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FRET between BODIPY Azide Dye Clusters within PEG-Based Hydrogel: A Handle to Measure Stimuli Responsiveness

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The purpose of this paper is to investigate the dynamics of the fluorescence mechanism of boradiazaindacene (BODIPY) dye molecules, which are covalently bound to a polyethylene glycol based hydrogel structure with different concentrations, using a picosecond time-resolved spectroscopic technique. Since the hydrogel structure is capable of absorbing a large amount of water, without dissolving and without losing its shape, upon swelling, the distance between the BODIPY azide dyes is controllably changed; it is observed that the intensity weighted fluorescence lifetime for the highly concentrated donor dye molecules embedded in the hydrogel cluster network changes from 2.03 to 7.14 ns. Calculations based on our experimental results suggest that the fluorescence dynamics of the BODIPY azide dye molecules confined within the hydrogel network obeys the Förster resonance energy transfer (FRET) rather than self (or contact) quenching. If the hydrogel is dry, in which the distance between donors and acceptors is minimum, the energy transfer efficiency is found to be about 72%, and the distance between the two dye molecules is calculated to be 4.59 nm. Such a close placement causes a significant reduction in the fluorescence intensity due to a strong dipole−dipole interaction of the dye molecules. As the separation increases upon hydrogel swelling, the FRET efficiency reduces to 2%, which corresponds to a separation of 10 nm between two BODIPY dyes and hence a considerable increase in the level of fluorescence intensity. For the dilute hydrogel samples, the distance between the dye molecules is larger than the critical Förster distance. Therefore, the energy transfer efficiency for this type of dilute samples is found to be much lower.

I. Introduction

BODIPY is a versatile fluorescence molecule widely used in imaging applications due to its smooth and almost location-fixed excitation and emission spectra contributing to overall brightness, which is superior to those of many fluorophores with its high quantum yield, often approaching over 90% in various solvents.[1−3]

In this work, a chemically cross-linked three-dimensional hydrogel network is doped with different concentrations of BODIPY dye molecules. They are covalently bound to a polyethylene glycol based hydrogel structure at two different concentrations: highly concentrated and dilute. The concentration of the dye molecules is controlled by the number of free reactive alkyne groups within the gel matrix. It is observed that the fluorescence intensity drastically decreases when the number of BODIPY azide dye molecules is increased within a cluster of a hydrogel. On the other hand, the fluorescence intensity significantly increases when the concentration of the dye molecules is reduced (e.g., see Figure 4). The rationale of this research is to reveal the transduction mechanism that governs such a molecular concentration-dependent fluorescence change of the BODIPY dyes by means of time-resolved fluorescence lifetime measurements. For example, the intensity weighted fluorescence lifetime of the concentrated BODIPY dye molecules increases more than 3-fold upon exposing the hydrogel structure to water swelling.

If the donor and acceptor fluorophores in the hydrogel clusters are the same species, like the BODIPY dye molecules used here in this work, two different types of mechanisms can be effective in quenching of the fluorescence intensity: self-quenching (or contact quenching) and fluorescence resonance energy transfer (FRET).[4] Self-quenching is the predominant mechanism of quenching if there is a physical contact between the donor and acceptor dye molecules. Otherwise, FRET becomes the predominant mechanism and the efficiency of the energy transfer is strongly sensitive to the distance between the donor and the acceptor,[5] with typical distance changes from ∼1 to 10 nm. It is possible to find many applications of the fluorescence self-quenching method in the literature. For example, recently, fluorescence quenching between two dye molecules has been employed to elucidate the movement of single kinesin molecules on microtubules in real time.[6] Calculations based on our experimental results, specifically, the overlap integral that represents the degree of the spectral overlap between the donor emission and the acceptor absorption, confirm that the fluorescence dynamics of the BODIPY azide dye molecules confined within the hydrogel network obeys Förster resonance energy transfer, which is an important photoprocess in which the excitation energy of an excited fluorophore (the donor) is transferred to a light absorbing molecule (the acceptor) without emission of a photon.[7] Efficiency of FRET is proportional to the inverse sixth power of the intermolecular distance between donor and acceptor pair due to the dipole−dipole coupling mechanism. Apart from separation distance, the spectral overlap

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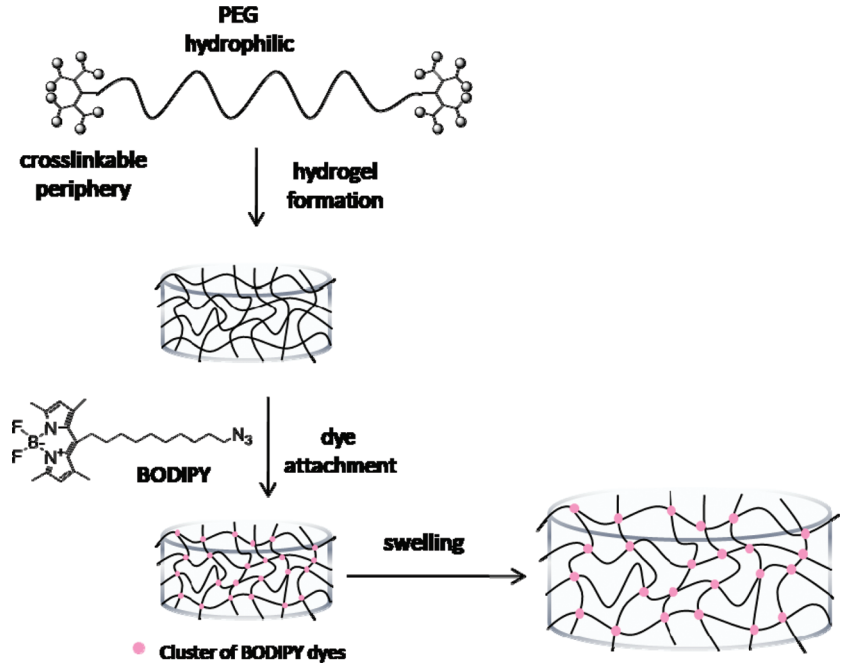

Figure 1. General illustration of stimuli responsive fluorescent hydrogel.

of the donor emission and acceptor absorption spectra, the quantum yield of the donor molecule, and the transition dipole orientations are also important parameters that can alter the rate of energy transfer between donors and acceptors.[8]

Hydrogels play an important role in biomedical applications such as drug delivery systems due to their high water content and low interfacial tension with the surrounding biological environment.[9,10] Generally, drug gets trapped in the hydrogel during the polymerization process. When this hydrogel is immersed in water, release occurs by outflow of drug from the hydrogel and inflow of water to the hydrogel.[11,12] Because the water uptake value of hydrogel directly depends on the cross-link density and conformation of the clusters in the hydrogel network, the drug release rate can be modulated by changing these parameters. The hydrogel network used as a host in our experiments is a hydrophilic structure, and therefore, the distance between the BODIPY dye molecules is controllably adjusted without deforming the network (see Figure 1). Understanding the displacement of entrapped molecules within the hydrogel matrix due to swelling allows one to probe the stimuli responsiveness of such systems.

In the following sections of this paper, the theory of Förster energy transfer is revisited. This is followed by the sample preparation, experimental results, and a discussion on the dynamic mechanism that governs the dipole−dipole interaction of the BODIPY dye molecules embedded in a hydrogel structure.

II. Theory

According to the Förster theory, for a fixed separation distance r, the rate of energy transfer from donor to acceptor is given by[13]

$$k_T = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 \tag{1}$$

where R_0 is the Förster distance and τ_D is the lifetime of the donor in the absence of the acceptor. The Förster distance is defined using the spectral properties of the donor and acceptor as

$$R_0^{\,6} = 8.8 \times 10^{-25}(\kappa^2 n^{-4} \phi_d J) \qquad \text{(in cm)} \tag{2}$$

where ϕ_d is the quantum yield of the donor in the absence of the acceptor and n is the refractive index of the medium. κ^2 is the orientation factor defined by the relative orientation of transition dipoles of the donor and acceptor, and its value generally equals to 2/3. J is the overlap integral which represents the degree of the spectral overlap between the donor emission and the acceptor absorption, and it is defined as

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

$F_D(\lambda)$ is the normalized fluorescence intensity of the donor and $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor at a given λ.

The efficiency of energy transfer (E) is an important parameter for the FRET process. It is defined as the proportion of the photons absorbed by the donor which are transferred to the acceptor. FRET efficiency can be written directly in terms of r distance as

$$E = \frac{R_0^{\,6}}{R_0^{\,6} + r^6} \tag{4}$$

When the distance between the donor and acceptor equals the R_0 Förster distance, the donor dye molecule transfers half of its energy to the acceptor molecule and the efficiency E becomes 50%. The efficiency of the energy transfer can also be determined from the time-resolved measurements. The energy transfer efficiency changes as a function of donor lifetime. Equation 5 shows this relation:

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \quad (5)$$

τ is the fluorescent lifetime of the donor in the absence (τ_D) and presence (τ_{DA}) of the acceptor.

III. Experimental Section

a. Synthesis of PEG-Based Hydrogel and BODIPY Dye. Synthesis of the polymer precursor for fabrication of the hydrogels and BODIPY was published elsewhere.[14] Formation of hydrogel with click chemistry was achieved according to the literature example with minor changes.[15] The detailed experimental procedure and structures of polymers utilized for the synthesis of hydrogels are provided in the Supporting Information.

b. Time-Resolved Lifetime and Fluorescence Intensity Measurements. Time-resolved fluorescence lifetime and fluorescence intensity measurements are performed using a TimeHarp 200 PC-Board system (Picoquant, GmbH) and a fiber optic spectrometer (USB4000-VIS-NIR Ocean Optics), respectively. Figure 2 shows the optical experimental setup. The excitation source used in the experiment is an ultraviolet pulsed diode laser head with a wavelength of 405 nm (LDH-C-D-470 Picoquant, GmbH). The separation of the fluorescence emission and the excitation occurs at a dichroic mirror. The excitation light is focused onto the sample using this microscope objective of 0.55 numerical apertures with a working distance of 10.1 mm (Nikon ELWD 100×).

For multiexponential fluorescence decay fitting, the FluoFit 4.2 computer program (Picoquant, GmbH) is used. The fluorescence intensity decays are recovered from the frequency-domain data in terms of a multiexponential model

$$I(t) = \sum_{i=1}^{n} A_i \exp(-t/\tau_i) \quad (6)$$

where A_i is the amplitude of each component and τ_i is its lifetime. The fractional contribution of each component to the steady-state intensity is described by

$$f_i = \frac{A_i \tau_i}{\sum_j A_j \tau_j} \quad (7)$$

The intensity weighted average lifetime is represented as

$$\langle \tau \rangle = \sum_i f_i \tau_i \quad (8)$$

and the amplitude-weighted lifetime is given by

$$\bar{\tau} = \frac{\sum_i A_i \tau_i}{\sum_i A_i} \quad (9)$$

IV. Results

Figure 3 shows the absorption and emission spectra obtained from the highly concentrated and dilute BODIPY attached

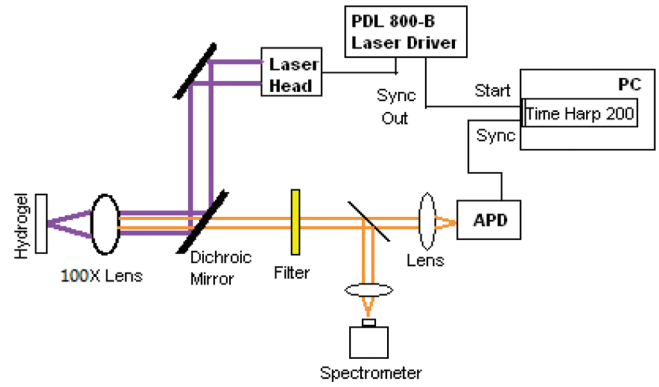

Figure 2. Optical setup.

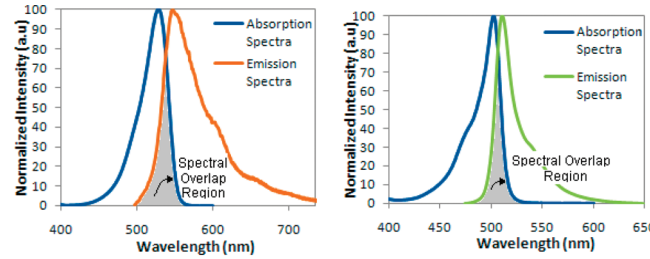

Figure 3. Normalized absorption and emission spectra for (a) highly concentrated BODIPY-dye-doped hydrogel and (b) dilute BODIPY-dye-doped hydrogel.

TABLE 1: Spectroscopic Characteristics of BODIPY Azide Dye in the Hydrogel Network

hydrogel type	λ_{abs} (nm)	λ_f (nm)	ε^a (M^{-1} cm^{-1})	$J(\lambda)$ (M^{-1} cm^3)	R_0 (nm)
concentrated	528	548	70000	1.763×10^{-13}	5.38
dilute	504	512	82000	1.0857×10^{-13}	4.96

[a] The molar extinction coefficient at the peak wavelength.

hydrogel samples. According to these spectra, fluorescence energy transfer is very likely to occur between two BODIPY dye molecules in both hydrogel networks. It is found that there is a good spectral overlap between the fluorescence and absorption spectra of the concentrated BODIPY, whereas the overlap between the fluorescence and absorption spectra of the dilute BODIPY is comparatively poorer. Concentration-dependent spectroscopic characteristics of BODIPY dye molecules in hydrogel networks and their Förster distances are given in Table 1. We are particularly interested in the case where the dyes have rapidly rotating dipoles; therefore, the κ^2 orientation factor value is taken to be 2/3. The refractive index of the medium is 1.4, and the quantum yield is equal to 0.9 in methanol.[16] The spectral overlap integral $J(\lambda)$ is calculated using eq 3. The value of the Förster distances R_0 is determined using eq 2.

Initially, a fiber optic spectrometer is used to monitor the effects of the dye concentration on the BODIPY-doped hydrogel photoluminescence. The number of the reactive alkyne groups in highly concentrated hydrogel is larger than that of the dilute one; as a result, BODIPY dyes aggregate in clusters in highly concentrated hydrogel matrix. It is observed that there are two important differences between concentrated and dilute hydrogels: the emission spectrum of highly concentrated hydrogel is red-shifted, and the fluorescence intensity of this gel severely decreases due to the high concentration of the dye molecules (see Figure 4).

To reveal the dynamics of the fluorescence mechanism, we decided to gradually increase the distance between the BODIPY

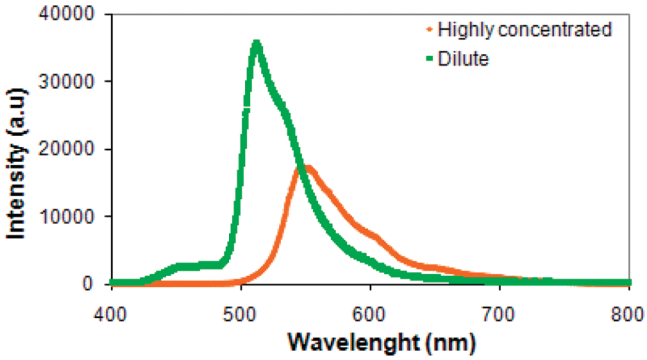

Figure 4. Fluorescence intensity of highly concentrated and dilute hydrogels.

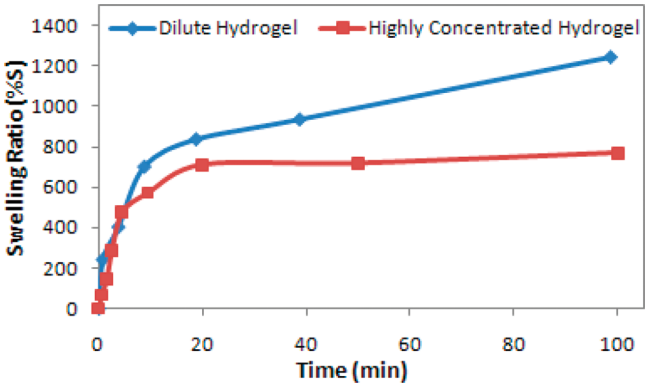

Figure 5. Dependency of swelling ratio (% *S*) of highly concentrated and dilute hydrogel on time.

dye molecules through exposing the hydrogels to water induced swelling. The water induced swelling process of a hydrogel can be controlled with the following parameters: the pH value of the hydrogel, the salt concentration in the hydrogel system, and immersion time in distilled water.[17−19] In our work, the swelling experiments for the hydrogels are carried out at room temperature in distilled water. Dried hydrogel is immersed in distilled water. By taking the sample out of the water periodically, the mass and the fluorescence lifetime of BODIPY dye are measured. The percentage swelling ratio (% *S*) is expressed as

$$\% S = \frac{W_t - W_0}{W_0} \times 100 \qquad (10)$$

where W_0 and W_t are the weights of hydrogels initially and at time t, respectively. Figure 5 shows the dependency of the percentage swelling ratio of hydrogels on swelling time. The equilibrium swelling value of concentrated BODIPY dye attached hydrogel is obtained after 100 min of swelling, reaching a maximum % *S* value of around 800. Moreover, the dilute BODIPY attached hydrogel saturates after 100 min and its maximum swelling ratio value is measured to be about 1200.

The time-resolved fluorescence lifetime of the BODIPY dye molecule is performed using the Timeharp 200 PC-Board system. The measurement of the fluorescence lifetime is based on the time correlated single photon counting (TCSPC) method. In this method, the time between the detected single photon of the fluorescence (start signal) and the excitation laser pulse (stop signal) is measured. The measured data is plotted as a fluorescence lifetime histogram. The fluorescence lifetime of BODIPY dye molecules attached to the dried and swollen hydrogel network are compared. The results for highly concentrated and dilute hydrogels are presented in Figures 6 and 7, respectively. As the hydrogel sample swells, the fluorescence lifetime of BODIPY dye molecule increases in both hydrogel networks. Decay parameters are determined using the double exponential tailfit model, and the best fits are obtained by minimizing the χ^2 values (see Figure 8). Decay times for concentrated and dilute type hydrogels are summarized in Tables 2 and 3, respectively. In the highly concentrated sample, we measured a fluorescence lifetime increase up to a factor of 3.5 at room temperature ($\langle\tau\rangle_{dry} = 2.0309$ ns and $\langle\tau\rangle_{swollen} = 7.1437$ ns). Moreover, the value of the intensity weighted average lifetime of dried dilute hydrogel increases from 5.6154 to 6.3292 ns.

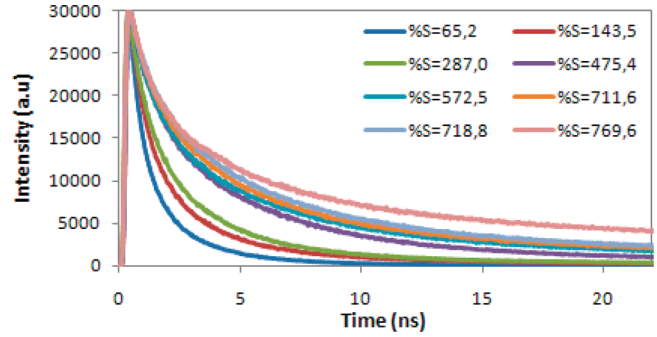

Figure 6. Decay plots of highly concentrated hydrogel for different swelling ratios (% *S*).

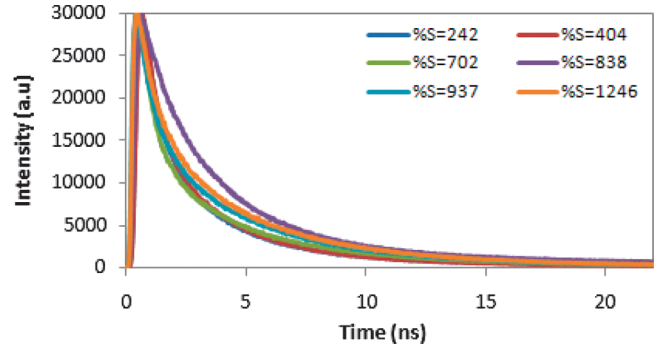

Figure 7. Decay plots of dilute hydrogel for different swelling ratios (% *S*).

It is seen that an efficient FRET is taking place between two BODIPY dye molecules that are both covalently bound to the same cluster of a hydrogel network. As the Förster resonance energy transfer theory states, FRET efficiency E depends on the distance between the donor−acceptor pairs (eq 4) and also the lifetime of the donor molecule (eq 5). The distance between two BODIPY dye molecules at different swelling ratios is calculated via fluorescence lifetime measurements. Experimental data points for r, shown in Tables 4 and 5 and also in Figure 9, are obtained through the experimental values of FRET efficiency E, which are obtained from the lifetime measurements, using eq 4. The theoretical solid curves in Figure 9 are generated for the FRET efficiency E versus r (again using eq 4) by simply giving arbitrary values to distance r from 0 to 15 nm. It is seen that our experimental data points fall on these theoretical curves when R_0 values are adjusted to 5.4 and 5.0 nm, which are quite close to our experimental R_0 values (i.e., 5.38 and 4.96 nm). Figure 9 is useful, since it shows where the data points lie on these curves; however, this shall not be interpreted as a perfect agreement between theory and experiment, as the experimental data points do not carry any more information than the tables. Such proper donor−acceptor distances are summarized in Tables

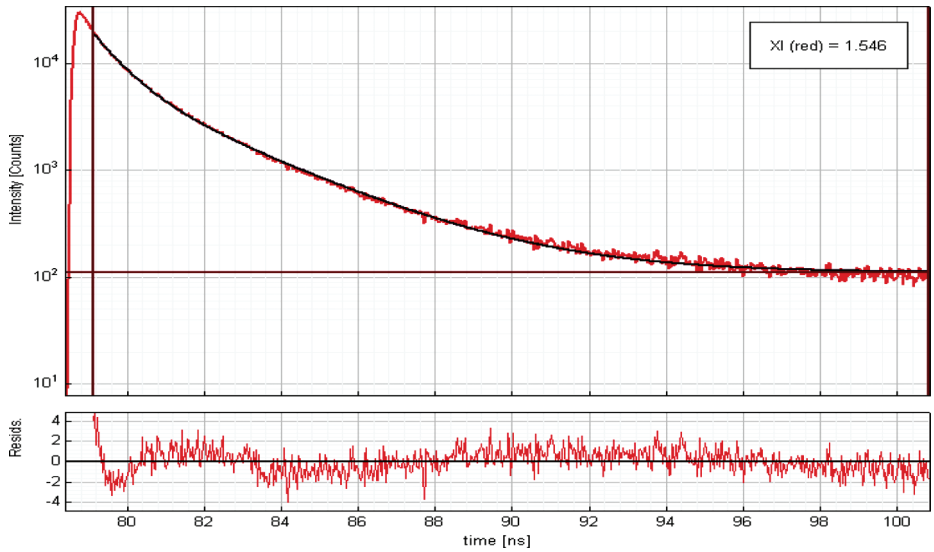

Figure 8. Decay fitting and calculation of the decay parameters of dried highly concentrated hydrogel (% $S = 0$).

TABLE 2: Decay Parameters for Highly Concentrated BODIPY-Azide-Doped Hydrogel

% S	A_1 (au)	τ_1 (ns)	A_2 (au)	τ_2 (ns)	χ^2	$\bar{\tau}^a$ (ns)	$\langle\tau\rangle^b$ (ns)
0	6540.2	2.7071	12454	0.7510	1.546	1.4245	2.0309
65.2	7038.0	3.9975	11965	1.1025	1.917	2.1747	3.0734
143.5	9239.3	4.0607	10363	1.2162	1.602	2.5569	3.3454
287.0	14883.9	5.3893	7657	1.1440	1.329	3.9472	4.9713
475.4	13466.5	6.0868	7313	1.1993	1.302	4.3667	5.6144
572.5	14065.4	6.2000	8188	1.4229	1.597	4.4423	5.6370
711.6	14608.7	6.5150	7557	1.4810	1.377	4.7987	5.9853
718.8	13113.3	7.2000	8204	1.3099	1.753	4.9332	6.5981
769.6	12746.0	8.0304	9939	1.6674	1.768	5.2426	7.1437

[a] The amplitude weighted average lifetime (eq 9). [b] The intensity weighted average lifetime (eq 8).

TABLE 3: Decay Parameters for Dilute BODIPY-Azide-Doped Hydrogel

% S	A_1 (au)	τ_1 (ns)	A_2 (au)	τ_2 (ns)	χ^2	$\bar{\tau}^a$ (ns)	$\langle\tau\rangle^b$ (ns)
0	17050.4	5.9648	7958	0.9591	1.386	4.3719	5.6154
242	16003.3	6.0365	7523	0.9625	1.896	4.1440	5.6827
404	16255.3	6.1556	7628	0.9325	1.956	4.4874	5.8089
702	16986.5	6.1100	6327	0.9218	1.527	4.7020	5.8340
838	13254.0	6.6274	6900	1.3695	1.181	4.8273	6.1167
937	15003.0	6.9520	7256	1.9620	1.856	5.1906	6.1618
1246	19025.0	6.3850	5826	2.0689	1.569	5.4270	6.3292

[a] The amplitude weighted average lifetime (eq 9). [b] The intensity weighted average lifetime (eq 8).

4 and 5, together with the percentage swelling and the FRET efficiency E.

From the absorption and emission spectra shown in Figure 4, the value of the Förster distance is determined to be 5.38 nm for the concentrated type hydrogel sample. When the hydrogel is in the deswollen state, the energy transfer efficiency equals 72% and the fluorescence lifetime of the BODIPY dye molecules is measured to be 2.031 ns. Using eqs 4 and 5, the distance between two dye molecules within the cluster is calculated as 4.59 nm. After immersion of the concentrated hydrogel in distilled water, its average lifetime is measured at definite intervals of time and the separation between two dye molecules is calculated for each swelling ratio. The lifetime measurements are iterated until the hydrogel achieves a constant weight. It is observed that the average lifetime of the concentrated hydrogel starts to increase. For a water-saturated hydrogel

TABLE 4: S, E, and r Values for the Concentrated Hydrogel Sample ($R_0 = 5.38$ nm)

% S	E	r (nm)
0	0.721	4.59
65.2	0.578	5.11
143.5	0.540	5.24
287.0	0.317	6.11
475.4	0.229	6.59
572.5	0.226	6.61
711.6	0.178	6.94
718.8	0.094	7.85
769.6	0.019	10.41

TABLE 5: S, E, and r Values for the Dilute Hydrogel Sample ($R_0 = 4.96$ nm)

% S	E	r (nm)
0	0.229	6.07
242	0.219	6.13
404	0.202	6.24
702	0.199	6.26
838	0.180	6.54
937	0.127	6.84
1246	0.043	8.31

state, average lifetime is measured up to 7.143 ns. In this case, the bonds within the cluster are widely separated from each other and the distance between the two close BODIPY dye molecules reaches 10.41 nm, which corresponds to a FRET efficiency of about 2%. On the other hand, the Förster distance of the BODIPY dye molecule, which is covalently bound to dilute hydrogel, is calculated as 4.96 nm. As far as the dried dilute hydrogel sample is concerned, the fluorescence energy transfer efficiency equals 23% and the fluorescence lifetime is measured to be 5.615 ns. After applying the same swelling process, the fluorescence lifetime of the BODIPY dyes increases up to 6.329 ns and the distance between the two close BODIPY dye molecules reaches 8.31 nm, which corresponds to a FRET efficiency of about 4.30%.

V. Discussion

It is well-known that a hydrogel consists of a network of polymer chains. Any two polymer chains are connected to each other with a cluster, that is, a cross-linking point. Each cluster is depicted with a red dot in Figure 1 and also has a flexible

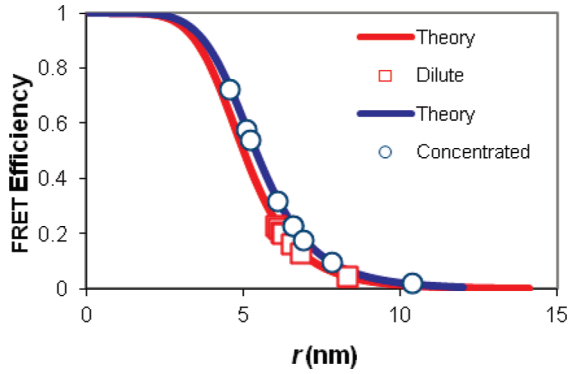

Figure 9. Normalized FRET efficiency and the separation between two dye molecules within one cluster of highly concentrated hydrogel and dilute hydrogel.

chemical structure. The method of cross-linking used here in this work for preparation of samples allows one to obtain hydrogels with a control over the degree of cross-linking and also control over the number of reactive groups in its interior. This control of reactive groups allows controlled dye loading using covalent dye immobilization. In this work, two different hydrogels are prepared, one with concentrated and the other with dilute BODIPY fluorophore density. The experimental results presented in this paper give the following physical insight of this work and help explain the significance of the measurements on commenting the fluorescence dynamics of the BODIPY dye molecules attached to hydrogel clusters: A BODIPY dye molecule is used as both a donor and acceptor fluorophore, which is covalently bound to the same cluster. As briefly stated above, when the donor and acceptor fluorophores are chosen to be the same species, two different types of mechanisms can be effective on the dynamics of the fluorescence intensity. These mechanisms are self-quenching (or contact quenching) and fluorescence resonance energy transfer.[4] Self-quenching is the predominant mechanism of quenching if there is a physical contact between the donor and acceptor dye molecules. In this kind of process, the typical donor−acceptor separation is less than 1 nm. If such a donor−acceptor separation is between 1 and 10 nm, fluorescence resonance energy transfer becomes the predominant mechanism and the efficiency of energy transfer is strongly sensitive to the distance between the donor and acceptor.[5] Since our hydrogels are designed to allow attachment of the dye molecules only at cross-linking points, one needs to clarify whether the fluorescence energy transfer or self-quenching is due to the interaction of the dye molecules within the same cluster or between the molecules in different clusters of the hydrogel network before deciding which mechanism is predominant for the fluorescence dynamics. As two clusters of the hydrogel are connected to each other with a long hydrophilic polyethylene glycol polymer chain, which has 134 repeating units of ethylene glycols, and the length of this polymer chain is expected to be longer than 40 nm when the hydrogel is fully swollen, it is obvious that the cluster−cluster interaction of the hydrogel has no contribution effects on self-quenching or resonance energy transfer. However, the spectral overlap shown in Figure 3 between the fluorescence and absorption spectra of the BODIPY together with the determined molecular separations of 5.38 and 4.96 nm strongly suggest that the underlying predominant fluorescence mechanism is based on the resonance energy rather than that of self-quenching. Such a large molecular separation implies that there is no physical contact between two dye molecules within one cluster of the hydrogel matrix. Furthermore, one more verification that our fluorescence dynamics does not obey self-quenching but conforms to resonance energy transfer is due to the concentration induced change in the lifetime of the dyes and the red-shift occurrence in the absorption spectrum. It is known that self-quenching takes place between the same type of dye molecules and is divided into two categories, namely, static self-quenching and dynamic self-quenching.[20,21] In the static self-quenching, the absorption spectrum of the dye molecules is red-shifted upon increasing the dye concentration in the network but the lifetime of the molecules is constant and experiences no change.[22,23] In the dynamic quenching case, the molecules' lifetimes change as the dye concentration is altered; however, no spectral shift occurs in the absorption spectrum.[24,25] Additionally, for very dilute dye concentrations, one may observe a complete self-quenching, that is, no fluorescence intensity at all, when two single molecules are touching each other.[6] In our experiments, fluorescence is never detected to be zero even for extremely dilute samples; moreover, both spectral shift and concentration induced change in the lifetime is observed for the BODIPY dye molecules used in the hydrogel network.

The separation displacement between dye molecules is studied via exposing the BODIPY-doped hydrogel samples to water induced swelling. As far as the physical mechanism is concerned, we may assume each cluster, depicted with a red dot in Figure 1, as a spherical polymer ball that contains covalently bound hydrogel BODIPY azide dye molecules. Water induced swelling of the hydrogel causes elongation in the network chains and hence introduces tensional forces onto the clusters, causing an increase in the volume of the cluster and consequently forcing attached BODIPY molecules to undergo a separation from each other. The level of fluorescence intensity is observed to clearly increase as the dye separation increases. When the hydrogel is in a dehydrated (or deswollen) state, BODIPY dye molecules are in close proximity and one BODIPY molecule can transfer its energy to another one. As a result, the decay time of the BODIPY dye molecules is quenched and their intensity weighted average lifetime decreases.

An alternative way of determining the separation distance between two dye molecules is to extract such information from the fluorescence intensity measurements. In our experiments, as seen in Figure 4, it is observed that the emission spectrum of the highly concentrated dye molecules is red-shifted and the fluorescence intensity is dramatically low when compared to the dilute sample. This is mainly due to formation of aggregates and hence the resonance energy transfer of the dyes in the hydrogel clusters, which significantly reduces the fluorescence intensity of these dyes. By changing the swelling ratio, one can increase the dye−dye separation without deforming the network and hence can partially avoid the effects of this phenomenon on the fluorescence intensity. However, for a dynamic system like ours, intensity based measurements may not be absolutely reliable, since each time one needs to monitor the fluorescence intensity over the same number of dye clusters through a confocal microscopic technique. As the hydrogel swells, the physical locations of these dye-doped clusters will not remain the same, and therefore, one may not guarantee the same number of dye clusters to get trapped within the spot size of the monitoring laser beam upon swelling. Therefore, we believe that the time-resolved technique presented in this paper is a much more accurate and reliable method and also does not suffer from the aforementioned deficiency.

VI. Conclusion

This paper describes the fluorescence dynamics of BODIPY azide dye molecules embedded within a hydrogel matrix

employing time-resolved fluorescence lifetime measurements. Due to the distance between the dye molecules being smaller than 10 nm, it is anticipated that the effective dynamical mechanism is based on the resonance energy transfer process. The conformational change in hydrogel clusters is analyzed, and the distance between the dye molecules inside one cluster is calculated with respect to the lifetime of the donor molecule in the presence of the acceptor for different swelling ratios. Förster distance measurements show that the energy transfer observed in these gels is due to dipole−dipole (or dye−dye) interactions that are confined in individual single clusters but not due to cluster−cluster interactions. Water induced swelling allowed us to continuously change the distance between the dye molecules. Upon swelling, the physical displacement between BODIPY-doped clusters, that is, the red color nodes shown in Figure 1, increases. However, this cluster displacement is far too big to affect the dipole−dipole interaction and consequently has no possessions on the fluorescence self-quenching and the Förster energy transfer. Our experimental results and theoretical calculations show that the mechanism that governs the Förster energy transfer is mainly due to dipole−dipole interaction within BODIPY-doped single clusters (nodes) but not due to cluster−cluster interaction. It is observed that FRET efficiency significantly decreases as the dipole−dipole distance increases within a single cluster upon water swelling. Such a large change in FRET efficiency can, for example, provide a handle for measuring stimuli responsiveness of hydrogels to be employed as a sensing mechanism for an application in concern.

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Supporting Information Available: General synthesis of PEG based hydrogel via the [3 + 2] Huisgen "click" reaction and functionalization of hydrogel with concentrated and dilute BODIPY dye. This material is available free of charge via the Internet at http://pubs.acs.org.

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