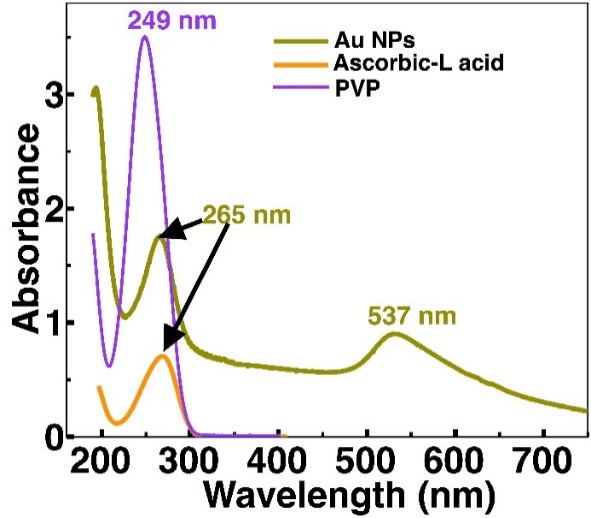
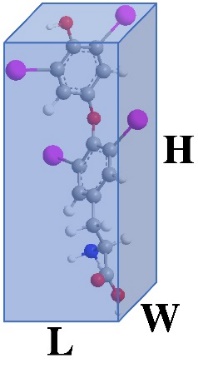
Figure S1 shows the absorbance spectra of Au NPs, L-ascorbic acid, and PVP. The Au NPs spectrum shows the surface plasmon band at 537 nm and other optical bands at 265 nm and below 200 nm. The L-ascorbic acid spectrum shows one band in 265 nm. The spectrum of PVP shows one band in 249 nm. The L-ascorbic acid and PVP were used during the synthesis of Au NPs. After four washes the band at 265 nm remains in the Au NPs spectrum. Therefore, we conclude that the L-ascorbic acid is functionalizing the Au NPs.



**Figure S1**. Absorbance spectra of the colloidal solution of Au NPs, L-ascorbic acid and PVP.

**Determination of NRaman and NSERS**

For NRaman determination, we assumed a uniform deposit of T4 on the Si wafer surface. We also assume each molecule occupies a volume of a rectangular prism (Figure S2). For the determination of the rectangular prism volume and area, ChemDraw ultra 12.0 software was used. A height (H) of 11.47 Å, a width (W) of 8.37 Å, and a length (L) of 8.06 Å, were obtained. The base area (LxW) occupied by one T4 molecule in vertical position is 67.46 Å2 (0.67 nm2).



**Figure S2.** Image of the Thyroxine molecule inside a rectangular prism, with H=11.47 Å, W=8.37 Å, and L=8.06 Å. The atoms of carbon, hydrogen, nitrogen, oxygen and iodine are distinguished by using the CPK coloration convention.

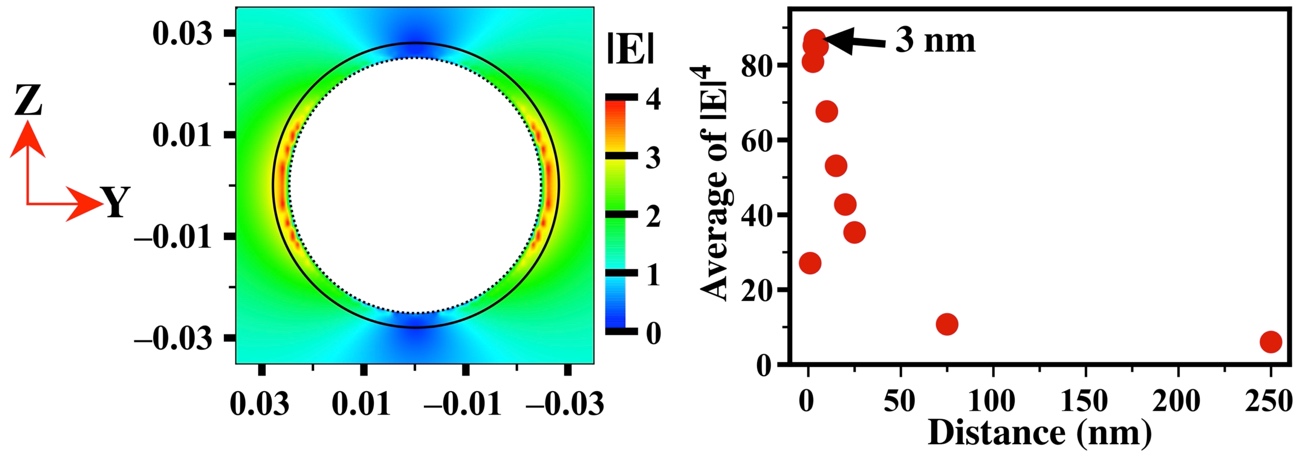
|  |
| --- |
|  |
| Address correspondence to Enrique Sánchez Mora, esanchez@ifuap.buap.mx |

The Si wafer area is 1 cm2 (1x1014nm2), therefore the maximum amount of molecules in the vertical position that covers the area is 1.48x1014. In table S1 we show the used concentrations of T4 and the number of molecules in each T4 solution concentration. As the last value is larger than 1.48x1014, we assume that the molecules are arranged in multi-layers (see column 3) with a total thickness shown in column 4. The number of layers for each concentration was estimated dividing the values of column 2 by of 1.48x1014. The thickness was calculated by the multiplication of the value column 3 by height of one molecule (1.147 nm). We only consider the excited molecules of T4 in the laser spot area (12.5X106 nm2). Finally, the NRaman value was calculated by using the next expression:

**Table S1.** Molecule concentration of T4, total number of molecules in each T4 solution concentration, number of molecule-layers on the Si wafer, and total thickness of the multi-layer array, and NRaman value.

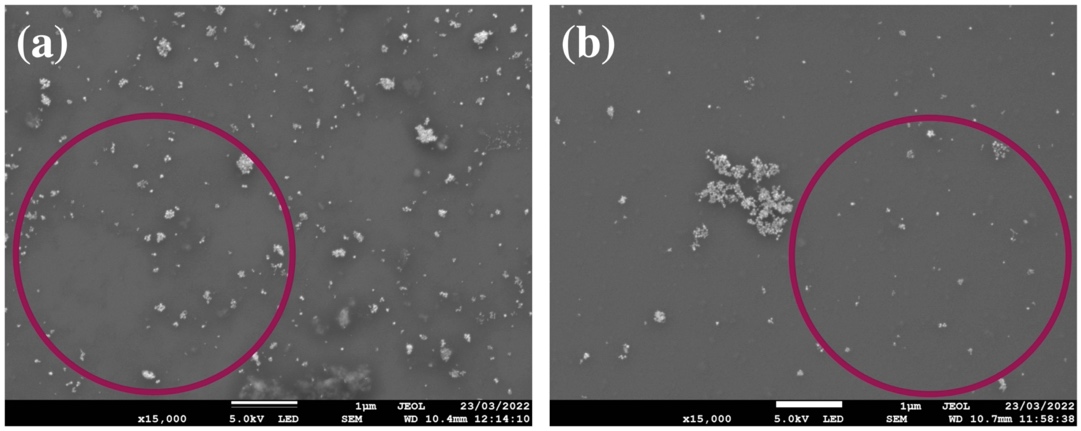
|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Concentration (mm) | Total number of molecules | Number of molecule-layers | Thickness (nm) | NRaman |
| 1.00 | 3.010x1016 | 203 | 233.7 | 3.78x109 |
| 0.30 | 0.900x1016 | 61 | 70.1 | 1.13 x109 |
| 0.10 | 0.300x1016 | 20 | 23.4 | 0.38 x109 |
| 0.05 | 0.015x1016 | 10 | 11.6 | 0.19 x109 |
| 0.01 | 0.003x1016 | 2 | 2.3 | 0.04 x109 |

In the case for calculating theT4 NSERS, we considered a homogeneous deposition of the T4 molecules on the surface of the NPs. Despite the thickness of molecule multi-layer array (see column 4 in Table S1), we decided to consider only the molecules in the first 3 nm thickness that are closer to the metal surface. The explanation is based on the theoretical-numerical modeling of a metal NP's near electric field intensity. On the left of Figure S3, an image of the near-electric field intensity of a spherical Au NP is shown, excited at 632 nm, with an electric field polarized in the Y-axis and propagating along the X-axis. As we can see, the field intensity decreases as we move away from the surface. Due to the dipole configuration, the electric field intensity is not uniform over the surface for a particular distance from the surface. To highlight this fact, we have drawn a circle 5 nm apart from the NP surface; the area enclosed between the circle and the surface contains hot spot areas and spots with lower intensities. We have calculated the average intensity for circles separated from different distances; the plot shown on the right of S3 figure indicates an exponential decrease of intensity as we move away from the surface. The maximum average is obtained at a distance of 3 nm.



**Figure S3.** Near electric field intensity image of an Au NP of 50 nm of diameter and plot of the mean |E|4 with increasing distance from the Au NP surface.

Again, we consider that each molecule occupies the volume of a rectangular prism and that only the excited T4 molecules are the ones on the metallic NPs located in the laser spot area. Several SEM images were analyzed to determine the average number of NPs under the laser spot area. Figure S4 shows representative SEM images of Au and Ag NPs; there, a red circle represents the laser spot area. A count of the number of particles inside the circle was made. A similar procedure was done for several SEM images. Finally, the average number of Au NPs is 150, and for Ag NPs is 70.



**Figure S4.** SEM micrograph at a magnification of 15,000x of the SERS substrate of (a) Au NPs and (b) Ag NPs,

Then, the total surface area occupied by the NPs was determined as follows. Under the laser spot several single NPs and clusters of them are excited. We assume that once the molecule solution drops are cast on the plasmonic substrate, and laser beam is on, the excited molecules are those that fall on the upper hemisphere of the metal NPs. The total area occupied by the NPs was determined by multiplying the average number of metal NPs times the upper hemisphere area. The values are contained in the table S2.

**Table S2**. Values used to estimate the total metal area occupied by the NP that are under the laser spot.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Metal nanoparticles** | **Diameter (nm)** | **Upper hemisphere area (nm2)** | **Average NPs under the laser spot** | **Atotal =Total area occupied by the NPs (nm2)** |
| Au NP | 53 | 4.41x103 | 150 | 6.62x105 |
| Ag NP | 47 | 3.32x103 | 70 | 2.33x105 |

We also considered that the T4 molecules, in a vertical position, totally cover the upper hemisphere of the NPs. Therefore, 9.81x105 molecules of T4 are absorbed on the Au metal surface and 3.45x105 on the Ag metal surface. For each T4 molecule concentration, again, we assume that molecule layers were formed and the first layers on the surfaces forming a 3 nm thickness contributed the most to the SERS-EF. Then, for the determination of the NSERS we follow the next equation:

.

Finally, the NSERS for Au NP substrate is 2.56 x106, and for Ag NP substrate is 8.99 x105. It is noteworthy to mention that these values are the same for all T4 concentrations, this is because we restrict the excited molecule layers to a thickness of 3 nm.