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Isocyanide and biotin-derivatized Ag nanoparticles: an efficient molecular sensing mediator *via* surface-enhanced Raman spectroscopy

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Silver nanoparticles capped with organoisocyanide and host or guest molecules are a very effective molecular sensing/recognition mediator *via* surface-enhanced Raman spectroscopy.

Noble metallic nanostructures exhibit a phenomenon known as surface-enhanced Raman scattering (SERS), in which the scattering cross sections are dramatically enhanced for molecules adsorbed thereon.[1] Thanks to the enormously large enhancement factor (EF) on the order of 10^6, one can readily acquire vibrational spectra from adsorbates on Ag, Au, and Cu nanoparticles.[1] In recent years, it has been reported that even single-molecule spectroscopy is possible by SERS, suggesting that the EF can reach as much as 10^{14}–10^{15};[2] at present, however, it is unclear what mechanisms and structural factors are responsible for single-molecule and single-particle SERS. At least in conventional SERS, it is believed that two enhancement mechanisms, one called a long-range electromagnetic (EM) effect and the other called a short-range chemical (CHEM) effect, are simultaneously operative. In either case, however, well-resolved vibrational spectra are rarely obtained for large molecules since the EF decreases very rapidly as the distance from the surface increases.[3] Although there are many reports on SERS of biological molecules,[4] this implies that SERS may not provide detailed information regarding the vibrational structure of large biological molecules.

Organoisocyanides readily adsorb on metals such as Ag and Au by forming a M–C bond.[5] This can be evidenced clearly from SERS from the high scattering intensity of the metal-bound isocyanide stretch, ν(–N≡C). One further noticeable thing for isocyanides is that their intrinsic stretching frequency observable at 2100–2300 cm^{-1} is substantially different from the usual group frequencies of organic compounds. These unique characteristics of organoisocyanides are expected to have wide applications in nanoscience and nanotechnology, particularly when they are anchored on SERS-active Ag and Au nanoparticles. We demonstrate in this communication that isocyanide and biotin-derivatized Ag nanoparticles can be used *via* SERS as very effective sensing units between avidin and biotin moieties. Avidin is a tetrameric protein that has symmetric biotin-binding pockets positioned in pairs at opposite faces of the protein.[6] The high binding affinity of avidin–biotin (K_a ~ 10^{13} M^{-1}) combined with diverse analytical methods (*e.g.* electrochemical method, fluorescence, radioactive species, optical spectroscopy and so on) has made the avidin–biotin system exceedingly useful in a wide range of biotechnical applications. SERS derived from nanoparticles that have been capped with isocyanide and appropriate host or guest molecules must add to the state-of-the-art means to probe biomolecular interactions without any prior fluorescent labeling.

As indicated schematically in Fig. 1, three different cases were considered to show the effective use of isocyanide-derivatized Ag nanoparticles to probe the avidin–biotin interaction by SERS. For cases I and II, self-assembled monolayers (SAMs) of *N*-(+)-biotinyl-6-aminocaproic acid (BACA) were fabricated on two silver plates; these are called Biotin-SAM-1 and Biotin-SAM-2.† For reference, SAMs of 12-hydroxydodecanoic acid (HDA) were manufactured on another silver plate as the third case; this is called Hydroxy-SAM. It is well known that carboxylic acids form stable SAMs on Ag, Cu, Pt, and metal oxides.[7] Silver nanoparticles (~15 nm in diameter), manufactured by laser ablation of pure silver plate in distilled water,[8] were derivatized with cyclohexyl isocyanide (CHIC) and/or BACA.‡ Due to the adsorbate-induced aggregation, the Ag sol, originally shown to absorb at *ca.* 395 nm, exhibited a broad UV/Vis absorption band spanning from 400 to 800 nm. This will be beneficial to SERS. It is also conceivable that Ag nanoparticles capped sparsely with BACA due to the co-adsorption of CHIC will interact more efficiently with the avidin molecules rather than the case of nanoparticles which are covered fully and exclusively with BACA.[9]

To induce the occurrence of the avidin–biotin interaction, the three SAMs on Ag plates, *i.e.*, Biotin-SAM-1, Biotin-SAM-2, and Hydroxy-SAM, were immersed in a 1 mM solution of avidin in phosphate buffered saline (PBS) solution for 1 h. After washing three times with pure PBS solution, these SAMs were immersed in three new PBS solutions. In the PBS solution containing Biotin-SAM-1, we added Ag nanoparticles which were capped with CHIC and BACA; see Fig. 1(a). In the PBS solution containing Biotin-SAM-2, Ag nanoparticles derivatized only with CHIC were added; see Fig. 1(b). In the PBS solution containing Hydroxy-SAM, Ag nanoparticles derivatized with CHIC as well as BACA were added; see Fig. 1(c). After waiting for 1 h, the three SAMs were thoroughly washed with triply distilled water to remove any physisorbed silver nanoparticles. These SAMs were finally subjected to optical microscopy and SERS analysis.§

Fig. 2A shows the optical microscope image of the Biotin-SAM-1 film after treating with Ag nanoparticles derivatized with BACA and CHIC, *i.e.*, case I. Silver nanoparticles are clearly seen in the image, indicating that there must exist two separate avidin–biotin interactions in the film, one involving the biotinylated Ag plate below and the other involving the biotinylated Ag nanoparticles; although not shown here, atomic force microscope (AFM) images from three different regions revealed that the Ag particles ranged in size from 150 nm to 3

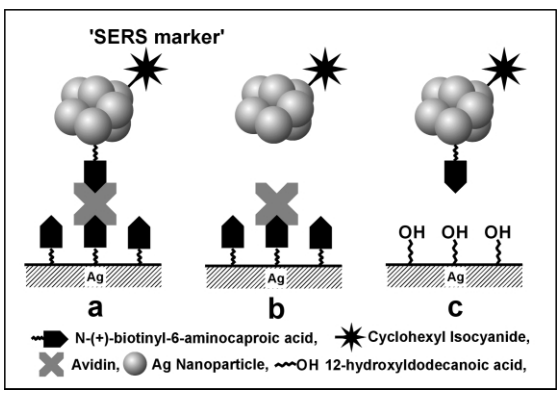

Fig. 1 The three different cases tested to probe the avidin–biotin interaction, called case I (a), case II (b), and case III (c).

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μm. In contrast, no Ag particle was identified at all in the optical microscope images of the films of case II and case III. This indicates that hydroxyl-terminated SAMs are indeed resistant to the non-specific adsorption of avidin and biotin molecules.[9]

The selective avidin–biotin interaction can be confirmed from the SERS spectral feature of the isocyanide stretching band. Fig. 2B shows the SERS spectra taken for the Biotin-SAM-1, Biotin-SAM-2, and Hydroxy-SAM films after the treatment with the Ag nanoparticles as mentioned above. The spectra of the Biotin-SAM-2 and Hydroxy-SAM films are featureless (see Fig. 2B(b) and B(c)), indicating that SERS-active Ag nanoparticles are not present on these films. This also implies that the Ag plate used to prepare SAMs of BACA or HDA is not SERS-active. However, an enhanced SERS spectrum is observed for the Biotin-SAM-1 film. A broad band spanning from 1700 to 1000 cm^{-1} and two sharp and intense peaks at ~2200 and 2900 cm^{-1} are clearly identified in Fig. 2B(a). These bands have nothing to do with any BACA and CHIC molecules that are not bonded to Ag nanoparticles since their concentrations in free state are too low to detect by ordinary Raman (OR) spectroscopy. Consulting the UV/Vis absorption spectrum and the optical microscope image, they must originate from the aggregated Ag particles. The broad band in Fig. 2B(a) may be composed of cathedral peaks from carboneous layers as well as carboxylate signatures. The sharp band around 2900 cm^{-1} is supposed, at least in this case, to arise mostly from the C–H stretching vibration of the cyclohexyl group. The intense peak at 2209 cm^{-1} is, however, exclusively due to the isocyanide stretching vibration; in the OR spectrum of neat CHIC, the isocyanide stretching peak appears at 2139 cm^{-1}, but it blue-shifts considerably upon surface adsorption due to the donation of the anti-bonding lone-pair electrons on carbon to silver. The isocyanide stretching peak is indeed isolated from other peaks of organic moieties, suggesting that Ag nanoparticles can be used as a very effective mediator to probe *via* SERS any kind of host–guest interaction once the particles are properly modified with organoisocyanide and host or guest moieties.

In summary, we have demonstrated using the SERS of CHIC that silver nanoparticles modified with BACA and CHIC can be anchored on a separate biotinylated substrate *via* the avidin–biotin interaction. Considering the high sensitivity of SERS and the ready functionalization of biomolecules (*e.g.*, antibodies, peptides, and nucleotides) with biotin, the present sensing method is believed to find wide application in numerous biomolecular systems. The method will be suitable for sensing 2-D patterned arrays in submicrometer size since large SERS effects can be derived from nano-sized Ag clusters; different analytes may be detected simultaneously using several isocyanides possessing different numbers of extra cyanide groups. Fluorescence spectroscopy using fluorescent molecular probes or semiconductor nanoparticles is in fact very effective for probing various biological systems,[10] but there are several characteristics of SERS that warrant the present method to be developed further for practical application.[11] Raman bands are generally 10–100 times narrower than most fluorescence bands, minimizing the potential overlap of different labels in a given spectral region. The optimum excitation wavelength for SERS is not strongly dependent on the adsorbed molecule, allowing the use of a single excitation source for multiple species. In addition, Raman scattering is not sensitive to humidity or affected by oxygen and other quenchers, facilitating applications in a variety of environments. The SERS signal is less subject to photobleaching, potentially enabling one to signal average for extended time periods to lower the limit of detection. On the other hand, it must be borne in mind that the use of semiconductors as biolabels is controversial, particularly because of general environmental concerns regarding the use of highly toxic cadmium compounds in biomedical diagnostics; there are other problems to be solved to enhance the solubility, physicochemical stability, and quantum efficiency of the particles.

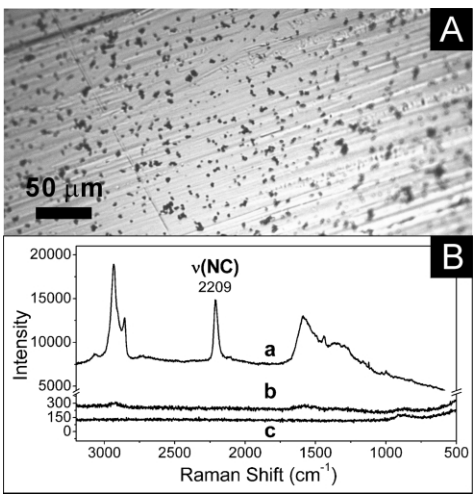

Fig. 2 (A) Microscope image seen for the sample of case I and (B) SERS spectra taken for the films corresponding to case I (a), case II (b), and case III (c).

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Notes and references

† Avidin from egg white was obtained from Sigma. 12-Hydroxydodecanoic acid (HDA) was obtained from Aldrich. *N*-(+)-Biotinyl-6-aminocaproic acid (BACA) was obtained from Fluka. These chemicals were used as received. Unless specified, organic solvents were all reagent grade, and triply distilled water (resistivity greater than 18.0 MΩ cm) was used when preparing aqueous solutions. SAMs of BACA or HDA on silver plates were made by dipping the plates in ethanolic solutions of each acid for 1 h.

‡ Ag nanoparticles derivatized with BACA and CHIC were obtained by adding 0.05 mL of BACA (2 mM) and 0.05 mL of CHIC (100 mM) mixture (1 : 50) solution in absolute ethanol to 0.9 mL of silver sol solution; the final concentrations of BACA and CHIC were thus 1×10^{-5} M and 5×10^{-4} M, respectively. The relative binding affinities of BACA and CHIC to silver have not been quantified, but the maximum SERS signal was observed for the ν(–N≡C) band when the ratio of BACA and CHIC was taken to be 1 : 50 among the four trials executed at ratios of 1 : 1, 1 : 10, 1 : 50, and 1 : 100.

§ SERS spectra were obtained using a Renishaw Raman system model 2000 spectrometer equipped with an integral microscope (Olympus BH2-UMA). The 514.5 nm radiation from a 20 mW air-cooled Ar^+ laser (Spectra Physics model 163-C4210) was used as the excitation source.

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