Making Gold Nanoparticles Glow: Enhanced Emission from a Surface-Bound Fluoroprobe

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In recent years, a burst of research activities has been seen in the synthesis and characterization of semiconductor and noble metal nanoclusters, since materials in the nanometer size regime display size-dependent optical, electronic, and chemical properties (see, for example, refs 1−8). There are numerous possible applications for such nanoscale materials in developing biological nanosensors and optoelectronic nanodevices.[9−11] Engineering of the nanocluster surfaces, with electro- or photoactive molecules, can provide three-dimensional molecular arrangements around the nanoparticles. Researchers have often used functional groups such as thiols, amines, or silanes to attach electroactive or photoactive molecules to the gold surface.[12−16] The ability of the gold surface to bind with specific functional groups has made it suitable for optoelectronic applications such as fluorescence patterning.[17] Colloidal gold particles adsorb strongly to some proteins, especially antibodies. Colloidal gold functionalized with specific binding groups can be used to label a wide variety of biologically active molecules, such as lipids, oligonucleotides, and peptides. Since fluorescence spectroscopy is a very sensitive technique, fluorophore-bound gold nanoparticles are useful probes for biomolecular labeling (e.g., as immunoprobes).[11,18,19] Such an inorganic−organic hybrid probe also offers the possibility of using complementary sensing via fluorescence spectroscopy (e.g., confocal microscope) and electron microscopy.

Efforts to extend the chemical functionalization concept to gold nanoparticles in aqueous and nonaqueous media have often encountered stability problems. Surface charge neutralization, which accompanies the binding of a thio or amine group to gold nanoparticles, induces aggregation and shifts the absorption to the infrared.[20] We have now succeeded in organizing fluoroprobe molecules on the gold nanoparticles without inducing such aggregation effects.

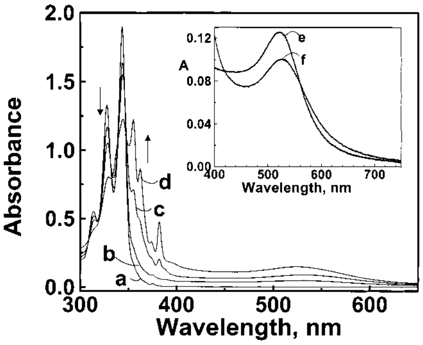

Figure 1. Effect of gold concentration, (Au) on the changes in the absorption spectrum of Py-CH_2NH_2 [60 μM] in THF: (a) 0; (b) 20.3; (c) 27, and (d) 47 μM of Au. Inset shows the absorption spectra of 34 μM of Au in the absence and presence of 20 μM Py-CH_2NH_2. All solutions contain 2.4 mM of tetraoctylammonium bromide (TOAB).

Highly concentrated (11 mM) gold nanoparticles, soluble in toluene, were prepared, according to the previously reported procedure.[21,22] The gold suspension in toluene was diluted with THF to achieve the desired concentrations (THF solutions used in these studies thus contained ∼0.5% of toluene, and the concentration of TOAB in the resultant solution is ∼2.4 mM). Transmission electron micrographs of gold solution in THF showed nearly spherical particles with a diameter of 5−8 nm. A known amount of gold was added to the Py-CH_2NH_2 (Molecular Probes) solution and allowed to stand at least 6−8 h to complete the surface complexation process.

The absorption spectrum of gold nanoparticles (34 μM) in THF (trace e in the inset of Figure 1) exhibits a surface plasmon absorption band (max. at 523 nm). The position and shape of the plasmon absorption of metal nanoclusters are strongly dependent on the particle size, dielectric medium, and surface-adsorbed species.[23−25] In the presence of monomeric Py-CH_2NH_2, we observe a dampening of the gold surface plasmon band at 523 nm (trace f in the inset in Figure 1). A more pronounced effect of surface binding is seen in the UV spectrum of the pyrene chromophore. THF solution of Py-CH_2NH_2 possesses well-defined absorption bands in the UV region (313, 327.5, and 343.5 nm) corresponding to the monomer form of the pyrene chromophore (trace a, Figure 1). Upon addition of gold colloids, new absorption bands appear at 354, 361, 373, and 382 nm with a concomitant decrease in the monomer absorption bands of Py-CH_2NH_2 (trace b−d, Figure 1). The changes in the absorption spectrum reflect the alteration of the electronic properties of the pyrene chromophore as it binds to the gold nanoparticle. The observation of isosbestic points at 319, 331, 338, and 347 nm in the absorption spectra recorded at varying gold nanoparticle concentrations confirms the existence of two forms of Py-CH_2NH_2, viz., free and bound forms of Py-CH_2NH_2 (Scheme 1). The absorption

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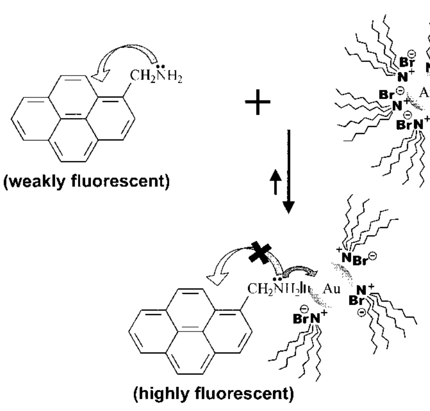
Scheme 1. Binding of Py-CH_2NH_2 to Gold Nanoparticles



changes resulting from surface binding occur with a relatively slow rate and all the spectra were recorded after 8 h of mixing of Py-CH_2NH_2 and gold nanoparticles in THF. Preliminary NMR studies carried out with ^{15}N-labeled compounds indicate a strong interaction between the amine functional group and the gold surface.[26]

An interesting observation of these Py-CH_2NH_2-bound gold nanoparticles in organic solvents is the appearance of a prominent blue emission upon illumination with UV-light. The gold nanoparticles are nonfluorescent and Py-CH_2NH_2 in THF containing 2.4 mM of TOAB exhibits weak florescence ($\phi_f = 0.09$) with emission maxima at 377 and 397 nm. The Py-CH_2NH_2 bound to gold nanoparticles exhibit strong emission bands at 383 and 403 nm (Figure 2B). The red-shift in the emission peaks parallels the shift in absorption bands (see Figure 1). These new electronic transitions of the pyrene chromophore become allowed as the amine group binds strongly to the gold particle. No such spectral shifts or enhanced emission could be seen when we added a THF solution of Py-CH_2NH_2 containing tetraoctylammonium bromide and treated with $NaBH_4$.

The origin of the broad emission in the visible was evaluated by recording the excitation spectrum (Figure 2A) of Py-CH_2NH_2 in the presence of gold nanoparticles. The excitation spectral features (excitation intensity peaks at 354, 361, 373, and 381 nm) of the Py-CH_2NH_2 (emission monitored at 410 nm) adsorbed on gold nanoparticles match the absorption features of Py-CH_2NH_2 bound to the gold surface (spectrum e in Figure 1). This further confirms that the observed emission in the gold suspension arises from the direct excitation of the bound Py-CH_2NH_2 and the contribution from the free (unbound) Py-CH_2NH_2 to the overall emission is negligible.

The fluorescence quantum yield of surface-bound Py-CH_2NH_2 is as high as 0.48 at a gold concentration of 30 μM.[27] The emission lifetimes of Py-CH_2NH_2 bound gold nanoparticles varied little. The unbound and bound species exhibited lifetimes of 18 ± 0.3 and 14 ± 0.3 ns, respectively. Although excimer emission of pyrene is commonly observed in heterogeneous media such as in silica or zeolites, aminomethyl pyrene bound to gold nanoparticles failed to exhibit such intramolecular interactions. On the basis of the absorption, steady-state emission, and singlet lifetime measurements, it is concluded that Py-CH_2NH_2 bound to gold nanoparticles is the only emitting species. Coexistence of TOAB around the gold nanoparticle is likely to isolate the pyrene moieties on the gold surface and thus prevent intermolecular interactions in the excited state. The triplet quantum yield values determined for bound and unbound forms of Py-CH_2NH_2 were found to be similar ($\Phi_T = 0.32 \pm 0.4$). These results further confirm that surface binding has a significant effect on the fluorescence yield, but it has no observable effect on the intersystem crossing efficiency.

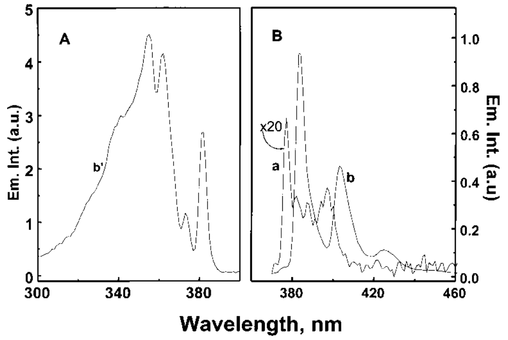


Figure 2. A. The excitation spectrum of Py-CH_2NH_2 bound gold nanoparticles (emission was followed at 410 nm). B. The emission spectra of Py-CH_2NH_2, Py-CH_2NH_2 bound gold nanoparticles in THF (excitation wavelengths were 321 and 361 nm, respectively. The intensity of the emission spectra of unbound Py-CH_2NH_2 has been multiplied by a factor of 20 for the purpose of comparison.) All solutions contain 2.4 mM of tetraoctylammonium bromide (TOAB).

The observed increase in the fluorescence yield therefore reflects the suppression of the nonradiative decay processes upon binding to gold nanoparticles. The low fluorescence yield of Py-CH_2NH_2 in THF is attributed to the intramolecular quenching of the singlet excited state. The photoinduced electron transfer between the lone pair of the amine and the pyrene moiety competes with the radiative and nonradiative decay of the singlet excited state. Upon binding of the amino group to the gold surface, the electron-donating ability of the amine group is decreased and this in turn suppresses the electron transfer from its lone pair to the pyrene moiety. A similar chelation-enhanced fluorescence has been reported earlier by Czarnick and co-workers[28,29] and de Silva and co-workers.[30] By binding metal cations to amine functional groups of probes (e.g., anthracene), they were able to demonstrate the suppression of intramolecular quenching.

The suppression of the electron-transfer process was also confirmed by protonating pyrenemethylamine using trifluoroactic acid. An enhancement of fluorescence quantum yield was observed on addition of trifluroacetic acid to a THF solution of 1-aminomethylpyrene (ϕ_f of pyrenemethylamine containing 1 mM of trifluoroacetic acid is 0.45). The fluorescence quantum yields obtained for protonated pyrenemethylamine and for the Py-CH_2NH_2 bound to the surface of gold nanoparticles are almost the same, further supporting the mechanism of enhancement of pyrene fluorescence via the inhibition of electron transfer from the lone pair of the amine group.

In conclusion, we have succeeded in achieving organization of pyrene chromophores around gold nanoparticles, through surface binding of the amine moiety. These inorganic−organic hybrid materials soluble in organic solvents are fluorescent and are potentially useful for the fabrication of biological nanosensors, biochemical labeling, and optoelectronic nanodevices.

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Supporting Information Available: Details of ^{15}N NMR spectroscopy (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(26) See Supporting Information.

(27) Fluorescence quantum yields were determined using quinine bisulfate in 0.5 M H_2SO_4 solution ($\Phi_{ref} = 0.55$) as reference. Fluorescence lifetimes were measured using a PTI lifetime measurement apparatus (Ex. 337 nm).

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