Electronic Supplementary Material (ESI) for Nanoscale.

Supplemental Information

for

Reliable and Quantitative SERS Detection of Dopamine Levels in Human Blood Plasma by Plasmonic Au/Ag Nanocluster Substrate

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The criteria for patient recruitment were as follows: (i) diagnosis of schizophrenia by the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV); (ii) age between 20 and 60 years; (iii) outpatients receiving maintenance therapy of antipsychotics; and (iv) diagnosis of antipsychotic-induced parkinsonism by DSM-IV.[1] Antipsychotic-induced parkinsonism was also assessed using the Drug-Induced Extrapyramidal Symptoms Scale (DIEPSS).[2, 3] Patients were excluded if they had medical or neurological conditions. Fifteen (6 men, 9 women; mean age: 37.9 ± 13.1 years; median age: 34.0 years) patients with antipsychotic-induced parkinsonism were enrolled in this study. The antipsychotics that patients were taking at the time of enrollment were paliperidone (n = 7, mean dose 8.6 ± 2.7 mg/day), olanzapine (n = 2, mean dose 17.5 ± 3.5 mg/day), a combination therapy of paliperidone and olanzapine (n = 2, mean dose: paliperidone 4.5 ± 2.1 mg/ day, olanzapine 7.5 ± 3.5 mg/day), paliperidone palmitate (n = 1, dose: 78 mg/month), aripiprazole (n = 1, dose: 10 mg/day), risperidone (n = 1, dose: 4 mg/day), and sulpiride (n = 1, dose: 400 mg/day). The mean DIEPSS score was 2.5 ± 0.5, indicating a relatively mild severity of antipsychotic-induced parkinsonism. To compare the plasma DA levels between the groups, 15 (6 men, 9 women; mean age: 34.3 ± 6.7 years; median age: 33.0 years) healthy control subjects who met the criteria of an absence of current or past psychiatric, neurological, or medical illness and an absence of current use of medications were also recruited and examined. The age ($t = 0.95$, $p = 0.35$) and sex distribution ($\chi^2 = 0$, $p = 1.00$) were not different between the two groups.

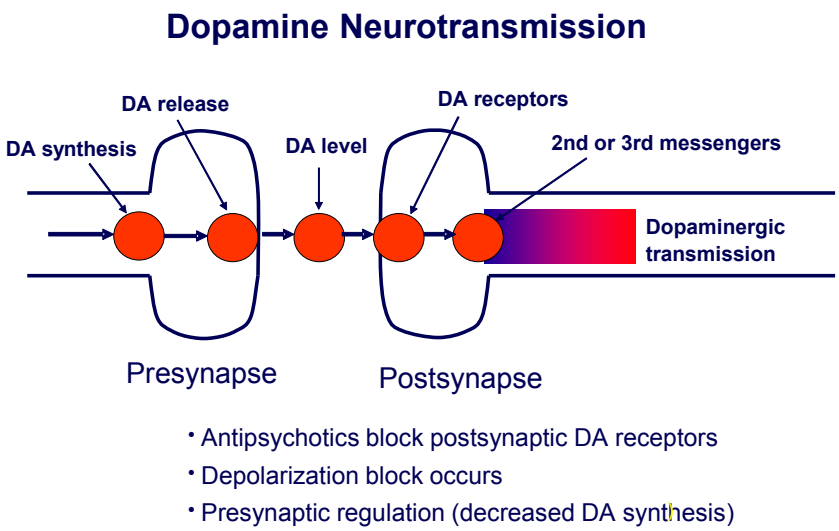


Figure S1. Schematic diagram of dopaminergic neurotransmission.

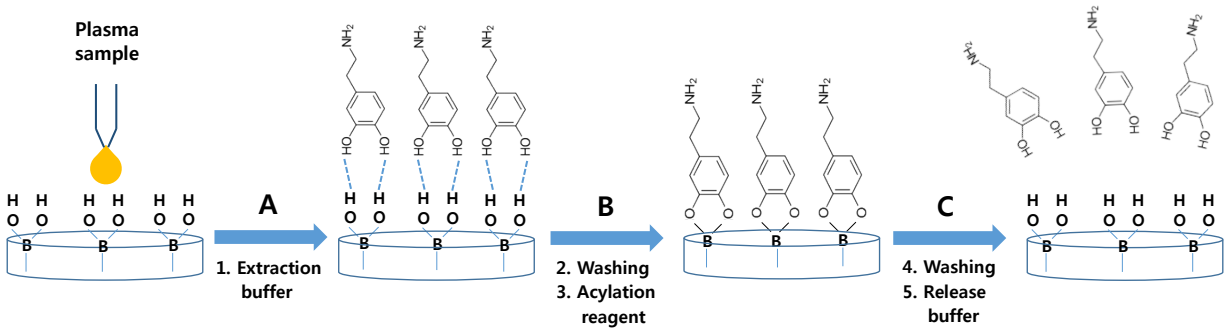


Figure S2. The schemes of dopamine extraction from blood plasma samples consisting of extraction, acylation, and release process. In Step A, 500 µL of the plasma sample was transferred to the respective wells of the extraction plate (RE59161, IBL international). 1 mL of extraction buffer was added to each well. The plate was covered by adhesive foil and shaken at room temperature for 30 min to anchor the DA onto the extraction plate. In Step B, the solution in each well was emptied and filled immediately with 2 mL of DI water. The well was washed by shaking at room temperature for 5 min. Subsequently, the water was emptied and filled immediately with 150 µL of extraction buffer and 50 µl of acylation reagent. The plate was gently shaken for 20 min. In Step C, the solution in each cell was emptied out and 2 mL of DI water was added to each well. The well was then emptied by removing the water, and 300 µL of release buffer was added to each well and the plate was shaken for another 30 min. The final product was collected and stored in a deep freeze refrigerator (– 70 ^{o}C) prior to a further characterization.

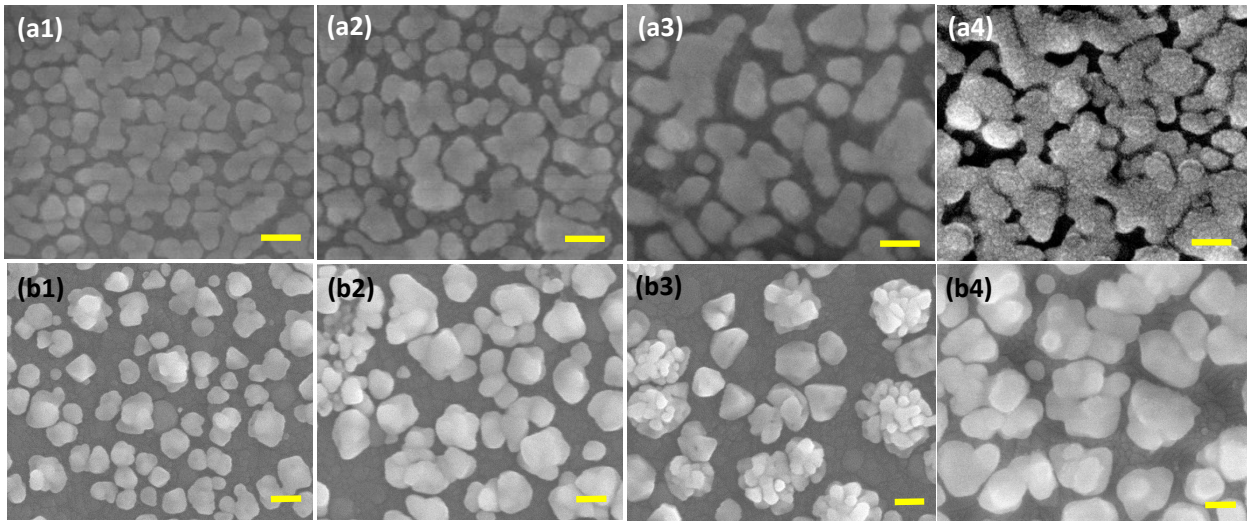
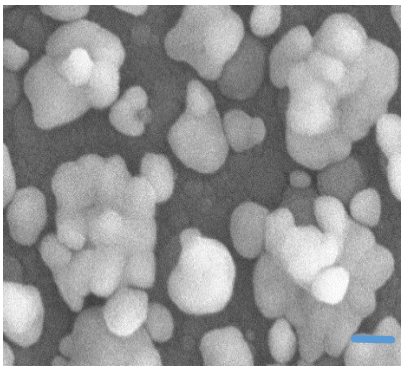
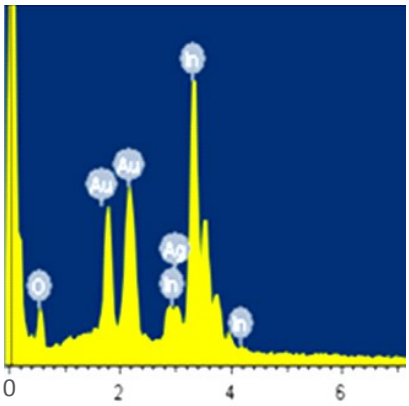


Figure S3. (a) SEM images of Au NPs deposited on the ITO glass (so-called Au_ITO) at different growth times via. one-cycle electrodeposition: a1) 500 s, a2) 1000 s, a3) 2000 s, a4) 3000 s. (b) SEM images of Au NPs deposited on the ITO glass (so-called Au_ITO) at different growth times via. two-cycle electrodeposition: b1) 1000 s, b2) 1500 s, b3) 2000 s, b4) 3000 s. The nucleation potential is E_1 = 0.7 V for 2 s and the growth potential is E_2 = -0.2 V. The scale bar is 100 nm.





Element	Weight%	Atomic%
O K	16.73	62.15
Ag L	1.74	0.96
In L	57.01	29.50
Au M	24.52	7.40
Totals	100.00	

Figure S4. SEM image and Energy-dispersive X-ray spectroscopy (EDX) analysis of the Au@Ag_ITO substrate. The EDX analysis indicates the presence of Au and Ag elements at different amount ratio. In the preparation of Ag@Au_ITO, Au NPs were first electrodeposited on the ITO glass with two cycles of elctrodeposition (nucleation potential of E_1 = 0.7 V for 2 s and growth potential of E_2 = -0.2 V for 1000 s). After then, the Au-deposited ITO (Au_ITO) was immersed into a solution which contained 40 µL of $AgNO_3$ (10 mM), 25 µL of ascorbic acid (0.1 M), and 0.25 µL of PVP (1.0 wt/wt %). The Au@Ag_ITO was finally formed by plating silver layer over the Au_ITO under gentle shaking for 3 h.

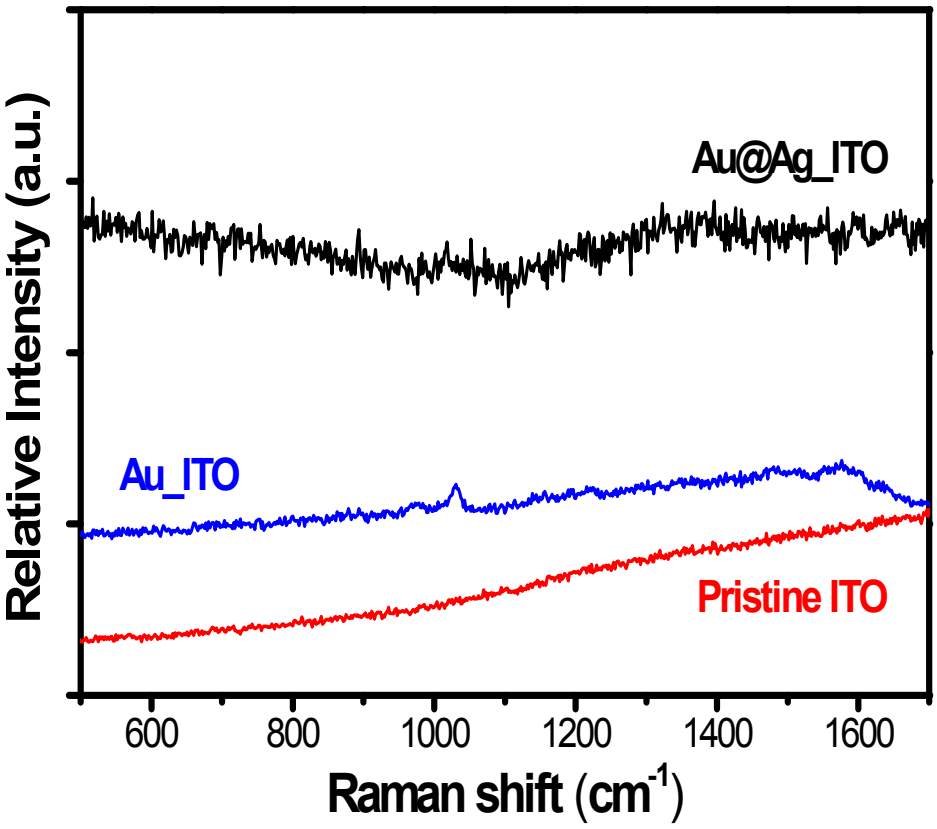


Figure S5. Raman spectra of as-prepared substrates (pristine ITO, Au_ITO, Ag@Au_ITO). The analyte-free substrates were analyzed by micro-Raman spectroscopy (ANDOR Monora500i, 633 nm) with an accumulation time of 5 s.

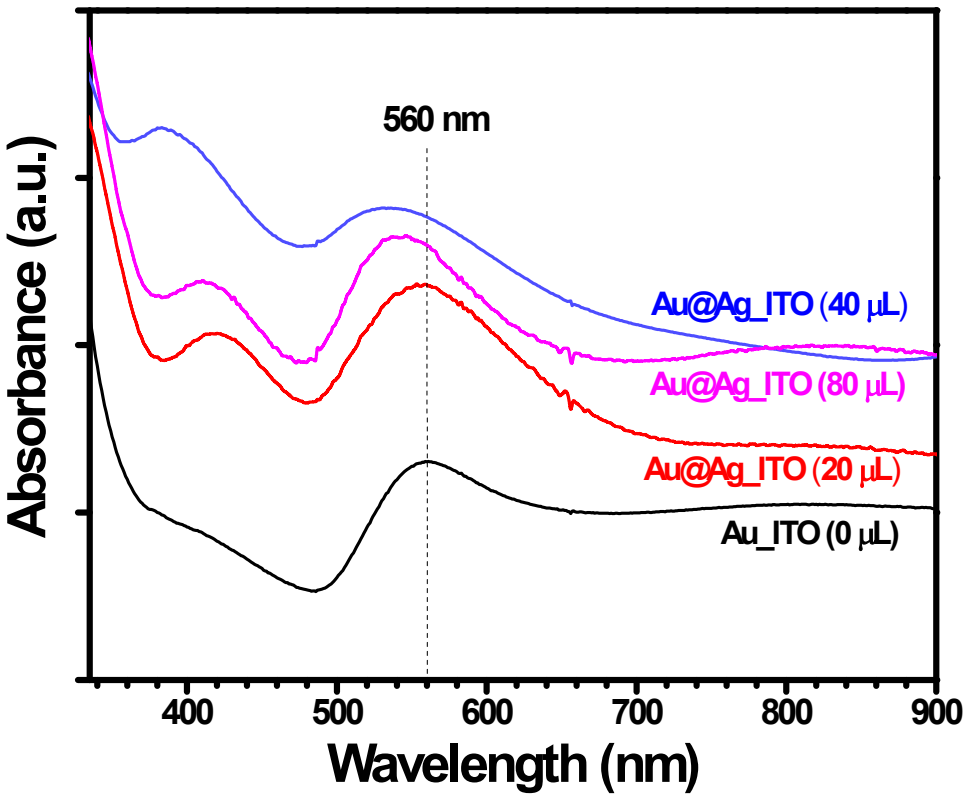


Figure S6. UV-vis spectra of Au_ITO and Au@Ag_ITO prepared with different amounts of $AgNO_3$ (20, 40, 80 μl).

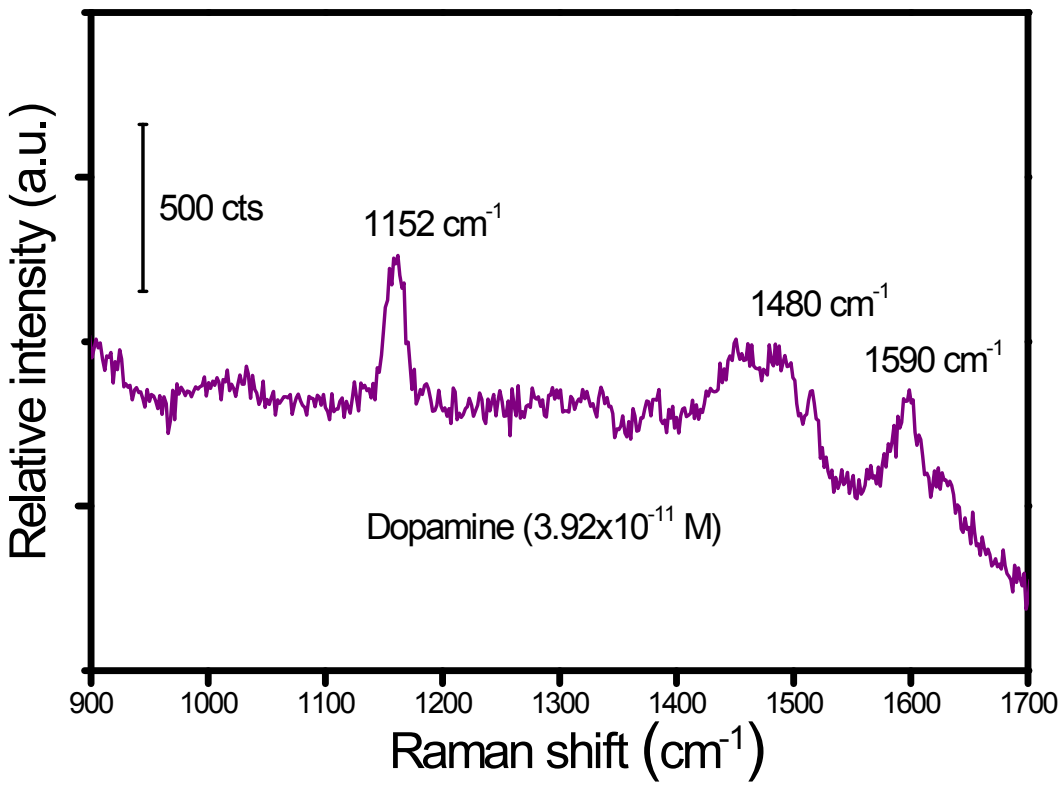


Figure S7. Raman spectra of DA adsorbed on the Au@Ag_ITO at the very low concentration of 3.92×10^{-11} M. The Raman spectra clearly displayed peaks at 1152, 1480, and 1590 cm^{-1} all of which are related to the presence of adsorbed DA molecules.

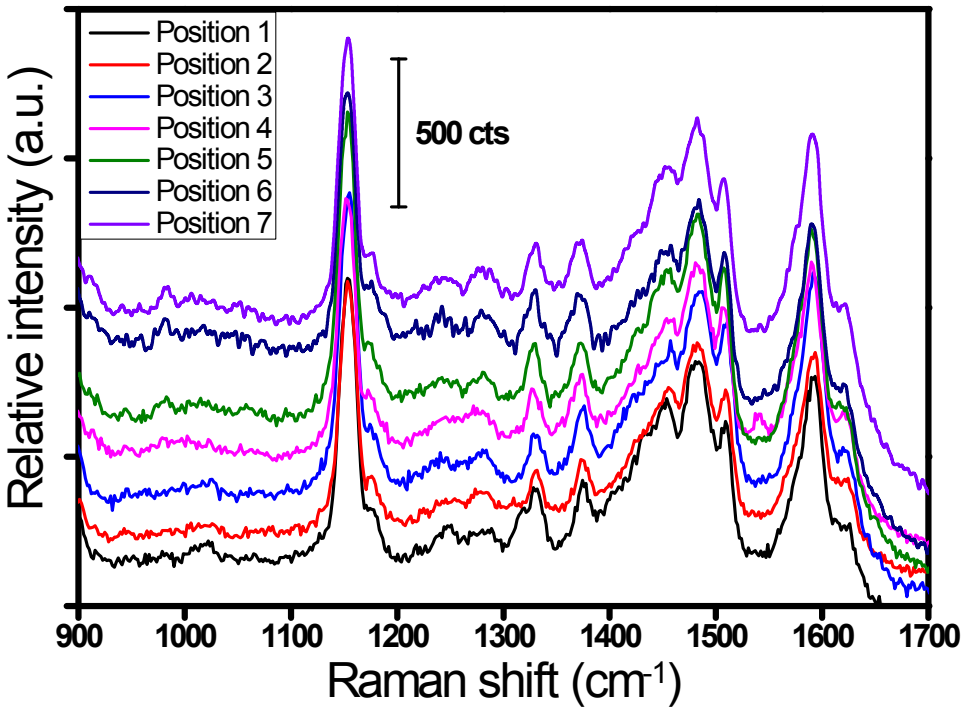


Figure S8. Raman spectra of standard DA (3.92×10^{-8} M) adsorbed on the Au@Ag_ITO (40 µl of $AgNO_3$) collected at different locations on the substrate. The Raman peaks show the similar intensity irrespective of the location on the substrate. These spectra were recorded by micro-Raman (ANDOR Monora500i) equipped with 633 nm He−Ne laser (12 mW) for 0.5 s of integration time and ten cycles of accumulation. The diameter of focused spot (50× objective) is approximately 1.0 µm of which area is enough to represent the average Raman intensity of the substrate.

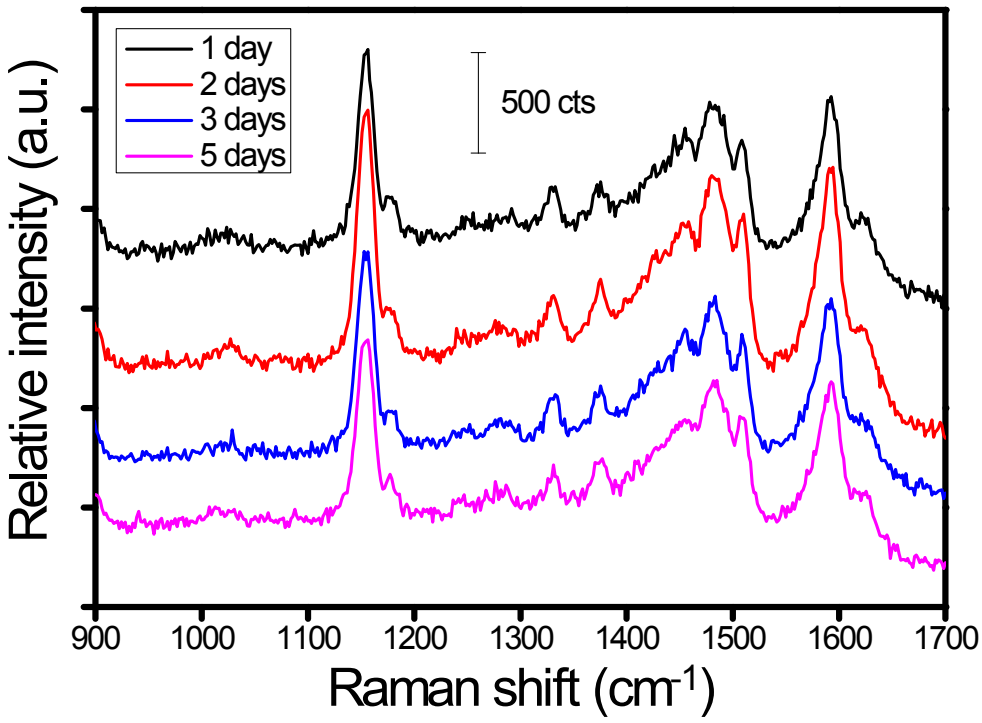


Figure S9. Raman spectra of standard DA molecules adsorbed on the substrates for different storage times. There were no significant changes of Raman intensities irrespective of storage times. The Au@Ag_ITO was stored in the desiccator of 310 mmHg prior to Raman analysis, which prevented the direct contact with an ambient atmosphere.

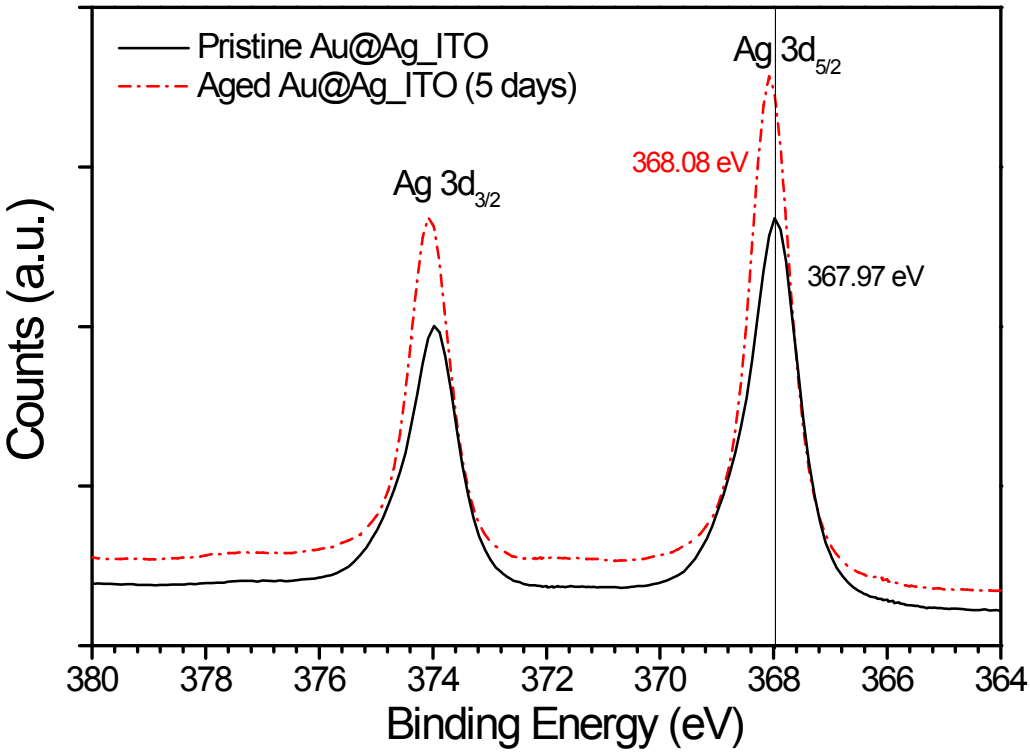


Figure S10. XPS analysis of core-level spectra of Ag 3d in Au@Ag_ITO sample before and after 5-day storage.

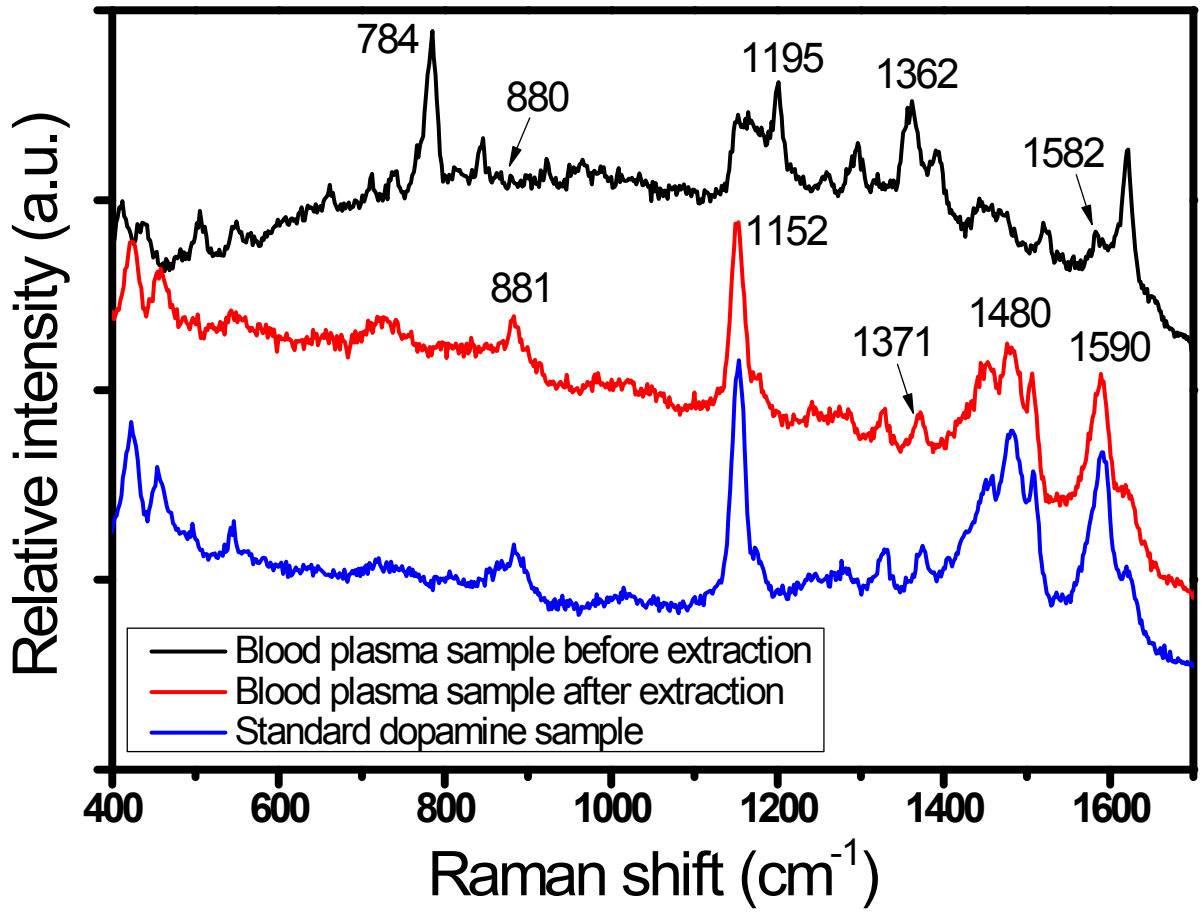


Figure S11. Comparative Raman spectra of blood plasma sample before and after the extraction process, including Raman spectra of standard dopamine sample.

Table S1. The summary of plasma DA levels in healthy subjects and patients with parkinsonism which were estimated by SERS technique using the optimized Au@Ag_ITO substrate

No.	Peak height at 1152 cm^{-1}						Average height	Concentration (M)	STDEV
N#1	1227	1030	988	969			1053.5	3.4424E-08	3.87E-09
N#2	1026	947	1159	1171	1030		1066.6	3.7993E-08	3.41E-09
N#3	993	1060	963	1019	949		996.8	2.2461E-08	1.00E-09
N#4	1108	952	976	940	920	1176	1012	2.5185E-08	2.60E-09
N#5	994	945	960	940	927	961	954.5	1.6334E-08	3.97E-10
N#6	611	600	604	608	628		610.2	1.2221E-09	2.16E-11
N#7	905	924	954	958	952	938	938.5	1.4480E-08	3.19E-10
N#8	1026	1039	1038	1135			1059.5	3.6015E-08	1.72E-09
N#9	793	792	816	749	851	867	811.3	5.5577E-09	2.95E-10
N#10	1085	1103	1093	1078			1089.8	4.5228E-08	4.46E-10
N#11	955	998	899	1015	943	1010	970	1.7572E-08	7.39E-10
N#12	834	792	848	866	814		830.8	6.4350E-09	2.24E-10
N#13	1037	1024	1057	1028	947		1018.6	2.6471E-08	1.09E-09
N#14	1061	1080	1050	986			1044.3	3.2108E-08	1.25E-09
N#15	974	941	946	1042	1038	1124	1010.8	2.4965E-08	1.74E-09
D#1	910	870	880	867	926		890.6	1.0095E-08	2.96E-10
D#2	708	791	753	742	761	741	749.3	3.4844E-09	1.27E-10
D#3	670	620	654	603	704		650.2	1.6517E-09	1.02E-10
D#4	656	686	698	684	695		683.8	2.1272E-09	5.17E-11
D#5	636	604	618	643	665		633.2	1.4533E-09	5.38E-11
D#6	687	600	621	626	681		643	1.5646E-09	9.43E-11
D#7	683	600	712	587	640		644.4	1.5811E-09	1.31E-10
D#8	789	721	756	776	814	824	780	4.3896E-09	2.14E-10
D#9	735	566	686	738			681.3	2.0868E-09	2.46E-10
D#10	696	763	851	701			752.8	3.5753E-09	3.43E-10
D#11	766	576	715	754			702.8	2.4535E-09	3.05E-10
D#12	576	611	847	618			663	1.8188E-09	3.40E-10
D#13	725	751	639	789			726	2.9230E-09	2.56E-10
D#14	645	614	667	699	989		722.8	2.8534E-09	6.00E-10
D#15	912	783	867	883	720		833	6.5426E-09	6.23E-10

* STDEV = Standard deviation; Avg. = Average; Conc. = Concentration

** N = Normal blood samples of healthy subjects; D = Disease blood samples of patients with drug-induced Parkinsonism.

*** The plasma DA levels were calculated by the following procedures. From the Raman spectra of standard DA molecules, the peak heights at 1152 cm^{-1} was recorded at different concentrations of DA. Based on linear relationship between logarithm of DA concentration (log C) and the peak height at 1152 cm^{-1}, the standard curve was calibrated by the linear equation with a reliability of $R^2 = 0.98$. The linearly regressed equation was used to estimate the plasma DA levels in human blood samples: $y = -305.8\ x + 3335.6$ (y = peak height at 1152 cm^{-1}, x = logarithm of dopamine concentration). The Raman spectra of plasma samples were measured more than three times to get an average peak height at 1152 cm^{-1}. Logarithm of DA concentration, x, was calculated by the following equation: $x = \frac{3335.6 - y}{305.8}$.

**** The STDEV was estimated based on the average values of peak heights at 1152 cm^{-1} with multiple measurements of the blood plasma samples.

***** Statistical analysis for DA levels in eight blood plasma samples tested more than three times: N#1: 3.88E-08 ± 1.75E-08 (44.97%), N#2: 2.53E-08 ± 1.16E-08 (45.98%), N#3: 2.50E-09 ± 7.89E-09 (31.55%), N#4: 3.53E-08 ± 1.09E-08 (30.83%), D#1: 8.90E-09 ± 1.41E-09 (15.81%), D#3: 2.74E-09 ± 0.94E-09 (34.47%), D#8: 3.66E-09 ± 0.64E-09 (17.37%), D#15: 5.69E-09 ±0.74E-09 (13.03%)

References

1. A. American Psychiatric and D.-I. American Psychiatric Association. Task Force on, *Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revision: DSM-IV*, American Psychiatric Association, Washington, DC, 2000.
2. T. Inada, *Nihon shinkei seishin yakurigaku zasshi = Japanese journal of psychopharmacology*, 1996, **16**, 181-185.
3. J. H. Kim, H. Y. Jung, U. G. Kang, S. H. Jeong, Y. M. Ahn, H. J. Byun, K. S. Ha and Y. S. Kim, *Movement disorders : official journal of the Movement Disorder Society*, 2002, **17**, 1354-1359.