Second Harmonic Generation Properties of Fluorescent Polymer-Encapsulated Gold Nanoparticles

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It has been known for some time that second harmonic generation (SHG) can be greatly enhanced ($\sim 10^4$) for molecules on a roughened versus an unroughened metal surface. This effect is generally thought to arise, through some combination of a surface plasmon resonance and the conona effect.[1] Similarly, very large enhancements ($\sim 10^6$) have been observed in surface enhanced Raman scattering (SERS) from organic dyes on several metallic surfaces.[2,3] More recently, these enhancements have been observed at the single-particle level as well.[4−9] There is a vast literature describing the utility of SHG in probing chemical and physical processes from bulk interfaces. However, only recently has SHG been used to probe particles on the micron and submicron size scale.[10] The minimum size limit for spherical particles has been shown by Eisenthal and co-workers to be approximately $\lambda/10$ for plastic beads and oil droplets.[10] SHG has been also observed in smaller, asymmetric gold particles in the 30 nm range.[9] There have also been recent theoretical treatments of the SHG properties of gold nanoparticles. [11,12]

In this work, we report on the SHG properties of a naphthyl styryl chromophore that is chemically linked to polymer-coated 100 nm gold nanoparticles. The particles were coated with a styrene/methacrylic acid copolymer[13] and linked to the dye via an activated succinimidyl ester. The structure of the dye JPW4041 and cartoon of a conjugated particle is shown in the left panel of Figure 1. This class of dye was chosen for two reasons: First, the chromophore possesses environmentally sensitive photophysics[14] and, second, it has a large second-order response ($\sim 10^{-27}$ esu).[15] These particles are novel because both the dye (membrane-bound) and the gold colloids will individually produce weak SHG, while the combination produces significantly enhanced second harmonic signals relative to either component. Using high-resolution optical imaging, we first demonstrate efficient SHG imaging from these particles in freely diffusing aqueous solution relative to conjugated latex beads (lower bound of 100 fold). In addition, we used these new constructs to selectively label the cellular membrane and show that an approximately 20-fold enhancement in SHG intensity is achieved relative to cells stained with the unconjugated chromophore.

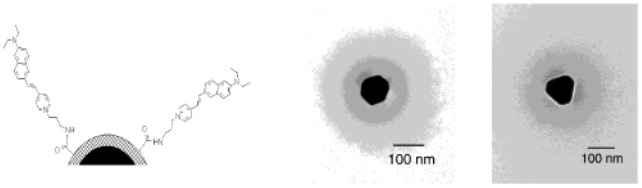

Figure 1. Left: A cartoon of the partial surface of a dye-conjugated gold particle with the chemical structure of JPW4041. Right: TEM image of a uncoated (left) and coated (right) nanoparticle where the white negative staining shows the uniform coverage of the polymer.

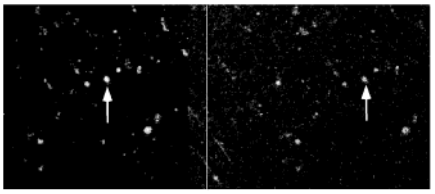

Figure 2. SHG (left) and TPEF (right) images of the same field of dye-conjugated nanoparticles in aqueous buffer. The arrows point to the same particles in each modality.

The preparation of the polymer-coated gold particles was based on a procedure for coating silver colloids with a mixture of styrene and methacrylic acid.[13] A detailed description of the synthesis is given in the Supporting Information. A TEM of one uncoated and coated 100 nm particle is shown in the left and right panels, respectively, on the right in Figure 1. The even coating of polymer appears as a white ring around the particle and is approximately 3 nm thick. The colloids were then linked to the amino group of the voltage-sensitive dye JPW4041 (prepared by a modification of the Pd-coupling procedure previously described)[16] via a succinimidyl ester.

The SHG/two-photon excited fluorescence (TPEF) imaging apparatus consists of a femtosecond titanium sapphire oscillator (Coherent 900-F) operating at 840 nm coupled to a modified laser-scanning confocal microscope.[17] In the left and right panels of Figure 2 are shown the respective SHG and TPEF images of the dye/nanoparticle conjugates in an aqueous environment. Each small bright point corresponds to a single particle ($\sim$100 nm diameter) or a small aggregate. As controls, first the laser was taken out of mode-locking operation, and both the SHG and TPEF signals disappeared, indicating the signals arose from a nonlinear process. Next, excitation of unlabeled gold particles in aqueous solution produced no observable SHG signals at the power densities utilized. In addition, photobleaching of the particle-bound dye eliminated SHG (and TPEF), indicating the signal depended upon the dye and gold particle (free dye produces no SHG). Similarly, 100 nm latex beads conjugated to JPW4041 (via the same chemistry as the 100 nm gold particles) had high TPEF in water, but produced no observable SH signals. These controls

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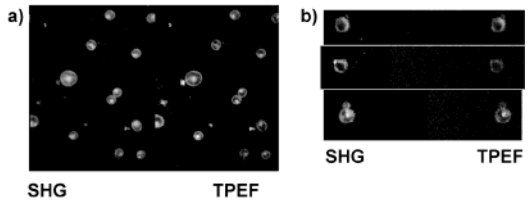

Figure 3. (a) SHG (left) and TPEF (right) images of the same field of L1210 lymphocytes stained with JPW4041. (b) SHG (left) and TPEF (right) images of the same field of L1210 lymphocytes stained with dye-conjugated nanoparticles. Each cell is approximately 10 micrometers in diameter.

indicate the second harmonic signals arose from the dye conjugated to the gold nanoparticle and that the observed SHG enhancement is due to the gold. Given the excitation wavelength, (~840 nm), we suggest this enhancement is due to a combination of a two-photon excited surface plasmon resonance as well as the corona effect.[1]

Since no measurable signals from the conjugated beads were observed, we can only put a lower-bound on the SHG enhancement factor relative to the conjugated gold nanoparticles. In an ensemble-averaged measurement our lower limit of detection is approximately 20 counts/s to achieve a signal-to-noise ratio of 1. The conjugated gold nanoparticles (of unknown concentration) produced SHG signals of approximately 2000 counts/s, allowing a lower-bound determination for enhancement of 100. A second method of examining SHG efficiency of these particles is to compare the SHG signals from the unconjugated naphthyl styryl dye vs the dye-conjugated gold nanoparticles. Freely diffusing dye will not produce SHG; however, the dye intercalated within the plasma membrane of cells does produce an SHG signal.[17] We therefore compared L1210 cells stained with the dye-conjugated gold nanoparticles to cells stained with the dye alone. A convenient method to quantitatively assay SHG intensity dependence between such samples is to normalize to the TPEF intensity. Such normalization is required to correct for the sample-to-sample variations inherent in measurements from single cells. A composite SHG (left panel) and TPEF (right panel) image of the former is shown in Figure 3a. The process was then repeated for L1210 cells stained with the dye-conjugated nanoparticles. A composite of 3 SHG and TPEF frames is shown in Figure 3b. In each case, following background subtraction, the SHG/TPEF ratio was measured on a cell-by-cell basis, and 20 were used for the average. The enhancement due to the gold particle is then given by:

$$\mathrm{SHG}_{\mathrm{enhancement}} = \frac{[\mathrm{SHG/TPEF}]_{\mathrm{gold}}}{[\mathrm{SHG/TPEF}]_{\mathrm{nogold}}} = 21 \pm 9$$

where the error bars express the absolute standard deviation.

A drawback of the ratiometric method described above is that it is not possible to directly distinguish between SHG enhancement and fluorescence quenching due to the gold. It is well-known that fluorescence quenching due to metals is highly distance-dependent[18] and that there can also exist a surface plasmon resonance fluorescence enhancement.[19] Indeed, in previous work, upon addition of freely diffusing gold particles, both SHG enhancement and TPEF decrease was observed.[20,21] To account for these uncertainties, the fluorescence lifetime was measured for both the unconjugated JPW4041 and the dye-conjugated nanoparticles. For convenience, these measurements were performed as a bulk suspension of PC liposomes stained with these dyes. The observed temporal decays (data not shown) were fit to single exponential curves, and the respective lifetimes for JPW4041 and the dye-conjugated nanoparticles were 2.6 and 2.3 ns. Attempts to fit the data to two exponential decays produced physically unrealistic values, indicating that the population of chromophores of each sample was relatively homogeneous. The relative fluorescence quantum efficiencies can then be expressed as the ratio of these lifetimes. Thus, the extent of fluorescence quenching is approximately 15%, relative to an approximately 20-fold SHG enhancement. An assumption of this treatment is that the oscillator strength of the dye and dye-conjugated nanoparticles remains unchanged. However, since the SHG signal is the sum of the nonresonant and resonant components,[22] and that the latter greatly dominates at the 840 nm excitation wavelength (at least by 10-fold), the SHG and TPE cross section should be equally affected by any change in oscillator strength. Therefore, the increase in SHG/TPEF ratio can be ascribed largely to SHG enhancement rather than fluorescence quenching.

While our observations of a 20-fold and a lower-bound of 100-fold SHG increase for the cell and aqueous imaging measurements, respectively, are small by comparison to prior work on SERS,[5] this represents a great increase in contrast level for microscope imaging, especially considering the already large first hyperpolarizabiltiy (~10^{-27} esu) of this class of chromophore.[15] Further, it is expected the SHG signal levels will depend greatly upon distance of the chromophore from the gold particle, particle size, and excitation wavelength. Future work will address these variables and determine dye coverage and orientation, as well to screen individual particles for high activity. Additionally, the SHG data shown here was collected in a forward collection geometry. Recent work by Eisenthal and co-workers[23] suggests that for submicrometer size particles, a significant portion of the scattering intensity is in the 90° direction. However, this arrangement is not easily feasible for high-resolution imaging, and our experiment would have been insensitive to such signals

These particles are novel both because dye−gold enhanced SHG response and because the functionalized polymer coating on the gold nanoparticles provides flexibility in chemical linkage to a variety of chromophores. The environmental sensitivity and enhanced signals of these dye−polymer−gold nanoparticles opens up the possibility of SHG-based sensors in living cells as well as other biologically relevant systems. It will be possible to elaborate the functionality of the nanoparticle surface to incorporate specific biomolecular recognition elements such as antibodies.

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Supporting Information Available: Details of the polymer coating procedure and dye conjugation (PDF). This material is available free of charge via the Internet at htttp://pubs.acs.org.

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