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Counting Single Rhodamine 6G Dye Molecules in Organosilicate Nanoparticles

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Abstract

Rhodamine 6G (R6G) dye molecules have been embedded into organosilicate nanoparticles to improve thermal and chemical stability of these marker molecules. We demonstrate that the well-established method of optical single-particle microscopy can be used to determine the number of dye molecules per nanoparticle in such hybrid materials. Analysing the fluorescence intensity of R6G in single nanoparticles, we obtain an average number of 1.3 – 1.7 dye molecules per nanoparticle as compared to 1 R6G per particle obtained from ensemble experiments. The blinking behaviour of embedded R6G can be described by a power law with an exponent $\alpha_{on/off} = 1.7$. Ensemble measurements complete the optical characterization of the nanoparticles, which reveals no pronounced R6G aggregate formation.

Keywords

organic-inorganic hybrid nanostructures; optical properties of nanoparticles; Fluorescence of molecules; photoluminescence Fluorescence intermittency (blinking)

1. Introduction

In various fields of life sciences fluorescence methods enable a deeper insight and understanding of underlying processes. For this purpose, the selection of appropriate markers is essential. On one hand, markers should not limit the sensitivity of the applied method, for example, due to a low quantum yield or photostability. On the other hand, the physical and chemical properties of the markers should match the investigated system; for instance, with respect to the solubility, toxicity and interaction with the system [1]. Therefore, tailoring adequate markers becomes more and more important, and with that the physical characterization of their properties.

Some of the most promising systems for tailored markers are hybrid nanoparticles, which are fabricated by embedding organic dye molecules in a solid particle [2]. This technique

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has several advantages. The fluorescence intensity of hybrid nanoparticles is considerably increased when embedding multiple dye molecules. Simultaneously an isolation of the dye molecules from the environment is obtained and related interactions can be considerably reduced. For example, in the case of in-vivo measurements a damage of the investigated cell through the toxicity of the fluorescent dye can be avoided. Furthermore, the surface properties of the nanoparticles can be modified by ligands. With this, the markers are adjustable to the investigated system and/or the related functions, for example, the solubility or labelling specific parts of the studied cells. A further outstanding advantage is the exchangeability of the embedded fluorescent dyes and the possibility of enclosing different dyes in one nanoparticle [3, 4].

To optimize markers it is essential to tailor the (optical) properties. This also includes the number of involved dye molecules. In the present work we will demonstrate that the number of enclosed Rhodamine 6G molecules in hydrophobic organosilicate nanoparticles (H3C-SiO_x-R6G) could be determined, depending on the approach, to be about 1.3 – 1.7 by using the technique of recording and analysing fluorescence time traces of single nanoparticles via widefield microscopy. Measurements of the absorption and emission spectra, as well as the obtained fluorescence lifetime complete the investigation of Rhodamine 6G doped organosilicate nanoparticles.

2. Experimental Section

2.1. Materials

Polymethyl silsesquioxane (PMSSQ, $M_w = 10,000$; 14% hydroxyl groups) and Rhodamine 6G (R6G) are from Techneglas, Inc. (P/N GR650F) and Exciton, respectively. Polypropylene glycol (PPG, $M_n = 425$) is from Sigma-Aldrich. The doped organosilicate nanoparticles were prepared by dissolving PMSSQ and R6G in ethanol. At high temperature, the polymer becomes cross-linked and R6G is embedded in the nanoparticles. A detailed description of the nanoparticle preparation can be found in [5]. In brief, PMSSQ and R6G (with a mass ratio of about 62) were dissolved in ethanol. The organosilicate nanoparticles were prepared by a polymer collapse of the molecules (transition of the molecule chain from the swollen phase to a collapsed globule phase) initiated by the addition of a low molecular weight PPG ($M_n = 425$), which is a poor solvent for PMSSQ [6, 7]. After aging for 3 days in the dark at room temperature, the solution was spin-coated on hydrogen-passivated low-doped p-type Si substrates. The obtained films were immediately calcined (by placing on a hot plate at 250°C for 35 s) and allowed to cool down. Presently, we do not know if R6G is partly destroyed during the calcination. However, this will not influence the determination of the still active number of dyes per nanoparticle, but chemical stability is an important issue and will be investigated in a next series of experiments. For removal of free R6G the films were extensively washed with water. Particle suspensions were prepared from the nanoparticle films without light exposure. The films were scraped off the substrate with a scalpel blade, while immersed in propylene glycol monomethyl ether acetate (PMA). The nanoparticle flakes were sonicated in PMA at high power (sonic wand, 50 W for 10 min) to obtain isolated single nanospheres. The nanoparticle solution was further diluted using PMA as obtained from ABCR GmbH & Co. KG.

2.2. Ensemble Measurements

For the ensemble measurements, we prepared solutions of R6G and doped nanoparticles in PMA with concentrations of 20.5 $\mu\text{mol/l}$ and 0.1807 mg/ml, respectively. Absorption and emission spectra were recorded with a UV-Vis spectrometer Cary 100 and a fluorescence spectrometer Cary Eclipse (both from Varian), respectively. For the fluorescence lifetime measurement a pulsed NdYVO -laser (Vanguard 2000-HM532) from Spectra Physics was

used at a wavelength of 532 nm. The fluorescence signal was detected with a microchannel plate-photomultiplier tube from Hamamatsu (R3809-U51).

2.3. Single-particle Measurements

Single-particle studies were carried out using a home-built widefield microscope. We use the 476 nm line of an argon/krypton mixed-gas laser (Innova 70 C from Coherent) to excite the single particles via a 100-x objective (Zeiss, Neofluar, 0.9 NA). The emission of the single particles is collected by the same objective and focused onto a charge-coupled device (EMCCD, iXon DU 897, Andor Technology). For a more detailed description see [8].

To study fluorescence time traces, R6G doped nanoparticles were diluted in PMA and spin-coated at 3000 rpm onto Si wafers with a 100 nm thick oxide layer (ZfM, Chemnitz University of Technology). Prior to this, the substrates were carefully cleaned with piranha solution (mixture of sulfuric acid and hydrogen peroxide) to minimize the amount of contaminations. The concentration of nanoparticles has been adjusted to receive after spin-coating less than 25 nanoparticles in an area of $21\ \mu\text{m} \times 21\ \mu\text{m}$. Because of the diffraction limit, fluorescent particles appear as bright spots with diameters of several hundred nanometres in widefield images. Thus, neighbouring nanoparticles with a distance of several tens of nanometres cannot be detected separately. With the here used concentration the average distance between neighboured nanoparticles is about $4\ \mu\text{m}$. For this reason, the statistical probability that two nanoparticles appear as one is negligible. Fluorescence images were recorded with an optical array of $21\ \mu\text{m} \times 21\ \mu\text{m}$, which corresponds to an image size of 200 pixel $\times$ 200 pixel. The recorded time traces consist of 7200 frames at a frame rate of 20 fps. This corresponds to a bin time of 50 ms between succeeding frames including a read-out delay of 1.74 ms. The centre positions and the intensity of fluorescence spots in each frame above the background noise are identified automatically by a software package developed in our lab (for more details see [9]). In this way emitters with a fluorescence intensity below a certain threshold are omitted. In the next step, the intensities of spots with the same position in succeeding frames are linked to a fluorescence intensity time trace. The analysis of the time traces will be discussed in section 3.2.

3. Results and Discussion

The studied organosilicate nanoparticles have an average diameter of $(3.3 \pm 0.9)\ \text{nm}$ [5]. With this, the intermolecular distance between embedded dyes is smaller than the molecular Förster radius, which is 5 nm for R6G [10]. Hence, energy transfer between the dye molecules is possible and also a dimerization of the molecules might occur. Calculations of the absorption spectra using density-functional theory have shown [11] that the formation of dimers with parallel-aligned molecular axes (H-dimers) yields a splitting of the optical singlet transition. The dominant transition is blue shifted, while a smaller one is red-shifted with respect to the monomer transition. The calculations also predict a splitting for dimers with head-to-tail aligned dipole moments (J-dimers). In contrast to the former ones, one transition remains at the same position as the singlet transition of the monomer and the other one is red-shifted [11]. Previous studies have demonstrated that with increasing size of the formed aggregates the absorption peak is blue-shifted in the range of several tens of nanometres [12]. Consequently, the existence of monomers, dimers and higher aggregates in the studied solution would lead to the formation of strong side bands and/or additional transitions. Furthermore, in previous studies a red-shift of the emission was observed if the intermolecular distance reaches the Förster radius [10].

To make sure what kind of (aggregated) dyes exist in our samples, we measured the absorption and emission spectra, as well as the fluorescence lifetime of the nanoparticle-dye composites. Because of the fact that the nanoparticles are hydrophobic and dissolved in

PMA, which was not yet been reported for R6G, we carried out all R6G measurements in PMA. These enables a comparison between the R6G doped nanoparticles and free R6G in the same solvent.

3.1. Ensemble Properties

The normalized absorption and emission spectra of R6G are shown in Figure 1 with maxima at 529 nm and 553 nm, respectively. The measured maxima belong to the transition between the ground state S_0 and the excited singlet state S_1 . A solvent-shift to longer wavelengths is observed in comparison to R6G in aqueous solution [13].

The spectra of R6G embedded in the nanoparticles show no significant deviation from free R6G spectra. Furthermore, no side bands or additional transitions, typical for the formation of aggregates or resonant energy transfer, are observed. Only a slight blue-shift of 1 nm in the absorption and 2 nm in the emission spectra is detected for R6G embedded in the nanoparticles. This effect is assigned to the silicon oxide shell around the dyes, which changes the dielectric environment of R6G. Therefore, we conclude that aggregation of dyes in the nanoparticles can be excluded.

This assumption is supported by fluorescence lifetime measurements (see Figure 2). The recorded R6G fluorescence signals are fitted with a decay time of (3.5 ± 0.1) ns and (3.55 ± 0.03) ns for R6G doped into nanoparticles and for R6G, respectively. These value are slightly smaller than those reported for R6G [14, 15]. Penzkofer et al. [14] determined for low R6G concentrations in methanol a fluorescence lifetime of 3.9 ns. They observed a lifetime shortening accompanied with a decreasing quantum yield upon increasing R6G concentration. They attributed this behaviour to Förster energy transfer between monomers and weakly fluorescent stable dimers at high R6G concentrations. In the work of del Monte et al. [15] a lifetime of 3.9 ns is obtained for R6G in silica gel. In contrast to the study of Penzkofer et al., del Monte et al. noticed a rising of the lifetime for increasing R6G concentration. They interpreted their observation with the formation of fluorescent J-dimers in the silica gel. Inspecting the fluorescence decay of the doped nanoparticles more closely, reveals a slight deviation from a mono-exponential decay which can be approximated by 2 decay times of about 2.8 ns (with a fraction of 70%) and 3.6 ns (with a fraction of 30%). This finding points towards a heterogeneity of the hybrid material. The particles have an average diameter of 3.3 nm, which is in the range of typical Förster transfer radii. Therefore, energy transfer should be present among (nearly) equivalent R6G molecules within one nanoparticle, which will not result in considerable lifetime shortening. However, whether Förster type energy transfer takes place in the doped nanoparticles, can presently not be decided from the performed ensemble experiment, since we did not vary the concentration within the particles.

The fluorescence spectra of doped organosilicate nanoparticles and R6G can be used to calculate the average number of dye molecules n_{R6G} in one doped nanoparticle following the procedure reported by Cho et. al [16] according to

$$n_{R6G} = \frac{I_{NP}/C_{NP}}{I_{R6G}/C_{R6G}} \quad (1)$$

where I_{NP} and I_{R6G} are the measured fluorescence intensities of the doped nanoparticles and R6G solution, respectively [5]. C_{NP} and C_{R6G} are the numbers of nanoparticles per unit volume and the number of R6G per unit volume, respectively. Taking the average diameter of (3.3 ± 0.9) nm of the nanoparticles and the known density of PMSSQ (before the polymer collapse) of about 1.08 g/cm^3 [17], the number of particles present in the stock solution was calculated to be $9.25 \cdot 10^{14}/\text{ml}$. The measured fluorescence intensity of the solution is $(1.17$

$\pm 0.11) \cdot 10^7$ a.u. for the doped nanoparticles. The fluorescence of R6G solution was measured to be $(5.45 \pm 0.23) \cdot 10^6$ a.u. from a $0.36 \mu\text{g/ml}$ solution, which corresponds to $4.53 \cdot 10^{14}$ molecules/ml. Using equation 1, the brightness of a single doped nanoparticle was calculated to be equal to the brightness of (1.05 ± 0.10) dye molecule. From this ensemble experiment we conclude, that on average 1 R6G molecule is enclosed in one nanoparticle [5].

3.2. Fluorescence Time Traces of Single Nanoparticles

In ensemble measurements, an averaging over all particles yields mean values for the studied parameters though the conditions might be quite heterogeneous. Upon averaging all information related to spectroscopic differences between individual molecules or particles is lost. Since the distribution of parameters contains more information than the average value, single particle and/or molecule detection became more and more important in the last two decades [18, 19, 20]. One of the most interesting behaviour of single emitters like dye molecules is the so-called fluorescence intermittency or blinking. Although the emitters are continuously excited, in most cases a fluctuation between on- and off-intensities is observed [21, 22], and has also been reported for R6G [23]. Blinking might have different origins, such as (photophysical) bleaching, charging or changes in the environment [21, 22, 23]. Nevertheless, it is a clear indicator that we are dealing with a single quantum system.

The base for the analysis of dye molecules embedded in a nanoparticle is the following simplified model. Each dye molecule exhibits one on- and off-state. Since the excitation is constant during the total observation time, we assume that each dye molecule has a constant intensity of the respective on- and off-level, whereby the off-level is given by the background intensity. The fluorescence intensity of a single dye molecule depends on the orientation of its electronic transition dipole moment with respect to the direction of observation and (linearly) polarised laser excitation. We assume that the molecules are randomly orientated resulting in a broad distribution of intensities for the single R6G molecules with an upper intensity I_{max} at an optimal orientation. However, since we identify only those molecules above a certain threshold, we neglect in a first step weakly emitting molecules. However, as soon as we have identified a single R6G, we take all intensity levels including the background-level into account. Thus, we select only nanoparticles, which contain at least one strongly emitting dye molecule.

Applying this model to the doped nanoparticles, we expect for nanoparticles containing one active dye molecule one on- and one off-level and for nanoparticles with two enclosed dye molecules three on-levels and one off-level. Thereby, two (different) on-levels are assigned to a situation, where only one of the two dyes fluoresces. The related on-intensities vary due to their different orientations. The third on-level is realized when both molecules fluoresce. In that case the intensity is the sum of the intensities of the two distinct on-levels. If both dye molecules have the same intensity, we cannot discriminate which of the dye molecules fluoresces and only one intermediate on-level is observed. A second on-level with the doubled intensity is recorded when both molecules emit at the same time. With respect to the later discussion we like to point out that we cannot discriminate by merely counting intensity levels, whether a given level is due to one emitting R6G or is the sum of two R6G.

For the analysis of $\text{H3C-SiO}_x\text{-R6G}$, time traces of 214 particles were examined. In doing so, three types of particles can be qualitatively distinguished which differ in the number of on-intensity levels. Type A is related to time traces with only one on-level, type B and type C with two and three on-levels, respectively. Figure 3 illustrates examples of these three types and the corresponding intensity histograms. Due to a low statistical occurrence, the highest on-level for type C nanoparticles is not very pronounced. In all three examples, the background is marked and the on- and off-levels are labelled. Corresponding to this

classification 34.6 % of the observed nanoparticles can be assigned to type A, 55.6 % to type B and 9.8 % to type C.

Comparing the three examples with each other reveals that the off-(background)-level (0) can be related to an intensity of (4800 ± 500) a.u. for all nanoparticles. During single fluorescence time traces the intensity drops from the highest on-level to a lower one or the background-level, as well as it recovers from the background-level or a lower on-level to a higher one. Thereby the on-times, also in the highest on-level can last up tens of seconds, like in Figure 3 type C. Both for type B and C, the first on-level (1) can be related to an intensity of (6300 ± 750) a.u. and the second one (2) to an intensity of (8800 ± 500) a.u. Furthermore, for type C the third on-level (3) can be assigned to a value of (11500 ± 800) a.u. The intensity differences $(0 - 1)$ correspond to (1500 ± 1250) a.u. and $(1 - 2)$ to (2500 ± 1250) a.u.

For type A it is remarkable that the on-level distribution is related to an intensity level (with a value of (9300 ± 1300) a.u.), which is comparable to the intensity of the second on-level (2) of type B and C. There are two possibilities to explain this observation. Firstly, such a time trace will show up, if one molecule reaches due to its orientation the maximal possible fluorescence intensity. Secondly, two embedded dye molecules are (strongly) coupled as can be concluded from the unique blinking behaviour (which show only a few intermediate intensities). Thereby both molecules would change from a fluorescent on-state to a non-fluorescent dark-state and back. At first glance, the explanation of strongly coupled dyes disagrees with the results of the ensemble measurements. However, correlated blinking does not imply that the dyes are directly coupled via their transition-dipole moments. The emission of both dyes might for example be quenched simultaneously by charge generation in the nanoparticles as it has been observed for example in semiconductor nanocrystals [21, 22].

As already outlined before, the embedded dye molecules are considered to be randomly orientated in the nanoparticle. As a result, the orientation of the optical transition-dipole moment differs from one molecule to the other. We exclude that the distinct intensity levels are caused by fluctuations between different positions of the transition-dipole moment or essential modifications of the configuration of the dye itself, since this is improbable due the embedding of the dyes into silicon oxide. However, we cannot exclude changes of the photophysical properties of the dyes due to variations in the physical-chemical nanoenvironment of the molecules.

Figure 4 shows a common intensity histogram for all analysed time traces including all three types A, B and C. Some structure is obviously seen in the intensity histogram. This is caused by two reasons. The first one is given by the selection of molecules above a certain threshold, which sets a lowest intensity. The second one is related to the fact that we expect in the case of isotropic orientation and identical emission properties for each molecule one upper limit for the emission intensity (sharp cut-off). For nanoparticles with one embedded dye molecule the histogram has an intensity cut-off at the intensity I_{max} , while for nanoparticles with two molecules the cut-off is at $2 I_{max}$. Since minimal alterations of the emission parameter, for example variations of the nanoenvironment, lead to a shift of the maximal intensity this upper limit is not pronounced and smeared out. Both effects will result in a non-vanishing structure in the intensity histogram, which will qualitatively reflect the number of invoked dye molecules. The background and the first two on-levels are fitted with a Gaussian function. The derivation from the Gaussian function might indicate the presence of the expected intensity cut-offs for each realisation of n dyes per nanoparticle. Though it is somewhat beyond the level of experimental accuracy we tempt to assign the positions of the cut-offs to intensities of 7500 a.u. and 9500 a.u. (12500 a.u. and 14500 a.u.

for the data shown on the enlarged scale) corresponding to the expected upper intensity level of an "ensemble" of single molecules. Since the fraction of nanoparticles with on-intensities higher than 9300 a.u. is very small, a magnification of the high intensity range is shown in the inset. The additional maxima might belong to cases, where more than two R6G molecules are enclosed in one nanoparticle. However, the related occurrence is at least by one order of magnitude smaller than for the incorporation of 1 or 2 molecules. According to these results, we conclude from the summed-up single particle measurements by comparing the integrals of the two fitted Gaussian functions, that each nanoparticle is on average doped with 1.3 R6G molecules. This result is very close to the value calculated from the ensemble fluorescence spectra.

From a statistic point of view we expect a distribution of the number of dye molecules embedded in a nanoparticle. Single particle microscopy can only detect fluorescent particles. Consequently, it is not possible to identify the fraction of non-fluorescent and/or undoped nanoparticles. But this fraction is included in the calculation (see section 3.1) of the average number of enclosed dyes from the ensemble fluorescence spectra. Therefore, the calculated value is smaller than the one determined from the single particle measurements. According to a Poisson distribution, we will have to assign type A molecules to nanoparticles with only 1 dye molecule, type B to 2 doped molecules and type C to 2 or even 3. However, our results can only be in very rough agreement with a Poisson distribution since bleaching of dyes (during synthesis and experiment) will strongly influence the statistics. If we assume that all nanoparticles belonging to type A are doped with one dye molecule and if at least two dye molecules are embedded in type B and C nanoparticles, we obtain the average number of enclosed dye molecules to be 1.7.

The fact that we could not observe time traces with only one on-level for type A, B and C, which is e.g. comparable to the intensity $I = (6300 \pm 750)$ a.u. of the on-level (1) of type B and C particles, is an evidence for the presence of interactions between the embedded dyes. Namely, if only one dye molecule is embedded in type A particles, the recorded fluorescence intensity with $I = (9300 \pm 1300)$ a.u. corresponds to the maximum possible intensity, which is by about a factor of 1.5 larger as compared to the same situation in type B or C particles. For particles belonging to type B and C with at least two enclosed dye molecules, intermolecular interactions such as energy transfer (among nearly equivalent molecules) or annihilation processes open up new non-radiative recombination channels which lead to a decreased fluorescence quantum yield. This results in a reduced intensity for the first on-level (1) of those nanoparticles. Surprisingly, the ratio of the two decay times observed in ensemble fluorescence decay experiments is close to 1.3 which implies a reduction of the fluorescence intensity by this factor in case that a second R6G is close by. However, the fit of the luminescence decay in Figure 2 is not very reliable because of noise and limited dynamic range. For this reason further time resolved single molecule experiments in the nanosecond regime are necessary to explain the exact nature of the interaction (energy transfer, annihilation) among dye molecules in nanoparticles of the three different types A, B and C.

In addition we like to point out that the calculation of the number of R6G per nanoparticle from ensemble spectra was carried out with a density value for PMSSQ of about 1.08 g/cm^3 , which is correct for the swollen polymer phase in a good solvent. In such a case, the interaction between monomer and monomer is repulsive. In contrast to this, in a poor solvent, like PPG, the monomer-monomer interaction is attractive, which leads to a collapse of the polymer chain resulting in a globule phase [24]. Consequently, each single chain requires a smaller volume than a swollen one. Furthermore, during the preparation the single polymer chains were cross-linked. Therefore, it is reasonable that the density in the prepared nanoparticles differs from the density of PMSSQ (in a good solvent). With respect to the

numerical calculation, the density is a major factor, which reduces or increases the number of dye molecules per nanoparticle. For example, an increase of the density leads to an increase of dye molecules per nanoparticle, which would approach values from single particle measurements even more closely.

Since the distributions of the on- and off-times of R6G depend on the environment [23] we analysed the blinking dynamics of H3C-SiO_x-R6G in more details. On- and off-times are obtained from the individual time traces and depicted in Figure 5 as function of their occurrence. As it is typical for blinking dynamics the obtained distributions can be described with a power law in the form of

$$P_{on/off}(t_{on/off}) = B \cdot t^{\alpha_{on/off}} \quad (2)$$

over several orders in time and occurrence [21]. Both power law exponents are $\alpha_{on/off} = - (1.7 \pm 0.1)$. In previous studies in our group on single R6G spin-coated on Si wafers covered with 100 nm silicon oxide the exponents were determined to be $\alpha_{on/off} = -2.0$ [23]. In both studies the values for the on- and off- exponents are identical. In recent experiments we found that the power-law exponents depend on the environment [21, 22]. For example, an increase of the solvent polarity leads to a decrease of the off-exponent α_{off} [25]. From this comparison, we conclude that the environment of R6G in nanopores of H3C-SiO_x-R6G is more polar than on a silicon oxide layer.

4. Conclusion

In the present paper, we suggest that the number of enclosed dye molecules in doped nanoparticles can be determined by simply counting the on-intensity levels of individual fluorescence time traces. The number of R6G molecules embedded into organosilicate nanoparticles was determined to be on average 1.3 – 1.7 as compared to 1 from ensemble experiments. The fluorescence time traces can be separated into three classes depending on the characteristics of the on-intensity levels. Particles of type A contain probably one R6G, while type B and C show well defined intensity levels related to two R6G molecules with as compared to type A reduced fluorescence intensity. Type B corresponds to a situation where most of the time only one R6G is "on", while for type C mostly both R6G are "on". We cannot completely exclude following the single particle experiments that one and the same R6G molecule might have two distinctly different environments causing different quantum yields (and fluorescence intensities). Then fluctuation of the environment will also result in blinking. However, in that case we should have seen in bulk experiments two very different fluorescence decay rates related to the quantum yields differing by a factor of 2. Measurements of the absorption and fluorescence spectra as well as the fluorescence lifetime of R6G and H3C-SiO_x-R6G solutions reveal that aggregation of the enclosed dyes and/or Förster energy transfer to aggregates can be excluded. Both effects would lead to a large shift and/or splitting in the absorption spectra or to a modification of the lifetime compared with free dye molecules.

Highlights

Hybrid nanoparticles are groundbreaking to tailored fluorescent markers. We examine organosilicate nanoparticles doped with Rhodamine 6G on a single particle level. The individual fluorescence time traces can be classified according to the number of on-levels. From this classification we can determine the average number of enclosed dye molecules to about 1.3 – 1.7.

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Figure 1. Normalized absorption (unfilled symbols) and emission spectra (filled symbols) of R6G and R6G doped organosilicate nanoparticles. The excitation wavelength was 529 nm. The molecular structure of R6G is inserted.

Figure 2.

Fluorescence lifetime measurement of R6G (bottom) and R6G embedded in organosilicate nanoparticles (top) dissolved in PMA at room temperature. Excitation wavelength is 532 nm. A monoexponential fit yields for the doped nanoparticles a lifetime of $\tau = (3.5 \pm 0.1)$ ns and for R6G of (3.55 ± 0.03) ns.

Figure 3.

Examples of single fluorescence time traces (left) of H3C-SiOx-R6G separated into 3 typical classes and related probability distributions (right). Particles of type A show only one pronounced on-level. Particles belonging to type B and C have two or more on-levels. The hatched area indicates the background-intensity (noise).

Figure 4.

Intensity histogram for all 214 time traces of H3C-SiOx-R6G. The distributions of the background-level and the first two on-levels are fitted by Gaussian functions. The inset shows a magnification of the high intensity part of the histogram with a Gaussian fit to a third on-level.

Figure 5.

Occurrence of on- and off-times $t_{\text{on/off}}$ of H3C-SiOx-R6G. The data are fitted with a power law yielding power law exponents $\alpha_{\text{on/off}} = -1.7$.