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Resonance Energy Transfer in Hydrogels Based on Quantum Dots and Capture Antibodies: A Prototype Nanophotonic Immunodiagnostic System

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In recent years, a growing number of studies have investigated the structural and optical properties of hydrogels based on various nanoparticles. Due to their high porosity and compatibility with living tissues, hydrogels provide a promising basis for the development of sensitive and specific biomolecule detectors (biosensors). This study has estimated the efficiency of Förster resonance energy transfer (FRET) in hydrogel systems containing CdSe/ZnS quantum dots, diamine derivatives of polyethylene glycol (PEG) with different molecular weights, and immunoglobulin molecules labeled with the AlexaFluor 633 fluorophore. The new system is a prototype nanophotonic diagnostic tool where immunoglobulins labeled with organic fluorophores serve as „identification tags" for detecting disease biomarkers. It has been shown that FRET occurs in this prototype between hydrogel quantum dots (energy donors) and AlexaFluor 633 fluorophores (energy acceptors) with an efficiency as high as 87%. The results demonstrate that the hydrogels based on quantum dots and diamine derivatives of PEG developed here can be used for highly sensitive and specific FRET-based immunohistochemical analysis of biomarkers providing a high signal-to-background ratio.

Keywords: nanocrystals, quantum dots, hydrogels, FRET, labeled molecules.

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Introduction

Various recently developed biosensor systems employ fluorescence detection due to its high sensitivity. The fluorescent detection methods include those based on Förster resonance energy transfer (FRET) between closely located donors and acceptors of energy characterized by a high degree of overlap of the fluorescence spectra of the former with the absorption spectra of the latter. These methods ensure a record-high signal-to-noise ratio with an efficiency of energy transfer as high as 100%. Semiconductor nanocrystals known as quantum dots (QDs) have a wide absorption range, a high photoluminescence (PL) quantum yield (QY), an increased photobleaching resistance, and a narrow fluorescence spectrum, whose maximum position depends on the particle size. These QD properties explain their wide use in designing biosensor system and in bioimaging [1].

QD-based sensor systems using the FRET effect have been previously developed for detecting nucleic acids, proteins, small molecules, and toxins [2]. Sensors based on fluorescent hydrogels consisting of QDs are advantageous in that they have a high capacity for molecule binding due to the three-dimensional structure of the hydrogel, tunability of the gel matrix properties for detecting different analytes, and the possibility of multiple use of the biosensors [3]. Many of these systems record the fluorescence quenching via FRET. These are, e.g., a progesterone biosensor based on hydrogel-immobilized CdSe/CdS/ZnS QDs and labeled DNA molecules [4], as well as sensors based on hybrid films containing CdTe QDs developed for detecting dopamine [5]. Apart from sensors recording FRET-mediated fluorescence quenching, there are FRET-based sensors recording fluorescence emitted by the acceptor. This is possibly if the donor and the acceptor have substantially overlapping fluorescence and absorption spectra, respectively, and they are located at distances between 1 and 10 nm from each other. For example, high efficiency of FRET has been shown in conjugates of CdSe/ZnS QDs with the organic fluorescent label AlexaFluor647 [6]. At the same time, few studies in this field address issues related to increasing the efficiency of FRET, which is the basis of the operation of many sensor systems.

Here, we studied the FRET efficiency in hydrogels with different morphologies based on CdSe/ZnS QDs that were modified with cysteine (CdSe/ZnS-Cys) using diamine polyethylene glycol derivatives with various molecular weights, where the QDs served as a donor and the conjugate of immunoglobulin G (IgG) with the fluorescent label AlexaFluor633 served as an acceptor. This

system is a prototype nanophotonic diagnostic tool where immunoglobulins labeled with organic fluorophores are used as „identification“ tags for detecting disease biomarkers.

Methods

Manufacturing gels based on CdSe/ZnS quantum dots

The CdSe/ZnS QDs were synthesized as described earlier [7]. The synthesized CdSe/ZnS QDs were solubilized using DL cysteine [8]. The aqueous solution of CdSe/ZnS-Cys QDs was transferred into a 0.05 M borate buffer solution, pH 8.5, using a chromatographic column containing Sephadex G-25.

Gel samples were prepared by adding EDC, s-NHS and PEG diamine with an average molar weight of 3400 g/mol (poly(ethylene glycol) bis(amine), average Mn 3400, Sigma-Aldrich, cat. no. P9906) (PEG3400) to 10 μL of the CdSe/ZnS-Cys QD solution in 0.05 M borate buffer (pH 8.5) to the optimal molar ratios determined previously (EDC/QD , 5000/1; NHS/EDC , 10/1; PEG/QD , 1000/1). To experimental samples, labeled immunoglobulins (goat anti-human IgG (H+L) cross-adsorbed secondary antibody, AlexaFluor^{TM}633, Invitrogen, cat. no. A21091) (IgG-AlexaFluor633) were added to IgG-AlexaFluor633/QD molar ratios of 0.1/1, 0.5/1, and 1/1. In the case of control samples, 0.05 MES buffer solution (pH 6.0) was added in the volume corresponding to that of the added labeled immunoglobulins, and the samples were incubated for 24 h at 25°C in the dark. After that, the liquid above the gel was withdrawn, and 30 μl of MilliQ water was added for washing the samples.

The hydrogel samples with predetermined optimal molar ratios of labeled immunoglobulins to QDs were prepared by mixing M 10 μ of the CdSe/ZnS-Cys QD solution in 0.05 M borate buffer (pH 8.5) with EDC, s-NHS, and PEG diamine with an average molar weight of 400 or 2000 g/mol (poly(ethylene glycol) diamine, average Mn 400, Sigma-Aldrich, cat. no. 909149; poly(ethylene glycol) diamine, average Mn 2000, Sigma-Aldrich, cat. no. 753084) (PEG400 and PEG2000, respectively), and IgG-AlexaFluor633 to the optimal molar ratios determined previously (EDC/QD = 5000/1, NHS/EDC = 10/1, PEG/QD = 1000/1, IgG-AlexaFluor633/KT = 1/1). In order to compare different methods of obtaining the gels, a sample was also prepared containing CdSe/ZnS QDs and IgG-AlexaFluor633, where gel formation was induced by adding 20 μl of the 0.1 M $MgCl_2$ solution. Each experimental sample had a matched control sample containing the same volume of the 0.05 MES buffer solution (pH 6.0) as the volume of the labeled immunoglobulin solution in its experimental counterpart. All the samples were incubated for 24 h at 25°C in the dark and then washed as described in the previous section.

Instrumental methods

The fluorescence spectra of the QD solutions were recorded using a Cary Eclipse spectrofluorimeter (Agilent Technologies). The fluorescence spectra of the gels based on the CdSe/ZnS-Cys QDs containing PEG diamine and IgG-AlexaFluor633 were recorded by means of an integrating sphere connected with a HR2000+ES spectrometer (Ocean Optics). The fluorescence QY of the samples was determined as described in [9] and the manual [10].

Time-resolved studies of fluorescence of the gel samples were performed using a system that consisted of a PicoQuant semiconductor diode laser with a wavelength of 398 nm, a Taiko PDL M1 laser module driver (PicoQuant), an M266 monochromator (SolarLaserSystems), and an avalanche photodiode (MPD). Fluorescence of the samples studied was excited at a pulse repetition rate of 100 kHz and an average pulse energy of 180 μW. The system response function was evaluated using a diffusion reflector; it was found to be ~560 ps. The obtained curves of fluorescence decay in the studied gel systems were approximated by the function $I(t)$ under the assumption that the fluorescence change law was biexponential [11]:

$$I(t) = I_0 + q_1 \cdot e^{-\frac{t}{\tau_1}} + q_2 \cdot e^{-\frac{t}{\tau_2}}, \quad (1)$$

where $I(t)$ is the intensity of fluorescence, t is time, I_0 is the approximation constant, q_1 and q_2 are the coefficients of the approximated function at the corresponding exponential factors, and τ_1 are τ_2 are the typical decay times corresponding to the fast and slow fluorescence decay kinetic components in the system.

The average luminescence decay time (τ_{cp}) was calculated using Equation (2) as the amplitude-weighted mean lifetime [12]:

$$\tau_{cp} = \frac{q_1\tau_1 + q_2\tau_2}{q_1 + q_2}. \quad (2)$$

Results and discussion

FRET is the physical process of nonradiative energy transfer from one excited molecular fluorophore (donor) to another fluorophore (acceptor) via long-range dipole — dipole intermolecular interaction. The FRET efficiency depends on the distance between the donor and the acceptor as a function of the reciprocal value of the sixth power of this distance [13]. The systems exhibiting effective FRET usually have optimal distances between the donor and the acceptor within the range of 1–10 nm [14]. One of the main parameters used for evaluation of the FRET efficiency in the systems studied is the Förster radius (R_0) , the distance at which half of the donor excitation energy is transferred to the acceptor. Usually, this distance is 3–10 nm [15]. Another important parameter used for evaluating the FRET efficiency in a specific system is the integral of the overlap $J(\lambda)$ between the donor PL emission spectrum and the acceptor optical absorption spectrum [16].

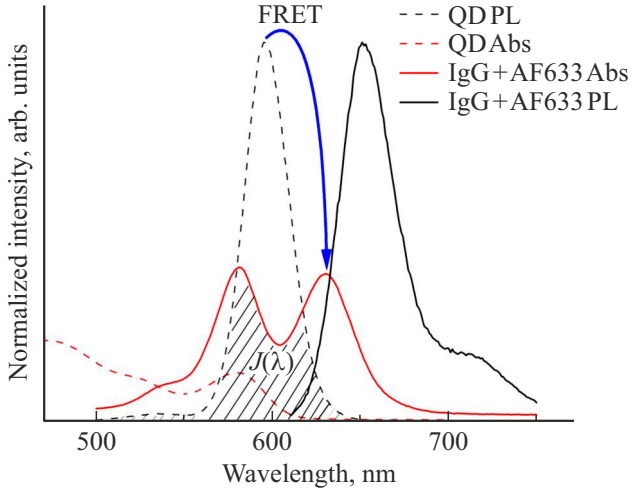

Figure 1. The PL (black curves) and optical absorption (red curves) spectra of the components of the gels studied: solutions of CdSe/ZnS-Cys QDs in 0.1 M NaOH (dashed curves) and immunoglobulins labeled with an organic dye (conjugate of IgG with AlexaFluor633, solid curves). The hatched area shows the degree of overlap of the donor PL and acceptor absorption spectra.

Figure 1 shows the corresponding spectra for the studied system through the example of the gel based on QDs, PEG diamine, and IgG-AlexaFluor633. Analysis of the spectra shown in Fig. 1 demonstrates that the overlap between the donor PL emission spectrum and the acceptor optical absorption spectrum is large enough for the FRET efficiency in the systems to exceed 50%. The FRET efficiency can be calculated using the values of the amplitude-weighted average time, according to the equation [12]

$$E_{\mathrm{FRET}} = 1 - \frac{\tau_{\mathrm{DA}}}{\tau_{\mathrm{D}}}, \qquad (3)$$

where τ_{D} and τ_{DA} are the average times of donor PL decay in the sample in the presence and absence of the acceptor, respectively.

In order to compare the FRET efficiencies, two series of samples of the hydrogels based on CdSe/ZnS-Cys QDs, PEG diamine, and IgG-AlexaFluor633 were fabricated. In the first series, the average molar weight of the polymer was the same in all samples (3400 g/mol), whereas the molar ratio between the acceptor (IgG-AlexAFluor633) and the donor (CdSe/ZnS-Cys QDs) varied (IgG-AF633/QD = 0.1:1, 0.5:1, or 1:1). The samples of the second series had the same molar ratio between the donor and the acceptor, but they were fabricated using PEG diamine with different average molar weights, 400 or 2000 g/mol.

The PL decay curves for the two series of samples are shown in Fig. 2. Measurements performed on the first series of samples showed that the FRET efficiency was the highest at the ratio of 1:1; hence, this ratio was selected for the second series of samples.

As seen in Fig. 2 *a*, the PL decay curve for the solution of CdSe/ZnS-Cys QDs in 0.1 M NaOH is located below the corresponding curve for the gel based on these QDs; i.e., the mean QD PL decay time in the solution (12 ns) was smaller than in the solid gel even in the presence of a small amount of the acceptor. This was possible because relatively short cysteine ligands were used for primary QD solubilization, which precluded effective isolation of the inorganic part of the QDs from the environment, resulting in partial PL quenching. In addition, the formation of hydrogen or electrostatic bonds between the surface groups of the cysteine-solubilized QDs may have resulted in partial aggregation, which also suppressed their PL [17,18]. In the course of further gel formation, the distance between neighboring QDs increased due to the inclusion of long PEG diamine molecules, which suppressed these quenching processes, thereby increasing the mean time of gel PL decay.

Then, Equations (2) and (3) were used to calculate the τ_{cp} and E_{FRET} values for each sample (Table).

As seen from the table, for the gels based on the QDs, PEG diamine, and immunoglobulins labeled with AlexaFluor633, it is possible to achieve high FRET efficiencies (up to 87%), which indicates that gels of this type constitute a promising basis for sensor systems. For comparison, the gel formed by adding a simple inorganic stitching agent, $MgCl_2$, to a solution of the QDs and the dye (sample gQD−AF633 (1:1), $MgCl_2$) exhibited a FRET efficiency as low as 31%, which may have been related to a low porosity of the gel insufficient for effective internalization of the IgG-AlexaFluor633 conjugate in the gel pores. It should also be noted that the length of the molecules of the PEG diamine derivative used in gel formation has almost no effect on the efficiency of energy transfer, which can be advantageous in using the gels for detecting biomolecules. For example, the pore size and, hence, pore permeability for specific biomolecules can be varied by using PEG diamine derivatives with different molecular weights, which enhances the selectivity of analysis.

In order to further confirm the FRET efficiencies estimated using the integrating sphere, the PL QYs for the system as a whole and for the acceptor separately were determined in each sample studied. This made it possible to estimate the proportion of the acceptor PL intensity in the total PL intensity of the entire system, thereby obtaining an alternative estimation of the energy transfer efficiency.

Figure 3 illustrates the procedure for determining the QY of the gel as a whole and the acceptor separately using the example of the PL spectrum of a gel based on the CdSe/ZnS-Cys QDs and the IgG-AlexaFluor633 (AF633) conjugate in a molar proportion of 1:1. In this case, the maxima of emissions of the QDs and the dye exhibit distinct spectral separation; therefore, the QYs can be calculated under the assumption that the integral emission of the gel as a whole is the sum of the integral emissions of its components.

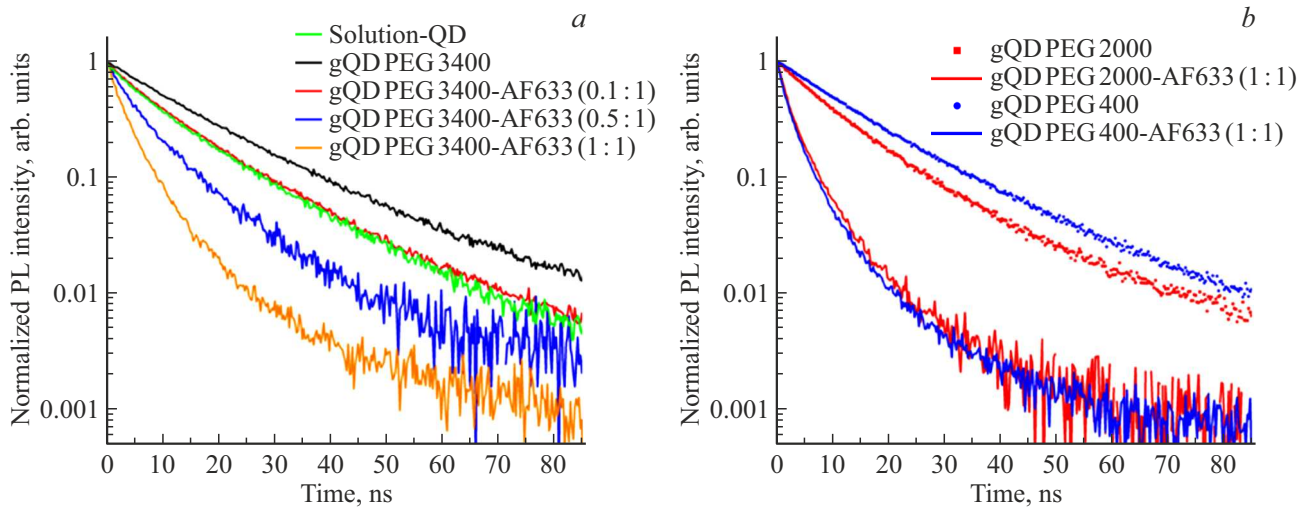

Figure 2. PL decay curves of the CdSe/ZnS-Cys QDs in a solution and in the samples of gels fabricated on their basis using (*a*) PEG diamine derivatives with a molar weight of 3400 g/mol, with IgG-AlexaFluor633 added at ratios of 0.1:1, 0.5:1, and 1:1 and (*b*) PEG diamine derivatives with molar weights of 400 and 2000 g/mol, with IgG-AlexaFluor633 added at a ratio of 1:1.

Characteristics of the PL of QD samples in a solution and in CdSe/ZnS-Cys QD hydrogels obtained using PEG diamine derivatives with different molar weights and IgG-AlexaFluor633 at different ratios. The measured values and measurement errors are indicated.

Sample	τ, ns	Efficiency of FRET, %	Quantum yield of fluorescence of the system, %	Quantum yield of the acceptor, %	Proportion of the emission of the acceptor, %
Solution-QD	11.84 ± 0.32	–	34.89 ± 3.49	–	–
gQD-PEG 3400	17.28 ± 0.85	–	17.09 ± 1.72	–	–
gQD-PEG 3400-AF633 (0.1:1)	12.22 ± 0.29	29.28 ± 3.85	16.67 ± 1.66	3.29 ± 0.33	19.73 ± 2.79
gQD-PEG 3400-AF633 (0.5:1)	5.54 ± 0.16	67.94 ± 1.82	11.34 ± 1.13	7.12 ± 0.72	62.73 ± 8.91
gQD-PEG 3400 -AF633 (1:1)	3.87 ± 0.09	77.59 ± 1.30	14.63 ± 1.46	12.72 ± 1.27	86.94 ± 12.27
gQD-PEG 2000	11.99 ± 0.37	–	25.04 ± 2.50	–	–
gQD-PEG 2000-AF633 (1:1)	3.27 ± 0.25	72.73 ± 2.23	17.26 ± 1.73	15.03 ± 1.50	87.08 ± 12.32
gQD-PEG 400	15.72 ± 0.46	–	22.76 ± 2.28	–	–
gQD-PEG 400-AF633 (1:1)	2.98 ± 0.12	81.04 ± 0.87	11.33 ± 1.13	9.94 ± 0.99	87.73 ± 12.37
gQD-$MgCl_2$	17.66 ± 0.57	–	17.20 ± 1.72	–	–
gQD-AF633 (1:1)-$MgCl_2$	3.65 ± 0.12	79.33 ± 0.92	5.97 ± 0.60	1.87 ± 0.19	31.32 ± 4.48

When using the integrating sphere, we calculated the QY of the whole system as

$$QY = \frac{E_{QD} + E_{AF633}}{S_{DE} - S_{QD}}, \quad (4)$$

where E_{QD} and E_{AF633} are the integral PL intensities of the QDs and the dye, respectively, and S_{DS} and S_{QD} are the integral intensities of the signals in the range of excitation radiation in recording the spectra of the diffusion reflector and the gel, respectively.

The PL QY of the acceptor was calculated by the following equation:

$$QY^* = \frac{E_{AF633}}{S_{DE} - S_{QD}}. \quad (5)$$

Then, the contribution of the acceptor, IgG-AlexaFluor633 (E_{AF633}), to the total PL is

$$\Omega_{AF633} = \frac{QY^*}{QY} \cdot 100\%. \quad (6)$$

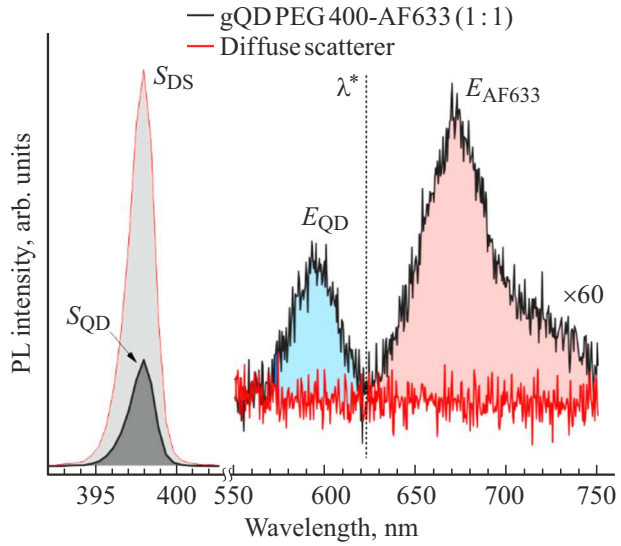

Figure 3. Schematics of the procedure for determining the absolute PL QY in the integrating sphere. λ^*, the characteristic wavelength of separation of the two PL spectra, which allows obtaining the numerical values of E_{QD} and E_{AF633}. In this study, λ^* was taken to be the wavelength corresponding to the local minimum between the PL maxima of the QDs and the AlexaFluor633 dye.

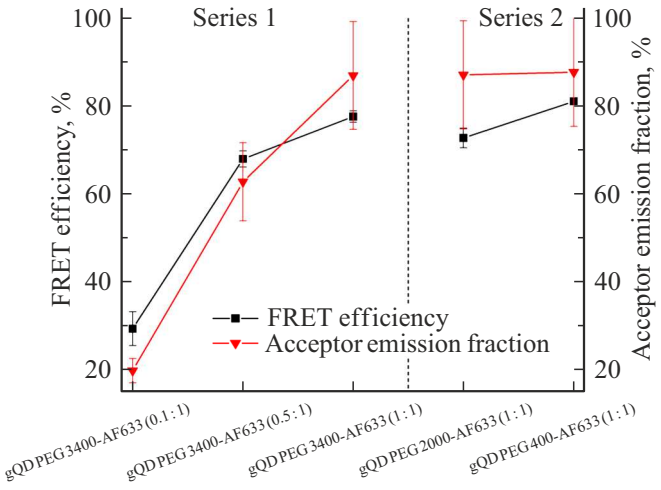

Figure 4. Correlation between the FRET efficiencies in two series of the samples of gels determined in the experiments on measuring the PL decay time (black curves) and estimating the proportion of acceptor emission in the total QY of the samples (red curves).

We used these calculations to obtain an alternative estimation of the FRET efficiency in the gels; the corresponding values are also shown in the table.

Figure 4 shows the correlation of the efficiency of FRET between the donor and the acceptor estimated from the experimental data on the PL decay time with the contribution of acceptor emission to the QY of the whole system for the series of gels based on QDs, PEG diamine, and IgG-AlexaFluor633 conjugates.

Thus, the results of our studies lead to the conclusion that FRET as efficient as 87% between the donor (the gel base) and the relatively small-sized PL label can be obtained in the systems studied. This suggests that the gels developed here can serve as a basis of biosensor systems using the PL of the acceptor as an analytical signal. Furthermore, our data show that the FRET efficiency is practically independent of the length of the linking agent, which makes it possible to vary the size of the gel pores and adapt the gel to different types of the analyzed molecules, from low-molecular-weight compounds to large protein molecules.

Conclusion

Thus, we present a method for obtaining gels from cysteine-modified CdSe/ZnS QDs using diamine derivatives of PEG and immunoglobulins labeled with the AlexaFluor633 dye. We have shown that the FRET efficiency of these gels is more than two times higher than that of the gels where low-molecular-weight linkers are used. In particular, the estimation of the proportion of the acceptor PL in this system has shown that, at a donor-to-acceptor ratio of 1:1, a FRET efficiency as high as 87% can be obtained. This result further confirms large prospects of using hydrogel materials in various fields of biomedical research. The capacity of these systems for highly efficient energy transfer makes it possible to develop biosensors for highly precise and sensitive detection of biomolecules and monitoring of biochemical processes, as well as technologies for fabricating next-generation biomedical therapeutic agents based on hydrogel systems.

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Conflict of interest

The authors declare that they have no conflict of interest.

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