Supplementary Material

Gold and silver nanoparticles assemblies obtained using living biotemplates for plasmon enhanced spectroscopy

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Recently, hybrid structures of microorganisms with inorganic elements received great interest, showing to be versatile templates for the organization of nanostructured functional materials in large scale and has been used as biotemplates aiming to nanotechnological applications. In this work, we are interested in the use of fungi as biotemplate to obtain self-assembled systems of gold nanoparticles forming stable mesostructures with potential use for sensors and biosensors via surface-enhanced Raman scattering. We also present a facile route for self-organization of colloidal gold nanoparticles on living filamentous fungi aiming to fabricate microtubular. The material nanostructured was prepared by adding the spores of a filamentous fungus *Cladosporium sphaerospermum* in the colloidal suspension of gold nanoparticles. During two months, fungi grew continuously consuming unreacted citrate ion remaining in the media and gold nanoparticles adhered to the cell wall, covering it in multiple layers resulting in uniform gold microtubules with controlled thickness.

All the chemicals used were of analytical grade and were used as received. Gold nanoparticles (AuNP) were synthesized from 100 mL of an aqueous solution of $HAuCl_4$ (1.0 mmol·L^{-1}, 99.9% Sigma-Aldrich) at 90 °C that was prepared with deionized water obtained from a commercial Millipore Elix 3 system. Then, 1 mL of a solution of sodium citrate was added (0.30 mol·L^{-1}, $Na_3C_6H_5O_4$) and a stable red colloidal suspension of AuNP was formed after 12 minutes (Figure S1)

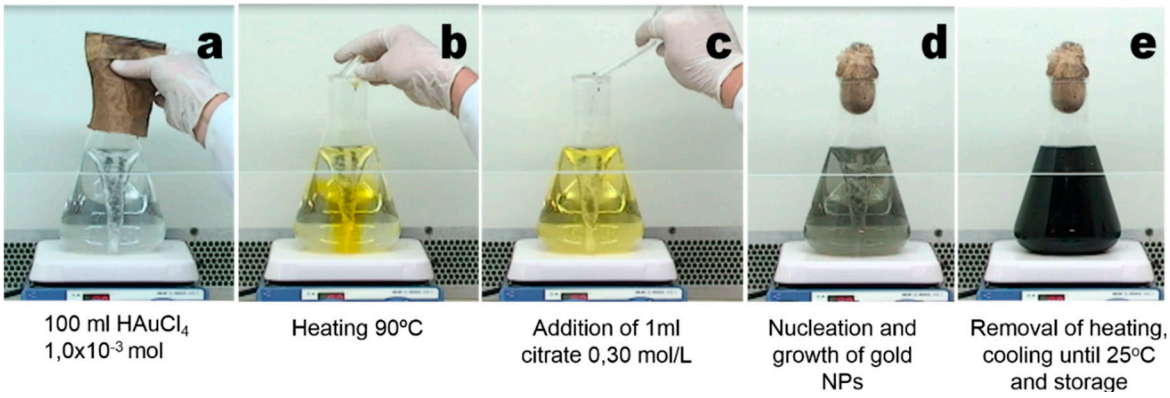

***Figure S1**: Synthesis of the gold colloids synthesized at pH 3.4 and 90 ºC.*

Silver nanoparticles (AgNP) were synthesized through the addition of 1 mL of an aqueous solution of $AgNO_3$ (0.10 mol·L^{-1}, 99.9% Sigma-Aldrich) in 100 mL of deionized water at 95 °C, followed by addition of 1 mL of sodium citrate (0.30 mol·L^{-1}). After approximately 12 minutes of reaction, the system was cooled to room temperature, resulting in a stable grayish yellow suspension of AgNP.

Gold and silver nanoparticles were deposited on silicon substrate by casting their respective colloidal suspensions on the substrate at room temperature to be characterized by X-ray diffraction (Rigaku Dmax 2500PC diffractometer) with CuKα radiation in the 2θ range from 20° to 120° degrees with the equipment operating at 40 kV and 40 mA. The UV-Vis spectra of gold and silver suspensions were obtained from 190 to 800 nm with a UV-Vis spectrophotometer (Shimadzu Multspec 1501). Images of scanning transmission electron microscopy (STEM) of the nanoparticles were recorded at 20 kV (FEG Zeiss Supra 35-VP). To prepare the STEM samples, the colloids were dripped on Cu grids of 400 mesh (PELCO®) and the solvent evaporated at room temperature. Images of scanning electron microscopy (SEM) (FEI Company – XL30 FEG) were obtained from nanoparticles and microtubules deposited on silicon plate (111). The contacts between the sample and the support were carried out with conductive silver ink (Degussa). Histograms were constructed using the public domain Image-J image processing software.

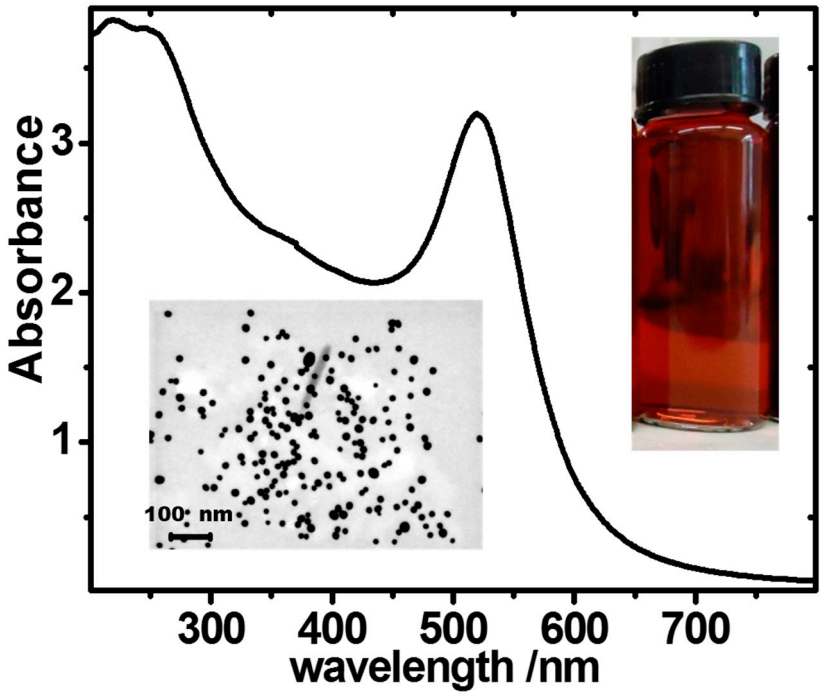

Figure S2: *UV-Vis spectra of the gold colloids synthesized at pH 3.4 and 90 °C. The TEM image show gold nanoparticles with an average size of 25 nm.*

A red coloration and the position of maximum absorption at 530 nm observed in the UV-Vis spectrum of Figure 02 indicates particles of about 20 nm in good agreement with the TEM image, The X-ray diffraction pattern (XRD) confirms that the gold nanoparticles have a cubic crystal structure of face-centered and diffraction peaks are consistent with the average particle size observed in the TEM image of 25 nm.

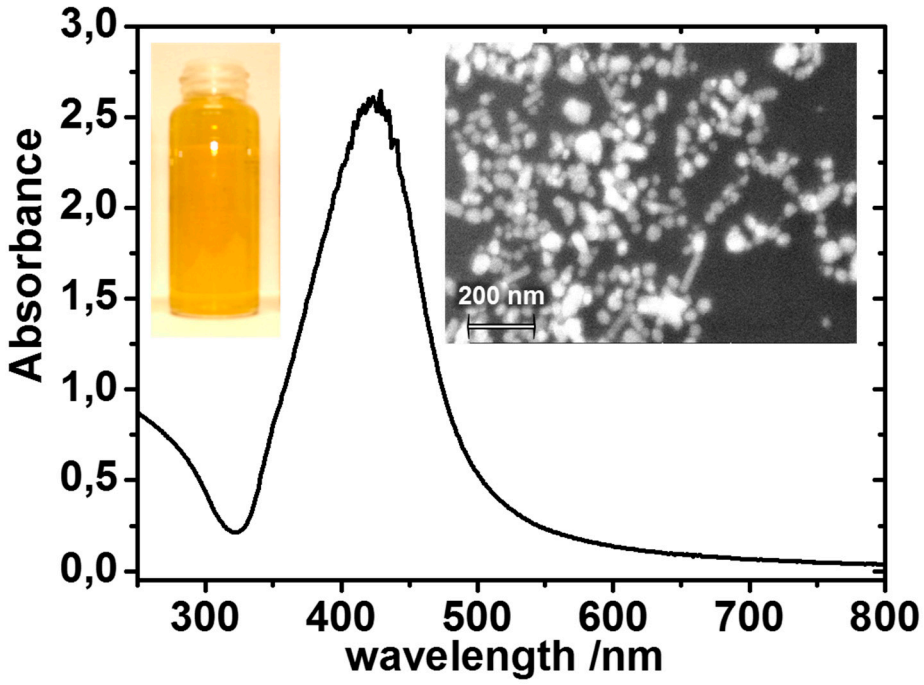

Figure S3: *UV-Vis spectra of the silver colloids synthesized at pH 8.3 and 90 °C. The SEM image show silver nanoparticles with a average size of 35 nm.*

A yelow coloration and the position of maximum absorption at 430 nm observed in the UV-Vis spectrum of Figure 3 indicates particles of about 30 nm in good agreement with the TEM image, The X-ray diffraction pattern (XRD) confirms that the gold nanoparticles have a cubic crystal structure of face-centered and diffraction peaks are consistent with the average particle size observed in the SEM image of 35 nm.

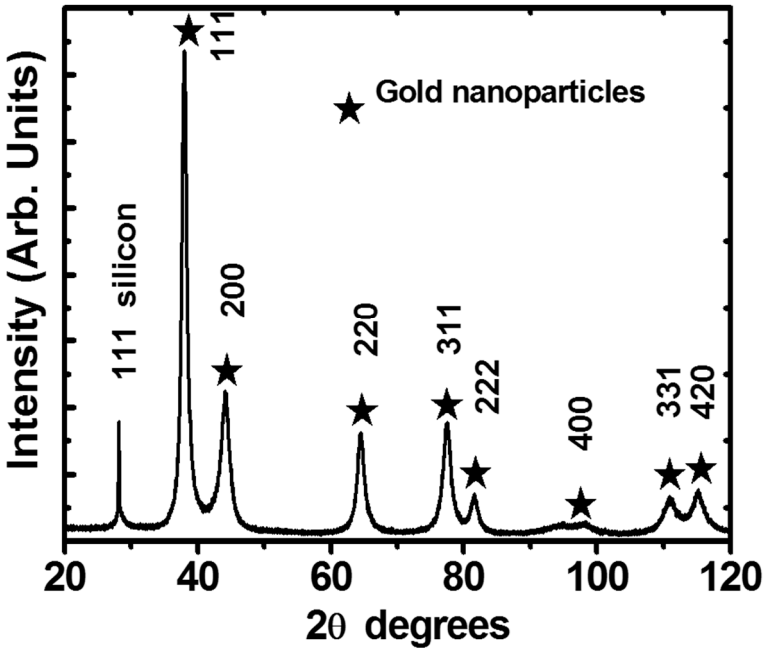

***Figure S4**: XRD pattern collected at room temperature of gold nanoparticles synthesized at 90°C.*

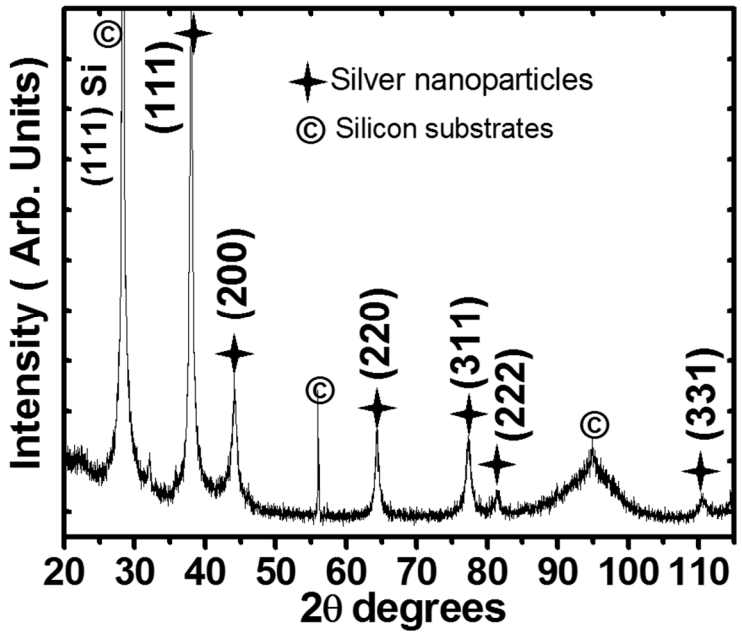

***Figure S5**: XRD pattern collected at room temperature of silver nanoparticles synthesized at 90 °C*

In the first experiment, spores of the filamentous fungus Cladosporium sphaerospermum were added in 200 mL of the colloidal suspension of AuNP at room temperature. After this inoculation, the culture media were stored during two months at room temperature protected from light. During this time, fungus grew

continuously and AuNP adhered to their walls, covering it in multiple layers and forming uniform microtubules of AuNP with controlled thickness. In a second experiment, the aqueous medium was partially removed from the system and the fungus-AuNP microtubules transferred to 200 mL of the colloidal dispersion of AgNP, which adhered on the surface of the gold microtubules, forming a complex microtubule of fungus-AuNP-AgNP.

Figure S6: *Erlenmeyer flasks containing mycelium of Cladosporium sphaerospermum and gold nanoparticles. a) Two days after the addition of spores, b) 30 days after the addition of spores, c) 45 days after the addition of spores, and d) 60 days after the addition of spore.*

Images show the colloidal suspension between 2 – 60 days of inoculation (Figure S6 a-d). It is evident the decrease in the red color of the media after 60 days of inoculation and the concomitant formation of the hybrid material, that can be visualized with naked eyes. Gold nanoparticles are easily observed decorating the fungal surface, forming a uniform surface on the cell wall (Figure S6 e and f).

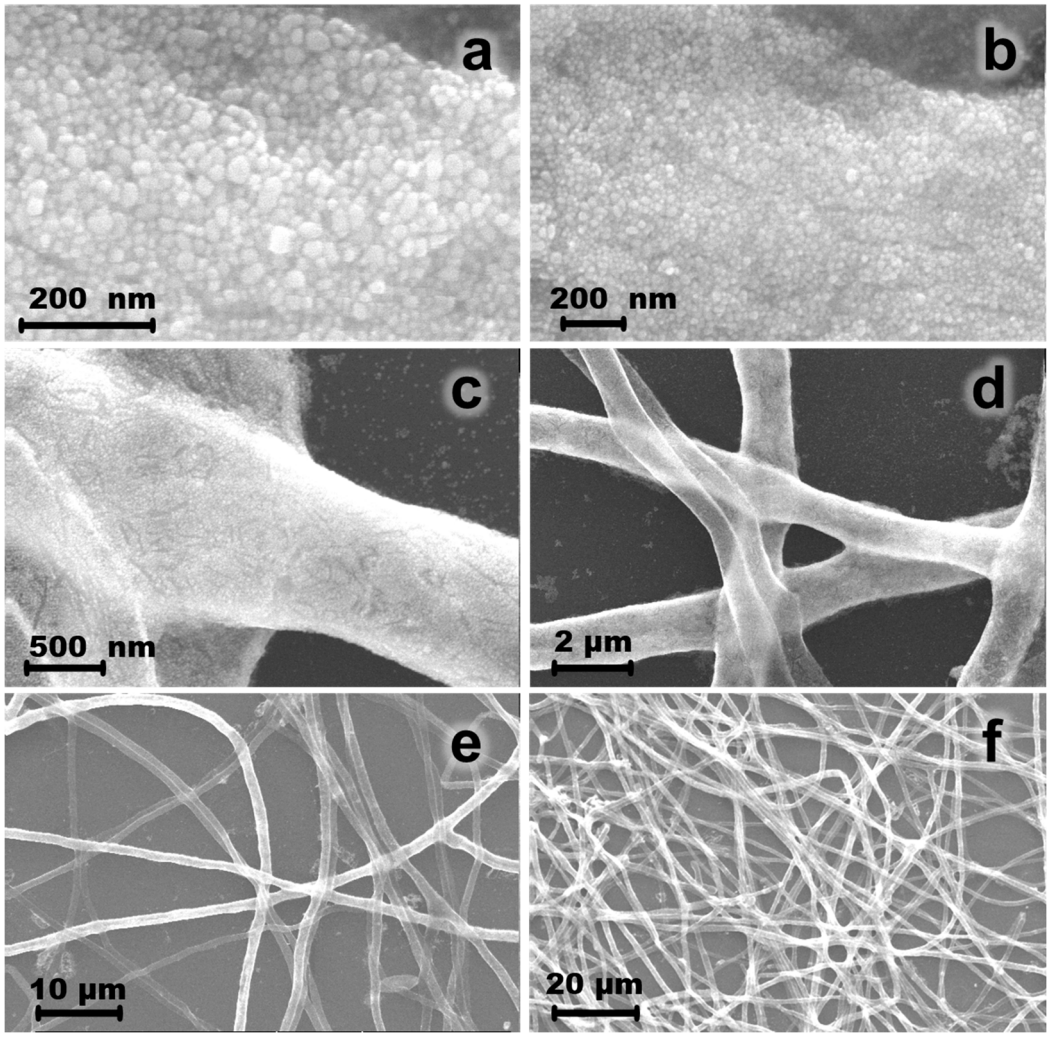

***Figure 7**: SEM images of the microtubules in different magnifications of the fungus Cladosporium sphaerospermum.*

SEM images of the microtubules obtained using fungi as biotemplate for the self-assembly of gold nanoparticles, showed uniformity of shape (Figure S7) and diameter (Figure S8) of the microtubule.

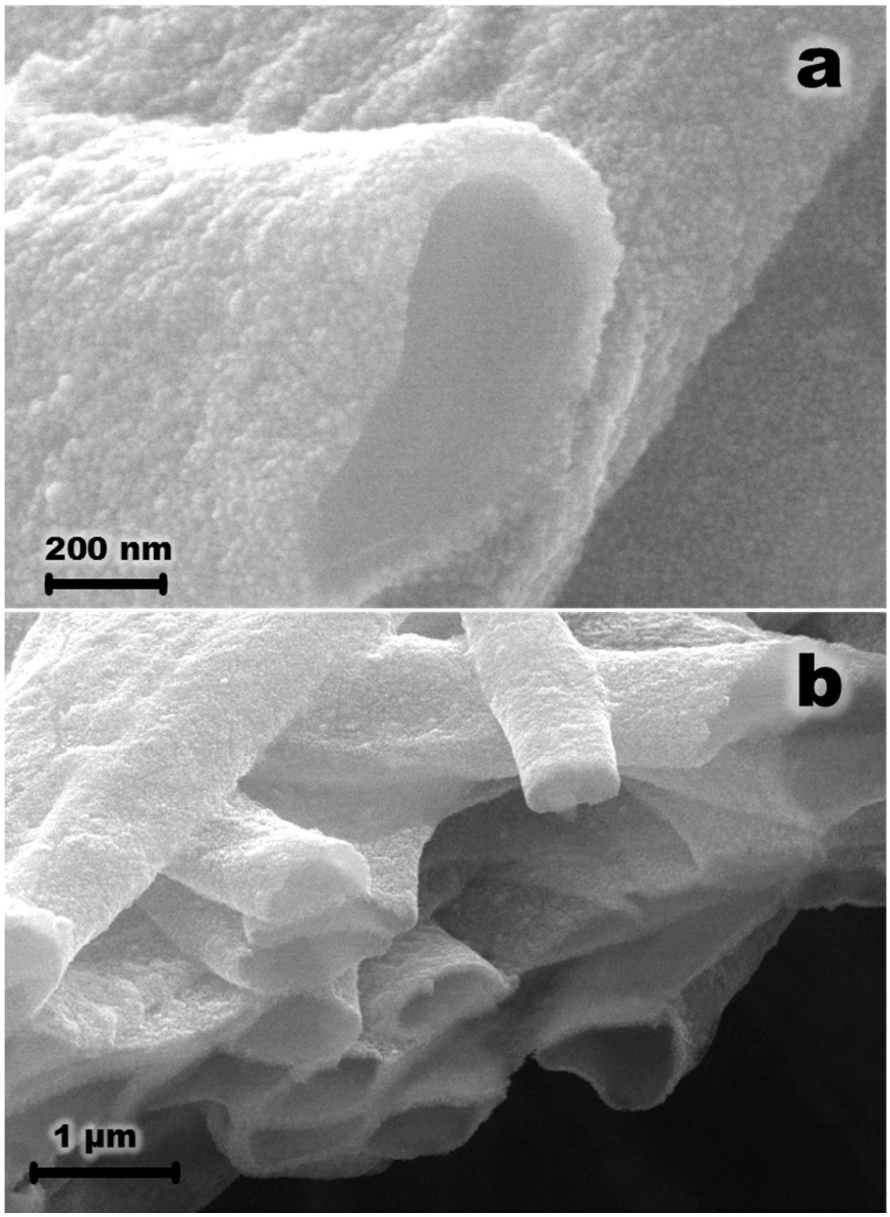

Figure S8*: SEM images of microtubules obtained using fungi Cladosporium sphaerospermum as biotemplates for the self-assembly of gold nanoparticles showing the wall thickness with 80 nm of the microtubule.*

The distribution of gold nanoparticles onto the fungi were confirmed by EDS analysis using 2D-mapping obtained by EDS chemical composition (Figure S9).

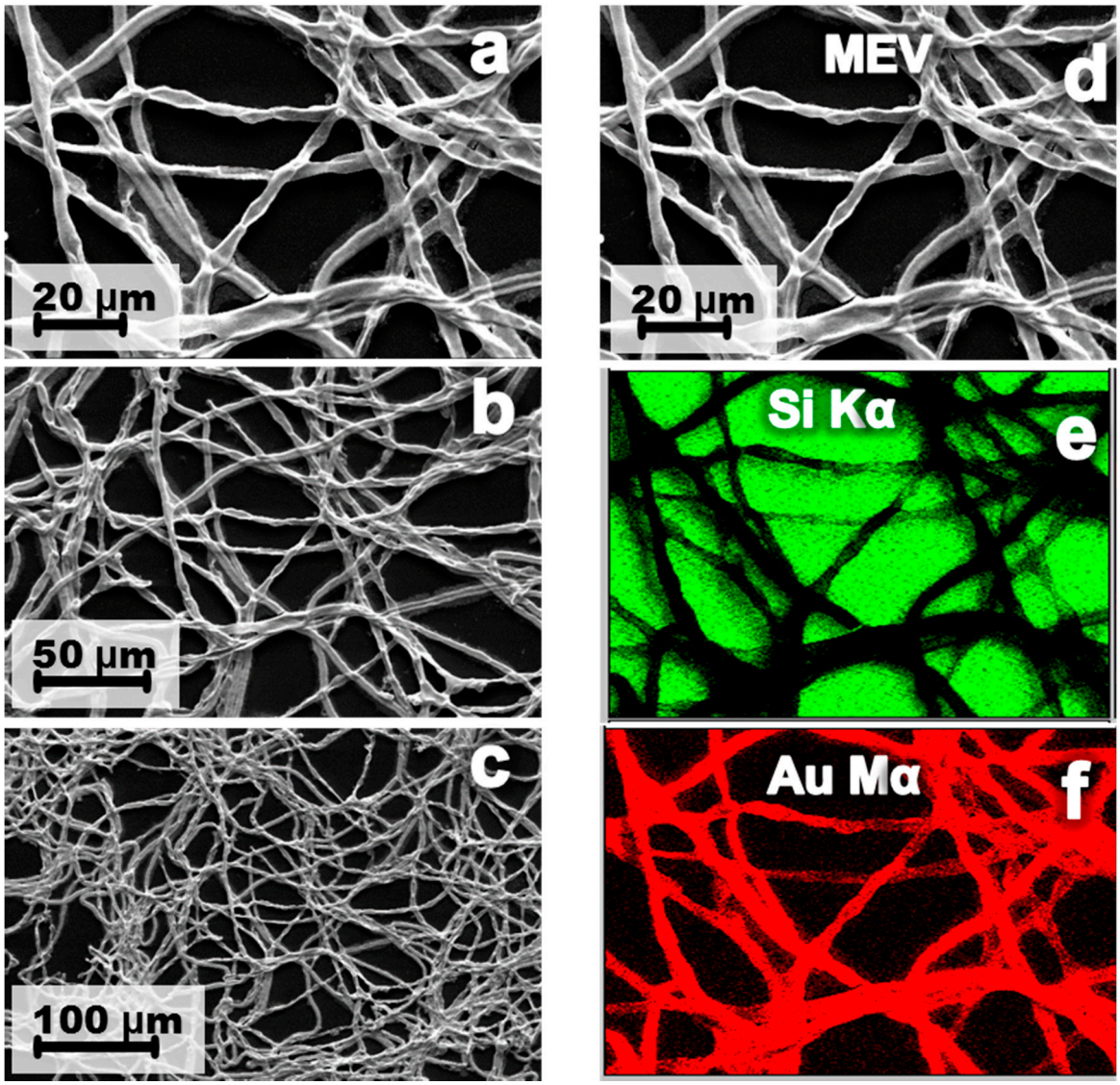

Figure S9: *SEM images of the fungus Cladosporium sphaerospermum recovered with multilayers of gold nanoparticles (a-d) in different regions and magnifications, 2D-mapping of the elements (e) Si and (f) Au, in false color related to the SEM image "d".*

Two-dimensional mapping were constructed by analyzing the energy released from the emission of the elements Si and Au, indicating the distribution of these elements on the demarcated area in the micrograph. We can clearly observe the outline of gold microwires highlighted in red color and the silicon substrate in green. The analysis of the element Si present in the substrate afforded the images contrast with the substrate and metal microtubules.

The detailed observation of the chemical mapping of Si indicates the presence of light and dark areas. Green regions are related to the electron beam was focused directly

on the substrate, since the dark regions where the beam focused on the hybrid material fungus + nanoparticles .

During the second step of our experiment, the FG microtubules previously fabricated were exposed to the second suspension of AgNP with average size of 35 nm and sharp size distribution, aiming to obtain microtubules with a core-shell structure of fungus-AuNP-AgNP (FGS). Their SEM images Figure S10 show the fungi biotemplates covered with the first layer of AuNP followed by second layer of AgNP. While the Figure 8 shows a multilayer and somewhat uniform covering of the fungi wall by AuNP, samples that received a second wall of silver nanoparticles (Figure S3) exhibit an irregular surface owing to AgNP with different sizes. The presence of both AuNP and AgNP in the FGS microtubules was confirmed by EDS.

The presence of gold and silver were confirmed by EDS analysis on the sample indicated a close values of Weight % of both gold nanoparticles in a rectangular area scan from 2D-mapping obtained by EDS chemical composition. (Figure S11 b and d).

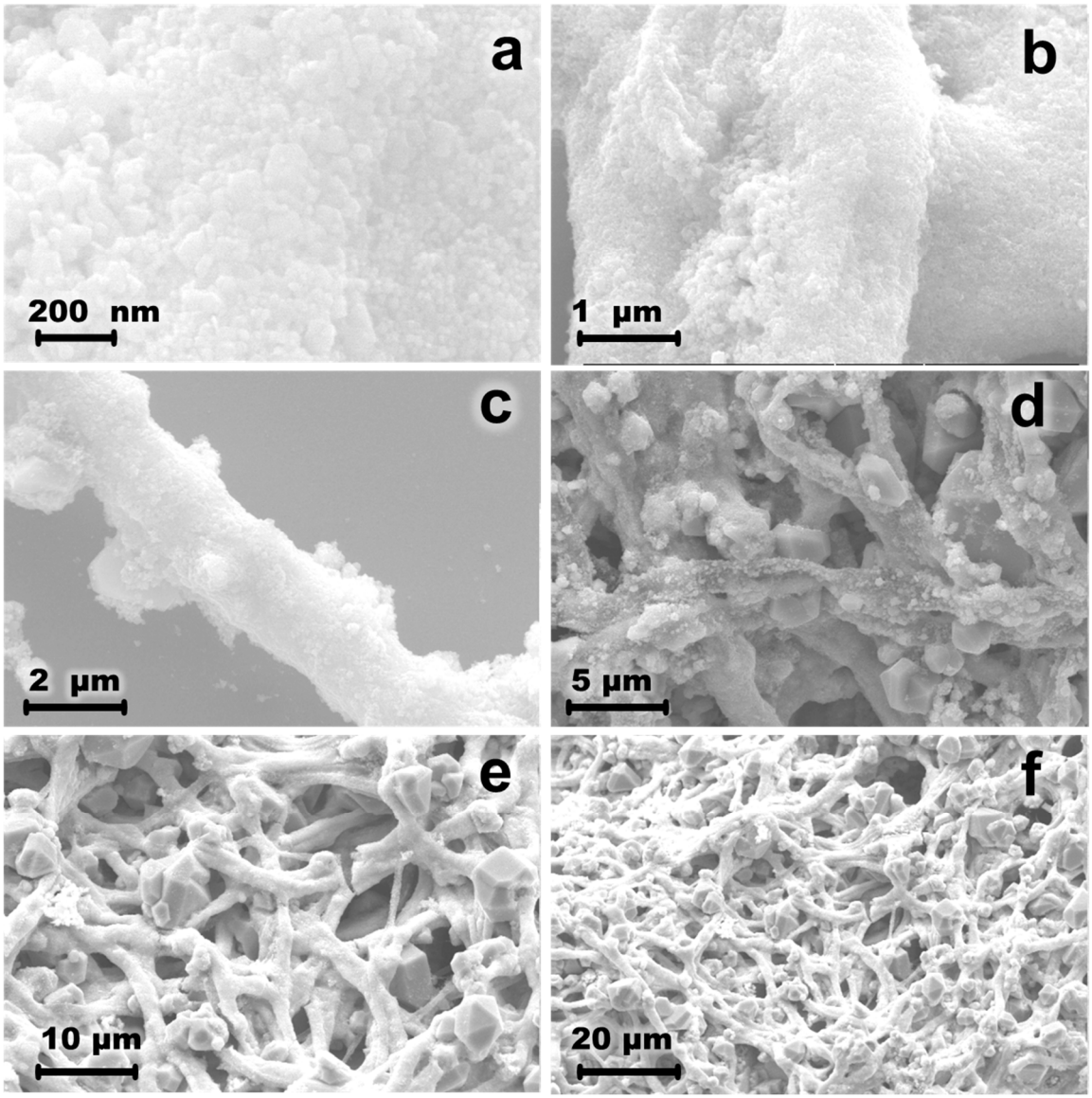

***Figure S10**: SEM images of microtubules obtained using fungi as biotemplates for the self-assembly of gold nanoparticles showing uniformity of shape.*

EDS with 2D-mapping analyzed, whether Si, Ag and Au elements were constituents of the delimited area by the SEM. The colors green, red and gray were chose to identify the silicone substrate, AgNP and AuNP, respectively and the dark region indicates the non-presence of the analyzed element. In our hybrid materials, gold and silver nanoparticles were present and well-dispersed throughout the hyphae.

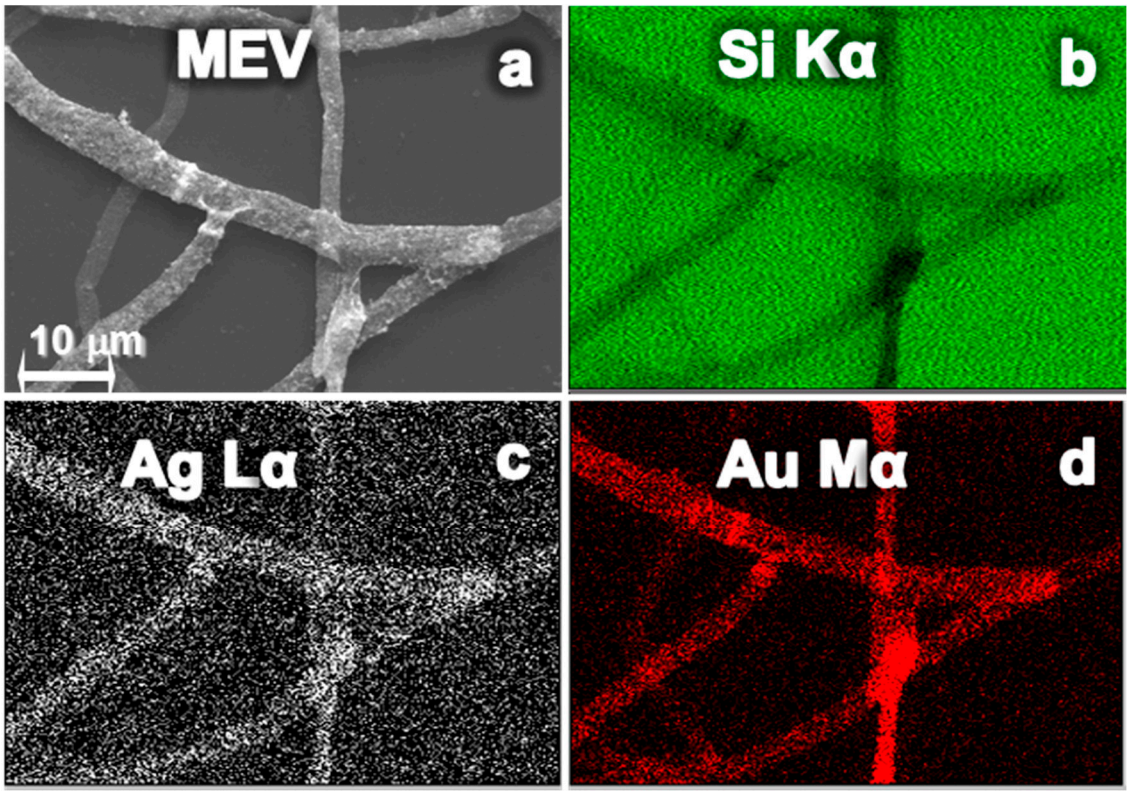

Figure S11*: SEM images and EDS with 2D-mapping of the elements Si, Ag and Au (false green, red and gray color, respectively) that compose the filamentous fungus Aspergillus aculeatus recovered with gold and silver nanoparticles. Hyphae were deposited on a silicon substrate.*

The SERS activity of the fungus-AuNP microtubules (thereafter referred to as FG) and fungus-AuNP-AgNP microtubules (FGS) were obtained by dipping them in a 1×10^{-4} mol·L^{-1} thiophenol solution for 3 minutes. A clean area of the same surface used for mounting the fungi was dipped in the same solution for the same duration prior to the microtubules wires dipping, the spectra of this surface was the control to determine enhancement factor of the Au/Ag biotemplates for the thiophenol molecule. The control spectra were taken with a laser power of 1.2 mW and 2.5 mW at the sample as a higher power was required to obtain a substantial signal of Raman scattering of the thiophenol. SEM analysis was done for sample FG and FGS microtubules. The microscope used was a The FEI Quanta 200 FEG microscope with a Field Emission Gun, an Everhart-Thornley Secondary Electron Detector and a Solid State Backscatter Detector. Atomic force microscopy (AFM) images were also obtained for the FS microtubules using Nanoscope IV instrument with tapping mode, aluminum coated (n^+) silicon tip, Model: TESPA (Bruker, Inc.).

Raman and SERS data were collected using a micro-Raman Renishaw InVia system, with laser excitation at 633 nm and power of 245 μW at the sample. The measurements were made in a backscattering geometry using a 50x microscope objective, where the probing area was ca. 1 μm^2. The measurements were collected as 2D mappings of a computer controlled 3-axis encoded (XYZ) motorized stage. The results show that the gold microtubules showed good enhancement of the SERS signal thyphenol, signal enhancement are close to that reported in the literature use only gold nanoparticles

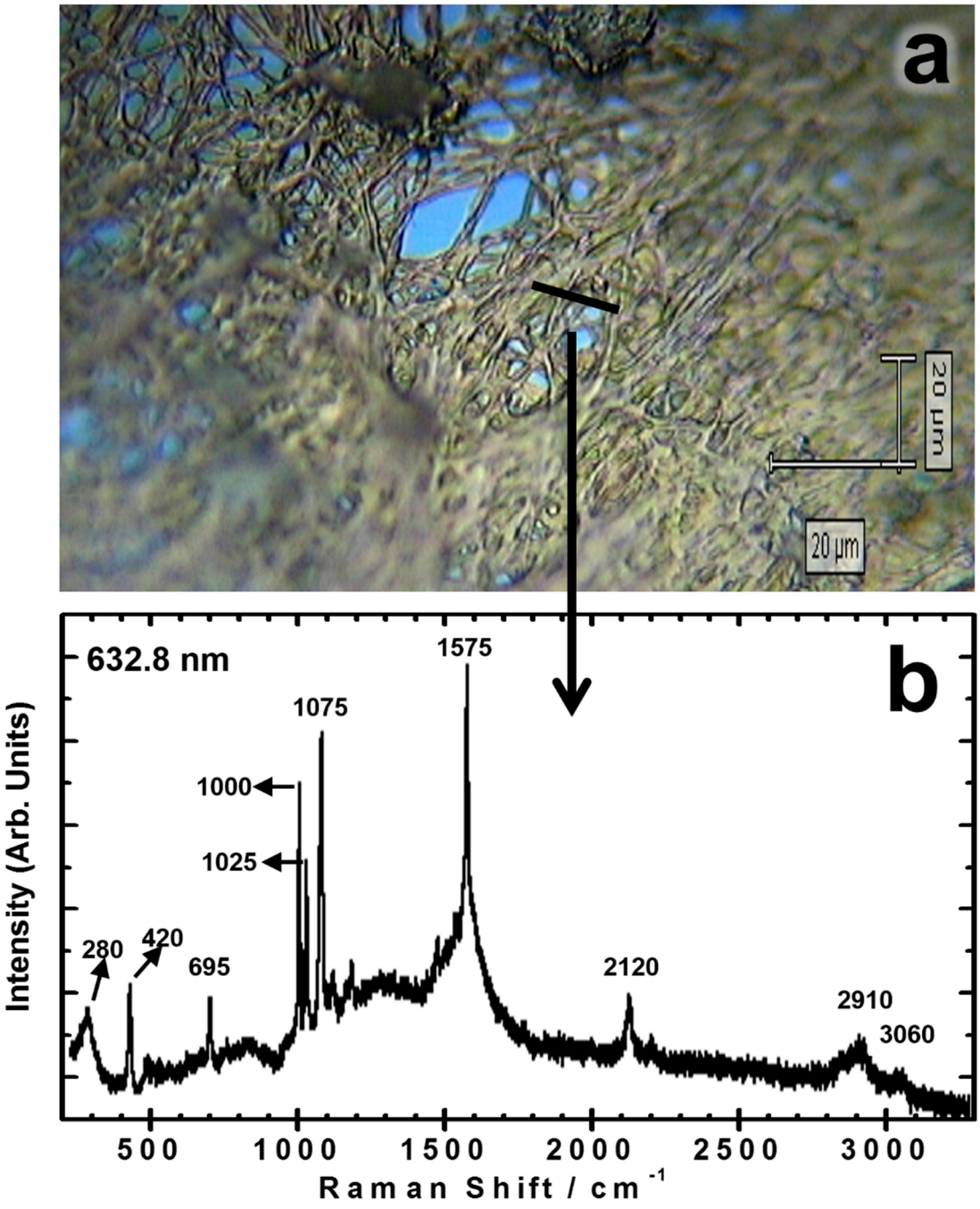

***Figure S12**: Optical images of (a) the FS microtubules used as SERS substrates. The short lines on the images indicate the region analyzed and (b) their respective SERS spectra of the thiophenol are shown below and were obtained using a laser at 633 nm.*

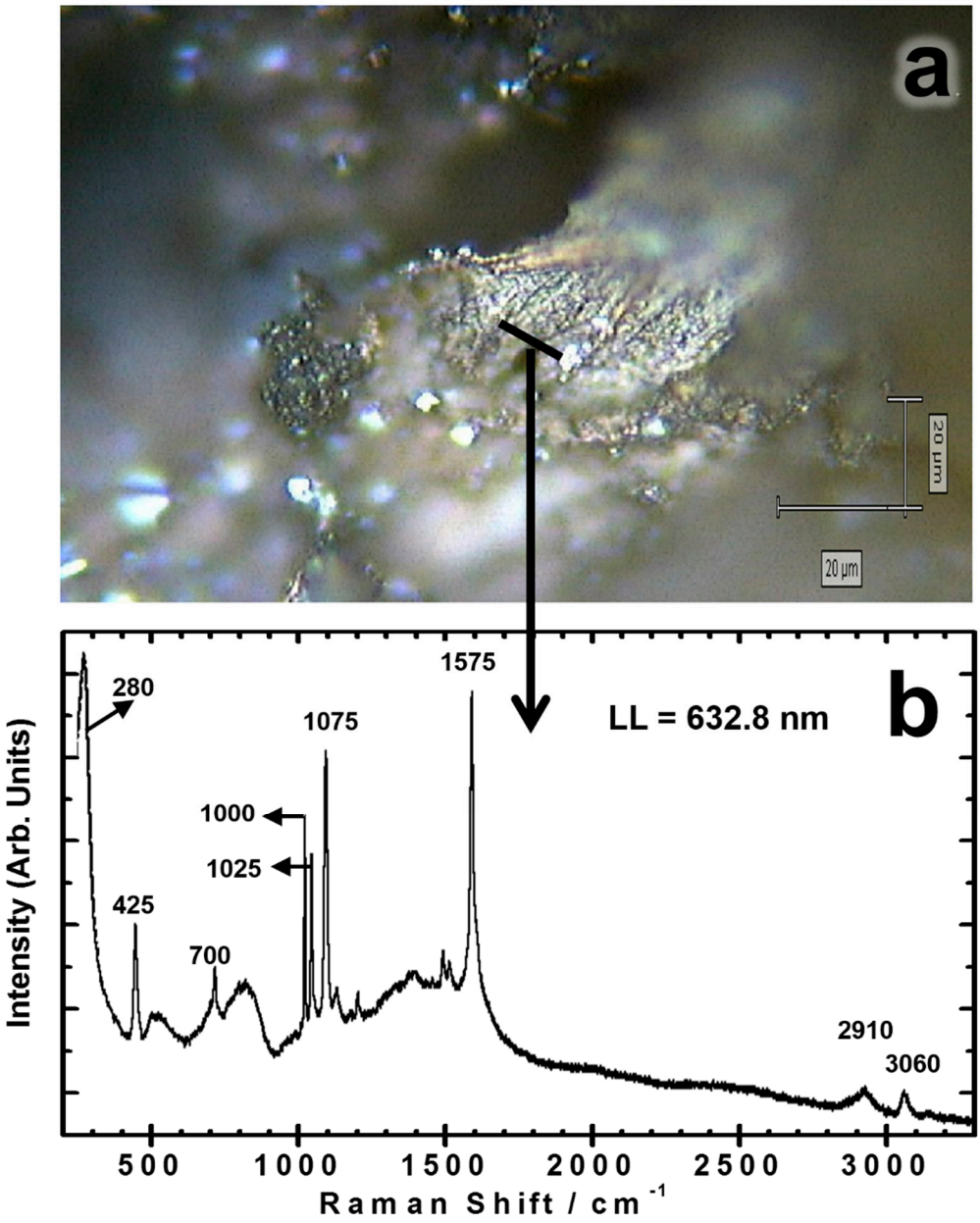

Figure S13: *Optical images of (a) the FGS microtubules used as SERS substrates. The short lines on the images indicate the region analyzed, and (b) their respective SERS spectra of the thiophenol are shown below and were obtained using a laser at 632.8 nm.*

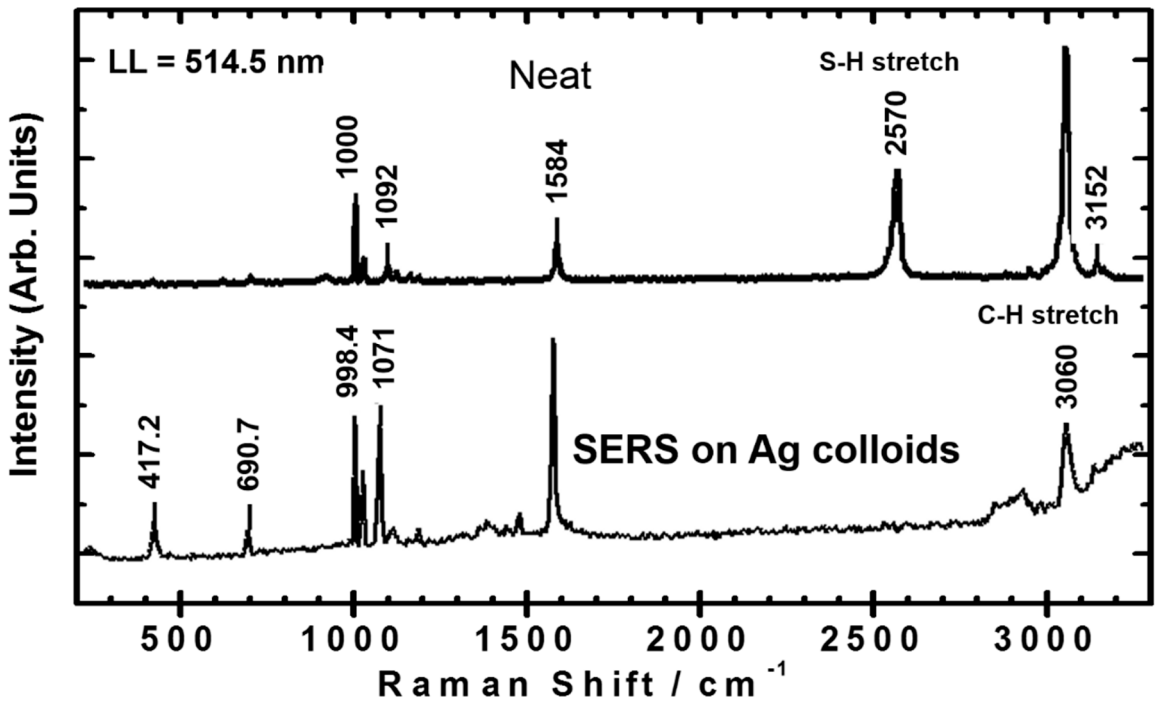

Figure S14: *Raman signal in 514 nm of benzene thiol reference spectra; Raman signal of benzene thiol neat and SERS of benzene thiol with silver nanoparticles.*