Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Enhanced Label-Free Detection of Proteins on Au Nanoparticles Micropatterns for Surface Enhanced Infrared Spectroscopy

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1. Experimental

1.1. Preparation of Au and Pt coated glass mirrors substrates

The IR reflective Au and Pt coated glass mirrors substrates were obtained through a vacuum vaporization technique (BAL-TEC, Liechtenstein), where a glass wafer of size 1 × 2 cm^2 was initially sputtered with Ti film of 10 nm to be used as adhesive layer followed by the sputtering of 20 nm film of either Au or Pt. The Au and Pt coated glass mirrors obtained were used without any further modification. The Si/SiO_2 substrate was obtained commercially (Graphene Supermarket) and was used as received.

1.2. Preparation of Au-NPs and Au-NPs micropatterns

Sodium borohydrite ($NaBH_4$) reduction method was used to synthesize the Au NPs. Initially, 2 µL of each $NaBH_4$ (5 mmol L^{-1}, VETEC) and chloroauric acid trihydrate ($HAuCl_4.3H_2O$) (5 mmol L^{-1}, Sigma Aldrich) solutions were mixed on IR transparent CaF_2 window, which quickly results in the formation of Au-NPs. In order to obtain the lithographic patterns of the Au-NPs, similar procedure was employed, but this time a lithographic mask (Cu grid (300 mesh, carbon film coated)), usually employed to characterize nanostructures in transmission electron microscopy (TEM), was placed on the CaF_2 window and equal amounts of the $NaBH_4$ and $HAuCl_4.3H_2O$ solutions (equimolar) were added. After about 10 minutes the lithographic mask was removed, leaving behind several micropatterns of Au NPs. The micropatterns were also fabricated on several other substrates, such as Au coated glass mirror, Pt coated glass mirror, and SiO_2/Si substrates. It is important to mention that for the uniform synthesis of the micropatterns, one should properly fix the Cu grid on the desired substrate using the scotch tape and should cover larger area from the four sides to leave few open spaces in the middle of the grid, as shown in Figure S1. This will ensure a uniform distribution of the nanoparticles within the micropatterns from the small volume of the solutions of both precursor salt and reducing agent used during the synthesis.

1.3. UV-Vis absorption measurements and Scanning Electron Microscopy (SEM) measurements

Au NPs were characterized by UV-Vis spectrophotometry technique, performed in a UV-Vis spectrophotometer (Jasco® V-670) by using a quartz cuvette with optical path length of 1 cm in a wavelength range of 190 – 800 nm. The Au NPs solution was obtained

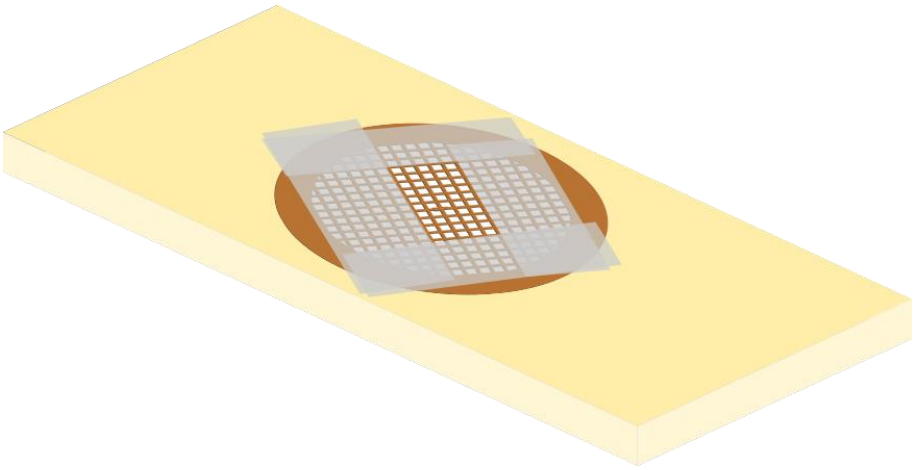

Figure S1: Schematic of fixing TEM Cu grid on the Au coated glass mirror using the scotch tap procedure.

by initially mixing 2.0 mL of $NaBH_4$ solution (5 mmol L^{-1}) and 1.0 mL of $HAuCl_4.3H_2O$ solution (5 mmol L^{-1}) in a test tube. After that, about 1.5 mL of the Au NPs solution was transferred in to an eppendorf® tube and centrifuged at 6000 rpm for 40 minutes. The supernatant was removed and the process was repeated three times using distilled water, and finally the NPs solution was stored in a glass vial and wrapped with aluminum sheets until analysis.

For SEM analysis, about 2 µL of each $NaBH_4$ (5 mmol L^{-1}, VETEC) and chloroauric acid ($HAuCl_4.3H_2O$) (5 mmol L^{-1}, Sigma Aldrich) were drop cast on Au coated glass mirror followed by drying at room temperature. The resultant Au NPs islands were characterized by using scanning electron microscope (SEM, model ZEISS LEO 440 with Oxford 7060 detector).

1.4. Micro-FTIR spectroscopy and FTIR chemical imaging

Micro-FTIR spectra and chemical images were recorded in both the transmission and reflectance modes using a spectral resolution of 4 cm^{-1} and 100 scans in the 4000–900 cm^{-1} spectral window using an FTIR spectrometer (Vertex 70v, Bruker) coupled with an FTIR microscope (Hyperion 3000, Bruker) equipped with liquid N_2 and cooled 64×64 elements focal plane array (FPA) detector. Each element of the FPA works as an individual detector, making possible the measurement of 4096 spectra from different regions of the samples in a single detection with a spatial resolution of about 2.5 µm. 2D and 3D Chemical images were obtained by integrating the areas under the peaks corresponding to specific vibrational modes in the FTIR spectra. For the determination of

the limit of detection (LOD), the FTIR experiments were performed in the ATR mode using the diamond crystal in the FTIR spectrometer coupled to mercury cadmium telluride (MCT) detector in the spectral range of 4000–600 cm^{-1} at a spectral resolution of 4 cm^{-1} and 32 scans.

1.5. Immobilization of enzymes and evaluation of SEIRAS

For SEIRA spectroscopic study, approximately 2 µL (1 mg mL^{-1} in phosphate buffer solution, pH 7.2) of cyt C (Sigma-Aldrich, *CAS Number*: *9007-43-6*), bilirubin oxidase (Sigma-Aldrich, *CAS Number:* 80619-01-8), glucose oxidase (Sigma-Aldrich, *CAS Number* 9001-37-0), bovine serum albumin solutions were drop-cast onto the Au-NPs and Au-NPs micropatterns, immobilized for about 10 minutes, subsequently dried, and analyzed using the FTIR microscope described above. The SEIRAS effect was evaluated by monitoring the Amide-I band of the proteins.

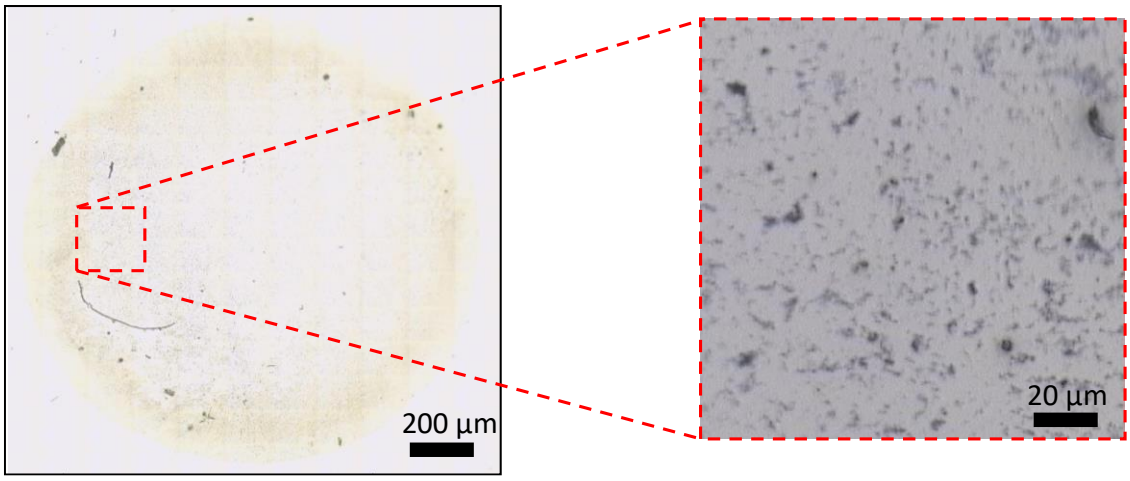

Figure S2: Optical microscopic image showing the formation of Au NPs on the IR transparent CaF_2 window. The zoomed image showed that nanoparticles are actually formed in the form of NPs islands.

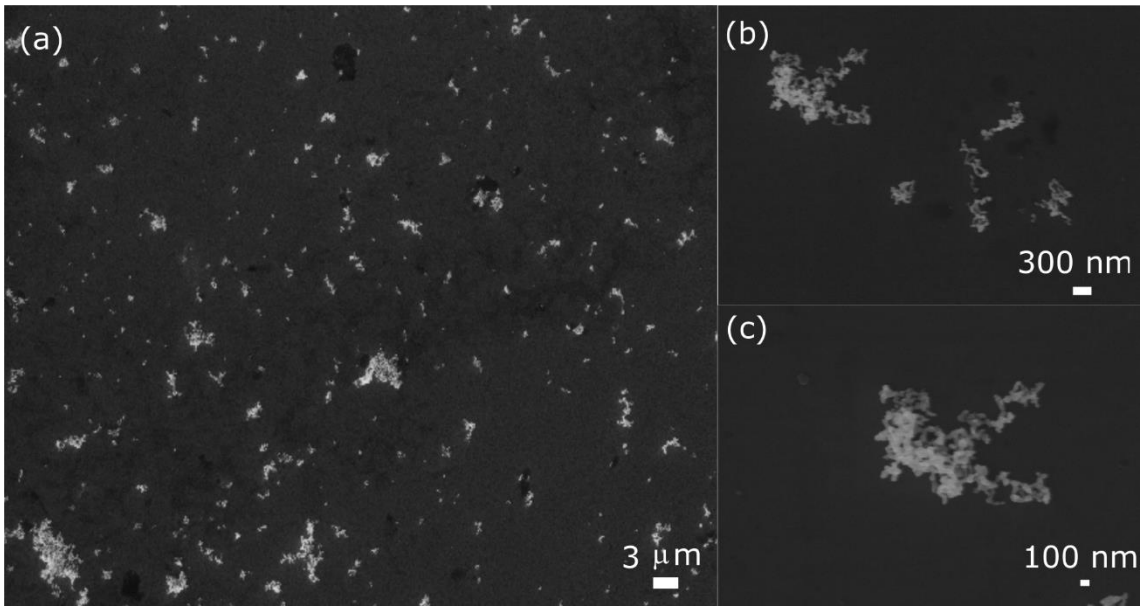

Figure S3: SEM images showing the formation of Au-NPs islands on Au coated glass mirror recorded at different magnification; (a) 3 µm, (b) 300 nm, and (c) 100 nm different magnification.

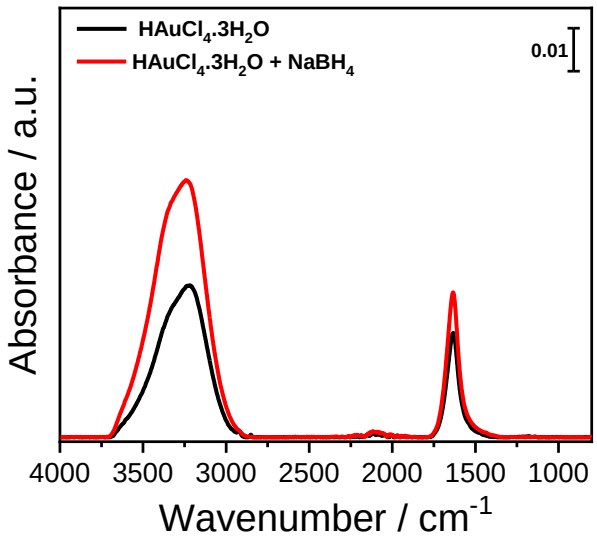

Figure S4: FTIR spectra of $HAuCl_4.3H_2O$ aqueous solution (—) and $HAuCl_4.3H_2O$ + $NaBH_4$ aqueous solution (—) recorded at the FTIR spectrometer in the ATR mode.

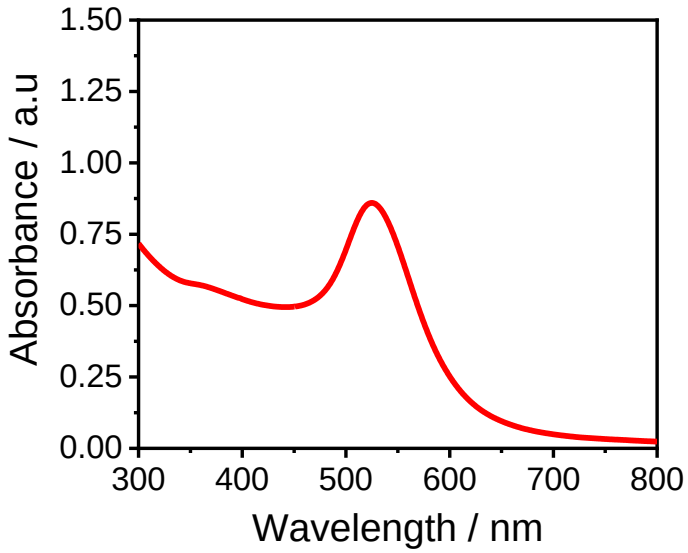

Figure S5: UV-Vis absorption spectrum of Au NPs solution recorded in the wavelength range from 300 – 800 nm.

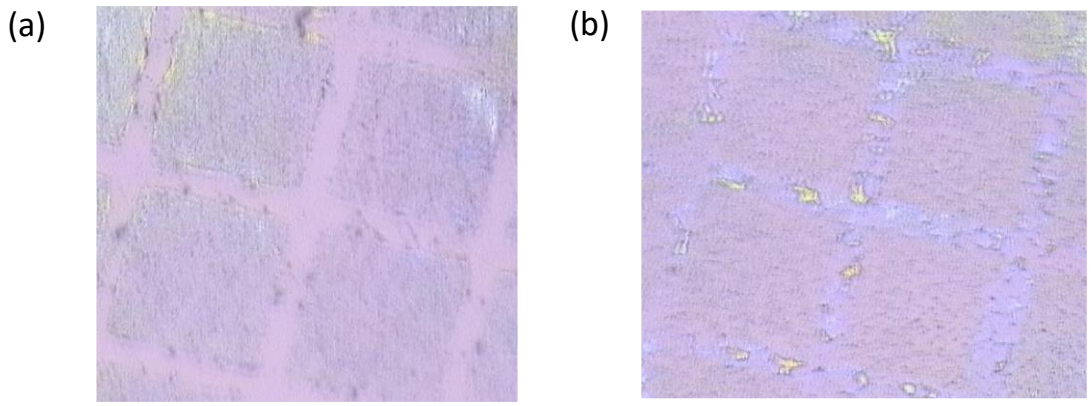

Figure S6: (a) Optical microscopic image of the Au NPs micropatterns obtained by directly drop casting about 2 µL of each $HAuCl_4.3H_2O$ (2 µmol L^{-1}) and $NaBH_4$ (2 µmol L^{-1}) aqueous solution and (b) Optical microscopic image of the Au NPs micropatterns obtained by drop casting about 4 µL of the previously synthesized Au NPs.

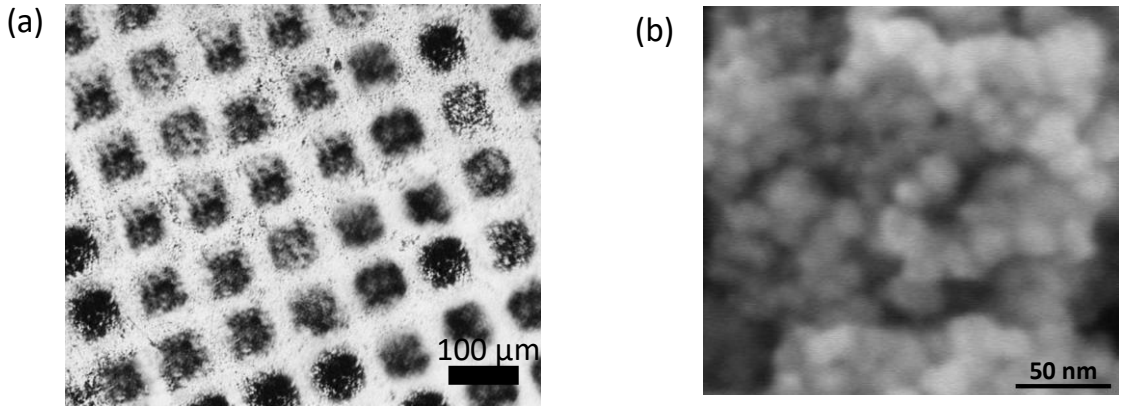

Figure S7: (a) Optical microscopic image showing the Au NPs micropatterns formed on IR transparent CaF_2 window, (b) SEM image showing the Au NPs within micropatterns.

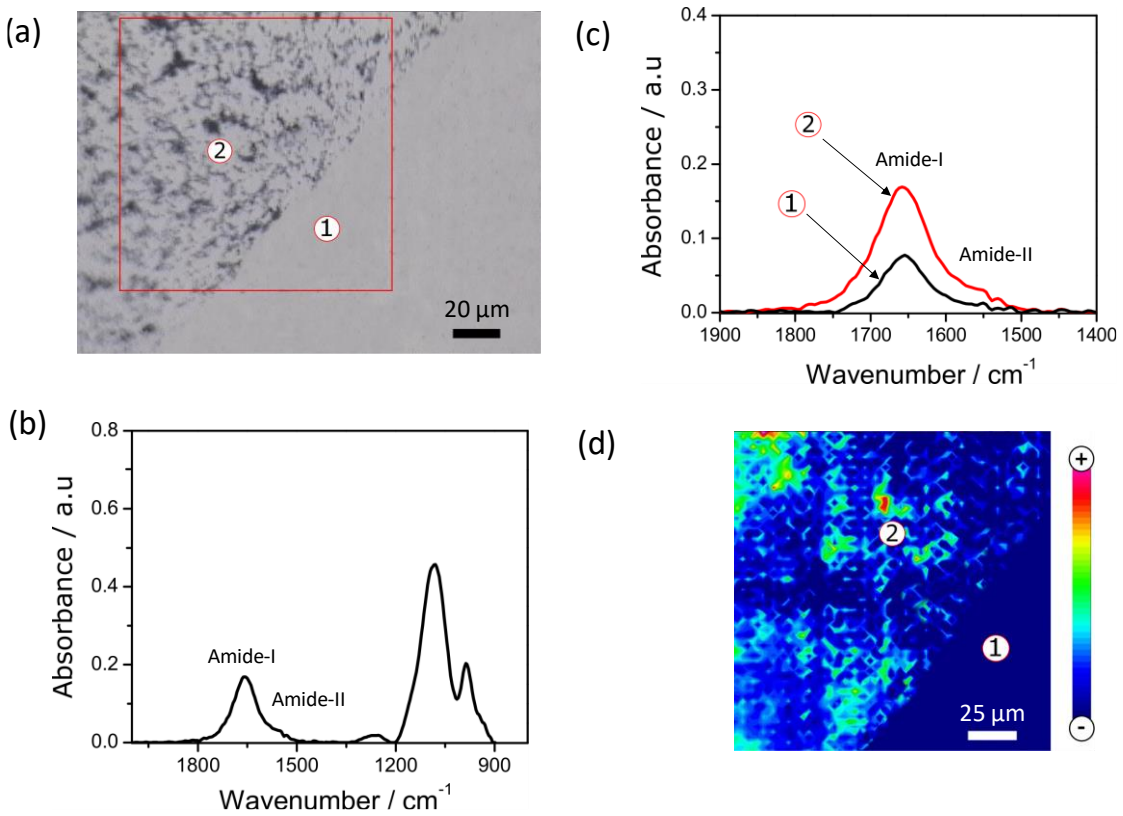

Figure S8: (a) Optical microscopic image showing the Au NPs islands formed on CaF_2 window, (b) FTIR spectrum of cyt C adsorbed to the Au NPs islands recorded in the mid IR region, (c) spectra showing the SEIRAS signal enhancement of Amide-I band when region 2 with Au NPs islands (—) was compared with region 1 without NPs islands (—), and (d) 2D chemical image showing the distribution of Amide-I band of cyt C. These regions correspond to 1 and 2 in (b).

Table S1: Secondary structure elements obtained after deconvolution of Amide-I band for different proteins evaluated in SEIRAS enhancement.

Protein	Protein secondary structure after interaction with Au NPs	
	α-helices	β-sheets
Cyt c	49	4
BOx	12	39
BSA	60	---
GOx	34	19

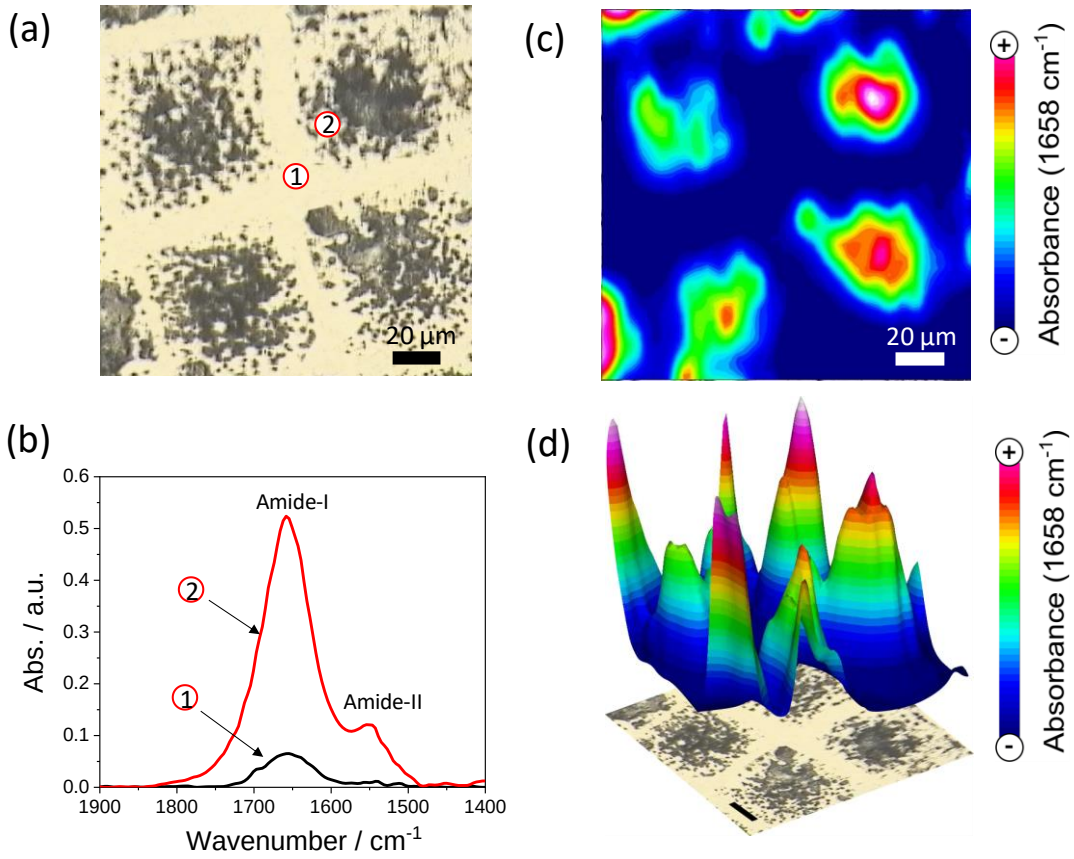

Figure S9: (a) Optical microscopic image showing the Au NPs micropatterns formed on Pt coated glass mirror substrate, (b) spectra showing the SEIRAS signal enhancement of Amide-I band of cyt C when region 2 with Au NPs micropatterns (—) was compared with region 1 with no NPs micropatterns (—), (c) 2D chemical image showing the distribution of Amide-I band of cyt C, and (d) 3D chemical image showing the distribution of Amide-I band of cyt C. The scale bar in (d) is 20 µm.

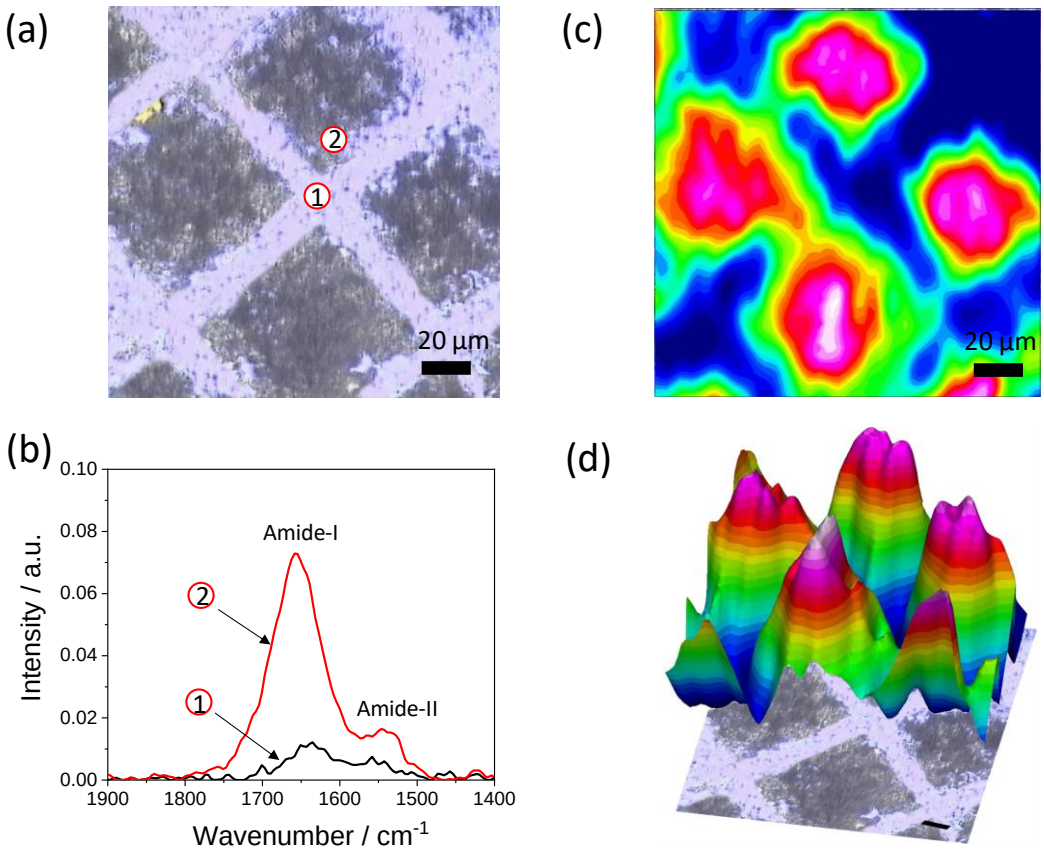

Figure S10: (a) Optical microscopic image showing the Au NPs micropatterns formed on Si/ SiO_2 substrate, (b) spectra showing the SEIRAS signal enhancement of Amide-I band of cyt C when region 2 with Au NPs micropatterns (—) was compared with region 1 with no NPs micropatterns (—), (c) 2D chemical image showing the distribution of Amide-I band of cyt C, and (d) 3D chemical image showing the distribution of Amide-I band of cyt C. The scale bar in (d) is 20 µm.

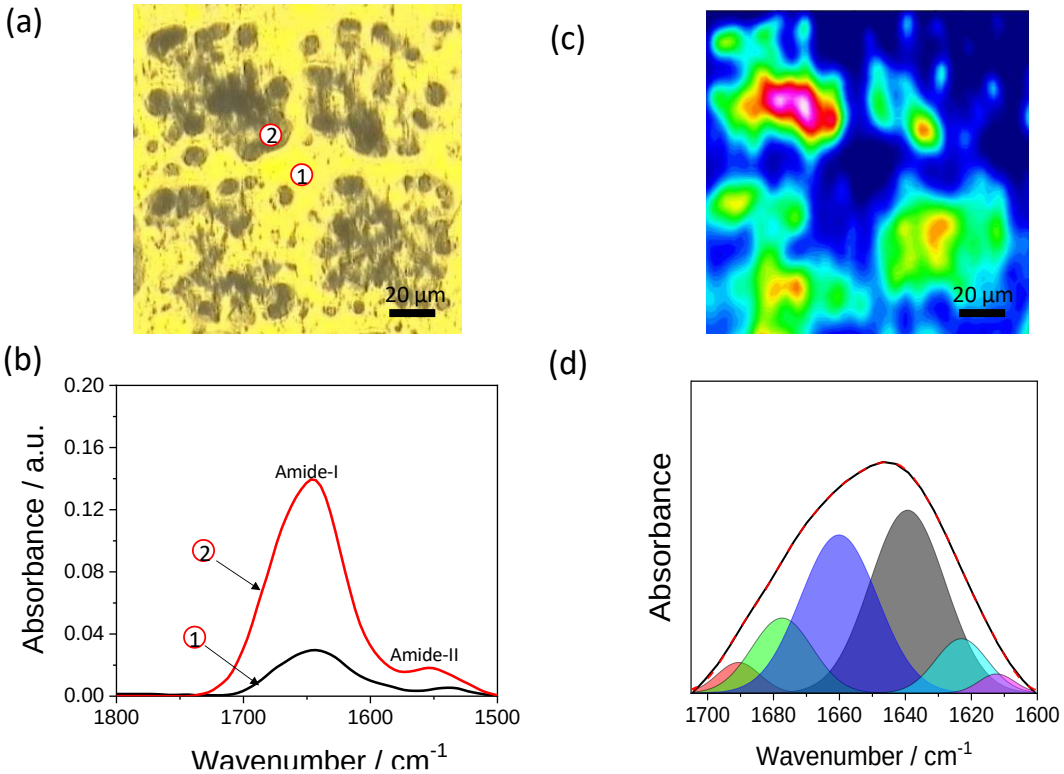

Figure S11: (a) Optical microscopic image showing the Au NPs micropatterns formed on Au coated glass substrate, (b) spectra showing the SEIRAS signal enhancement of Amide-I band of glucose oxidase (BOx) when region 2 with Au NPs micropatterns (—) was compared with region 1 with no NPs micropatterns (—), (c) 2D chemical image showing the distribution of Amide-I band of BOx, and (d) deconvoluted Amide-I band of BOx for the secondary structure analysis after interaction with Au NPs micropatterns.

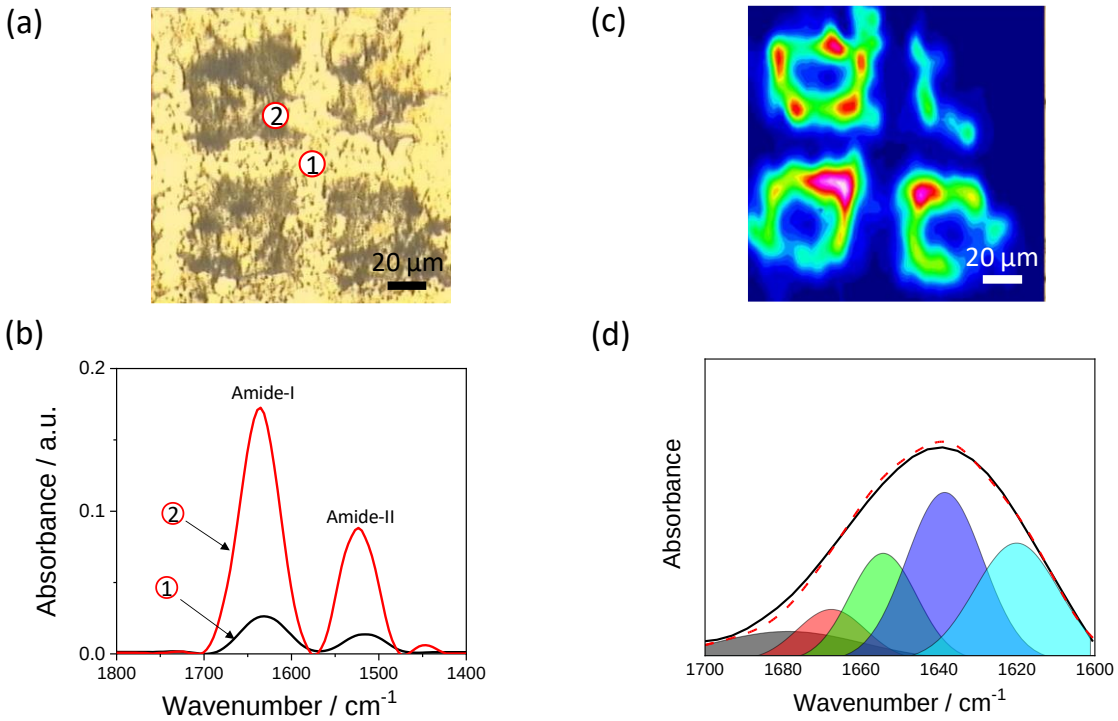

Figure S12: (a) Optical microscopic image showing the Au NPs micropatterns formed on Au coated glass mirror substrate, (b) spectra showing the SEIRAS signal enhancement of Amide-I band of glucose oxidase (GOx) when region 2 with Au NPs micropatterns (—) was compared with region 1 with no NPs micropatterns (—), (c) 2D chemical image showing the distribution of Amide-I band of GOx, and (d) deconvoluted Amide-I band of GOx for the secondary structure analysis after interaction with Au NPs micropatterns.

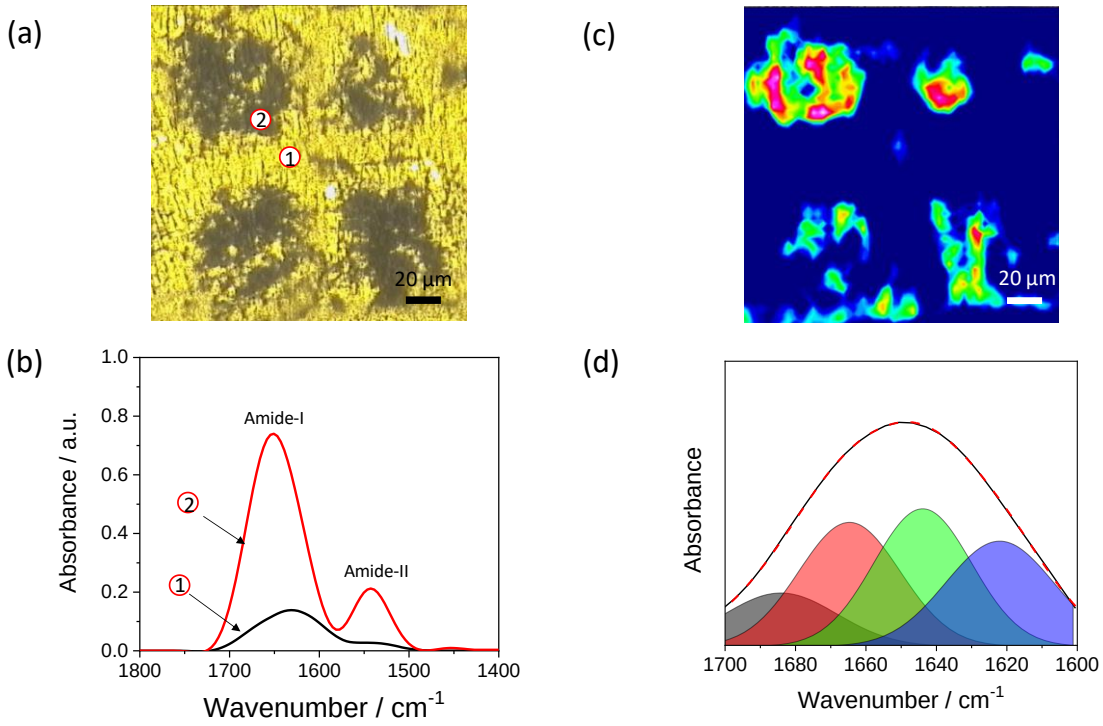

Figure S13: (a) Optical microscopic image showing the Au NPs micropatterns formed on Au coated glass mirror substrate, (b) spectra showing the SEIRAS signal enhancement of Amide-I band of bovine serum albumin (BSA) when region 2 with Au NPs micropatterns (▬) was compared with region 1 with no NPs micropatterns (▬), (c) 2D chemical image showing the distribution of Amide-I band of BSA, and (d) deconvoluted Amide-I band of GOx for the secondary structure analysis after interaction with Au NPs micropatterns.

Table S2: SEIRAS enhancement values for different proteins after adsorptions to Au NPs micropatterns and Au NPs aggregates on different IR substrates.

Probing protein/substrate	Absorbance (region 1)	Absorbance (region 2), SEIRAS	Enhancement
Cyt c on CaF_2 (NPs micropatterns)	0.02±002	0.25±0.05	9.06±1.82
Cyt c on CaF_2 (NPs aggregates)	0.08±0.02	0.25±0.06	2.99±0.66
Cyt c on Au	0.07±0.02	0.68±0.17	9.39±0.57
Cyt c on Pt	0.07±0.03	0.58±0.11	8.21±1.51
Cyt c on SiO_2/Si	0.02±0.01	0.18±0.10	7.03±0.36
BOx on Au	0.02±0.005	0.15±0.02	7.81±1.28
GOx on Au	0.03±0.003	0.17±0.01	6.04±0.34
BSA on Au	0.13±0.03	0.70±0.06	5.51±0.90