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Energy transfer processes under one- and two-photon excitation of nano-biohybrid structures based on semiconductor quantum dots and purple membranes

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Abstract

In this work we present the results of the resonance energy transfer study in bio-nanohybrid structures, engineered on the basis of semiconductor quantum dots (QD) and photosensitive membrane protein bacteriorhodopsin (bR) in purple membranes (PM) under one- and two-photon excitation. We showed the formation of bio-nanohybrid complexes between QDs and PMs and we also showed the Forster resonance energy transfer (FRET) from QDs to bR under one-photon excitation. The measured two-photon absorption cross-section (TPACS) of QDs was about two orders of magnitude larger than TPACS of bR and consequently the two-photon excitation of QDs in hybrid structure is highly selective. It was found that FRET in bio-nanohybrid system of QDs and bR under two-photon excitation is possible and the FRET efficiency was sufficient to initiation of bR photocycle. Studying of energy transfer between QDs and bR gives the perspective of considerable improvement of bR light absorption and consequently extends the possible applications of this photo-converting material. Additionally we showed that the two-photon excitation of QDs in QD-bR hybrid material makes possible the initiating of bR photocycle in the infrared spectral range.

Keywords: quantum dots; bacteriorhodopsin; energy transfer; two-photon absorption; nanomaterial.

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1. Introduction

**Nomenclature**

| Symbol | Definition |
|--------|------------|
| AFL    | average fluorescence lifetime |
| bR     | bacteriorhodopsin |
| FRET   | Forster resonance energy transfer |
| PM     | purple membrane |
| WM     | white membrane |
| QD     | quantum dot |
| QY     | fluorescence quantum yield |
| TPACS  | two-photon absorption cross-section |
| $\varphi$ | fluorescence quantum yield of hybrid system of quantum dots and purple membranes |
| $E$    | efficiency of Forster resonance energy transfer |
| $\varphi_0$ | fluorescence quantum yield of quantum dots without purple membranes |
| $R_0$  | Förster radius |
| $Q_D$  | donor fluorescence quantum yield |
| $N$    | Avogadro’s number |
| $\kappa^2$ | transition dipole orientation factor |
| $J(\lambda)$ | normalized overlap integral between the donor emission and acceptor absorption |
| $F_D$  | normalized emission of donor |
| $\lambda$ | wavelength |
| $\varepsilon_A$ | molar absorption coefficient of acceptor |
| $n$    | refractive index of the medium |
| $r$    | distance between the donor and the acceptor |
| $r_0$  | distance between the donor and the acceptor before dissolution |
| $C$    | concentration of the solution |
| $C_0$  | concentration of the solution before dissolution |
| GM     | Goeppert-Mayer unit is equal to cm$^4 \cdot s \cdot$ photon$^{-1}$ |

In recent time there is great interest to biological photosynthetic systems for novel photoelectronic devices. This is justified by the fact that the main function of such systems is the charge separation by using solar energy. An example of such system is the light-sensitive protein bacteriorhodopsin (bR), described in Oesterhelt et al. (1998), which can generate an electrical signal under visible light irradiation due to the proton transport through the plasma membrane. BR is a promising material for a new generation of biohybrid solar cells and optoelectronics devices. However, bR absorbs light mainly only in the visible spectral region, which limits the scope of this photosensitive protein. Fluorescent semiconductor nanocrystals, also known as quantum dots (QDs), can transfer energy to bR via Forster resonance energy transfer (FRET) and significantly improve the amount of absorbed by bR light energy due to its broad absorption spectrum and large extinction coefficient values in the blue and ultraviolet spectral regions (Bouchonville et al. (2011); Bouchonville et al. (2013); Rakovich et al. (2010); Nabiev et al. (1990)). This greatly increases the applicability of the BR in the fields of photovoltaics and optoelectronics. In addition, bR can’t absorb light in the infrared spectral region in the one-photon mode, and its maximum value of two-photon absorption cross section (TPACS) is only 290 GM (Birge et al. (1990)). At the same time QDs have TPACS values, which by orders of magnitude higher than that of the bR (Larson et al. (2003); Hafian et al. (2014)). This can significantly increase the amount of energy absorbed by bR in nano-biohybrid system under two-photon excitation. This means that two-photon excitation of the hybrid material allows to initiate a bR biological function in the infrared region, which opens up new possibilities of using this material. Furthermore, this means that two-photon excitation can provide a high selectivity of the QD excitation in QD-bR system. Recently, we have demonstrated the two-photon-induced Förster resonance energy transfer (FRET) from CdSe/ZnS QDs to the bR in purple membranes (PMs) from bacteria *Halobacterium salinarum* (Krivenkov et al. (2015)).
2. Experimental and theory

CdSe/ZnS QDs with a fluorescence maximum at 570 nm were selected because of the maximum spectral overlap between their fluorescence spectrum and absorption spectrum of bR and therefore the maximal effectiveness of FRET from QDs to the bR. They were synthesized by the method described in Baranov et al. (2003) and Stsiapura et al. (2006). Then QDs were made water-soluble by substitution the surface ligand trioctylphosphine oxide by SH-polyethyleneglycol-OH polymer described earlier in Sukhanova et al. (2012). We used bR in its native purple membranes (PMs) extracted from the bacteria *H. Salinarum* by the standard procedure described in Oesterhelt (1998). White membranes (WMs) were obtained by careful extraction of photosensitive chromophore retinal (FRET acceptor) from PM as described in Bouchonville et al. (2011). Membranes were purified as described in Ovchinnikov et al. (1988).

Absorption and luminescence spectra of samples were measured by Cary 60 UV-vis spectrophotometer (Agilent Technologies) and fluorescence spectrofluorimeter Cary Eclipse (Agilent Technologies), respectively. Microfluorescence spectra were recorded as described in Feofanov et al (1995) and Sharonov et al. (1993). One-photon excitation of the QD–PM complexes in aqueous solution was performed using a Ti:Sapphire femtosecond laser system operating at the wavelength of 395 nm, pulse energy of 0.5 nJ, and pulse duration of 300 fs. Two-photon excitation was performed using the same system operating at the wavelength of 790 nm, pulse energy of 5 nJ, and pulse duration of 300 fs. For two-photon excitation the laser beam was focused on the sample with a 10-cm focal length lens. For fluorescence decay measurements we used time correlated single photon counting system with a time resolution of 256 ps.

TPACS of QDs was estimated by a comparative measurement of the fluorescence of QDs and organic dyes with known TPACS under two-photon excitation, as described in Pu et al. (2006). Solutions of fluorescein in water buffer (pH = 11) and rhodamine 6G in methanol were used as a reference dyes with TPACS known from Makarov et al. (2008). QDs concentrations in the solutions were evaluated using the extinction coefficient in the first exciton absorption maximum of QDs defined in Jasieniak et al. (2009).

FRET efficiency in our experiments was determined from the expression, obtained by substituting of quantum yield (QY) parameter instead of fluorescence lifetime in the equation 13.14 from Lakowicz (2006):

\[ E = 1 - \frac{\varphi}{\varphi_0}, \tag{1} \]

where E is FRET efficiency, \( \varphi \) is QY of QD-PM system, and \( \varphi_0 \) is QY of QDs without PM.

For theoretical calculations, the FRET efficiency can be also determined using the following equation:

\[ E = \frac{n_j R_0^6}{(n_j R_0^6 + \nu^6)}, \tag{2} \]

where \( r \) is the distance between the donor and the acceptor, \( n_j \) is the number of acceptors accessible for energy transfer from the donor, and \( R_0 \) is the Förster radius defined as the distance between donor and acceptor at which the FRET efficiency is 50%. It can be calculated from the intrinsic optical and spectral parameters of donor and acceptor: \( R_0^6 = 9000 \ln(10) Q_D \kappa^2 (128\pi^3 N n^4)^{-1} J(\lambda), \) where \( Q_D \) is the donor fluorescence QY, \( \kappa^2 \) is the transition dipole orientation factor, \( N \) is Avogadro’s number, \( n \) is the refractive index of the medium, and \( J(\lambda) \) is the normalized overlap integral between the donor emission and acceptor absorption. The normalized overlap integral is calculated as follows:

\[ J(\lambda) = \int_0^\infty F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda, \tag{3} \]

where \( F_D \) is the normalized emission of donor, and \( \varepsilon_A \) is the molar absorption coefficient of acceptor at the wavelength \( \lambda. \)
3. Results and discussion

3.1. Energy transfer under one-photon excitation

First, we have shown the presence of FRET in the system of PM and QDs under one-photon excitation, by measuring the fluorescence quenching efficiency of QDs using the Eq. 1. It should be noted that the decrease in QDs QY may be explained not only by FRET from the QDs to the Br, but also by other mechanisms of quenching. To show that the FRET is the main quenching mechanism in our samples, we compared the effect of PM and white membrane (WM) on the fluorescence of QDs. WM and PM have the same physico-chemical and structural properties. Thus, the QD-WM system is structurally and chemically identical to the QD-PM, however, due to the absence of the acceptor, the FRET can't be realized in the QD-WM system, because the overlap integral (Eq. 3) is zero. Therefore QD-WM system were prepared in the same molar proportions as for the QD-PM system, and was used as a negative control.

In the experiment, we gradually added small amounts of PM or WM in an aqueous solution of QDs with a molar concentration of $2\times10^{-6}$ M. Then we measured the average fluorescence lifetime (AFL) and the fluorescence integrated intensity of the resulting solution, the data presented at Fig. 1. Increasing the protein concentration in the QDs-PM system provokes the reduction of fluorescence intensity and AFL. However, in the QDs-WM system the reduction was much less. This difference between samples containing PM and WM can be explained by fact that the overlap integral of the QDs-WM system is equal to zero. These data indicate the FRET presence in the QDs-PM system under one-photon excitation.

![Fig. 1. Integrated fluorescence intensity (a) and the average fluorescence lifetime (b) dependences of the Br to QDs molar ratio under one-photon excitation. The data obtained for the control (QDs-WM system) is also presented. Adapted with permission from Krivenkov et al. (2015). © 2015 Optical Society of America.](image)

3.2. Verification of existence of QD-PM complexes

We has not knew whether the FRET in our samples is due to the formation a QDs-PM nano-biohybrid complexes or to the proximity of freely floating particles of the donor and acceptor. To check it, we measured the FRET efficiency dependence of the solution concentration and compared with theoretical predictions for a system of freely floating particles.

The distances between the particles ($r$) are inversely proportional to cube root of the concentration ($C$):

$$r \propto \left( \frac{1}{C} \right)^{1/3}.$$  

(4)

Thus, in a free-floating particle system, the concentrations and, hence, the distances between the donor and acceptor in aqueous mixtures of QDs and PM can be changed by diluting the sample without changing the PM-to-QD molar
ratio. Under this assumption, we can found a theoretical dependence of FRET efficiency on the concentration of the solution. When the distance changes from \( r_0 \) to \( r \) the concentration changes from \( C_0 \) to \( C \), and their ratio equals to:

\[
\frac{r}{r_0} = \left( \frac{C_0}{C} \right)^{1/3},
\]  

(5)

Using the above reasoning, we can estimate the dependence of the FRET efficiency on the solution concentration \( C_i \) by the following equation by substituting \( r \) from (5) to the (2):

\[
E(C_i) = \frac{n_s R_0^6}{n_s R_0^6 + r_0^6 \left( \frac{C_0}{C_i} \right)^{1/3}},
\]  

(6)

We assume that \( n_s R_0^6 \) does not change with changing concentration; hence, it can be expressed in terms of \( r_0^6 \):

\[
n_s R_0^6 = r_0^6 \frac{E(C_0)}{1 - E(C_0)},
\]  

(7)

where \( C_0 \) is the initial concentration of the sample. Thus, by substituting (7) into (6), we obtain the final expression for the dependence of the FRET efficiency on the concentration of the solution:

\[
E = \left[ 1 + \frac{1 - E_0 \left( \frac{C_0}{C} \right)^{2/3}}{E_0} \right]^{-1}.
\]  

(8)

Next, we measured the FRET efficiency experimental dependence on the solution concentration to compare with the theoretical dependence and confirm our assumption that the system is not a set of freely floating particles, but is an aqueous solution of QDs-PM complexes. We used an aqueous mixture of QD with PM with initial FRET efficiency of 0.4. The stock solution was diluted to 16 times, and the QY has been measured for each dilution step. The comparison of the experimental and theoretical dependences shows that the FRET efficiency was much greater than the theoretically expected value for a system of freely floating particles in each experimental point (Fig. 2). This shows that FRET in our system has been associated with the formation of the complexes, because in otherwise case the experimental FRET efficiency dependence would be close to the theoretically calculated value. The experimentally observed decrease in the FRET efficiency (from 0.4 to 0.25) can be explained by the fact that the complexes are not rigidly connected and the system is in dynamic equilibrium between the desorbed QDs and formed QD–PM complexes. Thus, upon dilution of the solution, the rate of complex formation will decrease and the proportion of the complexes in the solution is reduced, leading to reduced FRET efficiency.
3.3. Energy transfer under two-photon excitation

Before the experiment to determine the two-photon induced FRET in QD-PM complexes, we measured the TPACS value of QDs. The measured TPACS value at an excitation wavelength of 790 nm was about 20,000 GM. Thus, the QDs exceeds the maximum TPACS of bR by two orders of magnitude, which theoretically allows selective excitation of quantum dots in the QD-PM system.

The experiment was performed in the same manner as in the one-photon excitation case, as described above in part 3.1. The measured dependences of the average fluorescence lifetime (AFL) and the fluorescence integrated intensity on the bR-to-QD molar ratio are presented in Fig. 3. Increasing the protein concentration in the QDs-PM system provokes the reduction of fluorescence intensity and AFL. However, in the QDs-WM system the reduction was much less. This difference between samples containing PM and WM can be explained by fact that the overlap integral of the QDs-WM system is equal to zero. These data indicate the FRET presence in the QDs-PM system under two-photon excitation. In addition, in our experiments the FRET efficiency was reached 40-60% at a molar ratio of BR to QDs is 6, which is sufficient to initiate bR photocycle under two-photon excitation of QDs in the infrared spectral region.

4. Conclusion

In this work we have studied the FRET from CdSe/ZnS QDs to the bR in its natural form of PM under one- and two-photon excitation. We have shown experimentally that QD-PM complexes are formed in the QD-PM water solution. It has been shown that the selective excitation of QDs in nano-biohybrid QDs-PM complexes in the
infrared region of the spectrum in two-photon mode was realized. In addition, the FRET efficiency is enough to initiate bR biological function under two-photon excitation of QDs in the infrared region of the spectrum. It could open up new ways to use this promising bio-hybrid material in the fields of solar energy conversion, biosensors, biocomputing technologies, optoelectronics, imaging and drug delivery (Montenegro et al. (2013)).

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References

Baranov, A., Rakovich, Y., Donegan, J., Perova, T., Moore, R., Talapin, D., Rogach, A., Matsumoto, Y., Nabiev, I., 2003. Effect of ZnS Shell Thickness on the Phonon Spectra in CdSe Quantum Dots. Physical Review B 68, 165306.

Birge, R. R., Fleitz, P., Lawrence, F., Mathay, M., Zhang, C. F., 1990. Nonlinear Optical Properties of Bacteriorhodopsin: Assignment of Second Order Hyperpolarizabilities of Randomly Oriented Systems Using Two-Photon Spectroscopy. Molecular Crystals and Liquid Crystals Incorporating Nonlinear Optics 189, 107-122.

Bouchonville, N., Le Cigne, A., Sukhanova, A., Molinari, M., Nabiev, I. 2013. Nano-Biophotonic Hybrid Materials with Controlled FRET Efficiency Engineered from Quantum Dots and Bacteriorhodopsin. Laser Physics Letters 10, 085901.

Bouchonville, N., Molinari, M., Sukhanova, A., Artemyev, M., Oleinikov, V., Troyon, M., Nabiev, I., 2011. Charge-Controlled Assembling of Bacteriorhodopsin and Semiconductor Quantum Dots for Fluorescence Resonance Energy Transfer-Based Nanophotonic Applications. Applied Physics Letters 98, 013703.

Feofanov, A., Sharonov, S., Valisa, P., Da Silva, E., Nabiev, I., Manfait, M., 1995. A New Confocal Stigmatic Spectrometer for Micro-Raman and Microfluorescence Spectral Imaging Analysis: Design and Applications. Review of Scientific Instruments 66, 3146-3158.

Hafian, H., Sukhanova, A., Turini, M., Chames, P., Baty, D., Pluot, M., Cohen, J. H. M., Nabiev, I., Millot, J.-M., 2014. Multiphoton Imaging of Tumor Biomarkers with Conjugates of Single-Domain Antibodies and Quantum Dots. Nanomedicine: NBM 10, 1701-1709.

Jasieniak, J., Smith, L., Van Embden, J., Mulvaney, P., Califano, M., 2009. Re-Examination of the Size-Dependent Absorption Properties of CdSe Quantum Dots. Journal of Physical Chemistry C 113, 19468-19474.

Krivenkov, V., Samokhvalov, P., Solovyeva, D., Bilan, R., Chistyakov, A., Nabiev, I., 2015. Two-Photon-Induced Förster Resonance Energy Transfer in a Hybrid Material Engineered from Quantum Dots and Bacteriorhodopsin. Optics Letters 40, 1440-1443.

Lakowicz, J. R., 2006. Principles of Fluorescence Spectroscopy, third ed., Springer, New York, pp. 954.

Larson, D. R., Zipfel, W. R., Williams, R. M., Clark, S. W., Bruchez, M.P., Wise, F. W., Webb, W. W., 2003. Water-Soluble Quantum Dots for Multiphoton Fluorescence Imaging in Vivo. Science 300, 1434-1436.

Makarov, N. S., Drobizhev, M., Rebane, A., 2008. Two-Photon Absorption Standards in the 550-1600 nm Excitation Wavelength Range. Optics Express 16, 4029-4047.

Montenegro, J.M., Grazu, V., Sukhanova, A., Agarwal, S., de la Fuente, J.M., Nabiev, I., Greiner, A., Parak, W.J., 2013. Controlled Antibody/(Bio-) Conjugation of Inorganic Nanoparticles for Targeted Delivery. Advanced Drug Delivery Reviews 65, 677-688.

Nabiev, I.R., Chumanov, G.D., Efremov, R.G., 1990. Surface-Enhanced Raman Spectroscopy of Biomolecules. Part II. Application of Short- and Long-Range Components of SERS to the Study of the Structure and Function of Membrane Proteins. Journal of Raman Spectroscopy 21, 49-53.

Oesterhelt, D., 1998. The Structure and Mechanism of the Family of Retinal Proteins from Halophilic Archaea. Current Opinion in Structural Biology 8, 489–500.

Ovchinnikov, Yu.A., Arystarkhova, E.A., Arzamazova, N.M., Dzhandzhugazyan, K.N., Efremov, R.G., Nabiev, I.R., Modyanov, N.N., 1988. Differentiated Analysis of the Secondary Structure of Hydrophilic and Hydrophobic Regions in Alpha- and Beta-Subunits of Na+,K+-ATPase by Raman Spectroscopy. FEBS Letters 227, 235-239.

Pu, S.-C., Yang, M.-J., Hsu, C.-C., Lai, C.-W., Hsieh, C.-C., Lin, S. H., Cheng, Y.-M., Chou, P.-T., 2006. The Empirical Correlation Between Size and Two-Photon Absorption Cross Section of CdSe and CdTe Quantum Dots. Small 2, 1308-1313.

Rakovich, A., Sukhanova, A., Bouchonville, N., Lukashev, E., Oleinikov, V., Artemyev, M., Gaponik, N., Bouchonville, N., Molinari, M., Troyon, M., Rakovich, Y. P., Donegan, J. F., Nabiev, I., 2010. Resonance Energy Transfer Improves the Biological Function of Bacteriorhodopsin within a Hybrid Material Built from Purple Membranes and Semiconductor Quantum Dots. Nano Letters 10, 2640–2648.

Sharonov, S., Chourpa, I., Morjani, H., Nabiev, I., Manfait, M., Feofanov, A., 1993. Confocal Spectral Imaging Analysis in Studies of the Spatial Distribution of Antitumour Drugs Within Living Cancer Cells. Analytica Chimica Acta 290, 40–47.

Stsiapura, V., Sukhanova, A., Baranov, A., Artemyev, M., Kulakovitch, O., Oleinikov, V., Pluot, M., Cohen, J.H.M., Nabiev, I., 2006. DNA-Assisted Formation of Quasi-Nanowires from Fluorescent CdSe/ZnS Nanocrystals. Nanotechnology 17, 581-587.

Sukhanova, A., Even-Desrumeaux, K., Kisseler, A., Tabary, T., Reveil, B., Millot, J. M., Chames, P., Baty, D., Artemyev, M., Oleinikov, V., Pluot, M., Cohen, J. H. M., Nabiev, I., 2012. Oriented Conjugates of Single-Domain Antibodies and Quantum Dots: Toward a New Generation of Ultrasmall Diagnostic Nanoprobes. Nanomedicine: NBM 8, 516–525.