that is very important when concerning dark toxicity and clearance issues of QD – Ce_6 complex. These promising results constitute a first step toward the application of such type QD–PS complexes alongside TP irradiation in PDT.

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References

1. X. Michalet et al., "Quantum dots for live cells, in vivo imaging, and diagnostics," *Science* **307**(5709), 538–544 (2005).
2. I. L. Medintz et al., "Quantum dot bioconjugates for imaging, labelling and sensing," *Nat. Mater.* **4**(6), 435–446 (2005).
3. P. Juzenas et al., "Quantum dots and nanoparticles for photodynamic and radiation therapies of cancer," *Adv. Drug Delivery Rev.* **60**(15), 1600–1614 (2008).
4. E. Yaghini, A. M. Seifalian, and A. J. MacRobert, "Quantum dots and their potential biomedical applications in photosensitization for photodynamic therapy," *Nanomedicine* **4**(3), 353–363 (2009).
5. A. C. S. Samia, X. B. Chen, and C. Burda, "Semiconductor quantum dots for photodynamic therapy," *J. Am. Chem. Soc.* **125**(51), 15736–15737 (2003).
6. R. Bakalova et al., "Quantum dot anti-CD conjugates: are they potential photosensitizers or potentiators of classical photosensitizing agents in photodynamic therapy of cancer?" *Nano Lett.* **4**(9), 1567–1573 (2004).
7. J. M. Tsay et al., "Singlet oxygen production by peptide-coated quantum dot-photosensitizer conjugates," *J. Am. Chem. Soc.* **129**(21), 6865–6871 (2007).
8. D. R. Larson et al., "Water-soluble quantum dots for multiphoton fluorescence imaging in vivo," *Science* **300**(5624), 1434–1436 (2003).
9. S. C. Pu et al., "The empirical correlation between size and two-photon absorption cross section of CdSe and CdTe quantum dots," *Small* **2**(11), 1308–1313 (2006).
10. L. M. Maestro et al., "Nanoparticles for highly efficient multiphoton fluorescence bioimaging," *Opt. Express* **18**(23), 23544–23553 (2010).
11. M. A. Albota, C. Xu, and W. W. Webb, "Two-photon fluorescence excitation cross sections of biomolecular probes from 690 to 960 nm," *Appl. Opt.* **37**(31), 7352–7356 (1998).
12. B. A. Reinhardt et al., "Highly active two-photon dyes: design, synthesis, and characterization toward application," *Chem. Mater.* **10**(7), 1863–1874 (1998).
13. C. Xu and W. W. Webb, "Measurement of two-photon excitation cross sections of molecular fluorophores with data from 690 to 1050 nm," *J. Opt. Soc. Am. B* **13**(3), 481–491 (1996).
14. P. Chen et al., "Two-photon excitation of chlorin-e(6)-C-15 monomethyl ester for photodynamic therapy," *Proc. SPIE* **5630**, 209–217 (2005).
15. A. M. Smith, M. C. Mancini, and S. M. Nie, "Bioimaging second window for in vivo imaging," *Nat. Nanotechnol.* **4**(11), 710–711 (2009).
16. S. Dayal and C. Burda, "Semiconductor quantum dots as two-photon sensitizers," *J. Am. Chem. Soc.* **130**(10), 2890–2891 (2008).
17. Y. N. Wen et al., "Activation of porphyrin photosensitizers by semiconductor quantum dots via two-photon excitation," *Appl. Phys. Lett.* **95**(14), 143702 (2009).
18. Z. D. Qi et al., "Biocompatible CdSe quantum dot-based photosensitizer under two-photon excitation for photodynamic therapy," *J. Mater. Chem.* **21**(8), 2455–2458 (2011).
19. J. Valanciunaite et al., "Complex of water-soluble CdSe/ZnS quantum dots and chlorin e(6): interaction and FRET," *Proc. SPIE* **7376**, 737607 (2010).
20. J. Valanciunaite et al., "Spectroscopic study of non-covalent complex formation between different porphyrin analogues and quantum dots with lipid-based coating," *Chemija* **22**(4), 181–187 (2011).
21. T. L. Jennings et al., "Reactive semiconductor nanocrystals for chemoselective biolabeling and multiplexed analysis," *ACS Nano* **5**(7), 5579–5593 (2011).
22. M. Drobizhev et al., "Extremely strong near-IR two-photon absorption in conjugated porphyrin dimers: quantitative description with three-essential-states model," *J. Phys. Chem. B* **109**(15), 7223–7236 (2005).
23. N. S. Makarov, M. Drobizhev, and A. Rebane, "Two-photon absorption standards in the 550–1600 nm excitation wavelength range," *Opt. Express* **16**(6), 4029–4047 (2008).
24. A. A. Frolov et al., "Chlorin e6-liposome interaction—investigation by the methods of fluorescence spectroscopy and inductive resonance energy-transfer," *J. Photochem. Photobiol. B* **7**(1), 43–56 (1990).
25. H. Mojzisova et al., "The pH-dependent distribution of the photosensitizer chlorin e6 among plasma proteins and membranes: a physico-chemical approach," *Biochim. Biophys. Acta* **1768**(2), 366–374 (2007).