Multimodal optical studies of single and clustered colloidal quantum dots for the long-term optical property evaluation of quantum dot-based molecular imaging phantoms

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Abstract: Understanding the optical properties of clustered quantum dots (QDs) is essential to the design of QD-based optical phantoms for molecular imaging. Single and clustered core/shell colloidal QDs of dimers, trimers, and tetramers are self-assembled, separated, and preferentially collected using electrospray differential mobility analysis (ES-DMA) with electrostatic deposition. Multimodal optical characterization and analysis of their dynamical photoluminescence (PL) properties enables the long-term evaluation of the physicochemical and optical properties of QDs in a single or a clustered state. A multimodal time-correlated spectroscopic confocal microscope capable of simultaneously measuring the time evolution of PL intensity fluctuation, PL lifetime, and emission spectra reveals the long-term dynamic optical properties of interacting QDs in individual dimeric clusters of QDs. This new method will benefit research into the quantitative interpretation of fluorescence intensity and lifetime results in QD-based molecular imaging techniques. The process of photooxidation leads to coupling of the QDs in a dimer, leading to unique optical properties when compared to an isolated QD. These results guide the design and evaluation of QD-based phantom materials for the validation of the PL measurements for quantitative molecular imaging of biological samples labeled with QD probes.

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1. Introduction

Colloidal nanoparticles (CNPs) are critical to commercial applications such as high efficiency solar cells, low-power solid state lighting, optoelectronic components for quantum computing, and high fidelity biomedical imaging [1–6]. CNPs and CNP-included hybrid materials are also interesting because they mimic molecular structures and interfaces allowing fundamental characteristics of atomistic systems to be reproduced and investigated [7–13]. For example, colloidal quantum dot (QD)-based solar cells have achieved increased efficiencies by generating multiple excitons (electron-hole pairs stimulated by photon absorption) within an individual dot [11]. QDs have also been employed in fluorescence imaging of cells and tissues and nanoscale biosensor applications as contrast-enhancing tags to image the distribution and the motion of molecular targets and as nanoscale sensors to probe local biochemical environments and detect biological targets [1,3,14–16].

The quantitative interpretation of photoluminescent (PL) properties of QDs in biomedical imaging is difficult because clusters composed of QDs in intimate contact have altered optical properties. Scaled-up manufacturing processes bring QDs into even closer proximity, substantially affecting their emission lifetime, emission spectrum, relative quantum efficiency, and the longevity of the bulk materials and devices they enable [17–20]. Yet, how clustering affects the optical properties of QDs including, for instance, the conversion and efficiency, and the longevity of the bulk materials and devices they enable [17–20]. Understanding the mechanisms that govern energy transport within clusters is essential to optimizing their structure and composition for eventual development of QD-included materials with long-term photostability. In an effort to enable the quantitative interpretation and calibration of PL intensities and lifetimes in these biological applications, optical phantom materials including colloidal QDs have been developed [22]. Furthermore, concerns linger regarding the long-term stability of QDs in physiologically relevant oxygen-rich environments characteristic of biological tissues. In these environments, photooxidation may lead to photodegradation, which will affect the energy transport of QDs and subsequently their emission properties including PL intensity, emission spectrum, and PL lifetime. Precise resolution of the detailed dynamics of the photodegradation mechanisms,
particularly for clustered QDs, is the key to engineering nanoassemblies of QDs with stable photophysical properties of QD-included materials [14,17,23–25]. Yet, how clustering affects photooxidation remains unknown. This article addresses these key knowledge gaps and measurement challenges.

An essential advance that enables this study is the ability to precisely tune the composition of small nanoparticle clusters, using a droplet induced clustering strategy developed by Pease, et al [26,27]. This highly tunable approach has not been used previously to generate nanoparticle clusters for optical/photonic characterization. As shown in a schematic of the experimental setup in Fig. 1, electrosprayed droplets confine a fixed number of QDs, which become a cluster in intimate contact as the droplet evaporates. The clusters pass through a bipolar neutralizer and differential mobility analyzer (DMA) that separate the particles based on their aerodynamic size [28–31]. Because a two-particle cluster (i.e. a dimer) of identical QDs has a different size than a three-particle cluster (i.e. a trimer) or a single particle (i.e. a monomer), the DMA’s subnanometer resolution allows for purification of clusters based on their size and agglomeration state. Clusters of a selected size are then deposited onto TEM grids or glass substrates for optical analysis. This is the only technique to date with the capability to precisely tune the composition of small clusters. The synthesis and stability of the clusters do not depend on chemical, biological (e.g. hybridized DNA or streptavidin-biotin), thermal or light sensitive linkers, which is critically important because the emission properties of QDs depend heavily upon their local environment.

In this study, we analyze these clusters of QDs with time-correlated spectroscopic confocal microscopy to enable multimodal optical characterization of fluorescence intermittency, spectral diffusion, and dynamic fluorescence lifetime [32] of individual and clustered QDs comprised of cadmium selenide (CdSe) cores and zinc sulfide (ZnS) shells functionalized by carboxyl (COOH) surface groups. This new approach opens an innovative opportunity to understand the excitonic charge transfer mechanism during photooxidation in single and clustered QDs. This fundamental study is critical to the design of QD-included optical phantom materials with tunable optical properties and long-term photostability.

2. Experimental section

2.1. Electrospray differential mobility analysis (ES-DMA)

A schematic of the ES-DMA setup is shown in Fig. 1, and the technique is described in detail elsewhere [26,27,31]. In brief, QD solutions containing individual COOH surface functionalized cadmium selenide (CdSe) core and zinc sulfide (ZnS) shell core-shell QDs (605 nm emission peak, Invitrogen, Carlsbad, CA) were electrosprayed using an electrospray

![Fig. 1. Setup to generate and isolate nanoparticle clusters of a specific number of particles per cluster.](image-url)
aerosol generator (TSI Inc., Shoreview, MN, #3480) through a nominally 25 μm inner diameter capillary with a tapered outlet. The stable cone-jet condition was obtained by varying the electrospray voltage from 0.2 kV to 3.6 kV and confirmed by monitoring both the current and visual appearance of the meniscus at the capillary tip. Electrospray sheath gas flow rates were 0.2 L/min of CO₂ and 1.0 L/min of air. Droplets containing the QDs were stabilized by passage through a bipolar charge neutralizer in which a Po-210 source reduced the charge on the droplet as described in detail by Wiedensohler using a modified Boltzmann distribution [33]. Droplets dried as they pass through the neutralizer chamber and approximately 1.0 m of plastic Tygon tubing (1.6 cm diameter) connecting the exit of the ES to the entrance of the DMA. The clusters form as the droplet dries. The DMA acted like a band-pass filter that for a given electrode voltage and gas flow rate enables a narrow size band of ions to be purified and exit for further analysis. The DMA operated with sheath gas flow rate of 30 L/min of nitrogen gas in an annular analysis chamber (TSI Inc., #3080). Electrostatic potentials as strong as −10 kV deflected positively charged particles toward collection slits at the distal end of the chamber. Because a negative bias was applied to aerosolized ions within the DMA, only nanoparticles that acquire a positive charge were detected (the fraction with positive charge is known and does not adversely bias the distribution). The monodispersed flow exiting the analysis chamber were either counted using a condensation particle counter or deposited on surfaces for additional analysis. For counting, the CPC (TSI Inc., #3025A) operated at 1.5 L/min with 1.0 L/min of flow exiting the DMA supplemented by 0.5 L/min of ambient air filtered through a HEPA filter. Within the CPC, particles passed through a saturated butanol environment and grew into droplets several microns in size, which can be counted individually as they obscure light impinging on a photodetector. The CPC reported the number of particles passing the detector per unit time after averaging for 20 s to minimize any transient responses. By varying the voltage in the DMA, nanoclusters in the range of interest in 0.2 nm increments were measured. Conversion to size was performed assuming the particles to be spheres with a Cunningham slip correction factor of \( C_c = 1 + Kn (\alpha + \beta \exp(\gamma/Kn)) \), where \( Kn = 2\lambda/d \), \( d \) is the particle’s diameter, \( \alpha = 1.257, \beta = 0.40, \gamma = 1.110 \), and the gas mean free path at room temperature is \( \lambda = 66 \) nm. The conversion from voltage to mobility size has been described in detail elsewhere [31]. The mean or number–average diameter is calculated as \( d = \Sigma d_i N_i / \Sigma N_i \), where \( N_i \) is the number of particles counted by the CPC of size \( d_i \).

2.2. Preparation of monomer, dimer, and trimer QDs on substrates for microscopy

The second part of this technique involved electrostatically depositing clusters of a selected size onto substrates. Monomer QDs and dimer and trimer QD clusters were identified in size distributions as described in Pease, et al [27]. Based on this analysis, the DMA voltage was set to that corresponding to peaks representing monomers, dimers, etc. They were then passed into an electrostatic deposition chamber (TSI, Inc., #3089) at a flow rate of 1.0 L/min-1.5 L/min under an electrostatic potential of −10 kV for a variable amount of time. The deposition time onto holey polymer-coated carbon TEM grids was selected to ensure that the product of the aerosol number density and time exceeded 3000 particle·hr/cc. For deposition onto cover slips pretreated in KOH and UVO-cleaner (Jelight Co.), this product appeared to be optimal at approximately 100 particle·hr/cm³.

2.3. Transmission electron microscopy

TEM samples were prepared as described above. The monomer, dimer, and trimer QDs were analyzed by bright-field transmission electron microscopy (TEM). The images were measured on a Philips EM 400T microscope operating at 120 KV equipped with a Soft Imaging System CD camera (Cantega 2K). More than 200 QD monomers or clusters were imaged.
2.4. Multimodal integrated confocal spectroscopic microscopy

Cover glasses supporting the monomer, dimer, and trimer QDs were placed on a holder attached to an XYZ closed-loop piezo stage (Mad City Labs). The sample was directly positioned above a high numerical aperture objective lens (Zeiss, 100X, 1.45 NA) mounted in an inverted fluorescence microscope (Zeiss Axiocover 135TV). A 471 nm pulse laser (Model Hamamatsu C 8898, operated with a 180 ps pulse width, 3 μW peak power, and 10 MHz repetition rate) was directed through a dichroic mirror (485 DRLP, Omega). The laser is then, coupled into the objective lens via the side port of the microscope, and focused onto a diffraction-limited spot with the objective lens. The emission signal reflected from the sample was collected with the same objective lens, filtered to remove residual laser excitation (LP02-488RS - Semrock), and detected by an avalanche photodiode detector (APD). After creating a fluorescence image by scanning a 10 μm × 10 μm area of the sample visualized in data acquisition software (RHK Technology), we focused the collimated laser onto a bright fluorescence spot in the image representing the isolated position of an individual QD or QD cluster. The reflected signal from the QD or QD cluster was simultaneously collected into a spectrometer (Princeton Instruments) and an avalanche photodiode (APD) through a 50/50 split mirror. The photon-counting pulses generated by the APD are fed to a photon-counting board of a RHK controller (RHK Technology) system for time-intermittency PL (or blinking) measurement. A single photon correlation spectrometer board (SPC830, Becker & Hickl GmbH) is used for lifetime measurement. A schematic of the instrument is presented in Fig. 2. To simultaneously acquire the blinking signal, the PL spectrum, and the lifetime data, we controlled the lifetime measurement system and the spectrometer with external pulses generated via a PCI LabVIEW board (National Instruments). The LabVIEW board generated two pulses every 2 s for 20 min: one pulse goes to the spectrometer and the other to the lifetime measurement system. The fluorescence spectra were acquired with an exposure time of 1 s and a delay time of 1 s, while continuously acquiring the blinking signals for 20 min. In addition, the lifetime data was simultaneously acquired using the external pulse with sync-in mode. Lifetime fitting, spectral analysis and display were performed in MATLAB (Mathworks, Natick, MA). Lifetimes were fit to both single and double exponentials and compared using least χ² fitting. Based on this χ² fitting comparison, the single exponential fits...
are justified. For the description of the lifetime trends presented here, the single exponential fitting proves to be sufficient.

3. Results and discussion

Figure 3 demonstrates the capability of ES-DMA to preferentially sort clusters containing small numbers of QDs, purify them based on their aerodynamic size, and place them on transmission electron microscope (TEM) substrates for further analysis of their structure and optical properties. To generate QD clusters, we use a technique invented by Pease [26]. This technique leverages the ability of electrospray droplets to encapsulate a small number of well dispersed QDs (typically 2 nm to 50 nm including shell and organic coating). As the droplets (120 nm to 250 nm in diameter) evaporate in a stream of nitrogen at atmospheric pressure, capillary forces drive the QDs to cluster. Balancing the QD solution concentration against the electrospray droplet volume tunes the number of QDs per cluster [26]. Increasing the QD solution concentration increases the fraction of larger clusters. Figure 3(e) shows a typical experimental size distribution. The accuracy of the ES-DMA size measurement falls between

Fig. 3. TEM images of QD clusters and their size distributions following DMA separation. TEM images of (a) monomeric, (b) dimeric, (c) trimeric, and (d) tetrameric QDs collected on TEM grids using the ES-DMA technique. (e) Distribution of single QDs and clustered QDs after ES-DMA assembly/screening with Gaussian fits for the distribution of each population. The averaged length and width of the each single QD including shell and functionalized coating is about 11 nm and 4 nm, respectively.
0.2 nm and 0.4 nm. This was estimated both from aerodynamic mobility theory [27,28,30] and experimentally from analysis of the size distributions of polymeric nanoparticles measured by a high resolution TEM [34]. Therefore, the width of the peaks in Fig. 3(e) represents primarily the heterogeneity of the QDs themselves. The clusters produced by evaporation possess a variety of sizes. We use DMA to separate clusters with the same number of QDs by selecting a very narrow size window (± 0.4 nm) in the mobility diameter distribution. The selected clusters may either be counted individually using a condensation particle counter (CPC) to generate the size distribution of Fig. 3(e) or electrostatically deposited onto a substrate. DMA was used to collect clusters of only a targeted number (1-4) of QDs on separate TEM grids. Figure 3(b) displays several dimeric QDs on a TEM grid. Likewise, Figs. 3(a)-3(d) displays typical TEM images of one, two, three, or four QDs produced by this technique. The distance between two QDs in the dimer QDs is estimated to be about 2 nm to 4 nm, which is due to the local thickness variation of functionalized coatings on the QDs [35].

Figure 4 displays typical time evolutions of the emission spectrum of a single QD, a dimeric cluster, and a trimeric cluster. These dynamic spectral diffusion patterns allow direct visualization of the number of QDs within a single cluster. A popular method to measure the number of QDs within a cluster is based on the analysis of a histogram of the occurrence versus PL intensities from the PL intermittency data, counting the number (n) of digitized intensity levels characterized by n QDs in on-states as an individual QD exhibits digitized blinking [20,36,37]. However, estimating the number of QDs in a cluster using this histogram-based method is often challenging because intensity peaks in the histogram broaden and decrease with photooxidation and because the on-state intensities of QDs in the cluster may be different. In our new approach, the number of QDs per cluster is directly measured. For single QDs, a single emission peak in each spectrum is measured at all times as Fig. 4(a) displays only one blue-shifted curve, indicating a single isolated QD undergoing photooxidation [23]. In contrast, two or three piecemeal curves, where each curve corresponds to a time vs. emission spectrum trajectory of a single QD in the cluster, indicate dimers or trimers, respectively. Dimers show a unique pattern in the spectral time-trace, where a blue down-shifting spectral time-trace appears to “branch” away from a steady, non-blueshifting PL spectral track (Fig. 4(b)). The blue-shifting PL spectrum is due to a faster blue-shifting QD, while the non-shifting one is from the other photostable QD in the dimer. In trimers, two blue down-shifted branches are observed (Fig. 4(c)), showing that photooxidation rates vary from QD to QD within the cluster.

The blue shift of the PL spectrum is reproducible in all the QD samples under the relatively high light energy density (≈3.1 x 10^−7 J/cm^2) in this study was estimated from a laser illumination with a ~1 ns pulse-width. This power density was used to induce the photooxidation of QDs in a few minutes under a continuous excitation, which would take much longer time period under a radiation power equivalent to the solar power density (2.5 x 10^−11 J/cm^2) at which the photostability of skin tissue phantoms would normally be tested. Remarkably, all QDs we observed did not photooxidize, as indicated by the spectral blue-shift, within the first 1 min of the continuous exposure time under this high power irradiation. Assuming a linear time-dependent PL response of the QDs under typical monochromatic exposure conditions with a power density equivalent to the solar power density, QDs would be photostable for up to seven days of continuous exposure. In addition, for phantoms with QDs included in a bulk material, oxygen diffusion into the bulk is reduced, further attenuating photooxidation, suggesting that included QDs would be photostable to PL measurement for long periods of time.

Figures 5(a)-5(c) display simultaneously measured multiple optical characteristics (PL intensity fluctuations, emission spectra, PL lifetimes) of a single QD isolated on a glass substrate after purification and deposition using ES-DMA. The emission intensity fluctuation during the early times exhibits the well-known quantized two-level (on and off) fluorescence.
Fig. 4. Spectral diffusion of single QDs in the cluster. Typical time evolution of the PL spectrum of (a) an isolated QD, (b) an isolated dimeric cluster, and (c) an isolated trimeric cluster. For clarity, the PL spectrum taken at each time point is normalized in order that all the maxima of all the spectra are of the same value.

Fig. 4 shows the spectral diffusion of single QDs in the cluster. The typical time evolution of the PL spectrum for (a) an isolated QD, (b) an isolated dimeric cluster, and (c) an isolated trimeric cluster is depicted. The PL spectrum at each time point is normalized to ensure that all the maxima of the spectra are of the same value.

Intermittency or “blinking” pattern of a single QD [38]. However, the on-time PL intensity gradually decreases as the PL spectrum blue-shifts due to photooxidation, resulting in the broadened histogram distribution for the on-time episodes over all three stages (Fig. 5(a)). Consequently, the initial bimodal distribution, where one peak arises from the background and the other from the emission from a single QD, in the intensity histograms smears out as photooxidation progresses. On the other hand, the solitary, non-branched spectral time-trace curve displayed in Fig. 5(b) provides direct evidence of the single QD. All single QDs, confirmed by the single time-traced spectral curve, share the following three distinct, dynamic stages (before, during, and after photooxidation denoted by i, ii, and iii, respectively in Fig. 5(a)) under continuous excitation with a confocal beam: (i) digitized intensity fluctuations with no noticeable change in the emission intensity, emission spectral peak position, and PL lifetime; (ii) gradually decreasing intensities indicative of photooxidation characteristics of blue-shifted PL spectra and decreasing PL lifetimes; and (iii) an irreversible dark state. Although some single QDs exhibit long-lasting PL emission, they all eventually undergo a blue-shift and irreversibly lose their PL.

In this report, regardless of blinking patterns, dimers consist of otherwise identical QDs where one QD exhibits a faster rate of blue-shifted PL than the other in the cluster. These dimers also exhibit a three-stage dynamic optical characteristic sequence, where each stage is uniquely defined as denoted by (i), (ii), and (iii) in Fig. 5(d): (i) initially, two independently
Fig. 5. Simultaneously measured multiple dynamic PL characteristics of a single QD and a dimeric cluster. (a) PL intensity fluctuations of the PL emission photon counts with a bin time of 10 ms. The signal is colored according to the fitted peak PL emission wavelength as denoted in the color bar, with black used for signals below the fitting threshold. A histogram of this intensity fluctuation is presented on the right side of the plot; (b) spectrum vs. time; (c) PL lifetime vs. time where the data points with arrows denote anomalous increased lifetimes. (d-f) Dynamic PL characteristics of a dimeric QD cluster comparable to results from the single QD (see panels a-c). The red dotted lines are visual guides for the time period of photooxidation.

blinking QDs show three digitized levels (both QDs in off-state, only one QD on, and both on); (ii) one QD exhibiting diminishing PL intensities and a blue-shifting spectral time-trace, while the other QD exhibits constant intensities and emission peaks during the 200 s – 300 s period shown in Fig. 5(d); and (iii) the blue-shifted PL from the blue-shifted QD irreversibly disappears, and the stable PL from the other QD remains for a long time, where this “residual” PL intermittency pattern broadens and slightly red shifts at the later times. These time-evolution characteristics of dimers are reproduced in all dimers we observed, although the blue-shift rates of QDs differ from cluster to cluster.

Further analysis of the simultaneously obtained set of data, including PL intermittencies, spectral time-traces, and PL lifetimes, provides insight and understanding into the mechanism of PL in single and dimeric QDs as photooxidation of QDs progresses. For the time period before photooxidation (section (i) in Fig. 5(a) and Fig. 5(d)), both single QDs and dimeric clusters exhibit positive correlation between the lifetime and the emission intensity or the quantum yield (QY) (i.e., the ratio of the radiative rate to the total, radiative plus nonradiative, rate) resulting in similar lifetime vs. intensity plots (see section (i) data in Figs. 6(a) and 6(c)). The similar positive correlation pattern for both dimers and single QDs, before photooxidation, implies the optical characteristics of QDs in the dimer before photooxidation...
are not significantly different from those of solitary QDs, suggesting that the PL of a dimer may be the linear superposition of PL from each non-interacting solitary QD. The positive correlation in the decrease of the excited state lifetime, with concomitant decrease in the PL intensity, is consistent with recent studies of the PL of single QDs, supporting the dominance of nonradiative decay channels [37,39]. Similarly, when the PL intensity increases, the PL lifetime also increases. These correlated characteristics have also been explained by the stabilization of trap states (i.e., increased PL lifetimes) involving radiative decay pathways, enhancing the probability of thermalization of trap-state exciton charge carriers back to the lowest emitting exciton state to increase QY [12]. This positive correlation observed before the photooxidation (i.e. blue-shift) of QDs suggests that the fluctuation of the nonradiative decay channels is not necessarily associated with the photooxidation process. Rather, they may be associated with external acceptor levels such as adsorbed molecules, dislocation, or defect traps on or near the surface of the QDs, which also may not directly involve the formation of CdSeO\(_x\) (\(x = 2, 3\)) at the core-shell interface, which would occur only during the photooxidation of a QD in the cluster.

During the photooxidation period (section (ii) in Fig. 5), the range of lifetime fluctuations of single QD emission is increased, weakening the positive correlation of PL intensities vs. lifetimes. Specifically, the red arrows in Fig. 5(c) denote increased lifetimes with no concomitant increase of corresponding PL intensities, resulting in data points above the standard deviation of the lifetime values (Fig. 6(a), scatter plot for section (ii)). Regardless of this weakened correlation, the overall trend of positive correlation is still noticeable in the

![Fig. 6. Quantitative analysis of the dynamic PL characteristics. (a) PL lifetimes vs. PL intensities and (b) the on-time probability plot analyzed from two different time periods of the single QD data set (section (i), and section (ii) shown in Fig. 5(a), corresponding to before and during the photooxidation period, respectively). (c, d) Same plots from three different time periods of the dimeric QD data set (sections (i), (ii), and (iii) in Fig. 5(d), corresponding to before, during, and after the photooxidation period, respectively). In panels (a) and (c), mean and standard deviation are displayed over scatter plots.](image)
single QD. On the other hand, a dimeric QD cluster does not exhibit positive correlation when the entire measurement time period is considered. Specifically, the lifetime vs. intensity scatter plots for sections (i), (ii), and (iii) in Fig. 6(c) demonstrate that the lifetimes of QDs decrease while QD$_1$ is photooxidized (period (ii)) but increase again after QD$_1$ is completely photobleached and loses its PL intensity (period (iii)). From (ii) to (iii), the average lifetime values substantially increase to the same or slightly higher level of period (i) although the average intensity remained same (compare scatter plots for section (i) in Fig. 6(c) and for section (iii) shown in the inset). This dynamic behavior of lifetime vs. intensity fluctuation in dimeric QD clusters may not be explained by a simple mechanism where fluctuating nonradiative decay rate is dominant.

In dimers, the PL episodes with both QDs emitting photons at the same time are rare for this time period (ii). Supporting this observation, Fig. 5(e) rarely shows PL episodes with two simultaneous PL spectra, implying that the underlying mechanism of the exciton decay kinetics during the photooxidation of one QD$_1$ in the dimer may be described by a transition from “decoupled” to “coupled” to “decoupled” states. Here, we denote QD$_1$ as the QD undergoing photooxidation (i.e. spectral blue-shift) at a faster photooxidation rate than QD$_2$, the other QD, whose emission peak position barely changes during the entire measurement time period. The two QDs in the dimer during this period are “coupled” so that the emission from one QD may be suppressed when the other QD emits photons, or excitons may be transferred from one to the other or the electron-accepting surface state so that the PL emission occurs preferentially in only one QD and is then “decoupled” after complete photooxidation of QD$_1$. Initially, as photooxidation of CdSe progresses, quasi-stable trap states at the interface may trap excitonic charge carriers allowing for interparticle or core-to-shell tunneling through the oxidized entities. Additional photooxidation of QD$_1$ further increases the interfacial tunneling barrier width to decrease the tunneling probability and eventually eliminates PL from QD$_1$ after irreversible photobleaching (period (iii)). To summarize this scenario, our results suggest that the “decoupled” dimer, before photooxidation, exhibits PL from each non-interacting single QD without charge transfer from one QD to the other; the “coupled” dimer, during photooxidation, allows for inter-particle or core-to-shell charge transfer by tunneling excitonic charge carriers, and the PL originates only from QD$_2$ after complete photooxidation of QD$_1$.

It is also noteworthy that, in our experiments, lifetimes shorter than a few nanoseconds were not observed, implying that photooxidation-induced surface states exist away from the core of the QD since PL involving near-core or core-confined excitons would result in much shorter lifetimes [12,23]. Accordingly, we believe that some episodes of photooxidation of QDs may induce radiative decay processes involving photooxidation-induced defects or electron-accepting surface state away from the QD core [38], which serve as trap states with extended lifetimes. For example, during the photooxidation period, some PL episodes are observed with substantially increased lifetimes despite decreased QYs (marked with red arrows in Fig. 5(c) and Fig. 5(f)). For radiative decay at a given temperature, the rate of exciton decay depends on the details of the band structure and crystal lattice, and the relatively long lifetime and lower QY are characteristic of an electron-accepting defect-related PL involving radiative decay.

The above scenario explains the low intensities and long lifetimes in the intensity vs. lifetime scatter plots for the time period when QD$_1$ undergoes photooxidation, during the period (ii). To explain this in a more quantitative way, we may need a “coupled” dimer model, depicting possible transition pathways involving multiple excitonic charge carriers in the dimer. This modeling is beyond the scope of this study. However, the following justifies the possibility of the multiexciton charge carrier generation or interparticle energy transfer in dimeric QD clusters.

In single QDs, photooxidation results in shortened on-time periods, supported by a substantial increase of exponent, $m$, $(1.33 \pm 0.05 \rightarrow 1.72 \pm 0.06)$ in the power-law time-
dependency form $P(\tau_{\text{on}}) \propto \tau_{\text{on}}^{-m}$ for the on-time probability distribution shown in Fig. 6(b). However, in dimers, when multiexciton generation or inter-particle energy transfer occurs, we would expect the on-time of PL intermittency to increase. Therefore we attempted to fit the probability distributions with the simple power-law function as was done for single QDs. In fitting the data with the power law, the simple power-law function which describes the PL of single QDs does not describe the on-time probability distribution of the dimer for the entire time range of the measurement. For the pre-photooxidation PL episodes, this power law still provides a reasonably good fit, consistent with our result from the positive correlation of lifetime vs. intensity, where two QDs in a dimer are not coupled and have no charge or energy transfer across the interparticle interface. However, during the time periods of photooxidation (period (ii)) and afterwards (period (iii)), noticeable downward curvatures in the on-time probability distribution are seen for all dimers measured, where a modified power law fitting function of the form, $P(\tau_{\text{on}}) = A\tau_{\text{on}}^{-m}\exp(-\tau_{\text{on}}/t_1/e)$, describes well the on-time probability distribution for longer on-time ($\tau_{\text{on}} \geq 200$ ms) PL episodes [36]. In recent studies, this exponential factor in the modified functional form is suggested to be the result of multiexciton creation or donor-acceptor energy transfer at the interface [36,40,41]. This modified fit measures, for episodes with short on-times ($\tau_{\text{on}} \leq 200$ ms), decreased ($m$) slope ($1.43 \pm 0.04 \rightarrow 1.24 \pm 0.02$, i.e. prolonged on-times) indicative of multiexciton or energy transferred PL (Fig. 5(e)). Further analysis for the post-photooxidation period provides an even smaller $m$ value ($0.94 \pm 0.02$) as shown in Fig. 6(d). Although multiexciton PL of QD$_2$ involving transferred charge from QD$_1$ is not possible, the multiexciton creation may be possible under the power level of our experiment, or further quenching of trap states by O$_2$ after photooxidation of QD$_1$ [23].

4. Conclusions

Dynamic PL properties of a single QD and a dimer of two QDs are compared after controlled assembly of dimers is achieved using the ES-DMA technique. TEM confirmed that the DMA technique enables the assembly of QDs in clusters with controlled number. Multimodal time-correlated hyperspectral confocal microscopy measured the time evolution of dynamic PL intermittencies, PL lifetimes, and spectral diffusion all at once to reveal multiexciton decay dynamics in PL of photooxidizing single and dimeric QDs. Our new technique reveals previously undescribed insight into the initial distribution of lifetimes of both species where the lifetimes significantly broaden as the QD undergoes a photooxidation process and exciton decay rates become quite dynamic during the photooxidation process. Episodes with prolonged lifetimes indicate that some PL kinetics are not necessarily associated not only with a conventional blinking model involving charging and discharging of the QD core but also with the creation of surface electron-accepting sites at the shell or the interface of QDs in a photooxidizing dimeric cluster. The approach demonstrated in this work opens new avenues to controllable fabrication and design of clustered QDs that may lead to a better understanding of the excitonic charge transfer mechanism during photooxidation in single and cluster.

These studies empower design of QD-included optical phantom materials with tunable optical properties and long-term photostability. For instance, clusters of QDs with controlled number of QDs may be collected and included in a matrix reagent to make phantoms including clusters of known number of QDs. As the local scattering and absorption properties of tissue phantoms are highly sensitive to the nanoscale size of clusters, it is crucial to assess the number of QDs in the cluster either individually or on average. The dynamic optical properties in a localized region of these phantoms may be compared with the results of this study to validate the number of QDs in clusters for specific matrix materials.

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