Supporting Information

SERS probe to oxidation of glutathione under plasma irradiation

Shanshan Ma [a], Qing Huang* [a,b]

Key Laboratory of Ion Beam Bio-engineering, Institute of Technical Biology and Agriculture Engineering, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei, 230031, China.

[b] *University of Science and Technology of China, Hefei, 230026, China.*

***Corresponding author:** huangq@ipp.ac.cn

This Supporting Information part includes:

Part 1: S1 Characterization of silver nanoparticles

The Ag nanoparticles were synthesized through the reduction of $AgNO_3$ by β-CD under alkaline condition. We performed zeta potential experiment to check the stability of the silver colloids.

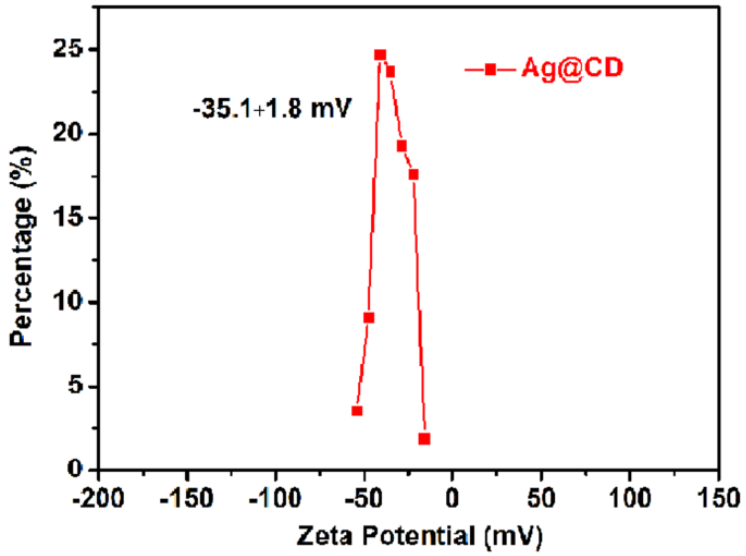

Fig.S1 The zeta potentials for silver nanoparticles.

Fig. S1 The zeta potential measurement of Ag NPs, showing that Ag NPs prepared by reduction of $AgNO_3$ with β-CD are negative charged.

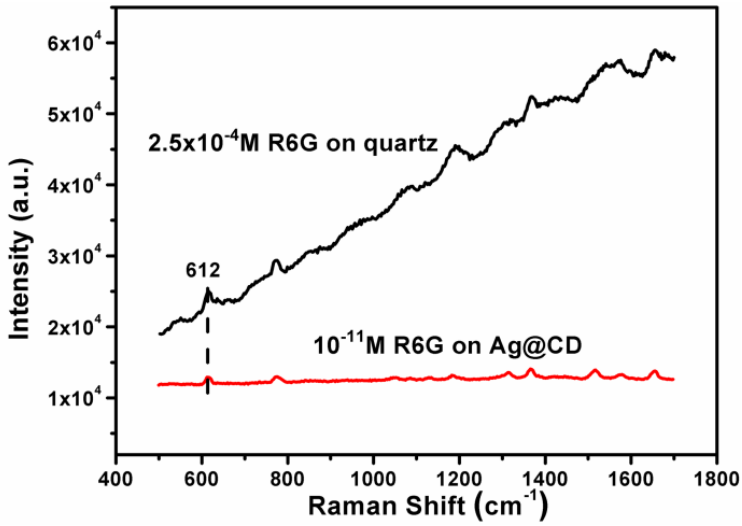

Fig.S2 Raman spectrum of $2.5x10^{-4}$ M R6G aqueous solution spread on the quartz plate (above) and SERS spectrum of 10^{-11} M R6G adsorbed on the Ag NPs SERS substrate (below).

We used the peak at 612 cm^{-1} for R6G to estimate the average enhancement factor (EF) of the Ag-NPs. The EF can be calculated by:

$$EF = \frac{I_{SERS} \times N_{Raman}}{I_{Raman} \times N_{SERS}}$$

where N_{SERS} and N_{Raman} are the numbers of R6G molecules probed on the SERS substrates and quartz plate respectively. I_{SERS} and I_{Raman} correspond to the intensities of the SERS signal on the Ag@CD and the normal Raman signal on the plate, respectively. Herein, the same volume of 10^{-11} and 2.5×10^{-4}M R6G was dropped on the SERS substrate and the quartz plate, and dried in air, the foregoing equation thus becomes:

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{S_{SERS}}{S_{Raman}} \times \frac{C_{Raman}}{C_{SERS}}$$

S_{SERS} and S_{Raman} are areas of R6G solution on the Ag-NPs substrates and the quartz, respectively. In our experiments, SERS and SRaman are about 7 mm^2 and 5 mm^2, respectively. Thus the average enhancement factor of the Ag NPs substrate (the band at 612 cm^{-1} is considered) is calculated to be $5x10^6$.

Part 2: Detection of Raman and SERS spectra of GSH, GSSG

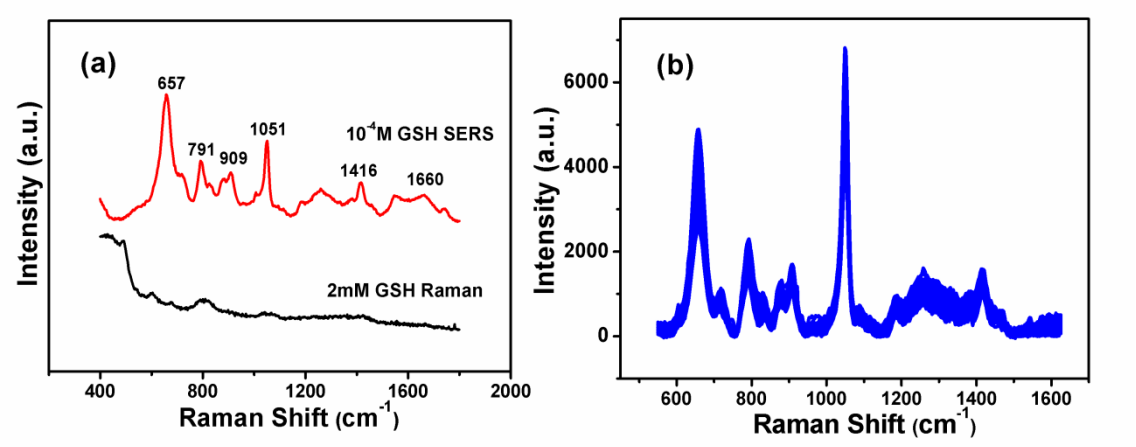

Fig S3. (a)The Raman and SERS of GSH under different concentrations. (b).The SERS mapping spectra of GSH.

We have measure the normal Raman spectrum of GSH, and even for the concentration of 2 mM, the Raman signal was still too weak to be clearly determined. However, the SERS signal of GSH at the concentration of 10^{-4} M could readily identified, showing the promising potential for the trace detection of GSH in cells (Fig S3a). Also, the spectral reproducibility was checked in the present work. As shown in

the Fig S3b for example, the spectra of GSH on the SERS substrate were quite uniform, confirming that our result is repeatable and reliable.

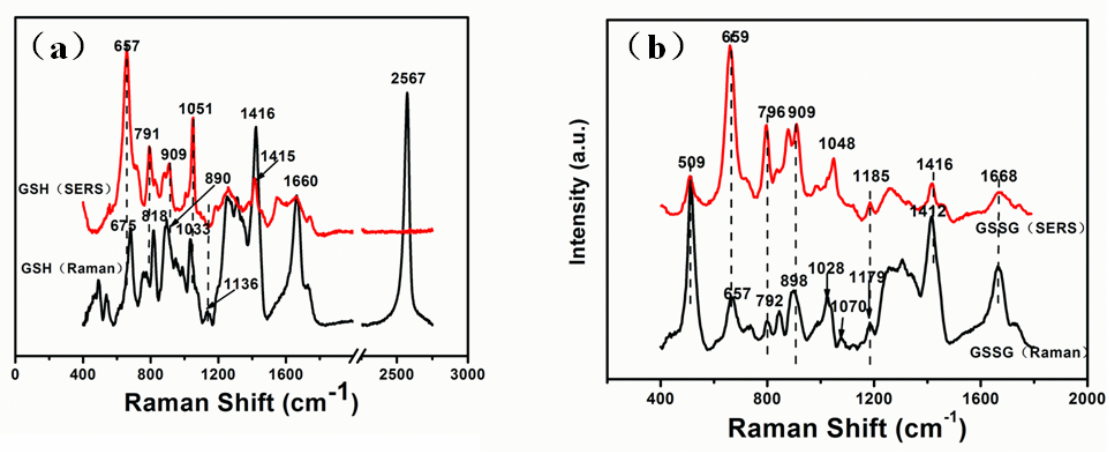

Fig S4. The Raman and SERS spectra of GSH (a) and GSSG (b).

Moreover, the SERS spectra of GSH and GSSH are compared with the normal Raman spectra, respectively. Most bands are distinguishable and consistent with each other. However, the 2568 cm^{-1} (-SH) in the normal Raman of GSH disappears in the SERS spectrum, indicating the conjugation of GSH onto the surface of Ag NPs.

Part 3: Influence of pH value and silver colloid concentration on the SERS measurements of GSH/GSSG

The SERS intensity of glutathione is sensitive to the pH variation. The silver nanoparticles used here are negatively charged. When the pH is lower than 5.93, the isoelectric point (PI) of GSH, the GSH is positively charged. The electrostatic adsorption between GSH molecules and Ag particles lead to the strong enhancement. In contrast, when the pH is higher than 5.93, GSH is negatively charged. The interaction between GSH and the surface of silver particles is weak, resulting in the lower enhancement. Moreover, as the pH of GSH solution is lower than 2, the stability of silver colloids will be destroyed in the mixture of GSH and silver particles. Therefore, the SERS intensity of GSH becomes lower. In our DBD-SERS experiments, the pH value falls within the range of 2-4, and in this region, the SERS intensity does not show big difference, as shown in Fig. S5.

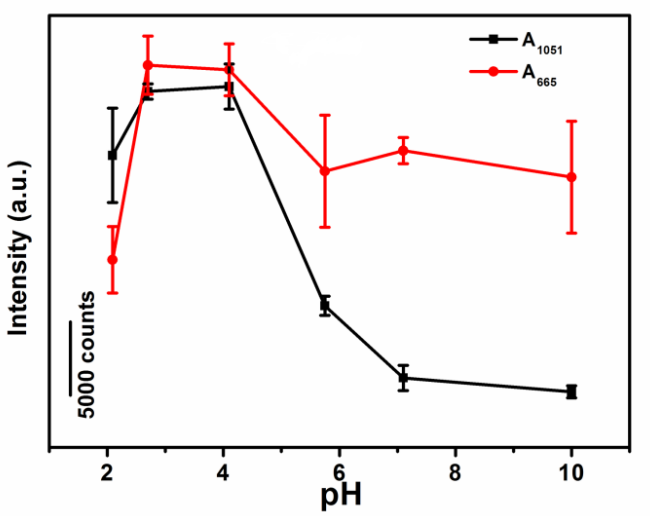

Fig S5.The SERS intensity of GSH changes with pH value. The concentration of glutathione is $5x10^{-5}$ M.

Another difficulty for quantitative evaluation is due to that the SERS intensity is actually dependent on the concentration of Ag NPs applied. To overcome this uncertainty or ambiguity, we also conducted SERS measurement of the GSH/GSSH mixtures using different concentration of Ag NPs.

Fig. S6(a) shows that the Raman intensity at 1051 cm^{-1} changes from 1.6×10^4 to 2.2×10^4 for C_{Ag}/C_{GSH} in the range of 2×10^{-5} - 2×10^{-4}. So when the concentration of Ag NPs is large enough, the SERS intensity becomes relatively steady. This means that if there are enough Ag NPs, all the GSH can be adsorbed to the Ag NPs and contributes to the overall SERS signal. In our experiment, the concentration of GSH is less than 10^{-4} M. Therefore, when we applied 2 nM Ag colloids, the observed SERS signal is not so sensitive to small variation of concentration of Ag NPs.

Fig. S6(b) shows the standard curves of GSSG/GSH of different concentration at different concentration of Ag NPs. It shows that when the concentration of GSSG C_{GSSG} is low or comparable with C_{GSH}, the three curves are almost overlapped; only when C_{GSSG} is much larger than C_{GSH}, the discrepancy of the curves becomes larger. For our DBD-induced oxidation experiments, we have $0< C_{GSSG}/C_{GSH}<2$, and in all the cases the same concentration of 2 nM of Ag NPs was applied, which thus ensured our measurements are reproducible and comparable with each other.

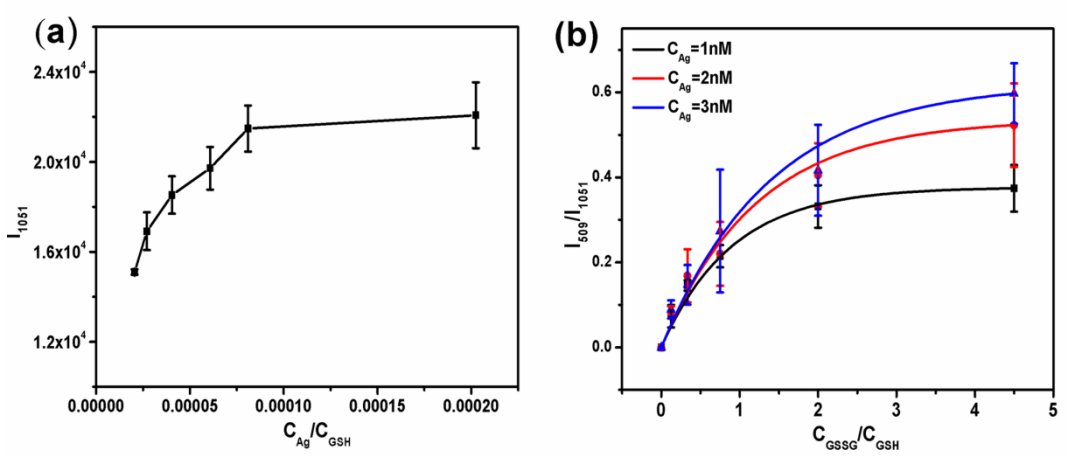

Fig .S6 (a) The SESR intensity of GSH（$5x10^{-5}$M） mixed with Ag colloids of varied concentrations. (b)The standard curves for the intensity ratio of 509 cm^{-1} (S-S) to 1051 cm^{-1} (C-N) changes with concentration ratio for the mixture of GSSG/GSH. The concentrations of Ag colloids are 1 nM, 2 nM, 3 nM for the black, red and blue curves, respectively.

Part 4: Validity of application of the band at 1051 cm^{-1} for quantitative assessment of GSH

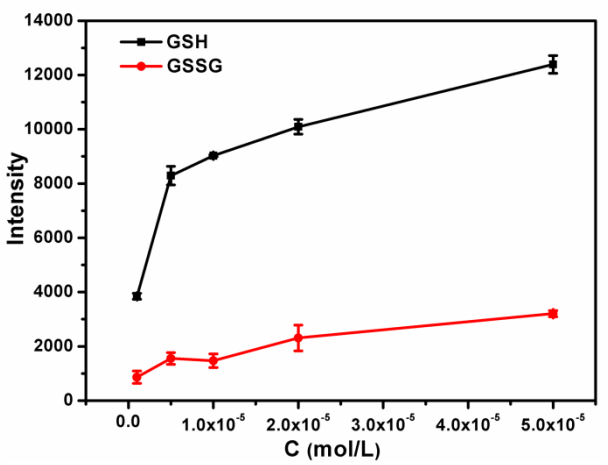

Fig. S7 The intensity of C-N bands in the SERS spectra of GSH and GSSG, respectively.

The ratio of the intensity of 509 cm^{-1} (S-S) to 1051cm^{-1} (C-N) was used to estimate the content of GSSG and GSH. This is because that the intensity of for C-N band at 1051 cm^{-1} from GSH is much larger than that of C-N band at 1048 cm^{-1} from GSSG as seen from Fig. S7. Therefore, the ratio of I_{509}/I_{1051} can be approximately applied as a standard curve to quantify C_{GSSG}/C_{GSH}, neglecting the contribution from the 1048 cm^{-1}.

Part 5: Estimation of the GSH-GSSG conversion efficiency

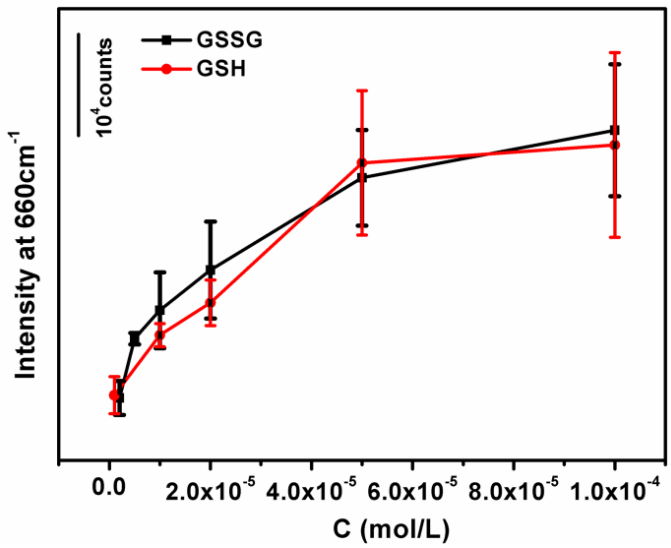

Fig. S8 The intensity of C-S bands around 660cm-1 in the SERS spectra of GSH and GSSG with differen concentrations.

From SERS measurements, we can estimate the conversion from GSH to GSSG quantitatively based on the intensity ratio $P=I_{S\text{-}S}/I_{C\text{-}S}$, which can be expressed as:

$$P=\frac{I_{\text{S-S}}}{I_{\text{C-S}}}=\frac{I^{o}_{509}\times C_{GSSG}}{I^{o}_{659}\times C_{GSSG}+I^{o}_{657}\times C_{GSH}} \qquad (1)$$

where C_{GSH} and C_{GSSG} are the concentrations for GSH and GSSG, respectively. I^{0}_{509} and I^{0}_{659} refer to the intensity per unit concentration for S-S (509 cm^{-1}) and C-S (659 cm^{-1}) vibrations of GSSG, respectively; while I^{0}_{657} refers to the intensity per unit concentration for C-S vibration of GSH. Note that for the SERS bands at 657 cm^{-1} of GSH and 659 cm^{-1} of GSSG, their Raman intensities are almost identical as shown in Fig.S7, so Eq. (1) can be simplified as:

$$P=\frac{I^{o}_{509}}{I^{o}_{659}}\times\frac{C_{GSSG}}{C_{GSSG}+C_{GSH}} \qquad (2)$$

As two GSH molecules can form one GSSG molecule given that the conversion is complete. But for real case, the conversion is not complete, so if the conversion rate is X, then we have:

$$C_{GSSG}=\frac{(C_{o}-C_{GSH})\times X}{2} \qquad (3)$$

Bring (3) to (2), so we obtain:

$$P = P^{o} \times \frac{(C_o - C_{GSH}) \times X}{(C_o - C_{GSH}) \times X + 2C_{GSH}} \qquad (4)$$

where $P^0 = I^0_{509} / I^0_{659}$ which is a constant (measured as ca. 0.25). Therefore, the conversion efficiency X can be estimated by the following equation:

$$X = \frac{2P \times C_{GSH}}{(P^{o} - P) \times (C_o - C_{GSH})} \times 100\% \qquad (5)$$

Part 6: HPLC measurements of the DBD-treated samples

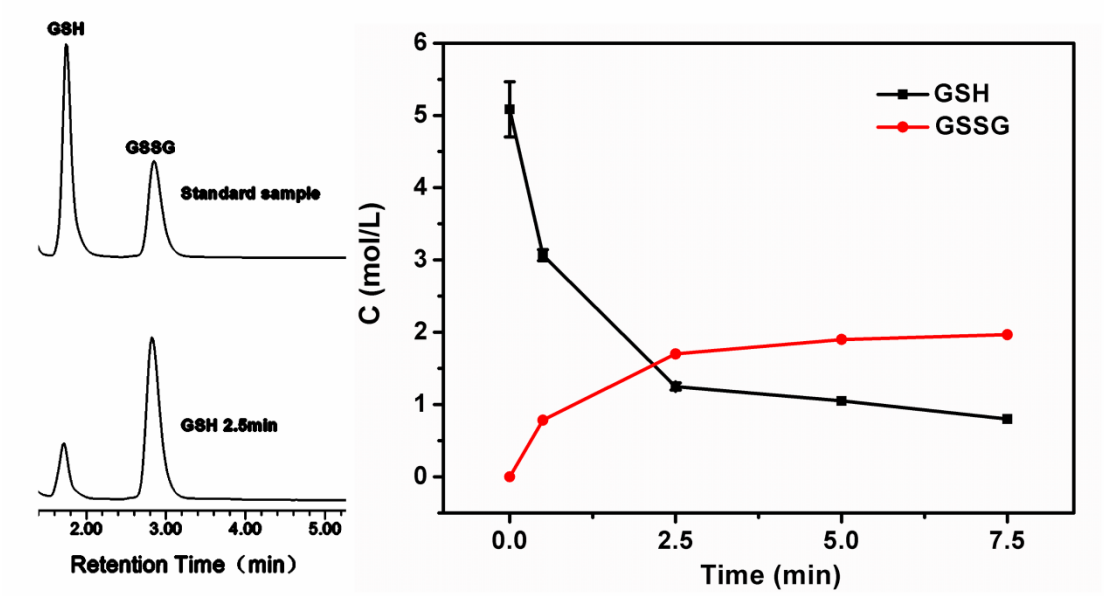

Fig. S9. HPLC analysis of GSH/GSSG samples. The data in the plot was obtained from the DBD-treated sample with discharge time 2.5 min.

In the standard samples, the peaks observed at 1.8 and 3 min are assigned to GSH and GSSG respectively. In the chromatogram of the DBD-irradiated sample, the peaks of GSH and GSSG can be identified clearly. Fig. S9 shows that the content of GSH decreased and content of GSSG increased with rise of discharge time.

Part 7: Measurement of H_2O_2 in the DBD-treated samples

Under the acidic condition, hydrogen peroxide can react with titanium sulfate (Ti^{4+}) and form a yellow polymer which shows a characteristic absorption peak near 407 nm. Thus,we can determine the content of hydrogen peroxide in the system over time,

according to change in absorbance changes detected by titanium sulfate reagent[2].

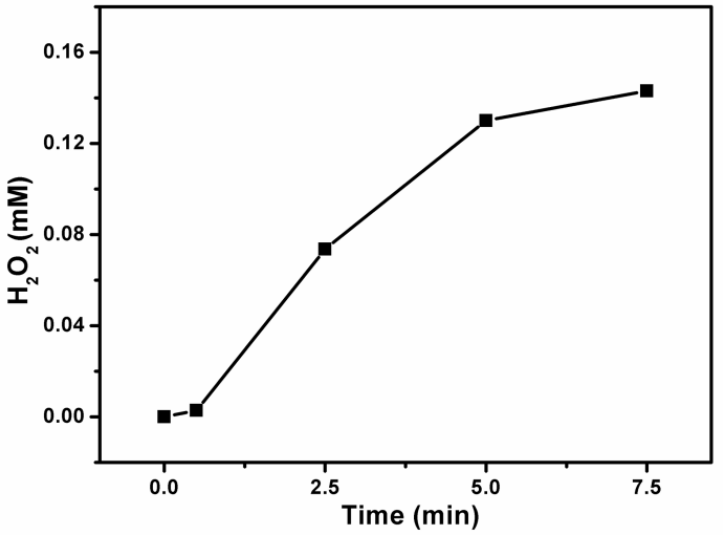

Fig S10. Content of H_2O_2 in the DBD-treated sample increases with discharge time.