Electronic Supplementary Information

Gold-nanostar-based SERS substrates for studying protein aggregation processes

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SERS enhancement via gold-nanostar-modified substrates

The enhancement factor observed for BSA protein was also calculated according to the following equation:

$EF = (I_{SERS}/I_{Raman}) \times (N_{Raman}/N_{SERS})$,

I being the intensity (i.e. the peak height of amide I band of the protein) whereas N represents the total number of analyte molecules deposited on the AuNSts modified SERS substrate and bare glass, which has been directly related to the protein concentration deposited on each substrate since the same analyte volume has been deposited on both substrates, thus, the area of Raman and SERS measurements is the same, as well as that of that of the laser spot. A 20-fold EF of the Raman signal of BSA protein was observed. Figure S1 depicts the Raman and SERS spectra of BSA protein on glass and the SERS substrate at concentrations of 25 $mg \cdot mL^{-1}$ and 10 $mg \cdot mL^{-1}$, respectively.

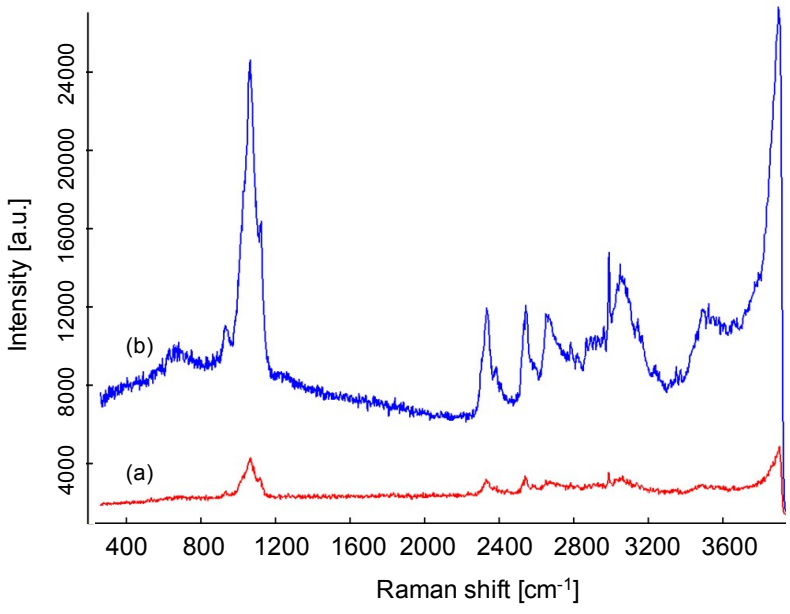

Figure S1. Comparison of the Raman spectra of CV at a concentration of 25 mg·mL^{-1} on bare glass substrate (a) with SERS spectra of BSA at a concentration of 10 mg·mL^{-1} on top of the SERS substrate (b).

Attenuated total reflection infrared spectroscopy study of protein aggregation induced by temperature

The effect of temperature on aggregation of proteins was also evaluated in a label free fashion via attenuated total reflection infrared spectroscopy (ATR-IR), which enables measurements of proteins in solution. For the measurements, a Tensor II (Bruker Optik GmbH, Ettlingen, Germany) spectrometer equipped with a Bio-ATR cell and DTGS detector was used. Spectra were recorded using a silicon ATR sampling plate connected to a ZnSe radiation coupling optics with 8-10 internal reflections. For each measurement 20 µL of sample were used acquiring 500 sample scans with a spectral resolution of 2 cm^{-1} within a spectral range of 4000-600 cm^{-1}.

Infrared spectra of proteins include several characteristic bands, namely amide I, amide II and amide III bands arising from vibrations from the peptide backbone. These bands are native to all proteins and provide information on the secondary structure of proteins. The amide I region between 1600 cm^{-1} and 1700 cm^{-1} is related mainly to C=O stretching vibrations, while the amide II vibration (1500-1600 cm^{-1}) arises from CN stretching and NH in-plane bending. The amide III region is associated with CN stretching, NH bending and CO in-plane bending. The amide I band is the most investigated region in the IR spectra of proteins because its shape is sensitive to changes in the secondary structure. Different secondary structure components (e.g. α-helices, β-sheets, random coils, loops) exhibit different absorption properties. They differ in their strength of hydrogen bonding from one peptide C=O group to an H-N group of adjacent amino acids. The hydrogen bonding environment is reflected in the adsorption frequency of the C=O vibration resulting in different regions within the amide I band that reflect different secondary structure elements. For instance, α-helices arise at approximately 1654 cm^{-1} and native β-sheets at approximately 1630 cm^{-1} [1-3].

Spectra of myoglobin protein at a concentration of 10 mg·mL^{-1} at different temperatures within 60 and 90ºC were recorded. At 60ºC no spectral changes were observed. Changes within the amide I band of the protein were observed starting at about 70ºC. Thus, two different temperatures, namely 70 and 80ºC were selected and measurements at different incubation times were performed. As can be observed in Figure S2, at 70ºC the observed changes already had started at 110 min, thus confirming that the incubation time used for the SERS experiments (120 min) was enough in order to achieve aggregation of proteins. Moreover, at 80ºC the increase in the region related to a major presence of β-sheet structures within the secondary structure of the protein had already started after 20 min of incubation.

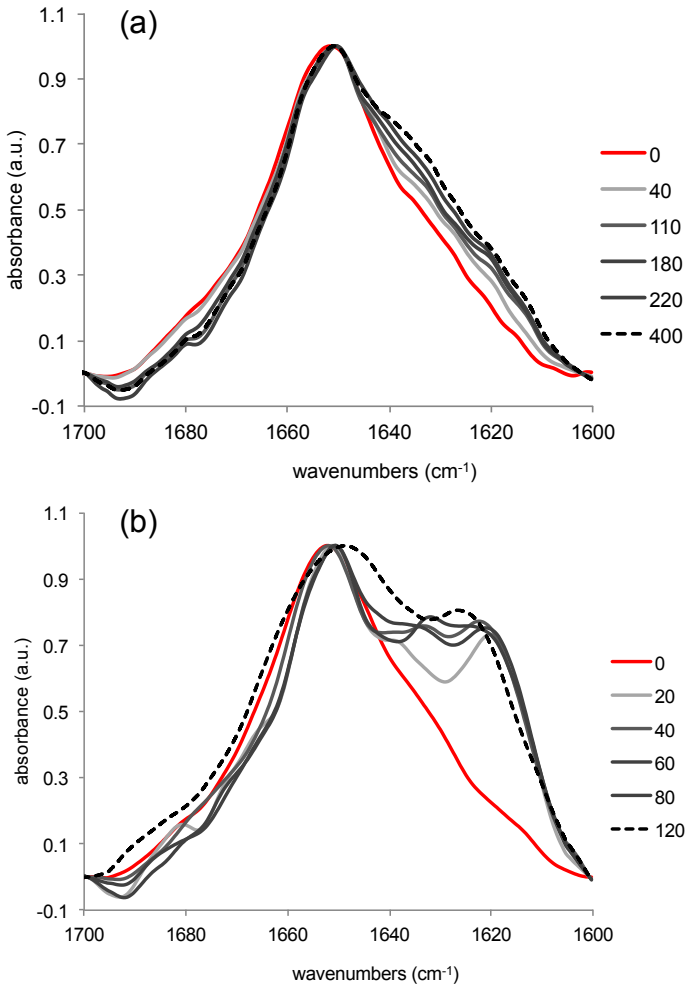

Figure S2. ATR-IR spectra showing the amide I band (1700-1600 cm^{-1}) after smoothing, baseline correction and normalization at different incubation times of a 10 $mg \cdot mL^{-1}$ myoglobin solution (a) at 70°C and (b) at 80°C.

Curve fitting analysis of the amide I band of the protein was performed by using second derivative Gaussian peak fitting. As shown in Figure S3, protein spectra is characterized by peaks at approximately 1651 cm^{-1}, which represent α-helix secondary structures. After aggregation of myoglobin protein an increase of the sub-peak at approximately 1620-1625 cm^{-1}, more evident in the case of incubation at 80ºC, is observed. The change at such frequency range is related to the formation of intermolecular crossed β-sheet structures [4].

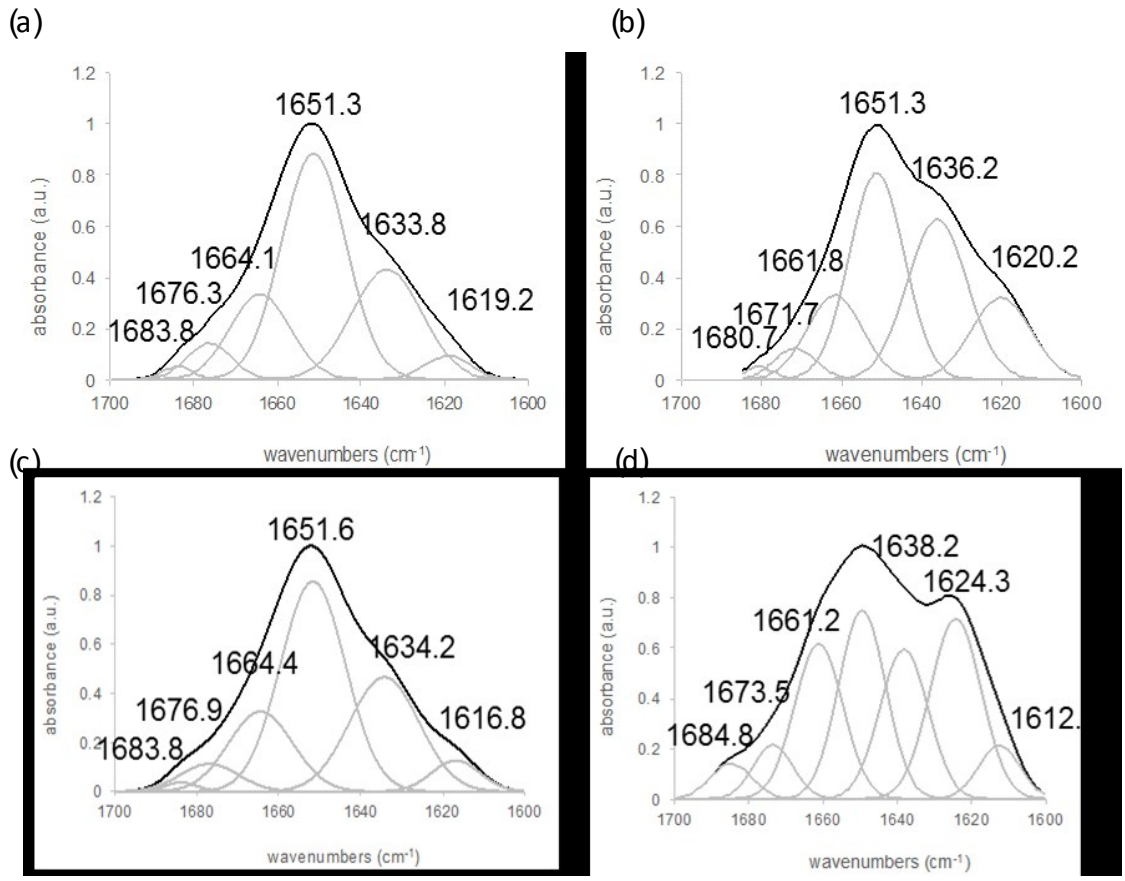

Figure S3. Curve fitting of amide I band of a 10 mg·mL^{-1} myoglobin solution after incubation at 70°C after (a) 0 min and (b) 400 min. Curve fitting of amide I band of a 10 mg·mL^{-1} myoglobin solution after incubation at 80°C after (c) 0 min and (d) 400 min.

2D ATR-IR correlation analysis showed similar results for the spectra at 70 °C and 80 °C (Figure S4). In both synchronous maps, one broad positive red autopeak at around 1620-1630 cm^{-1}, which reflects the increase in absorbance in this region, related to the increase in β-sheet structures, can be observed. The second weak autopeak at higher wavenumbers (1660-1680 cm^{-1}) shows the slight decrease that could be observed in this region with the time. As can be seen, the change within the region related to the formation of β-sheet structures is more intense at 80ºC.

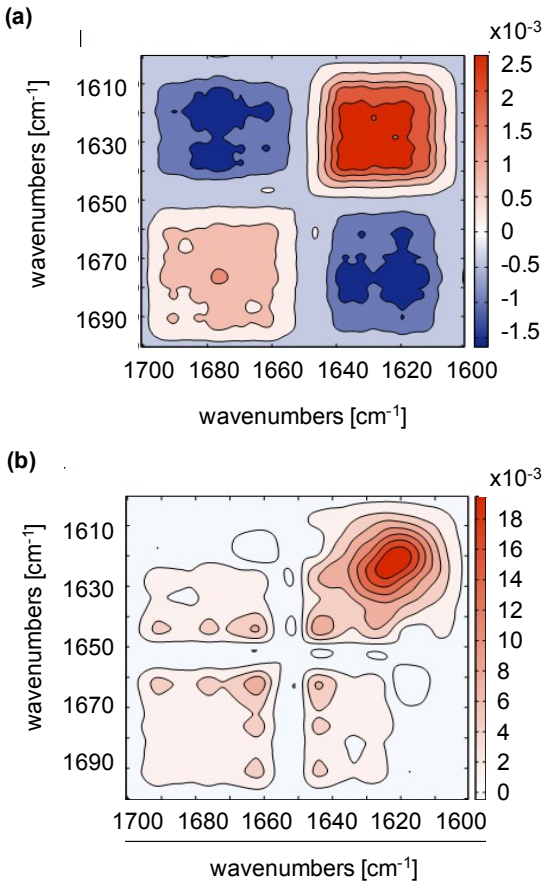

Figure S4. Synchronous 2D IR-ATR correlation spectra constructed from spectra of 10 mg·mL^{-1} myoglobin solution under incubation at (a) 70°C and (b) 80°C with time as external perturbation.

Solvent induced aggregation of BSA protein

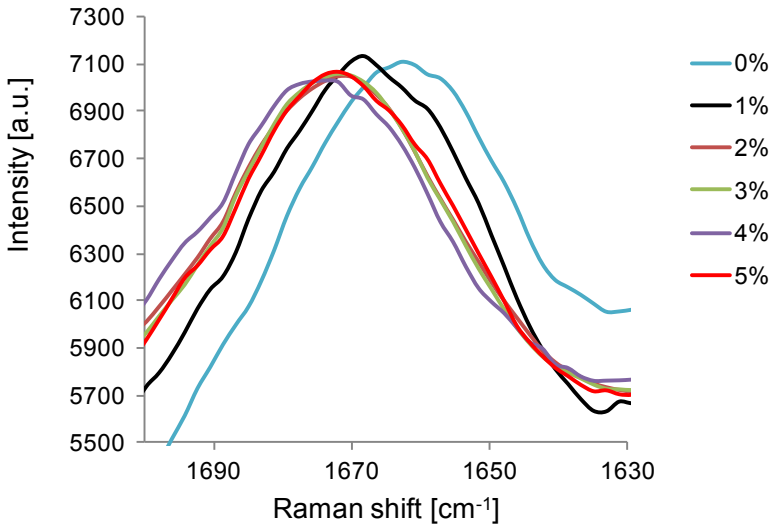

Figure S5. Raman spectra in the region 1700-1630 cm^{-1} of BSA protein after treatment with different amount of DMSO (1-5%) and without any solvent (0%).

Solvent induced aggregation of myoglobin protein

Solvent-induced aggregation of myoglobin protein was also investigated by using DMSO at different concentration in the range 1-5% in aqueous solution. As depicted in Figure S5, the tendency with increasing concentration of solvent is similar to that of temperature, i.e. an increase in the region 1520-1540 cm^{-1}, as reflected by an autopeak in the synchronous 2D correlation spectrum (Figure S6b), and a decrease in the maximum at about 1630-1640 cm^{-1}, which corresponds to the other autopeak that appears in the 2D correlation spectrum of Figure S5b.

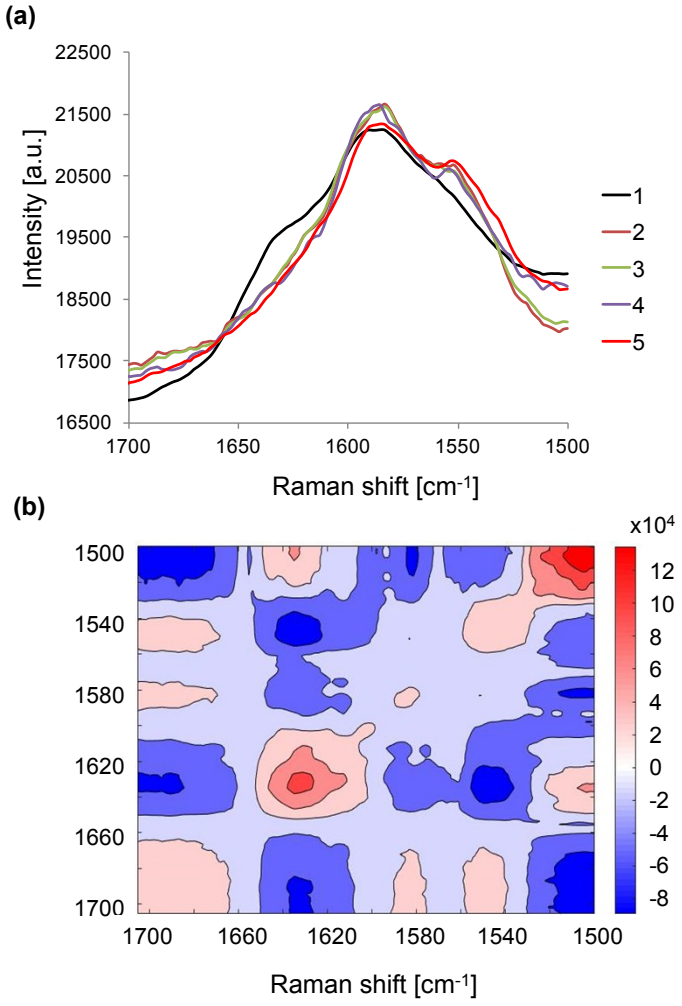

Figure S6. (a) Raman spectra in the region 1700-1500 cm^{-1} of myoglobin protein after treatment with different amount of DMSO (1-5%). Spectra were submitted to smoothing and normalization in PLS toolbox. (b) Corresponding synchronous 2D-correlation spectrum.

References

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