Ultrabright Supramolecular Beacons Based on the Self-Assembly of Two-Photon Chromophores on Metal Nanoparticles

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There is currently great interest in nanoscience due to the many potential applications in the fields of nanoelectronics[1] and ultrasensitive biomolecular detection.[2] Ligand-coated metal nanoparticles are very promising building blocks for applications in nanotechnology.[3] They are readily synthesized,[4] they show remarkable stability,[5] and they can be polyfunctionalized,[6] allowing for significant flexibility in the tailoring of their optical, electronic, and chemical properties. Two-photon absorption is a nonlinear optical property that is currently under extensive investigation for applications such as biological imaging,[7] 3-D microfabrication,[8] and optical limiting.[9] In biological imaging, there is a need for fluorescent labels with a large two-photon cross-section (δ) and high quantum efficiency (η) to enable detection at the single label level. One approach to the development of such high sensitivity labels is to concentrate a number of fluorophores into a supramolecular assembly.[10]

Here we report on the preparation of the first supramolecular assembly consisting completely of a self-assembled layer of chromophores on a metal nanoparticle core, which allows for packing of ~2500 chromophores within a sphere of <10 nm diameter. The resulting nanoparticles act as ultrabright two-photon fluorescent nanobeacons, with an effective δ of ~2500 times that of the individual chromophores, a large η, and a decreased sensitivity to photobleaching.

The chromophore-**1**-coated nanoparticles (**1-Np**) were synthesized in 71% yield using a modification of the method of Kim et al.,[11] involving reduction of $AgNO_3$ with $NaBH_4$ in the presence of chromophore-functionalized alkylthiol ligands (**1**, see Figure 1) in ethanol at 0 °C. The acceptor$-\pi-$acceptor (A$-\pi-$A) design of the chromophore was chosen to impart a high δ[12] and a moderate electron deficiency, such that it would neither reduce silver ions nor react with sodium borohydride. Ligand **1** possesses these properties and has a large $\eta\delta$ (see Supporting Information for the ligand synthesis).

Synthesis of the chromophore-coated silver nanoparticles using this approach allows for a large chromophore density[13] in the ligand shell (Figure 1) as compared to that typically achieved by place exchange[14] or sequential reactions.[15] For many purposes, nanoparticles that are polyfunctionalized with ligands that provide different functions, for example, specific binding or solubility properties, would be very useful.[6] To demonstrate the feasibility of the polyfunctionalization for these particles, we synthesized mixed-ligand-coated silver nanoparticles using **1**, 1-dodecylthiol, and 1-octylthiol. Such nanoparticles were prepared both by reduction of $AgNO_3$ in the presence of a mixture of the ligands and by a place exchange reaction starting with either octylthiol/dodecylthiol-coated nanoparticles or **1-Np**. We were able to prepare particles with varying numbers of chromophores in their ligand shell (~10
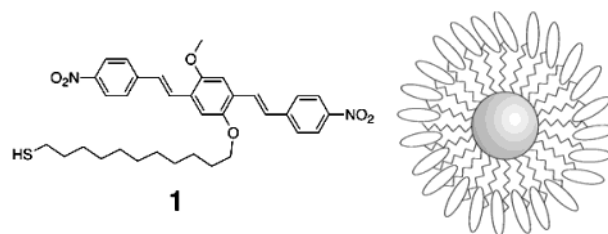
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Figure 1. Left: Structure of dye **1**. Right: Schematic representation of silver nanoparticles coated with densely packed chromophores.

to ~2500). To impart a specific chemical functionality to the particle, we performed a place exchange reaction of the pure **1-Np** with 11-mercaptoundecanoic acid[16] and obtained fluorescent particles that were soluble in water/ethanol (95:5 vol/vol).

1-Np was found to be relatively insoluble in common organic solvents and showed appreciable solubility (~10^{-5} M) only in 1,2-dichlorobenzene under reflux conditions (180 °C). In contrast, mixed-ligand nanoparticles with a 1.53:1 ratio of octylthiol and dodecylthiol to **1** (O-D/**1-Np**) were soluble in dichloromethane and toluene (~10^{-3} M). It is known that the ligands of alkylthiol-coated nanoparticles can strongly interdigitate.[17] We have found that the solubility of ligand-coated nanoparticles can be correlated with the free energy of interdigitation and that the mixed-ligand nanoparticles are typically more soluble than their monoligand counterparts.[18] An endothermic transition was observed by differential scanning calorimetry for **1-Np** at higher temperatures (200 °C) than that which is typical for the de-interdigitation of alkylthiol-coated nanoparticles (130 °C), but with a lower enthalpy change (2.2 kJ/mol ligand vs ~20 kJ/mol ligand for 1-octylthiol-coated particles). 1,2-Dichlorobenzene was chosen because its boiling point is near the onset of the endothermic peak at 200 °C for **1-Np**. Thus, if the transition involves breakup of interparticle interactions (such as $\pi-\pi$ stacking of the chromophores), it would facilitate dissolution of the particles at a temperature that is lower than the decomposition temperature (240 °C). The particles did not precipitate even after dilution with other solvents, such as dichloromethane and toluene.

The diameter distribution of the particles was obtained using transmission electron microscopy (TEM). The molar ratio of silver atoms to ligand molecules was determined by elemental analysis. Using the mean diameter and the molar ratio, we estimated the number of ligands per particle. For example, **1-Np** was found to have an average diameter of 6.5 $\pm$ 2 nm (1σ), see Figure 3. The average ratio of silver atoms to ligands is 3.4; thus the average particle has ~2500 ligands on the surface. These data indicate a coverage of ~90%, showing a very high density of dye in the outside shell of the particles.

Infrared spectroscopy showed that the benzene ring stretching modes at ~1595 and 1520 cm^{-1}, as well as a mode (nitro group stretch) at 1345 cm^{-1}, shifted to lower frequency (e.g., 1345 to
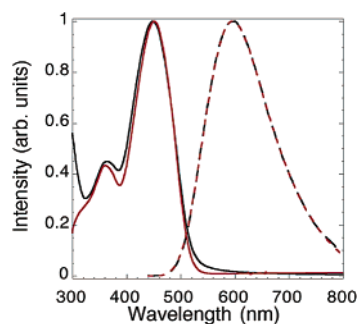
Figure 2. Absorption (solid line) and fluorescence (dashed line) spectra of **1** (red) and of **1-Np** (black) in 1,2-dichlorobenzene.
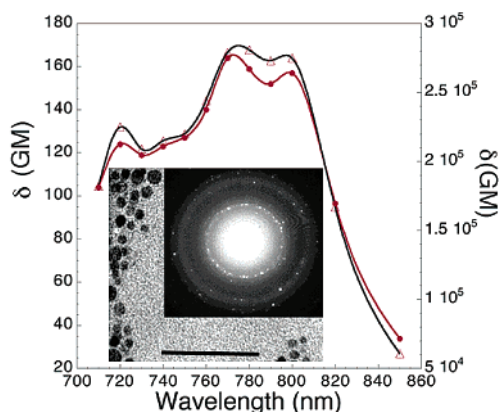

Figure 3. Two-photon absorption spectra of **1** (red, abscissa on the left) and of **1-Np** (black, abscissa on the right) in 1,2-dichlorobenzene. Inset: TEM image and electron diffraction pattern of **1-Np**. Scale bar 66 nm.

1330 cm^{-1}) on going from the free ligand **1** in chloroform solution to crystallites of **1** in KBr pellets. The band frequencies for the O-D/**1-Np** and the **1-Np** (both nanoparticle samples in KBr pellets) were intermediate to those for the ligand in the solution and solid state, see Supporting Information. These results suggest that the environment of the chromophores on the nanoparticles is intermediate to the solution and solid state of the unbound chromophore. Electrochemical measurements indicated that there was no change in reduction potential on going from the free dye to the dye bound to the nanoparticle (both in solution), ruling out a significant electronic coupling of the ligand molecular orbitals and the metal electron levels.

The absorption and fluorescence spectra of the **1-Np** in solution are nearly identical to those of free **1** (Figure 2).[19] Little difference was observed in the fluorescence quantum yield of the free dye ($\eta = 0.47$) and the dye-coated mono- or mixed-ligand nanoparticles ($\eta = 0.33$) in solution, consistent with the small spectral overlap of the dye fluorescence and the nanoparticle absorption. The same trend was observed for the fluorescence lifetime (2.13 ns vs 1.85 ns).[20] The optical spectroscopy indicates that there is little interaction between the bound chromophores for the nanoparticles in solution, in contrast to the IR evidence for the nanoparticles dispersed in the solid. These results suggest that a large degree of disorder in the arrangement of the chromophores is induced by the high temperature used to solvate the nanoparticles.

Two-photon fluorescence excitation spectra of **1** and **1-Np** have the same shape as shown in Figure 3, in accord with the one-photon spectra. **1** showed a peak δ value of 165 GM (1 GM = 10^{-50} cm^4 s photon^{-1}). The nanoparticles showed a huge per-particle δ value of 2.7×10^5 GM and an $\eta\delta$ of 0.9×10^5 GM. The value for the **1-Np** is consistent with a linearly additive contribution from the roughly 2500 chromophores and no significant enhancement induced by the nanoparticle.[21]

The two-photon action cross section ($\eta\delta$) of the **1**-coated nanoparticle is one of the highest ever reported[10b,12,22] and demonstrates the utility of using the particles as a tool for concentrating chromophores. The optical properties coupled with polyfunctionalization to provide water solubility may allow for applications in biological imaging. We believe that the use of nanoparticles as a carrier for large numbers of chromophores is promising and may have an impact on ultrasensitive detection.

Supporting Information Available: Synthesis, infrared, and optical spectra of **1** and of **1-Np** (mixed- and mono-ligand) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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