Supporting Information for

Glucose Sandwich Assay based on Surface-Enhanced Raman Spectroscopy

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This supporting information is a 17-page document, including 10 figures, 4 tables, and this cover page.

1. Calculation of enhancement factor

According to the most widely used definition of the average SERS enhancement factor (EF)[1], we calculated the SERS EF for R6G adsorbed on polyamide-Ag.

$$EF = (I_{SERS}/N_{surf})/(I_{RS}/N_{vol}) \qquad (S1)$$

where I_{SERS} and I_{RS} are the intensities of a particular peak of an analyte in the SERS and normal Raman (non-SERS) spectra measured under identical conditions, respectively. During a SERS experiment, N_{surf} represents the average number of molecules adsorbing in the scattering volume, while in a non-SERS experiment, N_{vol} represents the average number of molecules adsorbing in the scattering volume. Based on the assumption that the molecules were uniformly distributed on the polyamide-Ag, it was assumed that the density of R6G on the polyamide-Ag under SERS would be 1×10^{-9} mol $L^{-1} \times 10$ μL × NA/16 mm^2 (16 mm^2 stands for the surface area of the polyamide-Ag), which corresponds to 3.76×10^8 molecules per mm^2, whereas the density of folic acid on the silicon under non-SERS was assumed to be 1×10^{-3} mol $L^{-1} \times 10$ μL × NA/16 mm^2, which corresponds to 3.76×10^{14} molecules per mm^2. The diameter of the laser spot is approximately 1 μm, and its surface area is about 7.9×10^{-7} mm^2. Therefore, the N_{surf} value is 2.97×10^3, and the N_{vol} value is 2.97×10^8, respectively. In accordance with Equation (S1), the EF value is 3.95×10^5, and detailed data are provided in **Table S3.**

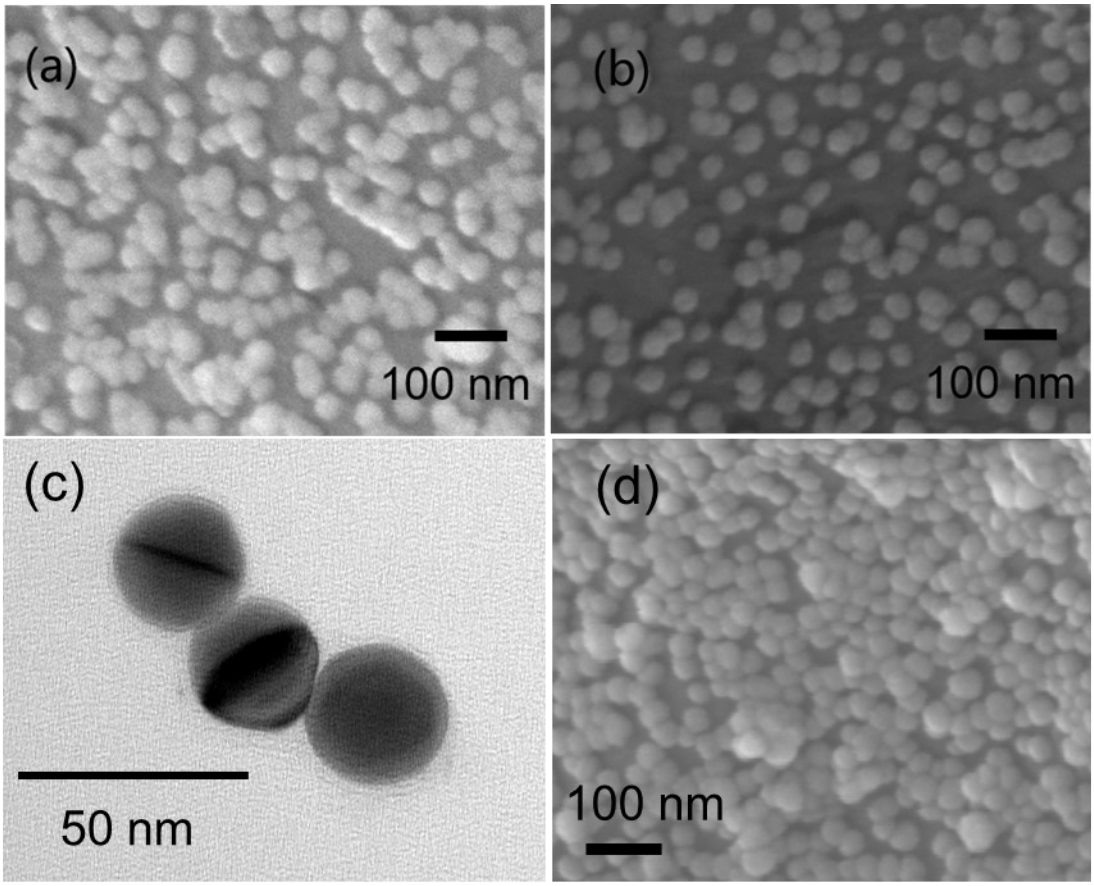

Fig. S1. SEM images of (a) polyamide-Ag, (b) polyamide-Ag-APBA thin film substrates, (c) TEM image of 30 nm Ag NPs and (d) SEM image of {polyamide-Ag-APBA}-glucose/PVP-[Ag-APBA/AEPO sandwich structure.

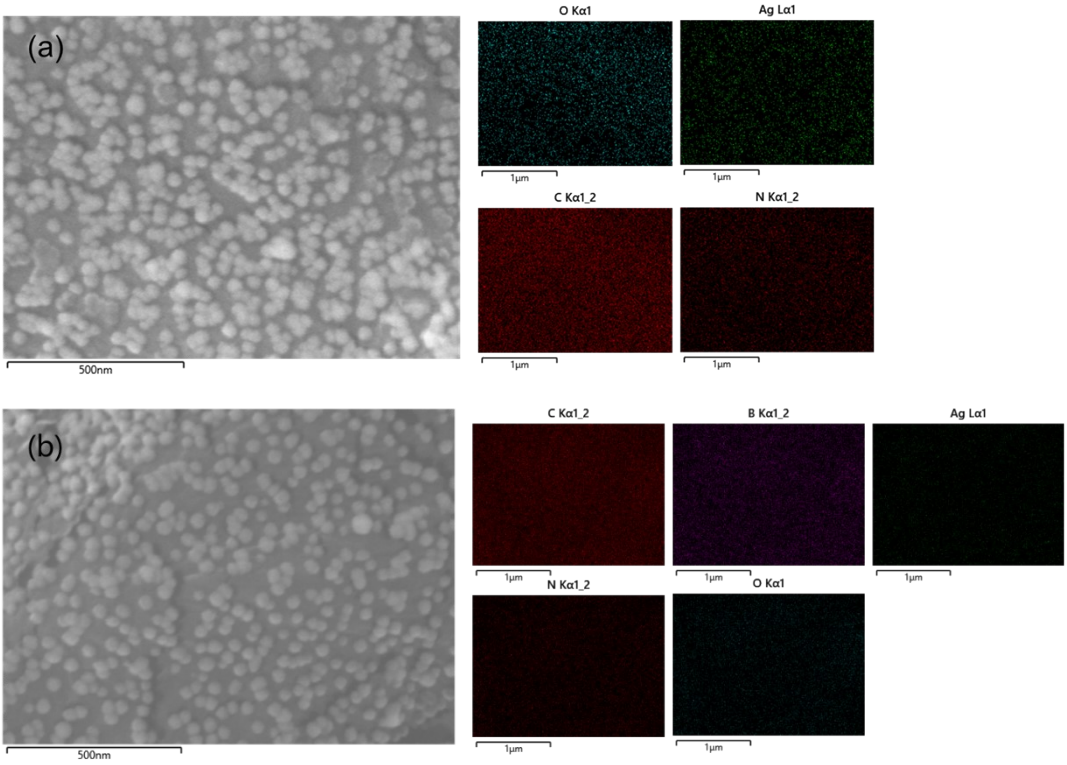

Fig. S2. Element surface distribution of (a) polyamide-Ag and (b) polyamide-Ag-APBA energy spectrum analysis.

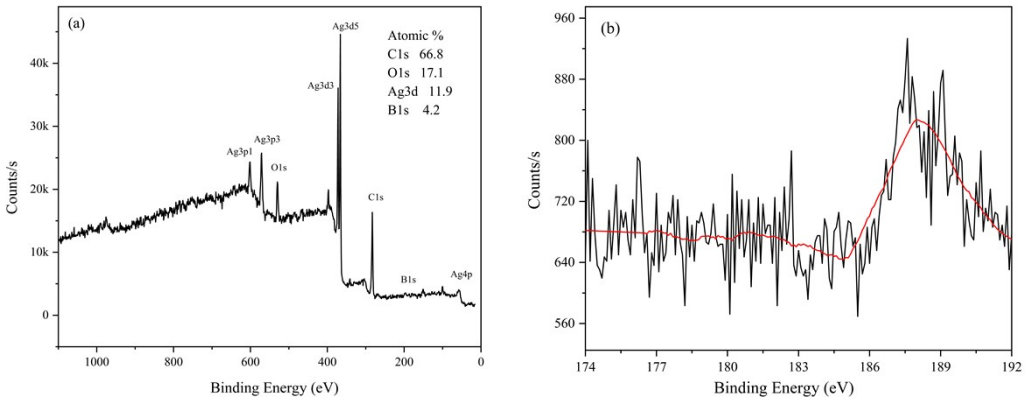

Fig. S3. XPS spectra of (a) polyamide-Ag-APBA and (b) boron element.

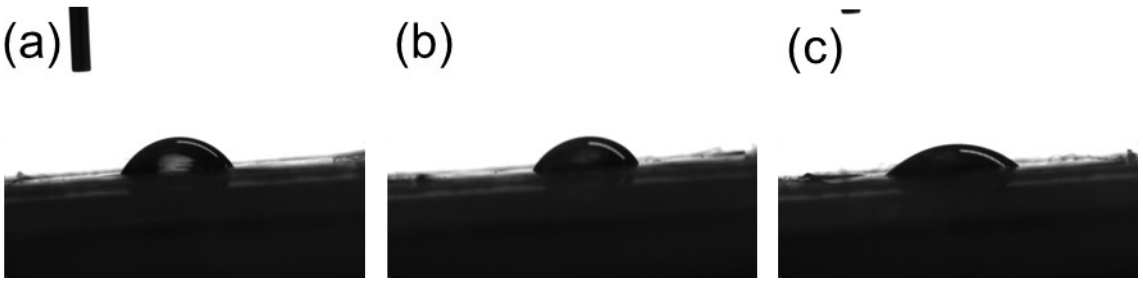

Fig. S4. The contact angle test of (a) polyamide-Ag, (b) polyamide-Ag-APBA and (c) polyamide-Ag-APBA-PVP.

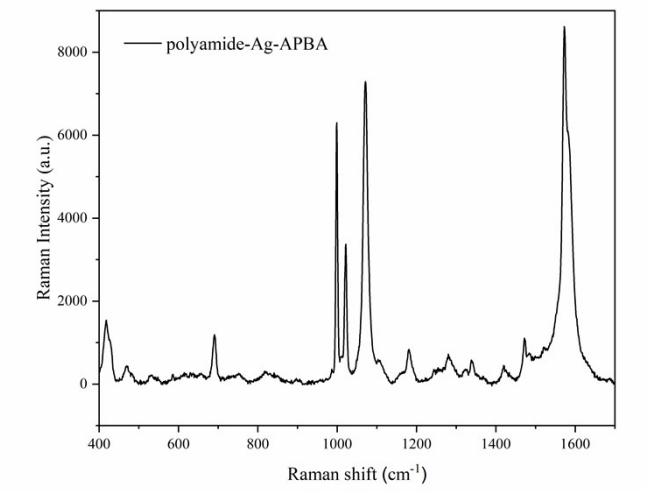

Fig. S5. SERS spectra of polyamide-Ag modified with APBA.

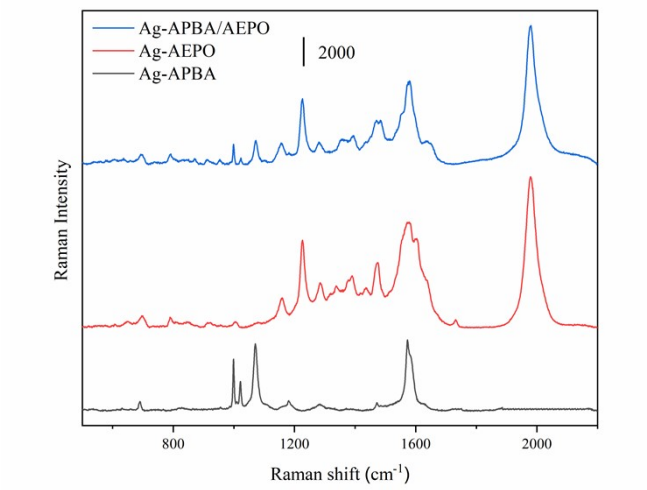

Fig. S6. SERS spectra of Ag nanoparticles modified with APBA, AEPO, and their mixtures with the ratio of 1:1.

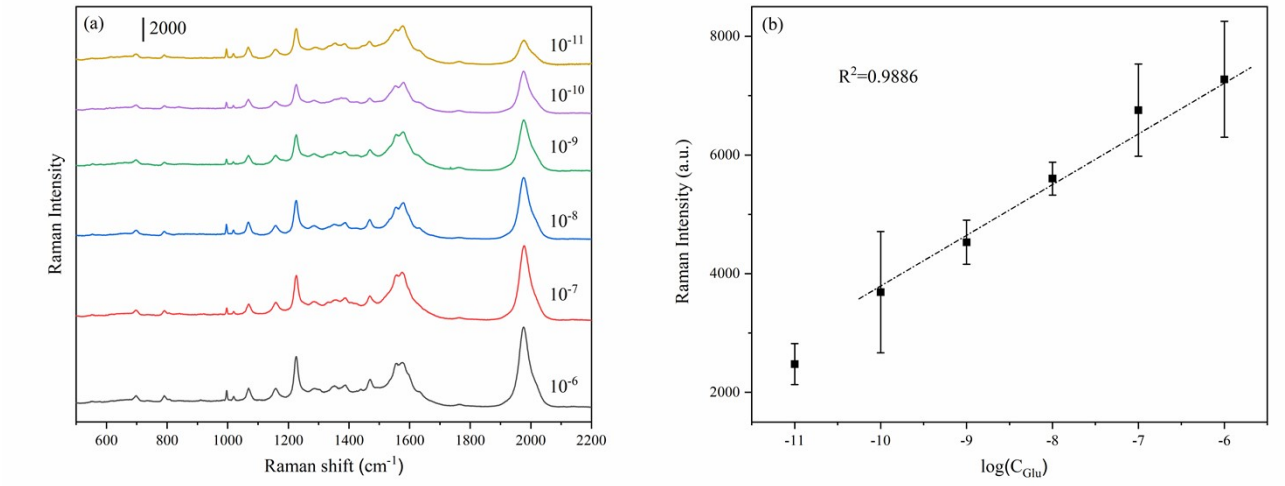

Fig. S7. (a) Evolution of Raman spectra with the increase of glucose concentrations from 10^{-11} M to 10^{-6} M by a factor of 10 and (b) the calibration curve of the Raman signal at 1977 cm^{-1} as a function of Glu concentration in aqueous solution.

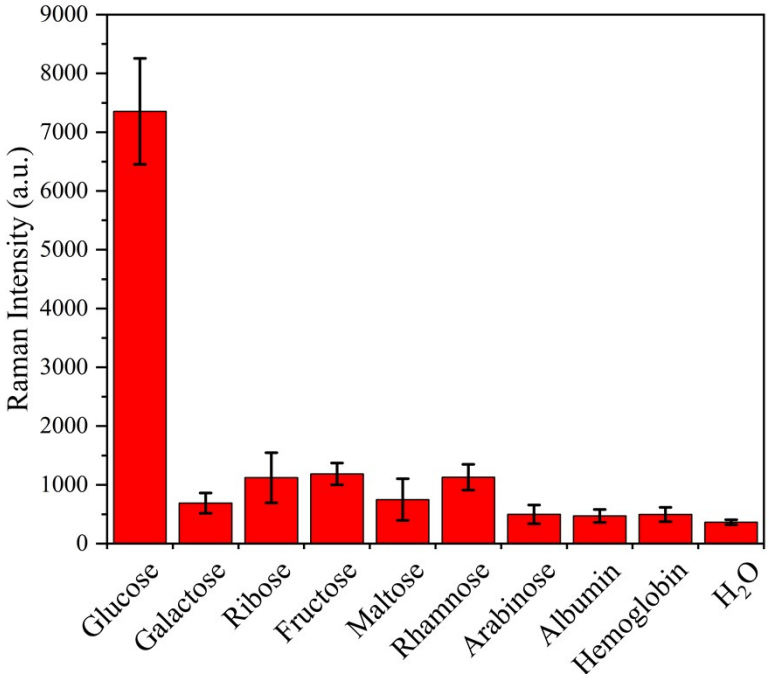

Fig. S8. Peak intensities at 1977 cm^{-1} of glucose, other saccharides, albumin and hemoglobin (10^{-6} M) in aqueous solution. Error bars represent RSD from three replicate samples, each of which was measured at six different spots.

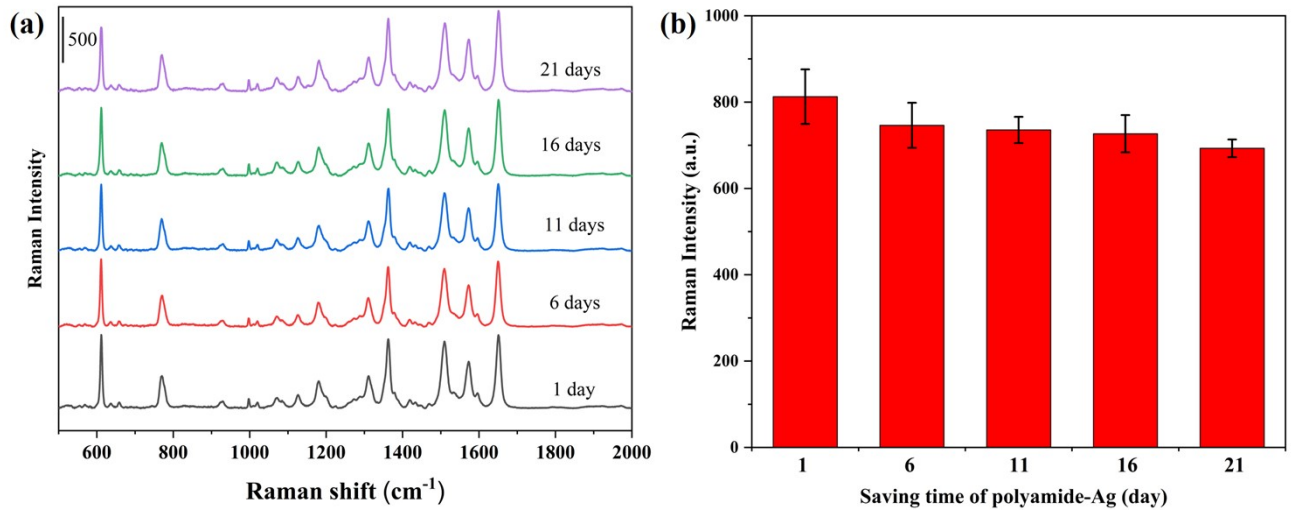

Fig. S9. (a) SERS spectra and (b) Peak intensities at 609 cm^{-1} of 10^{-9} M R6G collected from a freshly prepared polyamide-Ag substrate and the same substrate after different days of storage.

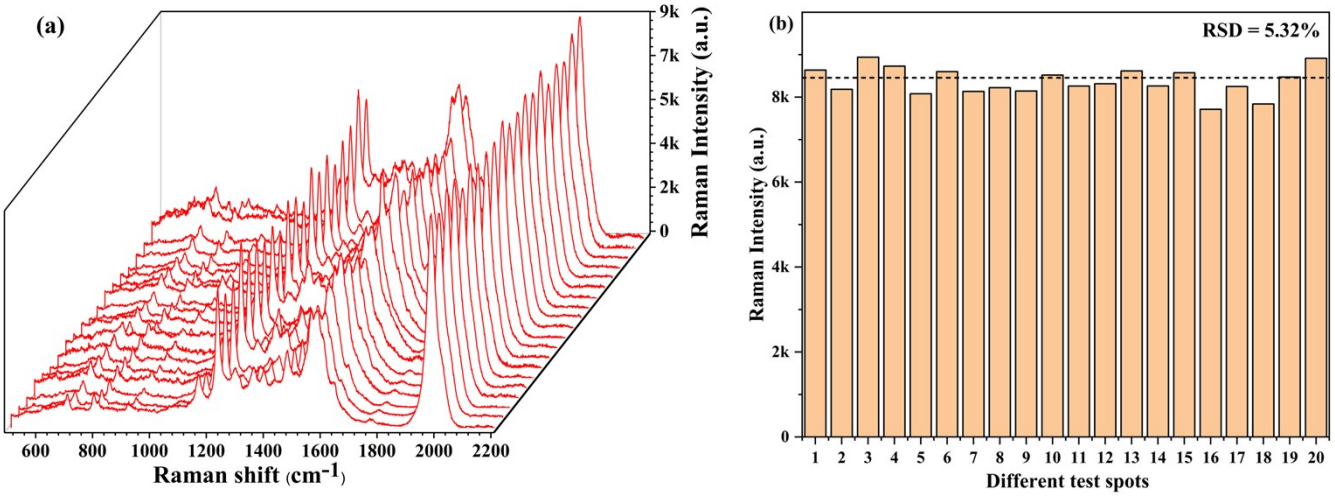

Fig. S10. (a) SERS spectra of and (b) peak intensities at 1977 cm^{-1} of 10^{-6} M glucose obtained from 20 random points on the same substrate.

Table S1. Composition of artificial urine medium.

component	Concentration (g/L)
Urea	25.00
KCl	1.60
NaCl	2.93
Na_2SO_4	2.25
NH_4Cl	1.00
KH_2PO_4	1.40
$CaCl_2 \cdot 2H_2O$	1.10
creatinine	1.10
Ovalbumin	0.05

Table S2. The Peak intensities at 1977 cm^{-1} of various glucose concentrations in aqueous solution and artificial urine.

Concentration	Glucose in aqueous solution						Glucose in artificial urine					
10^{-6} M	8834	8796	8165	7931	8024	7852	8712	8320	8130	8332	7785	7592
10^{-7} M	6626	6158	6895	6253	6985	6852	5608	6546	6822	7068	6835	6381
10^{-8} M	4670	4590	4835	4597	4362	4975	4682	4174	4518	4787	4407	4980
10^{-9} M	3419	3260	3550	3574	3230	3492	3207	3368	3498	3357	3526	3481
10^{-10} M	2063	2340	1860	1960	2040	2109	2763	1926	2231	2024	2306	2053
10^{-11} M	1606	1408	1760	1490	1730	1680	1921	1798	1596	1638	1723	1692

Table S3. The calculation of SERS EF for the R6G adsorbed on polyamide-Ag.

Parameter	Date
I_{RS}	1640
I_{SERS}	648
N_{vol}	2.97×10^8
N_{surf}	2.97×10^3
EF	3.95×10^5

Table S4. Comparison of developed methods and previously reported SERS methods for glucose detection.

Reporter molecule	Chemical bond	Raman peak (cm^{-1})	Limit of detection (M)	Real samples	References
Mercapto phenylboronic acid	B-OH stretching	1071	Physiologically relevant range	Vivo rabbit eyes	2
Triosmium Carbonyl Cluster−Boronic Acid Conjugates	C=O	2111	Physiologically relevant range	Human saliva	3
4,4′-dimercaptoazobenzene	N=N stretching vibrations	1436, 1140	10^{-5}	Urine	4
Malachite green	ring C-C stretching	1613	3.9×10^{-7}	Tears	5
4-Mercapto phenylboronic acid	B-OH stretching	1068	2.11×10^{-7}	Tears	6
4-Mercapto phenylboronic acid	B-OH stretching	1069	1.2×10^{-7}	Serum	7
3-Mercapto phenylboronic acid	B-OH stretching	1069	7×10^{-8}	Artificial urine and normal human serum	8
4-Mercaptophenylboronic acid-triosmium carbonyl cluster conjugate	C≡N	2111	10^{-10}	Human urine	9
3-amino-6-ethynylpicolinonitrile	C≡C	1977	10^{-11}	Artificial urine	This work

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