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UV Excitation of DNA Stabilized Ag Cluster Fluorescence via the DNA Bases

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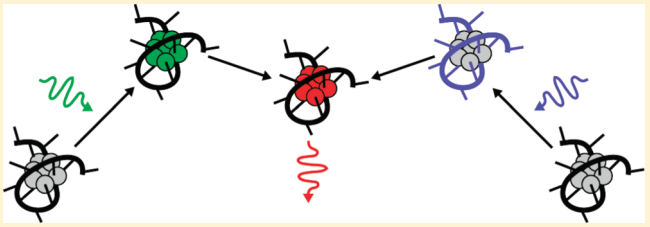
Supporting Information

ABSTRACT: We report UV excitation through the DNA bases as a universal pathway to visible emission from DNA stabilized silver cluster fluorophores (AgDNAs). AgDNAs with peak emissions throughout the visible spectrum all exhibit two peaks in fluorescence excitation: a visible peak at a wavelength 50−150 nm shorter than the emission peak and a UV peak between 260 and 270 nm depending on the specifics of the DNA strand. UV excitation produces the same emission spectrum as visible excitation with 2−4 times greater intensity depending on the specific emitter. The excellent agreement between AgDNA excitation and absorption spectra, the similarity between AgDNA and bare DNA absorption spectra, and the ubiquity of UV excitation for all the AgDNAs studied strongly suggest that UV excitation proceeds via the DNA bases rather than by direct excitation of higher energy excited states of the Ag cluster. We explore the possibility that UV excitation can provide information about the type, number, and orientation of cluster-bound bases. As an efficient and universal means of exciting AgDNA fluorescence, UV excitation should prove useful in detailed studies of AgDNA photophysics and in identification and purification of the most stable AgDNA fluorophores.



INTRODUCTION

DNA oligomers can stabilize few-atom Ag clusters in aqueous solution.[1,2] Some of the resulting AgDNAs are fluorescent with chemical stabilities, excitation and emission wavelengths, and quantum yields that depend strongly on the DNA sequence.[3−6] This sequence dependence has been exploited for detecting disease-related DNA mutations[7] and for identifying promising biolabels.[3,8,9] Rational design of AgDNA fluorophores for such applications will require a better understanding of how cluster−nucleobase interactions determine AgDNA properties.

Direct coordination of DNA bases with the Ag clusters is evident from the pH dependence of fluorescence, which implicates the N3 sites of thymine and cytosine in cluster binding.[10,11] However, many fundamental questions pertaining to the sequence dependence remain. For example, how many bases bind the cluster in a given AgDNA? Do bases that are not directly bound to the cluster affect its properties? How does DNA secondary structure influence the stability of AgDNAs?[5,12] Detailed structural characterization of these emitters awaits improved chemical yields and purification. Here, we explore such issues in the context of a striking feature of AgDNA fluorophores: resonant UV excitation of visible emission via transfer of excitation energy from the DNA bases that stabilize the cluster.

This work was motivated by previous studies which found that visible emission from AgDNAs could be excited both by visible light and by UV wavelengths.[1,11] Since then, despite numerous studies of AgDNA fluorescence, little attention has been paid to UV excitation. The early studies suggested energy transfer from the DNA bases as one possible mechanism; however, excitation spectra for the visible emission were not made to definitively test the role of the bases. Here, we use AgDNAs formed on cytosine and thymine homopolymers and on mixed-base strands to provide strong evidence that the DNA bases provide a general excitation pathway for AgDNA fluorescence.

EXPERIMENTAL SECTION

AgDNA solutions typically contain a mixture of dark and fluorescent products. For these studies, we chose DNA strands and synthesis conditions that produce just one fluorescent species or two that are spectrally well-separated. We selectively study a given fluorescent species by detecting at its peak emission wavelength and scan fluorescence excitation across UV and visible wavelengths.

Comparison of UV and visible excitation spectra of AgDNA fluorescence requires careful attention to effects of the strong absorbance of DNA at UV wavelengths. At the DNA concentrations used in prior studies (typical nucleobase concentrations of ∼100−300 μM), the UV extinction length falls short of the 1 cm excitation path length of standard fluorescence cells and limits the volume of UV excited solution. The extinction length is a sensitive function of wavelength in the 200−300 nm range, and as a result, the UV excitation peak of AgDNAs appears red-shifted and

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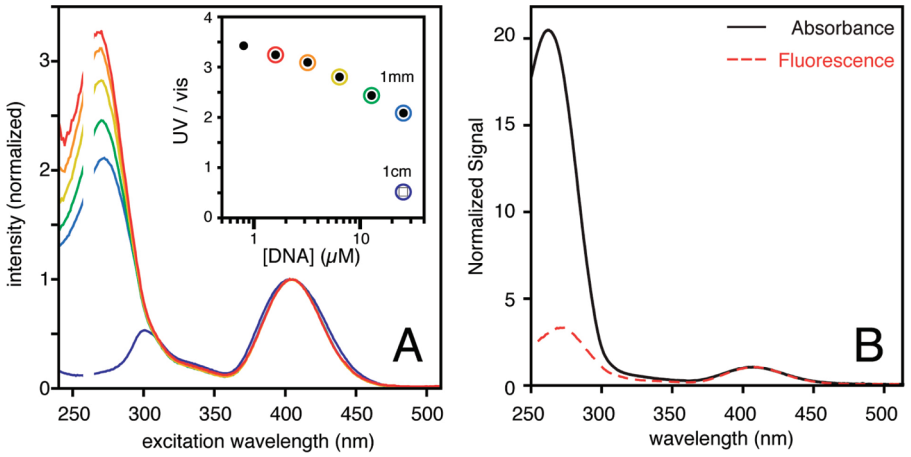

Figure 1. (A) Effect of path length and DNA concentration on the excitation spectra of a AgDNA fluophore. Excitation spectra were recorded at the peak emission wavelength of 525 nm for the green AgDNA made with the sequence 5′-TATCCGT-C_9-ACGGATA-3′. Measurement of the true UV peak excitation wavelength requires short path lengths and low DNA concentrations. At 25 μM DNA, 10 mm path length, the apparent UV excitation peaks near 300 nm. Reducing the path length to 1 mm reveals much stronger UV excitation peaked near 270 nm. As the concentration is reduced, the wavelength of the UV peak approaches a constant value as does the relative emission intensity for UV vs visible excitation (inset). Data points in the inset are circled with the color of the corresponding excitation curve. Curves are normalized to the visible excitation peak. Gaps in the data are from second-order scattering of the excitation light. (B) Excitation and absorption spectra for the same AgDNA solution as in A normalized to the visible peak. The large difference in magnitude of the UV peaks indicates that a significant portion of the energy absorbed in the UV is not transferred to the emissive state. This suggests that a large fraction of the nucleobases in solution are not able to transfer energy to the emissive state either because they are not part of the emissive Ag:DNA in question or because their position/orientation in the emissive Ag:DNA prevents coupling to the emissive core.

erroneously weak (Figure 1A). To obtain accurate UV excitation spectra, we ensured uniform excitation intensity over the path length by making fluorescence measurements on dilute solutions (~20−60 μM nucleobases) in short excitation path length cuvettes (1 mm).

Comparison of UV and visible excitation spectra also requires careful normalization of the wavelength dependence of lamp emissivities and detector sensitivity. The sensitivity of most commercial fluorimeters drops sharply in the UV, and instrument-specific correction factors are needed to produce accurate spectra. Fortunately, appropriate UV correction factors can be conveniently determined from excitation and absorption measurements of $CeCl_3$ solutions.[13] Combined with visible wavelength calibration using rhodamine B, $CeCl_3$ standards enable us to make quantitative comparison of the UV and visible excitation efficiencies of AgDNA fluorescence. Instrument specifications and correction factors, DNA sequences, and details of the AgDNA synthesis are provided in the Supporting Information.

RESULTS AND DISCUSSION

To accurately measure fluorescence excitation in the UV, we took care to control for the effects of strong absorbance by the DNA in solution and spectral dependencies in the fluorimeter (see Experimental Section). Figure 1A illustrates, for one fluorescent AgDNA species, how reducing the excitation path length and diluting the DNA solution reveals a peak in the excitation spectrum near the absorbance maximum for DNA bases. Figure 1B superimposes the excitation and absorption spectra of the solution containing this emitter normalized to their peaks in the visible region of the spectrum. These peaks are nearly identical both in location and in shape, which enabled us to determine the extinction coefficient of this emitter.[14] In this solution, the emitter of interest was about one-third as concentrated as the DNA (see the Supporting Information). This can explain, in part, the difference between the two spectra in the UV region of the spectrum.

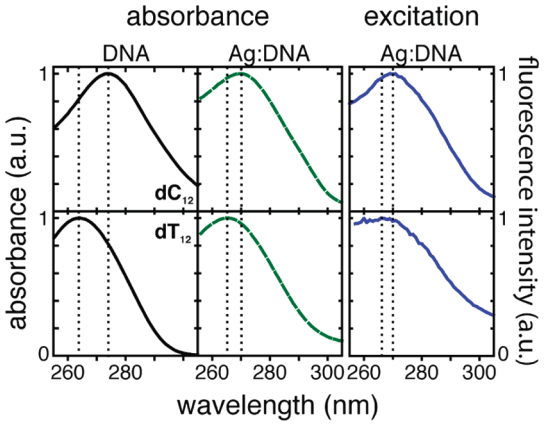

Figure 2. Left panels: UV absorbance spectra of the DNA strands dC_{12} (top) and dT_{12} (bottom). Middle panels: UV absorbance spectra of the same DNA sequences after AgDNA synthesis. Right panels: UV excitation spectra of the visible emission from the corresponding AgDNA solutions (emission detected at 490 nm for dC_{12} and at 550 nm for dT_{12}). The close correspondence between the fluorescence excitation and the absorbance spectra of the AgDNA solution suggests that the dominant absorbing transition is the same as the one that eventually excites the emission. That these are shifted only slightly from the absorption spectra of the bare DNA further suggests that UV excitation proceeds via the DNA bases. Vertical dashed lines highlight the small offset in peak wavelengths between the poly-C and poly-T solutions.

Both spectra display a single peak in the UV at nearly the same wavelength as the UV absorbance peak for pure DNA, but the absorbance peak is about 6 times greater in magnitude than the excitation peak. This suggests that the visible emission of the

AgDNA can be excited by the UV absorbance transition of the DNA albeit with limited efficiency.

Universality of UV Excitation Implicates the DNA Bases. To determine unambiguously whether energy transfer from the nucleobases can excite AgDNA fluorescence, we first asked whether the excitation spectrum of a AgDNA fluorophore matches the absorption spectrum of its DNA bases in detail. We expect such agreement only if all of the nucleobases in the DNA strand transfer energy to the visible emission center of the AgDNA complex in the same proportion as they absorb UV light. Of course, energy transfer from remote bases might be less efficient than energy transfer from bases that are proximate to the emission center. Thus, in mixed base strands, the ~20 nm difference among the peak absorbances of the different nucleobases may shift the UV excitation spectrum relative to the UV absorbance spectrum.

We avoided such complications by focusing on the homopolymers dT_{12} and dC_{12} with synthesis conditions chosen to stabilize different AgDNA species with emission at 550 nm and 490 nm, respectively.[10,11] Figure 2 shows the absorption spectra of poly(dT) and poly(dC) DNA solutions before and after Ag cluster synthesis and the fluorescence excitation spectra corresponding to emission at 550 nm and 490 nm, respectively. The emitter concentrations in these solutions are unknown, and various AgDNA species may contribute to the absorption spectrum following Ag cluster synthesis. Nonetheless, the fluorescence excitation spectra, which directly probe the AgDNA species of interest, match very well with the absorption spectra and are only slightly shifted with respect to the bare DNA absorption. Thus, the orbitals involved in the UV transition, while slightly perturbed by the Ag cluster, are likely still primarily confined to the DNA bases.

If UV excitation proceeds via the DNA bases, one would also expect that all fluorescent AgDNAs can excite in the UV. Figure 3 shows this to be true for AgDNAs synthesized with solution conditions and strand types chosen to stabilize a wide range of peak emission (490−665 nm) and excitation (340−585 nm) wavelengths. Despite the wide range of visible transition wavelengths, all UV excitation peaks lie within the narrow range 260−270 nm consistent with absorption by the DNA bases.

Because the fluorescence excitation spectrum exhibits peaks at energies corresponding to the initially excited electronic states, the peak energy does not provide information on what subsequent processes transfer energy to the visibly emissive excited state. For small chromophores, nonradiative decay by internal conversion and vibronic processes are a common route for relaxation from high energy electronic excited states to the lowest energy electronic excited state from which radiative transitions typically occur.[15] Thus, nonradiative decay from UV excited states of the silver cluster itself might, in principle, account for the excitation spectrum of Ag:DNA fluorophores. In this case, we would expect the Ag:DNA fluorescence excitation spectrum to resemble the silver cluster absorbance spectrum. Silver atom numbers identified by correlation of fluorescence and mass spectra range from $N = 11$ to 14 atoms[5] while elemental analysis of high-performance liquid chromotography (HPLC)-separated Ag:DNA products suggests 9−11 silver atoms per strand.[16,17] The UV absorbance spectra of matrix isolated silver clusters[18] in this size range, as well as calculated vacuum spectra,[19] typically show multiple excitations spread across 3−5 eV. The peak energies depend on cluster size and shape, but in all cases, the spectral distribution differs markedly from the relatively narrow fluorescence excitation peak we observe near 4.7 eV (265 nm) for all Ag:DNAs.

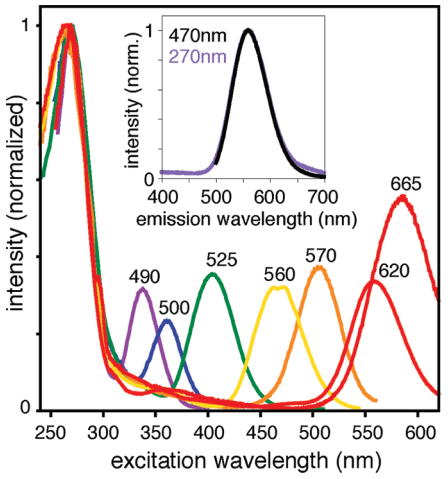

Figure 3. AgDNA species with a wide range of visible excitation peaks (340−585 nm) all exhibit UV excitation peaks in the 260−270 nm range suggesting that UV excitation via the DNA bases is a universal feature of AgDNA fluorophores. For each emitter, UV excitation produces the same emission spectrum as visible excitation indicating that both pathways feed the same radiative state. This is shown in the inset for the 560 nm emitter excited at the peak visible (470 nm) and UV (270 nm) excitation wavelengths. Peak emission wavelengths for all seven Ag:DNA emitters studied are displayed above the corresponding visible excitation peaks. All measurements were made in a 1 mm path length cuvette at DNA concentrations between 1.5 and 2.5 μM. DNA sequences (with corresponding emission wavelength): dC_{12} (490 nm), TATTAAT-C_9-ATTAATA (500 nm), TATCCGT-C_9-ACGGATA (525 nm), TATCCGT-C_7-ACGGATA (560 nm), TGCCTAT-G_5-ACGGATA (570 nm), TATCCGT-C_{12}-ACGGATA (620 nm), dC_{12} (665 nm). The 490 and 665 nm emitters were measured in the same solution, but their nonoverlapping emission spectra allowed for independent measurement of their excitation spectra throughout the UV and visible range. (See Supporting Information for sample preparation.)

Instead, the small variation observed in the peak UV excitation wavelength is more plausibly attributed to the different base composition of the DNA strands that stabilize the various emitters (see Supporting Information for strand compositions). Because every emission spectrum is the same for both UV and visible excitation, the electronic excitations of the DNA bases caused by UV light relax to the same emissive state of the AgDNA that is pumped directly by visible light (Figure 3, inset).

Mechanism of Energy Transfer Is Independent of Spectral Overlap. We consider the possibility that UV excited DNA bases may transfer their energy to the Ag cluster through either the well-known Förster[20] or Dexter[21] mechanisms keeping in mind that these processes are not exclusive.[22] Förster transfer is mediated by interactions between transition dipoles of the energy donor and acceptor and can act over distances up to ~10 nm. Dexter transfer, on the other hand, is an electron-exchange mechanism that requires spatial overlap of electron orbitals on the donor and acceptor and is typically only observed for distances <1 nm.

Both Förster and Dexter energy transfer require spectral overlap of the donor emission spectrum with the acceptor excitation spectrum.[23] Bare DNA bases emit in the ~300−400 nm range with very low quantum efficiencies,[24] but few AgDNAs exhibit excitation peaks in this range; those that do are not more efficiently excited in the UV than the rest (Figure 3). Compare, for example, the 490 nm and the 620 nm emitters. The visible

excitation peak for the 490 nm emitter centers on 340 nm overlapping significantly with the known emission spectra of DNA bases. The visible excitation peak for the 620 nm emitter centers on 560 nm, and no excitation occurs at wavelengths corresponding to DNA emission. Nonetheless, both emitters are excited about three times more efficiently at 260 nm than at their respective visible peak excitation wavelengths.

The apparent independence of the energy-transfer efficiency on the spectral overlap of AgDNA excitation peaks with the known DNA emission spectra argues against Förster or Dexter energy transfer as an explanation for UV excitation of AgDNAs. It suggests, instead, that the interaction energy associated with overlapping electron orbitals between the bases and the Ag cluster may be too large to treat perturbitively.[25] Förster's approach treats only cases where the interaction energy is negligible, while Dexter's approach treats it as a small perturbation.[23]

Extensive work in the past decade on excitation energy transfer in large complexes has shown that in many cases both Förster and Dexter models are inadequate to explain observed phenomena.[25] Very weak or even optically forbidden electronic transitions may participate in efficient energy transfer when the separation between molecules is small compared to the size of the molecules.[26] A strong interaction between the nucleobases and the silver cluster is qualitatively consistent with AgDNA emission wavelengths, which indicate the existence of states with energies far below those of clusters or bases alone, and with recent time-dependent density functional calculations of the vertical absorbance transitions of small silver clusters bound to one or two bases, which found low-energy transitions, far from those of the bases and the bare clusters, and a large number of UV transitions, significantly shifted (to both lower and higher energies) from those of the bare components.[27]

What Can UV Excitation Reveal about the Cluster-Bound Bases? A deeper understanding of the energy-transfer mechanism will require further research, but our results suggest that one can reasonably assume that DNA bases directly coordinated with the Ag cluster provide the main contribution to UV excitation of the visibly emissive moiety. Fluorescence measurements made with UV excitation may thus provide information specific to the bases that attach directly to the silver cluster. In the discussion that follows, we explore three aspects of such cluster-bound bases in the context of UV excitation: the type of cluster-bound bases, the number of cluster-bound bases, and the orientation of cluster-bound bases relative to the Ag cluster.

Base Type. We begin by asking whether UV excitation can identify the types of bases bound to a given Ag cluster. The four different DNA bases (adenosine, cytosine, guanine, and thymine) have distinct absorption peaks in the UV, so one might expect spectral shifts in UV excitation depending on which bases bind the cluster. As shown in Figure 2, the UV excitation peaks for AgDNA synthesized on C and T homopolymers exhibit slight but distinct shifts in wavelength in the same direction and of similar magnitude as the shift in peak UV excitation between the bare dT_{12} and dC_{12} strands. Larger shifts would be expected for clusters bound to A or G because those bases have absorption maxima that are blue-shifted (~15−20 nm) with respect to C.[28] Although no AgDNA fluorophores have been reported to form with A homopolymers, excitation spectra of heteropolymer-stabilized AgDNAs hint at the involvement of A's in cluster binding. For example, Figure 4 shows that the 490 nm and the 665 nm emitters stabilized by dC_{12} have nearly identical UV excitation peaks, but the UV excitation peak of the 500 nm emitter stabilized by a G-free sequence (5′-TAT TAA TCC CCC CCC CAT TAA TA-3′) is both shifted and broadened toward the blue. These spectral changes are consistent with the notion that not only C's but also A's contribute to the UV excitation of this cluster. As another example, the 620 nm emitter made with a 12C hairpin sequence (5′-TAT CCG TCC CCC CCC CCC CAC GGA TA-3′) is similarly blue-shifted specifically implicating A's or G's in its UV excitation.

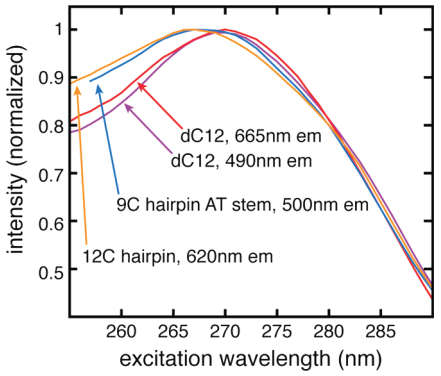

Figure 4. Blue-shifts in the UV excitation peaks for AgDNA made with heteropolymers relative to those made with dC_{12} suggest that purines (A, G) contribute to the UV excitation. In particular, a 620 nm emitter made with the 12C hairpin sequence (5′-TATCCGT-C_{12}-ACGGATA-3′) has a UV peak that is both blue-shifted and broadened at shorter wavelengths compared to both dC_{12} spectra consistent with contributions to UV excitation arising from absorption by A or G bases. If short-ranged energy transport between the bases and clusters is important for UV excitation, this could indicate that A's or G's participate in cluster binding. More direct evidence for adenine cluster binding comes from the UV excitation of a 500 nm emitter made with the 9C hairpin AT stem sequence (5′-TATTAAT-C_9-ATTAATA-3′), which contains no G's. All four measurements were made in a 1 mm path length cuvette at DNA concentrations between 1.5 and 2.5 μM.

The two heteropolymeric-DNA sequences cited above were originally chosen for their potential to form hairpins in which the Ag clusters might be confined to poly-C loops.[5] It is conceivable that bases not bound to the cluster can contribute to UV excitation, for example, by energy transport through the double-stranded stem. However, direct attachment of the cluster to A's in the designed stem, either by binding simultaneously to the loop and stem or by a reconfiguration of strand secondary structure by the cluster, is a more plausible explanation. Ag induced changes to the DNA secondary structure are in line with recent electrokinetic and diffusivity measurements that reveal distinct conformations of AgDNA stabilized by the same DNA sequence.[14]

Base Number. Sequence dependence of AgDNA fluorescence implicates multiple bases in cluster binding, but until now, there has been no experimental insight into the number of cluster-bound bases. Given that UV excitation proceeds via the DNA bases and results in the same emission as visible excitation, the relative fluorescence intensity produced by UV and visible excitation provides a starting point for estimating the number of cluster-bound bases. For the emitters studied here, UV excitation produces 2−4 times more fluorescence than visible excitation depending on the emitter species (Figure 3). Motivated by our observation that the UV excitation spectra resemble the absorption spectra of bare DNA (Figure 2), we asked the following question: If the total UV excitation cross section of a AgDNA is the sum of the absorption cross sections of its cluster-bound bases, how many cluster-bound bases are needed to

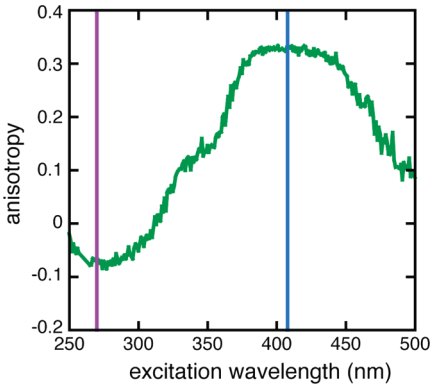

Figure 5. Room-temperature fluorescence polarization anisotropy of the 525 nm emitter indicates that transition dipoles corresponding to UV and visible excitation are at different angles. The anisotropy of 0.33 at 408 nm, the peak visible excitation wavelength (blue line), puts an upper bound of 20° on the angle between the visible excitation and the emission dipoles, while the value of −0.08 at 270 nm, the peak UV excitation wavelength (purple line), puts a lower bound of 63° on the angle between the UV excitation and the emission dipoles. The measurement was made in a 1 mm path length cuvette with 2.5 μM DNA.

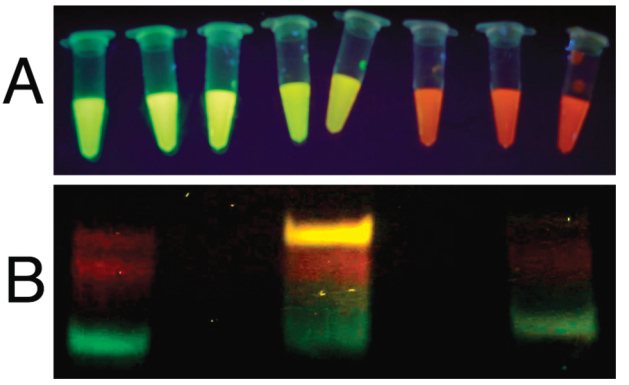

Figure 6. (A) A standard UV transilluminator (Spectroline TE-312S, 312 nm) excites fluorescence from a set of AgDNA solutions that also exhibit the same fluorescence colors when excited by visible light at wavelengths near 405 nm (green emission) and 560 nm (red emission). The same strand, 5′-TAT CCG TCC CCC CCC CAC GGA TA-3′, was used in all solutions with different Ag:DNA ratios to select the different colors. (B) UV excitation enables simultaneous visualization of different color AgDNA bands in an unstained polyacrylamide gel. In this gel, the three lanes represent AgDNA fluorophores synthesized with three different DNA sequences: TATCCGT-C_8-ACGGATA (left), TATCCGT-C_7-ACGGATA (middle), and TATCCGT-C_6-ACGGATA (right). All three sequences stabilize multiple AgDNA fluorophores with different colors and electrophoretic mobilities. Electrophoresis was performed for 30 min at 150 V/11 cm, 27 mA using 80 mM HEPES, pH 7.5, as the running buffer.

account for the 2−4 fold increase in fluorescence upon UV versus visible excitation?

Two factors could contribute to this increase: a larger cross section for UV excitation relative to visible excitation and a higher quantum yield for the subsequent emission. The presence of multiple species in our AgDNA solutions prevented us from comparing the UV and visible quantum yields. (Every DNA strand present absorbs UV light regardless of the number of attached silver atoms, while only a fraction of the strands form fluorescent AgDNAs.) We therefore proceed under the simplest assumption that the quantum yields for the two transitions are equal. In that case, the ratio of the UV and the visible maxima in the fluorescence excitation spectrum is given by the ratio of the extinction coefficients for the UV and visible peak absorbances. Thus, we compare extinction coefficients for both peak excitation wavelengths of the 525 nm emitter of Figure 3. This emitter has a visible extinction coefficient of 3.8 (±0.3) × 10^4 M^{-1} cm^{-1} at 408 nm, the peak visible excitation wavelength, as determined by fluorescence correlation spectroscopy (see the Supporting Information). Assuming equal quantum yields, the 3-fold greater fluorescence upon UV excitation (Figure 1) implies a UV excitation extinction coefficient of ∼11 × 10^4 M^{-1} cm^{-1}. Given that extinction coefficients of individual bases are close to 1 × 10^4 M^{-1} cm^{-1},[28] roughly 10 cluster-bound bases are required to account for this level of UV excitation.

Ten is a plausible estimate given that the DNA sequence has 23 bases and hosts an Ag_{11} cluster.[5,29] However, 10 is probably an overestimate because the UV extinction coefficients of bases contributing to UV excitation are likely to be larger than those of bare DNA bases as a result of cluster-enhanced absorption. Absorption enhancement has been reported for peptides bound to Ag clusters[30,31] and is predicted on the basis of time-dependent density functional theory (TDDFT) for Ag clusters bound to DNA bases.[27]

Base Orientation. Detailed structural information is outside the realm of fluorescence-based measurements; however, if the fluorescence lifetime is short compared to the rotational correlation time (as is the case for the 525 emitter, see the Supporting Information), information on relative orientations of transition dipoles can be gleaned from fluorescence anisotropy[32] because UV and visible excitation pathways of AgDNA yield the same emission. Figure 5 shows the room-temperature fluorescence anisotropy of the 525 nm emitter as a function of excitation wavelength. The anisotropy of 0.33 for 408 nm excitation places an upper bound of 20° on the angle between the visible excitation and the visible emission dipole moments, whereas the anisotropy of −0.08 for 270 nm excitation places a lower bound on the angle between the UV excitation and the visible emission dipoles of 63° (see the Supporting Information). Apparently, the contribution of multiple bases to the UV excitation results in very different orientations for the visible excitation dipole moment and the net UV excitation moment, information that could serve to test future models of AgDNA structures.

Applications of UV Excitation. In addition to serving as a probe of AgDNA structure and photophysics, UV excitation of AgDNA fluorescence is also poised to facilitate the purification and characterization of AgDNA fluorophores. For example, we have simultaneously imaged distinct AgDNA species with different emission colors and gel mobilities in unstained electrophoresis gels using a standard UV transluminator (Figure 6B). UV transillumination also enables rapid, qualitative comparisons of many solutions in parallel as illustrated in Figure 6A, which is especially convenient for selecting AgDNAs on the basis of chemical stability. Interestingly, some AgDNAs exhibit significant fluorescence loss within five minutes of exposure to the UV transluminator (e.g., our 570 nm emitter) while others exhibit no measurable change (e.g., our 525 nm emitter).

CONCLUSIONS

In conclusion, we have documented the universal nature of UV excitation of AgDNA fluorescence. The excellent agreement of the AgDNA fluorescence excitation spectra with the absorption

spectra of the DNA bases for all of the AgDNAs studied strongly suggests that UV excitation of AgDNA fluorescence proceeds via the DNA bases. This discovery opens a key new path toward unraveling the details of AgDNA photophysics and will be of practical use in discovery and purification of the most stable AgDNAs.

■ ASSOCIATED CONTENT

Supporting Information. DNA sequences, AgDNA synthesis details, generation of fluorometer correction factors, extinction coefficient measurements for the 525 nm emitter by FCS, quantum yield measurements of the 525 nm emitter, anisotropy details. This material is available free of charge via the Internet at http://pubs.acs.org.

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