Supporting Information

Highly uniform silver nanospheres self assembled monolayers for sensitive quantitative detection of glutathione by SERS

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In order to explore the repeatability of the Ag film@Si SERS platform, five batches of Ag NPs were compared to detect the SERS signal of SPDP and after mixed incubation of GSH and SPDP. The test conditions were the same as this article. According to the SERS signals of five batches of SPDP on the base surface in Fig. S1A and the intensity map in Fig. S1B, there was no great difference in the SPDP signals of each batch, and the relative standard deviation of the intensity value at 1000 cm^{-1} was 15.05% through quantitative calculation. Fig. S1C and Fig. S1D showed the SERS signal and intensity diagram after the mixed incubation of GSH and SPDP, with RSD value of 9.64%. The results showed that the SERS platform had good repeatability.

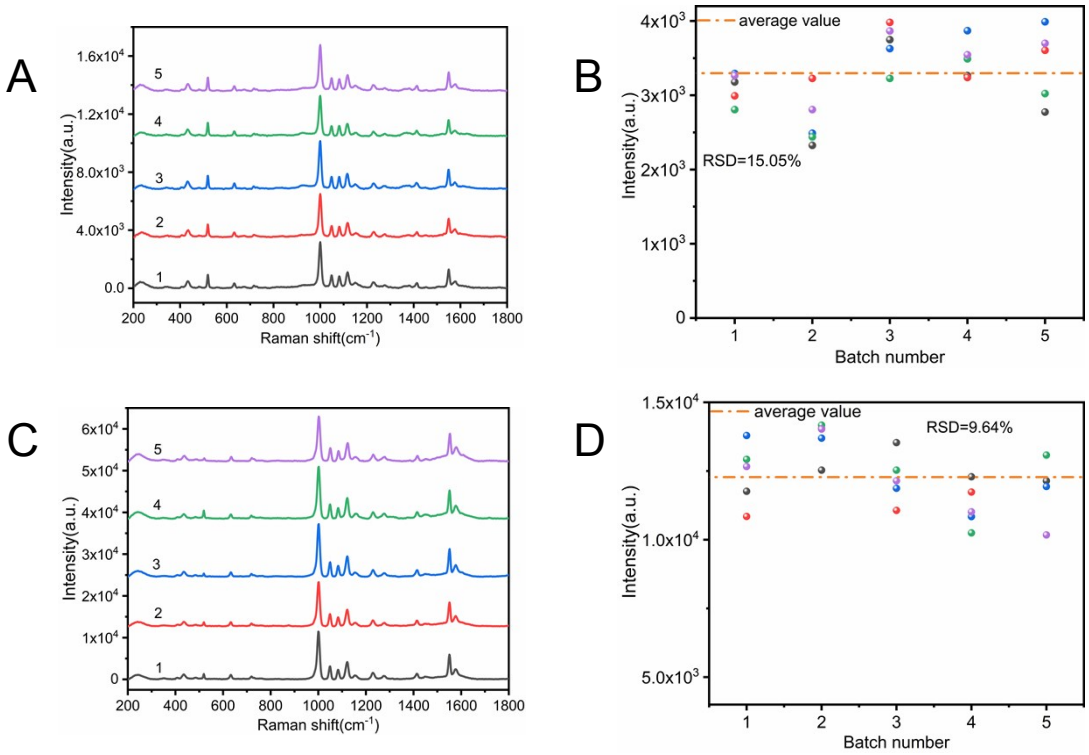

Fig. S1.(A) SERS spectra of 0.1 mM SPDP on different batches of Ag film@Si, (B) SERS intensity distribution maps of SPDP at 1000 cm^{-1} on different batches of Ag film@Si, (C) SERS spectra of 0.1 mM SPDP and 1 μM GSH on different batches of Ag film@Si, (D) SERS intensity distribution maps of SPDP and GSH after mixed incubation on 1000 cm^{-1} on different batches of Ag film@Si.

As a heterobifunctional crosslinking agent, SPDP reacts with sulfhydryl groups by its pyridine disulfide group. Research pointed out that when the disulfide group of SPDP reacts with thiol, the disulfide bond of the linking arm will be broken. (Fig. S2A). Reduced glutathione (GSH), a biological mercaptan, contains groups with active sulfhydryl groups. The pyridine disulfide group of SPDP and the sulfhydryl group of glutathione are exchanged by mercapto disulfide bonds to generate the NHS ester with sulfhydryl group and pyridine-2-sulfhydryl group or pyridine-2-thione group.

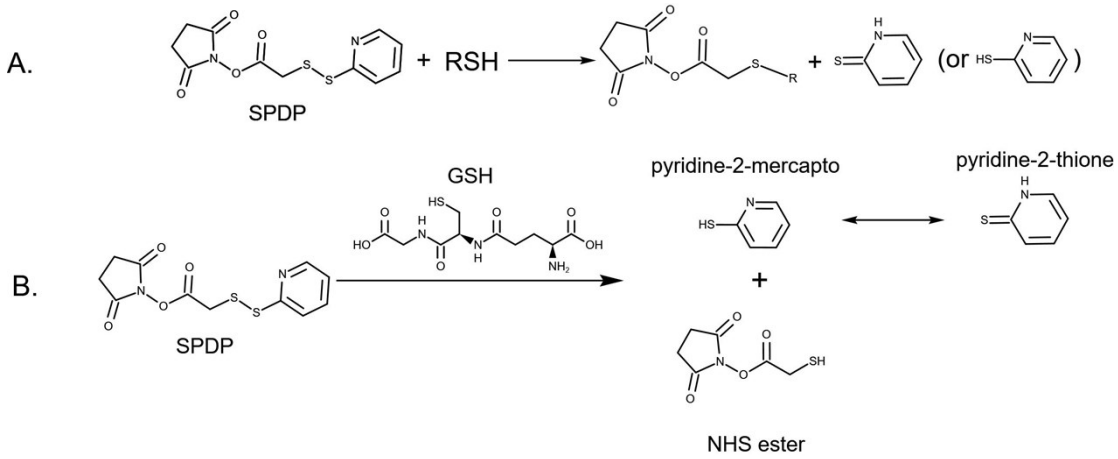

Fig.S2. (A) Reaction process of SPDP with thiol, (B) Reaction process of SPDP with GSH

Since sulfhydryl group is the active group of GSH, DTNB colorimetry, a classic method for detecting sulfhydryl group, was chosen here to make a comparison. A series of concentrations of GSH (same as used in the manuscript) and DTNB solution were mixed and incubated, and NADPH coenzyme was added to promote the reaction between the two. The absorbance at 412 nm at different time was measured by a microplate reader. As can be seen from the standard curve in Fig. S3A, the platform was reached by the chromogenation reaction of 50 μM GSH after 15-20 min by kinetic method. Fig. S3B is the line graph at reaction time of 15 min, and the linear relationship is good. However, when the detection concentration is 1 μM, the absorbance is close to blank. Fig.S3C and Fig.S3D are the detection of serum samples. It should be emphasized that the serum used was the same as before, stored at -80℃. The results showed that after the reaction of 90 min, the absorbance of the serum containing 1 μM GSH exceeded the maximum value of the standard value. At this time, the DTNB colorimetric method may have failed. Compared with the standard method, the SERS detection method based on Ag film@Si is a reliable, rapid and sensitive method for glutathione evaluation. The minimum detection sensitivity GSH is 10 nM, and a good

linear relationship of GSH is found in the range of 10 ~ 500 nM. On the other hand, the preparation of SERS substrate in this paper is simpler and easier to carry, which can avoid the possible data deviation caused by enzyme activity damage.

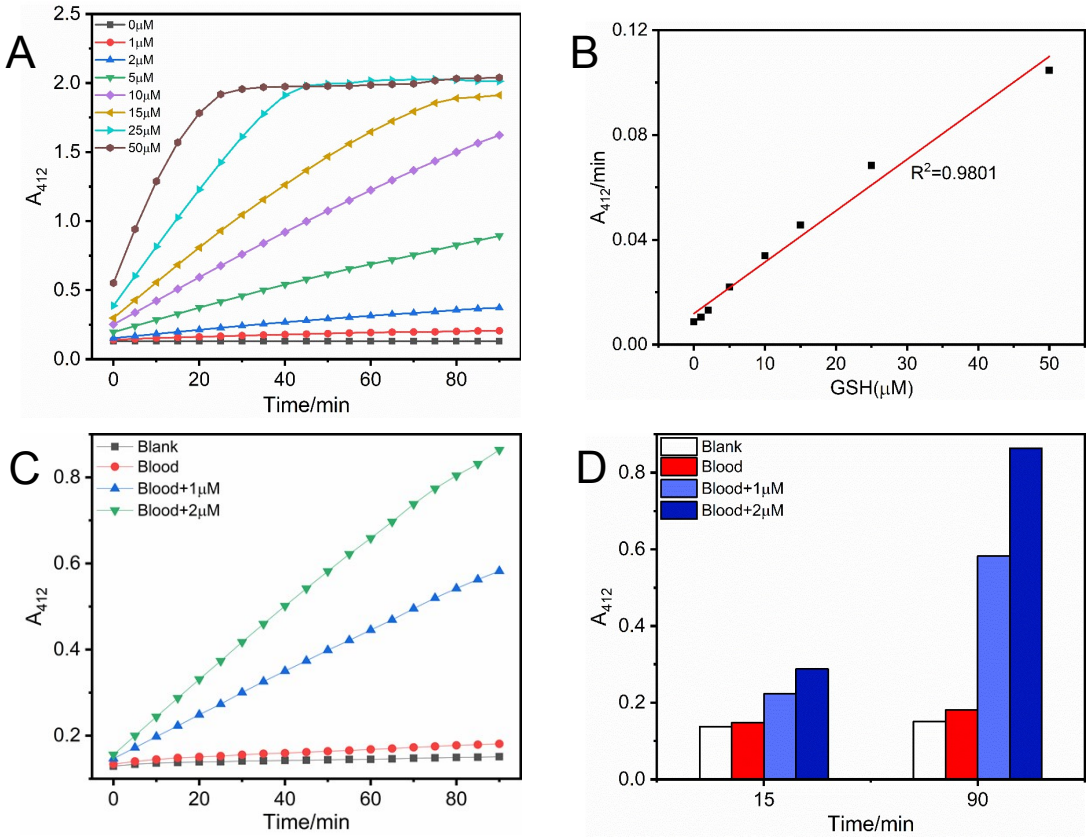

Fig.S3 (A) The standard curve of the absorbance of different concentrations of GSH within 90 min of reaction. (B) Line graph at 15 min of reaction. (C) The dynamic curve of blank, serum, serum containing 1 μM GSH and serum containing 2 μM GSH at 412 nm for 90 min. (D) The absorbance at 412 nm after the reaction of blank, serum, serum containing 1 μM GSH and serum containing 2 μM GSH for 15 min and 90 min.

In addition, compared with other methods for detection of glutathione, such as fluorometry, high liquid chromatography, colorimetry, etc., the results are shown in the following Table S1. The results show that the analysis cost of liquid phase detection is relatively high, the time is longer and the sensitivity is low. The solid phase detection

used in this paper is simple and quick to prepare, easy to transport and carry, and has high sensitivity. The SERS detection method based on Ag film@Si is a reliable, rapid and sensitive method for glutathione evaluation.

Table S1 Comparison of various detection methods for glutathione

Detection method	LOD	Linear range	References
Fluorometry	36.9 nM	0-26 μM	[1]
Fluorometry	1 μM	5-10 μM	[2]
Fluorometry	0.12 μM	0-60 μM	[3]
HPLC	1.1 ng/mL	10-1000 ng/mL	[4]
Colorimetry	10.9 nM	10.9-400 nM	[5]
Colorimetry	0.013 μM	1-40 μM	[6]
SERS	13 μM	13-2200 μM	[7]
SERS	0.56 μM	—	[8]
SERS	0.25 μM	0.25-1 μM	[9]
SERS	10 nM	10-500 nM	this work

References

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