Supplementary Information

Experiment

1. Materials and Instruments

1.1. Materials

$HAuCl_4 \cdot 4H_2O$, $AgNO_3$, ascorbic acid (AA), sodium citrate, hydroxylamine hydrochloride (NH2OH·HCl), ammonia (25%-28%), NaOH, HCl, HNO_3, 4-mercaptobenzoic acid (4MBA), cysteamine hydrochloride (CA, 98%), dodecanethiol (DDT) and methyl parathion were purchased from Sinopharm Chemical Reagent Co.,Ltd. Polyvinylpyrrolidone (PVP) were purchased from Aldrich. Ethanol was purchased from Shanghai Titan Scientific Co.,Ltd. 4-mercaptopyridine and methylene blue were purchased from Shanghai Macklin Biochemical Co.,Ltd. Rhodamine 6G (R6G) and crystal Violet (CV) were purchased from Shanghai Yuanye Ciological Co.,Ltd. Thiabendazole (TBZ) was purchased from Adamas Reagent Co.,Ltd. All chemicals were analytical reagent and used without further purification. Ultrapure water (18.2 MΩ·cm) was produced using Millipore water purification system.

1.2. Apparatus

The morphology, structure and properties of as prepared AuNP and Au@Ag sol were characterized by Shimadzu UV-2600 spectrometer, JEOL 2010 high-resolution TEM. Raman spectra were performed on B&W Tek portable Raman spectrometer at 785 nm laser excitation. The laser focal spot on the metal surface was about 100 μm in diameter with a measured power of 30 mW.

2. Synthesis of nanoparticles

2.1. Synthesis of AuNPs

AuNPs were synthesized according to the Frens' method[1]. Briefly, 15 nm Au seeds were first synthesized, 98.9 mL of water and 1 mL of trisodium citrate (30 mg/mL) were heated to boil with gentle stirring (200 rpm) for 7 minutes. Then 0.1 ml of 98.5 mg/mL $HAuCl_4 \cdot 4H_2O$ was quickly added and keep boiling under vigorous stirring for seven minutes. The solution changes from purple to red, indicating the formation of 15 nm AuNPs, then cooling on ice for subsequent use.

The bigger diameter AuNPs were synthesized via hydroxylamine hydrochloride reduction method[2]. 10 mL of above AuNP sols, 9.8 mL of water, 0.2 mL of 100 mM $NH_2OH \cdot HCl$ and 0.2 mL of 10 mg/mL trisodium citrate were mixed in a flask under magnetic stirring (650 rpm) during 5 min, then the 0.4 ml of 1% (w/v) $HAuCl_4 \cdot 4H_2O$ was added and keep stirring for 1 h. AuNPs with a particle size of about 25 nm were obtained.

In order to get AuNPs with the size of about 60 nm. 1 mL of above AuNP sols, 37.4 mL of water, 0.4 mL of 100 mM $NH_2OH \cdot HCl$ and 0.4 mL of 10 mg/mL trisodium citrate were mixed in a flask under magnetic stirring (650 rpm) during 5 min, then the 0.6 ml of 1% (w/v) $HAuCl_4 \cdot 4H_2O$ was injected and keep stirring for 1 h.

2.2. Synthesis of PVP-modified Au@Ag

Synthesis of Au@CA@Ag

50 nm AuNPs were synthesized according to previous method by changing the amount of $HAuCl_4 \cdot 4H_2O$. The synthesized AuNPs were diluted to 57.3 pM with 1% PVP solution. Then adjust the pH of the solution to about 9. CA (with the final concentration of 1.16 μM) was added to the solution before the silver ammonia solution (with the final concentration of 0.131 mM). Finally, 0.1 M ascorbic acid solution was added dropwise in 15 minutes to bring the final concentration to 0.185 mM. The color of Au@CA@Ag colloid is orange-yellow.

Synthesis of Au@CA+4MPY@Ag

The method is similar to the method of Au@CA@Ag. Changing the step of adding CA to adding the mixed solution of CA and 4MPY. (with the final concentration of 1.16 and 0.17 μM, respectively.)

Synthesis of Au@CA+4MPY@Ag-4MBA

the 4MBA solution (dissolved in ethanol) was injected into the prepared Au@CA+4MPY@Ag colloid with the modification density of 0.5 4MBA molecule per square nanometer.

All synthetic PVP-modified Au@Ag colloid requires further washing for subsequent use. The excess PVP was removed by centrifuge (5500 rpm/15 min), and then the nanoparticles were collected and dispersed into ultra-pure water, and repeat this procedure 2-3 times.

2.3. Synthesis of citrate modified Au@Ag

The synthesis method is basically the same as that of PVP-modified Au@Ag. The synthesized AuNPs were diluted to 57.3 pM with ultra-pure water, instead of PVP.

3. Characterization and self-assembly of core-shell nanostructures

The newly synthesized CMS colloid were washed twice by centrifugation (5500 rpm/15 min) and resuspended with water to remove excess PVP. First, the appropriate amount of PEG-SH was added to the CMS solution to stabilize the nanoparticles. The surface of the nanoparticles was modified by about 1.5 PEG-SH per nm^2. Subsequently, a chloroform solution containing dodecanethiol (DDT) was added to the aqueous CMS solution. The modification density is about 300 DDT per nm^2. Excessive DDT to ensure that 4MPY adsorbed on the surface of nanoparticles were replaced. After one hour of vigorous stirring, the CMS nanoparticles self-assembled to the chloroform-water interface. Remove the water phase part, and then add ultrapure water. Repeat several times to remove free molecules in the water phase.1 ml of liquid plasma array was transferred to cuvette for SERS detection.

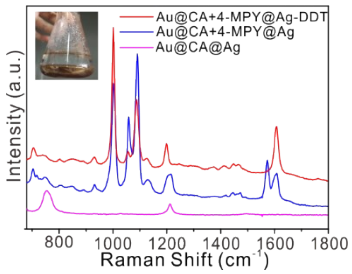

Figure S1. The SERS spectra of Au@CA@Ag (black line), Au@CA+4MPY@Ag (blue line), and Au@CA+4MPY@Ag replaced by DDT (red line). The illustration is the physical picture of the plasma nanoarray formed by self-assembly at the two-liquid interface during the DDT replacement process.

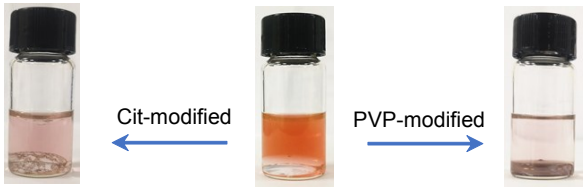

Figure S2. Self-assembly of citrate (left) and PVP (right) modified Au@CA@Ag at two-liquid interface.

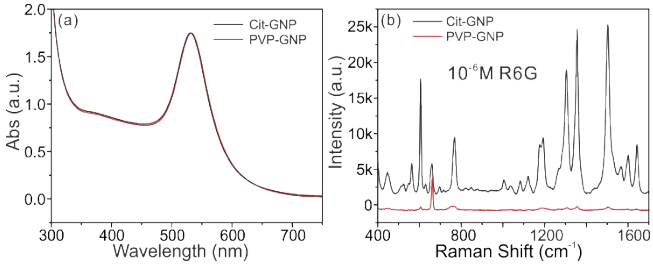

Figure S3. (a) UV/Vis spectra of the cit-GNPs (black line) and PVP-GNPs (red line). (b) The SERS spectra of 1 μM of R6G detected on two-liquid plasma arrays assembled with cit-GNPs (black line) and PVP-GNPs (red line), respectively. The PVP-GNPs were obtained by cit-modified GNPs added with excess PVP and incubated for 2 h.

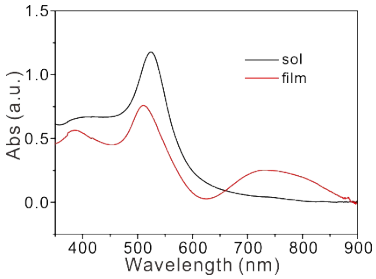

Figure S4. UV/Vis spectra of the CMS sol (black line) and CMS plasma array (red line).

4. Quantitative SERS analysis

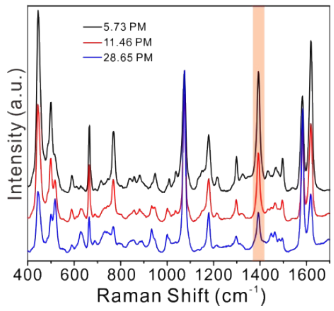

Figure S5. The SERS spectra of 1 μM of MB detected on a plasma array assembled of same concentration GNPs and Au@CA@Ag of different concentrations.

4.1. Spectral normalization of 5 μM R6G with different ISs and statistical analysis

Table S1. A table for RSDs of 10 replicates, 40 SERS spectra of 5 μM R6G collected at random sites on array were calculated by the use of 1359 cm^{-1}, $I_{1359/517}$, $I_{1359/999}$, $I_{1359/665}$ normalization.

IS	Group									
	1	2	3	4	5	6	7	8	9	10
Uncorrected	13.12	7.29	16.37	11.06	11.65	8.71	9.67	10.01	12.90	12.34
4-MBA	8.04	5.51	3.79	7.53	9.05	8.37	13.73	12.00	10.51	4.93
4-MPY	8.76	8.06	11.35	9.43	11.35	11.48	7.35	11.67	11.97	6.51
$CHCl_3$	26.43	11.86	16.46	14.01	17.45	12.12	13.72	6.49	13.78	16.88

4.2. Spectral normalization of 1 μM MB with different ISs and statistical analysis

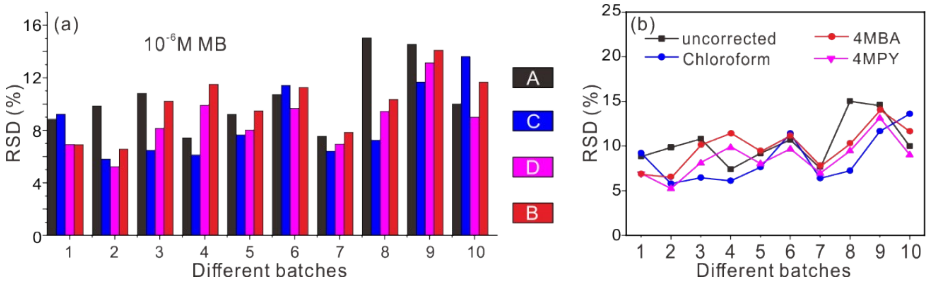

Figure S6. (a) Histograms of RSDs of 40 peaks at 1390 cm^{-1} of MB with the concentration of 1 μM calibrated with three different ISs. (A) (B) (C) (D) represents uncalibrated, calibrated by 4MBA modified on the surface of core-shell NPs, calibrated by 4MPY molecules embedded in the core-shell NPs and organic solvent chloroform, respectively. (b) Line graph of RSDs calculated by uncorrected and corrected by different ISs.

Table S2 A table for RSDs of 10 replicates, 40 SERS spectra of 1 μM MB collected at random sites on array were calculated by the use of 1390 cm^{-1}, $I_{1390/1581}$, $I_{1359/999}$, $I_{1359/665}$ normalization.

IS	Group									
	1	2	3	4	5	6	7	8	9	10
Uncorrected	8.84	9.86	10.81	7.41	9.21	10.72	7.54	15.03	14.54	10.00
4-MBA	6.91	6.56	10.22	11.51	9.48	11.26	7.84	10.35	14.10	11.66
4-MPY	6.91	5.23	8.15	9.91	8.02	9.67	6.95	9.45	13.15	9.00
$CHCl_3$	9.23	5.81	6.47	6.12	7.65	11.42	6.41	7.25	11.67	13.61

Table S3. One-way ANOVA results related to RSDs obtained from 40×10 peaks of 1 μM MB relation to the Different IS calibration.

The Source of Variance	Sum of Squares	df	Mean Square	F	*P*
Between Groups	26.094	3	8.368	1.417	.254
Within Groups	220.034	36	6.137		
Total	247.037	39			

Table S4. Group statistics of uncorrected and 4MBA calibration independent sample t-test.

IS		N	Mean	Std. Deviation	Std. Error Mean
RSD	uncorrected	10	10.3960	2.58775	.81832
	4-MBA	10	9.9890	2.35515	.74476

Table S5 T-test results of related to different levels of RSD depending on uncorrected and 4MBA calibration.

		Levene's Test for equality of variances		t-test for Equality of Means					
		F	Sig.	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	90 % Confidence Interval
									Lower / Upper
RSD	Equal variance assumed	.011	.918	.368	18	.717	.40700	1.10649	-1.51172 / 2.32572
	Equal variance not assumed			.368	17.84	.717	.40744	1.10649	-1.51264 / 2.32664

Table S6. Group statistics of uncorrected and 4MPY calibration independent sample t-test.

Group Statistics					
IS		N	Mean	Std. Deviation	Std. Error Mean
RSD	uncorrected	10	10.3960	2.58775	.81832
	4-MPY	10	8.6440	2.15770	.68232

Table S7. T-test results of related to different levels of RSD depending on uncorrected and 4MPY calibration.

		Levene's Test for equality of variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	90 % Confidence Interval	
									Lower	Upper
RSD	Equal variance assumed	.215	.649	1.644	18	.117	1.75200	1.06546	-.09558	3.59958
	Equal variance not assumed			1.644	17.44	.118	1.75200	1.06546	-.09882	3.60282

Table S8. Group statistics of uncorrected and $CHCl_3$ calibration independent sample t-test.

IS		N	Mean	Std. Deviation	Std. Error Mean
RSD	uncorrected	10	10.3960	2.58775	.81832
	$CHCl_3$	10	8.5640	2.76594	.87467

Table S9 T-test results of related to different levels of RSD depending on uncorrected and $CHCl_3$ calibration.

		Levene's Test for equality of variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	90 % Confidence Interval	
									Lower	Upper
RSD	Equal variance assumed	.437	.517	1.529	18	.144	1.83200	1.19778	-.24503	3.90903
	Equal variance not assumed			1.529	17.92	.144	1.83200	1.19778	-.24553	3.90953

Table S10. Group statistics of 4MBA and 4MPY calibration independent sample t-test.

IS		N	Mean	Std. Deviation	Std. Error Mean
RSD	4-MBA	10	9.9890	2.35515	.74476
	4-MPY	10	8.6440	2.15770	.68232

Table S11. T-test results of related to different levels of RSD depending on 4MBA and 4MPY calibration.

		Levene's Test for equality of variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	90 % Confidence Interval	
									Lower	Upper
RSD	Equal variance assumed	.159	.695	1.332	18	.200	1.34500	1.01007	-.40653	3.09653
	Equal variance not assumed			1.332	17.86	.200	1.34500	1.01007	-.40725	3.09725

Table S12. Group statistics of 4MBA and $CHCl_3$ calibration independent sample t-test.

IS		N	Mean	Std. Deviation	Std. Error Mean
RSD	4-MBA	10	9.9890	2.35515	.74476
	$CHCl_3$	10	8.5640	2.76594	.87467

Table S13. T-test results of related to different levels of RSD depending on 4MBA and $CHCl_3$ calibration.

		Levene's Test for equality of variances		t-test for Equality of Means					
		F	Sig.	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	90 % Confidence Interval
									Lower / Upper
RSD	Equal variance assumed	.739	.401	1.24	18	.231	1.42500	1.14879	-.56707. / 3.09653
	Equal variance not assumed			1.24	17.6	.231	1.42500	1.14879	-.56982 / 3.41982

Table S14. Group statistics of 4MPY and $CHCl_3$ calibration independent sample t-test.

IS		N	Mean	Std. Deviation	Std. Error Mean
RSD	4-MPY	10	8.6440	2.15770	.68232
	$CHCl_3$	10	8.5640	2.76594	.87467

Table S15 T-test results of related to different levels of RSD depending on 4MPY and $CHCl_3$ calibration.

		Levene's Test for equality of variances		t-test for Equality of Means					
		F	Sig.	T	df	Sig.(2-tailed)	Mean Difference	Std. Error Difference	90 % Confidence Interval
									Lower / Upper
RSD	Equal variance assumed	1.607	.221	.072	18	.943	.08000	1.10933	-1.84365. / 2.00365
	Equal variance not assumed			.072	17.0	.943	.08000	1.10933	-1.84984 / 2.00984

4.3. Spectral normalization of 10 mM TBZ with different ISs and statistical analysis

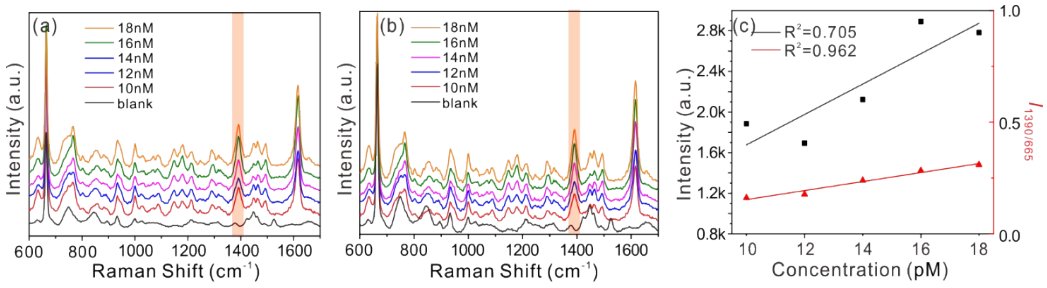

Figure S7. (a) SERS spectra of MB with the concentrations of 0, 10, 12, 14, 16, 18 nM. (b) SERS spectra of MB with the concentrations calibrated by the peck of 665 cm^{-1} belongs to chloroform. (c) A plot of the SERS intensities of 1390 cm^{-1} ascribed to MB as the concentration increases (black curve) and after (red curve) the IS calculation by 665 cm^{-1}, assigned to chloroform.

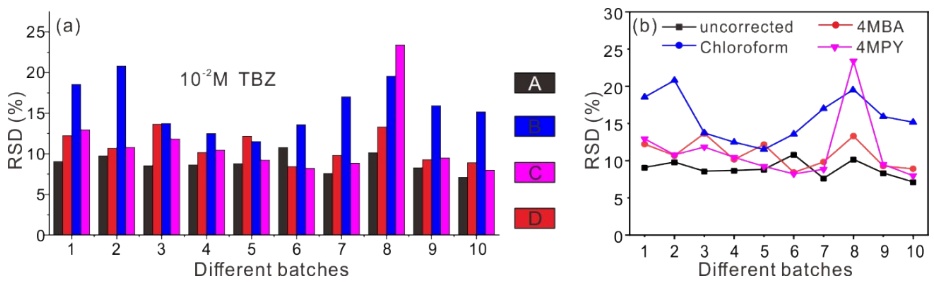

Figure S8. (a) Histograms of RSDs of 40 peaks at 775 cm^{-1} of TBZ with the concentration of 10 mM calibrated with three different ISs. (A) (B) (C) (D) represents uncalibrated, calibrated by 4MBA modified on the surface of core-shell NPs, calibrated by 4MPY molecules embedded in the core-shell NPs and organic solvent chloroform, respectively. (b) Line graph of RSDs calculated by uncorrected and corrected by different ISs.

Table S16 A table for RSDs of 10 replicates, 40 SERS spectra of 10 mM TBZ collected at random sites on array were calculated by the use of 1359 cm^{-1}, $I_{1359/517}$, $I_{1359/1610}$, $I_{1359/665}$ normalization.

IS	Group									
	1	2	3	4	5	6	7	8	9	10
Uncorrected	9.04	9.75	8.53	8.63	8.79	10.77	7.58	10.13	8.29	7.09
4-MBA	12.22	10.69	13.65	10.16	12.17	8.42	9.82	13.31	9.28	8.91
4-MPY	12.94	10.78	11.84	10.45	9.23	8.21	8.84	23.39	9.49	7.98
$CHCl_3$	18.54	20.80	13.72	12.49	11.51	13.57	17.01	19.57	15.92	15.17

Table S17. Descriptive statistics of One-way ANOVA related to RSDs obtained from 40×10 peaks of 10 mM TBZ relation to the Different IS calibration.

	N	Mean	Std. deviation	Std. Error	99 % Confidence Interval		Min	Max
					Lower	Upper		
Uncorrected	10	8.8600	1.12291	.35510	8.0567	9.6633	7.09	10.77
4-MBA	10	10.8630	1.86205	.58883	9.5310	12.1950	8.42	13.65
4-MPY	10	11.3150	4.52552	1.43110	8.0776	14.5524	7.98	23.39
$CHCl_3$	10	15.8300	3.11353	.98458	13.6027	18.0573	11.51	20.80
Total	40	11.7170	3.83581	.60649	10.4902	12.9438	7.09	29.39

Table S18. One-way ANOVA results related to RSDs obtained from 40×10 peaks of 10 mM TBZ relation to the Different IS calibration.

The Source of Variance	Sum of Squares	df	Mean Square	F	*P*
Between Groups	259.701	3	86.567	9.921	.001
Within Groups	314.123	36	8.726		
Total	573.824	39			

Table S19. Findings of multiple comparisons related to the different ISs calibration (Dunnet-C) (*) the difference is significant at the level of 0.01.

RSD			Mean Difference (I-J)	Std. Error	Sig.	99 % Confidence Interval	
						Lower limit	Upper limit
IS	Uncorrected	4-MBA	-2.00300	1.32103	0.138	-5.5955	1.5895
		4-MPY	-2.45500	1.32103	0.071	-6.0475	1.1375
		$CHCl_3$	-6.97000	1.32103	0.000	-10.5625	-3.3775
	4-MBA	Uncorrected	2.00300	1.32103	0.138	-1.5895	5.5955
		4-MPY	-0.45200	1.32103	0.734	-4.0445	3.1405
		$CHCl_3$	-4.96700	1.32103	0.001	-8.5595	-1.3745
	4-MPY	Uncorrected	2.4500	1.32103	0.071	-1.1375	6.0475
		4-MBA	0.45200	1.32103	0.734	-3.1405	4.0445
		$CHCl_3$	-4.51500	1.32103	0.002	-8.1075	-0.9225
	$CHCl_3$	Uncorrected	6.97000	1.32103	0.000	3.3775	10.5625
		4-MBA	4.96700	1.32103	0.001	1.3745	8.5595
		4-MPY	4.51500	1.32103	0.002	0.9225	8.1075

*. Mean difference is significant at 0.01 level.

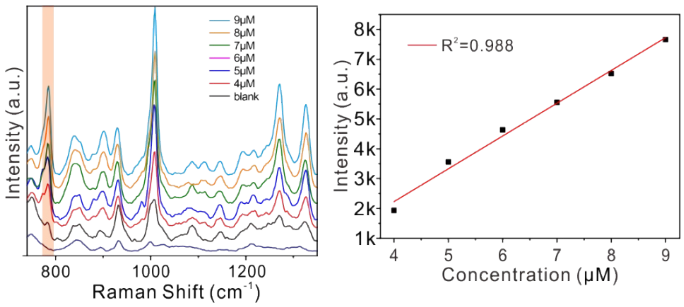

Figure S9. (a) SERS spectra of TBZ with the concentrations of 0, 4, 5, 6, 7, 8, 9 μM. (b) A plot of the SERS intensities of 785 cm^{-1} ascribed to TBZ as the concentration increases.

[1] G. Frens, *Nature (Physical Science)* **1973**, *241*, 20-22.

[2] W. Haiss, N. T. K. Thanh, J. Aveyard, D. G. Fernig, *Analytical Chemistry* **2007**, *79*, 4215-4221.