

Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Detection of SARS-CoV-2 and its S and N proteins using Surface Enhanced Raman Spectroscopy

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Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

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Experimental section:

Chemicals and Materials.

Hydrogen tetrachloroaurate(III) hydrate (HAuCl₄), copper(II) chloride (CuCl₂), oleylamine (OLA), and ethanol were purchased from Sigma-Aldrich, all reagents were analytical grade and were used for the synthesis without any further purification, the synthesis comprises three steps:

Synthesis of Au Seeds.

In a 20 mL glass vial, 7 mL of OLA was combined with 50 μL of HAuCl₄ (0.1 M) dissolved in ethanol with stirring by vortex and placed on a hot plate. Then, the temperature was increased to 120 °C. The color of the solution changed gradually from pale yellow to light pink and then to wine red, the final color. The wine-red color indicates the formation of the small Au seeds. The solution was then allowed to cool to room temperature.

Synthesis of Concave Pentagonal Nanoparticles

In a 20 mL glass vial, 5 mL of OLA and 2 mL of Au seeds were mixed at room temperature. Then, 75 μL of HAuCl₄ (0.1 M in ethanol) and 50 μL of CuCl₂ (0.1 M in ethanol) were sequentially injected into the mixture and heated to 120 °C for 10 min.

Synthesis of Concave Nanostars.

In a 20 mL glass vial at room temperature, a growth solution was prepared by adding 600 μL of CuCl_2 (0.1 M in ethanol) and 200 μL of HAuCl_4 (0.1 M in ethanol) to 7 mL of OLA (3:1). Subsequently, 400 μL of freshly prepared CPNPs were added to the mixture. The solution was heated at 120 $^\circ\text{C}$ for 30 min and then cooled at room temperature. The color of the solution at this last step was brown-green.

Characterization.

Structural features of the produced NPs were characterized by electron microscopy. SEM was performed on a Hitachi 5500 high-resolution microscope operating at 30 kV, equipped with bright-field and annular dark-field detectors, and a Bruker Quantax 200 EDS system.

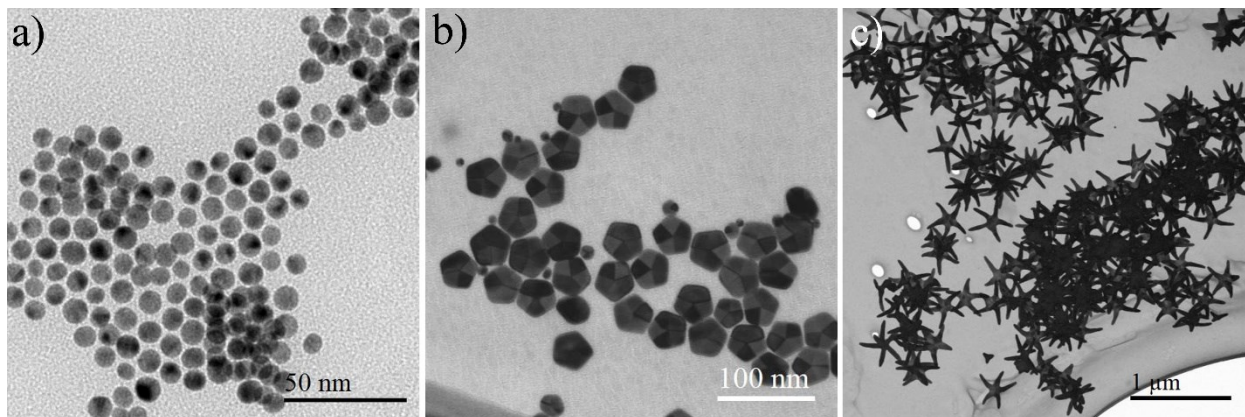


Figure S1: SEM image of the Au, Au-Cu nanostar used as SERS substrate (a) Au seeds, (b) Au-Cu seeds, (c) Au-Cu nanostars.

Results and discussion:

3D protein structures and region of interest, simulated through using Gaussian [5] to obtain the vibrational frequencies based on the Hartree-Fock approximation are shown in Figure S2 –S4.

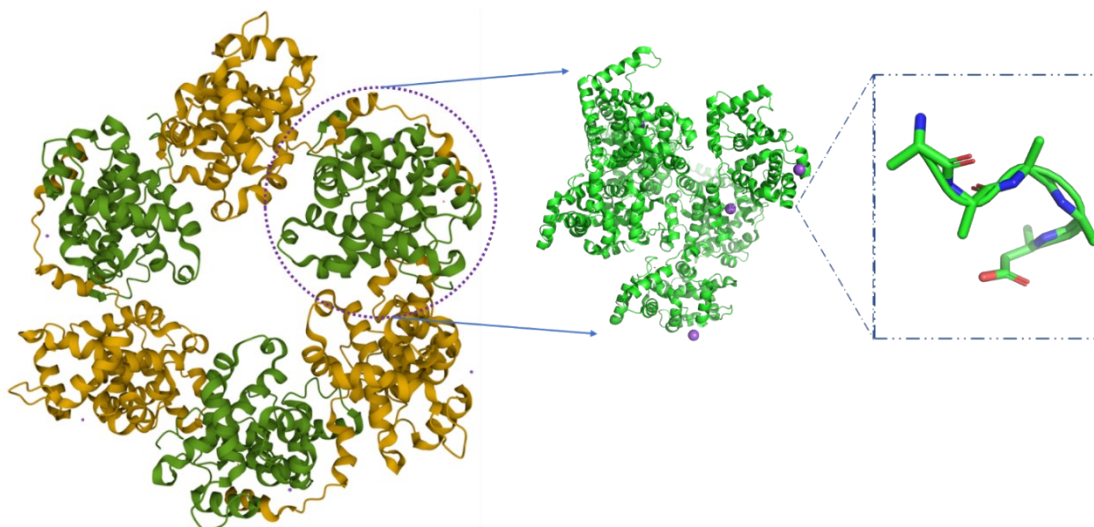


Figure S2. 3D structure of the protein - N (5fva [1]) asymmetric unit 9625 atoms and the region simulated for Raman frequencies [5].

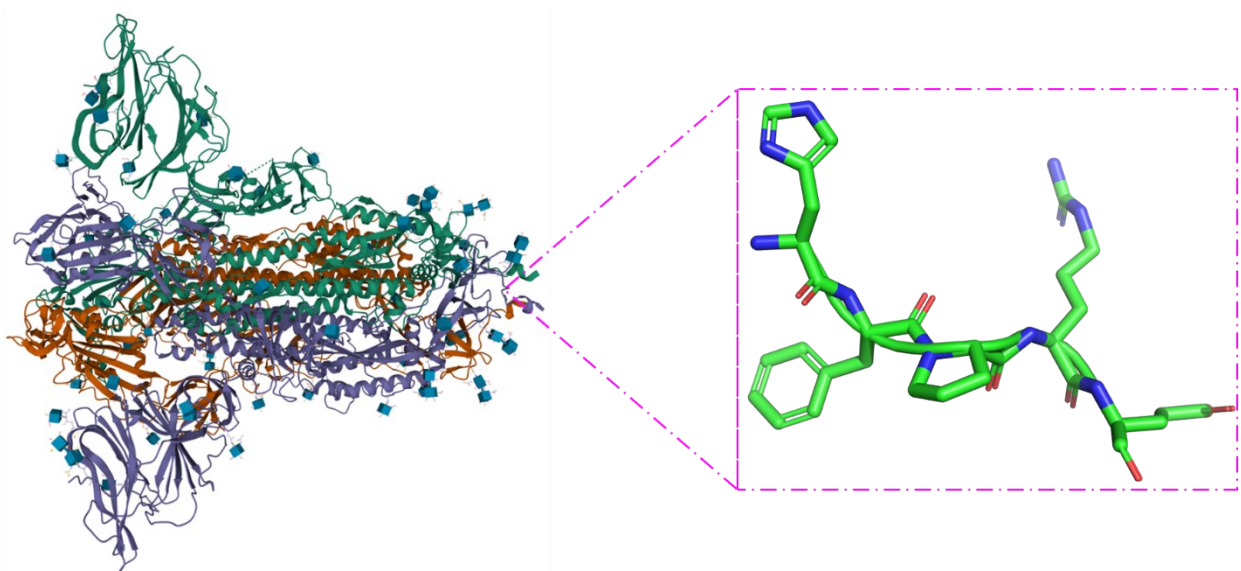


Figure S3. 3D structure of the Closed conformation of D614G SARS-CoV-2 spike protein (7bnm [2]) asymmetric unit 3207 atoms and the region simulated for Raman frequencies [5].

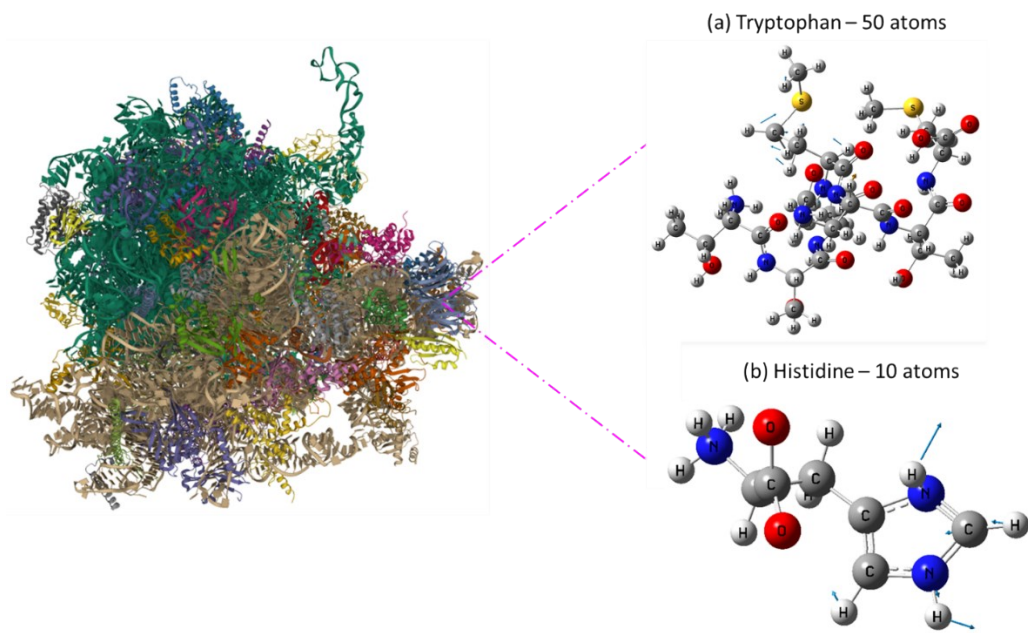


Figure S4. 3D structure of the SARS-CoV-2 Nsp1 bound to the human CCDC124-80S-eRF1 ribosome complex (6zme [3]) asymmetric unit 233375 atoms and the region simulated for Raman frequencies [5].

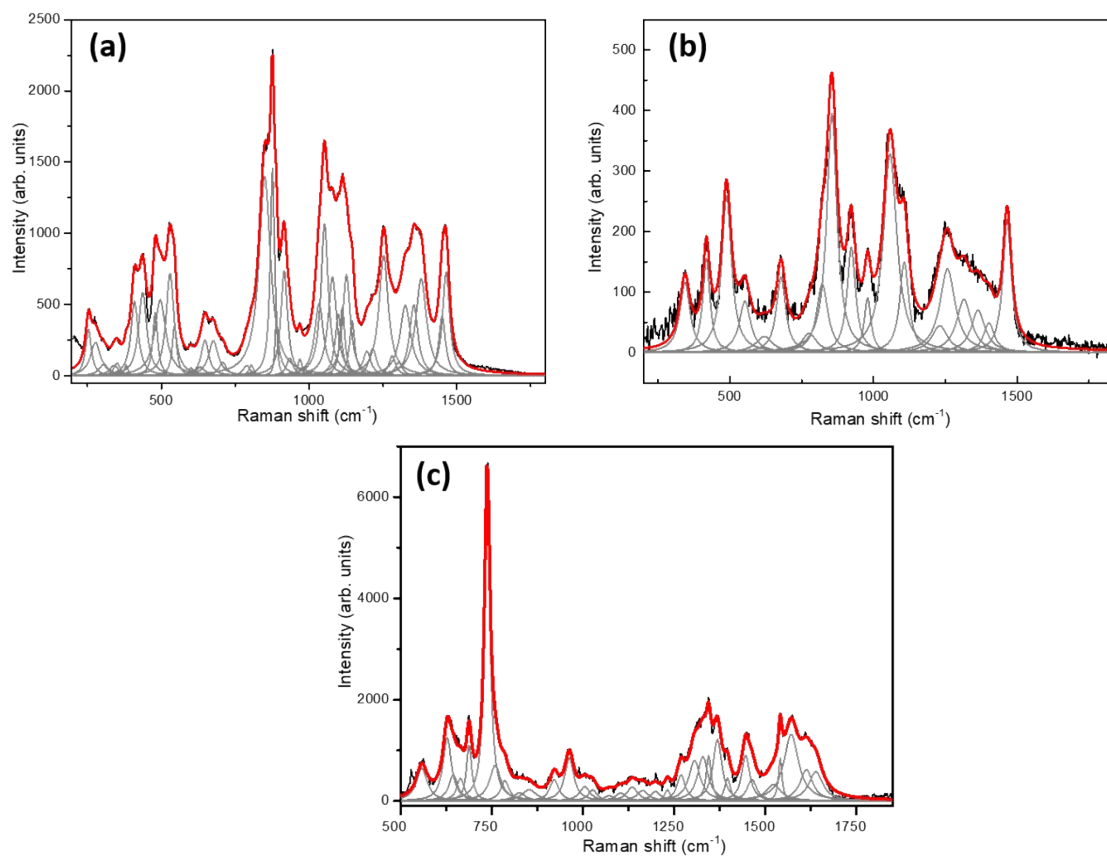


Figure S5. (a-c) examples of the deconvolution (red line) of the Raman peaks for S, N protein and NL-63, respectively, all spectral details, originating of the side-chains of the amino-acids, as well as from the peptide bonds (amide fingerprints) constitutive of the virion proteins are expected to be found in this spectral region [4]. The deconvolution data was processed by averaging 30-50 different spectra and after suppressing the background of the Raman signal by fitting the spectra curve using the cyclic approximation method, i.e., a polynomial (degree varying from 2 - 11) fitting is used in order to get a low-order S/N signal and a smoothness spectrum without little to nothing data treatment.

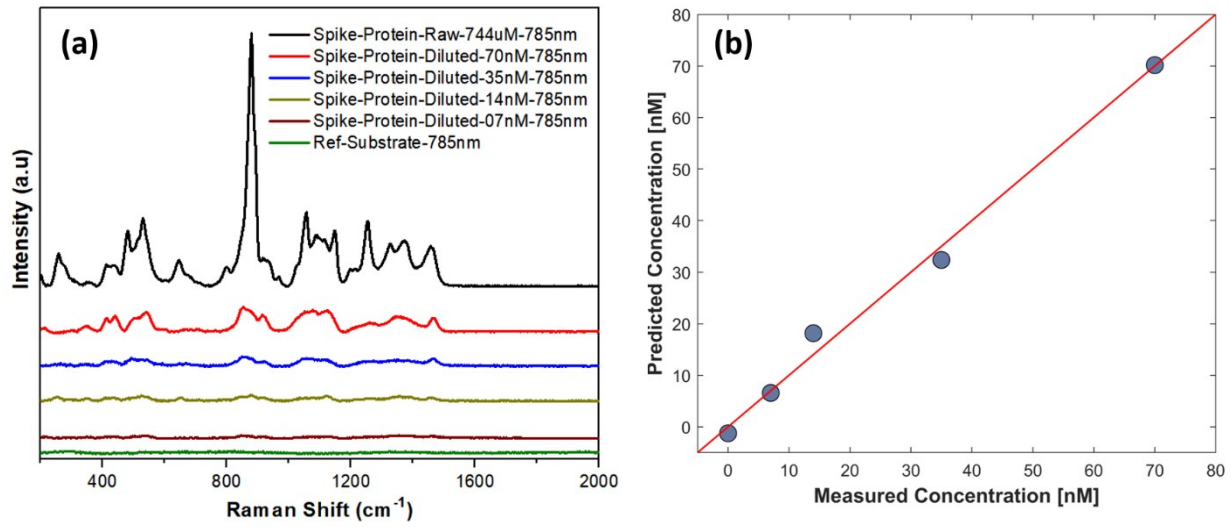


Figure S6. calibration curve and limit of detection for the S protein. (a) raw SERS spectra of the InBios-Spike-Protein for concentrations ranging from 744μM – 7nM. (b) calibration curve obtained by using PLSR (Partial Least Squares Regression) with two principal components. The variance for the two components was calculated to be 99.9%. Similarly, the detection limit was found to be 8.89×10^{-9} M with Root Mean Square Error (RMSE) = 2.27×10^{-9} .

Dimensions related. SARS-CoV-2 virion	Length (nm)	Copies (Number)
SARS-CoV-2 virion	100	—
SARS-CoV-2 - Spike protein	9 - 12	—
Membrane Protein	—	2000
Nucleoprotein	—	1000
Envelope Protein	—	20

Table S1. Dimensions of S, N and NL-63 proteins.

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