

Surface-Enhanced Infrared Spectra of Manganese (III) Tetraphenylporphine Chloride Physisorbed on Gold Island Films

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ABSTRACT: Surface-enhanced infrared absorption (SEIRA) spectra of manganese (III) tetraphenylporphine chloride (Mn(TPP)Cl) on metal island films were measured in transmission mode. Dependences of the enhancement factor of SEIRA on both the sample quantity and the type of evaporated metal were investigated by subsequently increasing the amount of Mn(TPP)Cl on gold and silver substrates. The enhancement increases nonlinearly with the amount of sample and varies slightly with the thickness of metal islands. In particular, the SEIRA transmission method presents an anomalous spectral enhancement by a factor of 579, with substantial spectral shifts, observed only for the physisorbed Mn(TPP)Cl that remained on a 3-nm-thick gold film after immersion of the substrates into acetone. A charge-transfer (CT) interaction between the porphyrinic Mn and gold islands is therefore proposed as an additional factor in the SEIRA mechanism of the porphyrin system. The number of remaining porphyrin molecules was estimated by calibration-based fluorescence spectroscopy to be 2.36×10^{13} molecules (i.e., $\sim 2.910^{-11}$ mol/cm²) for a 3-nm-thick gold film, suggesting that the physisorbed molecules distributed very loosely on the metal island surface as a result of the weak van der Waals interactions. Fluorescence microscopy revealed the formation of microcrystalline porphyrin aggregates during the consecutive increase in sample solution. However, the immersion likely redistributed the porphyrin to be directly attached on the gold surface, as evidenced by an absence of porphyrinic microcrystals and the observed SEIRA enhancement. The distinctive red shift in the UV-visible spectra and the SEIRA-enhanced peaks indicate the presence of a preferred orientation in the form of the porphyrin ring inclined with respect to the gold surface.

KEYWORDS: tetraphenylporphine, surface-enhanced infrared absorption, metal island film, atomic force microscopy; fluorescence.

INTRODUCTION

To date, considerable efforts have been focused on improvement of the sensitivity obtained by the conventional infrared (IR) spectroscopy, in order to achieve more reliable IR spectra of tiny amounts of samples. Starting in 1980, Hartstein et al.¹ first discovered that IR absorption of molecules adsorbed on rough metal island surfaces is remarkably enhanced by a strong electromagnetic (EM) field amplified through the excitation of collective electron resonance in the metal islands. This phenomenon, known as surface enhanced infrared absorption (SEIRA), has gained a significant interest over the past years as a result of the excellent improvement in sensitivity. Although the SEIRA effect was originally explored with the attenuated total reflection (ATR) technique,^{1,2} it was later reported that

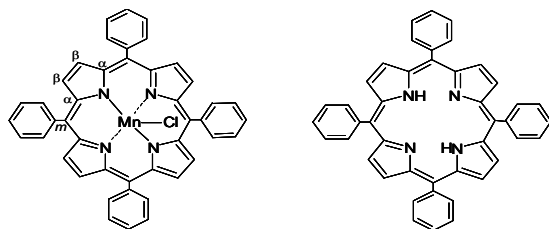
SEIRA spectra were successfully observed with both the transmission^{3,4} and external reflection⁵ techniques. Due to its remarkable absorption enhancement and the availability of versatile sampling methods, SEIRA spectroscopy has become a novel surface-sensitive technique for detecting a minute quantity of samples with high sensitivity. In particular, the SEIRA technique has been extensively applied to micro- and trace-analyses of molecules and/or monolayers on a variety of metal surfaces.^{6,7}

In addition to the EM mechanism,^{3,4,6} several studies have suggested an additional enhancement caused by the chemical interaction between the adsorbed molecule and the metal island surface.⁸⁻¹⁰ However, the extent of the chemical contribution has not yet been attributed definitely, and in fact the chemical interaction has been reported to be only an optional condition for

observing the SEIRA phenomenon.^{2,5,10} Therefore, it is possible to readily perform SEIRA measurements for *in-situ* characterization of molecules physically bound on the metal islands, through van der Waals interactions, without preparations of either a Langmuir-Blodgett (LB) film or self-assembled monolayer (SAM).

Accordingly, the current study was conducted for applying the SEIRA technique to detect such a small amount of surface species adsorbed on metal island films by means of physisorption. A metalloporphyrin and a free-base porphine were selected as sample molecules due to their significant roles in biology and chemistry, including many spectacular applications that are currently available.^{12,13} Enormous efforts have been made to study porphyrins in the form of a chemisorbed (i.e., self-assembled) monolayer.¹⁴⁻²⁰ Considering the chemical structures shown in Fig. 1, the porphyrin molecules used in this study, on the other hand, tend to physisorb on a metal island surface by van der Waals forces—no chemical interaction produced. We thus attempted to develop a SEIRA technique for detecting nanogram quantities of the physisorbed porphyrin molecules in the conventional transmission geometry by simply spotting the sample solution onto the metal-evaporated CaF₂ substrate. For each sample used, substantial enhancements were successfully achieved as compared to those with a bare CaF₂ substrate. The detection limit improved by the SEIRA-transmission technique was in the nanogram range, which is less than the monolayer quantity.

In this paper, we report an anomalous enhancement observed with some absorption bands in the SEIRA-transmission spectra of manganese (III) tetraphenylporphine chloride (Mn(TPP)Cl) physisorbed on a gold island surface. Such enhancement associated with the spectral shifts was present only after the immersion of the gold substrate into acetone. The spectral comparison of Mn(TPP)Cl and the free-base tetraphenylporphine (H₂TPP) suggests a significant influence of the porphyrinic metal on the SEIRA mechanism of porphyrin systems. Dependences



Mn (III) tetraphenylporphine chloride (Mn(TPP)Cl) **Free-base tetraphenylporphine (H₂TPP)**

Fig 1. Chemical structures of manganese (III) 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine chloride (Mn(TPP)Cl) and the analogous free-base 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (H₂TPP).

of the enhancement factor on type (gold vs. silver) and thickness of the evaporated metal were also investigated. In addition, the combination of spectral results obtained with UV-visible and SEIRA techniques suggests that the physisorbed Mn(TPP)Cl molecules possess a preferred orientation of an inclined ring on the gold surface, even though the physisorption is in theory unable to approach any specified alignment of adsorbed molecules.

MATERIALS AND METHODS

Materials and Reagents

Manganese (III) 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine chloride (Mn(TPP)Cl) and the analogous free-base 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine (H₂TPP) were obtained from Frontier Scientific, Inc. (Logan, UT) and used without further purification. The reagents used in this study were obtained from Sigma-Aldrich Co. Ltd. (St. Louis, MO). Gold (Au) and silver (Ag) wires 0.25 mm in diameter, utilized for the evaporation process, were purchased from Nilaco Corporation (Tokyo, Japan), and their purity was 99.98%.

Preparation of Metal Island Films for Metal Substrates

Pre-cleaned glass slides (Iwaki Clinical Test Ware, Tokyo, Japan) used for UV-visible transmission measurements were cleaned with a "piranha solution" (H₂SO₄ + H₂O₂ = 3:1), followed by flushing with plentiful water and purging with nitrogen gas. For IR experiments, a CaF₂ window with a diameter of 20 mm (Pier Optics Co. Ltd., Gunma, Japan) was selected. The CaF₂ surface on which metal islands were deposited was first polished with alumina slurries with successively finer particle sizes from 0.3 μm down to 0.05 μm, prior to successive sonications in acetone and milli-Q water. The evaporation approach was performed under high-vacuum at a pressure of <5 × 10⁻⁴ Pa (ca. 3.75 × 10⁻⁶ torr). The deposition rate was kept constant at 0.02 Å s⁻¹, using a quartz crystal microbalance (TM-350, Maxtek Inc., Santa Fe Spring, CA). The evaporation procedure was stopped at a particular thickness of metal deposited, as mentioned individually in each figure. To achieve a uniform metal morphology, the metal-evaporated substrates were further kept inside the vacuum bell jar for ~30 min to slowly cool down the system. The metal uniformity of obtained substrates was further explored by use of atomic force microscopy (AFM) in a tapping mode (SPM-9500, Shimadzu Corporation, Kyoto, Japan). It should be kept in mind for the substrate preparation that insufficient pre-cleaning of a substrate might cause

spotting, and thereby inhomogeneity of evaporated metal films, which would yield results with poor reproducibility. Such substrates were discarded.

Sample Preparations and Experimental Parameters

UV-visible Spectroscopy. A pre-cleaned glass slide and a freshly prepared gold-deposited glass slide with a gold thickness of 3 nm were immersed in an acetone solution of 100 μM Mn(TPP)Cl or H_2TPP , for 36 h. The substrates were then dipped into acetone, in order to remove excess adsorbed molecules. All the substrates were immediately employed for the spectral measurements.

The UV-vis transmission spectra were collected with a Shimadzu UV-3101PC UV-Vis-NIR scanning spectrometer. The UV-vis spectra of Mn(TPP)Cl and H_2TPP on a cleaned glass slide and a 3-nm-thick gold/glass slide were both recorded under the same experimental conditions. In order to achieve the spectrum of only porphyrins adsorbed on the gold-evaporated side of the glass slide, half of the spectrum observed with a bare glass slide was subtracted from the porphyrin spectrum measured with the gold island substrate.

IR/SEIRA Spectroscopy. The porphyrin solution was placed onto a metal-evaporated and a cleaned CaF_2 window, using a microsyringe. A spectral collection was performed repeatedly until identical spectra were obtained, to assure the complete evaporation of solvent (i.e., acetone), so that no spectroscopic evidence of solvent remained. A change in the enhancement factor with increasing sample volume was then investigated. In the last step, the substrate was immersed into acetone to reduce the excess number of physisorbed molecules before a final IR data collection.

The IR and SEIRA spectra of both Mn(TPP)Cl and H_2TPP were measured with a Nicolet 550 FTIR spectrometer equipped with an HgCdTe detector. All the IR spectra shown in this paper were acquired under the same experimental parameters by using a gain of 1, 4-cm^{-1} resolution, and a 7800-cm^{-1} bandwidth, with a mirror velocity of 1.8988 cm s^{-1} , giving an optical retardation velocity (ORV) = 100 kHz for the HeNe reference beam. Happ-Genzel apodization and Mertz phase collection were set as default acquisition parameters under Omnic software, without applying zero-filling applied. The number of coadded scans was 1024. Note that a higher number of scans does not always produce a significant improvement in spectral quality. The enhancement factor was estimated by comparing the absorption intensity of a particular band in the SEIRA spectrum with that of the corresponding band in the reference IR spectrum.

Fluorescence spectroscopy. The fluorescence emission spectra were recorded with a FluoroMax-2 Spectrofluorometer (Jobin Yvon-SPEX, Instruments S.A. Inc., Japan) at the excitation wavelength of 414 nm. The free-base H_2TPP used for producing the calibration curve was in dilute solution with the concentrations ranging from 10^{-3} to 100 μM . The spectrum of the H_2TPP sample solution was subsequently collected with the same experimental procedure, and the concentration was then determined based on the calibration curve obtained.

RESULTS AND DISCUSSION

Ultraviolet-Visible Spectra

UV-vis transmission spectra of Mn(TPP)Cl and H_2TPP deposited on 3-nm-thick gold substrates are shown in Fig. 2, where those of Mn(TPP)Cl and H_2TPP in acetone solutions are also presented for comparison purpose. The spectrum of the Mn(TPP)Cl solution shows a Soret band at $\lambda_{\text{max}} \sim 468\text{ nm}$ and two weak Q-bands at 571 and 609 nm, which is typical of a metalated porphyrin. On the other hand, the UV-vis spectrum of

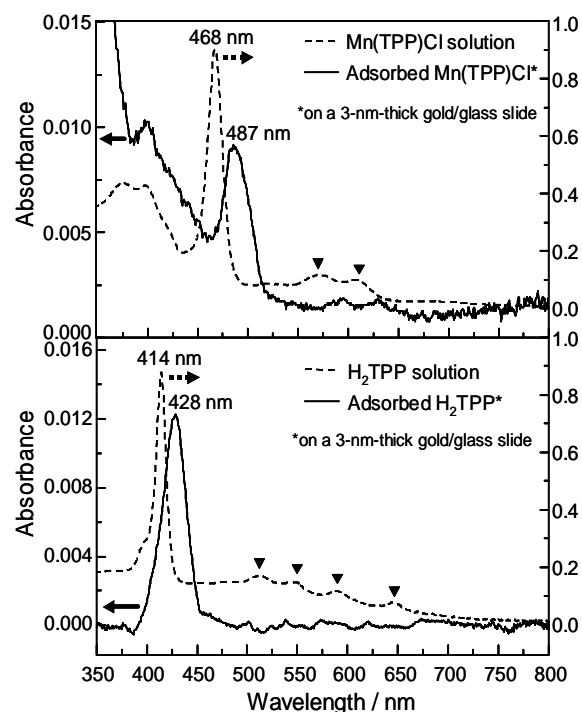


Fig 2. UV-visible transmission spectra of Mn(TPP)Cl (top) and H_2TPP (bottom) deposited on a 3-nm-thick gold substrate (solid line), and in an acetone solution (dashed line). For both samples, the y-axis at right hand side represents the scale for the solution form, whereas the left-side one indicates the scale for deposited molecules on the gold island film, as directed by the dashed and solid arrows, respectively.

the analogous H_2 TPP solution exhibits a strong Soret band at $\lambda_{\text{max}} \sim 414$ nm followed by four weak Q-bands, which is consistent with previous reports.^{14,16} For both porphyrin samples, differences in spectral features are clearly illustrated by comparing the spectra obtained with the adsorbed porphyrins to those in the solution forms. In particular, the spectra of deposited molecules yield a distinct red shift ($\Delta\lambda$) of the Soret band compared to the solution spectra (i.e., $\Delta\lambda = 19$ and 14 nm for $\text{Mn}(\text{TPP})\text{Cl}$ and H_2 TPP, respectively). Remarkable band broadening of the Soret band is also observed for the spectra of the deposited molecules (i.e., $\Delta\text{FWHM} = 11$ and 15 nm for $\text{Mn}(\text{TPP})\text{Cl}$ and H_2 TPP, respectively). These observed red shift and band broadening of the Soret band arise from the cofacial intermolecular p-electron interaction of the adsorbed porphyrins, which have commonly been explored with porphyrin chromophores in the forms of thin films.¹⁴⁻¹⁶ Accordingly, the adsorbed porphyrin molecules in this study likely aligned their macrocyclic rings parallel to each other in order to produce an effective π -electron interaction between the neighboring molecules. Therefore, the physisorbed porphyrin molecules are assumed to possess a preferred orientation on the gold islands, although the molecules in fact bind on the metal surface by only weak van der Waals forces in which a random orientation is expected.

Regarding the spectral shift, Schick et al.¹⁴ first reported a structural model for the arrangement of a porphyrin macrocycle in a monolayer on the basis of both experimental and theoretical observations. The experiment was carried out with some metalated 5,10,15,20-tetrakis[4-(1-octyloxy)phenyl] porphyrins on glass substrates using the LB method. They deduced the red-shifted Soret band as a result of off-edge configuration, which was later ascribed to the head-to-tail dipolar interactions between the neighboring porphyrin molecules.¹⁵ Similar red shifts were additionally noted in other covalently attached porphyrins in the form of SAMs,¹⁶⁻¹⁸ as well as in a solid-state (i.e., evaporated) porphyrin film.¹⁹ With respect to the excitonic coupling theory of the electronic transitions in porphyrin π systems, the face-to-face π - π stacking interaction responsible for a perpendicular (i.e., stack-of-cards) orientation has been well-known to produce a blue-shifted Soret band with or without band splitting.¹⁴ The observed red shift with the lack of split Soret bands thus supports the formation of head-to-tail aggregates—the plane of the porphyrin rings is coplanar with the surface of the metal islands.

Fluorescence Spectroscopy

Intensities of IR bands (*vide infra*) in the range of milli-absorbance units (10^{-3} a.u.) generally correspond to the thickness of a typical porphyrin monolayer (~ 20

Å).¹⁵ In contrast, the porphyrin molecules physisorbed on the metal film in this study do not form a uniform monolayer. The total number of molecules deposited is expected to be less than that necessary to form a monolayer. In an attempt to estimate the number of porphyrin molecules deposited on the gold substrate after immersion of the substrate into acetone, fluorescence spectra of standard H_2 TPP solutions with various concentrations, ranging from 10^{-3} to 10 nM, were collected to produce a calibration curve. The resulting emission spectra are presented in Fig. 3. Apparently, the fluorescence intensity increases with the concentration. As shown in the inset of Fig. 3, the plot of observed intensity at 648 nm as a function of concentration (in nM) was fitted very well (i.e., with an excellent correlation coefficient $R^2 = 0.9999$) to a linear relationship with the equation, $y = (3.4067 \times 10^8) x + 7573.53$, within the concentration range of 10^{-3} to 10 nM. In fact, the calibration curve experimentally elucidated a longer range of an acceptable linearity ($R^2 = 0.9997$) up to 100 nM (data not shown). At higher concentrations, the curve presented a deviation from linearity that matches a polynomial relationship.

For the sample preparation, we first applied $50 \mu\text{L}$ of $10 \mu\text{M}$ H_2 TPP onto gold substrates with a thickness of 3 and 10 nm. The total number of H_2 TPP molecules on both substrates was originally equivalent to 3.01×10^{14} (see Table 1). After immersion, the concentrations of porphyrin molecules desorbed into the acetone solution, obtained with the 3 - and 10 -nm-thick gold substrates are consequently plotted at 4.61 and 4.95 nM, respectively. In the case of the 3 -nm-thick substrate, the number of porphyrin molecules deposited

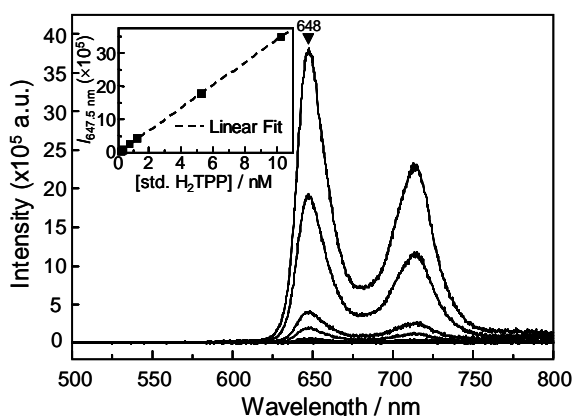


Fig 3. Fluorescence spectra of standard H_2 TPP solutions with the concentrations ranging from 10^{-3} to 10 nM. All the spectra were collected under the same conditions. **Inset:** Plots of the intensity at 648 nm as a function of the concentration of the standard H_2 TPP solutions, which are fitted very well ($R^2 = 0.9999$) by the linear equation of $y = (3.4067 \times 10^8) x + 7573.53$.

on the metal surface was estimated to be in total $\sim 2.4 \times 10^{13}$ molecules and the surface coverage of $\sim 3.0 \times 10^{-11}$ mol/cm² was consecutively acquired by assuming a circular area of sampling with a diameter of 1.30 cm. Therefore, the calculated molecular area of the adsorbed porphyrin molecules was about 1 macrocycle per 562 Å². According to the previous study of *meso*-tetrakis(4-pyridyl)porphyrin,²⁰ the molecular area and the surface coverage of a densely packed monolayer were 262 Å² and 7.0×10^{-11} mol/cm² when the porphyrin is coplanar to the gold surface. By a comparison of our calculated molecular packing density with this, the physisorbed porphyrin yields less surface coverage by a factor of 2.4, relative to that of the chemisorbed monolayer. It is, thus, very likely that the present porphyrin molecules are distributed very loosely on the metal island substrates because of the weak van der Waals interaction of the physisorption. In the experiments, the values of the surface coverage obtained with the physisorbed porphyrin are not highly reproducible. The factors involved mainly include uncertainty in binding strength acquired by physisorption, and partly, the difference in the morphology of the gold islands in the individual films even at the same metal thickness. However, the estimated number provides a quantitative range of the surface density observed for physisorbed molecules.

As shown in Table 1, the 10-nm-thick gold substrate yielded a substantial decrease in the surface coverage, by factors of 8.4 and 20.0, in comparison to the values obtained with the 3-nm-thick gold film and the *meso*-tetrakis(4-pyridyl)porphyrin monolayer, respectively. This can be explored further by examining the contrast of surface morphology between the two substrates with the different metal thickness.

Atomic Force Microscopy

Regarding the difference in the number of deposited molecules observed on the substrates with the different metal thicknesses, AFM topographic investigations were carried out. Figure 4 displays $1 \times 1 \mu\text{m}$ AFM images of gold island films with 3 and 10 nm in thickness, evaporated on mica substrates. The individual films reveal a good uniformity achieved by the described

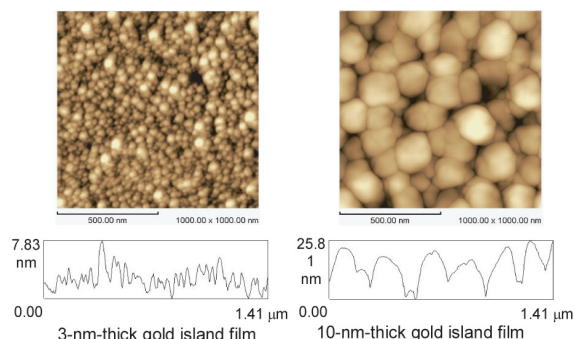


Fig 4. AFM images of the 3-nm-thick (right) and 10-nm-thick (left) gold island films deposited on mica substrates with the same evaporation conditions as those for a CaF₂ plate used for IR measurements. Both images were observed with the same size of scanning area (i.e., $1 \times 1 \mu\text{m}$) in a tapping mode. The roughness profiles shown below an individual image were specified by a diagonal line from upper left to lower right corners.

evaporation conditions. However, it should be noted that they exhibit distinct differences in surface morphology. The 3-nm-thick gold film perceptibly consists of small round islands with an average diameter of 20–30 nm and 3–4 nm in height. When the metal thickness is increased to 10 nm, the particles tend to join together and merge into larger islands producing a relatively smooth surface with a larger diameter in the range of 90–120 nm. Presumably, the porphyrin molecules retained after the immersion into acetone were mostly accommodated by cavities/channels between metal particles (i.e., at terrace edges and islands boundaries), rather than on the metal hill. As a consequence, the higher number of the remaining molecules was obtained with a thinner metal island film where the fine distribution of many small metal particles yields more cavity sites for the adsorbed porphyrin molecules than those of thicker films.

Surface-Enhanced Infrared Absorption Spectra

***Mn(TPP)Cl*.** We first investigated the sensitivity achieved with the SEIRA technique by using gold and silver substrates with an identical thickness (i.e., 3 nm), in comparison to that acquired with a bare CaF₂

Table 1. Calculated number of molecules and ng quantity of the free-base H₂TPP deposited on gold island substrates with the thickness of 3 and 10 nm, measured both before and after the immersion in acetone.

Thickness of gold island films	Before the immersion		After the immersion	
	Number of adsorbed molecules	ng quantity	Number of remaining molecules	ng quantity
3 nm	3.01×10^{14}	307.38	2.36×10^{13}	24.06
10 nm			2.80×10^{12}	2.86

substrate in a conventional IR transmission technique. Figure 5(A) shows an IR spectrum of Mn(TPP)Cl in a KBr pellet used as a reference, whereas Fig. 5(B) displays IR spectra of a Mn(TPP)Cl solution applied on gold and silver island films with the same thickness of 3 nm and on a bare CaF₂ substrate. In the cases in Fig. 5(B), only 1 μ L of 10 μ M sample solution was placed onto the substrates. The total sample amount on the substrates could then be calculated to be \sim 7 ng. As can clearly be seen in Fig. 5(B), both the gold and silver substrates yield substantially larger spectral enhancement than the bare CaF₂ substrate. The spectral patterns of these enhanced spectra are very similar to that of the KBr-pellet spectrum, showing that the porphyrin molecules physisorbed on the metal island surfaces exhibit the SEIRA effect. The numerical enhancement factor cannot be determined precisely, since the spectrum of the Mn(TPP)Cl solution on the bare substrate displays no observable signal above the noise level. However, based on the peak intensity of an intense band at 1010 cm⁻¹, which is fully characteristic of metalated porphyrin and assignable to the C _{β} -H rocking vibration of the pyrrole ring,²¹ it is roughly estimated to be 96 and 50 for the gold and silver island films, respectively. Apparently, the gold substrate gives rise to about twice as high of an enhancement as the silver one with the identical metal thickness.

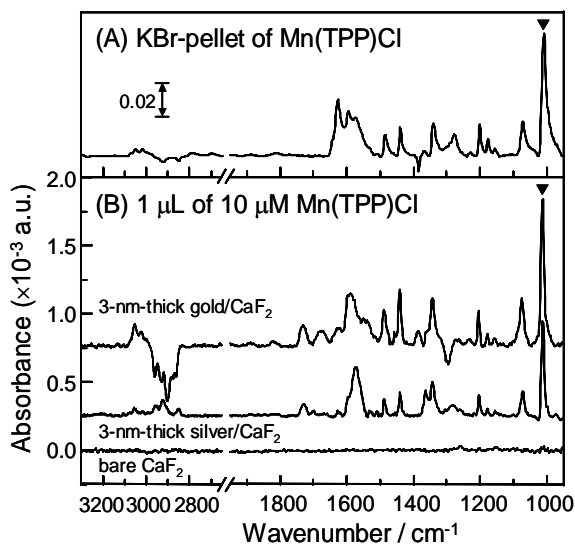


Fig 5. (A) An IR transmission spectrum of Mn(TPP)Cl powder dispersed in a KBr pellet used as a reference. (B) IR transmission Spectra of Mn(TPP)Cl physisorbed on a 3-nm-thick gold/CaF₂, a 3-nm-thick silver/CaF₂, and a bare CaF₂ substrate as presented from the top to the bottom lines, respectively. The samples were introduced by putting a 1 μ L/10 μ M Mn(TPP)Cl solution on the substrates. All the spectra were measured under the same experimental conditions.

According to the previous investigations with secondary electron microprobe (SEM) and SEIRA techniques,^{22,23} it was suggested that the degree of the absorption enhancement depends on the distribution and size of the metal islands. In more detail, an optimized packing density of the islands with definite channels surrounding each metal particle gives rise to a larger absorption enhancement. We, therefore, conducted an experiment to investigate a change in the enhancement factor as a function of metal thickness by using gold island films with increasing thickness from 1 to 10 nm. Although some previous publications on the SEIRA phenomenon reported the optimum thickness of a metal film to be in a range of 7-10 nm,^{6,24} we, on the contrary, gained only a slight improvement in the enhancement factor for thicker metal island films (>5 nm; data not shown). Particularly, the use of thicker films yielded deteriorated spectral quality due to a distinctive decrease in energy throughput, thereby causing a poorer signal-to-noise ratio (SNR). We have been unable to achieve additional improvement in the spectral SNR with longer scans because the resulting spectra with >1024 scans demonstrated intense peaks due to water vapor, which interfere the appearance of weak sample signals. In an attempt to compromise between the enhancement factor and the spectral quality, the substrates with the 3 nm metal thickness were employed for further experiments.

Figure 6 (a)-(e) shows the dependence of the SEIRA intensity on sample volume applied on a gold island film with the thickness of 3 nm. According to the Beer's law, absorbance is linearly increased with the added sample amount. However, as can be seen from the inset of Fig.

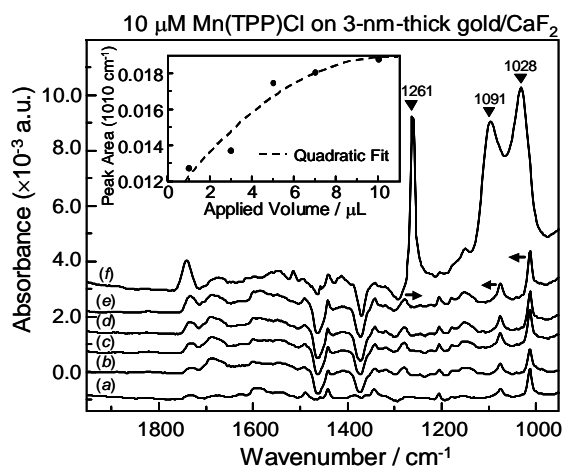


Fig 6. A series of spectra of 10 μ M Mn(TPP)Cl applied on a 3-nm-thick gold/CaF₂ with subsequently increasing the sample volume (i.e., 1, 3, 5, 7, 10 μ L, and after immersion) present from (a) – (f). **Inset:** Plot of the peak area at 1010 cm⁻¹ as a function of applied sample volume, which tends to fit by a quadratic relationship.

6, the increase in absorbance at 1010 cm^{-1} tends to be linear only for the first few μL applied. The plot in the inset is presented in terms of peak area, instead of the absorption intensity, in order to minimize the band-shape effect. After $5\ \mu\text{L}$, the fitted curve tends to approach an asymptotic value monotonically which matches well to a quadratic relationship.

Figure 6(f) illustrates the SEIRA spectrum obtained after the immersion of the metal substrate into acetone. Of note is that anomalously enhanced peaks are clearly observed at 1028 , 1091 and 1261 cm^{-1} , as marked by inverted triangles in the figure. The first two enhanced bands, which are due to the $C_{\beta}\text{-H}$ rocking vibration of the pyrrole ring and the asymmetrical $C_{\beta}\text{-H}$ deformation, respectively,²¹ are considerably shifted to a higher frequency by $\sim 20\text{ cm}^{-1}$ in comparison to the corresponding bands in the spectra of $\text{Mn}(\text{TPP})\text{Cl}$ measured before the immersion and $\text{Mn}(\text{TPP})\text{Cl}$ in the KBr pellet. In contrast, the enhanced peak at 1261 cm^{-1} , which is assigned to the symmetrical stretching of pyrrole half ring, exhibits a shift to a lower frequency with the same magnitude (i.e., $\sim 20\text{ cm}^{-1}$), as directed by arrows in the figure. Regarding band shifts in metalloporphyrin systems, Thomas and Martell²¹ reported that a shift is often observed for bands near 1000 cm^{-1} . It has been generally accepted that the bands in this region are sensitive to the nature of the central metal ion, and are directly related to the strength of the porphyrinic metal-nitrogen bond in metallotetraphenylporphines. A change in the Mn-N bond strength is accordingly proposed for $\text{Mn}(\text{TPP})\text{Cl}$ molecules remaining after the immersion, to explain the observed spectral shift and the unusual absorption enhancement. In particular, the charge-transfer (CT) interaction between Mn and gold islands is considered as a key factor responsible for changes in the bond strength, since such an interaction is the fundamental feature existing in porphyrin systems, which is capable of altering the charge distribution in the adsorbed porphyrins.

Despite the enhanced peaks investigated, the SEIRA effect thereby exhibits a strong influence mainly on the porphyrin ring, rather than the substituted groups at meta-carbon positions. However, the enhanced peaks indicate a difference in the enhancement factor after the immersion as compared to those corresponding intensities obtained before the immersion. In particular, the enhancement factor of the band at 1261 cm^{-1} appears to be higher than those of the two bands at lower wavenumbers. The different enhancement factor can be theoretically explained based on the surface selection rule—*only the vibrations that give a portion of dipole changes perpendicular to a metal surface are SEIRA active, and their absorption intensities are greatly enhanced.*⁶ Generally speaking, SEIRA intensity depends strongly

on the orientation of a particular vibration relative to the perpendicular axis of the metal surface. If the vibration orients close to the perpendicular axis of the metal surface, this results in stronger SEIRA effect and thereby a higher enhanced intensity. In contrast, no enhanced intensity is observed in the case that the vibration aligns itself parallel to the metal surface. Our spectral observations suggest that, after the immersion, the band at 1261 cm^{-1} (i.e., the symmetrical stretching of pyrrole half ring) re-oriented closer to the axis perpendicular to the metal surface, giving rise to a higher enhancement factor than those of the bands at 1091 and 1028 cm^{-1} . By applying the rule to the observed SEIRA spectra with a combination of the UV-vis results, the molecular orientation is more consistent with an inclined configuration—the adsorbed porphyrin molecules aligned the rings parallel to each other, but the molecular plane is likely tilted, at least to some extent, with respect to the gold island surface.¹⁷ However, a clearer analysis of the ring alignment must await some additional in-depth spectroscopic studies.

Free-base H_2TPP . The IR and SEIRA measurements of the metal-free tetraphenylporphine are very important for the comparison with the IR and SEIRA spectra of the metalated $\text{Mn}(\text{TPP})\text{Cl}$. Accordingly, we further investigated the SEIRA effect for H_2TPP by the same experimental procedure as that for $\text{Mn}(\text{TPP})\text{Cl}$. Figure 7(A) shows a transmission IR spectrum of H_2TPP

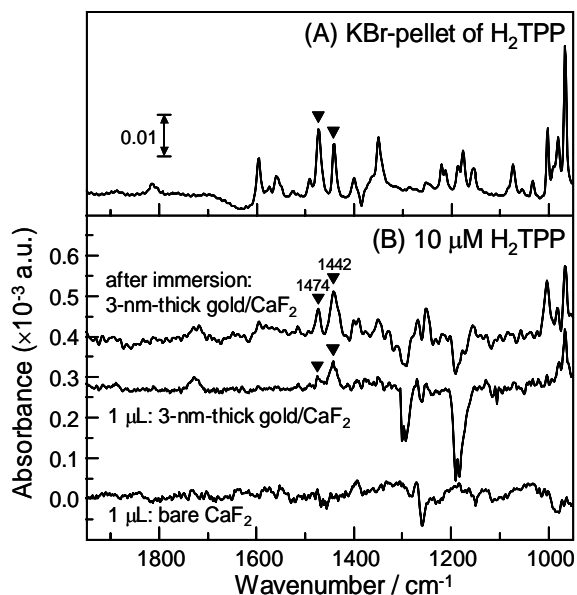


Fig 7. (A) ATR transmission spectrum of H_2TPP in a KBr matrix. (B) SEIRA spectra of $1\ \mu\text{L}/10\ \mu\text{M}$ H_2TPP applied on a $3\text{-nm-thick gold}/\text{CaF}_2$ (before and after the immersion, as shown from the bottom to the top lines) in comparison to that observed with $1\ \mu\text{L}/10\ \mu\text{M}$ H_2TPP on bare CaF_2 .

in a KBr matrix and Fig. 7(B) presents SEIRA spectra of 1 $\mu\text{L}/10 \mu\text{M}$ H_2TPP applied on a 3-nm-thick gold film, both before and after the immersion, in comparison to that obtained with 1 $\mu\text{L}/10 \mu\text{M}$ H_2TPP on bare CaF_2 . It can be seen from Fig. 7(B) that the SEIRA-active gold substrate exhibits some detectable signals, while the bare substrate displays only noise all over the frequency range investigated. By using the 3-nm-thick gold film with only a total amount of 6.15 ng sample deposited (i.e., less than the quantity for a monolayer), characteristic bands of the metal-free porphyrins are observed at 1442 and 1474 cm^{-1} , as marked by inverted triangles. These two bands arise from the symmetrical $\text{C}_\alpha\text{-C}_m$ and $\text{C}_\beta\text{-C}_\beta$ stretching vibrations of the porphyrin skeleton.²¹ The enhancement factors achieved with the gold substrate were estimated to be 7 and 1.5 for the corresponding bands at 1442 and 1474 cm^{-1} , as compared to that of a bare substrate. Once again, their intensities increase noticeably in the spectrum collected after the gold substrate was immersed in acetone, by factors of 11 and 3.5 relative to those in the spectrum of H_2TPP on the bare substrate. However, neither a peak shift nor an unusual absorption enhancement takes place in the case of the metal-free porphyrin.

Regarding the spectral comparison of $\text{Mn}(\text{TPP})\text{Cl}$ and H_2TPP , it is noted that $\text{Mn}(\text{TPP})\text{Cl}$ gives more highly enhanced intensities than H_2TPP in all the cases. Moreover, the remarkable spectral shifts and the anomalously enhanced peaks were observable only with $\text{Mn}(\text{TPP})\text{Cl}$. These observations thus support the contribution of the proposed CT interaction between the porphyrinic Mn-gold islands, which may be considered as an additional factor influencing the SEIRA mechanism in porphyrin systems, in addition to the well-defined mechanism of the enhanced EM field. However, further attempts must be made to clarify the effect of the porphyrinic metal that is attributed to play a role in the enhancement mechanism. In retrospect, another possibility can be adopted based on the fact that the metal-free porphyrins reportedly adhere just poorly on the metal surface.¹⁶ Accordingly, a considerable decrease in the number of the adsorbed molecules remaining after immersion is expected for H_2TPP , as compared to $\text{Mn}(\text{TPP})\text{Cl}$. However, fluorescence spectroscopy could not be employed for determining the number of remaining $\text{Mn}(\text{TPP})\text{Cl}$ molecules, due to the lack of emission at any excitation wavelength.

Furthermore, the SEIRA spectra of both $\text{Mn}(\text{TPP})\text{Cl}$ and H_2TPP measured after the immersion exhibit substantially higher enhancements than the corresponding spectra collected before the immersion with 1 μL sample solution applied, though the latter case, in fact, possessed a larger number of porphyrins deposited. In this respect, fluorescence microscopy

revealed the formation of microcrystalline porphyrin aggregates during a gradual increase of sample volume on the gold substrate. In other words, the porphyrins preferentially cling together, rather than adhering directly on the gold surface, upon increasing the sample amount. It should be noted that the enhancement phenomena, based on the exponential decay of the induced EM field on a metal particle, is theoretically restricted within a narrow region ($\sim 5 \text{ nm}$) in contact with the metal surface.² As a result, only a small portion of porphyrin molecules in direct contact with the gold surface is involved in the enhanced EM field of the SEIRA phenomenon. However, the immersion of gold substrates helps re-distributing the porphyrin aggregates to be directly attached on the gold islands, as evidenced by an absence of microcrystalline aggregates in the observed fluorescence image. Therefore, the remarkably stronger enhancement obtained after the immersion could be considered as a result of an improved adsorption between the porphyrin molecules and the gold surface.

Finally, it is noteworthy to report that, for both porphyrin samples, the negative bands in the region of 1400-1200 cm^{-1} in the SEIRA spectra of gold substrates arise from saturated hydrocarbon contaminants occurring during the evaporation process.²⁵ The negative direction indicates that the hydrocarbons were later replaced by the applied porphyrin molecules. To remove these negative bands and get rid of the contamination, Osawa and his co-workers²⁵ suggested the use of electroless deposition instead of the high-vacuum evaporation. However, the method is currently limited to only the deposition of gold film onto a silicon substrate, and moreover, a gradual change in metal thickness is unlikely to be deposited accurately, compared to the use of a quartz crystal microbalance in the evaporation technique.

CONCLUSION

Applications of SEIRA technique to the porphyrins physisorbed on metal island films have been demonstrated. Nanogram quantities of $\text{Mn}(\text{TPP})\text{Cl}$ and H_2TPP adsorbed on metal substrates exhibited substantial intensity enhancements with respect to the results with a bare substrate. Gold films, particularly, have yielded ~ 2 -times higher enhancement than silver films, and the enhanced intensity increases nonlinearly with increasing sample solution. The SEIRA spectra of both porphyrins have shown considerably higher intensities after the immersion process, which are possibly caused by the re-distribution of the adsorbed porphyrin molecules to adhere directly onto the gold surface.

In an essence, the anomalously enhanced peaks

associated with the spectral shifts were observed only for the metalated Mn(TPP)Cl, not for the metal-free H₂TPP. Accordingly, the additional CT interaction between the porphyrinic Mn and gold islands is proposed to contribute in the SEIRA mechanism of porphyrin systems, in addition to the generally accepted EM mechanism. The UV-vis results in combination to the observed SEIRA spectra have suggested a preferred orientation of the physisorbed porphyrins on gold film in an inclined configuration. However, an analysis of orientation of the porphyrin physically bound on the gold islands must await for additional in-depth spectroscopic studies.

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