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Anchoring Molecular Chromophores to Colloidal Gold Nanocrystals: Surface-Enhanced Raman Evidence for Strong Electronic Coupling and Irreversible Structural Locking

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Supporting Information

ABSTRACT: High-affinity anchoring groups such as isothiocyanate (ITC, —N═C═S) are often used to attach organic chromophores (reporter molecules) to colloidal gold nanocrystals for surface-enhanced Raman scattering (SERS), to atomically smooth gold surfaces for tip-enhanced Raman scattering, and to scanning tunneling microscopy probes (nanosized electrodes) for single-molecule conductance measurements. However, it is still unclear how the attached molecules interact electronically with the underlying surface, and how the anchoring group might affect the electronic and optical properties of such nanoscale systems. Here we report systematic surface-enhanced Raman studies of two organic chromophores, malachite green (MG) and its ITC derivative (MGITC), that have very different functional groups for surface binding but nearly identical spectroscopic properties. A surprise finding is that, under the same experimental conditions, the SERS signal intensities for MGITC are nearly 500-fold higher than those of MG. Correcting for the intrinsic difference in scattering cross sections of these two dyes, we estimate that the MGITC enhancement factors are ∼200-fold higher than for MG. Furthermore, pH-dependent studies reveal that the surface structure of MGITC is irreversibly stabilized or "locked" in its π-conjugated form and is no longer responsive to pH changes. In contrast, the electronic structure of adsorbed MG is still sensitive to pH and can be switched between its localized and delocalized electronic forms. These results indicate that ITC is indeed an unusual anchoring group that enables strong electronic coupling between gold and the adsorbed dye, leading to more efficient chemical enhancement and higher overall enhancement factors.

The ability to attach organic molecules to nanoparticles such as gold nanocrystals is of major interest in developing advanced nanosystems for use in molecular electronics,[1−3] chemical sensing,[4,5] biomedical diagnostics,[6−8] and solar energy conversion.[9] Due to their high affinities for gold, thiol-containing molecules are widely used to form self-assembled monolayers on flat surfaces, to coat the surface of nanoparticles, and to connect nanoelectrodes for studying molecular junctions.[10−12] Thiolated linkers with conjugated π-electrons are especially interesting because they could allow more efficient electron transfer and molecular orbital overlapping than saturated alkanethiols.[13] In addition, conjugated groups such as isothiocyanate (ITC, —N═C═S, a reactive group for covalent conjugation of organic dyes with biomolecules) have enabled the stable anchoring of reporter molecules to atomically smooth gold surfaces for tip-enhanced Raman scattering[14,15] and to colloidal gold nanoparticles for surface-enhanced Raman scattering (SERS).[16,17] Recent work by Halas et al.[18] has further shown that the electronic conductance and surface-enhanced Raman spectra of conjugated thiol molecules can be measured simultaneously from nanosized junctions. However, it is still not clear how the attached chromophores interact electronically with the underlying surface, and how the anchoring group might affect the electronic and optical properties of such nanohybrid systems.

Here we report detailed SERS studies of two organic chromophores, malachite green (MG) and its ITC derivative (MGITC) (Figure 1A), to examine how the anchoring group might affect the surface enhancement efficiency and the electronic structure of surface-bound molecules. As shown in Figure 1B−D, MG and MGITC have nearly identical spectroscopic properties including UV−vis absorption, normal Raman scattering, and SERS but very different chemical groups for surface adsorption. In fact, MG is known to adsorb via a single dimethylamino group in a tilted upright configuration, whereas MGITC is believed to adsorb via a nearly flat configuration involving π-electrons (more discussion later).[14,15,19−21] In this context of chemical adsorption and spectroscopic signatures, recent work by Van Duyne et al.[22,23] used isotope-substituted organic dyes (deuterated rhodamine 6G and deuterated crystal violet) for competitive adsorption at surface "hot spots" in single-molecule SERS studies. The rationale is that such isotopologues have identical surface adsorption properties but distinct spectroscopic features that allow identification of each dye from their composite SERS spectra. In this work, MG and MGITC are used for the opposite reason—that is, this pair of dyes has nearly identical spectroscopic signatures but different chemical groups for surface adsorption.

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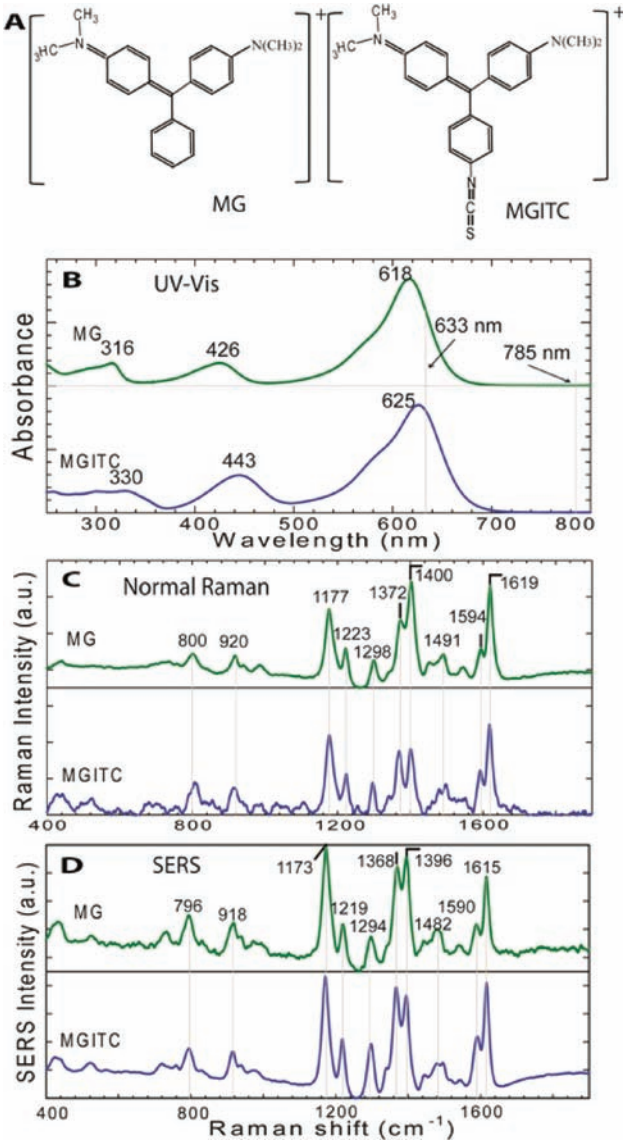

Figure 1. Malachite green (MG) and its isothiocyanate derivative (MGITC), two organic chromophores that have very different functional groups for attaching to gold surfaces but nearly identical spectroscopic properties. (A) Schematic chemical structures of MG and MGITC in their delocalized electronic forms (with delocalized π-electrons). (B) Comparison of UV–vis absorption spectra between MG and MGITC. (C) Normal Raman spectra and (D) SERS spectra of MG and MGITC. The normal Raman spectra were obtained from MG (2.5 mM) and MGITC (1 mM) in water solution with 2-s integration time and plotted on the same intensity scale. The SERS spectra were obtained from a colloidal gold solution (60-nm particle diameter, 14 pM concentration) mixed with 2.3 μM MG or 0.2 μM MGITC at room temperature, 1-s integration time. All Raman measurements were acquired by using 785-nm laser excitation, 40-mW laser power. Note that the SERS signal intensities were normalized to highlight the spectroscopic features of MG and MGITC.

Both MG and MGITC are found to stably adsorb on colloidal gold in a quantitative manner (>95% of the added dye is rapidly adsorbed to the gold particles, see Supporting Information (SI) Figure S1). Thus, the number of dye molecules per particle can be calculated within an error of only a few percent. Furthermore, the normal Raman data in Figure 1 C indicate that MG and MGITC have not only similar vibrational modes but also similar scattering cross sections, allowing a direct comparison of their SERS signal intensities and enhancement factors. In fact, at 785-nm nonresonant excitation, the normal Raman scattering intensities are similar for 1 mM MGITC and 2.5 mM MG under otherwise identical experimental conditions. These data indicate that the intrinsic scattering cross sections of the ITC derivative are likely higher than those of the parent dye by a factor of 2–3.

Figure 2 shows SERRS and SERS spectra of MG and MGITC obtained at resonant and nonresonant excitation

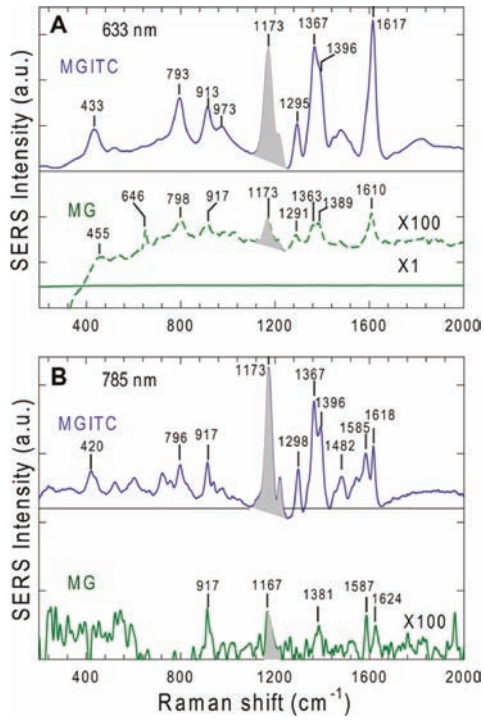

Figure 2. Surface-enhanced Raman (SERS) and resonance Raman scattering (SERRS) spectra of MG and MGITC obtained under the same experimental conditions at resonant and nonresonant excitation wavelengths. (A) SERRS spectra obtained at 633-nm (in-resonance) laser excitation (3 mW, integration time = 1 s). (B) SERS spectra obtained at 785-nm (off-resonance) laser excitation (40 mW, integration time = 20 s). The MG and MGITC concentrations were the same (100 nM), and the concentration of the colloidal gold was ~14 pM. Under these conditions, there were ~7000 dye molecules per particle. In both (A) and (B), the MG spectra are expanded by a factor of 100 for spectral details. By using the intensity of the 1173 cm^{-1} peak (shaded area), the MGTIC signals are calculated to be nearly 500-fold higher than those of MG at both 633- and 785-nm excitation wavelengths.

wavelengths (633 and 785 nm, respectively). A surprise finding is that the absolute signal intensities of MGITC are dramatically higher (by ~500-fold) than those of MG, even though their spectral patterns (relative intensities) and peak frequencies are similar. Since both MG and MGITC have electronic transitions at 633 nm, the observed signals contain a resonance enhancement and are thus surface-enhanced resonance Raman scattering (SERRS). To ascertain whether this resonance enhancement effect could be responsible for the observed difference, we have obtained the SERS spectra of these two dyes at 785-nm excitation, a wavelength that is not in resonance with the electronic transitions of MG or MGITC. The results in Figure 2B show that the MGITC SERS signals are also more intense than those of MG by a large factor under

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nonresonant excitation conditions. When the SERS intensities are plotted as a function of dye concentration (which is directly related to surface coverage before adsorption saturation), the ITC-derivative data show a fitted slope of 170 (over a roughly linear concentration range), while the MG data show a slope value of only 0.35 (see SI Figure S2). The ratio of these slope values is roughly 500, nearly the same as the absolute intensity ratio. This convergence is not a coincidence but represents a fundamental difference in the SERS cross sections between MG and MGITC. By correcting for the intrinsic difference in the intrinsic scattering cross sections between MGITC and MG, we estimate that the MGITC enhancement factors are ~200-fold higher than those for MG. This dramatic difference is believed to arise from efficient chemical enhancement mediated by the ITC anchoring group and strong electronic coupling, as discussed in more detail below.

To further investigate how surface absorption could alter the electronic structures of MG and MGITC, we have taken advantage of their pH-responsive properties to examine how their SERS signals change as a function of pH. Figure 3 shows

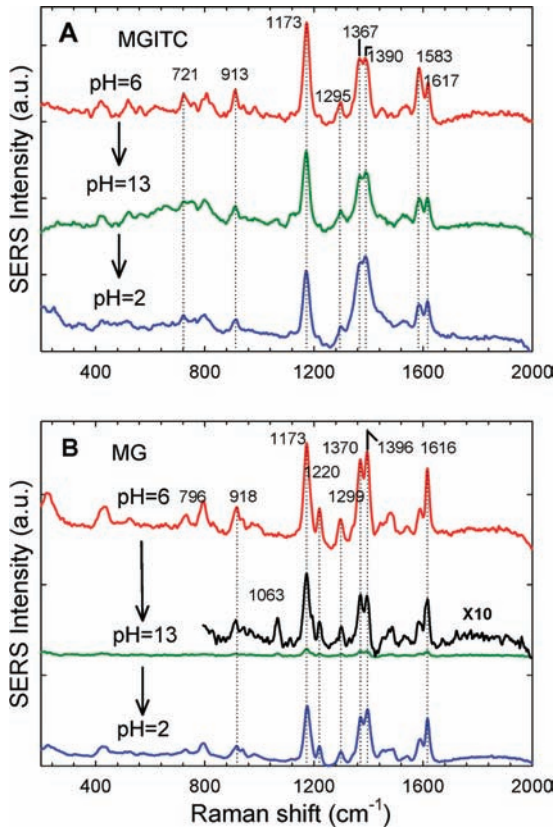

Figure 3. SERS spectra of MGITC (A) and MG (B) obtained with 785-nm laser excitation as a function of pH. As the pH was changed sequentially from 6 to 13 and then to 2, the ITC dye was found irreversibly stabilized or "locked" in its π-conjugated structure, whereas the adsorbed MG was still sensitive to pH and could be switched between its localized and delocalized forms. Laser power = 40 mW; integration time = 20 s.

the SERS spectra of MG and MGITC obtained sequentially at pH 6, 13, and then 2. Similar to other pH indicator dyes,[24] both MG and MGITC change from a deeply colored (green) solution (due to delocalized π-electrons) to a nearly colorless solution (due to a change in its electronic structure and the disappearance of optical absorption in the visible spectrum) (SI Figure S3). In free solution, these pH-induced structural changes give rise to completely different normal Raman spectra (see SI Figure S4). However, for the adsorbed MGITC, similar SERS signal intensities and frequencies are observed under both basic and acidic conditions, indicating a stabilized or locked surface structure that is no longer sensitive to pH. In contrast, adsorbed MG is still responsive to pH, and its SERS signals largely disappear when the pH is changed from 6 to 13. Interestingly, a trace of the original SERS signals (very weak but reproducible) remains at pH 13, and the residual SERS spectrum corresponds to adsorbed MG with a delocalized electronic structure (the same structure at neutral and acidic pH's). It is likely that a tiny fraction of the MG molecules is adsorbed at high-affinity sites on faceted gold nanocrystals or at the junction of two particles[25,26] and that its structure is stabilized in the delocalized form, similar to MGITC. Also, it is remarkable that the MG SERS signals can be reversibly turned on and off by switching the pH between 2 and 13 (Figure 3B), indicating that the MG molecules are not lost or desorbed from the nanoparticle surface at pH 13. In other words, this reversible behavior suggests that the surface coverage is approximately constant at pH's 2 and 13 (note that there are no free dye molecules in solution, so any lost or desorbed MG molecules at pH 13 would not be recovered at pH 2).

At resonant laser excitation (633 nm), similar pH-dependent behaviors are observed for MGITC and MG (see SI Figure S5). These data confirm that the electronic structure of MGITC is irreversibly locked on gold nanoparticles, but MG is still responsive to pH, and its SERS signals are turned off by changing the pH to 13. These intriguing results cannot be explained by a resonance enhancement effect alone, although resonance enhancement contributes to the SERS signals at 633-nm excitation. Instead, we believe that the observed pH dependence arises largely from a chemical enhancement effect[27−30] that is modulated (turned on and off) by pH-induced structural changes and electronic coupling. That is, efficient chemical enhancement is observed for conjugated MG at acidic or neutral pH, but this chemical effect is greatly reduced when the dye is converted to its localized electronic structure at pH 13. The electromagnetic field enhancement could be involved,[27] but its contributions to the observed pH dependence must be minor because the colloidal nanoparticles do not aggregate and have nearly identical surface plasmon absorption peaks with and without the reporter dyes (see SI Figures S6 and S7).

It is believed that triarylmethane dyes such as crystal violet and MG adsorb on gold surfaces via only one nitrogen atom in a tilted upright configuration.[19] The observation of strongly enhanced in-plane vibrational modes is consistent with this adsorption geometry.[31] Surface binding via a single dimethylamino group does not sufficiently stabilize or lock the dye's electronic structure. As a result, MG on gold is still responsive to pH, and its electronic structure can be reversibly switched by pH changes, similar to pH-induced structural changes in solution. In contrast, surface adsorption via the ITC group irreversibly locks the adsorbed molecule in its delocalized electronic structure. As a result, its SERS and SERRS spectra of MGITC are no longer responsive to pH, as schematically illustrated in Figure 4. Electrical conductance measurements have also shown that stronger headgroup−surface coupling can improve contact conductance and that ITC is an effective anchoring group due to its rich π-electrons.[32,33] Density

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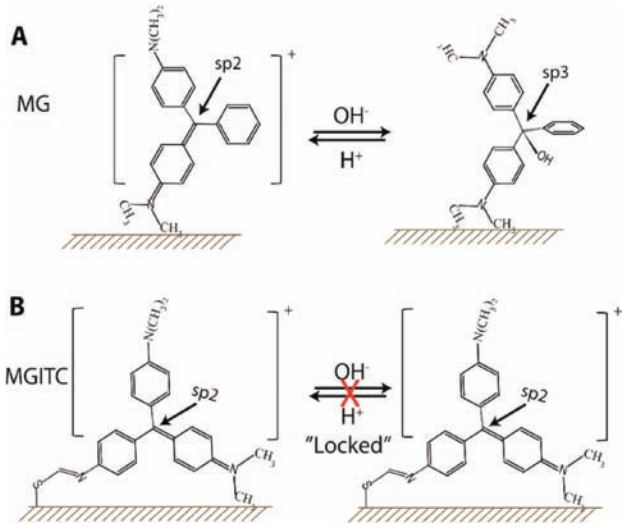

Figure 4. (A) Schematic diagram showing that MG is adsorbed on gold via a single dimethylamino group in a tilted configuration. In this geometry, the central carbon atom can change from sp^2 orbital hybridization (delocalized π-electrons, planner structure) at acidic pH to sp^3 orbital hybridization (localized π-electrons, octahedral structure) at basic pH. (B) Schematic diagram showing that MGITC is adsorbed on gold via two attachment sites. As a result, its electronic structure is locked in the π-conjugated form and is no longer responsive to pH changes.

functional theory calculations have further revealed that the ITC anchoring group binds to gold with a bent angle (∠Au−S−C) of ∼103°, in contrast to the nearly linear (standing) geometry for ITC adsorption on platinum (that is, ∠APt−S−C ≈ 180°).[34]

We also note that molecules with stronger binding affinities to metal surfaces are known to give larger enhancement in SERS due to both Franck−Condon and Herzberg−Teller (vibronic) mechanisms, as predicted by theory.[35,36] Our results thus provide further evidence for chemical enhancement in SERS, an elusive and often controversial topic in the literature.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures including preparation of spectrally encoded SERS nanoparticles, normal Raman spectra, and a series of characterizations in Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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