

Plasmon-Resonance-Enhanced Absorption and Circular Dichroism**

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The excitation of surface plasmons at rough noble-metal surfaces is responsible for the enhancement of several optical phenomena observed for molecules adjacent to these surfaces, such as surface-enhanced Raman scattering,^[1] surface-enhanced infrared absorption,^[2] and surface-enhanced fluorescence (SEF).^[3] We report herein on a new application of plasmon-enhanced absorption: circular dichroism (CD).^[4] CD enhancement of two orders of magnitude arises from a comparable enhancement of the overall electronic absorption of specific probe molecules on colloidal silver nanoparticles (NPs), where the metal surface plasmons are in resonance with the molecular electronic transition. Significant enhancement of the sensitivity of CD spectroscopy, which is used to study conformations of biological molecules,^[5] would help in probing samples of lower concentrations and would enable CD measurements of biomolecules adsorbed on surfaces.

Direct observation of resonant surface-enhanced electronic absorption is difficult owing to the large extinction of metal nanostructures at visible wavelengths compared to rather weak molecular extinctions. Alternatively, absorption enhancement has been frequently inferred from SEF or enhanced photocurrent in photovoltaic devices.^[6] Previously, Inoue and Hatta deduced an electronic absorption enhancement of >20 in a 7,7,8,8-tetracyano-*p*-quinodimethane (TCNQ) layer over planar Ag films in reflection experiments,^[7] and Dintinger et al. measured an absorption enhancement in an Ag nanohole array, in a wavelength range for which the array had a peak in transmission.^[8] In the present study the absorption enhancement was measured directly in solution absorption experiments and was further elaborated by CD spectroscopy where the silver nanoparticles used for enhancement had a zero average contribution to the CD signal. This study follows previous work on surface-enhanced magnetic CD in magnetic nanoparticles.^[9]

Molecular CD is of relatively low sensitivity and thus limited to solution studies of samples of moderate molecular concentrations; the lower concentration limit for proteins is of the order of about 0.1 mg mL^{-1} , or 0.1 mM of single amino acids. In our work the chiral molecule selected for the

molecular CD enhancement study needed to have a chromophore absorbing at the surface-plasmon-resonance wavelength of small silver spheres ($\approx 400 \text{ nm}$), a chiral center adjacent to the chromophore, and a functional group for attaching the molecule to the silver surface. Such a system was realized by binding a bimane chromophore^[10] to L-glutathione (L-GSH)-coated colloidal silver nanoparticles; this was generated by reducing a silver nitrate solution in the presence of L-GSH.^[11] Bimane is commonly used to label the thiol group of GSH,^[10] and formation of a derivative was verified by thin layer chromatography. The absorption, fluorescence, and CD spectra of aqueous solutions of the silver nanoparticles, L-GS-bimane, and their conjugates were measured.

Transmission electron microscopy of the silver nanoparticles formed in the presence of the L-GSH with an Ag/ligand concentration ratio of 50:1 showed a broad particle-size distribution in the range of 2–50 nm, with no apparent change in size distribution on addition of the bimane to the Ag-L-GSH.

Figure 1 depicts the absorption spectra of the various samples. The absorption spectrum of the Ag particles coated with L-GS-bimane includes a contribution from the Ag particle plasmon absorption (maximum at about 400 nm) and the (enhanced) bimane $n-\pi^*$ absorption^[12] (maximum at $\approx 390 \text{ nm}$). The absorbance of 0.05 mM free L-GS-bimane at 390 nm is ≈ 0.02 (thick black line) and the plasmon absorbance of the GSH-coated Ag nanoparticles is ≈ 0.4 (thick gray line), while the absorbance of the Ag nanoparticles coated with 0.05 mM L-GS-bimane is ≈ 1.8 (thick red line). Since the absorption coefficient of the plasmons in such Ag nanoparticles cannot be highly sensitive to the attachment of the bimane moieties to the capping GSH, the extra absorbance

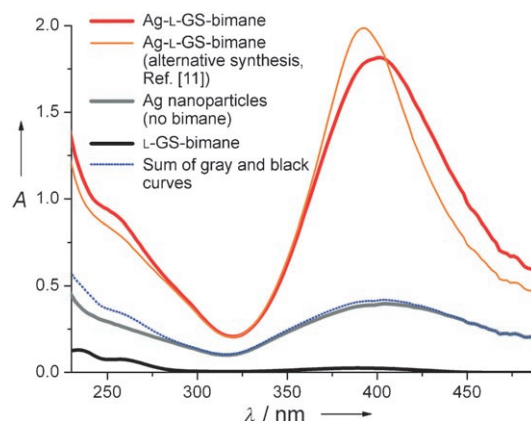


Figure 1. Absorption spectra of various solution samples. In all solutions the bimane concentration was 0.05 mM . The blue curve is the sum of the separate absorptions of L-GS-bimane and the Ag nanoparticles.

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must be due to plasmon-enhanced absorption of the bimane chromophores. The extra ≈ 1.4 absorbance units indicate an average enhancement factor of ≈ 70 . An alternative preparation of the Ag nanoparticles coated with L-GS-bimane produced a similar result (thin orange line).^[11] The true enhancement factor per attached molecule is larger, since a fraction of the L-GS-bimane molecules is free in solution owing to incomplete coating of the Ag surface.^[13] The second $n-\pi^*$ absorption band of the bimane at ≈ 260 nm appeared to be enhanced by a smaller factor (≈ 5), probably reflecting its detuning from resonance with the surface plasmon. The two relevant electronic transitions of the bimane moiety were assigned as $n-\pi^*$ transitions and originate in the splitting of the nonbonding levels of the two central nitrogen atoms.^[12]

Figure 2 (black curves) shows that the binding of the bimane moiety to the L-GSH produced a very weak CD signature at the 390 nm absorption band of the bimane

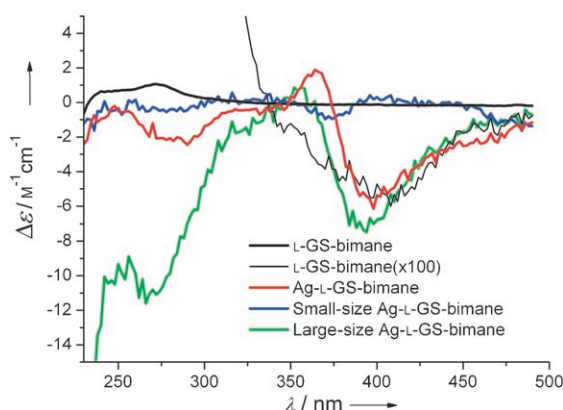


Figure 2. CD spectra of various solution samples. The small- and large-particle samples were separated from the Ag-L-GS-bimane by centrifugation and their concentration estimated as half of the original (0.05 mM). The thin black curve is the measurement for L-GS-bimane, with $\Delta\epsilon$ multiplied by 100.

induced by the chiral centers of the GSH, while the second band at 260 nm had a stronger induced CD. The induced CD signal at 390 nm, with $\Delta\epsilon \approx 10^{-2} \text{ M}^{-1} \text{ cm}^{-1}$, was enhanced by a factor of ≈ 100 (Figure 2, red curve). Both the achiral bromobimane precursor and solutions of GSH-coated Ag nanoparticles showed no detectable CD signal between 250–450 nm. The attachment to the silver nanoparticles appeared to strongly modify the internal structure of the induced CD bands of the L-GS-bimane; this is ascribed to the interaction of the L-GS-bimane with the silver surface and a concomitant change in conformational distributions. Like the absorbance, the CD band at the higher energy absorption band of the bimane was less enhanced.

The broad size distribution of the Ag-L-GS-bimane nanoparticles could be separated by centrifugation at 12000 rpm into two fractions with different characteristic sizes. The small-particle population (mostly 2–5 nm) remained in the supernatant. These small Ag-L-GS-bimane particles showed a negligible CD signal (Figure 2, blue curve), while the precipitate that was redispersed in solution, which contained larger particles and particle aggregates (5–50 nm), repro-

duced the CD enhancement of the original solution around 390 nm (Figure 2, green curve). It should also be noted that a dried L-GS-bimane (no silver) film showed no CD enhancement. This rules out the possibility of CD enhancement by induced supramolecular chirality upon aggregation of the molecules as has been observed for chiral porphyrins.^[14]

The fluorescence emission spectra shown in Figure 3 indicate that the emission is enhanced for the small-particle fraction and quenched for the large-particle fraction of Ag-L-

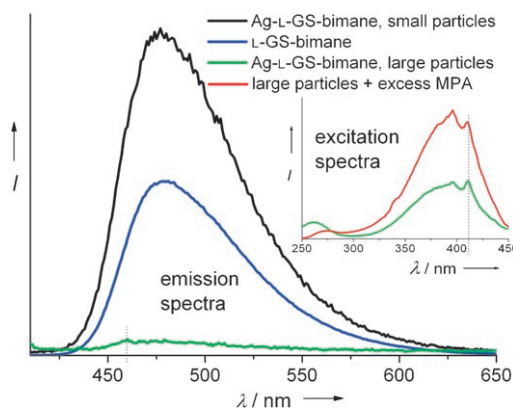


Figure 3. Fluorescence emission spectra of various solution samples. The inset shows the excitation spectrum of the large Ag-L-GS-bimane nanoparticles (green) and of the same solution after the addition of MPA (red) to displace the L-GS-bimane ligands from the Ag surfaces. The vertical dotted lines mark Raman bands of water.

GS-bimane. These changes in fluorescence intensity are the combined result of two effects: 1) Absorption enhancement and 2) quantum yield change due to increased excitation decay rates. The inset of Figure 3 displays fluorescence excitation spectra of the large-particle dispersion before and after the addition of excess mercaptopropionic acid (MPA) to the sample. The MPA displaced most of the L-GS-bimane bound to the surface of the nanoparticles. This resulted in an immediate twofold increase in emission intensity and changes in the excitation spectrum. Thus, it can be concluded that in the case of the larger particles, the emission quenching was about two times greater than the absorption enhancement.

The attachment of L-GS-bimane to the surfaces of the Ag nanoparticles resulted in enhancement of the CD spectra (enhanced $\Delta\epsilon$), which can be simply explained by the plasmon-induced resonant absorption enhancement (enhanced ϵ) arising from the larger particles and particle aggregates. The two orders of magnitude enhancement enabled the observation of the very weak induced CD of the bimane chromophore by bringing the $\Delta\epsilon$ up to typical values of the usual UV CD of amino and nucleic acids. The contribution of the larger particles to the enhancement indicates that this effect is related to the plasmon-induced electromagnetic enhancement responsible for the other surface-enhanced optical phenomena.^[1,15] Simultaneous measurements of absorption and fluorescence enhancement enabled us to estimate that the emission quenching roughly balanced the absorption enhancement, with both effects

changing with particle size. The absorption/CD enhancement is sensitive to spectral overlap with the plasmon resonance. Thus, bimane derivatives of biological molecules with their moderate intensity and CD-favorable $n\text{--}\pi^*$ transitions are promising candidates for further study. However, most biological molecules have absorption bands further into the UV, and an examination of metal nanostructures with shorter wavelength plasmon resonances would be desirable, in particular for cases in which labeling is not feasible.

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