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Electrochemical surface-enhanced Raman spectroscopy (EC-SERS) study of the interaction between protein aggregates and biomimetic membranes

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ABSTRACT

Human diseases characterized by the uncontrolled deposition of insoluble extracellular protein aggregates are collectively referred to as amyloidoses. Such diseases include Alzheimer's, Parkinson's, Huntington's, and prion disease. In Alzheimer's disease, it is believed that amyloid-β proteins may be responsible for pore and defect formation within cellular membranes, leading to a breakdown of cellular homeostasis causing eventual neuronal death. This theory is referred to as the amyloid pore hypothesis of Alzheimer's disease. In this work, the molecular level interaction between a model amyloid-forming protein (insulin) and a biomimetic membrane was studied. Protein at different stages of aggregation was allowed to interact with a biomimetic membrane formed on a nanostructured substrate using Langmuir-Blodgett / Langmuir-Schaefer deposition. Electrochemical surface-enhanced Raman spectroscopy (EC-SERS) was used to monitor the molecular level changes occurring as a result of this interaction. Based on the results it was observed that oligomers and protofibrils caused the most significant membrane deterioration whilst native protein appeared to play a protective role. To the best of our knowledge, this work represents the first EC-SERS investigation of protein aggregate – biomembrane interactions, and highlights the usefulness of this tool for studying complex biomolecular interactions.

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Supporting information

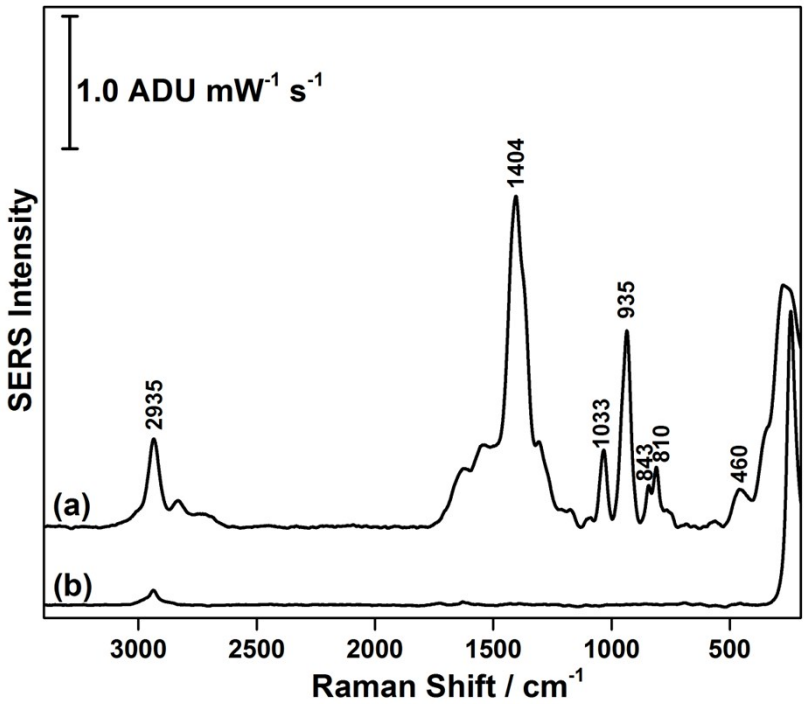

Figure S-1: SERS spectral comparison measured in air of a) AgNP electrode and b) AgNP electrode treated with 0.5 M KCl for 30 minutes. Spectra were measured at 2 mW for a time interval of 30 seconds using 532 nm excitation.

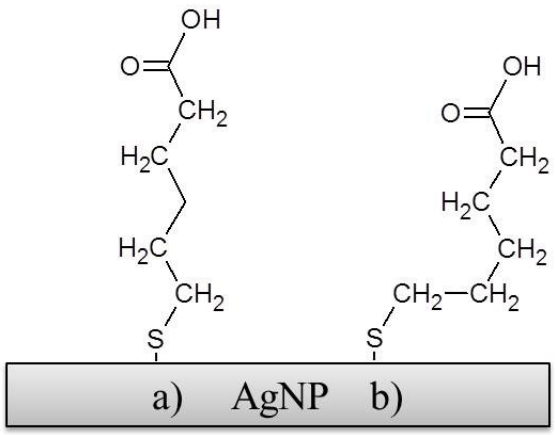

Figure S-2: The two surface orientations of 6-mercaptohexanoic acid on AgNP substrates: a) trans conformation, or b) gauche conformation.

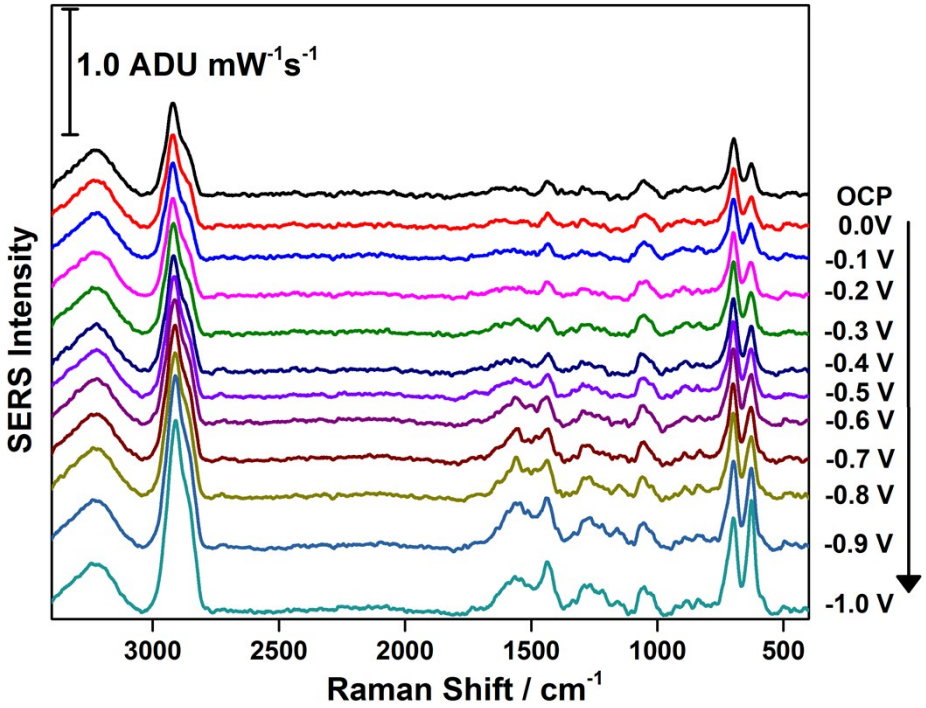

Figure S-3: EC-SERS signal of 6-MHA SAM on a AgNP-modified electrode in 0.1 M NaF supporting electrolyte. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

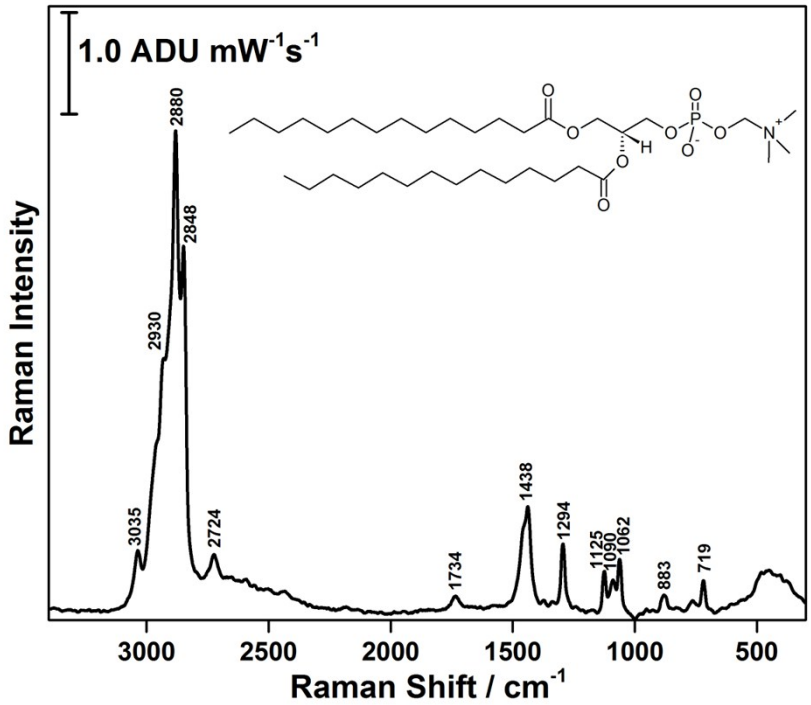

Figure S-4: Raman spectra of solid 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) collected for 30 seconds at a laser power of 4 mW at 532 nm.

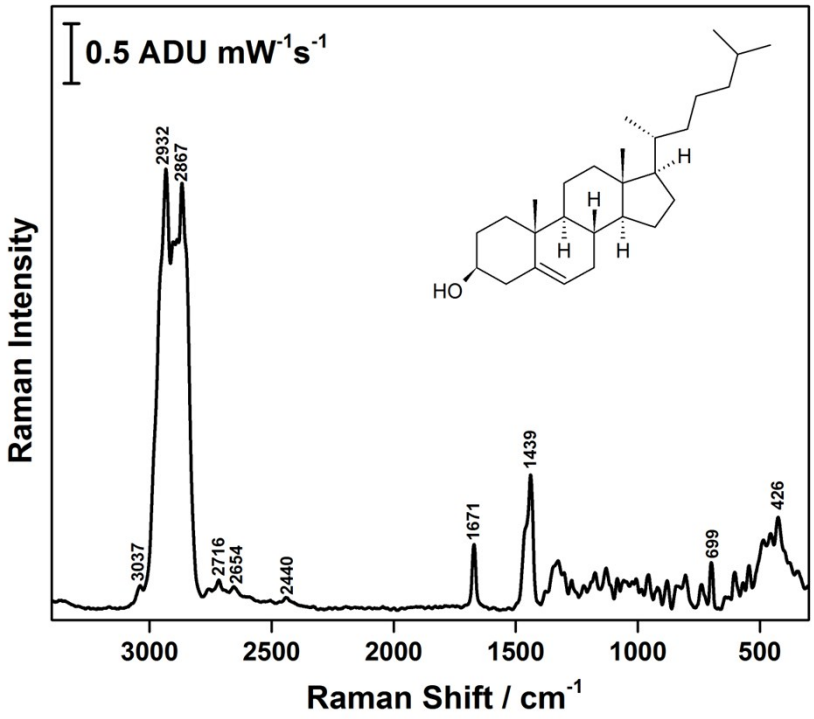

Figure S-5: Raman spectra of solid cholesterol collected for 30 seconds at a laser power of 6 mW at 532 nm.

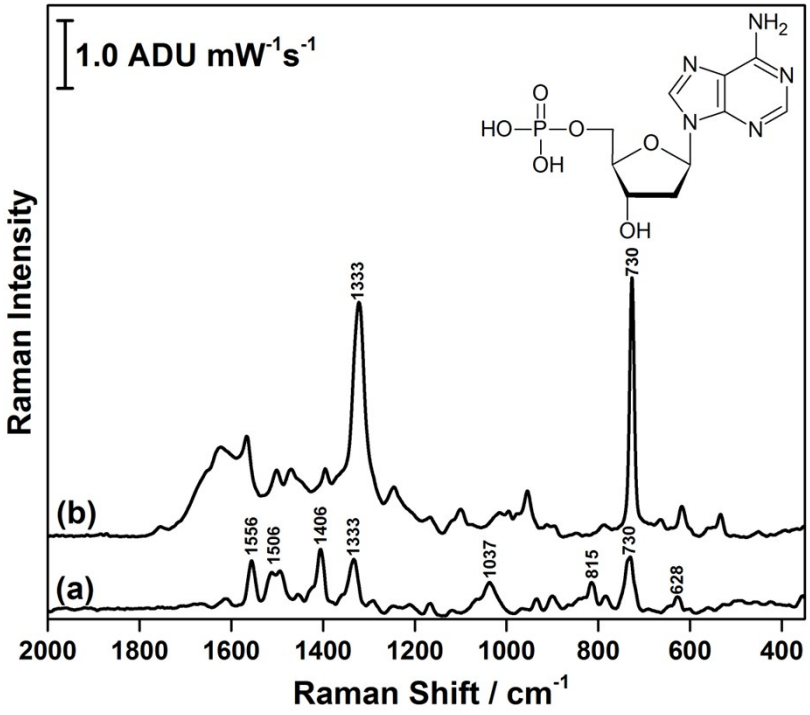

Figure S-6: (a) Raman spectrum of dAMP powder, (b) SERS spectrum of 1.0 mM dAMP solution at -1.0 V. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

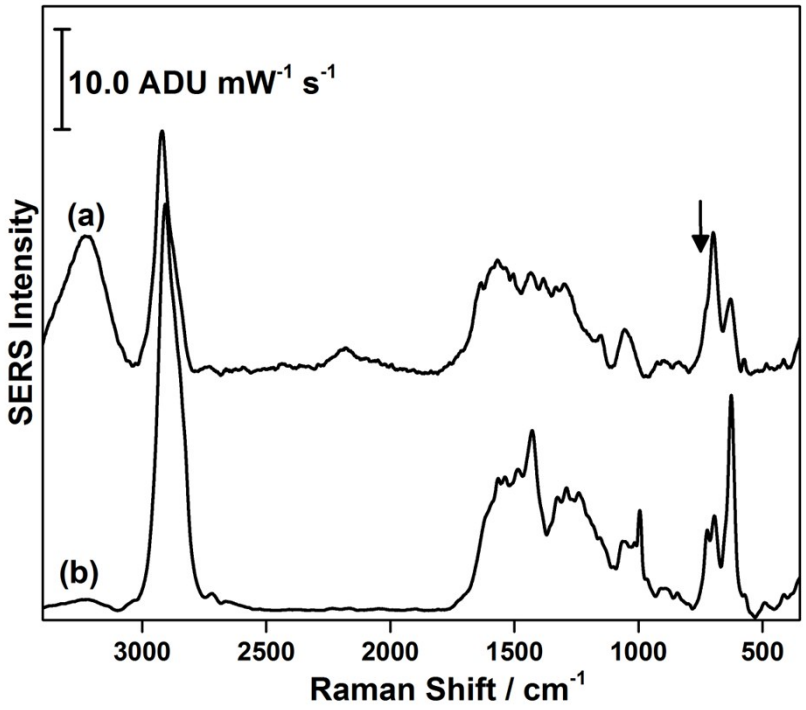

Figure S-7: Comparison between DMPC / cholesterol (70:30) bilayer on a AgNP electrode in 1.0 mM dAMP / 0.1 M NaF solution at a) OCP and b) -1.0 V. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

Spectroscopic characterization of native insulin protein

This research work focused on characterising the interaction between s-BLM and aggregated protein to better understand the mechanism of protein aggregation disorders such as Alzheimer's disease. Recombinant human insulin was chosen as a model of amyloid-β for this purpose. In literature, insulin is used as a model protein for various amyloid diseases because it forms amyloid fibrillar structures under certain conditions.[1-3] Prior to studying the protein aggregate-biomembrane interaction using EC-SERS, characterization of the aggregation process for human insulin was completed using various techniques as outlined below.

Raman Spectroscopy

The normal Raman signal of human insulin powder is shown in Figure S-8 (a). For proteins, the vibrational spectra are dominated by features arising from the peptide backbone, notably the amide vibrational modes.[4] In the Raman spectrum for insulin it is evident that the Amide III (1267 cm^{-1}) and Amide I (1656 cm^{-1}) modes are present and Amide II is not, as it is not Raman active. Other peaks that are present are due to side chains of amino acids: phenylalanine (1002, 1029 cm^{-1}), proline (1338 cm^{-1}) and tyrosine (849, 1206, 1609 cm^{-1}).[5] While Raman spectra of solutions can be difficult to obtain, a Raman spectrum of an aqueous 2 mg/ mL human insulin solution was successfully obtained in this work. Many of the peaks that were present in the normal Raman spectrum of human insulin powder sample are also observed in the solution spectrum as shown in Figure S-8 (b).

Electrochemical Surface-Enhanced Raman Spectroscopy (EC-SERS)

Native human insulin was characterized using EC-SERS to observe any vibrational changes occurring for the protein as the potential was varied. A bare AgNP electrode was incubated in 2 mg / mL human insulin solution for two hours, rinsed with ultrapure water, transferred to the spectroelectrochemical cell containing 0.1M NaF supporting electrolyte, and the EC-SERS data was recorded, as seen in Figure S-9. At OCP, several peaks due to the protein are present, and as the potential was stepped negatively the signal increased significantly and additional peaks appeared. The maximum SERS signal for human insulin was obtained at -0.9 V. Some of the peaks that were present include peaks for cysteine, phenylalanine, and glutamic acid at 542 cm^{-1}, 1100 cm^{-1}, and 1590 cm^{-1} respectively.[6] Other peaks include C-N asymmetric stretching (1303 cm^{-1}), CH_2 bending and stretching (1454 cm^{-1} and 2933 cm^{-1}, respectively), phenylalanine ring breathing (1080 cm^{-1}), and CH_2 wagging vibration from proline side chain (1202 cm^{-1}).[4,6] The EC-SERS signal obtained was very useful

in providing a spectral profile of native human insulin on the AgNP electrode. In the majority of cases, SERS analysis of proteins is difficult due to their large molecular size, weak scattering by the majority of the amino acids and their weak physisorption onto metallic surfaces.[7] However, application of a voltage can help in analyzing protein through manipulation of the surface charge which can in turn influence surface adsorption for proteins depending on their charge. These results show that EC-SERS can be useful for analyzing non-heme protein adsorption at electrified interfaces.

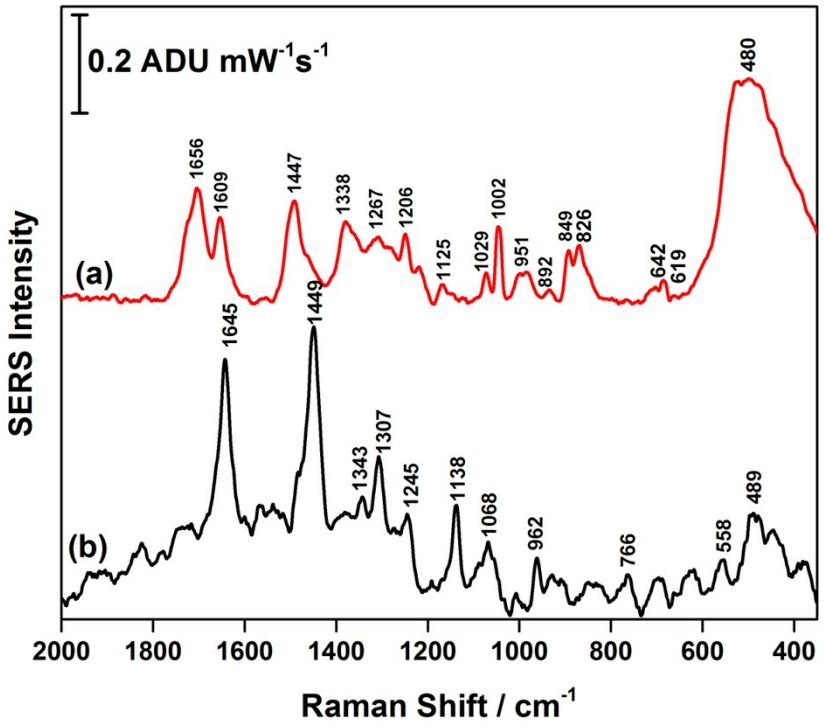

Figure S-8: (a) Normal Raman spectrum of recombinant human insulin powder collected for 30 seconds at a laser power of 4 mW at 532 nm excitation. (b) Raman spectrum of an aqueous 2 mg / mL recombinant human insulin solution collected for 30 seconds at a laser power of 4 mW at 532 nm excitation.

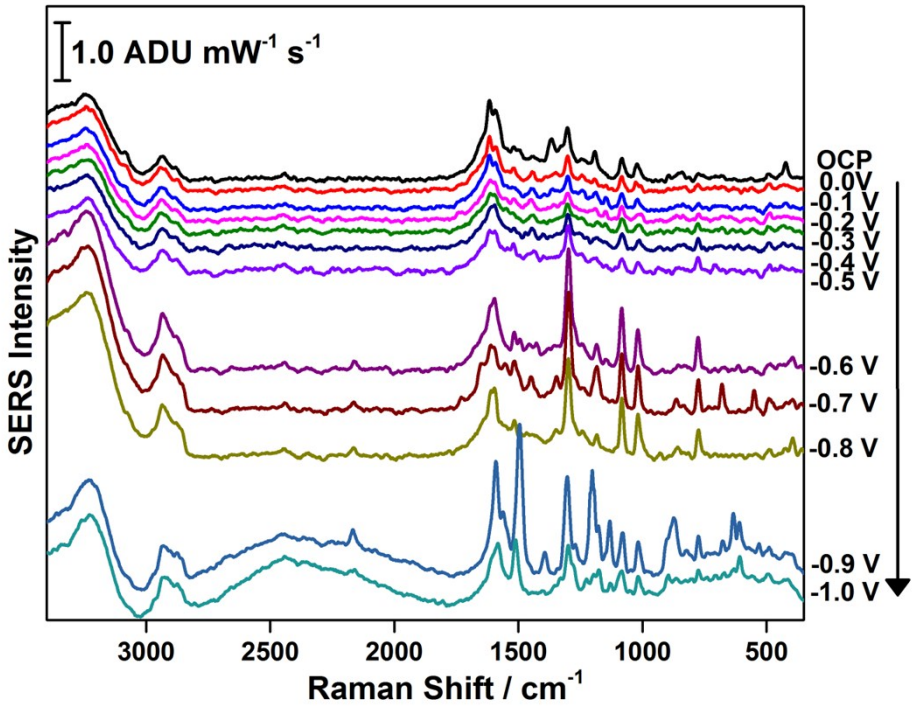

Figure S-9: EC-SERS signal of native human insulin on the AgNP surface, recorded in 0.1 M NaF solution. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

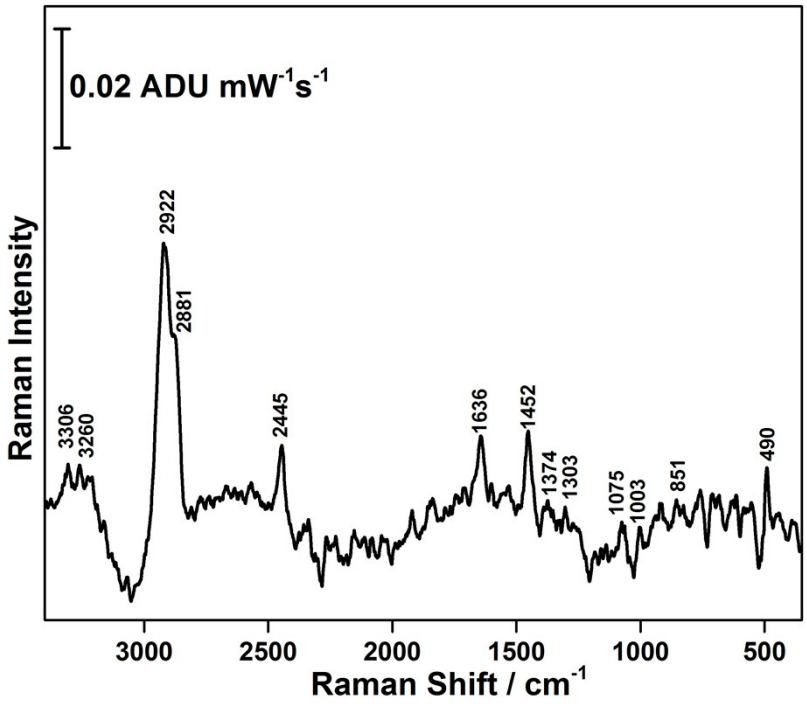

Figure S-10: Normal Raman spectrum of aggregated recombinant human insulin solution collected for 30 seconds at a laser power of 4 mW at 532 nm excitation.

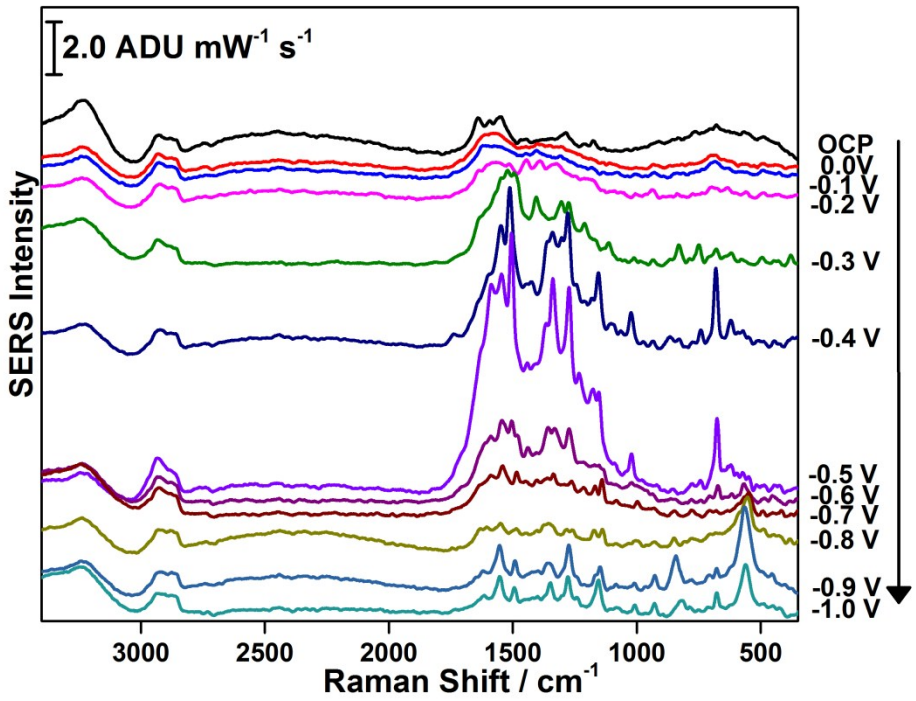

Figure S-11: EC-SERS cathodic signal of aggregated human insulin in 0.1 M NaF solution. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

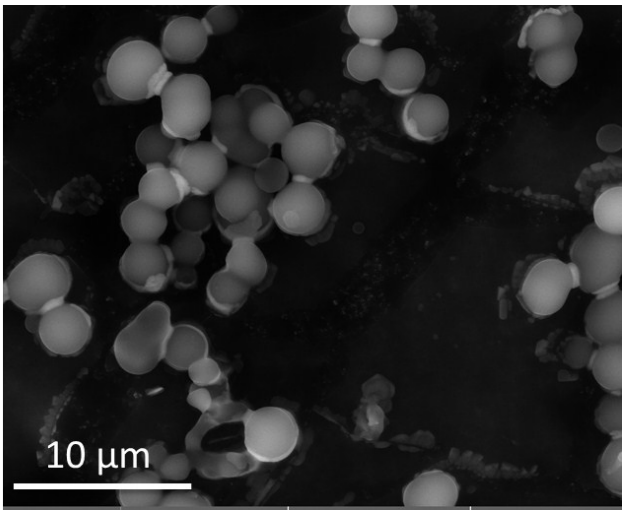

Figure S-12: SEM image of native human insulin. SEM images were conducted using Tescan MIRA3 LMU Full Emission at a high vacuum mode at 10 kV, and at a scanning speed of 32.00 µs/pixel.

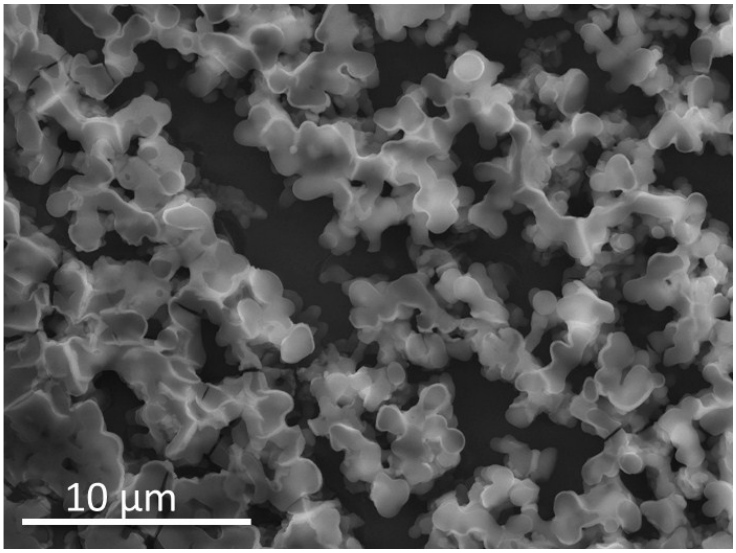

Figure S-13: SEM image illustrating a 2 mg mL^{-1} native human insulin solution of pH 1.6 stored at room temperature for a month, which was drop-cast and dried onto a silicon wafer prior to imaging. SEM images were conducted using Tescan MIRA3 LMU Full Emission at a high vacuum mode at 10 kV, and at a scanning speed of 32.00 µs/pixel.

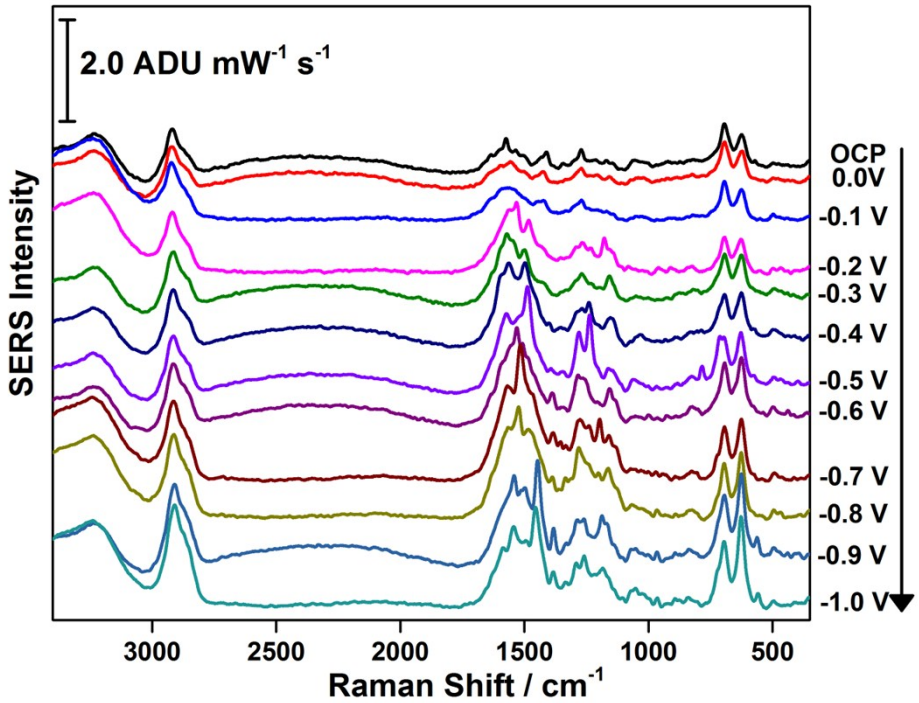

Figure S-14: EC-SERS cathodic signal of 6-MHA modified s-BLM + native human insulin, measured in 0.1 M NaF solution. The spectra were collected at 4 mW for a time interval of 30 seconds using 532 nm excitation.

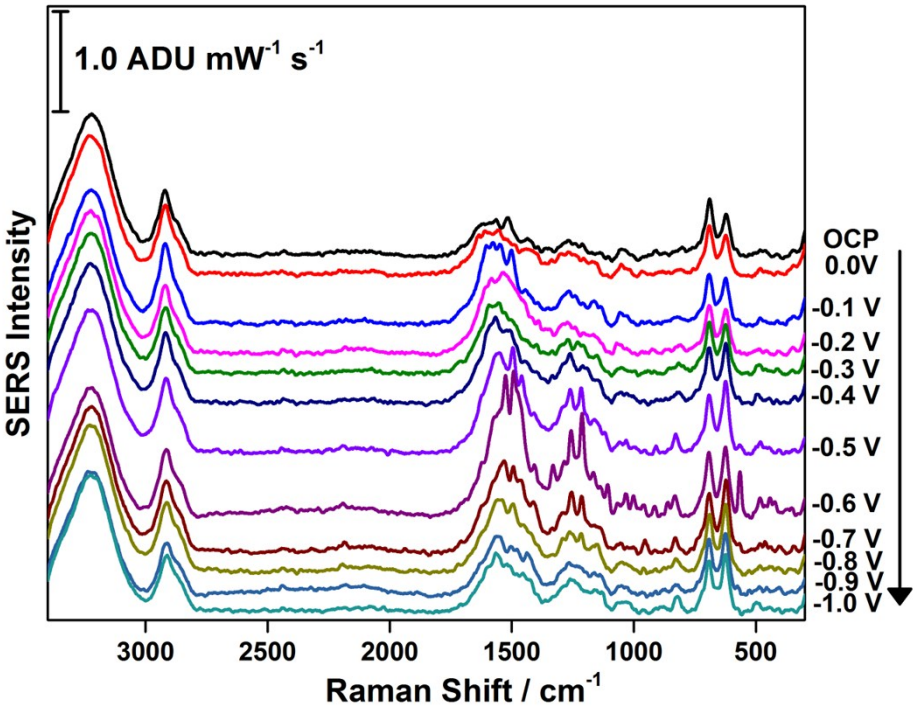

Figure S-15: EC-SERS cathodic signal of s-BLM + oligomeric human insulin in 0.1 M NaF solution. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

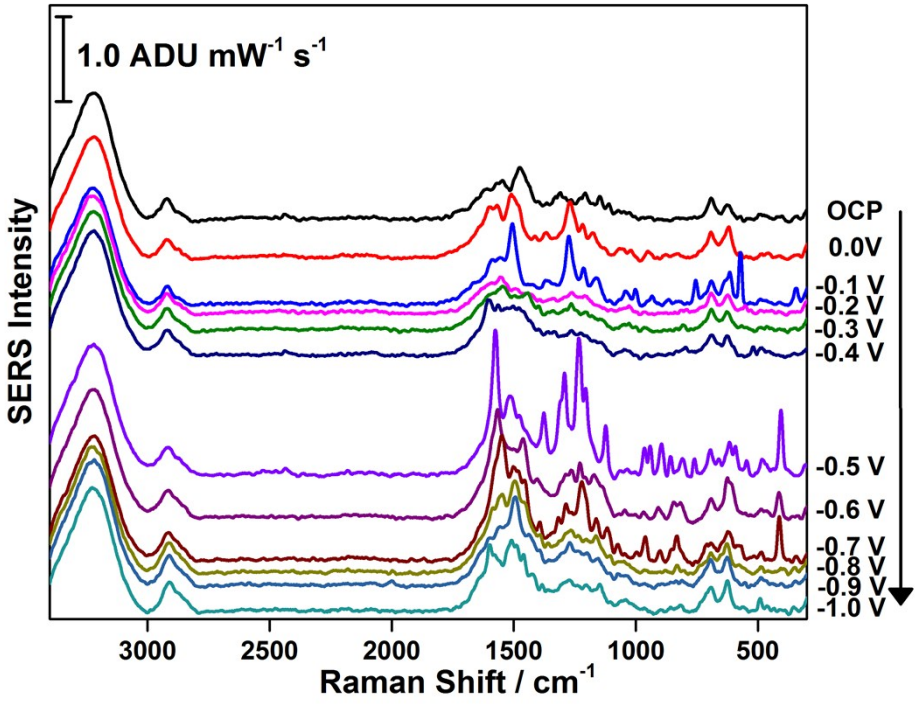

Figure S-16: EC-SERS cathodic signal of s-BLM + protofibrillar human insulin in 0.1 M NaF solution. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

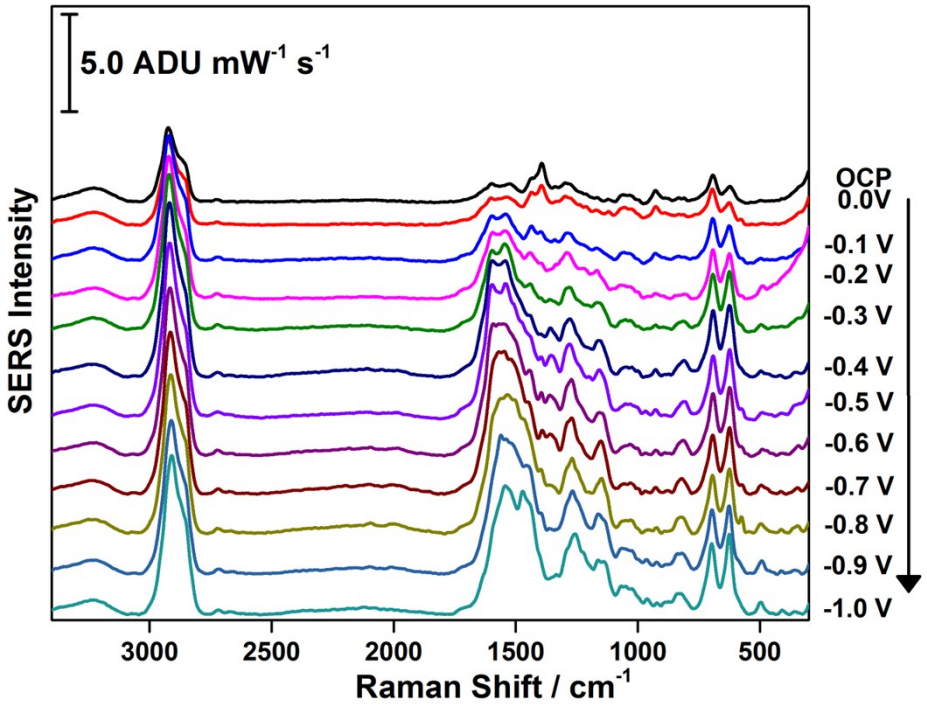

Figure S-17: EC-SERS cathodic signal of s-BLM + fibrillar human insulin in 0.1 M NaF solution. The spectra were measured at 4 mW for a time interval of 30 seconds using 532 nm excitation.

References

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