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Individual Water-Soluble Dendrimer-Encapsulated Silver Nanodot Fluorescence

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Single molecule fluorescence microscopies[1] have the power to unravel many mysteries underlying the heterogeneous dynamics characteristic of materials[2,3] and biological[1] systems. Although many fluorophores can be utilized in single molecule microscopy, only semiconductor nanocrystals[4,5] have sufficiently strong absorptions to be easily observed with weak lamp excitation. Such materials can potentially enable facile, inexpensive single molecule studies, but syntheses require toxic compounds and high temperature methods. Nontoxic noble metal nanoclusters composed of only a few atoms also show very strong, robust, discrete, size-dependent emission,[6−8] but at much smaller sizes than do either semiconductor quantum dots (hundreds of atoms, 2−6 nm diameters)[5] or Raman-enhancing metal particles (10−100 nm diameters).[9] Thus, Au and Ag nanoclusters are attractive candidates for making the smallest possible labels with strong oscillator strengths. Recently, we found that small silver nanoclusters (Ag_2−Ag_8) are readily photoproduced from room-temperature silver oxide films to yield strong multicolored fluorescence.[10,11] While potentially useful as optical data storage elements, very little control could be effected over individual surface-bound nanoclusters as continued irradiation further modifies nanocluster size and therefore emission color.[11] Consequently, while absorption strengths are comparable to those of much larger quantum dots, studying individual species proved very difficult. Additionally, the surface-bound nature of these highly fluorescent species precludes application as fluorescent labels or as volumetric optical data storage elements. Thus, synthesis of stable, water-soluble individual silver nanoclusters will greatly facilitate the use of these photoactivated nanomaterials, both as optical storage elements and as small, extremely bright, photostable fluorophores, while simultaneously expanding the accessibility of single molecule methods.

Known to sequester metal ions from solution,[12] PAMAM G4-OH and G2-OH dendrimers (fourth- and second-generation OH-terminated poly(amidoamine), 4.5 and 2.9 nm in diameter, respectively, Aldrich) were utilized to concentrate, stabilize, and solubilize Ag nanoclusters in both aerated and deaerated aqueous solutions. By dissolving 0.5 μmol of G4-OH and 1.5 μmol of $AgNO_3$ into 1 mL of distilled water (18 MΩ) and adjusting to neutrality with acetic acid, silver ions readily interact with the dendrimer. Usually used to create small nanoparticles (>3 nm diameter), literature preparations generally add small amounts of reducing agents such as $NaBH_4$.[12] To create dendrimer-encapsulated nanoclusters ("nanodots"), not nanoparticles, no reducing agents were added to our reactions. The fluorescence of these solutions was probed by placing a 10 μL drop of the solution on a clean coverslip in ambient air, nitrogen, and/or evacuated (10^{-5} Torr) environments and irradiating it with blue light (450−480 nm) from a band-pass-filtered mercury lamp through a standard epifluorescence microscope. The results were unaffected by the degree of oxygenation or dendrimer generation.

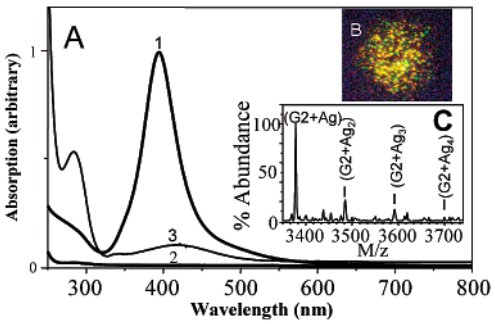

Figure 1. (A) UV−vis spectra of aqueous Ag/dendrimer solutions. (1) Strong plasmon absorption (398 nm) characteristic of large, nonfluorescent dendrimer-encapsulated silver nanoparticles prepared through $NaBH_4$ reduction of silver ions in the dendrimer host (1:12 dendrimer:Ag), (2) absorption spectrum of nonfluorescent 1:3 (dendrimer:Ag) solution before photoactivation, and (3) the same solution after photoactivation to yield highly fluorescent silver nanodots. (B) Fluorescence image from solution 3 (250×, 1 s exposure on Kodak DCS620X color digital camera, 476 nm excitation). (C) Electrospray ionization mass spectrum of photoactivated G2-OH PAMAM (MW: 3272 amu) − $AgNO_3$ solution. Ag_n nanodot peaks are spaced by the Ag atomic mass (107.9 amu) and only appear in the fluorescent, photoactivated nanodot solutions.

Initially, no visible absorption or fluorescence is observed from these solutions, but photoactivation is clearly demonstrated by the solution absorption spectra before and after exposure to white light (Figure 1A). Initially, only the dendrimer contributes to the spectrum with a single absorption at 284 nm. After photoactivation, the solution exhibits two new peaks (345 and 430 nm) due to the absorption of small, photoreduced silver nanodots (Ag_2−Ag_8).[7,8] The size and geometry differences of the small silver nanoclusters simultaneously created during photoactivation yield multicolored fluorescence throughout the visible region (Figure 1B). Confirmed by mass spectrometry of our fluorescent nanodot solutions (Figure 1C), silver nanoclusters of this size are the only ones known to have strong visible absorption and emission.[7,8] Contrary to our Ag nanodot preparation, $NaBH_4$ reduction yields larger Ag nanoparticles (3−7 nm) with a characteristic strong surface plasmon absorption at 398 nm, but with essentially no fluorescence (Figure 1A).

Correlated with absorption changes, fluorescence grows in with increasing irradiation time as silver ions are photoreduced inside the dendrimer host. Within ~6 s, the field of view is filled with individual blinking fluorescent species, with little subsequent photoactivation (Figure 2A). These very bright, stable fluorescent features are all highly polarized and exhibit well-defined dipole emission patterns[13] (Figure 2B) and blinking dynamics[1,2] (not shown) characteristic of individual emitters. After completion of photoactivation in this silver-limited environment, the fluorescent silver−dendrimer nanodots remain very stable both in average

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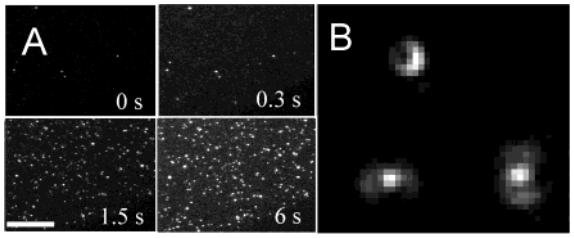

Figure 2. (A) Hg-lamp excited (450−480 nm, 30 W/cm^2, scale bar = 15 μm) epifluorescence microscopy images showing time-dependent photoactivation of aqueous dendrimer-encapsulated Ag nanodots. Each 300 ms CCD frame shows increasing fluorescence with illumination time. (B) Surface-bound silver nanodot emission patterns in aqueous solutions. Indicative of single molecules, blinking and anisotropic emission patterns are easily observable under weak Hg-lamp excitation.

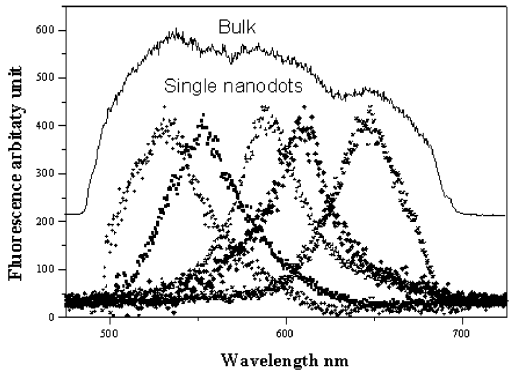

Figure 3. Room-temperature single nanodot fluorescence spectra (476 nm Ar^+ laser excitation, 496 nm long-pass filter, dispersed by a 300 mm spectrograph). Emission maxima for the five typical nanodots shown are 533, 553, 589, 611, and 648 nm. Indistinguishable from that on AgO surfaces, the ensemble fluorescence spectrum of bulk silver nanodot solutions (top) largely consists of these five spectral types.

emission intensity and in spectral characteristics. The dendrimer thereby stabilizes the nanoclusters and enhances their optical properties relative to those on AgO films.[10,11] Necessary because the binding energy of small Ag nanoclusters is less than the excitation energy, the cage effect of the dendrimer acts similarly to that of rare gas matrices[7] to stabilize and enhance nanocluster fluorescence by preventing photodissociation. While water is known to quench Ag nanocluster fluorescence on AgO films,[14] dendrimer-encapsulated silver nanodots are highly fluorescent and quite stable in aqueous solution. Thus, the photochemically produced Ag nanoclusters are also protected inside the dendrimer, thereby preventing interaction with quenchers in solution.

Contrary to studying nanoclusters on AgO films,[10,11] single nanocluster spectroscopy is readily performed on these soluble dendrimer-encapsulated silver nanodots. While the bulk spectra of Ag_n on AgO and of our aqueous nanodot solutions (Figure 3, top) are indistinguishable, individual Ag nanodots have much narrower and more stable emission spectra (Figure 3) than do individual Ag nanoclusters on AgO films.[10,11] Because nanocluster size on AgO films is continually modified with excitation, individual nanoclusters were observed to exhibit large spectral shifts.[10,11] In contrast, five stable and easily distinguished fluorescence spectra are obtained from these highly dispersed dendrimer-encapsulated silver nanodots (Figure 3), suggesting that the bulk spectrum is dominated by as few as five nanocluster sizes. Considerably narrower than those of bulk nanodot films or solutions, room-temperature single nanodot fluorescence spectra exhibit no obvious spectral diffusion. Because no additional silver can be incorporated into the nanodot and the dendrimer stabilizes the nanodot fluorescence, single nanodot emission is quite stable and robust with maxima at 533, 553, 589, 611, and 648 nm, although fluorescence intermittency is readily observed. Comparable to II−IV nanoparticles, these nanodots are very photostable with ~80% of individual features remaining fluorescent for >30 min of continuous 514.5 or 476 nm excitation at 300 W/cm^2. The nanodot photoactivation, blinking, dipole emission patterns, spectral stability, mass spectrometry, and fluorescence only at small size further confirm that individual dendrimer-encapsulated Ag_n nanoclusters less than 8 atoms in size give rise to the observed emission. No fluorescence is observed in similarly prepared solutions without the dendrimer or those without the silver. Crucial to solubility and stabilization, the dendrimer enhouses and protects the nanoclusters, yielding strong emission and a silver-limited environment that prevents further nanocluster growth.

In conclusion, very photostable, water-soluble silver nanodots have been successfully created in dendrimers through direct photoreduction in ambient conditions. Such silver nanodots are quite stable and highly fluorescent both in aqueous solutions and in films and are readily observed on the single molecule level with weak mercury lamp excitation (30 W/cm^2). With synthetic control of dendrimer attachment,[15] such simple nanomaterials are likely to find use as biological labels, thereby making single molecule studies much more widely accessible without expensive laser sources. The intense photoactivated emission and very long life before photobleaching make these attractive new nanomaterials for studying chemical and biological systems.

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(15) For example, thiol-reactive species can be made by coupling the dendrimer hydroxyl group to the isocyanate end of the bifunctional cross-linker, *N*-(*p*-maleimidophenyl)isocyanate, leaving a thiol-reactive maleimide for coupling to proteins or derivatized surfaces.

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