EFFECT OF GOLD NANOPARTICLES ON AN AEROSIL SURFACE
ON THE FLUORESCENCE AND RAMAN SPECTRA
OF ADSORBED TRYPTOPHAN

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We discuss the fluorescence and Raman spectra of the amino acid tryptophan (Trp) in the presence of gold nanoparticles in solution and on the surface of highly dispersed silica (aerosil) containing gold nanoparticles (Au-SiO$_2$). The fluorescence of Trp is efficiently quenched in the presence of gold nanoparticles both in solution and on the SiO$_2$ surface. The fluorescence and excitation spectra contain bands for molecular Trp and a charge transfer complex between Trp and the nanoparticles. In the Raman spectra of Trp with gold nanoparticles, considerably enhanced intense vibrations appear for the carboxyl and amino groups and also for the benzene and pyrrole rings. The effect of gold nanoparticles on the Raman spectra of Trp in a heterogeneous system is considerably weakened due to strong light scattering by the dispersed silica.

Key words: *gold nanoparticles, tryptophan, silica, fluorescence, enhancement of Raman scattering.*

In recent years, extensive experimental and theoretical data has been accumulated on the electronic spectra of biomolecules and Raman light scattering by such biomolecules: amino acids, proteins, etc., located near the surface of nanosized metal particles ([1] and citations therein, [2, 3]).

In studying fluorescence or surface-enhanced Raman scattering (SERS) by molecules in the presence of nanoparticles of noble metals in biomedical research, the starting hypothesis is that "labeled" particles are formed, marking the materials by direct addition of marker molecules to the surface of the nanoparticles. Study of the SERS phenomenon is of considerable theoretical and practical value both for understanding the mechanisms of interaction between biomolecules and metallic nanoparticles in each specific system and for solving applied problems in surface chemistry, analytical applications, in biochemistry, biomedicine, etc. In the literature, two mechanisms are considered for enhanced Raman scattering when molecules (including biomolecules) are adsorbed on metallic surfaces: electromagnetic and molecular. Electromagnetic enhancement is due to resonant incident or scattered electromagnetic radiation with characteristic electronic plasma oscillations localized near the surface of the nanoparticles. The increase in the local electromagnetic field near the surface of a

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metallic particle leads to an increase in the induced dipole moment of the molecule. The molecular mechanism for enhancement of Raman scattering occurs when the molecule chemically reacts with the metal atoms and new excited states are formed in such a complex [4-6]. In this case, the excited states are due to electron transfer from the molecule to the metal or vice versa.

In colloidal solutions of nanosized metals, application of electronic spectroscopy and Raman scattering in studying biomolecules is limited due to instability of the metallic particles during storage. In order to avoid precipitation of the nanoparticles, stabilizers are used: surfactants, polymers, metal oxides, etc. [7-9]. Conjugation of proteins with nanoparticles not only promotes their stabilization but more importantly is accompanied by their chemical reaction with the nanoparticles and formation of new functional composite materials for future biomedical application [10-14]. In this case, the toxic effect of nanoparticles on the cells in the body is suppressed.

In this work, as a biomolecule having high affinity for gold nanoparticles and simultaneously capable of acting as a stabilizer and reducing the toxic effect of the nanoparticles on living cells, we used tryptophan (Trp): an essential amino acid in the human diet. The aim of this work was to use electronic spectroscopy and Raman light scattering to study the interaction between gold nanoparticles, anchored on the surface of dispersed SiO_2, and adsorbed Trp molecules.

EXPERIMENTAL

Colloidal solutions of gold nanoparticles were obtained by reducing tetrachloroauric acid in the presence of tryptophan. The components were reacted in the mole ratio $v(Au^{3+}) : v(Trp) = 1 : 1$ as in the procedure described in [2], but without additional heating of the reagents. The concentrations of gold and tryptophan in the resulting solution were $C(Au) = C(Trp) = 10^{-3}$ mol/L.

Heterogeneous composites were obtained by two methods: 1) the colloidal solution of the complex between the gold nanoparticles and tryptophan was quantitatively adsorbed on the surface of SiO_2 that had been calcined at 500 °C (Au-Trp/SiO$_2$), where the ratio $C(Au) = C(Trp) = 10^{-5}$ mol/g (Au 0.2%); 2) samples of Au-SiO$_2$ (Au 0.02% and 0.2%) were obtained by quantitative adsorption of gold nanoparticles stabilized by cetyltrimethylammonium bromide (CTAB) from colloidal solution and dried at 85 °C. After drying, the Trp was adsorbed on the Au-SiO$_2$ surface (Trp/Au-SiO$_2$). The colloidal solution of gold nanoparticles was synthesized by reduction of tetrachloroauric acid by sodium tetrahydroborate (sodium borohydride) in aqueous solution. The metal concentration in solution was $1.5 \cdot 10^{-4}$ mol/L, the CTAB content in solution was 6 times higher than the molar amount of metal. The gold nanoparticles were coated with a stabilizer shell to determine the effect on the Raman spectra from the nanoparticles on the biomolecule when they were not in direct contact on the SiO_2 surface.

In this work, we used highly dispersed silica (aerosil) A300 with specific surface area 300 m^2/g, produced by the Kalush Research and Experimental Plant of the Institute of Surface Chemistry (National Academy of Sciences of Ukraine).

The morphology of the samples was studied on a LEO 1530 electron microscope. The fluorescence and excitation spectra of the colloidal solutions of Au-Trp and the heterogeneous composites were recorded on an Eclipse (Varian) spectrofluorimeter. The Raman spectra were excited by the emission from a cw Ar$^+$ laser at wavelengths 514.5 or 488 nm, power ~50 mW. The spectra were recorded using an automated DFS-24 dual monochromator, equipped with a cooled photomultiplier and a detection system operating in photon counting mode, and also on a Thermo Scientific/Nicolet Almega XR dispersive Raman spectrometer, spot size 2.0 μm, objective 10×80.

RESULTS AND DISCUSSION

According to electron microscopy data, the gold nanoparticles mainly form nanospheres when reduced in the presence of Trp. On the surface of SiO_2, the gold nanoparticles are uniformly distributed over the surface in the form of spheres. The average size of the nanoparticles is 30-40 nm.

The optical spectra of the gold nanoparticles in solution and in the adsorbed state contain characteristic surface plasmon resonance (SPR) bands with maximum at 560 nm in solution (Fig. 1a) and at 530 nm on the SiO_2 surface (Fig. 1b).
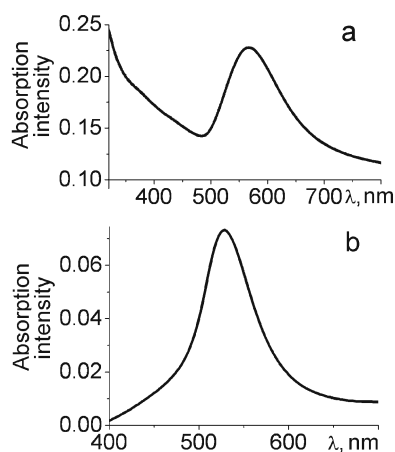
Fig. 1. Optical spectra of a colloidal solution of tryptophan-stabilized gold nanoparticles diluted 1 : 10 (a) and an Au-SiO$_2$ dispersion (b).

Fluorescence of Trp Molecules in Solution and on the Au-SiO$_2$ Surface

In the opinion of the authors of [15], when gold ions are reduced by Trp molecules, oxidative polymerization of the indole groups of the Trp bound to the nanoparticles occurs, as well as crosslinking of some portion of the gold nanoparticles to form polytryptophan. The fluorescence spectrum of Trp in aqueous solution is represented by a broad structureless band with maximum at 360 nm; the excitation spectrum has a maximum at 298 nm (Fig. 2, curves 1 and 2 respectively). In the presence of gold nanoparticles in solution, the fluorescence spectrum of Trp consists of two bands: a luminescence band for molecular Trp with λ_{max} = 360 nm, and a new band which we assigned to luminescence of a charge-transfer complex between Trp and the nanoparticles with λ_{max} = 450 nm (Fig. 2, curve 1′). The luminescence excitation spectrum of Trp bound to gold nanoparticles also consists of two bands: one band red-shifted by 5 nm belonging to the free molecule in solution, with λ_{max} = 303 nm; and a band for the charge-transfer complex with λ_{max} = 373 nm (Fig. 2, curve 2′). Possibly weak bands in the 230-260 nm region in the same spectrum (Fig. 2, curve 2′) can be assigned to Trp oxidation products, but we did not identify them in this work. The intensity of Trp luminescence is dramatically decreased in the presence of the nanoparticles (curve 1′ in Fig. 2 is magnified 15 times), which suggests quenching processes due to (as we hypothesize) chemical reaction: electron transfer from an excited molecule to a gold nanoparticle. The new band in the emission and excitation spectra of Trp with gold nanoparticles was assigned in Figs. 2 and 3 based on the chemical nature of the components and the spectral signs of donor–acceptor complex (charge transfer complex) formation, occurring in the ground state of the system: the appearance of new bands in the emission and absorption (excitation) spectra [16].

Adsorption of Trp on the Au-SiO$_2$ surface is accompanied by dramatic quenching of the fluorescence of Trp and a change in the spectrum. As in the solution of Trp with the gold nanoparticles, on the SiO$_2$ surface we detect an emission band for a charge-transfer complex between Trp and gold nanoparticles with maximum at 450-490 nm. The short-wavelength shift of the main emission maximum for adsorbed Trp on the original SiO$_2$ and on Au-SiO$_2$ (334 nm) compared with Trp in solution (360 nm) (Fig. 3, curves 1, 3) occurs due to protonation of the Trp amino group by weakly acidic OH groups of the aerosil surface, and is typical for the electronic spectra of aromatic molecules containing an amino group in acid medium [17]. Despite the weakly acidic function of the OH groups of silica, it is well known that the OH groups are capable of dissociation and ion exchange [18]. The emission spectra of Trp in the presence of the nanoparticles suggest the existence of several adsorption states for the amino acid on the surface: the OH groups of the SiO$_2$ surface, the gold nanoparticles on the silica surface, defects at the nanoparticle/SiO$_2$ boundary. In this case, we should point out that the adsorbability of Trp on the silica surface is
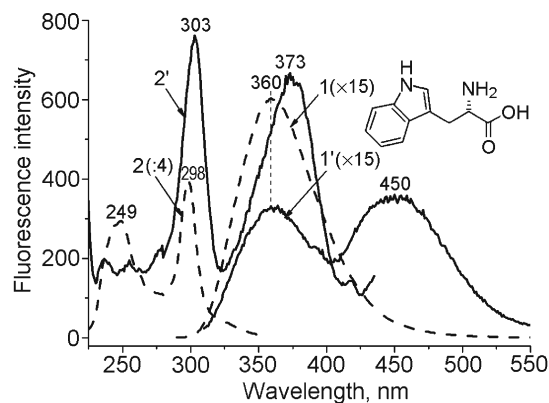
Fig. 2. Fluorescence and excitation spectra of Trp in aqueous solution (1, 2) and in the presence of stabilized gold nanoparticles (1', 2'). Excitation by light at $\lambda = 280$ nm; 2' is detected at the emission $\lambda = 450$ nm; the formula for the tryptophan molecule is given.
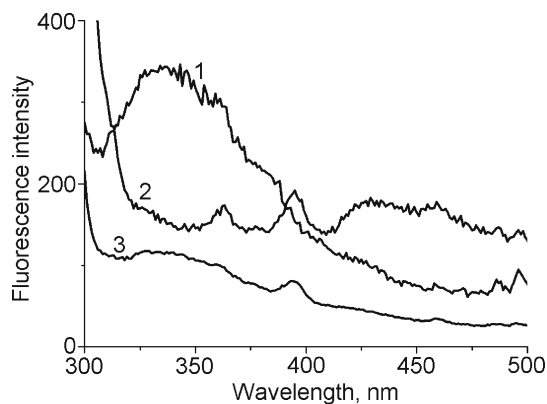

Fig. 3. Comparison of the fluorescence spectra of the following systems ($\lambda_{ex} = 290$ nm): 1) Trp, adsorbed on the surface of aerosil (Trp/SiO$_2$); 2) Au-Trp complex adsorbed on SiO$_2$ (Au-Trp/SiO$_2$); 3) Trp, adsorbed on the surface of Au(0.2%)-SiO$_2$ (Trp/Au-SiO$_2$), C(Trp) = 10^{-5} mol/g.

appreciably higher (by a factor of 1.5-2) in the presence of gold nanoparticles, possibly due to formation of new active centers and/or formation of electron-acceptor surface defects. The adsorption capacity can also increase due to the appearance of secondary porosity on contact between the aerosil and the Trp solution and subsequent drying. Broadening of the fluorescence spectrum of Trp in the 330-370 nm region is a consequence of superposition of the spectra for several emitting states of the adsorbed molecule.

In the case when Trp is adsorbed on an aerosil surface previously modified by gold nanoparticles (Trp/Au-SiO$_2$), the Trp fluorescence quenching effect is more pronounced than when the Au-Trp complex is adsorbed on the aerosil surface (Au-Trp/SiO$_2$) (Fig. 3, curves 2 and 3). This may be evidence for preferential localization of Trp molecules during adsorption near centers formed by gold nanoparticles on the aerosil surface and possibly for stability of a complex between the amino acid and the gold nanoparticles, not breaking down on adsorption.

The appearance of a band with maximum at about 395 nm (a shoulder at 392 nm in the case of the colloidal solution, cf. Figs. 2 and 3) in our opinion is connected with luminescence of a chelate compound between Trp and the gold ions, formed on the surface of the nanoparticles on contact with the environment and existing in dynamic equilibrium with the metal atoms of the nanoparticle. This hypothesis needs further study.

The fluorescence studies allow us to follow the processes involving quenching of Trp fluorescence by the metal nanoparticles. In [19], based on theoretical calculations, the authors discuss the nature of the interaction between Trp and the gold nanoparticles, including the amino, carboxyl, and indole groups, with formation of metal–molecule hybrid orbitals (the d orbital of gold and the atomic orbitals of Trp) having a mixed character. Nonradiative energy transfer from the excited Trp to such a hybrid state is accompanied by quenching of Trp fluorescence. In [20], the authors discuss the hypothesis that the carboxyl group of Trp loses an electron and is converted to a carbonyl group, and reduces the gold anion down to a nanoparticle, which may be the reason for quenching of the Trp fluorescence. Participation of the Trp amino group in the interaction with the gold nanoparticle is confirmed by the short-wavelength shift of the fluorescence spectrum on adsorption. The role of the π-electrons of the aromatic ring in the donor–acceptor interaction between the components on formation of the Trp-Au charge-transfer complex, accompanied by quenching of the fluorescence of the Trp molecules, also may be significant. Study of the Raman spectra of Trp in the heterogeneous system will help identify which functional groups are responsible for chemisorption of the Trp molecule.

Study of the Raman Spectra of Trp in the Presence of Gold Nanoparticles

The Raman method is a powerful tool for studying the molecular structures of Trp when it interacts with silver and gold nanoparticles. The Raman and SERS spectra of powders and aqueous solutions of Trp were studied in detail and presented in [21-23]. The authors of those papers, based on the fact that the COO— and C—COO— vibrations and the amino group vibrations are enhanced, showed that the preferred model for the interaction between the Trp molecule and the surface of silver nanoparticles is participation of the carboxyl and amino groups. In [23, 24], SERS was observed for Trp in silver colloids, depending on the nanoparticle aggregation time. The authors of [25] say that for low Trp concentrations, its interaction with the surface of silver nanoparticles occurs via the indole ring, positioned parallel to the surface.

In this work, the Raman spectra for Trp in the presence of gold nanoparticles were measured with the aim of detecting possible enhancement of the Raman scattering for Trp, and in the case of the heterogeneous system also taking into account localization of some of the adsorbed Trp molecules (not bound to gold nanoparticles) on the aerosil surface.

Figures 4 and 5 show the Raman spectra of Trp in solution and in the adsorbed state. Enhancement of the Raman scattering of Trp in the presence of gold nanoparticles is pronounced in solution (Fig. 4). With the aim of a more complete interpretation of the spectra obtained, we performed theoretical calculations for the normal modes of vibration for the Trp molecule in an aqueous medium (Gaussian 03, Revision D.01; B31yp/6-31G) (Table 1). From Fig. 4 we see that groups of lines are enhanced in the 850, 1080-1160, and especially 1350-1500 cm^{-1} regions. Analysis of the normal modes of vibration for the molecule showed that this involves stretching and librational vibrations of the C—COO—, CH$_2$, and NH$_3^+$ groups and also the benzene and pyrrole rings (about 1480 cm^{-1}) (see Table 1). Since enhancement of the vibrations occurs in the SERS spectra for the atoms and groups located closer to the surface, it is reasonable to assume that in this case interaction occurs between the surface of the gold nanoparticles and precisely the carboxyl and amino groups. According to the data in [6], chemisorption of Trp on gold nanoparticles occurs via the unshared electron pair on the nitrogen atom, and also as a result of the formation of a complex between the metal and the π-electrons of the indole ring of Trp, which probably participate in the process of reduction of the gold ions and formation of the nanoparticles.

Interpretation of the Raman spectra of Trp in the presence of gold nanoparticles when localized on the surface (Fig. 5) is very complicated compared with the spectra in solutions, due to the strong light scattering ability of silica, the presence of a layer of the stabilizer CTAB around the nanoparticle (preventing direct contact between the Trp and the gold nanoparticles), and the contribution to the Raman spectra from molecules adsorbed on the SiO$_2$ surface outside the sphere of influence of the electromagnetic field of the nanoparticles. We should point out the similarity in the Raman spectra of the heterogeneous systems both in the presence of CTAB and in the absence of CTAB (not presented in this paper). In the Raman spectrum of Trp
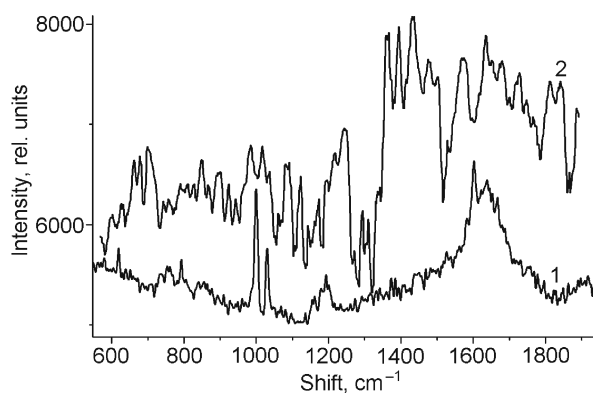
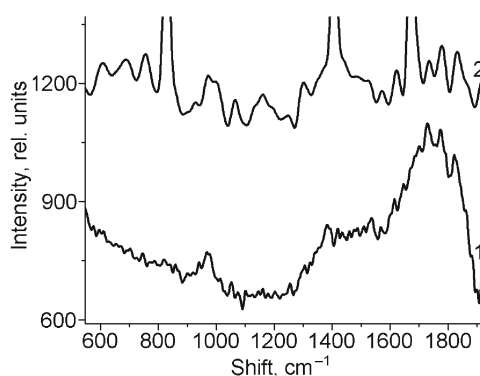Fig. 4. Raman spectra of tryptophan in aqueous solution (1) and in the presence of gold nanoparticles (2).

Fig. 5. Raman spectra of tryptophan, C(Trp) $= 10^{-5}$ mol/g, adsorbed on samples of SiO$_2$/CTAB (1) and Au(0.02%)-SiO$_2$/CTAB (2).

with gold nanoparticles, an enhanced signal appears with maximum at 1081-1091 cm^{-1} (in Au-Trp solution) (Fig. 4) and 1065 cm^{-1} (Trp/Au-SiO$_2$) (Fig. 5), belonging to a metal–nitrogen coordinate bond, which suggests direct interaction between Trp and Au. Furthermore, a frequency shift of 10-15 cm^{-1} on the average occurs for lines in the 900-1065 cm^{-1} region, characterizing stretching vibrations of the C—COO— bond and librational vibrations of the CH$_2$ and NH$_3^+$ groups, which also indicates they interact with the surface of the gold nanoparticles. In the 1100-1250 cm^{-1} region of the spectrum for the Trp-Au/SiO$_2$ complex, we detected enhanced lines at 1065, 1132, 1160, and 1194 cm^{-1}, which do not have analogs in the control spectrum. Based on analysis of the normal modes of vibration for the Trp molecule, we assigned them to vibrations of the CH$_2$ and NH$_3^+$ groups. The line at 1247 cm^{-1} is assigned to the CH$_2$ bending vibration. At the same time, the frequency of the 1305 cm^{-1}, corresponding to librations of the benzene and pyrrole rings, does not change. In the Raman spectra of adsorbed Trp, we do not detect any substantial changes in the vibrations of the indole ring in the 1520-1500 cm^{-1} region; the vibration of the protonated N—H group is detected at about 1254 cm^{-1}. The symmetric stretch of the COO group in pure silica is weakened. In this case, the vibrations of the carboxyl group (1635 cm^{-1} in solution and 1609 cm^{-1} in the heterogeneous system, asymmetric stretch) and the amino group (1123 cm^{-1} in solution and 1132 cm^{-1} in the heterogeneous system, rotational-vibrational mode) are considerably enhanced. Probably these groups are responsible for interaction between the Trp molecules and the gold nanoparticles. The data in [23], in which the SERS effect is experimentally observed for the amino and carboxyl groups due to chemical reaction between the Trp molecules and silver nanoparticles, are support for this hypothesis.

TABLE 1. Comparison of Raman Spectra of Trp in the 700-1700 cm^{-1} in the Presence of Gold and Silver Nanoparticles Given in the Literature with Spectra Obtained in This Work for the System Trp/Gold Nanoparticles (boldface indicates vibrations enhanced in the presence of gold nanoparticles)

Aqueous tryptophan solution		SERS		Calculated spectrum		Assignment of vibrations
Literature	Experiment	Data in [23] with Ag nanoparticles	Experiment (with Au nanoparticles)	Data in [23] with Ag nanoparticles	This work	
759 [23], 758 [25]	759	758	759	754	754	Stretching vibrations of benzene ring, carboxyl group
868 [23]	856	866	868	873	863	Bending vibrations of benzene and pyrrole rings
929 [25]		927	924	930	948	Stretching vibrations of carboxyl group
971 [23], 981 [25]	998	974	984	974	979	Stretching vibrations of CCO, CC pyrrole
1010 [1], 1015 [23]		1013	1017	1006	998	Bending vibrations of benzene ring
	1030		1034		1042	Stretching vibrations of carboxyl group, benzene and pyrrole ring
			1065		1053	Metal–nitrogen coordinate bond, CN stretching vibrations
1127 [1], 1135 [23], 1128 [25]			**1132**	1129	1112	Librations of NH_3^+
1215 [23], 1207 [25]	1202	1213	1217	1214	1203	Stretching vibrations of CN in the pyrrole ring, bending vibrations of the benzene ring
1250 [1], 1243 [23], 1247 [25]			1247		1235	Bending vibrations of the benzene and pyrrole rings, librations of the carboxyl group
1340 [1], 1347 [23], 1351 [25]		1348	1336	1351	1316	Carboxyl group
1356 [1], 1368 [23], 1362 [25]		1362	**1362**	1367	1340	Stretching vibrations of CC in the carboxyl group and the benzene ring
1392 [1], 1396 [25]			**1395**		1388	Stretching vibrations of CO_2^-
1440 [23], 1440 [25]		1442	**1432**	1418	1397	CN (pyrrole ring), CC (benzene ring)
1455 [1], 1467 [23], 1469 [25]		1463	**1477**	1473	1434	CC of benzene ring, CN of pyrrole ring
1499 [23]			**1499**	1486	1452	CH$_2$ of carboxyl group
			1573		1555	Bending vibrations of benzene and pyrrole rings
1608 [25]	1602	1605	**1609**	1608	1599	Asymmetric stretch of the COO$^-$ group

Thus the fluorescence of Trp bound to gold nanoparticles is efficiently quenched in solution and on the surface of dispersed silica. The fluorescence spectra contain luminescence bands for molecular Trp and charge-transfer complexes between Trp and the nanoparticles, with corresponding bands in the excitation spectrum. We discuss the hypothesis that a luminescent chelate compound is formed between Trp and gold ions, formed on the surface of the nanoparticles in the homogeneous and heterogeneous phases.

From the Raman spectra, we have concluded that chemical reaction occurs between Trp and the gold nanoparticles, with participation of the amino and carboxyl groups, which suggests a chemical mechanism for SERS. The effect of the gold nanoparticles on the Raman scattering of Trp in the heterogeneous system is considerably weakened due to strong light scattering by the dispersed silica, the presence of the nanoparticle stabilizer, and the presence of several adsorption states of Trp on the Au/SiO_2 surface.

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