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ARTICLE

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Monitoring the levels of pesticides on plant tissues is important for achieving effective protection on crops after application, as well as ensuring low levels of residues during harvest. In this study, a simple, rapid, and fieldable colorimetric method for detecting the pesticide glyphosate (Gly) on the plant tissues *in situ* using cysteamine-modified gold nanoparticles (AuNPs-Cys) has been developed. The aggregation of AuNPs-Cys in the presence of Gly results in a consequent color change from red to blue (or purple), which could be observed visually on the surface of plant tissues. By the naked eyes, we successfully detected Gly spiked on the surface of spinach, apple, and corn leaves *in situ*. Further verification and quantification were achieved using surface-enhanced Raman spectroscopy (SERS) which uses AuNPs-Cys as the substrate. Moreover, application of this method was demonstrated through the evaluation of the Gly distribution on plant tissues which could greatly facilitate the development of precision agriculture technology.

Introduction

The Precision agriculture (PA) is a modern concept that is aimed to re-organize the total system of agriculture towards a low-input, high-efficiency, sustainable agriculture.[1] PA technology has been promoted and implemented around the world in recent decades. The emerging field of PA intends to provide tools, such as distributed plant health sensors, to enable growers to make informed decisions about how best to use their resources.[2] The factual base of PA is the spatial and temporal variability of soil and crop factors between and within fields. For example, hyperspectral sensing is a relatively new technology that is capable of providing information over a nearly continuous spectrum in the visible, mid-infrared, and near-infrared wavebands. Images acquired from hyperspectral sensors have been used for estimation of crop vigor and yield prediction; discrimination between crops, weeds, pesticide residue, and soil; and quantitative measurements of crop water content and leaf area index.[3] Traditionally, crops have been treated under the assumptions of 'uniform' soil, nutrient, moisture, weed, insect, and growth conditions. This has led to over- or under-applications of herbicides, pesticides, and other treatments. PA leads to more effective pesticide application (reduce under or over dose application), thus enhancing production and profits, and food safety.

To facilitate the PA, it is important to have a simple, rapid, and fieldable technology to monitor the pesticide application to ensure effective protection on crops and the low level of pesticide residue during harvest. The golden standard methods for pesticide residue analysis are liquid or gas chromatography combined with mass spectrometry.[4,5] These methods offer accurate and sensitive detection results, however, are complicated, time-consuming, and have to be performed in a laboratory by highly trained technicians. Recently, emerging detection methods for pesticide were applied, such as immunoassay,[6] surface plasmon resonance sensors[7] and diffuse reflection spectrophotometric,[8] which have simpler procedure, less work load and higher efficiency. Saylan et al.

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Electronic Supplementary Information (ESI) available: [The photograph of AuNPs modified with different concentrations of cysteamine; The photographs of AuNPs-Cys at different pH, before and after the addition of glyphosate; CIELAB and colorimetric value of each sample point on spinach leaf; CIELAB and colorimetric value of each sample point on apple peel; CIELAB and colorimetric value of each sample point on corn leaf; Recovery for glyphosate on corn leaf]. See DOI: 10.1039/x0xx00000x

fabricated molecularly imprinted nanofilms and integrate them with surface plasmon resonance sensors for sensitive and selective detection of multiple pesticides.[9] Bala et al. detected organophosphorus pesticide in environmental samples using peptide and aptamer based nanoprobes.[10] However, all of them cannot be used for fieldable monitor.

The goal of our study is to develop an approach that can realize simple, rapid, and fieldable monitoring pesticide levels on plant tissues *in situ* (on plant surfaces directly without extracting the pesticides) after deploying pesticides in the field. Visual detection based on the color change of AuNPs drawn intense attention due to the ease of the method and the instrument-free requirement.[11,12,13] For example, Chen et al. Developed a colorimetric sensor based on citrate-stabilized AuNPs for the rapid pesticide residue detection of both terbuthylazine and dimethoate.[14] Zhao et al. used AuNPs-based colorimetric assays to sensitive determination of atrazine in apple juice.[15] Meanwhile, AuNPs is the most commonly used analytical substrate in surface enhanced Raman spectroscopy (SERS), by which Raman scattering can be enhanced more than one million times by using noble metal nanostructure.[16] The advantages of SERS include high sensitivity, fast and nondestructive signal acquisition and molecular fingerprinting.[17] Our strategy is to integrate these two techniques, colorimetric-SERS, to realize *in situ* detection of pesticide levels and its distribution on plant tissues (Figure 1). The colorimetric method is used for rapid screening and fieldable estimation of the pesticide level and distribution on plant tissues at farm *in situ*. The SERS method can serve as a verification and quantification method when needed.

The model pesticide we studied is glyphosate (Gly). Owing to its high herbicidal activity, in the past decades, Gly has been widely used as a plant killer in agriculture for the control of weeds, shrubs, and grasses,[18] especially after the introduction of Gly-resistant transgenic crops.[19] Due to the wide application of this pesticide, residues of Gly in food have raised concern, although this pesticide has proven to be relatively less toxic compared to many other chemical pesticides.[20] Our method served as a quick method to determine the cross-contamination level of Gly on crop tissues, which may further enable development of strategies to reduce residues on the crop. The model plant tissues include apple peels, corn leaves and spinach leaves which represent different background colors for testing the capability of observing color change of AuNPs with different background interferences. To highlight the innovation of our approach as compared to other colorimetric sensors that are measured in vials or tubes, our approach is an *in situ* analysis, where we directly placed the AuNPs on the leaf surface and performed the analysis in the several microliter volume on the surface. The unique advantage of this approach besides less volume required and vial/tube-free is the capability for us to study the distribution of analyte on the surface. The method will assist effective protection on crops and the low level of pesticide residue during harvest and greatly facilitate the development of PA technology.

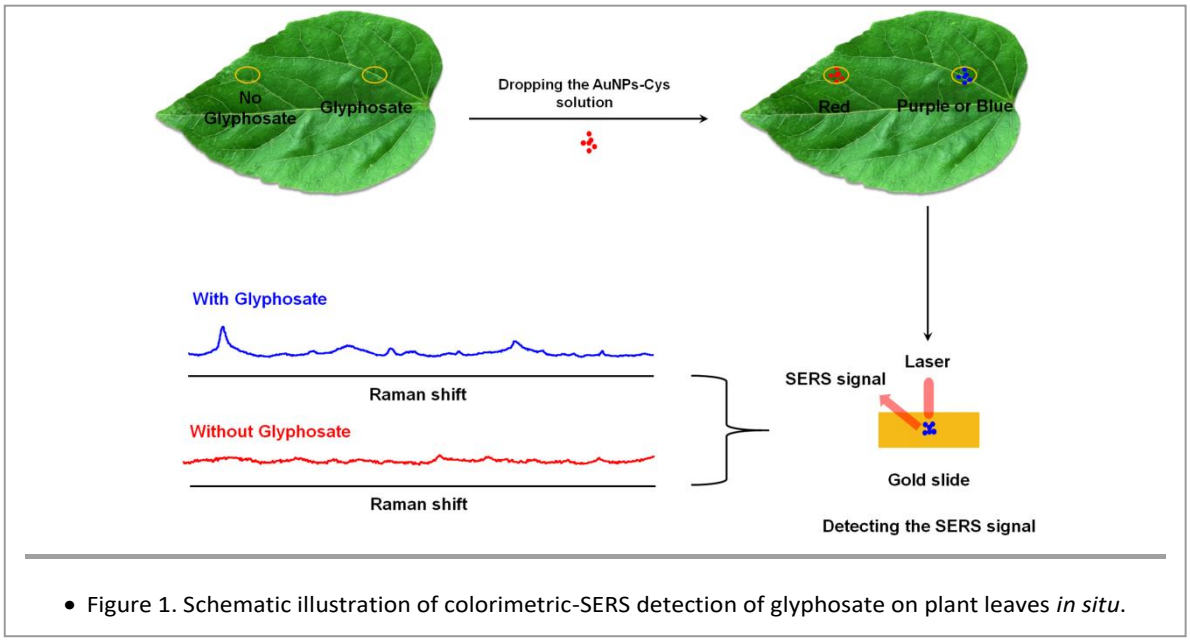
Exprimental

Chemicals and materials

Cysteamine hydrochloride (≥ 98%) and glyphosate (analytical standard) were obtained from Sigma Aldrich (St. Louis, MO). Citrate-capped AuNP colloids (50 nm, 0.05 mg mL^{-1}) were purchased from NANO PARTZ Inc. (Loveland, CO). Apple, corn, and spinach leaf (organic) were purchased from the local supermarket. All solutions were prepared using ultra-pure distilled water obtained from a Thermo Fisher Scientific Barnstead ultra-pure water purification system.

Modification of AuNPs

Cysteamine-modified AuNPs (AuNPs-Cys) were prepared by mixing equal volumes of AuNPs (0.5 mg mL^{-1}) and cysteamine



- Figure 1. Schematic illustration of colorimetric-SERS detection of glyphosate on plant leaves *in situ*.

hydrochloride.[21] The mixture was allowed to further react for 15 minutes at room temperature, after which the AuNPs-Cys were fully prepared for further use. To optimize the AuNPs-Cys, the effects of a range of cysteamine concentrations were evaluated. The highest concentration of cysteamine that did not cause any distinct color change prior to the addition of Gly was chosen for further testing. The optimized pH of AuNPs-Cys were further investigated by adjusting 0.01 mol L^{-1} HCl. 1000 mg L^{-1} of Gly was added into test samples to confirm the optimal pH for further Gly detection.

In tube and *in situ* visual detection

To test Gly in water, different concentrations of Gly (0.001 mg L^{-1}, 0.01 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1}, 100 mg L^{-1}, and 1000 mg L^{-1}) and AuNPs-Cys were mixed with a ratio of 1:1 (v/v). The mixture was allowed to react for 15 minutes, and the red-to-blue (or red-to-purple) color change was observed. When detecting Gly on the plant tissues, three tissues with different background colors were chosen: corn leaf, apple peel, and spinach leaf. Detection of Gly on plant tissues was carried out according to the method as follows. A 2 μL at aliquot of the different concentration of Gly (0.001 mg L^{-1}, 0.01 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1}, 100 mg L^{-1}, and 1000 mg L^{-1}) was pipetted onto the detached corn leaf, apple peel, or spinach leaf respectively, and then allowed to dry at room temperature for about 5 minutes. A 2 μL aliquot of the 0.25 mg mL^{-1} AuNPs-Cys solution was then pipetted onto the tissue surface with Gly exposure and mixed briefly by gentle pipetting for 5 seconds. The color change of the AuNPs-Cys solution was observed. Each test was repeated three times.

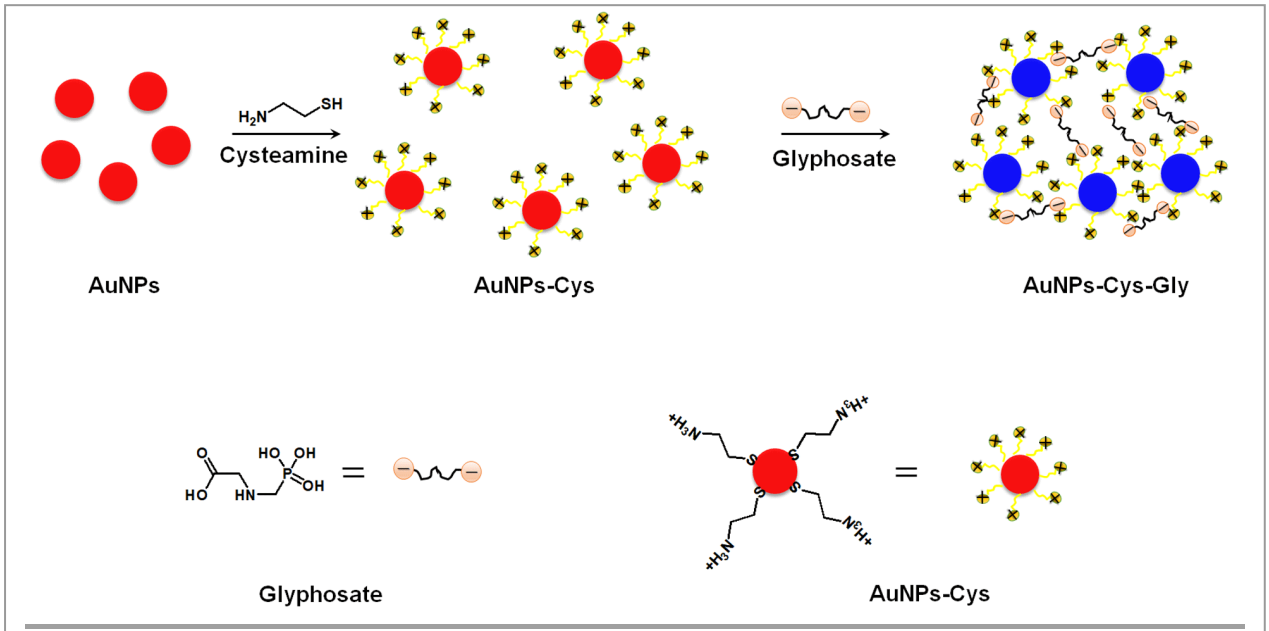
SERS analysis

To determinate Gly in water, a 20 μL aliquot of Gly (0.001 mg L^{-1}, 0.01 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1}, 100 mg L^{-1}, and 1000 mg L^{-1}) solution in water was transferred into different tubes (1 mL). respectively, then a 20 μL aliquot of the 50 nm AuNPs-Cys solution (at 250 mg mL^{-1}) was added into each tube and mixed briefly by gentle pipetting for 15 minutes. A 2 μL aliquot of solution from each tube was transferred onto the gold-coated microscope slide and allowed to dry at room temperature before Raman spectroscopy measurement. For detection of Gly on plant tissues, a 2 μL aliquot of the different concentrations of Gly (0.001 mg L^{-1}, 0.01 mg L^{-1}, 0.1 mg L^{-1}, 1 mg L^{-1}, 10 mg L^{-1}, 100 mg L^{-1}, and 1000 mg L^{-1}) was pipetted onto the detached tissues respectively, and then allowed to dry at room temperature. Then a 2 μL aliquot of the 250 mg mL^{-1} AuNPs-Cys solution was pipetted onto the plant tissue surface with Gly exposure and mixed by gentle pipetting for 90 seconds. AuNPs-Cys solution was then transferred onto the gold-coated microscope slide and allowed to dry at room temperature for Raman spectroscopy measurement.

A DXR Raman microscope (Thermo Scientific, Madison, USA) equipped with a 780 nm laser and a 20 × microscope objective was used in this study. All SERS spectra were obtained with a 2.0 mW laser power and a 50 mm slit aperture for 2 seconds acquisition time. OMINC 9.0 software (Thermo Scientific) was used for Raman data acquisition and analysis. For each sample, five spots were selected randomly and scanned with the range of 500–2000 cm^{-1}. The mean and standard deviation were analyzed. All the experiments were repeated three times.

Study the distribution of Gly on plant leaves

Three different plants tissues were chosen: corn leaf, apple peel, and spinach leaf. After cleaning these tissues with water



- Figure 2. Schematic illustration of the procedure and mechanism for the detection of glyphosate using cysteamine-modified gold nanoparticles.

and dried at room temperature, Gly solution (1000 mg L^{-1}) was sprayed by a small spray bottle on corn leaf, apple peel, and spinach leaf respectively. After drying at room temperature for 5 minutes, a series of positions on the tissues were selected where we pipetted AuNPs-Cys (3 μL, 250 mg mL^{-1}) solution on each position. The color change of the AuNPs-Cys solution were showed and based on the color change, two representative sample points (one blue sample point and on red sample point) were selected for SERS measurement as a validation for visual detection.

Data analysis

The calibration curves for quantification assays were obtained by using log 10–log 10 model:

$$\log Y = a \log X + b$$

where X (mg L^{-1}) is nominal concentration of Gly and Y (a.u.) is Raman intensity. a and b were determined by linear fitting of the calibration curves.

The limit of detection (LOD) is expressed as: LOD = 3.3 σ/S, where σ is the standard deviation of the blank, S is the slope of the calibration curve.[22]

The CIELAB L*, a*, b*, color values of each sample on the tissues were calculated using the Image-J software. We took the photos using a smart phone (iphone 7 plus). Then we transferred the photos to the computer and analysted them using the Image-J software. The three-dimensional scatter plot of L*, a*, and b* was analyzed using the MATLAB software.

The CIELAB system has the properties of an euclidean space. The distances between any two (or more) color points represents their colorimetric difference (Δ E*) and is calculated from the differences of its components[23], ΔL*, Δ a*and Δb*:

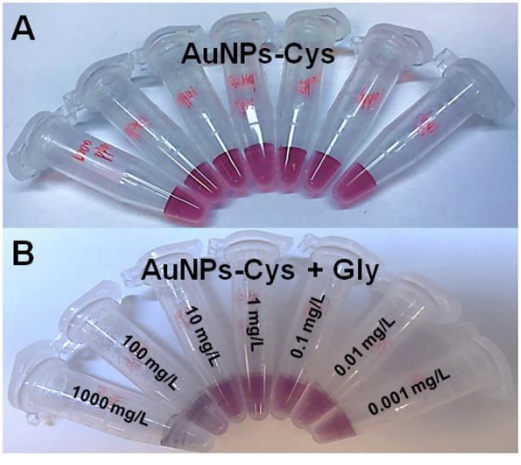
$$\Delta L^* = L^*_1 - L^*_0$$
$$\Delta a^* = a^*_1 - a^*_0$$
$$\Delta b^* = b^*_1 - b^*_0$$

L^*_1 is the L^* value of each sample point, and L^*_0 is the L^* value of control sample point (without Gly). a^*_1 is the a^* value of each sample point, and a^*_0 is the a^* value of control sample point (without Gly). b^*_1 is the b^* value of each sample point, and b^*_0 is the b^* value of control sample point (without Gly).

Results and Disscussion

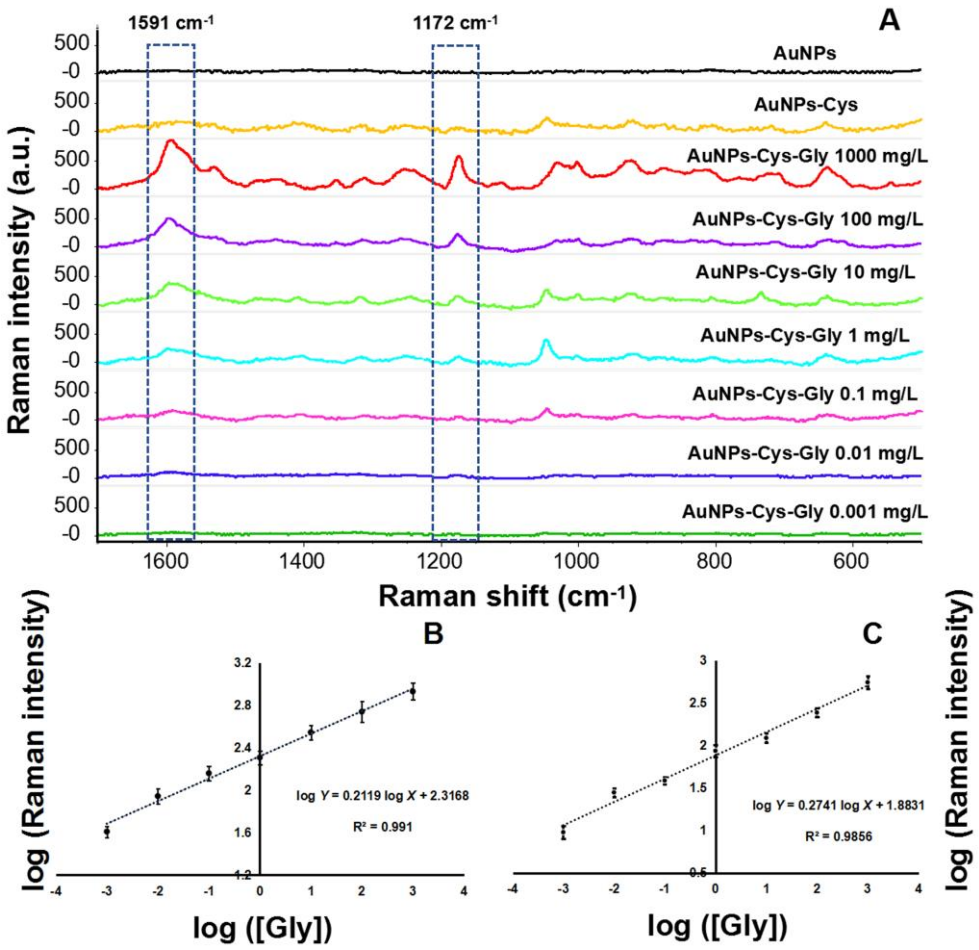
In tube colorimetric-SERS detection of Gly

Illustration of the colorimetric detection of Gly. As shown in Figure 2, cysteamine-modified AuNPs can interact with Gly and the color changed from red to blue/purple. The detailed mechanism was illustrated below. The thiol group of cysteamine can be easily attached to the surface of AuNPs by forming a strong Au-S bond, causing the amine groups were exposed on the outer surface of the AuNPs-Cys. The AuNPs-Cys are positively charged with the superficial $-NH_3^+$ groups, resulting in high stability against aggregation due to the electrostatic repulsion force[24,25]. Gly molecule contains functional groups of carboxyl ($-COOH$) and phosphonyl ($-PO_3H_2$) in its structure. Thus, Gly showed strong affinity to cysteamine modified AuNPs through electrostatic adsorption interactions and induced the inter-particle cross-linking of AuNPs[24]. Cross-linked AuNPs have a blue shift in the UV-Vis absorption[26], resulting in appreciable color changes from red to blue/purple.



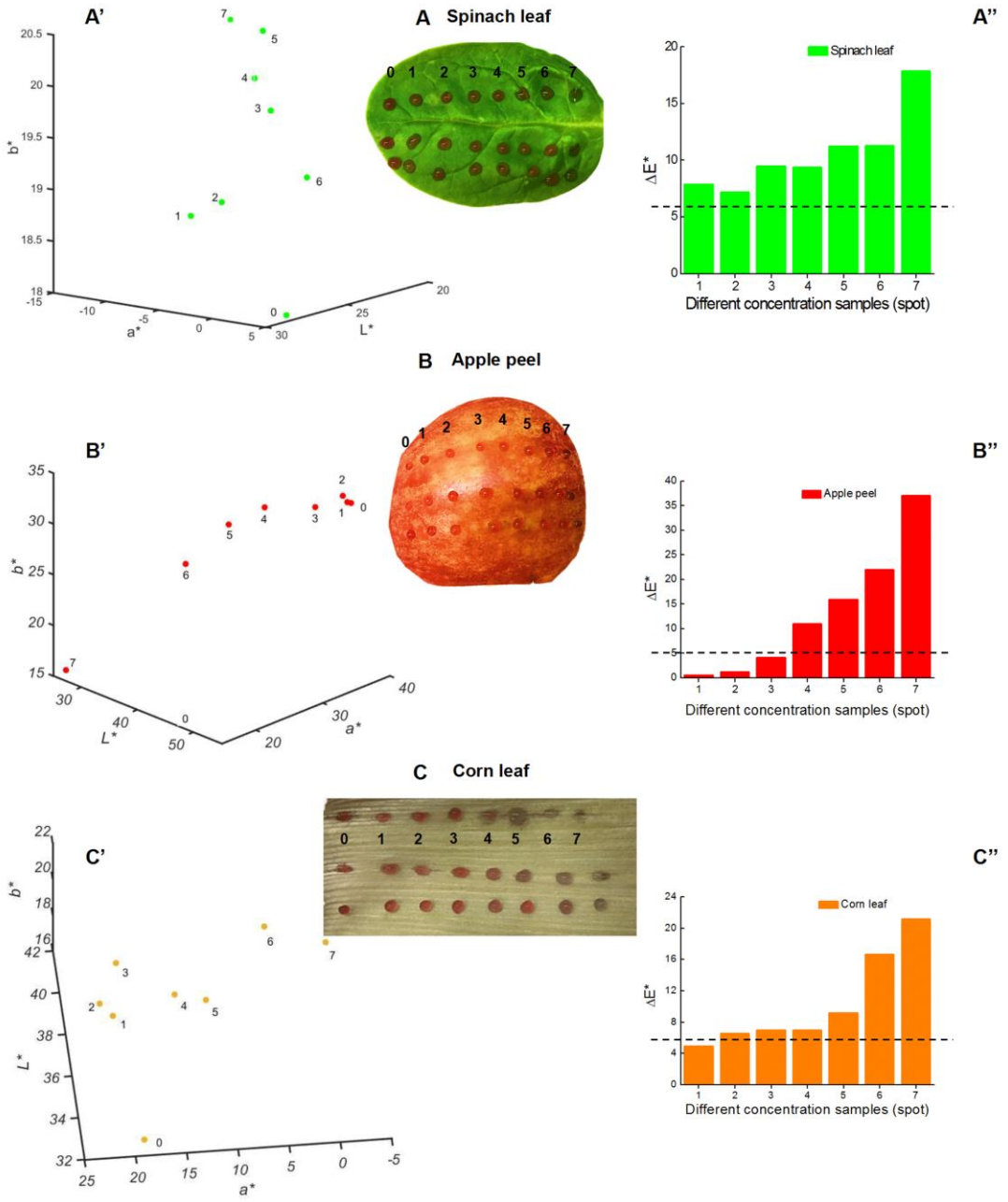
- Figure 3. The photograph of AuNPs-Cys, before (A) and after (B) the addition of different concentrations of glyphosate.

Optimize the experimental conditions for colorimetric detection of Gly. Zhao et al. have reported that large-AuNPs (40-45 nm) provided the highest sensitivity in colorimetric analysis.[15] So in the study, we chose 50 nm citrate-capped AuNP colloids as the material. To optimize the experimental conditions for the colorimetric detection of Gly, the concentration of cysteamine and media pH were investigated. Without cysteamine, the AuNPs did not change color upon adding Gly. However, it is important to control the concentration of cysteamine in the modified AuNPs since too much cysteamine attachment on the surface of AuNPs would decrease their stability and cause a visible color change[21]. Figure S1A shows the photograph of AuNPs with increasing cysteamine concentrations. When the final concentration of cysteamine was increased from 5×10^{-5} to 5×10^{-4} mg mL^{-1}, the light pink color of AuNPs turned darker and appeared



- Figure 4. (A) SERS spectra of AuNPs-Cys-Gly with various concentration; (B) Calibration curve for quantification of glyphosate based on 1591 cm^{-1} peak of AuNPs-Cys-Gly; (C) Calibration curve for quantification of glyphosate based on 1172 cm^{-1} peak of AuNPs-Cys-Gly.

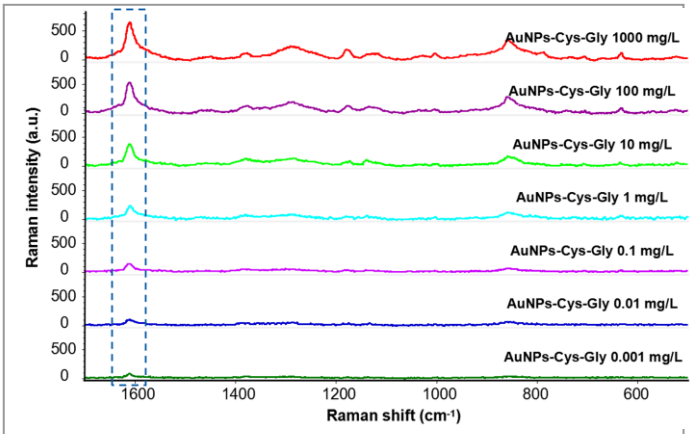
slightly violet. When the concentration was increased to 5 × their pink color in the pH range of 4.0-6.0, but they turned



- Figure 5. The photographs of AuNPs-Cys with different concentrations of glyphosate on spinach leaf (A), apple peel (B), and corn leaf (C), Number 0-7 represents different Gly concentrations (0, 0.001, 0.01, 0.1, 1, 10, 100, 1000 mg L^{-1}); (A' -C') the three-dimensional scatter spots of the average value L*, a*, and b* of three same concentration samples in (A), (B), and (C); (A'' -C'') the histogram of the colorimetric difference (Δ E*) between the sample spots on spinach leaf (A), apple peel (B), and corn leaf (C) and the control spot, the black dot line in (A'' -C'') represents Δ E* is 6.

10^{-3} mg mL^{-1} and higher, the color changed to darker purple and then blue. Therefore, AuNPs modified at a cysteamine concentration of 5 × 10^{-5} mg mL^{-1} were chosen for use throughout the following experiments. To optimize the pH condition, we investigated the effect of media pH in the range of 2.0-6.0 by adjusting different amounts of 0.1 mol L^{-1} HCl. Figure S1B shows that AuNPs-Cys without Gly could maintain blue at pH 3.0 due to the reduced electrostatic repulsion induced by hydrogen ions. At the presence of 1000 mg L^{-1} Gly, AuNPs-Cys in the range of pH 4.0-6.0 changed to blue (Figure S1C), which demonstrates this pH range can work well. The original pH of the system was about 4.56, therefore, we do not need to adjust the system pH during the subsequent experiments.

Determination of the sensitivity of the colorimetric detection of Gly. Upon the optimization, we evaluated the sensitivity of the colorimetric detection in the tube. Compared with the color of AuNPs-Cys without Gly (Figure 3A), The color of AuNPs-Cys gradually changed from wine-red to purple and finally to dark blue with gradually increasing Gly concentrations (Figure 3B). We can observe the difference from the control by the naked eye even at the lowest concentration (0.001 mg L^{-1}), which demonstrates the great sensitivity of the colorimetric detection for Gly in the tube and the potential for rapid screening of the presence of Gly.



- Figure 6. SERS spectra of AuNPs-Cys-Gly with various concentration on corn leaf.

SERS detection of Gly. Follow up the colorimetric screening, SERS was explored for rapid identification and quantification of Gly by detecting SERS signals of the AuNPs-Cys-Gly complex that formed in the colorimetric assay. Figure 4A showed the corresponding Gly concentration-dependent SERS spectra of AuNPs-Cys-Gly. With the increase of Gly concentrations, the stronger AuNPs aggregation occurs due to more interaction between Gly and AuNPs-Cys. Therefore, the characteristic SERS peaks of Gly at 1172 and 1591 cm^{-1} were largely enhanced. At concentrations as low as 0.001 mg L^{-1}, Gly can be clearly detected based on Raman peaks of AuNPs-Cys-Gly. The trend for enhanced SERS intensities at 1172 cm^{-1} and 1591 cm^{-1} varying with Gly concentrations were shown in Figure 4A. Based on the peak at 1172 cm^{-1}, a reasonable linear response was achieved in the concentration range from 0.001 mg L^{-1} to 1000 mg L^{-1} with an R square (R^2 = 0.9856). The regression equation is log Y = 0.2741 log X + 1.8831 (Figure 4C). At 1591 cm^{-1} (Figure 4B), a better linear response (R^2 = 0.991) in the concentration range from 0.001 mg L^{-1} to 1000 mg L^{-1} was obtained with the regression equation as log Y = 0.2119 log X + 2.3168. In the following experiment, therefore, we chose the peak at 1591 cm^{-1} for quantifying quantification of the Gly on plant leaves. According to the standard curve and LOD calculation formula, the LOD in the standard curve is 0.026 mg L^{-1}. The difference between the LOD value and the value which Gly can be detected based on SERS peaks of AuNPs-Cys-Gly is due to the relatively large standard deviation of the blank. Nevertheless, the maximum residue limit for glyphosate on various commodities range from 0.1-100 mg kg^{-1}. Taking the lowest 0.1 mg kg^{-1}, transferring to the extraction based on 10 mL water for a spinach leaf that is 30 g, the solution is 0.3 mg L^{-1}. Our detection limit is way below this level.

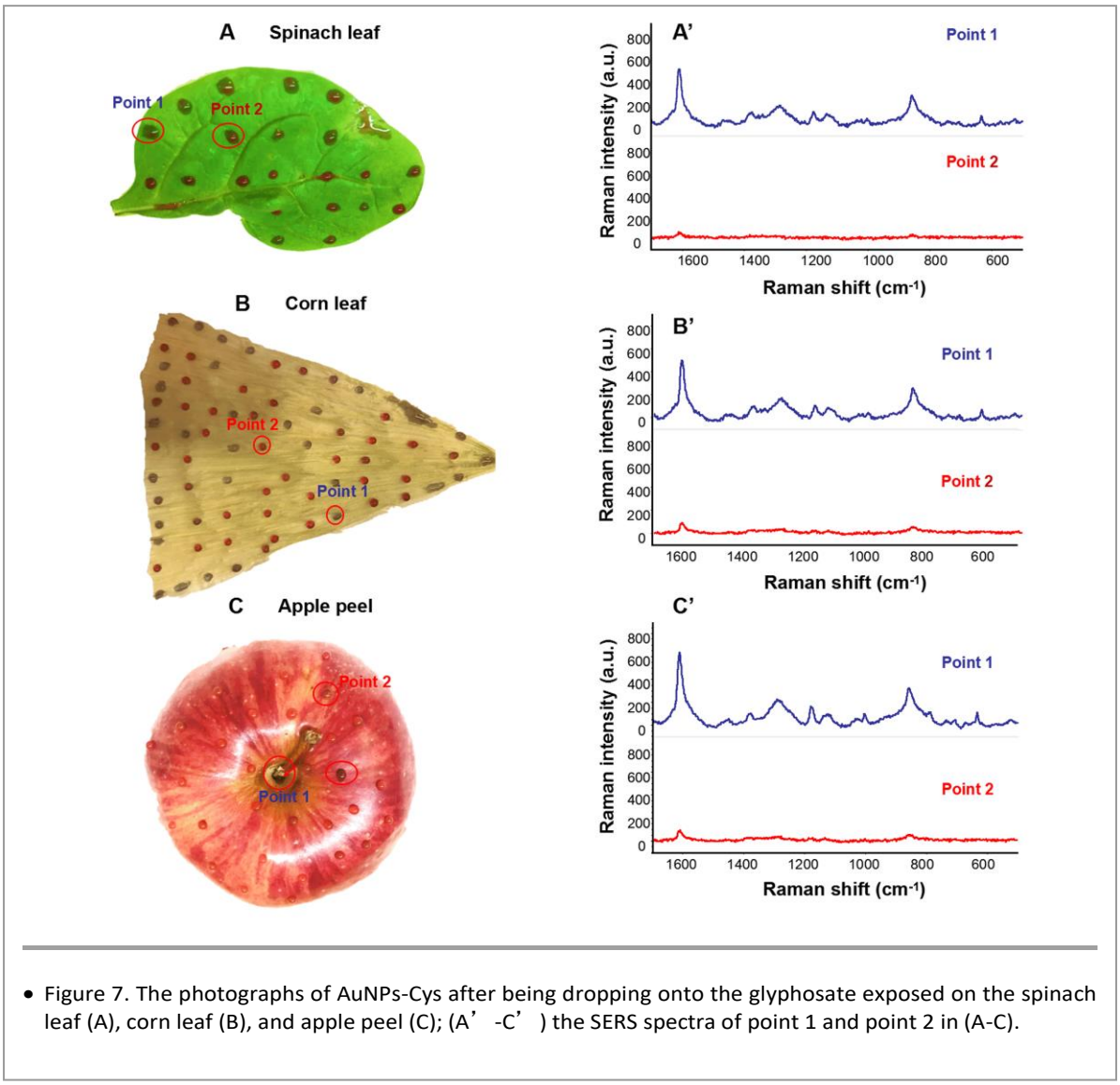
***In situ* colorimetric detection of Gly on plant surfaces**

After determining the capability of colorimetric-SERS detection of Gly in the tube, we explored the potential of this strategy for *in situ* detection of Gly on plant surfaces. Three different plant tissues, including spinach leaves, apple peels and corn leaves, were chosen for representing different background colors. The results are displayed in Figure 5. On the spinach leaf, we can clearly observe the color of AuNPs-Cys changing from wine-red to dark purple with gradually increasing concentrations of Gly. Even at the lowest concentration (0.001 mg L^{-1}), we can still observe clear color difference from the control (0 mg L^{-1}). This result demonstrated the green background from spinach leaves did not interfere the colorimetric detection. We further analyzed the CIELAB L*, a*, b* value and the colorimetric difference (ΔE^*) between the sample spots and the control spot (Figure 5A'' and Table S1). With increasing concentrations of Gly from 0.001-1000 mg L^{-1}, the value of L* which represents the lightness decreased gradually. The value of a* which represents the green-red had a dramatic decrease at the concentration of 0.001 mg L^{-1} compared to the control and gradually reduced over the range of 0.01-100 mg L^{-1}. The value of a* finally dropped obviously at the concentration of 1000 mg L^{-1}. The value of b* which represents the blue-yellow component did not change apparently among different concentrations. The ΔE^* for all the concentrations were all above 6, which was a threshold for human eyes to differentiate between colors[27]. These calculations supported the visual observation that we can clearly see the color difference from 0.001 mg L^{-1} with the control. The following spots with other concentrations appeared darken slowly with a more noticeable change at

1000 mg L^{-1}. On the apple peel, naked eyes were not able to differentiate between control and sample spots with Gly concentration from 0.001 mg L^{-1} to 0.l mg L^{-1} due to the background color interference. The lowest concentration that we can see difference was 1 mg mL^{-1}, and the ΔE^* value supported this finding well (Figure 5B'' and Table S2). Starting from 1 mg mL^{-1}, all values of L*, a*, b* decreased gradually. On the corn leaf, the naked eye observation and the ΔE^* calculation was not in a good agreement. By our naked eyes, we can only observe the difference starting from the 1 mg L^{-1}, however, the ΔE^* value suggested the detection limit at 0.01 mg L^{-1} (Figure 5C'' and Table S3). However, when looking into each value of L*, a*, b*, only the value a* which represents green-red shows gradually decrease when the concentration of Gly increased. The values of L* only changed significantly at higher concentrations. To reduce the background interference, the spot solutions can be transferred by using a pipette to a white background surface for observation. In addition, as human eye sensitivity can vary between individuals, this suggests the use of a computer or smartphone-based color recognition tool may be a direction to explore in the future to aid the eye observation.

SERS analysis

Following the visual observation, we used SERS for further identification and quantification. Initially, we tried to detect the spots after they dried on the tissue surface directly using SERS. However, we were not able to obtain good signals due to the weaker scattering from the tissue surface and some background interference. Then, we transferred the spot solution from the leaf onto a gold coated glass slide and dried for analysis to enhance the sensitivity and reliability of the SERS measurement. Taking the SERS detection of Gly on corn leaf as an example, as compared to the pure Gly in tube analysis, the recovered Gly from the leaf surfaces exhibited similar pattern except a little shift from1591 cm^{-1} to 1609 cm^{-1} which may due to the different condition on tube and on tissue surface (Figure 6). The recovered Gly was quantified using the standard curve established and shown in Figure 4. As shown in Table S4, the recoveries of the Gly spiked at seven concentration



- Figure 7. The photographs of AuNPs-Cys after being dropping onto the glyphosate exposed on the spinach leaf (A), corn leaf (B), and apple peel (C); (A'-C') the SERS spectra of point 1 and point 2 in (A-C).

levels of 0.001 to 1000 mg L^{-1} on corn leaf were in the range of 32.60% to 152.68%. It is worth noting that as low as 0.001 mg L^{-1} of Gly can be detected after recovery (Figure 6).

Study the distribution of Gly on plant leaves

Furthermore, we applied the *in situ* colorimetric-SERS detection method to study the distribution of Gly on plant tissues after carefully spraying the Gly on the leaf surfaces. As seen in Figure 7, it is clear to observe the variations of different AuNPs spots which indicates the distribution of Gly on plant tissues was not uniform. On the spinach leaf (Figure 7A), we observed the most spots in the middle of the leaf appeared red, while the blue spots were more around the edge of the leaf. This demonstrates the Gly tends to accumulate along the edge of a spinach leaf after surface spray. On the corn leaf (Figure 7B), the Gly appeared to be more uniform than on the spinach leaf, which may be resulted from the different surface structure of corn leaf which allows more retention of Gly on the leaf surface. On the apple peel (Figure 7C), it was more difficult to tell due to the red background color. One spot circled appeared to be dark/blue color, and the lower area around the handle also showed dark blue/purple color indicating higher amount of Gly in this position. Further verification was done using SERS on selected spots and confirmed that the blue/purple spots had higher concentrations of Gly as compared to the red spots (Figure 7A'-7C'). The *in situ* colorimetric method provides a facile and rapid way to determine the pesticide distribution on the surface of plant tissues to facilitate an on-site estimation of the effectiveness of pesticide protection after field application or cross contamination on field. Further verification and quantification can be done using a portable Raman on the field or a bench-top Raman in the lab.

Conclusions

In summary, a simple, rapid, fieldable, and colorimetric method for detecting Gly on the plant tissues *in situ* is reported. The Gly on the plant tissues could be monitored *in situ* by the color change of the AuNPs-Cys. As low as 0.001 mg L^{-1} of Gly can be observed by naked eyes on spinach leaves. Apple peels have interfering background which resulted in eye differentiation starting at 1 mg L^{-1}. For corn leaves, although our naked eyes can only tell 1 mg L^{-1} and higher, the L*, a*, b* color system was able to differentiate from 0.001 mg L^{-1}. The quantitative determination of Gly was based on the SERS signal of AuNPs-Cys-Gly at 1591 cm^{-1}, and it is able to detect as low as 0.001 mg L^{-1} of Gly that was recovered from plant tissues. Furthermore, we demonstrated the application of the in situ colorimetric and SERS method to determine the distribution of Gly on different plant tissues. The *in situ* colorimetric method we developed in this study can facilitate the fieldable monitoring of Gly on crops after deploying this pesticide on the field. Further verification and quantification can be realized using SERS if needed. Future study will focus on the integration of portable color analysis tool (e.g. a smartphone) for aiding the naked eye observation on field.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Table of contents

A simple, rapid and fieldable colorimetric method for detecting the pesticide on the plant tissues *in situ* has been developed.