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Quantum Ensembles of Silicon Nanoparticles: Discrimination of Static and Dynamic Photoluminescence Quenching Processes

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Abstract

Porous silicon photoluminescence is characterized by a broad emission band that displays unusually long (tens to hundreds of micro-seconds), wavelength-dependent emissive lifetimes. The photoluminescence is associated with quantum confinement of excitons in silicon nanocrystallites contained within the porous matrix, and the broad emission spectrum derives from the wide distribution of nanocrystallite sizes in the material. The longer emissive lifetimes in the ensemble of quantum-confined emitters correspond to the larger nanocrystallites, with their longer wavelengths of emission. The quenching of this photoluminescence by aromatic, redox-active molecules aminochrome (AMC), dopamine, adrenochrome, sodium anthraquinone-2-sulfonate, benzyl viologen dichloride, methyl viologen dichloride hydrate, and ethyl viologen dibromide is studied, and dynamic and static quenching mechanisms are distinguished by the emission lifetime analysis. Because of the dependence of the emission lifetime on emission wavelength from the silicon nanocrystallite ensemble, a pronounced blue shift is observed in the steady-state emission spectrum upon exposure to dynamic-type quenchers. Conversely, static-type quenching systems show uniform quenching across all emission wavelengths. Thus, the difference between static and dynamic quenching mechanisms is readily distinguished by ratiometric photoluminescence spectroscopy. The application of this concept to imaging of AMC, the oxidized form of the neurotransmitter dopamine that is of interest for its role in neurodegenerative diseases, is

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.jpcc.9b04334](https://doi.org/10.1021/acs.jpcc.9b04334). Stern–Volmer analysis of additional quinone and viologen type quenchers (PDF)

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demonstrated. It is found that static electron acceptors result in no ratiometric contrast, while AMC shows a strong contrast, allowing ready visualization in a 2-D imaging experiment.

Graphical Abstract

INTRODUCTION

Luminescence quenching has been widely deployed for both practical and fundamental reasons: practical applications include sensing or detection of a particular analyte, and fundamental motivations generally involve providing insights into the structure and function of protein or cellular systems.¹ Quantum-confined semiconductors offer several advantages over fluorescent dyes when used as the luminescent species in a quenching experiment.² Most relevant to the present case, silicon quantum dots derive their photoluminescence from small (<5 nm), quantum-confined silicon nanocrystallites, typically protected by a thin oxide shell.^{3,4} Porous silicon nanoparticles (pSiNPs) are a unique subset of silicon quantum dots, where the ~3 nm quantum domains exist in a larger silicon framework, usually ~100 nm in size, such that each pSiNP contains an ensemble of a relatively large number of quantum dots. These quantum-confined silicon emitters display an emission lifetime that scales with emission energy,⁵ each displaying its own distinct emission energy and lifetime (Figure 1). The emission lifetime is on the order of many tens of microseconds,⁶ which extends the effective distance over which diffusion of a quencher can be monitored to dimensions comparable to the size of proteins and membranes.¹ In the pSiNPs used in this work, the emission lifetime ranges from 70 μs at $\lambda_{\text{em}} = 600$ nm to 180 μs at $\lambda_{\text{em}} = 900$ nm. Thus, each pSiNP can be considered to be a nanoscale probe of both static and dynamic quenching processes—because of the ensemble of emission lifetimes and emission energies contained within each 100 nm nanoparticle.

Luminescent porous silicon is sensitive to a host of molecular quencher species.⁷ Previous works have shown that the ensemble of emissive centers in photoluminescent porous Si is quenched when exposed to molecules that undergo either energy transfer^{8,9} or charge transfer^{10,11} mechanisms. In some cases, the material displays a shift in the wavelength of

the spectral maximum upon quenching,¹²⁻¹⁵ while in others quenching results in a uniform decrease in photoluminescence intensity,¹⁴⁻¹⁷ with no shift in the energy distribution of the spectrum. To date, there has been no satisfactory description or predictive model for these disparate spectral shifts. In this work, we find that the spectral shifts are a consequence of the trade-off between dynamic and static Stern–Volmer quenching. We adapt a model to understand the balance between these two quenching modes, and we validate the model using a variety of viologen and quinone molecules. We find that the dependence of emission lifetime on emission wavelength from the silicon nanocrystallite ensemble allows the separation of dynamic-type (collisional) from static-type (adsorbate-based) quenchers. Finally, we exploit this concept by distinguishing these two classes of quenchers using fluorescence microscope-based, ratiometric imaging of the steady-state emission spectrum from a single-particle probe.

EXPERIMENTAL SECTION

The two types of luminescent particles used in this study, porous Si nanoparticles (pSiNPs) and porous Si microparticles (pSiMPs), were prepared in a similar fashion from highly boron-doped ($\rho \approx 1 \text{ m}\Omega \text{ cm}$), p-type $\langle 100 \rangle$ silicon wafers (Virginia Semiconductors). The wafers were contacted on the back side with aluminum foil and mounted in a Teflon cell that exposed 8.6 cm^2 of the wafer. They were anodized in a two-electrode configuration using a platinum counter electrode at a constant current density of 50 mA/cm^2 in an electrolyte consisting of 3:1 (v/v) aqueous 49% HF/ethanol (**CAUTION:** HF is highly toxic and corrosive, and proper care should be exerted to avoid contact with skin, eyes, or lungs). This generated a porous silicon layer with a porosity of 49%, as measured by the spectroscopic liquid infiltration method.¹⁸ For luminescent nanoparticle (pSiNPs) preparations, this porous layer was removed from the bulk substrate using a constant electropolishing current density of 5 mA/cm^2 in a 1:13 (v/v) aqueous 49% HF/ethanol solution. Luminescence was then activated in the free-standing porous layer by immersion of 30 mg of the porous Si film for $\sim 10 \text{ h}$ in 10 mL of a pH 7.4 aqueous solution of $1\times$ Tris buffer (Fisher Scientific) containing ethanol (20% by volume). The resulting Si–SiO₂ core–shell film was washed with water and subjected to ultrasonic fracture in ethanol (50T ultrasonic bath, VWR International) for $\sim 10 \text{ h}$ to generate the pSiNPs of approximately 200 nm diameter. Nanoparticles were characterized for the hydrodynamic size and zeta potential by light scattering (ZetaSizer Nano, Malvern Instruments). The pSiMPs (microparticle) materials were prepared similarly, except that the porous Si films were subjected to an ultrasonic fracture for only 1 h, to yield particles of approximately $50 \mu\text{m}$ in diameter.

All steady-state luminescence measurements were performed using a TE-cooled CCD spectrometer (OceanOptics QE65 Pro). A 375 nm light source (Horiba DeltaDiode) was used for excitation, and pSiNP emission was passed through a 535 nm long pass optical filter prior to spectral acquisition. Time-resolved photoluminescence measurements were collected using an intensified charge-coupled device (iCCD) detector (iSTAR 334T, Andor Technology Ltd) fitted to a 0.275 m focal length monochromator (Acton Corp). The 375 nm light source was driven by a function generator (Keithley 3390) to output pulses of $500 \mu\text{s}$ duration with a 0.5% duty cycle. Photoluminescence measurements and Stern–Volmer titrations used 1.25 mL aliquots of photoluminescent pSiNPs (0.36 mg/mL) suspended in

deionized (18 M Ω) water in a standard UV-grade PMMA optical cuvette (Globe Scientific). Quenchers were added to the luminescent pSiNP suspensions dropwise (0.02 mL) under rapid stirring. Quencher molecules tested in this work include aminochrome (AMC), adrenochrome (ADC), sodium anthraquinone-2-sulfonate (AQS), benzyl viologen (BV) dichloride, methyl viologen (MV) dichloride hydrate, and ethyl viologen (EV) dibromide. All quenchers, with the exception of AMC, were purchased from Sigma-Aldrich and dissolved in deionized water for the Stern–Volmer quenching measurements. To generate AMC, a solution of 350 μ M dopamine hydrochloride (Sigma-Aldrich) was prepared in deionized water and bubbled with pure O₂ for 5 min before addition of 300 units/mL mushroom-derived polyphenol oxidase (Worthington Biochemicals). The enzyme solution was allowed to stand for 15 min. to generate AMC and then diluted with deionized water to the desired concentration prior to titration. Quantum yield measurements were performed using an integrating sphere (OptoSigma LabSphere) using the two-equation method described elsewhere.¹⁹

Photoluminescence images of the pSiMPs were obtained using a fluorescence microscope (Eclipse LV150, Nikon) equipped with a thermoelectrically cooled CCD camera (CoolSNAP HQ², Photometrics). Particles were excited using 360 nm light and an exposure time of 5 s and observed through bandpass filters (750 $\pm$ 40 or 850 $\pm$ 40 nm, as indicated). To obtain ratiometric images, the value of raw fluorescence intensity measured in the 750 nm channel was divided by the intensity in the 850 nm channel using the Image Calculator function in the ImageJ software package (NIH), with no additional modifications or processing.

RESULTS AND DISCUSSION

Preparation and Characterization of Luminescent Porous Si Micro- and Nanoparticles.

We first characterized the emission properties of the porous Si micro- and nanoparticles (pSiMPs and pSiNPs, respectively) used in this study. The particles were fabricated through a top-down process that involves the electrochemical etching of electronics grade p-type silicon wafers to generate the porous nanostructure, followed by ultrasonic fracture of the resulting porous layer to generate the particles. The microparticles had approximate dimensions of 50 μ m (by optical microscopy), and the nanoparticles displayed an average size (hydrodynamic diameter, by dynamic light scattering) of 236 nm with a polydispersity index of 0.206 (see Figure S1 of the Supporting Information). Porous silicon prepared in this fashion is generally not luminescent because of the low prevalence of quantum-confined silicon domains and the high prevalence of nonradiative surface traps. Therefore, luminescence was activated by treatment in an aqueous amine-based buffer solution, which resulted in a Si–SiO₂ core–shell type of the structure.²⁰ Particles prepared and activated using the above processing conditions were found to be sufficiently stable in the aqueous media and conditions used to perform the quenching experiments described in this work. Figure 2 shows scanning electron microscopy (SEM) images of a representative microparticle and the porous texture of the material both before and after oxidation. The aqueous treatment has two simultaneous outcomes: it reduces the size of many of the silicon features in the material to the quantum size regime, and it generates a passivating surface oxide coating, increasing efficiency of photoluminescence.^{20–22} As depicted in Figure 1, the

process resulted in a relatively broad size distribution of quantum-confined silicon domains contained within a single particle, which led to correspondingly broad emission spectra (Figure 3).^{23,24}

Although an open mesopore morphology was readily observed on the as-etched porous Si material (Figure 2b), plan-view SEM images obtained after the photoluminescence activation processing step revealed a smoother texture, with no obvious open mesopores (Figure 2c). As reported previously,²⁵ aqueous activation generally involves oxidation of Si to SiO₂ and then an equilibrium between SiO₂ and soluble silicates (the sol–gel reaction), under the appropriate conditions silicon dissolved during activation precipitates onto the surface and into the pores of porous Si.²⁵ In the present case, Fouriertransform infrared spectroscopy–attenuated total reflection confirmed the increase in silicon oxide content, as well as the presence of liquid water captured within the pores during the activation step (see Figure S2 of the Supporting Information).

The measured quantum yield for these particles was 18.6%. Each of the emissive quantum dots in the porous silicon ensemble is expected to display its own distinct emission energy and lifetime. The emission wavelengths spanned by the ensemble in the present work ranged from 550 to 900 nm; the useable range (where photoluminescence intensity was sufficiently intense for analysis) was 700–850 nm (Figure 3). Based on the literature measurements of single, isolated quantum dots, the characteristic feature size for the silicon lumophores in this ensemble likely spanned 3–6 nm;²⁶ this corresponds to crystallites containing from 700 to 6000 silicon atoms.

Because of the indirect band gap nature of silicon, luminescence originating from quantum-confined silicon displays a relatively long excited state lifetime.²⁷ Additionally, this lifetime depends on the size of the individual silicon nanocrystallite, resulting in wavelength-dependent lifetimes when an ensemble of Si nanocrystallites is measured as is the case here. In general, the time-resolved photoluminescence spectra from porous silicon do not follow monoexponential decays.²⁸ However, in the present work, the deviations from single-exponential behavior were not excessive (see Figure S3 of the Supporting Information), and so for purposes of comparison in this work, an effective lifetime (τ) was determined by dividing the measured half-life ($t_{1/2}$, time at which integrated intensity was half of the total decay integral) by $\ln(2)$. In the pSiNPs used in this work, the calculated emission lifetimes ranged from 70 μ s at $\lambda_{\text{em}} = 700$ nm to 180 μ s at $\lambda_{\text{em}} = 850$ nm (see Figure S3 of the Supporting Information). The emission lifetimes measured here are comparable to average lifetimes extracted from measurements on isolated, dodecene-capped silicon quantum dots (~ 30 μ s at $\lambda_{\text{em}} = 700$ nm, 200 μ s at $\lambda_{\text{em}} = 850$ nm).⁶

Photoluminescence Quenching of Porous Si Nanoparticles by Quinones.

We next studied the effect of quinone-type quencher molecules on the photoluminescence of the pSiNPs. Molecules that can participate in electron-transfer processes are of great interest to the biomedical community because of the wide range of biochemical pathways and diseases that involve redox-active species,^{29–32} and quinones are one member of this class that are prevalent in many biological systems. In particular, we were interested in the neurotransmitter dopamine and its oxidized form, AMC. When dopamine is oxidized to

AMC in vivo, some neurons cannot perform the two-electron reduction needed to regenerate dopamine, and instead, the molecule undergoes a one-electron reduction resulting in the cytotoxic radical semiquinone species.^{33,34} AMC has been investigated for its role in neurodegeneration and Parkinson's disease and illustrates the need for probes sensitive to redox-active biomolecules.³⁵⁻³⁷

As expected from previous work with organic quenchers of porous Si photoluminescence,^{8,24,38,39} AMC exhibited strong quenching of the photoluminescence from porous Si nanoparticles (Figure 3). A concern with quinone-like quenchers is the reversibility of the photoluminescence quenching process because quinones have been shown to be competent oxidizing agents for silicon and will attack bare or hydrogen-terminated Si surfaces irreversibly.⁴⁰ We hypothesized that the surface oxide generated in the preparation used in the present work would form a protective shell that would be sufficiently thick to protect the silicon nanocrystallites from chemical attack by the quinone, but be sufficiently thin to allow interfacial photo-induced electron transfer, as has been seen in other Si nanoparticle core-shell systems.^{41,42} To evaluate the reversibility of the process, we fabricated the luminescent Si ensemble following the same etching and activation process as used for the particles described above, but we omitted the electro-polishing/ultrasonic fracture steps that remove the porous Si layer from the bulk silicon substrate. This generated a photoluminescent porous layer that was still physically anchored to the silicon substrate, such that the ensemble could be readily added to and removed from the quinone solutions for characterization. The porous Si chip was then mounted in a flow cell, and emission spectra were recorded as the chip was cycled between pure deionized water and aqueous AMC (see Figure S4 of the Supporting Information). The experiment showed that the irreversible chemistry was indeed suppressed with the core-shell preparation; both the photoluminescence intensity and wavelength of the emission maximum could be recovered upon removal of the quencher. However, there was a gradual decrease in both the peak emission wavelength and the total photoluminescence intensity upon additional cycling between the AMC and pure solvent. This finding suggests that the quenching behavior was primarily caused by a reversible interaction of the electron acceptor with the emitter ensemble, although some loss in signal due to irreversible degradation of the interface also occurred upon repeated dosing.

Next, we evaluated the quenching of the steady-state photoluminescence from pSiNPs dispersed in water by AMC. Exposure to AMC generated a strong quenching of the steady-state photoluminescence from the pSiNPs, and it followed the steady-state Stern-Volmer relationship

$$\frac{I_0}{I} = K_{SV}[Q] + 1 \quad (1)$$

where I_0 represents the intensity of photoluminescence measured in the absence of quencher, I is the intensity of photoluminescence measured in the presence of a quencher of concentration $[Q]$, and K_{SV} is the Stern-Volmer constant, which can also be represented as K_D or K_S for dynamic or static quenching events, respectively. The values of the Stern-Volmer constant measured for AMC quenching ranged from 10^5 to 10^6 M^{-1} , depending on

the emission wavelength at which the Stern–Volmer constant was measured (Figure 3). This quenching efficiency, as measured by the Stern–Volmer constant, increased with increasing pSiNP emission wave-length, such that the family of emission spectra taken at various concentrations of AMC appeared to blue shift with increasing quencher concentration. Two other quinone-based electron acceptors, ADC and AQS, were tested, and they displayed similar behavior (see Figure S5 of the Supporting Information). In small molecule fluorophores, this type of energy dependence has been attributed to the activation of a new emissive pathway.^{43,44} However, in the case of pSiNP systems, we found that the observed results can be interpreted as arising from the ensemble nature of the emissive centers.

The steady-state Stern–Volmer constant, K_D , for quenching of photoluminescence from pSiNPs by AMC was quantified as a function of emission wavelength from the pSiNPs, Figure 4. The data of Figure 4 are presented as a function of emission lifetime for the pSiNPs measured in the absence of quencher, which corresponds to the specific emission wavelengths indicated (see Figure S3 of the Supporting Information). As can be seen in these data, the efficiency of quenching increased with the increasing emission lifetime; the longer-lived portion of the ensemble (corresponding to the longer wavelengths of emission and the larger nanoparticles in the ensemble) was more efficiently quenched relative to the shorter-lived portion. The measured K_D values ranged from $5.5 \times 10^5 \text{ M}^{-1}$ at 700 nm to $3.1 \times 10^6 \text{ M}^{-1}$ at 850 nm.

Time-resolved measurements indicated that the quenching mechanism for AMC followed a dynamic or collisional quenching model with pSiNPs.^{1,24,45} Effective emission lifetime measurements on the pSiNPs (τ , as described above) made while titrating AMC into a pSiNP solution showed a general decrease in τ with increasing [AMC] (Figure 5), and the steady-state (I_0/I , Figure 3) and lifetime-based (τ_0/τ , Figure 5) Stern–Volmer plots were similar. Steady-state and time-resolved quenching constants of the two related quinones, ADC and AQS, were measured under the same conditions and found to be similar to AMC (see Figure S5 of the Supporting Information and Table 1). These results are consistent with prior photoluminescence quenching studies performed by Ceroni and co-workers using luminescent silicon nanoparticles that were prepared by a different, bottom-up type of synthesis. Those prior studies were performed in the toluene solvent rather than water, but they also observed a dynamic quenching mechanism for a range of quinone molecules.⁴¹

Both static and dynamic photoluminescence quenching mechanisms require molecular proximity between the emissive species and quencher.¹ In the case of dynamic quenching, often referred to as collisional quenching, the quencher must diffuse to the emitter during the lifetime of the excited state. Therefore, observation of collisional quenching requires the correct combination of the emission lifetime, quenching constant, molecular diffusion coefficient, and quencher concentration. Static quenching usually involves binding of the luminescent species to its quencher prior to excitation, resulting in a dark, nonluminescent state. The two mechanisms are discriminated by luminescence lifetime measurements as a function of the quencher concentration: static quenching displays a constant luminescence lifetime (corresponding to the lifetime of the remaining unquenched luminescent species), whereas with dynamic quenching, the lifetime is inversely proportional to the quencher concentration. This proportionality arises from the diffusional component of the quenching

process. In the present case, the observation that AMC, ADC, and AQS decreased the emission lifetime of the pSiNPs at all wavelengths measured is consistent with a collisional quenching mechanism. As in Ceroni's studies of quenching of luminescent silicon nanoparticles by quinones, we interpret the quenching of pSiNPs by AMC, ADC, and AQS to proceed via an electron-transfer pathway.⁴¹

Photoluminescence Quenching of Porous Si Nanoparticles by Viologens.

We next tested the quenching behavior of benzyl viologen (BV) toward pSiNPs. BV is readily reduced at silicon electrodes,^{46,47} and BV and AMC have similar reduction potentials (AMC = −350 mV vs NHE, BV = −359 mV vs NHE).^{48,49} Thus, we assume that BV also undergoes an electron-transfer quenching pathway with pSiNPs. Both BV and AMC do not display significant overlap between their absorption spectra and the emission spectrum of pSiNPs (see Figure S6 of the Supporting Information) and therefore, energy transfer is not a likely pathway for either of these molecules. The measured steady-state K_S value at $\lambda = 800$ nm emission for BV was $2.78 \pm 0.56 \times 10^6 \text{ M}^{-1}$ (Figure 6), somewhat larger than the corresponding values for AMC, indicating that BV is a more efficient quencher of photoluminescence from pSiNPs.

In contrast to the behavior of AMC, the benzyl viologen (BV) molecule exhibited no wavelength dependence in its quenching of photoluminescence from pSiNPs, at least for I_0/I values of between 1 and 2 (Figure 6). Consequently there was no observed change in the emission lifetime of pSiNPs upon exposure to this range of quencher concentrations (Figure 5). Similar behavior was seen for the related molecules methyl viologen and ethyl viologen (see Figure S7 of the Supporting Information), and this is consistent with a static Stern–Volmer quenching mechanism. At higher concentrations of BV, however, a blue shift was observed, consistent with the onset of a dynamic quenching component (Figure 7a). The measured Stern–Volmer quenching constant thus increased substantially at higher BV concentrations, and this increase was strongly dependent on pSiNP emission wavelength (Figure 7b). By contrast, at lower BV concentrations, the emission spectrum displayed a decrease in intensity that was independent of the emission wavelength. The optical absorbance of BV, measured at $\lambda = 375$ nm, was undetectable at the highest concentration used in this work (690 nM). Thus, spectral absorbance of the quencher is not a complicating factor in the analysis. We conclude that the quenching mechanism for BV transitions from primarily static at low BV concentrations to primarily dynamic at higher BV concentrations.

Static Versus Dynamic Photoluminescence Quenching.

The observed mechanistic differences in Stern–Volmer quenching behavior between the viologens and quinones can be attributed to differences in their adsorption affinity for the pSiNP surface. The viologens in this work are cationic species, whereas AMC and the other quinones are neutral or negatively charged molecules in the pH neutral aqueous solutions used in this investigation. The pSiNP preparation consisted of a silicon/silicon oxide core/shell structure, and the oxide of this shell displayed a negative surface charge in water, with a measured zeta potential of approximately −21 mV. Because of their positive charge, the viologens can be expected to strongly adsorb to the pSiNP surface via electrostatic interactions.

A static adsorption process is expected to follow the Stern–Volmer relationship of eq 1, where K_{SV} can be considered to be equal to K_S , the static quenching constant (or association constant) of the quencher with the nanoparticle surface. Similarly, for a dynamic (collisional) quenching process, K_{SV} can be considered to be equal to K_D , the dynamic quenching constant. These two expressions have been combined to account for a system where both static and dynamic photoluminescence quenching mechanisms operate in parallel and independent of each other, leading to a quadratic dependence on the quencher concentration¹

$$\frac{I_0}{I} = (K_D[Q] + 1)(K_S[Q] + 1) \quad (2)$$

When applied to the pSiNP quenching data, this model yielded imaginary values for both K_S and K_D , indicating that this is an inaccurate representation of the system. Lee et al.⁵⁰ encountered a similar problem with photoluminescence quenching in porous metal–organic frameworks, and these researchers developed a mathematical model based on the binding of multiple copies of a quencher to a single porous particle⁵⁰

$$\frac{I_0}{I} = \frac{(1 + K_S[Q]^n)(1 + K_D[Q])}{1 + (1 - f)K_S[Q]^n} \quad (3)$$

where n represents adsorption strength of the various lumophore sites within the porous nanoparticle and f represents the fraction of lumophores available for quenching. Using this model, self-consistent K_S and K_D values could be obtained for the present BV-based quenching systems; they are summarized in Table 2. All parameters were fit using a least squares model employing a trust-region algorithm, and all coefficient values were obtained using 95% confidence bounds.

The fits to the BV titration data using the above model revealed a few interesting features. First, all f values were found to be 1, indicating that there was no population of lumophores that was inaccessible to the BV quencher. Second, the K_S values determined for BV in the concentration regime where moderate quenching was observed (I_0/I values less than 2) are in good agreement with the linear Stern–Volmer model (Table 1). Additionally, meaningful K_D values were only observed for BV concentrations greater than ~250 nM. These results can be interpreted in terms of a model in which the surface becomes increasingly occupied with tightly bound, cationic BV (corresponding to a static quenching regime) until at some point the surface coverage is sufficient that a collisional (dynamic) quenching process begins to dominate. This is supported by the relatively smaller K_S and larger K_D values measured at the higher BV concentration range (for measurements made at 22 °C, Table 2). It should be noted that a standard Stern–Volmer model assumes constant values for K_S and K_D . To further validate this finding, BV titrations were performed at various temperatures. As previously established in other emission quenching systems,^{51,52} it is expected that at low temperatures (4 °C), surface interactions are more strongly favored, creating a bias toward the static quenching mechanism. Conversely, collisional events are more favored at elevated temperatures (50 °C), thus biasing the system toward dynamic quenching. The present work

is consistent with this behavior; a decrease in efficiency of static quenching (K_S and n , the two parameters which relate to quencher binding) and increase in efficiency of dynamic quenching (K_D) were observed when transitioning from 4 °C to room temperature (Table 2, Figures 8a and S8 of the Supporting Information). On the other hand, at the higher temperature, in which BV is expected to become more dissociated from the surface, there was a dramatic decrease in static quenching efficiency and distinct wavelength dependence of quenching was observed for all quencher concentrations tested (Figure 8b). It should be noted that while the model of eq 3 yielded a closer fit than eq 2, it is still an incomplete model, as the fit lines underestimated the curvature of the Stern–Volmer plots for high [BV] values (Figure 7).

Experiments were performed to investigate the effect of ionic strength on the quenching process. Increasing ionic strength tends to suppress dynamic quenching rate constants because of the screening effect of the inert ions separating the emitter–quencher pair in the solvent medium.⁵³ We performed analogous Stern–Volmer quenching experiments with BV, but in a solution of phosphate-buffered saline (PBS, pH = 7.4) rather than pure deionized water. We found that the presence of the buffer ions changed the behavior of the Stern–Volmer quenching response such that BV showed static quenching behavior over the entire concentration range studied; very little shift in the wavelength maximum of emission was observed, even for high concentrations of BV (Figure S9 of the Supporting Information). Thus, the presence of spectator ions in the solution retained the static quenching modality while suppressing dynamic quenching in the system.

Interestingly, Ceroni et al. recently investigated the electron-transfer reaction between quinones and luminescent silicon nanocrystals (SiNCs) and did not observe a blue shift upon quenching.⁴¹ This is in agreement with the work of Veinot et al., who also saw no blue shift during the dynamic quenching of SiNCs by quenchers.⁵⁴ We can attribute this difference in quenching behavior to differences between the two nanoparticle types—in the work of Ceroni and Veinot, isolated SiNCs with hydrophobic capping species were used. In the present case, the pSiNPs contained a negatively charged surface oxide, an ensemble of emitters in a single nanoparticle, and nanoscale voids on the order of the molecular dimensions of the quencher molecules.

Ratiometric Imaging To Distinguish Static Versus Dynamic Quenchers.

The wavelength dependence of steady-state photoluminescence quenching of pSiNPs presents the possibility for these nanoparticle ensembles to be used in a unique type of ratiometric sensing strategy, to differentiate static from dynamic quenchers for a set of related molecules of analytical interest. The separation of static and dynamic luminescence quenching mechanisms has important practical applications in the fields of DNA^{55–57} and protein analysis.^{58–60}

Ratiometric strategies^{61,62} are often deployed to overcome some of the inherent shortcomings of fluorescence imaging and sensing associated with angle, coherence, or scattering environment.^{63,64} A ratiometric approach typically involves sampling two or more discrete emission wavelengths and dividing one by the other. This strategy can remove instrumentation-based factors while providing useful information on the local chemical

environment being sampled by the fluorescent probe.⁶⁵ One drawback of the ratiometric approach is that it requires the probe to possess two or more spectral emission features that interact differently with their chemical environment. This is commonly achieved by covalently linking two small molecule organic dyes.^{66,67} Nanoparticle-based systems have also been used for ratiometric sensing following similar strategies, as many types of nanoparticles are readily modified to incorporate elements of a ratiometric emission scheme.⁶⁸ A typical application involves conjugation of an organic dye molecule to an emissive semiconductor quantum dot.⁶⁹ The two distinct emissions are influenced separately by the analyte, enabling the ratiometric measurement. Researchers have shown that a heterogeneous mixture of quantum dots of different sizes (and hence different spectral characteristics) can be used for ratiometric sensing,^{70,71} although for many applications the nonuniform size distribution substantially complicates the interpretation of such data. Of particular interest to this work is the detection of dopamine oxidation using two colors of quantum dots performed by Su et al.⁷² Quantum dot systems based on toxic heavy metals such as cadmium or lead pose challenges for translation of ratiometric sensing systems.^{73,74} The use of nontoxic^{75,76} silicon nanostructures as part of a ratiometric sensing probe has recently been demonstrated, in which emission from quantum-confined silicon and a fluorescent molecule were used; the emission ratio was mediated by energy transfer between the two.⁷⁷ Here, we capitalized on the insights gained from the present ensemble quenching experiments to design and demonstrate a ratiometric nanoparticle-based sensor for redox-active molecules that discriminates static from dynamic quenchers as a class, using a simple steady-state fluorescence microscope.

To better resolve single particles, we prepared luminescent porous Si in the form of larger microparticles (pSiMPs). The particles were suspended in a Petri dish in pure water and imaged in the $\lambda = 750$ nm and the $\lambda = 850$ nm channels of the fluorescence microscope, using a fixed excitation wavelength of 360 nm. Under unquenched conditions, the relative emission from pSiMPs in these two channels was approximately equal. Thus, when the image obtained in the $\lambda = 750$ nm channel was divided by the image from the 850 nm channel, the pixel value across the image was approximately unity, matching the background signal (Figure 9, “blank”). When the dynamic quencher AMC was added to the solution, the emission in the $\lambda = 850$ nm channel was significantly quenched relative to the $\lambda = 750$ nm channel, and high contrast was observed in the ratiometric image (Figure 9). As discussed above, benzyl viologen behaves as a static quencher at low concentrations, and when this quencher was added, it resulted in minimal contrast in the ratiometric image. A threshold ratio of 1.2 was selected to minimize background signal stemming from the slight variation in filter bandwidths and transmission rates. An important result from this experiment is that it shows a convenient means to distinguish static from dynamic quenchers using a single porous silicon particle.

CONCLUSIONS

This work identified a key feature of porous silicon nano- and microparticles that can be used to discriminate static from dynamic photoluminescence quenching processes. Because of the array of emissive centers in a single luminescent porous Si particle, each with a wavelength-dependent excited-state lifetime, the steady-state spectrum from a single porous

Si particle probe can identify the quenching process. Photoluminescence quenching follows a model in which static adsorption events dominate until the surface becomes sufficiently cationic, at which point collisional quenching occurs. The hallmark of a dynamic (collisional) quencher in these porous SiNC ensembles is a pronounced blue shift in the steady-state spectrum, whereas a static quencher yields a uniform decrease in intensity of photoluminescence that is independent of the emission wavelength. We found this blue shift sufficient to generate contrast using simple steady-state fluorescence microscopy, which allowed the discrimination of benzyl viologen from AMC, two molecules with very similar reduction potentials but different affinities for the silicon oxide surface due to their different charge states. For analytes that quench pSi photoluminescence via a static quenching mechanism, it was shown that contrast is only generated once the surface occupancy increases to the point that collisional quenching behavior occurs. Using the above insights, we demonstrated a single-particle, single component ratiometric sensor using luminescent porous silicon. Although the static quenching mechanism was activated in the present case via electrostatic interactions between the porous Si surface and the positively charged quencher, the concept should also apply for systems where a specific capture probe is introduced at the silicon surface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

NP	nanoparticle
AMC	aminochrome
ADC	adrenochrome
AQS	anthraquinone-2-sulfonate
BV	benzyl viologen
MV	methyl viologen
EV	ethyl viologen

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Figure 1.

Schematic depicting the ensemble of quantum-confined photoluminescent centers that comprise a porous Si particle. The smaller features are blue-shifted relative to the larger features. The photoluminescence spectrum represents the sum of emission from all of the nanocrystallites in the ensemble; the illustration gives three representative sizes. The characteristic emission wavelength (λ) and emission lifetime (τ) for a given feature size (d) from ref 6.

Figure 2.

Scanning electron micrographs of (a) representative porous silicon particle, after activation of photoluminescence by oxidation in tris buffer. (b) Surface of a porous silicon film prior to formation of nanoparticles or oxidation, revealing the porous nanostructure; (c) surface of a porous silicon film after activation of photoluminescence. All images collected at 500 000 $\times$ magnification. Scale bar represents 200 nm.

Figure 3.

AMC quenching of photoluminescence from pSiNPs. (a) Steady-state photoluminescence spectra of an aqueous dispersion of pSiNPs upon sequential addition of small aliquots of AMC (molecular structure is shown in the inset). (b) Stern–Volmer plots of the data from (a), plotted as intensity ratio I_0/I vs [AMC], measured at the indicated emission wavelengths. Points represent the average of three trials; error bars represent one standard deviation.

Figure 4. Steady-state Stern–Volmer dynamic quenching constant (K_D) as a function of (unquenched) excited-state lifetime measured at the wavelengths indicated. Points represent an average of $n = 3$ trials and error bars represent one standard deviation.

Figure 5.

Stern–Volmer lifetime plots for pSiNPs exposed to increasing concentrations of AMC (top) and benzyl viologen (bottom), measured at the indicated emission wavelengths. Data are presented as τ_0/τ , where τ_0 is the effective luminescence lifetime measured in the absence of a quencher. Points represent the average value of three trials, with error bars representing one standard deviation.

Figure 6.

Quenching of photoluminescence from pSiNPs by benzyl viologen (BV). (a) Steady-state photoluminescence spectra of an aqueous dispersion of pSiNPs upon sequential addition of small aliquots of BV (molecular structure is shown in the inset). (b) Stern–Volmer plots of the data from (a), plotted as intensity ratio I_0/I vs [BV], measured at the indicated emission wavelengths. The steady-state Stern–Volmer quenching constant (K_S) is $2.78 \pm 0.56 \times 10^6 \text{ M}^{-1}$ at 800 nm. Points represent the average of three trials; error bars represent one standard deviation.

Figure 7.

Quenching of photoluminescence from pSiNPs by benzyl viologen (BV), measured over a larger concentration range than in Figure 6. (a) Steady-state photoluminescence spectra of an aqueous dispersion of pSiNPs upon sequential addition of aliquots of BV (molecular structure shown in the inset). (b) Stern–Volmer plots of the data from (a), plotted as the intensity ratio I_0/I vs [BV], measured at the indicated emission wavelengths. Points represent the average of three trials; error bars represent one standard deviation. Dashed lines represent functions generated by eq 3, using parameters outlined in Table 2.

Figure 8.

Stern–Volmer plots recorded at (a) $T = 4\text{ }^{\circ}\text{C}$ and (b) $T = 50\text{ }^{\circ}\text{C}$, plotted as intensity ratio I_0/I vs [BV], measured at the indicated emission wavelengths. Points represent the average of three trials; error bars represent one standard deviation. Dashed lines represent fit lines from eq 3, using parameters outlined in Table 2.

Figure 9.

Fluorescence micrographs of luminescent porous Si microparticles in the presence of pure water (top, control), 0.22 μM benzyl viologen (middle), and 4 μM AMC (bottom). Scale bar represents 50 μm . Fluorescence images were collected using 360 nm excitation with bandpass filters (THORLabs FB750-40 and FB850-40) to observe emission at 750 and 850 nm. The final column represents the signal from the 750 nm channel divided by the 850 nm channel, with a calibration bar ranging from a pixel value of 1.3 (black) to 1.8 (white).

Table 1.Quenchers and Their Corresponding Stern–Volmer Constants (K_{SV})^a

	K_{SV} (750 nm) ($M^{-1} \times 10^5$)	K_{SV} (800 nm) ($M^{-1} \times 10^5$)	K_{SV} (850 nm) ($M^{-1} \times 10^5$)	mechanism (K_D or K_S)
AMC	1.16	2.02	4.04	dynamic
ADC	1.33	1.78	2.60	dynamic
AQS	0.0359	0.0601	0.0921	dynamic
BV	26.8	27.8	28.7	static
MV	193	234	278	static
EV	905	971	1040	static

^aCalculated at three unique luminescent pSiNP emission wavelengths. The proposed mechanism of quenching in the case of relatively low quencher concentrations (I_0/I values between 1 and 2) is given. All K_{SV} are reported in units of M^{-1} and represent average values from $n = 3$ trials. All linear Stern–Volmer fits (eqn (1)) have R^2 values > 0.99.

Table 2.

Summary of Stern–Volmer Constants and Fitting Parameters for Benzyl Viologen Titrations on pSiNPs

conditions	K_S (M^{-1}) $\times 10^5$	K_D (M^{-1}) $\times 10^5$	f	n	R^2
BV, $I_0/I < 2$ (22 °C)^a					
$\lambda_{em} = 750$ nm	18.3	0.000987	1	0.9757	0.996
$\lambda_{em} = 800$ nm	22.0	0.000994	1	0.9859	0.996
$\lambda_{em} = 850$ nm	19.4	0.00100	1	0.9764	0.994
BV, $I_0/I > 2$ (22 °C)^a					
$\lambda_{em} = 750$ nm	2.81	7.30	1	0.9577	0.9274
$\lambda_{em} = 800$ nm	4.21	11.3	1	0.9516	0.9377
$\lambda_{em} = 850$ nm	6.39	17.2	1	0.9516	0.9422
BV, $I_0/I > 2$ (4 °C)^a					
$\lambda_{em} = 750$ nm	60.5	0.974	1	1.087	0.9766
$\lambda_{em} = 800$ nm	60.9	4.84	1	1.072	0.9704
$\lambda_{em} = 850$ nm	131	9.89	1	1.105	0.9723
BV, $I_0/I > 2$ (50 °C)^a					
$\lambda_{em} = 750$ nm	0.00080	8.99	1	0.7171	0.969
$\lambda_{em} = 800$ nm	0.00044	11.2	1	0.7507	0.9407
$\lambda_{em} = 850$ nm	0.00011	13.8	1	0.722	0.9268
BV, $I_0/I > 2$ (in 1× PBS, 22 °C)^b					
$\lambda_{em} = 750$ nm	7.34				0.999
$\lambda_{em} = 800$ nm	9.47				0.999
$\lambda_{em} = 850$ nm	12.0				0.999

^aFit using eq 3. Notations $I_0/I < 2$ or $I_0/I > 2$ indicate regions of the Stern–Volmer curve that were used in the fits. Titrations were performed in deionized H₂O.

^bFit using eq 1. Titrations performed in the presence of high concentration of PBS solution.