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Analysis of Quantum Rod Diffusion by Polarized Fluorescence Correlation Spectroscopy

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Abstract To measure the polarization dependence of fluorescent probes, a confocal-microscope-based polarized fluorescence correlation spectroscopy system was developed, and the polarization dependence on the rotational diffusion of well-defined quantum rods (Qrods) was investigated and characterized. The rotational diffusion region of the Qrods was observed over a time range of less than 10^{-5} s in a water solution, and the rotational diffusion parameters were extracted using a rotational diffusion model in which the viscosity of the solution media was varied. Our work demonstrated that polarized fluorescence correlation spectroscopy (FCS) is useful for investigating both the rotational and translational diffusion of fluorescent probes.

Keywords Polarization · Spectroscopy · Fluorescence · Luminescence · Correlators

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Introduction

Fluorescence correlation spectroscopy (FCS) was developed to study molecular diffusion dynamics and the photochemical properties of various fluorescent probes. FCS is based on the analysis of time-dependent fluorescence fluctuations from diffusing fluorescent probes. Since the late 1990s, FCS has progressed due to advances in confocal systems and correlators as well as the development of detectors with high quantum efficiencies [1–5]. The recent developments in FCS systems are opening up several new research areas, especially in the biological sciences, including the analysis of biomolecular diffusion, interactions, and gene expression in both in vitro and in vivo cells [6, 7].

The dynamic properties of the molecules and particles in aqueous media include both translational and rotational diffusion. In principle, rotational diffusion is directly proportional to the molecular weight (Mw), but translational diffusion is proportional to $Mw^{1/3}$ [1]. Therefore, a change in rotational diffusion will be much more sensitive to molecular interaction processes than to translational diffusion, even though both diffusion parameters are applicable during analysis. In previous studies using FCS, translational diffusion of fluorescent probes has often been investigated, but few studies on rotational diffusion have been published [8–12]. The timescale of the rotational diffusion of small probes introduces technical challenges because the detectable time ranges of the correlator, photon antibunching, and triplet formation occur on a timescale similar to that of diffusion. However, larger fluorescent probes, such as nanometer or sub-micrometer-sized fluorescent particles like quantum dots (Qdots) and rods (Qrods), have different time scales for antibunching and lifetime, and they have no triplet state, which makes the diffusion analysis less complicated. Moreover, fluorescent particles such as Qdots and Qrods are useful as probes due to their broad absorption bands, narrow emission bands, and high quantum

yields [13, 14]. In particular, Qrods with a well-defined size and shape are useful as they can be used as a standard to experimentally verify the diffusion properties of various rod-like molecules and particles. The blinking property observed in Qrods interferes with the observation of the rotational diffusion of the rods [15–18]. Nevertheless, the rotational diffusion of Qrods can be measured by fluorescence methods that take advantage of the dependence of a fluorescent particle's absorption and emission of light on its dipole direction.

In this study, we developed a simple confocal-microscope-based polarized FCS system using CW-laser excitation to investigate the rotational and translational diffusion of a well-defined Qrod with a diameter of 5 nm and a longitudinal length of 50 nm. By cross-correlation analyses of various combinations of polarization and media viscosities, the dependence of the rotational and translational diffusion of Qrods on polarization was characterized using the amplitude and characteristic time of the correlation function.

Theory

Observation Region The beam from a light source forms a focal volume of a few femtoliters in a solution containing fluorescent particles, as shown in Fig. 1(a). If the observation region is the same as the focal volume of a microscope, its effective volume (V_{eff}) in Gaussian form can be calculated as approximately $V_{eff}=\pi^{3/2}w^2z$, where z and w are the half-axial length and the waist radius, respectively [see Fig. 1(b)] [1]. As shown in Fig. 1(a), when the particles enter the focal volume, they become excited and fluoresce. The fluorescence intensity of the particles depends on the number, speed, and size of those diffusing into and out of the volume. This diffusion of particles in the focal volume causes time-dependent fluctuations in fluorescence intensity. The fluctuations reflect information about the size, shape, number, and diffusion speed of the particles in the solution.

Dynamic properties, including the translational and rotational diffusion, can be analyzed by measuring the diffusion coefficients, which are represented using Eqs. (1.1) and (1.2) [8]:

$$D_{tran}=\frac{\langle w\rangle^2}{4\tau_{trans}} \tag{1.1}$$

$$D_{rot}=\frac{1}{6\tau_{rot}}, \tag{1.2}$$

where τ_{trans} is the average diffusion time, and τ_{rot} is the rotational relaxation time.

Fluorescence Correlation Function The fluctuations caused by the random diffusion of the fluorescent particles can be analyzed by the correlation function given in Eq. (2) [16]:

$$G(\tau)=\frac{\langle \delta F_1(t)\delta F_2(t+\tau)\rangle}{\langle F_1(t)\rangle\langle F_2(t)\rangle}, \tag{2}$$

where $\delta F_1(t)$ is the fluctuation deviation in fluorescence intensity on one detector at time t, $\delta F_2(t+\tau)$ is the fluctuation deviation in fluorescence intensity on the other detector at time $(t+\tau)$, and $<F_1(t)>$ and $<F_2(t)>$ are the average fluorescence intensities of the time series signals at each detector. The various properties of the fluorescent particles, such as triplet state, translation and rotation diffusions, and blinking, can be analyzed by the correlation function given in Eq. (2).

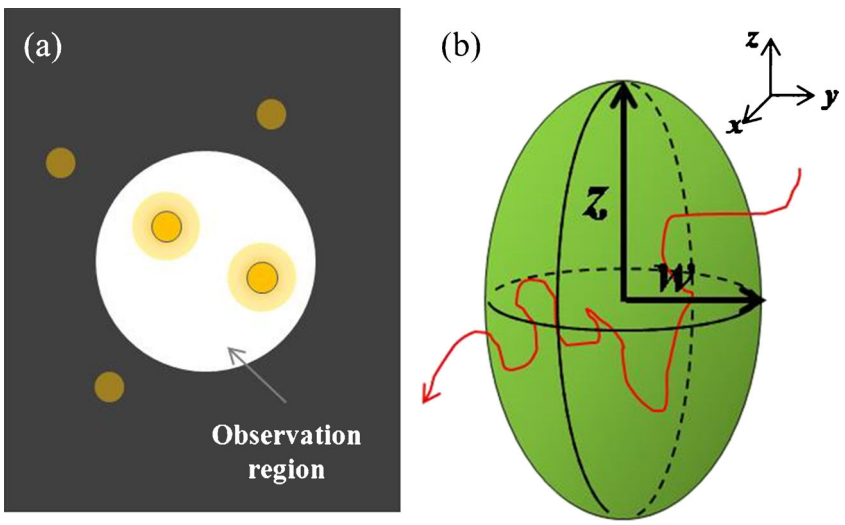

Fig. 1 **a** Observation of particles in a small region, and **b** the size and shape of the focal volume

A translational diffusion model for the normal diffusion of the particles in the focal volume is given by:

$$G_{tran}(\tau) = G(0)\left(1+\left(\frac{\tau}{\tau_{trans}}\right)\right)^{-1}\left(1+\left(\frac{w}{z}\right)^2\left(\frac{\tau}{\tau_{trans}}\right)\right)^{-1/2}, \quad (3)$$

where the characteristic time (τ_{trans}) is the average time passing through the focal volume, and $G(0)$ is the reciprocal of the particle number existing in the volume [1]. The normal diffusion model is applied in the case of very small particles for which size and shape are negligible compared with the focal volume and can therefore be ignored. However, if the size and concentration of the particles in solution increases and the shape changes from the globular form, the translational diffusion of the particles shows anomalous diffusion with sub-diffusions. Then, the normal diffusion model should be modified as follows [19]:

$$G_{tran}(\tau) = G(0)\left(1+\left(\frac{\tau}{\tau_{trans}}\right)^{\alpha}\right)^{-1}\left(1+\left(\left(\frac{w}{z}\right)^2\left(\frac{\tau}{\tau_{trans}}\right)\right)^{\alpha}\right)^{-1/2}, \quad (4)$$

where α is the degree of anomalous sub-diffusion. If $\alpha=1$, the anomalous diffusion becomes normal diffusion. In the case of $\alpha<1$, the slope of the translational diffusion in the correlation function is more gradual than that of normal diffusion.

In the focal volume, the intensity fluctuations emitted from the particles can be analyzed by models of rotational diffusion or a triplet state. The models are expressed as Eqs. (5 and 6) [1, 18]:

$$G_{rot}(\tau) = G_{tran}(\tau)\left[1+R\exp\left(-\frac{\tau}{\tau_{rot}}\right)\right] \quad (5)$$

$$G_{tri}(\tau) = G_{tran}(\tau)\left[1+\frac{F\exp\left(-\tau/\tau_{triplet}\right)}{1-F}\right]. \quad (6)$$

These models include the amplitude fractions R and F, and the characteristic times τ_{rot} and $\tau_{triplet}$ as intensity fluctuations occurring in the focal volume. The fractions R and F depend on the geometry and degree of polarization of the particles. Using these models, we can analyze the polarization dependence of the rotational diffusion of non-spherical particles.

Experiments

Optical Setup The FCS setup is shown in Fig. 2. A DPSS laser (Cobolt, Samba; 25 mW) with a wavelength of 532 nm and an single-photon counting module (SPCM; Id Quantique, id100-50) were used as an excitation source and a detector, respectively. Alignment of the optical components was accomplished using a commercial optical microscope (Olympus IX71). The optical components used in the FCS setup were as follows: polarization beam splitter (PBS), quarter wave plate (QWP), excitation filter (ExF), dichroic mirror (DM), objective lens (OB), cover glass (CG), emission filter (EmF), lens (L), and mirror (M).

The laser beam from the light source passed through the PBS and entered the optical microscope. The beam in the microscope passed the ExF (Semrock, FF01-531/40-25) and was then reflected from the DM (Semrock, FF545/650-Di02). Then, the reflected beam passed through the OB (Olympus, 60×, 1.2 NA), immersion water, and CG in series, and then formed a focal point with a very small volume inside the solution. The fluorescent particles in the focal volume were excited by the laser beam, and then emitted fluorescent light. The light emitted from the particles entered the OB. After the light passed through the DM, EMF, and L, it escaped the microscope by the inner mirror. The light emerging from the microscope reached the PBS and was split into two beams, and the beams were detected by two SPCMs.

The beam splitters in our FCS system were a non-polarized beam splitter (NPBS) and a PBS. These were used to select the polarization state of the fluorescent light. The fluorescent light detected at the SPCMs was converted into TTL signals, and the signals were then converted into a correlation function using the TCSPC system (PicoQuant, Picoharp300). The correlation function was monitored by the FluoFit program (PicoQuant), and the correlation time was limited to a range between 1.5 μs and 0.1 s.

Preparation of Probe Sample To investigate the rotational diffusion of rod-like particles, we used quantum rods (Qrods; GSH-Qrod612) with a length of 50 nm and a width of 5 nm (aspect ratio of 10) as the fluorescent particles [20]. The Qrods consisted of the core and shell; the core material was CdSe and the shell material was CdS. Growth of the Qrod is described in detail in Ref. [21]. The Qrods have a maximum emission peak centered at 612 nm and strongly absorb light below 600 nm. Therefore, these Qrods could be sufficiently excited by our laser, which had a wavelength of 532 nm. To characterize the rotational diffusion of Qrod612 particles, they were diluted with distilled water to a concentration of 1 nM. In addition, Qrod solutions with viscosities of 1 mPa·s, 2 mPa·s, 4 mPa·s, and 8 mPa·s were prepared in order to study the dependence of rotational diffusion on viscosity and then compare it with that of translational diffusion on viscosity. Glycerin was used

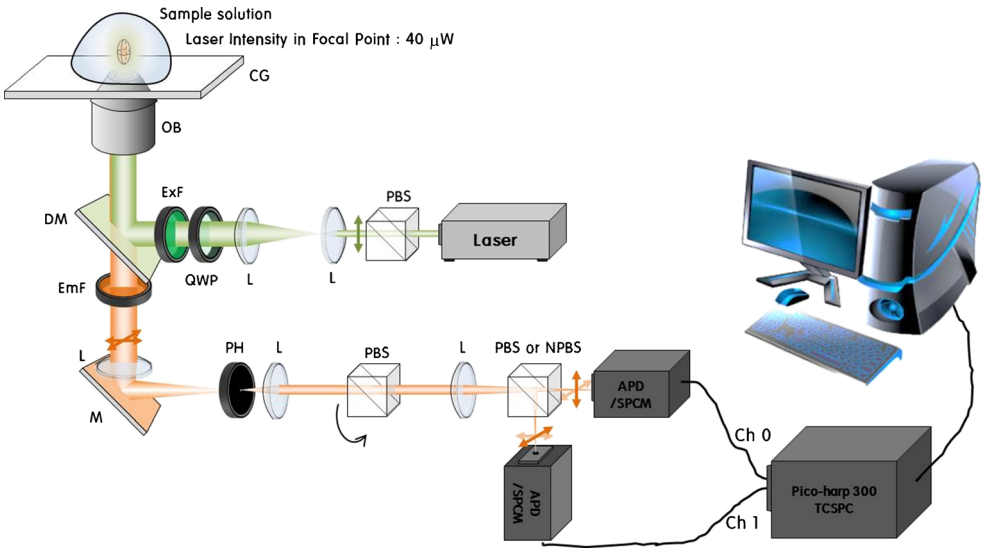

Fig. 2 The polarization FCS setup based on an optical microscope

to change the viscosity of the solutions. Rhodamine 6G (R6G) and Qrods in 1 nM solutions at the final viscosity were prepared at room temperature.

Combination of Polarization States To examine the dependence of excitation and fluorescence on polarization states, independent polarization states of the excitation light, i.e., linear (X) and circular (C), were generated using the QWP in front of the laser. The fluorescent light was split into two beams for cross correlation. The fluorescent light split by the NPBS or PBS was polarized into three states: N, X, and Y, where N is a non-polarization state, and X and Y denote the polarization state parallel to the excitation light and the polarization state perpendicular to the light, respectively. Table 1 shows the combinations of the polarization states of the excitation and fluorescent light entering the two SPDMs [18, 22]. In Table 1, the combination XXX denotes linearly polarized excitation and fluorescent light. The combination XYY indicates that the polarization states of the linearly polarized excitation light and the fluorescent light crossed. In the same manner, the third, fourth, and fifth combinations were generated by relative combinations of the polarization states of the excitation and fluorescent light. In a polarization combination, the first letter indicates the laser source polarization and the second and third letters are associated with the fluorescent light. These last 2 letters were used to obtain the cross correlation function.

Table 1 Classification of the polarization states of excitation and fluorescent lights

Mark	Polarization state of excitation light (First letter)	Polarization state of fluorescent light	
		Detector 1 (Second letter)	Detector 2 (Third letter)
XXX	$Linear_x$	$Linear_x$	$Linear_x$
XYY	$Linear_x$	$Linear_y$	$Linear_y$
XXY	$Linear_x$	$Linear_x$	$Linear_y$
CXY	Circular	$Linear_x$	$Linear_y$
XNN	$Linear_x$	Depolarization	Depolarization

Results and Discussion

Diffusion Dependence on Viscosity The diffusion coefficient of particles in solution depends on the viscosity of the solution, and is lower in a solution with high viscosity. This is the well-known Stokes-Einstein relation [23]. To analyze the translational and rotational diffusions of Qrods, a standard solution is needed for comparison. In our study, we used R6G prepared under the same conditions as the Qrod solution.

Figure 3 (a) shows the correlation functions of R6G in aqueous glycerin solutions with different viscosities (scattered symbol and line). The measured correlation functions were fitted by Eq. (6) (bold line). When the viscosity of the solution was 0.9 mPa·s or 1 mPa·s, the translational diffusion of R6G corresponded to the normal diffusion of Eq. (3). However, as the viscosity of the solution increased, the correlation functions of R6G could not be fitted by Eq. (3). Therefore, the experimental data were fitted by Eq. (6) with the triplet term, and Eq. (4) was used to describe the translational diffusion reflected as anomalous diffusion, even though R6G blinking could be the reason for the inconsistency in the fit. As shown in Fig. 3(a), the correlation coefficients of the triplet state region below 10^{-5} s, and the translational diffusion region above 10^{-5} s increased slightly with increasing viscosity. Our triplet state result is in good agreement with References [24, 25].

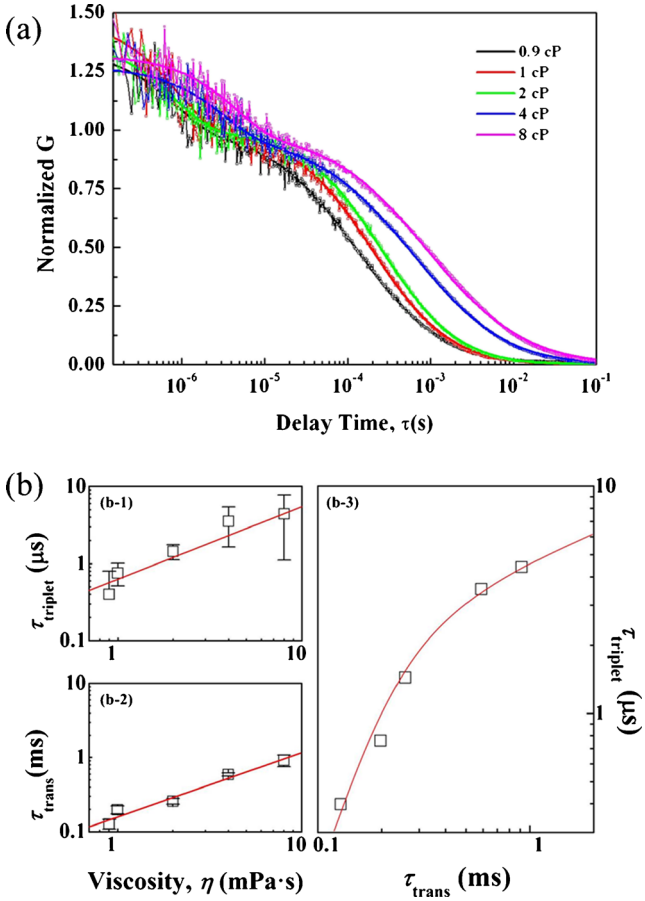

Fig. 3 **a** Fluorescence correlation functions of R6G in glycerin aqueous solutions with different viscosities; the *points* and the *solid line* indicate the experimental data and fitting results, respectively. **b-1** Triplet state and **b-2** translational diffusion time of R6G as a function of viscosity. The triplet state and translational diffusion showed a linear dependence on the viscosity of the glycerin solution. **b-3** Triplet state time vs. translational diffusion revealed a proportional relationship

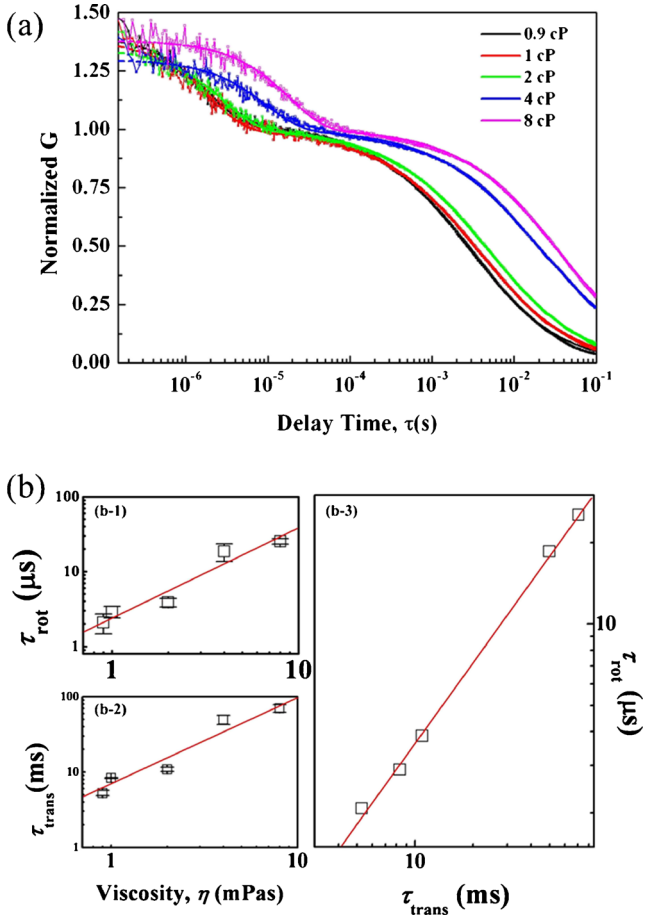

Fig. 4 **a** The fluorescence correlation functions of Qrods in aqueous glycerin solutions with different viscosities. The experimental data were fit by Eq. (6). **b-1** Rotational and **b-2** translational diffusion time of Qrods as a function of viscosity; rotational and translational diffusion showed a linear dependence on the viscosity of the glycerin solution. **b-3** Rotational diffusion time vs. translational diffusion time revealed a proportional relationship

To confirm the viscosity dependence of the triplet state and the translational diffusion of R6G, the triplet state and translational diffusion times of the R6G were analyzed as a function of viscosity (Fig. 3(b-1) and (b-2)). The translational diffusion times were 0.13±0.02 ms, 0.20±0.02 ms, 0.26±0.02 ms, 0.60±0.03 ms, and 0.92±0.17 ms, and the times for the triplet state were 0.40±0.40 μs, 0.76±0.25 μs, 1.44±0.32 μs, 3.53±1.89 μs, and 4.41±3.30 μs at viscosities of 0.9 mPa·s, 1 mPa·s, 2 mPa·s, 4 mPa·s, and 8 mPa·s, respectively. The triplet state and translational diffusion showed a linear dependence on the viscosity of the glycerin solution. The relationship between the triplet state and translational diffusion time was also confirmed (Fig. 3(b-3)), suggesting a non-linear relationship.

Figure 4(a) shows the correlation function for the diffusion of Qrods in solutions with different viscosities. The translational diffusion times of nanoparticles such as quantum rods and quantum dots were longer than those of R6G because the particle sizes were bigger. As shown in Fig. 4(a), the correlation functions of Qrods did not include the triplet state that was demonstrated with R6G. The lifetime of the Qrod is 3.0 ns at a temperature of 10 K [21], and the lifetimes of the CdSe core material do not exceed 100 ns at RT, which is the minimum delay time in our system [26]. Although the time between the rotation of a particle and the fluorescence lifetime can result in irregular emission of light in quantum dot materials, these properties also can produce negligible results because the correlation curve does not decay exponentially in a narrow delay time band (below a few microseconds) [15]. So, the correlation functions address rotational diffusion. The experimental data for the Qrods were thus fit by the rotation model of Eq. (5).

The diffusion time parameters were 5.27±0.44 ms, 8.31±1.00 ms, 10.85±0.70 ms, 49.73±7.10 ms, and 70.54±8.20 ms for translational diffusion, and 2.08±0.62 μs, 2.90±0.50 μs, 3.86±0.50 μs, 18.56±5.00 μs, and 25.40±2.10 μs for rotational diffusion at the same viscosities as the R6G solutions, respectively. The translational and rotational diffusion times

of the Qrods in solution are shown as a function of viscosity in Fig. 4(b-1) and (b-2), and both diffusion times were linearly proportional to the viscosity of the solution. The results are in agreement with the Stokes-Einstein relationship, which states that the rotational and translational diffusion coefficients are reciprocally proportional to the viscosity of the solution. The relationship between both diffusion times was also found to be linear, as shown in Fig. 4(b-3). This result indicates that the dynamic properties of Qrods, such as translational and rotational diffusions, depend on the viscosity of the solution. From this result, we conclude that the rotational diffusion of Qrods can be analyzed using our FCS system.

Rotational Diffusion Dependence on Polarization We performed polarized FCS measurements on the Qrods to study the dependence of rotational diffusion on polarization. For comparison, we also used R6G as the standard sample in the polarized FCS measurements.

Figure 5(a) shows the correlation functions of R6G with various combinations of excitation and emission (or fluorescent) polarization states. The various combinations of polarization states of both lights are listed in Table 1. Amplitudes of the correlation functions were normalized to the translational diffusion amplitude ($G(0)$), which is 1, for comparison with the triplet state amplitude (the fraction of the triplet state). The combinations XXX and XYY provided the same emission light polarization state for both detectors in this sample, so the combination XYY was neglected in this measurement. As shown in the inset graph, the triplet state did not depend on the polarization state.

Figure 5(b) represents the correlation functions of the Qrods for various combinations of excitation and emission polarization states. The sample viscosity was 0.9 mPa·s at 25 °C. In the rotational diffusion region, the rotational fraction was related to the amplitude of the intensity fluctuation as in the translational diffusion results. The rotational diffusion region can be generated by a difference in the polarization directions of the excitation light and the emission light, which can be regarded as a collection of dipoles. If a Qrod is excited by linearly polarized light in the dipole direction, it will emit light in nearly the same direction as the absorption dipoles. If the dipole direction changes due to rotation of the Qrods, the emitted light will then have a different polarization compared with that of the excitation light [17, 23]. This polarization difference due to rotation of the Qrods causes the intensity fluctuation in the focal volume. As the polarization difference approaches 90°, the absorption and emission of the dipoles gradually decreases. In our study, data obtained from all combinations of polarization states were fitted by Eq. (5). Equation (5) approximately expresses the correlation function for the rotational diffusion of Qrods as a collection of dipoles. As shown in Fig. 5(b), all data were in good agreement with Eq. (5), confirming the correlation was due to the rotational diffusion of Qrods. At times below 10^{-5} s, the amplitude of

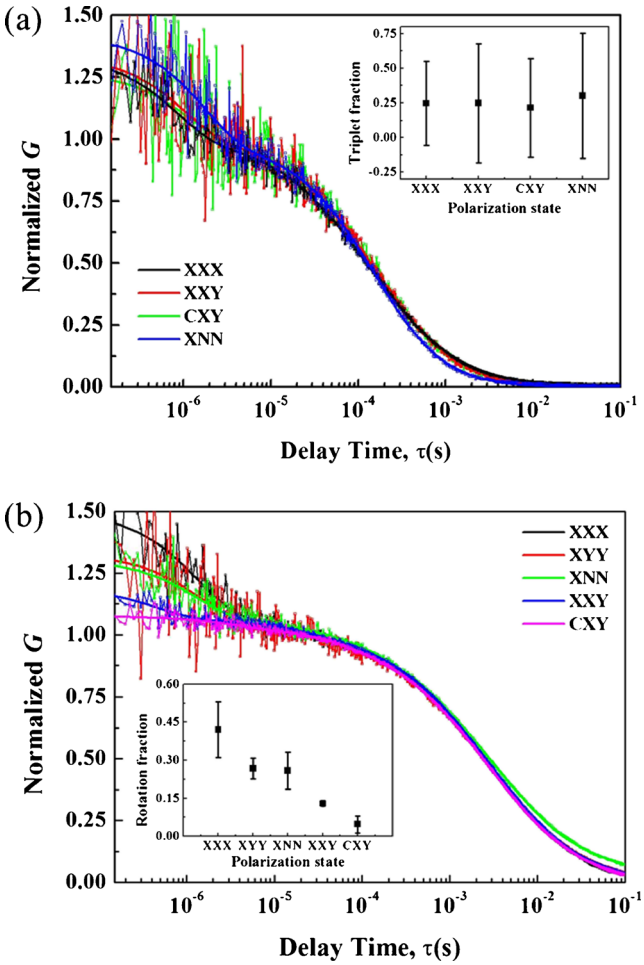

Fig. 5 **a** Fluorescence correlation functions of R6G with various combinations of excitation and emission polarization states. The inset shows the triplet fraction with various combinations of polarization states. **b** Fluorescence correlation functions of GSH-Qrod612 with various combinations of excitation and emission polarization states. The inset shows the rotational fraction with various combinations of polarization states

correlation functions with various combinations of polarization states began to diverge. This result indicates that the rotational diffusion of Qrods depends on the polarization states of the excitation and emission light.

As shown in the inset in Fig. 5(b), an autocorrelation with a combination of XXX had the largest rotational diffusion

Table 2 Rotational diffusion parameters obtained from the correlation functions of the rotational diffusion of Qrods

Polarization states	Rotation time (μs)	Error (μs)	Rotation fraction	Error
XXX	1.65	0.43	0.42	0.11
XYY	1.36	0.70	0.27	0.04
XNN	1.31	0.80	0.26	0.07
XXY	1.15	1.50	0.13	0.01
CXY	5.21	11.00	0.05	0.03

Solvent: water, Temperature: 25 °C

fraction of 0.42. The fractions of autocorrelations with combinations of XYY and XNN were 0.27 and 0.26, respectively. Cross-correlations with combinations of XXY and CXY were found to be 0.13 and 0.05, respectively. This indicates that the rotational diffusion of Qrods in solution depends on the excitation and emission polarization states. Data obtained from the various combinations of polarization states were used to estimate the rotational diffusion parameters [9]. The parameters are listed in Table 2. As listed in Table 2, the rotation time of Qrods in solution showed a dependence on polarization, except when the combination CXY was the rotation fraction. This result demonstrates that the rotational diffusion of Qrods is dependent on polarization.

Conclusions

A confocal-microscope-based polarized FCS system was developed to investigate the rotational diffusion of GSH-Qrod612 composed of CdSe/CdS core/shell in water and in aqueous glycerin solutions. For comparison, Qrods and R6G were used as large, rod-like particles and as standard, small, spherical molecules, respectively. The dependence of rotational diffusion on polarization was characterized and compared with that of R6G, which shows no polarization dependence, by analyzing cross-correlation functions for various combinations of polarization states. Moreover, the dependences of rotational and translational diffusion on aqueous viscosity were consistent. The rotational diffusion region of the Qrods was observed to be in the time range of 1.9 μs to 27.1 μs and in the viscosity range between 0.9 mPa·s and 8 mPa·s; the rotational diffusion parameters were extracted using a rotational diffusion model. The parameters revealed that the rotational diffusion of Qrods in solution is dependent both on polarization and viscosity. Our work demonstrates that polarized FCS analysis based on a conventional CW-laser excitation setup is applicable to the investigation of rotational and translational diffusion of fluorescent molecules and particles in vitro media with various viscosities and in compartments inside of live cells [25, 27].

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References

1. Lakowicz JR (2006), Principles of Fluorescence Spectroscopy, Third ed., Springer Science + Business Media. Chapter 24
2. Magde D, Elson E, Webb WW (1972) Thermodynamic fluctuation in a reacting system-measurement by fluorescence correlation spectroscopy. Phys Rev Lett 29:705
3. Elson EL, Magde D (1974) Fluorescnece correlation spectroscopy. I. Conceptual basis and theory. Biopolymers 13:1
4. Magde D, Elson E, Webb WW (1974) Fluorescence correlation spectroscopy. II. An experimental realization. Biopolymers 13:29
5. Rigler R, Mets Ü, Widengen J, Kask P (1993) Fluorescence correlation spectroscopy with high count rate and low background: analysis of translational diffusion. Eur Biophys J 22:169
6. Brazda P, Szekeres T, Bravics B, Tóth K, Vámosi G, Nagy L (2011) Live-cell fluorescence correlation spectroscopy dissects the role of coregulator exchange and chromatin binding in retinoic acid receptor mobility. J Cell Sci 124:3631
7. Pack C, Saito K, Tamura M, Kinjo M (2006) Microenvironment and effect of energy depletion in the nucleus analyzed by mobility of multiple oligomeric EGFPs. Biophys J 91:3921
8. Ehrenberg M, Rigler R (1974) Rotational Brownian motion and fluorescence intensity fluctuations. Chem Phys 4:390
9. Kask P, Piksarv P, Pooga M, Mets Ü, Lippmaa E (1989) Separation of the rotational contribution in fluorescence correlation experiments. Biophys J 55:213
10. Ehrenberg M, Rigler R (1976) Fluorescence correlation spectroscopy applied to rotational diffusion of macromolecules. Q Rev Biophys 9:69
11. Mets Ü (2001) Fluorescence correlation sepctroscopy. Springer, New York
12. Kask P, Piksarv P, Mets Ü, Pooga M, Lippmaa E (1987) Fluorescence correlation spectroscopy in the nanosecond time range: rotational diffusion of bovine carbonic anhydrase. Eur Biophys J 14:257
13. Alivastos AP (1996) Semiconductor clusters, nanocrystals, and quantum dots. Science 271:933
14. Michalet X, Finaud FF, Bentolia LA, Tsay JM, Doose S, Li JJ, Sundaresan G, Wi AM, Gambhir SS, Weiss S (2005) Quantum dot for live cells, in vivo imaging, and diagnostics. Science 307:538
15. Yao J, Larson DR, Vishwasrao HD, Zipfel WR, Webb WW (2005) Blinking and nonradiant dark fraction of water-soluble quantum dots in aqueous solution. PNAS 102:14284
16. Liedl T, Keller S, Simmel FC, Radler JO, Parak WJ (2005) Fluorescent nanocrystals as colloidal probes in complex fluids measured by fluorescence correlation spectroscopy. Small 1: 997
17. Wang S, Querner C, Emmons T, Drndic M, Crouch CH (2006) Fluorescence blinking statistics from CdSe core and core/shell nanorods. J Phys Chem B 110:23221
18. Aragon SR, Pecora R (1975) Fluorescence correlation spectroscopy and Brownian rotational diffusion. Biopolymers 14: 119
19. Weiss M, Elsner M, Karberg F, Nilsson T (2004) Anomalous subdiffusion is a measure for cytoplasmic crowding in living cells. Biophys J 87:3518
20. Ohmachi M, Komori Y, Iwane AH, Fujii F, Jin T, Yanagida T (2012) Fluorescence microscopy for simultaneous observation of 3D orientation and movement and its application to quantum rod-tagged myosin V. PNAS 109:5294
21. Deka S, Quarta A, Lupo MG, Falqui A, Boninelli S, Giannini C, Morello G, Giorgi MD, Lanzani G, Spinella C, Cinglani R, Pellegrino T, Manna L (2009) CdSe/CdS/ZnS double shell nanorods with high photoluminescence efficiency and their exploitation as biolabeling probes. J Am Chem Soc 131:2948
22. Tsay JM, Doose S, Weiss S (2006) Rotational and translational diffusion of peptide-coated CdSe/CdS/ZnS nanorods studied by fluorescence correlation spectroscopy. J Am Chem Soc 128:1639
23. Cooper A (2005) Biophysical chemistry. Life Science Chapter 4

24. Widengren J, Mets Ü, Rigler R (1995) Fluorescence correlation spectroscopy of triplet states in solution: a theoretical and experimental study. J Phys Chem 99:13368
25. Shin HS, Okamoto A, Sako Y, Kim SY, Kim SW, Pack CG (2013) Characterization of the triplet state of hybridization-sensitive DNA probe by using fluorescence correlation spectroscopy. J Phys Chem A 117:27
26. de Mello Donegá CM, Bode M, Meijerink A (2006) Size-and temperature-dependence of exciton lifetimes in CdSe quantum dots. Phys Rev B 74:085320
27. Pack CG, Song M, Lee E, Hirochima M, Byun K, Kim J, Sako Y (2012) Microenvironments and different nanoparticle dynamics in living cells revealed by a standard nanoparticle. J Control Rel 163: 315