Surface-Enhanced Raman and Surface-Enhanced Hyper-Raman Scattering of Thiol-Functionalized Carotene

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ABSTRACT: A thiol-modified carotene, 7'-apo-7'-((4-mercaptopentyl)phenyl)-β-carotene, was used to obtain nonresonant surface-enhanced Raman scattering (SERS) spectra of carotene at an excitation wavelength of 1064 nm, which were compared with resonant SERS spectra at an excitation wavelength of 532 nm. These spectra and surface-enhanced hyper-Raman scattering (SEHRS) spectra of the functionalized carotene were compared with the spectra of nonmodified β-carotene. Using SERS, normal Raman, and SEHRS spectra, all obtained for the resonant case, the interaction of the carotene molecules with silver nanoparticles, as well as the influence of the resonance enhancement and the SERS enhancement on the spectra, were investigated. The interaction with the silver surface occurs for both functionalized and nonfunctionalized β-carotene, but only the stronger functionalization-induced interaction enables the acquisition of nonresonant SERS spectra of β-carotene at low concentrations. The resonant SEHRS and SERS spectra are very similar. Nevertheless, the SEHRS spectra contain additional bands of infrared-active modes of carotene. Increased contributions from bands that experience low resonance enhancement point to a strong interaction between silver nanoparticles and electronic levels of the molecules, thereby giving rise to a decrease in the resonance enhancement in SERS and SEHRS.

INTRODUCTION

Carotenoids are widespread in nature and have important functions in plants for light harvesting and for protection against excess light.1 In humans and other mammals, carotenoids, especially β-carotene, serve as precursors for vitamin A, which is a part of photoreceptors for the visual process.2,3 The versatile natural functions and the underlying structural properties of carotenoids, particularly the large π-electron system generating energetically low excited states,4 make them interesting objects for spectroscopic studies.5

Resonance Raman spectroscopy has been extensively used to characterize β-carotene6–9 as well as the influence of the different molecular structures of many carotenoids on their vibrational spectra.6–9,10,11

On the basis of this fundamental knowledge, Raman spectroscopy, both in- and off-resonance with the π → π* electronic transition in the molecules, has been suggested as a tool to investigate the composition of carotenoid components and their behavior in naturally occurring matrices, such as plants and lipid membranes.12–16 In this context, hyper-Raman scattering can be useful, as it provides the possibility to combine near-infrared two-photon excitation, which has little impact on biological samples, with resonance-enhanced scattering at the second harmonic of this excitation in the visible. Hyper-Raman microspectra17 of β-carotene crystals have been reported, and an influence of β-carotene on the hyper-Raman signals of surrounding solvent molecules has been discussed.18

Under truly nonresonant conditions, measurements in dilute solutions are desirable, because when solid samples or solutions of carotenoid molecules at high concentration are investigated the strong electronic transition of carotenoids can also qualitatively influence their Raman spectra upon excitation in the near-infrared, far away from resonance.19 However, in contrast to the resonant case excited in the visible,20 inherent Raman scattering cross sections upon excitation in the near-infrared are too small to obtain spectra at low concentration. Plasmonic nanostructures can increase the signal intensities of Raman scattering by several orders of magnitude in surface-enhanced Raman scattering (SERS) and surface-enhanced hyper-Raman scattering (SEHRS),21 thus improving sensitivity in dilute solutions. Furthermore, SERS and especially SEHRS spectra are influenced by the presence of the metal surface, such as by the adsorption geometry, and by the changing chemical environment.22,23 In particular, for dyes with absorption maxima close to the plasmon band of the metal nanostructures, the interaction between the molecules and the metal additionally results in changes of the electronic properties.24,25

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Carotene can also take part in such a coupling when it is placed close to a silver surface. Here, we use SERS and SEHRS under molecular resonant and also nonresonant conditions to characterize the interaction between carotenoid molecules and silver nanoparticles.

The adsorption of carotenoids to silver nanoparticles in aqueous solution is only straightforward for carotenoids with hydrophilic end groups. β-Carotene and other nonpolar carotenoids are poorly soluble in water and have low affinity to the silver surface. In earlier work, to obtain SERS spectra, the affinity between the silver surface and the carotenoids was increased by functionalization of the silver nanostructures with organic layers. In the work reported here, we increase the affinity between carotene and silver nanoparticles by using 7′-apo-7′-(4-mercaptomethylphenyl)-β-carotene, which was shown to adsorb to gold surfaces via its thiol group. As organothiols in general strongly chemisorb to silver surfaces in a similar way as to gold, we expected a similar interaction between this functionalized carotene and silver nanoparticles. To investigate this, we compare the resonant SERS and SEHRS spectra of the thiol-functionalized to those of the normal, nonfunctionalized β-carotene (for a comparison of the molecular structures, see Figure 1a). As will be demonstrated, the stronger interaction between the functionalized carotene and the silver nanoparticles enables the investigation of the nonresonant SERS spectrum of carotene.

**MATERIALS AND METHODS**

Silver nanoparticles with an average diameter of 42 nm were produced by the reduction of silver nitrate with hydroxylamine hydrochloride, resulting in an aqueous solution with a particle concentration of 7 × 10^-10 M.

The carotenethiol 7′-apo-7′-(4-mercaptomethylphenyl)-β-carotene was synthesized following a procedure reported by Gust et al. and Leatherman et al. The final product and all of the intermediates were stored under argon at -20 °C, and reaction mixtures and products were shielded from light. 1H NMR spectroscopy confirmed the formation of 7′-apo-7′-(4-mercaptomethylphenyl)-β-carotene. For determining the actual amount of the carotenethiol, absorbance spectra of a defined solution of the synthesis product in dichloromethane were measured. From these spectra, the concentration of the carotenethiol in the measured solution, and thus its proportion in the synthesized product, was calculated using the extinction coefficient reported in ref 32.

Solutions of 7′-apo-7′-(4-mercaptomethylphenyl)-β-carotene and of nonfunctionalized β-carotene (Fluka, >97%) in ethanol of different concentrations were added to the nanoparticles and nanoaggregates in a volume ratio of 11:1 (aqueous nanoparticle solution/carotene solution) to yield the different final carotene concentrations (described in the respective figure captions). Samples for SEHRS experiments, and also for accompanying SERS experiments with 532 nm excitation (Figure 5), were prepared in the same way but were finally diluted with 10% (v/v) aqueous ethanol to obtain a ten times lower nanoparticle concentration than in the other experiments and a final carotene concentration of 3 × 10^-7 M.

Hyper-Raman spectra and near-infrared excited Raman spectra were excited at 1064 nm with a mode-locked laser producing 7 ps pulses with a repetition rate of 76 MHz. The peak photon flux density at the sample was between 3 × 10^27 and 6 × 10^28 photons cm^-2 s^-1 (average power at the sample ranging from 50 to 500 mW). Excitation at 532 nm was provided by a CW laser with a photon flux density between 1 × 10^23 and 6 × 10^24 photons cm^-2 s^-1 (average power ranging from 2 to 10 mW) at the sample. The excitation light was focused on the samples through a microscope objective. The scattered light was collected in backscattering geometry, dispersed in a grating spectrograph, and detected by a liquid nitrogen-cooled CCD (hyper-Raman spectra and 532 nm excited Raman spectra) or a liquid nitrogen-cooled InGaAs detector (1064 nm excited Raman spectra). For SERS experiments with 514.5 nm excitation, a LabRam HR800 (Horiba Jobin-Yvon) setup coupled to a microscope and equipped with an argon ion laser was used. This setup was used because it enables the observation of relatively low Raman shifts in the range 200–250 cm^-1. The spectral resolution in the full spectral range was 2–3 cm^-1 for the 1064 nm excited SERS and Raman spectra, 6–8 cm^-1 for the hyper-Raman spectra, SEHRS spectra, and 532 nm excited SERS and Raman spectra of solutions, 9–13 cm^-1 for the 532 nm excited Raman spectra of solid samples, and 11–15 cm^-1 for the 514.5 nm excited SERS spectra.

The SEHRS and near-infrared excited SERS spectra were baseline corrected using a method developed by Zhang et al.

**RESULTS AND DISCUSSION**

**SERS Spectra Obtained Off-Resonance.** The surface-enhanced Raman and surface-enhanced hyper-Raman spectra of the thiol-functionalized carotene 7′-apo-7′-(4-mercaptomethylphenyl)-β-carotene (1 in Figure 1a) and nonfunctionalized β-carotene (2 in Figure 1a) were obtained with colloidal silver nanoparticles using visible and near-infrared excitation wavelengths. The enhancing plasmonic nanostructures are silver nanoaggregates in water produced by the reduction of silver nitrate with hydroxylamine, which are well-known for producing high SERS enhancement. The broadening of...
the absorbance band of the silver nanostructures in the
presence of the carotene solutions (Figure 1b) indicates the
presence of nanoaggregates due to an interaction between the
molecules and the metal, and superimposes the molecular
absorption of the carotene molecules. We expected the
 carotenethiol to chemisorb to the silver nanoparticles via the
thiol group similar to previous experiments reported with the
same carotenethiol on a gold surface. However, different from
the previous work with the molecule, we use here an aqueous
solution instead of nonpolar organic solvents, which could have
an impact on the interaction of the carotene molecules with the
metal surface. As an example, in the presence of water, molecular aggregates of the molecules can form.

Figure 2a shows the nonresonant SERS spectrum of the
carotenethiol excited at 1064 nm. The most prominent bands
may indicate a very different SERS enhancement in the case of
resonant and nonresonant Raman signatures.

As illustrated in Figure 2b, compared to the carotenethiol
spectrum (black trace), the near-infrared excited SERS
spectrum of the nonfunctionalized carotene (blue trace),
shows only very weak signals even at integration times of a
few minutes. The band at 1155 cm$^{-1}$ in the spectrum of
carotenethiol in Figure 2b (black trace) is nine times more
intense than the same band in the spectrum of the
nonfunctionalized carotene (blue trace in Figure 2b), even
though both spectra were acquired under the same
experimental conditions. There are two possible explanations
for this intensity increase in the carotenethiol. First, the thiol
group could cause a higher affinity between the functionalized
carotene and the silver surface, resulting in a higher SERS
enhancement for the thiolated carotene due to a smaller
molecule–metal distance. Second, the modification of the
molecular structure also includes the enlargement of the
mesomorphic system by an additional phenyl ring (Figure 1a);
this could increase the Raman scattering cross section, which
would affect both SERS and normal Raman spectra.

Interactions of Carotenethiol with the Silver Surface.
The quantification of the enhancement in the SERS spectra of
the two carotenes is interesting when discussing differences in
the interaction with the silver surface. As it was not possible to
quantify the surface enhancement in the SERS spectra excited
at 1064 nm because, off-resonance, no reference Raman spectra
of carotenoid solutions without silver nanoparticles are
obtained, we estimate surface-enhancement factors for the
two carotenes from SERRS and RR spectra that were excited at
532 nm (Figure 3). This excitation wavelength is in resonance
with the strongest electronic transition of the carotenes with an
absorption maximum between 450 and 480 nm.

The SERRS spectra in Figure 3a show bands with similar intensity
for the two carotenes even though the concentration of the
nonfunctionalized carotene (Figure 3a, lower trace) is 1 order
of magnitude higher than that of the thiol-modified one (Figure
3a, upper trace). This is in accordance with the observation of
the weaker signals from the nonfunctionalized carotene for the
nonresonant case (Figure 2b). By comparing the respective
intensities of the bands at 1520 and 1156 cm$^{-1}$ in the SERRS
and RR spectra (Figure 3) and taking into account the different
concentrations, the SERS enhancement factors were estimated
to be $2 \times 10^3$ for the functionalized and $4 \times 10^2$ for the
nonfunctionalized carotene. This relatively low SERS enhance-
ment would be in agreement with previous observations of a
diminished surface enhancement in some cases of strong
molecular resonances$^{43,44}$ and furthermore, indicates a weak
interaction between the molecules and the silver surface for
both carotenes. Nevertheless, the enhancement is 1 order of
magnitude larger for the carotenethiol than for the non-
functionalized carotene. Therefore, there must be a difference
in the interaction of the two carotenes with the silver
nanoparticles, which can include a higher tendency of the
carotenethiol to adsorb to the silver surface.

To characterize the kind of interaction between the carotene
molecules and the silver surface, we examined the region of low
Raman shifts in 514 nm excited SERS spectra (Figure 4).
Chemical bonds between the silver and adsorbed molecules can
be observed in SERS spectra by the appearance of modes
between 200 and 250 cm$^{-1}$ related to stretching vibrations
between the molecules and the silver surface.$^{45,46}$ As shown
in Figure 4 (green spectrum), the silver nanostructures used in
this study exhibit a band at 235 cm\(^{-1}\), which can be explained
by surface-bound chloride or hydroxylamine from the synthesis
of the silver nanoparticles. Upon addition of carotene solutions,
the band at 235 cm\(^{-1}\) strongly decreases (Figure 4, blue
spectrum), and for the thiolated carotene, the band disappears
completely (Figure 4, black spectrum). This suggests that the
adsorbates, which are present on the silver surface from the
synthesis of the nanoparticles, are exchanged by carotene
molecules. The exchange seems to be more complete for the
thiolated carotene than for the nonfunctionalized carotene, as is
indicated by the small band at 235 cm\(^{-1}\) remaining in the SERS
spectrum of the nonfunctionalized carotene (Figure 4, blue
spectrum). Considering the high affinity between sulfur and
silver,\(^{33,34}\) and also because the carotenethiol was reported to
adsorb on gold surfaces via the thiol group,\(^{32}\) formation of an
Ag–S bond would have been expected. It is possible that such a
bond has formed without being detected in the SERS spectra
because the Ag–S stretching band would not be resonance
enhanced in contrast to the carotene bands. On the other hand,
the absence of a band in the 200–250 cm\(^{-1}\) region in the SERS
spectrum of the carotenethiol could also indicate that no
chemical bond between the thiol group of the carotenethiol and
the silver surface is formed at all. In this case, the interaction
between the carotenethiol and the silver surface would happen
via the \(\pi\)-electrons in the polyene chain. However, this
interaction would be weaker than a chemisorption of the
thiol group and would take place in a similar fashion for the
nonfunctionalized carotene. In such a case, the higher
enhancement in the SERS spectra of the functionalized
carotene would result from the larger part of the molecule
interacting with the silver nanostructure.

**SEHRS and SERRS Spectra.** To characterize the molecules
at the nanoparticle surfaces, we also discuss their surface-
enhanced hyper-Raman (SEHRS) spectra. Figure 5a shows the
SEHRS spectra of the carotenethiol and of nonfunctionalized
\(\beta\)-carotene obtained at an excitation wavelength of 1064 nm.
Hyper-Raman scattering, as a nonlinear process, follows
symmetry selection rules different from those of linear
Raman scattering,\(^{47}\) especially for centrosymmetric molecules
where the allowed modes in the two processes are mutually
exclusive.\(^{48}\) Upon adsorption to a metal surface, molecular
symmetry can be altered\(^{49}\) such that SERS spectra of a chemical
compound can be different from the corresponding Raman
spectra.\(^{23}\) This effect is even more pronounced for SEHRS,
which has been observed to strongly depend on the orientation
of a molecule on the metal surface and on the chemical
environment.\(^{22,50–52}\) Additionally, as the second harmonic of
the wavelength that we use to excite SEHRS spectra is close to
the electronic transition of carotene, a contribution of
resonance enhancement can be expected.\(^{53,54}\)

The exchange of one of the carotene end groups when
functionalizing the \(\beta\)-carotene should cause qualitative changes
in the SEHRS spectra. Nevertheless, the spectra of the two
carotenones are similar (Figure 5a) except for a few details: The
Figure 5. (a) SEHRS spectra excited at 1064 nm and (b) SERRS spectra excited at 532 nm of 7′-apo-7′-(4-mercaptopropylphenyl)-β-carotene (upper spectra in both panels) and of the nonfunctionalized β-carotene (lower spectra in both panels). Final concentrations of the carotenes in the sample solutions: 3 × 10⁻⁷ M; excitation: (a) 1064 nm; peak photon flux density 6 × 10²⁸ photons cm⁻² s⁻¹; 120 s acquisition time; (b) 532 nm; 7 × 10²⁸ photons cm⁻² s⁻¹; 100 ms acquisition time. The spectra in (a) are averages of 10 baseline corrected spectra. Bands marked with an asterisk in (b) are due to the ethanol in the solvent.

Figure 6. (a) Nonresonant Raman spectra, (b) hyper-Raman spectra, and (c) resonant Raman spectra of 7′-apo-7′-(4-mercaptopropylphenyl)-β-carotene (upper spectra) and of the nonfunctionalized β-carotene (lower spectra) measured from solid samples. Excitation: (a) 1064 nm; peak photon flux density 3 × 10²⁷ photons cm⁻² s⁻¹; acquisition time 20 s; (b) 1064 nm; peak photon flux density 1 × 10²⁸ photons cm⁻² s⁻¹; acquisition time 600 s; (c) 532 nm; peak photon flux density 1 × 10²³ photons cm⁻² s⁻¹ (upper spectrum); 5 × 10²³ photons cm⁻² s⁻¹ (lower spectrum); 1 s acquisition time.
bands at 1190 and 1271 cm\(^{-1}\) in the spectrum of the nonfunctionalized carotene (lower spectrum in Figure 5a) are shifted to 1173 and 1258 cm\(^{-1}\), respectively, in the spectrum of the carotenethiol (upper spectrum in Figure 5a). The same is observed for the SERRS spectra, which were obtained quasi-simultaneously from the same samples as the SEHRS spectra (Figure 5b). In the spectra of nonfunctionalized \(\beta\)-carotene, the two bands are assigned to C–C stretching and in-plane C–H bending vibrations, respectively, in the polycene chain. The shift of these two bands to 1173 and 1258 cm\(^{-1}\), respectively, in the spectra of the carotenethiol can be explained by an influence on the mesomeric system of the polycene chain by the insertion of the phenyl ring in the carotenethiol (1 in Figure 1a). The two frequency shifts are observed in the normal Raman and normal hyper-Raman spectra of powder samples of the two carotenes as well (Figure 6). Therefore, we attribute them to the different structures of the molecules as such and not to changes in the interaction with the silver surface.

While normal Raman and hyper-Raman spectra of carotene molecules differ greatly,\(^{35}\) there is a high similarity of the SEHRS and SERRS spectra because, due to the interaction with the silver surface, the symmetry of the molecules is lowered. Nevertheless, two differences can be observed between the respective one- and two-photon excited spectrum in both carotenes (Figure 5): (i) A shoulder band at 1051 cm\(^{-1}\) appears in the SEHRS spectra (Figure 5a), which is absent in the SERRS spectra (Figure 5b). At the same position, a band in the infrared absorption spectrum of \(\beta\)-carotene has been reported, which is due to a vibration of the ionone ring and which is not observed in one-photon Raman spectra.\(^{8}\) The appearance of this band in our SEHRS spectra is in accordance with the fact that the symmetry selection rules for two-photon excited Raman spectra are usually expected to be more similar to those for IR-spectra than to those for one-photon excited Raman spectra.\(^{47}\) (ii) The weak band at 1591 cm\(^{-1}\) in the SERRS spectra (Figure 5b) that is quite obvious in the nonresonant SERS spectra of the carotenethiol (Figure 2a) appears as a shoulder next to the band at 1520 cm\(^{-1}\) instead of as a separate band in the SEHRS spectra (Figure 5a). It seems that, in the SEHRS spectra, this band is either shifted to a lower Raman shift value or contains contributions from an additional vibration at lower frequency. The band at 1591 cm\(^{-1}\) can be assigned to a C=C stretching vibration of a double bond at the end of the polycene chain and has been observed to coexist with a weaker band of another C=C stretching vibration at around 1580 cm\(^{-1}\).\(^{19}\) It is possible that the band around 1580 cm\(^{-1}\) is selectively enhanced in the SEHRS spectra but is too weak to be observed in one-photon excited SERS spectra due to different surface selection rules in the two excitation regimes. An additional contribution to the shoulder at 1591 cm\(^{-1}\) can be made by an IR-active but Raman-inactive C=C stretching vibration, which was previously observed as an intense band at 1569 cm\(^{-1}\) in hyper-Raman spectra of \(\beta\)-carotene upon excitation at shorter wavelengths, together with additional IR-active modes.\(^{45,46}\) The presence of such a contribution from the IR-active C=C stretching vibration is suggested by the second derivative of the SEHRS spectrum (data not shown here) and is also supported by the hyper-Raman spectra of solid nonfunctionalized \(\beta\)-carotene, where we observe a small band at 1559 cm\(^{-1}\) (Figure 6b, lower trace).

### Relative Intensities in SEHRS, SERS, and SERRS Spectra

Comparison of the SERRS (Figure 5b) and SEHRS spectra (Figure 5a) shows relatively strong contributions in the SEHRS spectra from bands that experience a high SERS enhancement but low resonance enhancement. Specifically, the band at 1591 cm\(^{-1}\) is more intense than the band in the SERRS spectra (Figure 5b). In a similar fashion, the relative intensity of the band at 1178 cm\(^{-1}\) shows high relative intensity in the SEHRS spectrum of the carotenethiol (Figure 5a, upper trace). Conversely, describing this as a relative decrease in intensity of the bands at 1520 and 1155 cm\(^{-1}\) that experience strong resonance enhancement, the SEHRS data (Figure 5a) would support the theoretical consideration and SERS experimental observation of decreased molecular resonance enhancement due to electromagnetic and/or electronic coupling of the molecules and the metal nanoparticles.\(^{43,44}\)

In the nonresonant SERS spectrum of the carotenethiol (Figure 2a), the band at 1591 cm\(^{-1}\) is more intense than in the SEHRS (Figure 5a) and SERRS (Figure 5b) spectra, as seen by the intensity ratio between this band and the band at 1520 cm\(^{-1}\). A similar change in intensity ratio is observed for the bands at 1178 and 1155 cm\(^{-1}\), which are due to C=C and C=C vibrations at the end of the polycene chain in the spectra at visible and near-infrared excitation. The reason for the different intensity ratios between these bands and those at 1591 and 1178 cm\(^{-1}\) is more intense than in the SEHRS spectrum of the carotenethiol (Figure 5a, upper trace). Nevertheless, two differences can be observed between the respective one- and two-photon excited spectra (Figure 5a and b), respectively, also indicates that hyper-Raman scattering benefits from electronic resonances due to 2-photon excitation, and the same electronic transition is responsible in both one- and two-photon excitation.\(^{37}\) Nevertheless, this similarity of the respective one- and two-photon excited spectra differs for the SERS (Figure 5) and “non-SERS” (Figure 6b and c) cases: We observe high similarity of the SEHRS (Figure 5a) and SERRS (Figure 5b) spectra because the interaction with the silver surface lowers the symmetry of the adsorbed molecules. In contrast, the hyper-Raman spectra (Figure 6b) and RRS spectra of the solid samples (Figure 6c) differ greatly due to the different selection rules that become very evident for molecules with high symmetry. Other studies, working with lower excitation wavelengths,\(^{30}\) have reported even greater dissimilarities of HRS and RRS spectra of \(\beta\)-carotene. In SEHRS, similar to previous discussion of SERS,\(^{30}\)
the role of such different contributions from molecular resonance effects that act in addition to the altered symmetry of the adsorbed molecules will have to be studied in excitation profiles in the future.

**CONCLUSIONS**

In conclusion, β-carotene modified with a thiol group was used to obtain SERS and SEHRS spectra at 1064 nm excitation as well as SERRS spectra at 532 nm excitation using silver nanoparticles. Spectra of the nonfunctionalized β-carotene were also obtained. The functionalization leads to a strengthening of the interaction between carotene and the surface of silver nanoparticles, which is seen by higher SERS and SERRS signals. The improved interaction with the silver surface of the thiol-modified molecules can be utilized to obtain nonresonant SERS spectra of carotene in dilute aqueous solution, which show a different intensity pattern than normal Raman spectra of solid samples or solutions of high concentration. Comparison of the nonresonant SERS, resonant SEHRS, and SERRS spectra suggests that the resonance enhancement plays an important role in the 1064 nm excited SEHRS spectra. The SEHRS spectra of the thiol-modified and the nonfunctionalized carotene are very similar. In addition to bands that are also found in the SERRS spectra, they show several contributions from infrared-active vibrational modes of the polyene chain. Relatively strong contributions in the SEHRS spectra by bands with low resonance enhancement point to an influence of the electromagnetic and/or electronic coupling of the molecules and the silver nanoparticles on the enhancement in the resonant hyper-Raman process. Despite these small differences, the SEHRS and SERRS spectra are highly similar, as opposed to the more severe differences between the hyper-Raman spectra and resonant Raman spectra of carotenes in solids or in organic solvents. This is in accordance with the assumption that the symmetry of the molecules is lowered upon adsorption to the silver nanoparticles.

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**Notes**

The authors declare no competing financial interest.

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