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Diffusion Behaviors of Water-Soluble CdSe/ZnS Core/Shell Quantum Dots Investigated by Single-Particle Tracking

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As is known to us all, quantum dots (QDs), the fluorescent semiconductor nanocrystals, have many excellent optical properties which make them attractive fluorescent tags in single-molecule tracking in live cells. Because the intracellular environment is so complex, this paper aimed at simulating the intracellular solution environment in vitro and investigated the influence of the solution environment on the diffusion of water-soluble core/shell CdSe/ZnS QDs. Single-particle tracking (SPT) was applied to measure the diffusion coefficients of two water-soluble core/shell QDs, CTAB-modified CdSe/ZnS QDs (CTAB-QDs) and octylamine-modified poly(acrylic acid)-modified CdSe/ZnS QDs (OPA-QDs). The exposure time was optimized to be 29.95 ms. Then the paper was focused on the effects of pH value, salt concentration, and solution viscosity on the diffusion coefficients of the two water-soluble QDs. The results demonstrated that the pH value had a great influence on the diffusion coefficient of CTAB-QDs but little on that of OPA-QDs. The difference should be mainly due to the distinguishment of the charge and structure of surface ligands on the two water-soluble QDs. The diffusion coefficient of either CTAB-QDs or OPA-QDs was hardly affected by the salt concentration of the solution. Furthermore, for both CTAB-QDs and OPA-QDs, the diffusion coefficients decreased as the solution viscosity increased, which obeyed the Stokes−Einstein relation. In summary, OPA-QDs have more promising applications in single-molecule tracking in live cells, as compared with CTAB-QDs. The obtained results would benefit the further applications of QDs in single-molecule tracking in live cells. This system could also serve as a model system for studying the diffusion behavior of nanoparticles.

Introduction

To understand the molecular mechanisms of complex biological processes at the molecular level, even single-molecule level, is one of the main goals in modern biology. The single-particle tracking (SPT) technique, one of the most frequently used approaches for in situ and real-time study of single-molecule events in live cells, could provide detailed information on distributions and time trajectories of biomolecules.[1] Therefore, SPT has been used to monitor, in real time, the behavior of individual biological molecules in live cells, thus providing a clearer understanding of these processes.[2−8] G. Seisenberger and his co-workers[9] used SPT to describe the real-time visualization of the infection pathway of single adeno-associated viruses (AAV) labeled with only one dye molecule in living HeLa cells, and they also gained detailed information about various modes of motion of AAV during the whole infectious entry pathway. With the aid of SPT, G. Ruan and his co-workers[10] observed the internalization of the Tat peptide, which is derived from the HIV-1 Tat protein and could serve as a novel cellular delivery vector, into living HeLa cells. They also studied the transport of the Tat peptide in the cytoplasm.

SPT is an effective technique to investigate the diffusion behaviors of individual molecules within live cells and resolve modes of motion of individual molecules. In addition, it can be used to obtain information on biological processes. When an individual molecule diffuses in free solution, the mode of its motion is merely pure diffusion; that is, Brownian motion. Nevertheless, when it diffuses in a live cell, several other modes of motion, such as anomalous diffusion, directed motion with diffusion, and corralled diffusion, would appear due to the interactions between the molecule and some materials in the live cells. Therefore, it is of vital importance to study the diffusion events of objective molecules or complexes. Fluorescent tags are usually used to label and track them, and signals from the objective molecules or complexes could be obtained from the fluorescence information of the fluorescent tags. At present, organic fluorophores are the most commonly used fluorescent tags; however, they are subject to certain limitations: First, they cannot fluoresce continuously for long periods because they are not photostable enough and are easily photobleached. Second, they are not easily optimized for multicolor applications. This limitation comes from two factors: each fluorophore can be excited only by the light of a given wavelength to achieve the optimal condition, which often makes it necessary to use different excitation wavelengths for different types of fluorophores, and each fluorophore has a relatively broad emission spectrum, which usually causes the signals from different fluorophores to overlap. When compared with traditional organic fluorophores, a new kind of semiconductor fluorescence nanoparticle, quantum dots (QDs), have some excellent properties, such as high fluorescence quantum yield, large absorption cross sections, exceptional photostability, broad absorption spectra, narrow emission spectra, and a large Stoke's shift. These features make them have great potential to replace organic fluorophores as fluorescent tags in the field of biological imaging that requires long-term, multitarget, and highly sensitive imaging.[11−18]

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Some groups were engaged in the diffusion events of oil-soluble QDs in a polymer matrix,[19−21] and some researchers began to label biomolecules with water-soluble QDs and tried to study their diffusion events.[10,22−24] Nevertheless, there is still much work to do to understand the diffusion behaviors of QDs, for most experiments performed now are about the diffusion events of oil-soluble QDs and QD-labeled molecules or complexes. It is really of great necessity to first make clear the diffusion behaviors of water-soluble QDs themselves before they are labeled with other molecules or complexes and applied to biological applications. Since QDs have desirable fluorescence properties, fluorescence methods have usually been used to measure their diffusion coefficients. Fluorescence recovery after photobleaching, an ensemble-average method, averages over hundreds or thousands of diffusing molecules to measure the diffusion coefficient, but the spatial resolution of the method is low.[25] Fluorescence correlation spectroscopy, established by Madge et al.,[26] is another method to measure the diffusion coefficient of fluorescent particles, but it has not been used as frequently as SPT in real-time live-cell experiments. Therefore, SPT is fairly suitable for investigating the diffusion behavior of QDs, especially for real-time, live-cell experiments.

According to the classic Stokes−Einstein relation, the diffusion coefficient, D, of a particle in the solution is described as follows, where η is the pure solvent viscosity, T is the absolute temperature, k is the Boltzmann constant, and d_{eff} is the effective hydrodynamic diameter.[27]

$$D = \frac{kT}{3\pi\eta d_{\text{eff}}} \quad (1)$$

From equation 1, we can know that D is mainly related to η, d_{eff}, and T. It is stated that D is related to d_{eff}, the effective hydrodynamic diameter, which means D is related not only to the size of the CTAB-QDs (or OPA-QDs) themselves but also to the electric double layer surrounding CTAB-QDs (or OPA-QDs). The structure of the electric double layer could be influenced by the surface charge density of the particles. Therefore, as the pH value varies, the surface charge of the CTAB-QDs (or OPA-QDs) may change, resulting in a variation in the structure of the electric double layer. So d_{eff} may change if the size of the QDs themselves remains the same, while the structure of their electric double layer may vary. Thus, the pH value may affect the D of the QDs. Accordingly, the salt concentration may affect the structure of the electric double layer, as well, thus influencing the change in the d_{eff} values. In addition, the solution viscosity, η, is a factor in equation 1. Furthermore, concerning the difference between pure water and the intracellular solution, the pH value, salt concentration, and solution viscosity were considered as three factors that could possibly influence the diffusion behavior of water-soluble QDs, since our experiments were all performed in a room where the temperature is constant.

In this paper, a system based on an inverted fluorescence microscope equipped with an intensified charge-coupled device (ICCD) as a detector was established to effectively measure the diffusion coefficient of water-soluble CdSe/ZnS core/shell quantum dots. Then by gradually decreasing the exposure time, the most appropriate exposure time for further research was achieved. For the purpose of simulating an intracellular solution environment in vitro, we explored the effects of pH value, salt concentration, and solution viscosity on the diffusion behaviors of two kinds of water-soluble QDs. The results indicated that OPA-QDs were more suitable for application to single-molecule tracking in live cells compared with CTAB-QDs.

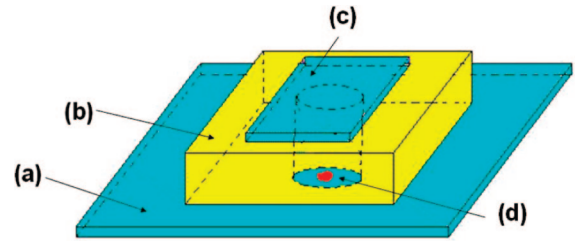

Figure 1. Schematic diagram illustrating the PDMS−coverslip configuration placed under the objective of the microscope: (a) a 24 × 50 mm^2 coverslip, (b) a piece of PDMS with a column-shaped hole in the middle, (c) an 18 × 18 mm^2 coverslip, (d) the sample.

Materials and Methods

Materials. Oil-soluble CdSe/ZnS QDs were purchased from Wuhan Jiayuan Quantum dots Co. Ltd. (China). CTAB-modified CdSe/ZnS QDs (CTAB-QDs) were prepared as follows: First, 0.5 mL of oil-soluble CdSe/ZnS QDs were centrifugated at 12 000 rpm for 5 min to remove deposition and then mixed with 1.5 mL of ethanol and centrifugated at 12 000 rpm for 5 min to remove the excess TOPO ligands on the surface of the QDs by discarding the supernate. Second, the deposition was dissolved in 1.0 mL of chloroform containing ~1.5 mg of CTAB. The solution was then transferred to a 5 mL beaker and heated to 60 °C with constant agitation so that chloroform volatilized gradually. A 1.0 mL portion of chloroform was added into the beaker after it was totally volatilized, and three repeated operations were performed. Finally, pure water was added to the beaker, and the solution was centrifugated at 12 000 rpm for 5 min to remove deposition; thus, the final product was obtained. Octylamine-modified poly(acrylic acid) (OPA)-modified CdSe/ZnS QDs (OPA-QDs; the terminal group is carboxyl) were prepared according to the reported method.[28] The fluorescence properties of these QDs can be found in the Supporting Information (Figures SI-1, SI-2). PEG 200 was of chemical grade and was purchased from Sinopharm Chemical Reagent Co. Ltd. (China). Other chemical reagents were of analytical grade and were purchased from Sigma or local reagent suppliers. All aqueous solutions were prepared with ultrapure water purified on a Labconco water system (Water Pro Plus, Kansas City, MO) unless otherwise mentioned.

Fluorescence Microscopy. All coverslips and slides were cleaned very carefully, and the concentrations of both CTAB-QDs and OPA-QDs were optimized so as to achieve the optimal concentration to observe single QDs under a fluorescence microscope. The specimen was observed with an Olympus IX70 inverted fluorescence microscope with an arc mercury vapor lamp. An oil immersion 100× objective (Olympus, N.A. = 1.30) was used to collect fluorescence from single QDs. The filter set 330−385/400/420 nm was used to excite fluorescence from single QDs. An intensified charge-coupled device (ICCD, 512 × 512 pixel^2, I-PentaMAX GeneIII, Roper Scientific Co., USA) was mounted beside the left side of the microscope. The exposure time of the ICCD can be selected from zero to several seconds, and its readout time is 61.44 ms.

Experimental Set-Up. Figure 1 shows the schematic diagram of the experimental setup with a PDMS−coverslip configuration placed under the objective of the microscope. A piece of PDMS with a column-shaped hole in the middle was first made and placed onto a 24 × 50 mm^2 coverslip, then the sample was added into the hole before an 18 × 18 mm^2 coverslip was finally put onto the PDMS. This setup was designed to form a closed system, since the coverslip and PDMS can attach to each other easily. It was used to perform all the experiments in this paper.

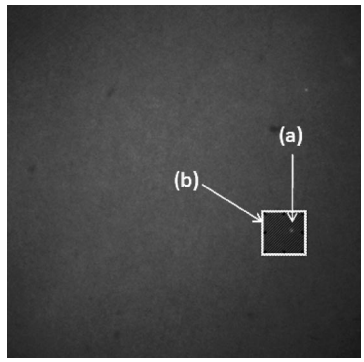

Figure 2. One of fluorescence images of a single QD in a single-particle tracking movie (Supporting Information movie-1): (a) a single QD in the movie, (b) the diffusion area of the single QD.

Results and Discussion

Measurement of the Diffusion Coefficient. Concerning the requisition of movies describing the movements of single QDs, we must take fluorescence intermittency into account because it could make us sometimes unable to capture single QDs. Due to the high fluorescence intensity of single QDs, it is possible to track the movement of single QDs in our experiments when the image stacks contain no more than 100 frames. In addition, if single QDs could not be captured in some frames of a given movie, such movies were not used for calculating the diffusion coefficients of the QDs. A movie was utilized for the calculation of diffusion coefficients of single QDs only if the movement of single QDs could be indentified in all frames of the movie (more than 30 frames). Our experiments were all performed in free solution, so the mode of motion of the particle is normal diffusion; that is, Brownian motion. Therefore, the diffusion coefficient D in our experiments can be described by the following equation,[29] where MSD is the mean-squared displacement, and t is time.

$$D = \mathrm{MSD}/4t \quad (2)$$

To determine a diffusion coefficient from a single trajectory, it is necessary to calculate the MSD. There are two methods to calculate the MSD for a given time lag, t: one is to average over independent pairs of points, and the other is to average over all pairs of points.[29] Herein, the latter method was used to obtain the value of MSD according to reference 29 because this method used all the data and could be more accurate. The inverted fluorescence microscope combined with an ICCD detector was used to track single QDs in real time; thus, a series of movies describing the diffusion trajectories of single QDs in a given time lag were acquired. As a result, the diffusion area for a single QD could be achieved from one of the movies (as shown in Figure 2). A homemade program in MATLAB was used to acquire two columns of data, the MSD and t. The MSD/4∼t curve could then be created. As shown in Figure 3a, it was found that the linear relationship of the curve is good for short time lags and rather scattered for long time lags, which was similar to the reported work.[29] It is therefore necessary to specify a cutoff time; that is, a maximum time lag used for the linear fit of the curve.

The selection of the cutoff time was based on the following two principles: the square linear correlation coefficient (R^2) of the fitted line should be as close to 1 as possible, and in the fitted line, the y value should be as close to zero as possible when x equals to 0. After the selection of the cutoff time lag, we could finally get the slope of the curve (Figure 3b). In this way, a diffusion coefficient from a single trajectory was calculated according to eq 2. The diffusion coefficients were then calculated from 45−70 single trajectories in most cases in the method identical to that mentioned above, forming the histogram of the diffusion coefficients. The statistical diffusion coefficient was attained after a Gaussian fit of the histogram.

Effect of the Exposure Time. With the decrease in the exposure time, the simulated trajectories are closer to the real ones, but the S/N ratio of the movies captured by the ICCD will decrease, so we gradually decreased the exposure time so as to find out the optimal exposure time. Figures 4 and 5 are the histograms of the diffusion coefficients of CTAB-QDs and OPA-QDs under five different exposure times: 150.00, 99.84, 49.92, 39.94, and 29.95 ms, respectively. Figure 4a−e shows the when the exposure time was set at 150.00, 99.84, 49.92, 39.94, and 29.95 ms, the statistic diffusion coefficients of CTAB-QDs were 0.77 ± 0.03 ($n = 36$), 0.55 ± 0.03 ($n = 45$), 0.77 ± 0.03 ($n = 66$), 0.85 ± 0.04 ($n = 42$), and 0.82 ± 0.06 $\mu m^2/s$ ($n = 62$), respectively. Figure 4f was the $D \sim t$ curve representing how the diffusion coefficients D of CTAB-QDs varied with time. The results told us that the exposure time did not significantly affect the diffusion coefficients measured. Figure 5a−e shows the statistical diffusion coefficients of OPA-QDs obtained at the five different exposure times, which were 0.78 ± 0.04 ($n = 55$), 0.83 ± 0.05 ($n = 46$), 0.95 ± 0.04 ($n = 55$), 0.88 ± 0.04 ($n = 51$), and 0.78 ± 0.04 $\mu m^2/s$ ($n = 44$), respectively. Figure 5f shows the variation of diffusion coefficients D of OPA-QDs corresponding to time. The results told us that the exposure time also did not have great effects on the diffusion coefficients of OPA-QDs. Therefore, the diffusion coefficients of both kinds of QDs were not affected greatly under the different exposure times selected in our experiments. When the exposure time was less than 29.95 ms, it was difficult for us to observe single QDs for both QDs, since the S/N ratio was too low. Thus, 29.95 ms was the optimal exposure time for our experiments.

The Effect of pH Value. As the pH value varies, the surface charge of QDs may change, and the structure of the electric double layer of QDs may also be different, so the value of d_{eff} may change. Therefore, D of QDs can be influenced by the pH value. Generally, the pH value of the intracellular solution is around 7; however, the pH value may be about 5 in the early stage of some endosomes. That is to say, the pH value varies in live cells. Therefore, it is necessary to investigate the effect of pH, especially for the application of QDs in single-molecule tracking in live cells. The pH range selected for our experiments should contain the pH values of the intracellular solution, so the series of pH values were 3.60, 4.86, 5.78, 6.88, 7.81, 8.18, and 9.34. Figure 6 illustrates the effect of pH value on the diffusion coefficients D of CTAB-QDs and OPA-QDs. The respective diffusion coefficients D of CTAB-QDs were 0.74 ± 0.02 ($n = 50$), 0.57 ± 0.01 ($n = 65$), 0.56 ± 0.03 ($n = 64$), 0.71 ± 0.03 ($n = 61$), 0.93 ± 0.02 ($n = 56$), 0.98 ± 0.02 ($n = 64$), and 1.33 ± 0.07 $\mu m^2/s$ ($n = 64$) when the pH values varied from 3.60 to 9.34 (The original histograms of the diffusion coefficients are shown in Figure SI-3 of the Supoporting Information). Figure 6a clearly describes how the diffusion coefficients D of CTAB-QDs changed with the pH value. From the curve, we could see that D was minimal when pH = 5.78, and D increased gradually no matter if the pH value decreased or increased from 5.78. The possible reason was that the surface charge of the CTAB-QDs was minimal when pH = 5.78, so the repulsive force from the ions in the solution was minimal, making the structure of the electric double layer the loosest. Because the structure of CTAB is rather simple, the surface charge increased gradually, and the repulsive force from ions

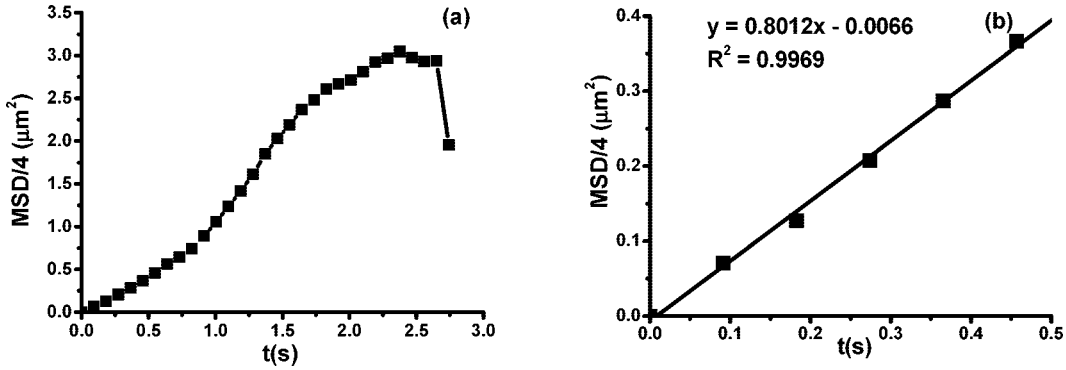

Figure 3. Typical MSD/4 $\sim t$ curve (a) and the linear fit of the curve after selecting the cutoff time lag (b).

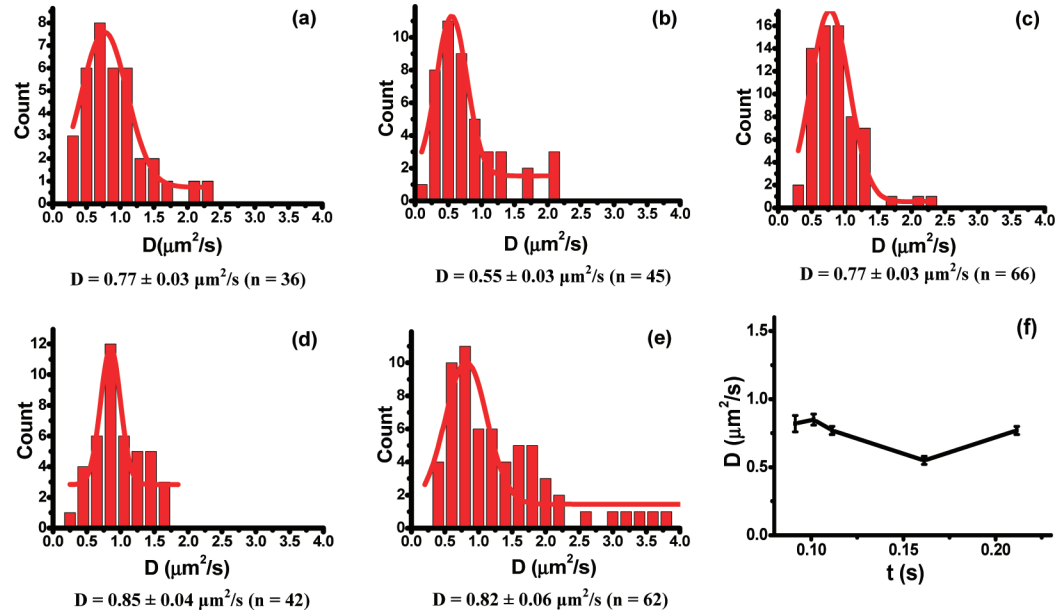

Figure 4. The effect of the exposure time on the diffusion coefficients of CTAB-QDs in pure water. (a−e) Histograms of the diffusion coefficients of CTAB-QDs in pure water under exposure times of 150.00, 99.84, 49.92, 39.94, and 29.95 ms, respectively (the respective D value was below the respective histogram). (f) The $D \sim t$ curve of CTAB-QDs (t is the exposure time plus the readout time of the ICCD).

in the solution increased gradually with the pH value, increasing or decreasing gradually from 5.78. Therefore, the structure of the electric double layer would be more and more compact. When the pH values were 3.60, 4.86, 5.78, 6.88, 7.81, 8.18, and 9.34, the respective diffusion coefficients D of OPA-QDs were 0.83 ± 0.02 (n = 55), 0.68 ± 0.02 (n = 58), 0.89 ± 0.03 (n = 64), 0.77 ± 0.03 (n = 58), 0.86 ± 0.02 (n = 61), 0.78 ± 0.03 (n = 53), and 0.90 ± 0.03 μm^2/s (n = 57). (The original histograms of the diffusion coefficients can be found in Figure SI-4 of the Supporting Information.)

Figure 6b shows the relationship between the diffusion coefficient D of the OPA-QDs and the pH value. It was found that D was not influenced greatly by the pH values. This was probably because the pK_a of the OPA-QDs with carboxyl terminal groups should be less than 3.60, and OPA is a kind of polymer whose structure is relatively complex. Therefore, as the pH value increased, the surface charge of the OPA-QDs varied little, and the structure of the electric double layer of the OPA-QDs changed to only a tiny extent, resulting in the pH-inert property of D of the OPA-QDs. Therefore, considering the results and discussion above about the two water-soluble QDs, from the perspective of the pH value, OPA-QDs were undoubtedly a better choice to be used as a fluorescent tag in further live-cell tracking experiments.

The Effect of Salt Concentration. Figure 7 shows the influence of the salt concentration of the solution on the diffusion coefficients of the two kinds of water-soluble QDs. When the NaCl concentrations were 0, 20, 50, 100, 150, and 300 mM, the respective diffusion coefficients of the CTAB-QDs were 0.79 ± 0.04 (n = 59), 0.77 ± 0.02 (n = 63), 0.62 ± 0.02 (n = 48), 1.01 ± 0.02 (n = 60), 0.72 ± 0.01 (n = 59), and 0.70 ± 0.01 μm^2/s (n = 67) (the original histograms of the diffusion coefficients can be found in Figure SI-5 of the Supporting Information). The variation of the diffusion coefficients of the CTAB-QDs, which changed with the salt concentration, is shown in Figure 7a. It was found that the diffusion coefficients were similar in the range of investigated NaCl concentrations, which indicated that the salt concentrations ranging from 0 to 300 mM had little influence on the diffusion coefficient of the CTAB-QDs. A similar conclusion could be deduced from Figure 7b and Figure SI-6 of the Supporting Information, that the diffusion coefficient of OPA-QDs was not affected greatly by the salt concentration. The isosmotic saline solution for live cells is similar to a 10 mM phosphate buffer solution containing 150 mM NaCl. The stability of the diffusion coefficients of QDs to the salt concentration could benefit their application in live-cell research.

The Effect of Solution Viscosity. Solution viscosity, η, is a factor in eq 1, so it will definitely influence D of QDs. The intracellular environment is molecularly crowded with macromolecules of up to 40% (w/v) in concentration,[30] and 400 g/L PEG 200 is widely used to mimic the intracellular solution

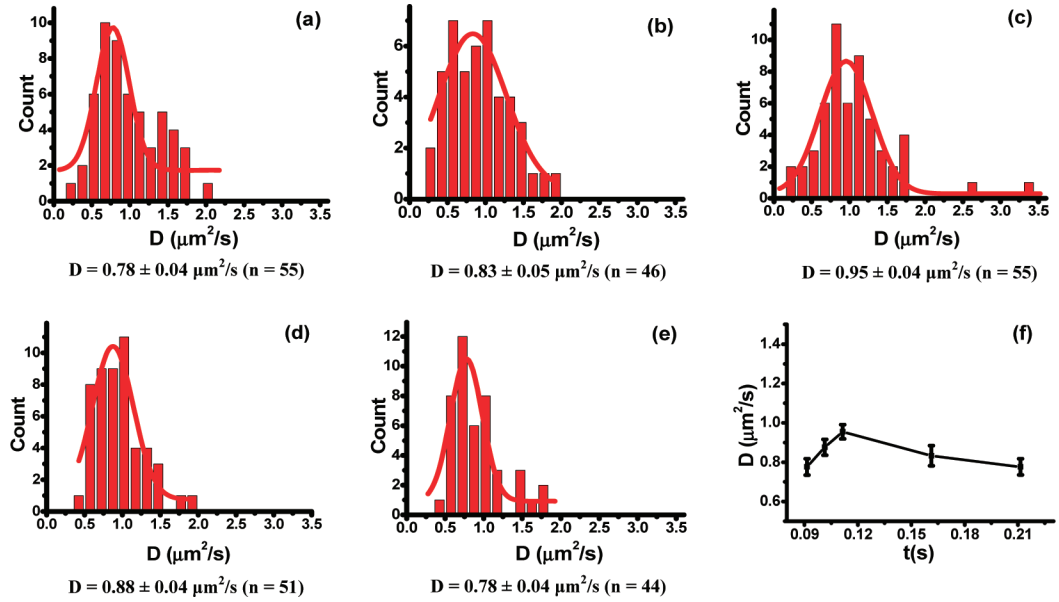

Figure 5. The effect of the exposure time on the diffusion coefficients of OPA-QDs in pure water. (a−e) Histograms of the diffusion coefficients of OPA-QDs in pure water under exposure times of 150.00, 99.84, 49.92, 39.94, and 29.95 ms, respectively (the respective D value was below the respective histogram). (f) The $D \sim t$ curve of OPA-QDs (t is the exposure time plus the readout time of the ICCD).

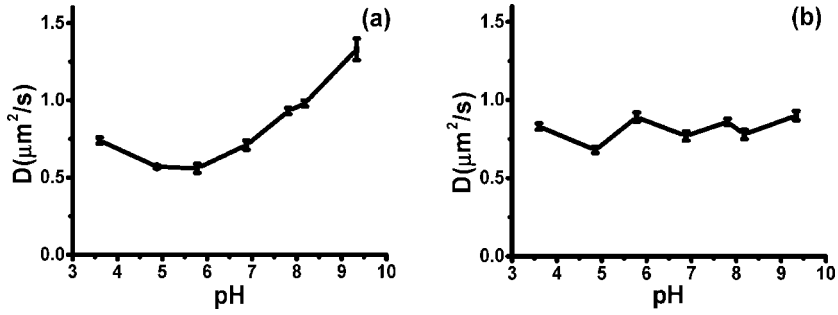

Figure 6. The effect of pH value on the diffusion coefficients D of both QDs: (a) CTAB-QDs and (b) OPA-QDs. (Histograms of the diffusion coefficients of CTAB-QDs and OPA-QDs in different pH values are shown in Figures SI-3 and SI-4, respectively, of the Supporting Information.)

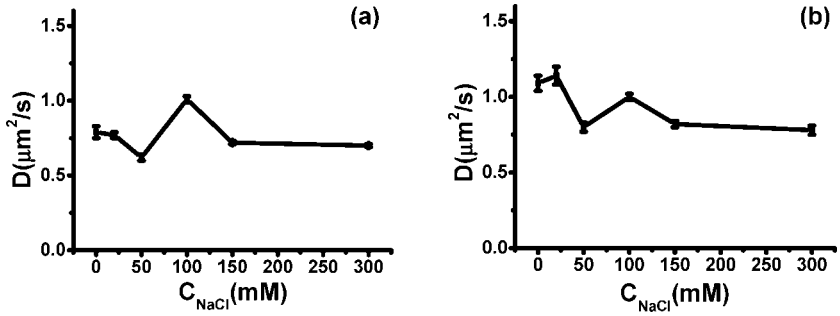

Figure 7. The effect of salt concentration on the diffusion coefficients D of both QDs: (a) CTAB-QDs and (b) OPA-QDs (histograms of the diffusion coefficients of CTAB-QDs and OPA-QDs in different NaCl concentrations are shown in Figures SI-5 and SI-6, respectively, of the Supporting Information).

viscosity.[31] Therefore, different concentrations of PEG 200 were selected to study the effect of solution viscosity on the diffusion coefficients of the two water-soluble QDs. The relative viscosity of the series of PEG 200 aqueous solution against pure water was determined to be 1.000, 1.346, 1.892, 2.765, and 3.909, for 0, 100, 200, 300, and 400 g/L PEG 200 aqueous solutions, respectively. The diffusion coefficients of the CTAB-QDs varied with the viscosity of solutions with concentrations of PEG 200 from 0.76 ± 0.06 (n = 46), 0.65 ± 0.02 (n = 63), 0.48 ± 0.02 (n = 51), and 0.29 ± 0.01 (n = 49) to 0.24 ± 0.01 μm^2/s (n = 59) (the original histograms of the diffusion coefficients can be found in Figure SI-7 of the Supporting Information). It was found that D values were proportional to $1/\eta$ with the equation of $D = 0.7385/\eta + 0.0573$ (as shown in Figure 8a). The diffusion coefficients of CTAB-QDs can be described by the Stokes−Einstein relation. Similar results that the diffusion coefficient decreased as the solution viscosity increased were obtained for the OPA-QDs. When the PEG 200 concentrations were 0, 100, 200, 300, and 400 g/L, the respective diffusion coefficients of OPA-QDs were 0.82 ± 0.03 (n = 40), 0.67 ± 0.01 (n = 55), 0.58 ± 0.02 (n = 58), 0.43 ± 0.02 (n = 51), and 0.34 ± 0.03 μm^2/s (n = 60) (the original histograms of the

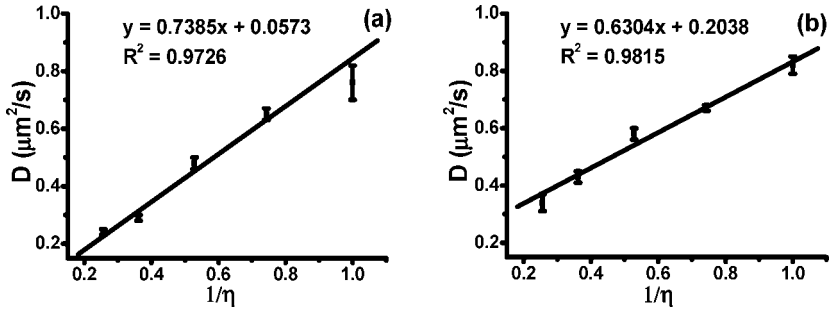

Figure 8. The effect of solution viscosity on the diffusion coefficients *D* of both QDs: (a) CTAB-QDs and (b) CTAB-QDs (histograms of the diffusion coefficients of CTAB-QDs and OPA-QDs in PEG−water solution with different PEG 200 concentrations are shown in Figures SI-7 and SI-8, respectively, in the Supporting Information).

diffusion coefficients can be found in Figure SI-8 of the Supporting Information).

The linear relationship between *D* and $1/\eta$ is shown in Figure 8b. The results for both CTAB-QDs and OPA-QDs indicate that the solution viscosity was an important factor that had great influence on the diffusion behavior of QDs, so when using QDs as fluorescent tags to study the single-molecule events in live cells, such as the infectious pathway of virus or the transport of biomacromolecules in the cytoplasm, the variety of solution viscosities in live cells or the differences between the intracellular and extracellular environments must be considered.

Conclusion

In this paper, the diffusion behaviors of two water-soluble core/shell CdSe/ZnS QDs were investigated using a system based on an inverted fluorescence microscope combined with an intensified charge-coupled device as the detector. The optimal exposure time for our experiments was found to be 29.95 ms. Considering the complex intracellular environment and to simulate intracellular solution environment in vitro, pH value, salt concentration, and solution viscosity as three factors to influence the diffusion behaviors of QDs were investigated. The results demonstrated that pH value had significant influence on the diffusion coefficients of CTAB-QDs, but nearly no influence on those of OPA-QDs. The difference was mainly because the charge and structure of surface ligands on the two water-soluble QDs were so different. In addition, the salt concentration could affect neither the diffusion coefficiencts of CTAB-QDs nor those of OPA-QDs greatly. Furthermore, for both CTAB-QDs and OPA-QDs, *D* decreased as the solution viscosity η increased, and the relationship between *D* and η could be described by the Stokes−Einstein relation. When compared with CTAB-QDs, OPA-QDs have more promising applications in single-molecule tracking in live cells, but much work still needs to be done. It was also indicated that the diffusion behaviors of QDs could be tunable by changing the structure and charge of surface ligands on them. Research on the diffusion behaviors of QDs and the influencing factors could pave the way for their applications. On the basis of the understanding of the diffusion behaviors of QDs, QDs could be used as the fluorescent labels to track viruses or biomolecules in live cells by SPT. Furthermore, this research provided a model system for studying the influence of solution environment on the diffusion behaviors of water-soluble QDs or other fluorescent nanoparticles.

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Supporting Information Available: (1) The absorption and the fluorescence spectra of oil-soluble CdSe/ZnS QDs, CTAB-QDs, and OPA-QDs; (2) the bright-field image and the fluorescent image of CTAB-QDs and OPA-QDs; (3) the effect of pH values on the diffusion coefficients *D* of CTAB-QDs; (4) the effect of pH value on the diffusion coefficients *D* of OPA-QDs; (5) the effect of salt concentrations on the diffusion coefficients *D* of CTAB-QDs; (6) the effect of salt concentrations on the diffusion coefficients *D* of OPA-QDs; (7) the effect of solution viscosity on the diffusion coefficients *D* of CTAB-QDs; (8) the effect of solution viscosity on the diffusion coefficients *D* of OPA-QDs; and (9) a single-particle tracking movie of a single QD. This material is available free of charge via the Internet at http://pubs.acs.org.

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