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Supplementary Information Available

Materials Silver nitrate, sodium citrate and citrate acid were obtained from Wako Co. Ltd. (Osaka, Japan). Lysozyme from chicken egg white and insulin from porcine pancreas were from ICN Biomedicals Inc (Aurora, Ohio) and Sigma (St Louis, MO, USA), respectively. All the materials were used without further purification. Eppendorf tubes were purchased from Eppendorf Company (Hamburg, Germany). Triply distilled water was used throughout the study.

Preparation of silver colloid. The citrate-reduced silver colloidal solution used in this study was synthesized according to a modified Lee and Meisel method.[1, 2] In brief, 90 mg of silver nitrate was dissolved in 500 ml of water and was refluxed to boil under stirring. Ten milliliters of 1% (w/v) sodium citrate was added to the solution right after the boiling started. The solution was kept boiling for 15 min. Then, the silver colloidal solution was placed in an ice bath to stop immediately the reduction. An UV absorption peak of the silver colloid was observed at 445 nm. This batch of silver colloid was aged for 6 months at 4 °C before used. All the study in the present work used this batch of colloidal silver, except for the semi-quantitative study of insulin in Figure 3, where a new batch of silver colloid (UV absorption peak is at 431 nm) was used freshly (within 24 h after preparation).

Sample preparation for SERS study. 500 μL of 5 mM citrate buffer solution （pH=3.7） containing 12 mM $NaNO_3$ was added to 500 μL of the prepared silver colloid solution in 2 mL EP tube (No obvious aggregation was observed in this mixture).

Then, 10 μL of protein solution obtained by dissolving proteins in pure water was added to the above 1 mL mixture (The pH of the final protein-colloid solution was 4.0. The aggregation of the colloidal silver was observed upon the addition of protein). Then, the protein-colloid solution was immediately shaken bottom to up by hand for 40 seconds during which one cycle of bottom to up and up to bottom spent 1 s. After that, the mixture was standed for 40s. Subsequently, removal of 30 μL of the mixture on an aluminum pan plate (0219-0062, Perkin Elmer) followed by heating at 100°C. The SERS sample was completely dry after 210 seconds heating. For quantitative study in Figure 3, 60 ul of the sample was used to dry.

Notes: In Figure 3(C), when the concentration is much lower than monolayer coverage which is estimated to be 1.4×10^{-7} M, the spectra ((e), (f)) suffer from citrate bands (1393, 1031, 947, 838, 798 cm^{-1}) [2], whether this background is related to the freshness of the silver colloid is still under investigation in our group.

SERS and normal Raman measurements. All SERS and normal Raman spectra were measured by a compact macroscopic Raman spectrometer (λex=785 nm), HoloProbe VPT system manufactured by Kaiser Optical Systems (Ann Arbor, MI, USA). The spot size of laser beam at the sample position was 10 μm. The laser power was 6 mW at the sample position, and the exposure time for each SERS measurement was set to be 20s with 1 accumulation. As for SERS spectra, each datum indicates an average of 15 measurements (in 3 plates, each plate was measured at 5 different points) and each error indicates the standard deviation.

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UV absorption measurements. Every absorption spectrum was obtained 5 min after addition of protein solution or pure water (control) into silver colloid with a Shimadzu UV-3101 UV/vis spectrometer at room temperature.

Calculation of lysozyme concentraion for monolayer coverage of colloidal silver. The UV plasmon peak of the prepared silver colloidal particles is at 445 nm, corresponding to the size of 60 nm in average. By approximation of the silver colloid to an ideal sphere, the cross section of each silver particle, ε was calculated to be 1.264×10^{-10} cm^2 using Mie theory. According to the equation.

absorption = ε (cm^2) × particle number (cm^{-3}) × length of a UV cell used (cm),

the number of silver particle was obtained to be 5.55×10^{10} cm^{-3}. This silver colloid solution was diluted by two fold using sodium citrate buffer. Therefore, the particle number becomes 2.78×10^{10} cm^{-3} (2.78×10^{13} L^{-1}), that is, 4.61×10^{-11} M. As the size of lysozyme is ca. 4.6×3×3 nm, there are two positions for lysozyme to adsorb to the surface of silver particle, namely, lying down and standing up. As for the lying down position, each 60 nm silver particle could coverage 1210 lysozyme molecules while the number is 1760 for the standing up position. Accordingly, the monolayer coverage concentration of lysozyme is estimated to be 5.6×10^{-8} M and 8.1×10^{-8} M, respectively, for the lying down and standing up positions. The real case should be between these two values. The size of insulin is ca. 2×2.5×3 nm which was approximated to be a 2.5 nm sphere and the concentration of monolayer coverage is calculated to be 1.4×10^{-7} M.

Table 1 Wavenumbers and band assignments for normal Raman spectra of solid lysozyme and inulin and SERS spectra of lysozyme and insulin (the assignments were based on ref. 3-8).

Assignment	Normal Raman lysozyme	SERS lysozyme (solution)	SERS lysozyme (100°C heating)	Normal Raman Solid insulin	SERS insulin (100°C heating)
Amide I and or H_2O	1676		1669	1681	1665
	1654	1646		1659	
Trp^{w1},Tyr,and/or Phe(ν_{8a})	1619		1618	1613	**1618**
				1606	1607
Trp^{w2} and/or Phe(ν_{8b})	1580	NO	1579	1587	
Trp^{w3} and/or Amide II	1553	1545	**1553** [a]	1556	1556
His	1492			1517	
$\delta(CH_2)$	1458,			1461	
	1449		1449	1449	1449
Tyr and/or $\delta(CH)_{indole\ ring}$	1422	1424	1420	1423	
				1415	
Trp^{w7}	1360		**1359**	**NO**	**NO**
Trp^{w7} and/or δ(CH)	1337		**1340**	1342	
				1319	
Amide III	1273		1273	1281	
	1254			1267	
	1245	1237	1236	1244	1234
Tyr and/or Phe(ν_{7a})	1207		1208	1206	**1207**
Tyr and/or Phe(ν_{9a})	1177		1182	1174	**1177**
ν(CN)	1154			1158	
	1128	1121	1128	1128	1128
	1106	1101	1101	1111	
	1078			1082	
				1063	
NO3		1049	1049		1049
Phe(ν_{18a})	1031			1032	1032
Trp^{w16}	1011				
Phe(ν_{12})	1006	1007	1007	1004	1005
ν(CC)	979				989
	960	951	958	960	954
	933	935 [a]	934 [a]	946	944, 934 [b]
	904		904	895	904
Trp^{w17}	876	878	**878**	**NO**	**NO**
Tyr	859	846	853	853	**853**
Tyr	837	836	829	829	**829**
			809		809
				768 (?)	
Trp^{w18}	761	760	**761**	**NO**	**NO**
ν(CS) and/or Trp^{w19} and/or NO_3^-				744	
	722	716	722	723	**722**
ν(CS)	695		680		
	666	659	662	665	662
Tyr	645	643	646	644	646
Phe(ν_{6b})	623		NO	622	623
Trp	575	581	NO		
Trp	537		538		
				561(?)	
ν(SS)	508	525	520	512	**515**
Phe or Tyr	428	438 (?)	426	422	430

a: Red, green and blue represent bands of trp, tyr and ν(CS)/ ν(SS), respectively.

b: Partly from ν_s (C-COO$^-$).

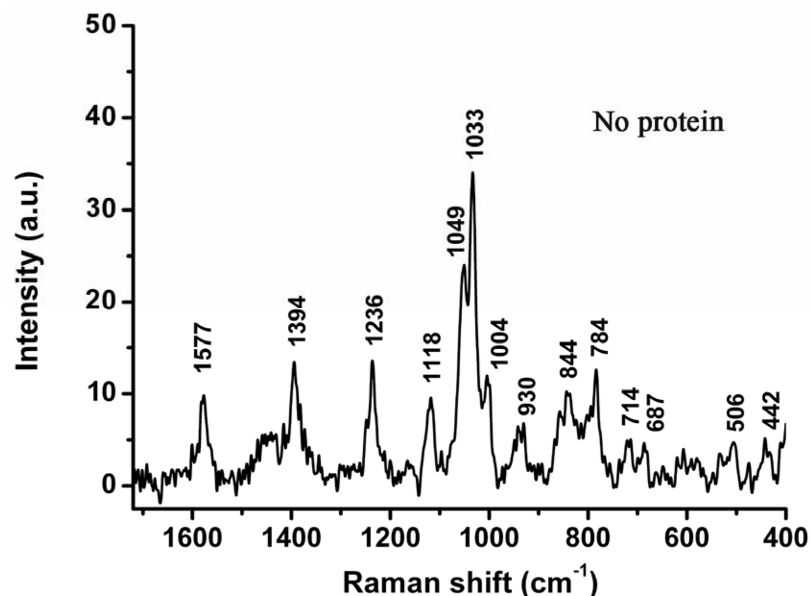

Figure I. A SERS spectrum of a dry film of a solution containing 6mM $NaNO_3$. There was no protein in the sample. Drying temperature was 100 °C. The $NaNO_3$ was dissolved in a 5 mM citrate buffer. Except for the peak at 1049 cm^{-1} due to NO_3^- ion, all other peaks are assignable to citrate ion (ref 2).

Table 2 The number and appearance probability of the amino acids whose peaks dominate in SERS spectra

	Lysozyme (129 amino acids)		Insulin (51 amino acids)	
	Number	Appearance probability	Number	Appearance probability
Trp	6	0.047	0	0
Tyr	3	0.023	4	0.078
Phe	3	0.023	3	0.059
S-S	4	0.031	3	0.059

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