Published in final edited form as:

J Phys Chem C Nanomater Interfaces. 2011 ; 115(41): 20105–20112. doi:10.1021/jp204836w.

Wavelength Dependence of the Fluorescence Quenching Efficiency of Nearby Dyes by Gold Nanoclusters and Nanoparticles: The Roles of Spectral Overlap and Particle Size

Sanchari Chowdhury, Zhikun Wu¹, Andrea Jaquins-Gerstl², Shengpeng Liu, Anna Dembska, Bruce A. Armitage, Rongchao Jin, and Linda A. Peteanu*

Department of Chemistry, Carnegie Mellon University, 4400 Fifth Ave, Pittsburgh, PA 15213

Abstract

The efficiency of the glutathione monolayer-protected gold nanocluster (NC) Au₂₅ (1.2 nm metal core diameter (d)) in quenching the emission of dyes intercalated into DNA is compared to that of 2 and 4 nm gold nanoparticles (NPs). In all cases, the DNA/dye moieties and the gold particles are not covalently attached but rather form non-covalent ground state complexes. Under these conditions, steady-state measurements reveal that the quenching efficiency of Au₂₅ is a factor of 10 lower than that of plasmonic 4 nm gold NPs but comparable to that of 2 nm particles which do not show a distinct plasmon band. Nonetheless, significant emission quenching is observed even at very low (nM) concentrations of Au₂₅. The quenching efficiency of the 4 nm NPs is significantly higher for dyes emitting near the wavelength of the plasmon peak whereas that of the 2 nm gold NPs is well described by the nano-surface energy transfer (NSET) model proposed by the Strouse group (*J. Am. Chem. Soc.* 127, 3115 **2005**). Interestingly, for Au₂₅ the maximum quenching efficiency occurs for dyes emitting in the same wavelength range as that of the 2 and 4 nm NPs (490–560 nm), where it shows no discrete absorption features, rather than for wavelengths coincident with its HOMO-LUMO, intra-band or inter-band transitions. The fluorescence quenching properties of Au₂₅ NCs are therefore found to be distinct from those of larger NCs and NPs but do not appear to conform to theoretical predictions advanced thus far.

Introduction

A well-known characteristic of metals and metallic nanoparticles (NPs) is their ability to alter the emission properties of fluorophores within several nm of the surface. Over the last few decades, there has been extensive research on these effects^{1–7} which fall into two categories: those that quench the dye emission due either to energy transfer,^{8–11} electron transfer,^{12–13} or by decreasing the radiative rate of the dye¹⁴ and those that enhance emission by increasing the dye's radiative rate and/or absorbance.¹⁵ These effects depend on factors such as the identity of the metal, the distance of the fluorophore from the NP and the

*Corresponding authors. peteanu@andrew.cmu.edu.

¹current address: Key Laboratory of Materials Physics, Anhui Key Laboratory of Nanomaterials and Nanotechnology, Institute of Solid State Physics, Chinese Academy of Sciences (CAS), Hefei 230031, Anhui, China. (zkwu@issp.ac.cn)

²current address: Department of Chemistry, University of Pittsburgh, Pittsburgh PA 15260

Supporting Information

The fluorescence lifetime data for YOYO-1, POPO-1 and the Click dyes before and after adding gold NPs/NCs are included in Table S1. A set of curves summarizing the calculated variation in the fluorescence lifetime with dye-particle distance based on the FRET and NSET models is shown in Figure S1. The calculations are described in Section S1. The emission spectra of YOYO-1/DNA before and after the addition of 4 nm gold NPs and of the supernatant following precipitation of the NPs from the solution by centrifugation are shown in Figure S2. The procedure used to estimate the trends in electron transfer energetics with dye wavelength is summarized in Section S2. This material is available free of charge via the Internet at <http://pubs.acs.org>.

orientation of its transition dipole relative to the NP surface.¹⁵ In particular, quenching is thought to be favored for particle-dye distances less than 6 nm whereas enhancement has been reported primarily for distances between ~6-10 nm.^{2,15}

The observation that smaller gold NPs (diameter (d) < 2 nm) and NCs can also be efficient quenchers is especially important because of their promising use as labels in *in-vivo* biophysical measurements. With the recent advances in the synthesis and characterization of ultrasmall gold NCs,¹⁶⁻²⁰ the metal NC-fluorophore interaction can also be exploited for the development of new sensor applications. However the mechanism of quenching for these smaller particles is expected to be different from that in larger NPs and bulk metal in several regards. For larger NPs ($d > 2$ nm), the absorption spectrum is dominated by the surface plasmon,²¹ which is indicative of metallic character as only free electrons can be collectively excited to create a surface plasmon. For small NCs such as Au₂₅, quantum confinement effects instead produce discrete electronic transitions due to the excitation of a single electron between two molecular-like orbitals.^{19,22} These transitions lie at wavelengths distinct from that of the plasmon band. In the size regime intermediate between NCs and larger NPs are gold particles ~ 1.4–2 nm in diameter for which the absorption spectrum shows no pronounced peaks due to the broadening of surface plasmon band and the presence of many overlapping one-electron transitions.²³ Here we term these particles small NPs with the understanding that their properties differ from those of the larger ($d > 2$ nm) NPs. Specifically, the dominant resonance in the absorption spectrum of these smaller particles is much broader, weaker, and somewhat red shifted as compared to the plasmon band in larger metallic NPs.²³⁻²⁴

The distinct properties of the electronic transitions that characterize these three size regimes (large NPs, small NPs, and NCs) would be expected to affect the efficiency and perhaps even the mechanism by which they quench nearby fluorophores. Though there have been a few recent studies devoted to understanding the interactions between nonplasmonic metal particles and nearby fluorophores, the field is still in its infancy. The existing studies are suggestive but the results sometimes appear inconsistent with one another. For example, whereas efficient fluorescence quenching was observed for dyes covalently bound to 1.4 nm gold nanoclusters (commercial Nanogold®),²⁵ Lu *et al.* reported minimal quenching of Alexafluor 514 covalently bound to 1.8 ± 0.4 nm monolayer-protected gold NCs.²⁶ Other workers found that 1.3 nm and 1.8 nm gold particles are comparably efficient in quenching the dye [Ru(bpy)₃]²⁺ following the formation of an electrostatic complex.²⁷

Recent studies by Strouse and coworkers have suggested that the energy transfer process in non-plasmonic particles follows a fundamentally different mechanism from that in larger plasmonic NPs.^{11,28} They examined the energy transfer efficiency of commercially available 1.4 nm Nanogold® particles on the emission quenching of dyes covalently attached by various lengths of DNA to the particle surface as a function of dye-particle distance (R) and dye emission properties such as the wavelength maximum and quantum yield. The distance dependence of the quenching efficiency was found to be $1/R^4$ rather than $1/R^6$ as would be expected for a mechanism based on Förster resonance energy transfer (FRET). Their proposed energy transfer mechanism for these non-plasmonic particles, termed nano-surface energy transfer (NSET), was described as energy transfer from a molecular dipole to the nanometal surface similar to that which would occur to a bulk metal.²⁹ In addition to predicting a weaker dependence of the energy transfer efficiency on distance than does FRET, the NSET mechanism also predicts that the increase in efficiency will be linear in the quantum yield of the donor dye but quadratic in its emission wavelength.¹¹ Another recent study from this group showed that energy transfer between 2 nm gold NPs and dyes is most efficient when there is overlap between the donor photoluminescence and the calculated absorption spectrum (400-700 nm) of the particle.³⁰ Within

this wavelength range, the NSET model adequately explains the quenching behavior of these particles.

Turning to the properties of smaller NCs, a recent time-dependent density functional theory (DFT) study by Muñoz-Losa *et al.*³¹ predicted that increasing the overlap of the donor dye emission with specific absorption transitions in Au₂₅ would yield enhanced quenching. Moreover, it was proposed that non-plasmonic small metal clusters such as Au₂₅ would be similarly effective as plasmonic particles in quenching the emission of dyes having emission wavelengths coincident with specific electronic transitions of the metal cluster. In summary, based on the studies described here, the dependence of the quenching efficiency on the dye emission wavelength is clearly an important parameter that distinguishes the behavior of plasmonic NPs from smaller non-plasmonic particles and NCs. This comparison is the focus of the current study.

We have found relatively few experimental studies in the literature devoted to examining the dependence of the quenching of gold NCs as a function of donor emission wavelength.²⁷ Here we report the wavelength dependence of the collisional quenching efficiency of Au₂₅ NCs with that of 4 (± 0.2) nm and 2 (± 0.2) nm NPs. These species were chosen to represent three distinct size regimes, *viz.* molecular clusters with distinct HOMO-LUMO gaps (Au₂₅), plasmonic particles (4 nm), and intermediate-sized particles (2 nm) which exhibit a broader and weaker absorption resonance than do plasmonic NPs.³⁰ The Au₂₅ NCs used are unique in that they are atomically precise (or mono-disperse), and have been fully characterized by mass spectrometry, thermogravimetric and elemental analyses, and by X-ray crystallography.¹⁹⁻²⁰ The optical absorption spectrum of Au₂₅ exhibits multiple discrete absorption bands which have been assigned using DFT.¹⁹ The Au₂₅ NCs and the 2 and 4 nm NPs used here were formed in a single pot and surface functionalized using the identical glutathione ligand (surface charge -1 at the pH used which is 8.4). The fact that the surface characteristics and synthetic history of all three species are the same allows the focus to remain specifically on the dependence of the quenching properties on particle size.

A simple assay was used that measures the quenching efficiency of these gold particles on dyes bis-intercalated within 30-mer double stranded (ds) DNA when both are mixed together in solution at concentrations of 1-100 nM. The quenching effects are due to the formation of ground-state non-covalent complexes between the DNA-dye constructs (*vide infra*) and the gold particles and are therefore short-range in nature. The dyes used strongly bis-intercalate into dsDNA (*eg.* the dissociation constants (K_d) for TOTO-1 is ~ 1 nM³²) and their emission is enhanced by several orders of magnitude upon binding to dsDNA but not to single stranded (ss) DNA.³²⁻³³ As a total of five dyes were used (Chart 1) to span the wavelength range over which transitions are observed in the absorption spectra of the NPs and NCs (434-642 nm), the wavelength dependence of the quenching efficiency is mapped with good resolution. Moreover, the brightness of the multiply-labeled DNA-dye constructs provided high sensitivity even at the low (~ 10 – 100 nM) concentrations used in these measurements. For comparison, we also examined the quenching of model systems consisting of either one or three thiazole orange (TO) dyes covalently attached to DNA *via* a polyethylene glycol (PEG) chain (Click dyes, Chart 2).³⁴ The Click dyes are structurally related to the other dyes used here and also require intercalation to show efficient emission. From the standpoint of the current study, the important distinction between these dyes and the others used is that the Click dyes cannot completely de-bind from the dsDNA. However, only a single emission wavelength is available at present (490 nm) and the number of dye labels is either one (mono-Click) or three (tri-Click) rather than the 15 used for the non-covalent case.

The studies reported here show that the fluorescence quenching properties of Au₂₅ NCs are distinct from those of larger NCs and NPs but do not appear to conform to theoretical predictions advanced thus far. Therefore, the effects of gold particles on nearby dyes provide a sensitive probe of the transition to metallic behavior with particle size.

Experimental

HPLC purified ssDNA 30 base pairs long (5' TTT TTTTTC CTC TCG CGC GTT TTT TTTTTC 3', MW = 9342.2) and its complementary strand (MW = 9242.2) were purchased from Integrated DNA Technologies Inc. as lyophilized powders. Synthesis and characterization of the TO-conjugated DNA strands will be described elsewhere.³⁴ The sequences used were: 5'-GCG CTG TXC ATT CGC G-3' (mono-Click) and 5'-TAC TAA XTA CAT TTG CTA GXC CAG ATC GGC AGXGCA GGC-3' (tri-Click) where X corresponds to a uracil residue with a TO dye covalently attached to C-5 of the base. These TO-modified strands were annealed with their respective complementary strands to form stable duplexes as described below.

The intercalating dyes POPO-1, YOYO-1, LOLO-1, YOYO-3, and TOTO-3 were purchased from Molecular Probes (Eugene, Oregon) and used without further purification. Tris-borate EDTA buffer (TBE) was obtained from Fisher Scientific. Glutathione-monolayer-protected Au₂₅ NCs, 2 nm and 4 nm particles were synthesized in one pot following the procedure described in detail elsewhere.¹⁸

The DNA-dye constructs were assembled by first annealing two complementary strands of ssDNA at 95° C for 10 min followed by cooling to room temperature. For the complexes with intercalating dye, the dye was added in a 15:1 ratio to the dsDNA in order to saturate the binding sites and incubated overnight in the dark at room temperature.³⁵ The fluorescence intensities of these complexes were stable at room temperature for at least 3 days.

The quenching experiments were performed by adding a solution of Au NPs/NCs (concentration range ~1-100 nM) to solution of the dye/DNA complexes in varying mole ratios of particles to DNA, incubating for 30 min and monitoring the emission spectrafluorometrically. The incubation time was chosen to allow the emission intensity to reach a stable plateau. All solutions were prepared and diluted using 0.5xTBE buffer (pH 8). Fluorescence lifetime measurements of the dye/DNA complexes with and without Au particles were performed *via* time correlated single photon counting (TCSPC) using the output from a pulsed diode laser at 440 nm (LDH-PC-440, PicoQuant GmbH) and detecting the emission through band-pass filters (Chroma) using an actively quenched photodiode detector (PDM series SPAD detector, Micro Photon Device) with ~300 ps time resolution.

Results and Discussion

The overlap of the emission peaks of the different dyes used in this study with the absorbance spectra of all three Au particles is shown in Figure 1. The 4 nm particles show the expected plasmon band centered at 525 nm while no distinct absorbance peak is seen for 2 nm particles (Figure 1(a)). On the other hand, Au₂₅ exhibits multiple discrete absorption bands (Figure 1(b)) that have been assigned using time-dependent density functional theory (TDDFT).¹⁹ Briefly the lowest-energy optical transition of Au₂₅ (670 nm, Figure 1(b)) has been assigned as a LUMO→HOMO transition wherein the HOMO and LUMO orbitals are comprised almost exclusively of atomic orbital contributions from the thirteen Au atoms in the icosahedral core. The peak at 450 nm arises from mixed intraband (sp←sp) and

interband (sp $\leftarrow$ d) transitions (Figure 1(b)) while the peak at 400 nm arises principally from an interband transition (sp $\leftarrow$ d).

The magnitude of the fluorescence quenching of each dye as a function of varying ratios of gold particles to DNA-dye complexes was computed using a Stern-Volmer (S-V) plot^{1,27,36} (Eqn. 1). The ratio of the fluorescence intensity in the absence of (I_0) and in the presence of (I) the quencher is related to the concentration of the quencher ($[Q]$) by a coefficient K_{sv}

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

Linear S-V plots were found in the experiments reported here (Figure 2), indicating that a single quenching mechanism is operative. The K_{sv} constants are reported in Table 1 and the wavelength dependences of the quenching efficiencies for all three particles are shown graphically in Figure 2(d-f).

The quenching process studied here requires the formation of ground-state complexes between the gold and the dye-labeled DNA. This follows because the gold concentrations used are at least an order magnitude too small to quench emission by diffusion to the DNA/dye complexes while the dyes are in their excited states given their 1-5 ns emission lifetimes.³⁷ Moreover, fluorescence lifetime measurements using TCSPC showed no change in the decay kinetics of the dye complexes upon addition of gold particles though their emission yield decreased substantially (see Table S1 for TCSPC data). This implies that the quenching process is faster than the temporal resolution of the TCSPC measurements. To determine whether this high quenching rate would be expected given our experimental parameters, we calculated the predicted lifetimes of dye complexes (YOYO-1/DNA and TOTO-3/DNA) at varying separation distances from 2 and 4 nm gold particles assuming that these follow NSET and FRET theory, respectively.

The results (Figure S1) clearly show that, for a separation distance less than 3 nm, the predicted lifetimes of these dye after quenching are too short to be resolved by our TCSPC instrument. Given that the gold NPs interact strongly with DNA (*vide infra*) and the DNA is saturated with the bis-intercalating dyes,³⁸ the separation between the nearest dye molecule and the gold NP/NC is likely to be much less than 3 nm. As we argue below that electron transfer is *not* the likely quenching mechanism operative here, the dominant process is instead attributed to fast energy transfer from dyes to gold NPs/NCs.

The K_{sv} values (Table 1) measured here clearly demonstrate high short-range quenching efficiencies even for the non-plasmonic particles. This is particularly striking when one considers that the surface charge of the particles is negative at the pH (8.4) of the solution as is the DNA backbone. In fact, these K_{sv} values are similar to those previously reported for positively-charged dyes with negatively charged (citrate coated) Au nanoparticles.²⁷ The large K_{sv} 's measured for the systems studied here are also in line with numerous other studies that found evidence for specific association of gold NPs with DNA though the detailed nature of the interaction is still somewhat controversial.³⁹⁻⁴¹

In order to further characterize the DNA-gold interaction and to confirm that dissociation of the dyes from DNA does not contribute significantly to the observed quenching, the following experiments were performed. First, 4 nm gold NPs were added to a 10 nM YOYO-1/DNA in molar ratio 1:1 (NP:DNA) resulting in a ~40% decrease in the emission. This is roughly the magnitude of quenching expected given the K_{sv} constant and its reported error limit (Table 1). After centrifuging at 15000 rpm for 1h to precipitate the Au NPs, the level of fluorescence of the supernatant was found to be the same as that prior to centrifugation (see Figure S2). This indicates that the quenched DNA/dye complexes

interact strongly with the Au NPs and that the two co-sediment on centrifugation. Centrifugation does not perturb the DNA/dye interaction itself as evidenced by the fact that centrifuging a 10 nM YOYO-1/DNA solution with no added NPs produces no change in emission intensity of the solution and no visible precipitate (data not shown). Moreover, adding either excess DNA or excess YOYO-1 to the supernatant following precipitation of the Au NPs did not recover any fluorescence intensity. This confirms that the amount of either free dsDNA or free YOYO-1 remaining in the solution following sedimentation of the gold is minimal. This experiment does not address whether or not the dyes dissociate from the dsDNA and then bind to the particle surface along with the DNA in either its double or single stranded forms.⁴² However, several factors argue against these processes being the predominant source of quenching in our experiments. The first is that these mechanisms would be expected to be equally efficient for all of the dye complexes used whereas the quenching efficiencies of all three particles show a distinct wavelength dependence. Secondly, efficient quenching was observed for the DNA/Click dye complex for which complete de-binding of dye from the DNA is prevented by the covalent attachment (see below). Finally, the complexes between bis-intercalating dyes and DNA are known to be very stable as evidenced by their high binding constants.⁴³ In fact Albinsson *et al.*⁴⁴ showed that YOYO-1 and TOTO-1 DNA complexes remain bound even during electrophoresis. Therefore it is likely that the majority of the dyes remain bound to DNA under the experimental conditions used here. Nonetheless, experiments are underway to further probe the nature of the interactions between DNA and these NPs and NCs.

In addition to the specific DNA-gold interactions which lead to large measured K_{sv} 's, the presence of multiple intercalating dyes on each DNA strand appears to enhance the efficiency with which they are quenched relative to that for singly-labeled strands. Evidence for this is seen by comparing the K_{sv} 's measured for mono-Click versus the tri-Click DNA complexes. For all three particles, the K_{sv} 's for the tri-Click complex are ~ 2-3 times larger than these for the mono-Click labeled DNA. The simple approximately linear scaling of the K_{sv} with the number of dyes on each DNA strand suggests that the quenching probability increases when there are more dyes potentially near the region of the DNA where the gold particle binds. One possible reason why greater quenching is observed for multiply-labeled complexes is that, even if the gold NPs interacts with only one of the dyes, rapid energy transfer among them may render the entire complex dark. This would be consistent with the findings of Albinsson⁴⁵ who showed a significant increase in the efficiency of energy transfer between two dyes covalently bound to DNA when intercalating dyes were inserted to form an intermediary donor-acceptor chain between them. Another possibility is that, in the ground-state complex formed between Au and DNA, all the dyes are close enough to the particle to undergo efficient direct energy transfer. The quenching properties of multiply-labeled DNA structures will be explored further in another contribution from our group.

Turning to the main focus of the current study, which is on the dependence of quenching efficiency on particle size and on the dye emission wavelength, comparison of the measured K_{sv} values (Table 1) shows that the 4 nm particles are at least an order of magnitude more efficient quenchers than either the 2 nm particles or the Au₂₅ NCs for dyes emitting near the wavelength of the plasmon band (LOLO-1 and YOYO-1) or at higher energy (POPO-1). However, the 4 nm particles are much less efficient quenchers for the two longer wavelength dyes, YOYO-3 and TOTO-3, which have K_{sv} 's closer to those measured for the smaller particles in this wavelength range. This striking dependence of the quenching efficiency on dye emission wavelength for the 4 nm particles is evident in Figure 2(d) which shows that the maximum quenching coincides with the plasmon peak. This is to be expected for an energy transfer mechanism. However, this comparison neglects the fact that the fluorescence yields of the two longest wavelength dyes are significantly lower than those of the others which would also cause them to be less efficiently quenched *via* a FRET-type mechanism.

We attempted to account for this effect by dividing the K_{sv} values for each dye by its quantum yield (Table 1). In so doing, the wavelength dependence of the quenching efficiency becomes less strongly peaked though its maximum remains coincident with that of the plasmon band.

Compared to what is seen for the larger plasmonic particles, the quenching efficiency of the 2 nm particles increases less steeply and more monotonically with decreasing dye emission wavelength. As described earlier, particles in this size range were shown by Strouse and coworkers to follow the predictions of NSET theory.^{24,28,46} From various definitions of energy transfer efficiency and the expressions of NSET theory (eqns 1, 2 and 5 in the SI Section S1), the following relation between K_{sv} and both dye emission wavelength (λ_{dye}) and quantum yield (ϕ_{dye}) is readily derived.^{10,28}

$$K_{sv} \propto \phi_{dye} \lambda_{dye}^2 \quad (2)$$

This expression predicts that normalizing K_{sv} to the product of the dye quantum yield and its emission wavelength squared will eliminate the observed variation in K_{sv} with wavelength. This is indeed the case for 2 nm Au particles (Table 1). Their quenching behavior is in a striking agreement with the predictions of NSET theory, except for the longest wavelength dye (TOTO-3). This may be due to the insufficient overlap of emission spectrum of this dye and the absorption of the 2 nm particles.³⁰

In contrast to what is observed for the larger particles, the quenching properties of the Au₂₅ clusters measured under our conditions are not well modeled either by NSET nor by predictions that have been made based on electronic structure theory.^{28,31} In particular, the Au₂₅ NCs quench emission at least an order of magnitude less efficiently than do the plasmonic NPs at *all* wavelengths measured. Moreover, the efficiency does not increase for dyes having emission wavelength coincident with any of the electronic transitions of the NCs. In fact, the peak quenching efficiency lies in the same wavelength region for the Au₂₅ NCs as for the 2 and 4 nm particles (490–560 nm). Moreover, Au₂₅ is a very poor quencher at wavelengths coincident with its HOMO-LUMO transition (λ_{max} =670 nm, Figure 1(b)) contrary to what has been predicted in the literature.³¹ The poor quenching of POPO-1 by Au₂₅ is also surprising, given that the emission band of this dye overlaps the strongest absorption transition in the absorption spectrum of the NC (Figure 2(f)). The NSET model¹¹ explains the variation in the efficiency of quenching by Au₂₅ for all the dyes *except* POPO-1 which deviates substantially from prediction. As this theory successfully predicts the trend in the quenching efficiency of 2 nm particles with all dyes *including* POPO-1, it appears unlikely that any vagaries in the properties of the dye itself would explain the deviation observed for Au₂₅.

As, to our knowledge, none of the existing energy-transfer-based models appear to fully explain the quenching dynamics of Au₂₅, it is worth considering whether electron transfer is a valid alternative mechanism. This is particularly true as the experiments reported here probe very short range dye-NP interactions. A few examples of emission quenching of dyes bound to gold NPs as a result of electron transfer from dyes to NPs have been described in literature.^{1,47} One relevant example is the recent report by Devadas *et al.* of the fluorescence quenching of pyrene attached to Au₂₅ NCs due to electron transfer with Au₂₅ acting as the electron donor.⁴⁸

To examine the possible role played by electron transfer in the quenching process, we calculated the expected free energies for excited state electron transfer quenching, $\Delta G^0(ET)$, for three of the dyes studied here using the electrochemical data of the oxidation (dye^+ /

dye^{*2+}) and reduction (dye⁺/dye^{*}) of their mono-cationic cyanine dye analogues as given in ref 37. Comparing the reduction potential of the 4 nm Au NPs (~ 0.25 V *vs.* SCE, assumed to be the same as the Fermi level of gold)⁴⁷ to those of the dye analogs reported in the literature (-1.70 V, -1.3 V and -1.08 V for POPO-1, YOYO-1 and YOYO-3 analogs, respectively, all *vs.* SCE)³⁷ suggests that these particles can quench dye emission by accepting an electron. In contrast, Au₂₅ may act as an electron donor as its oxidation potential (assumed to be the same as that of anionic phenylethylthiolate [(SCH₂CH₂Ph)₁₈] capped Au₂₅ (Au⁻/Au, -0.085 V *vs.* SCE)⁴⁹⁻⁵⁰) is higher than those reported for mono-cationic analogues of POPO-1 (-1.1 V), YOYO-1 (-1.14 V) and YOYO-3 (-0.72 V).

With these data, the ΔG^0 values for electron transfer from POPO-1, YOYO-1 and YOYO-3 to 4 nm Au NPs are calculated to be -1.94 eV, -1.59 eV and -1.53 eV respectively (details in SI Section S2). Similarly, the ΔG^0 values for electron transfer from Au₂₅ nanoclusters to POPO-1, YOYO-1 and YOYO-3 are -1.01 eV, -1.10 eV and -0.84 eV respectively.

Assuming that all three dyes have similar electronic coupling and reorganization energies, the calculated ΔG^0 values should correlate with the expected relative electron transfer quenching rates for these dyes.⁵¹ Though the negative values of the ΔG^0 indicate that electron transfer between the various Au particles and dyes used here is energetically feasible, the trend in the observed quenching efficiencies of these dyes by gold NPs/NCs is not in agreement with the predicted ΔG^0 values for these processes. For example, based on the calculated ΔG^0 values, the quenching of POPO-1 by 4 nm Au NPs is predicted to be more favorable than for YOYO-1 though the reverse is seen. Similarly, the electron transfer mechanism is inconsistent with the fact that the quenching efficiency of Au₂₅ is one order magnitude higher for YOYO-1 than that for POPO-1 as both processes have similar calculated ΔG^0 values. It is certainly true that accurately predicting whether or not these dyes will undergo electron transfer with Au NPs and NCs requires information that is currently unavailable on the geometries, electronic couplings, and reorganization energies of the complexes.³⁷ Moreover, even the dye reduction potentials used to estimate the ΔG^0 values are based on those of model compounds. Nonetheless, given the available data, the electron-transfer mechanism does not explain the wavelength dependence of the quenching efficiencies measured here.

Summary and Conclusions

The quenching efficiencies of both Au₂₅ NCs and 2 nm NPs were determined to be roughly one order of magnitude less than that of plasmonic 4 nm particles in an assay based on the concentration-dependent quenching of non-covalently bound DNA/dye complexes. Analysis of the dependence of the quenching efficiency on the emission wavelength and quantum yield of the dyes revealed three different regimes of behavior. The 4 nm NPs were the most efficient quenchers of dyes having emission bands overlapping the plasmon resonance. For 2 nm particles, the quenching decreases essentially monotonically with decreasing dye emission wavelength, a trend which fits well to the NSET theory put forward by Strouse, *et al.*^{28,46} The wavelength dependence of the quenching efficiency of the Au₂₅ particles, however, could not be explained by the NSET relationship nor by considering the effect of spectral overlap with the discrete electronic transitions of the NC as proposed in ref. 31. Moreover, a mechanism based on electron transfer is also inconsistent with the observed trends. Understanding the emission quenching behavior of NCs may therefore require a model different from those which successfully describe larger NPs and metallic species. Further mechanistic studies are underway in our laboratory to explore this phenomenon.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

B. A. A. acknowledges the NIH (R01GM080994 and the donors of the American Chemical Society's Petroleum Research Fund (44470-AC4) for financial support of this research. L. A. P. acknowledges the NSF (CHE-079112 and CHE-1012529).

References

- (1). Nikoobakht B, Burda C, Braun M, Hun M, El-Sayed MA. Photochem. Photobiol. 2002; 75:591. [PubMed: 12081320]
- (2). Malicka J, Gryczynski I, Fang J, Kusba J, Lakowicz JR. Anal. Biochem. 2003; 315:160. [PubMed: 12689825]
- (3). Gueroui Z, Libchaber A. Phys. Rev. Lett. 2004; 93:166108/1. [PubMed: 15525013]
- (4). Liu N, Prall BS, Klimov VI. J. Am. Chem. Soc. 2006; 128:15362. [PubMed: 17131988]
- (5). Tam F, Goodrich GP, Johnson BR, Halas HJ. Nano Lett. 2007; 7:496. [PubMed: 17256995]
- (6). Chen Y, Munechika K, Jen-La Plante I, Munro AM, Skrabalak SE, Xia Y, Ginger DS. App. Phys. Lett. 2008; 93:053106/1.
- (7). Chen F-C, Wu J-L, Lee C-L, Hong Y, Kuo CH, Huang MH. App. Phys. Lett. 2009; 95:013305/1.
- (8). Chance RR, Prock A, Silbey R. Adv. Chem. Phys. 1978; 37:1.
- (9). Gersten JI, Nitzan A. Surf. Sci. 1985; 158:165.
- (10). Persson BNJ, Lang ND. Phys. Rev. B. 1982; 26:5409.
- (11). Yun CS, Javier A, Jennings T, Fisher M, Hira S, Peterson S, Hopkins B, Reich NO, Strouse GF. J. Am. Chem. Soc. 2005; 127:3115. [PubMed: 15740151]
- (12). Ghosh SK, Pal A, Kundu S, Nath S, Pal T. Chem. Phys. Lett. 2004; 395:366.
- (13). Fan C, Wang S, Hong JW, Bazan G, Plaxco KW, Heeger AJ. Proc. Nat. Acad. Sci. 2003; 100:6297. [PubMed: 12750470]
- (14). Dulkeith E, Morteaux AC, Niedereichholz T, Klar TA, Feldmann J, Moller M, Gittins DI. Phys. Rev. Lett. 2002; 89:203002/1. [PubMed: 12443474]
- (15). Lakowicz JR. Anal. Biochem. 2005; 337:171. [PubMed: 15691498]
- (16). Qian H, Eckenhoff WT, Zhu Y, Pintauer T, Jin R. J. Am. Chem. Soc. 2010; 132:8280. [PubMed: 20515047]
- (17). Qian H, Jin R. Nano Lett. 2009; 9:4083. [PubMed: 19995083]
- (18). Wu Z, Chen J, Jin R. Adv. Funct. Mater. 2011; 21:177.
- (19). Zhu M, Aikens CM, Hollander FJ, Schatz GC, Jin R. J. Am. Chem. Soc. 2008; 130:5883. [PubMed: 18407639]
- (20). Zhu M, Lanni E, Garg N, Bier ME, Jin R. J. Am. Chem. Soc. 2008; 130:1138. [PubMed: 18183983]
- (21). Kelly KL, Coronado E, Zhao LL, Schatz GC. J. Phys. Chem. B. 2002; 107:668.
- (22). Zhu M, Eckenhoff WT, Pintauer T, Jin R. J. Phys. Chem. C. 2008; 112:14221.
- (23). Alvarez MM, Khoury JT, Schaaff TG, Shafiguillin MN, Vezmar I, Whetten RL. J. Phys. Chem. B. 1997; 101:3706.
- (24). Singh MP, Strouse GF. J. Am. Chem. Soc. 2010; 132:9383. [PubMed: 20560666]
- (25). Dubertret B, Calame M, Libchaber AJ. Nat. Biotech. 2001; 19:365.
- (26). Lu Y, Dasog M, Leontowich AFG, Scott RWJ, Paige MF. J. Phys. Chem. C. 2010; 114:17446.
- (27). Cheng PPH, Silvester D, Wang G, Kalyuzhny G, Douglas A, Murray RW. J. Phys. Chem. B. 2006; 110:4637. [PubMed: 16526695]
- (28). Jennings T, Singh MP, Strouse GF. J. Am. Chem. Soc. 2006; 128:5462. [PubMed: 16620118]
- (29). Persson BNJ, Lang ND. Phys. Rev. B. 1982; 26:5409.
- (30). Singh MP, Strouse GF. J. Am. Chem. Soc. 2010; 132:9383. [PubMed: 20560666]
- (31). Munöz-Losa A, Vukovic S, Corni S, Mennucci B. J. Phys. Chem. C. 2009; 113:16364.
- (32). Bowen BP, Woodbury NW. Photochem. Photobiol. 2003; 78:582. [PubMed: 14743866]

- (33). Rye H, Yue S, Wemmer DE, Quesada MA, Haugland RP, Mathies RA, Glazer AN. *Nucleic Acid Res.* 1992; 20:2803. [PubMed: 1614866]
- (34). Stadler AL, Delos Santos JO, Stensrud ES, Dembska A, Silva GL, Liu S, Shank NI, Kunttas-Tatli E, Sobers CJ, Gramlich PME, Carell T, Peteanu LA, McCartney BM, Armitage BA. *Bioconjugate Chem.* 2011; 22:1491.
- (35). Orden AV, Cai H, Goodwin PM, Keller RA. *Anal. Chem.* 1999; 71:2108. [PubMed: 21662745]
- (36). Gierlich J, Burley GA, Gramlich PME, Hammond DM, Carell T. *Org. Lett.* 2006;3639. [PubMed: 16898780]
- (37). Netzel TL, Nafisi K, Zhao M, Lenhard JR, Johnson I. *J. Phys. Chem. B.* 1995; 99:17936.
- (38). Günther K, Mertig M, Seidel R. *Nucl. Acids Res.* 2010; 38:6526. [PubMed: 20511588]
- (39). Cárdenas M, Barauskas J, Schillén K, Brennan JL, Brust M, Nylander T. *Langmuir.* 2006; 22:3294. [PubMed: 16548591]
- (40). Gearheart LA, Ploehn HJ, Murphy CJ. *J. Phys. Chem. B.* 2001; 105:12609.
- (41). Sandström P, Boncheva M, Åkerman B. *Langmuir.* 2003; 19:7537.
- (42). Yang J, Pong B-K, Lee JY, Too H-P. *J. Inorg. Biochem.* 2007; 101:824. [PubMed: 17368547]
- (43). Glazer AN, Rye HS. *Nature.* 1992; 359:859. [PubMed: 1436062]
- (44). Larsson A, Carlsson C, Jonsson M, Albinsson B. *J. Am. Chem. Soc.* 1994; 116:8459.
- (45). Hannestad JK, Sandin P, Albinsson B. *J. Am. Chem. Soc.* 2008; 130:15889. [PubMed: 18975869]
- (46). Jennings T, Schlatterer JC, Singh MP, Greenbaum NL, Strouse GF. *Nano Lett.* 2006; 6:1318. [PubMed: 16834403]
- (47). Barazzouk S, Kamat PV, Hotchandani S. *J. Phys. Chem. B.* 2004; 109:716. [PubMed: 16866432]
- (48). Devadas MS, Kwak K, Park J-W, Choi J-H, Jun C-H, Sinn E, Ramakrishna G, Lee D. *J. Phys. Chem. Lett.* 2010; 1:1497.
- (49). Wu Z, Gayathri C, Gil RR, Jin R. *J. Am. Chem. Soc.* 2009; 131:6535. [PubMed: 19379012]
- (50). Parker JF, Kacprzak KA, Lopez-Acevedo O, Hakkinen H, Murray RW. *J Phys. Chem. C.* 2010; 114:8276.
- (51). Moser CC, Keske JM, Warncke K, Farid RS, Dutton PL. *Nature.* 1992; 355:796. [PubMed: 1311417]
- (52). Haugland, RP. *Molecular Probes*, Inc; Eugene OR: 1996.

Figure 1.

Absorption spectra of glutathionate (-SG) coated (a) 2 nm and 4 nm particles, and (b) Au₂₅(SG)₁₈ clusters. Normalized emission spectra of various intercalating dyes used in the present study are shown in both (a) and (b). Spectra are arbitrarily scaled.

Figure 2.

S-V plots for quenching of various dyes intercalated in 30 bp dsDNA by (a) 4 nm (b) 2 nm and (c) Au₂₅ particles. I_0 and I are defined in the text. Comparison of the quenching of various intercalating dyes in DNA after the addition of (d) 10 nM 4 nm NPs (e) 100 nM of 2 nm Au NPs and (f) 100 nM Au₂₅ NCs. The DNA to gold particle ratio is ~1:1 in all cases. Solid lines represent the emission spectrum of the dye/DNA complex in the absence of quencher with the intensity normalized to 1. Dotted lines represent the emission spectrum of dye/DNA complex with added NPs or NCs scaled to show the intensity relative to that of the dye/DNA complexes alone. Absorption spectra are scaled arbitrarily

Chart 1.

Chemical structures of the intercalating dyes and of the ligand used to stabilize the gold NPs and NCs.

Chart 2.

The chemical structure of the Click dye TO and a schematic of the dye/DNA construct. The dye is represented by the blue ellipsoid.

Table 1

Collisional quenching constants for various dyes by 2 nm and 4 nm NPs and Au₂₅ NCs.

Dye Properties														
Dyes	λ_{abs}	a	ϵ_{max}	b	ϕ_{dye}	a	λ_{em}	a	$K_{SV} \times 10^{-8} \epsilon$				$\frac{K_{SV}}{\phi_{dye} \lambda_{em}^2} \times 10^{-3}$	
									Au ₂₅	2 nm	4 nm	Au ₂₅	2 nm	4 nm
POPO-1	434		92400		0.60	456			0.01	0.1	0.6	0.01	0.1	0.5
YOYO-1	491		99000		0.52	513			0.2	0.1	2	0.4	0.1	1
LOLO-1	566		108000		0.40	589			0.2	0.2	2	0.5	0.1	1
YOYO-3	612		167000		0.15	628			0.04	0.06	0.4	0.3	0.1	0.7
TOTO-3	642		154000		0.06	663			0.02	0.05	0.1	0.3	0.1	0.4
mono-Click	490		62800		0.20	530			0.01	0.05	0.1	-	-	-
tri-Click	490					530			0.02	0.1	0.3	-	-	-

^a Absorption (λ_{abs}) and fluorescence emission (λ_{em}) maxima in nm.^b Molar extinction coefficients (ϵ_{max}) in $\text{cm}^{-1} \text{M}^{-1}$ and quantum yields (ϕ_{dye}) of non-covalent dyes are taken from ref. 52.^c The standard deviation for SV constants (K_{SV}) in M^{-1} is ~ 25%.