Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A Straw-Housed Paper-based Colorimetric Antibody-antigen Sensor

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This file includes

Protein expression and purification, chitosan pre-treatments, antigen immobilization, incubation of target antigen, investigation of buffer pH, investigation of chitosan-glutaraldehyde ratio, investigation of glutaraldehyde toxicity in protein crosslinking, investigation of chitosan-glutaraldehyde modification on protein immobilization, and supplementary figures.

Supplementary Methods

1. Protein expression and purification

All antibodies and antigens were cultured in 2xYT media at 37 °C with shaking at 200 rpm until OD_{600nm} 0.6. Protein expression was induced with 1mM IPTG at 25 °C and shaking at 160 rpm for overnight. All proteins were then purified with his-tag column (GE Healthcare and Life Sciences; UK) and buffer exchanged with 1x PBS buffer. Buffer exchanged protein fractions were then analysed with 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel to confirm the size. All proteins were stored at -20 °C until use.

2. Chitosan pre-treatments

0.25 mg/mL of chitosan solution was prepared by dissolving 25 mg of chitosan flakes in 100 mL of 0.1 M acetic acid. Solution was heated at 55° C overnight with stirring until all chitosan flakes completely dissolved. The pH of solution was then adjusted to 4.5 with NaOH solution and stored at 4 °C until use.

3. Antigen immobilization on sensor

3.1 For dipstick:

10µL of 250µg/mL of antigen in 1x PBS buffer (pH 7.4) was coated to the corresponding paper disk on the sensor and dried at room temperature (rt). Then, 10µL of 1% BSA was applied to the paper disk to block any remaining active sites which would cause unspecific binding. Excess BSA was removed by washing with 200µL of 1 X PBST (0.1% Tween) for 2 min with shaking at 600rpm. All the washes were performed in 2mL round bottom microcentrifuge tube. Then, the paper disk was allowed to dry and ready for use.

3.2 For dipstick in straw:

Three pieces of chitosan-glutaraldehyde treated paper disks were assigned as positive control (+), sample (S), and negative control (-) as shown in Figure S-7. The (+) paper disk was coated with 2 µg of target antibody while (S) was coated with 5µg of target antigen in 1X PBS buffer while (-) was not coated with anything. All three paper disks were blocked with 10µL of 1% BSA and left to dry at rt. The paper disks were then washed with 200 µL of 1 x PBST to remove excess BSA on disk surface. The rigid film was cut to 2 cm x 0.5 cm (length x width) in dimension. The three paper disks were then positioned onto the plastic strip with double sided tape.

4. Incubation of target antibody

4.1 For dipstick:

2 µg of target antibody in 1% of BSA was deposited to the modified paper disk on the sensor and left to incubate until the paper was dried. Unbound antibody was removed by washing with 200 µL of 1 X PBST (0.1% Tween) for 2 mins with shaking at 600rpm. After the paper

disk was dried, 5 µL of Protein L HRP in 1% BSA was dispensed and followed by 2 mins of incubation. The sensor was washed with 500µL of 1 X PBST (0.1% Tween) for 5 mins with shaking at 600 rpm. The sensor was dipped into 200 µL of ABTS solution and allowed to develop in the dark for 10 mins.

4.2 For dipstick in straw:

The sensor was immersed in 300 µL of unknown sample inside the drinking straw of 5 cm length and stapled at the edges after folding. The sensor was incubated inside the straw for 10 mins with shaking by hands. Subsequently, the sensor was washed with 300µL of 1 x PBST for 2 mins with shaking in the straw. The sensor was then incubated with 200 µL of Protein L HRP solution inside the straw for 2 mins. Finally, the sensor was washed with 500 µL of 1 x PBST for 5 mins with vigorous shaking, followed by incubation in 300 µL of ABTS solution in the straw. A waiting period of 10 mins was used to allow the assay to develop on the paper disks as shown in Fig. 3A and 3B . Detection limit of the straw sensor was carried out by incubating the sensor inside the straw with different concentrations of target antibody (0.5 nM, 1 nM, 2 nM, 3 nM, 5 nM, and 15 nM).

5. Investigation of buffer pH on protein immobilization on cellulose paper

0.25 mg/mL of chitosan solution was prepared as mentioned previously. The pH of solution was then adjusted to 3, 4, 4.5, 5 and 6 respectively with 1 M of NaOH solution. 5 µL of chitosan solution with different pH was pipette onto the paper disks and left to dry. Number labelled on paper disk represents the pH of chitosan solution. Next, 5 µL of 2.5 % glutaraldehyde was deposited onto the chitosan modified paper surface and washed with 10µL of 1 x PBS buffer. After the paper disks dried, 2 µg of fluorescent protein (cherry/ eGFP) was pipette onto the chitosan-glutaraldehyde modified paper and incubated for 1 minute. The paper was then washed vigorously with 300 µL of 1 X PBST at 800 rpm for 10 minutes. Documentation of results was carried out by smart phone camera. Quantification of intensity of fluorescent protein on paper was performed by Adobe Photoshop CS2 software using 'Red' channel for cherry and 'Green' channel for eGFP.

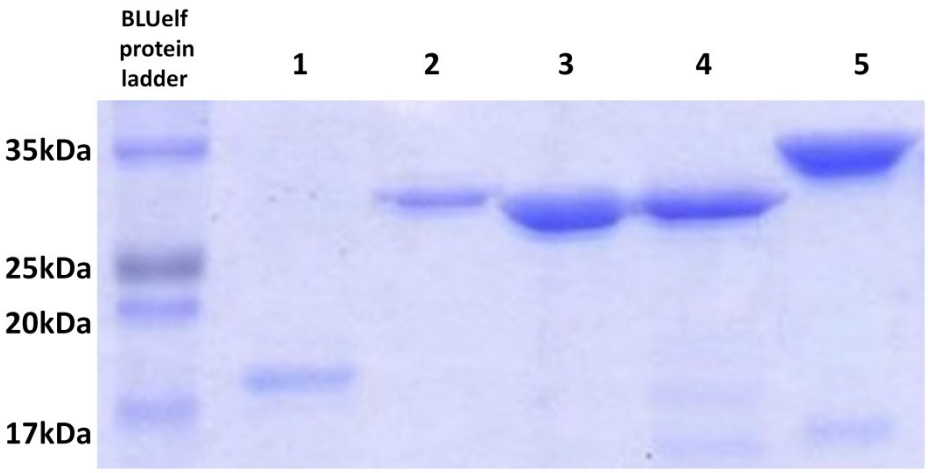
6. Investigation of ratio of chitosan and glutaraldehyde on protein immobilization on cellulose paper

A total volume of 10 µL chitosan and glutaraldehyde solution was applied onto cellulose paper. Control paper disk without pre-treatment by chitosan and glutaraldehyde was labelled as (C) for cherry and (e) for eGFP. Paper disks were labelled with C_x or e_x where x represents volume of chitosan applied onto cellulose paper. For example C1 indicates the paper was pre-treated with 1 µL of chitosan and 9 µL of glutaraldehyde. Pre-treated paper disks were washed with 10 µL of 1 x PBS buffer and excess buffer was removed by a piece of normal tissue paper by contact with the paper disk. 2 µg of each fluorescent protein was applied onto pre-treated cellulose paper and incubated for 1 minute. Then, the paper disks were washed vigorously in 300 µL of 1 X PBST for 10 minutes at 800 rpm. Documentation was performed under UV_{302nm} and intensity of fluorescent was measured by Adobe Photoshop CS2. The mean intensity of fluorescent was obtained by setting the 'Channel' to 'Red' for cherry coated paper disks and 'Green' for eGFP coated paper disks.

Supplementary Figures

Label	Membrane
1	CF 6
2	Whatman filter paper 1#
3	Fusion 5
4	VF 2

Figure S-1. Selection of suitable membrane for fabrication of paper based sensor. All the membranes above except for Whatman filter paper 1# were obtained from GE Healthcare Sdn Bhd. The membranes were named after the product name or commercial name. 5 µL of pink dye was pipette onto the punched out membranes to compare the permeability, absorbency and wicking rate of membrane.



1	Ubiquitin antigen
2	Anti-ubiquitin antibody
3	Enhanced green fluorescent protein (eGFP) antigen
4	Anti-eGFP antibody
5	Cherry protein

Figure S-2. 12 % SDS-PAGE gel showing the size of purified proteins used in this work.

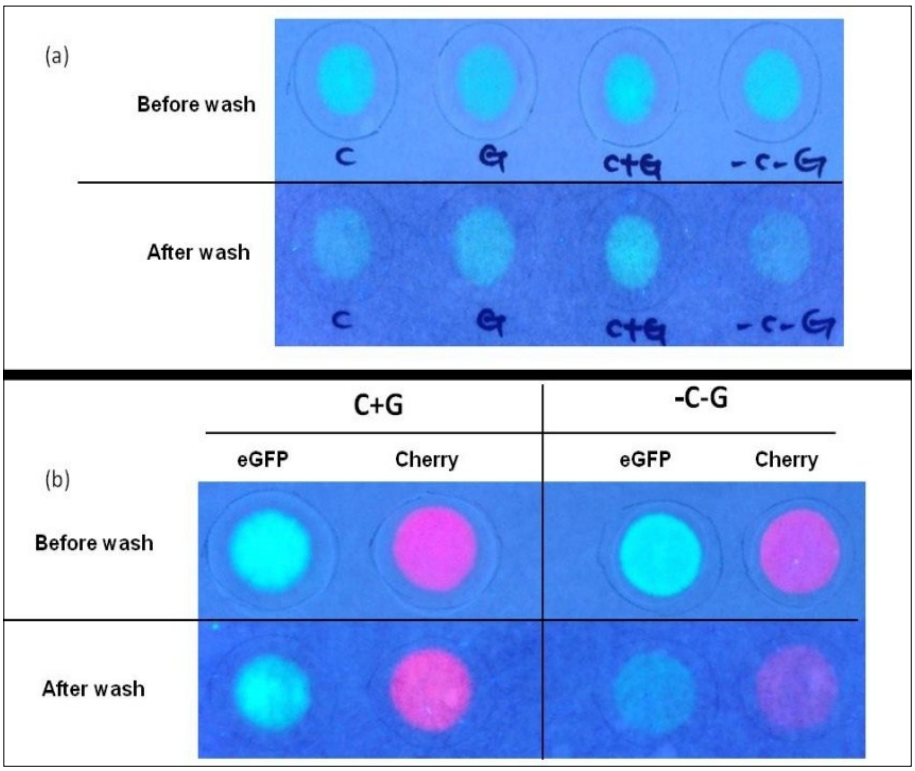


Figure S-3. Immobilization optimization and analysis. (a) Intensity of enhanced green fluorescent protein (eGFP) before and after washed under UV_{302nm} with different combination of chitosan and glutaraldehyde. (b) Comparison of protein immobilization on paper with and without chitosan and glutaraldehyde by using eGFP and cherry protein. Labels used are (C): chitosan; (G): glutaraldehyde; (C+G): chitosan and glutaraldehyde; (-C-G): without both chitosan and glutaraldehyde.

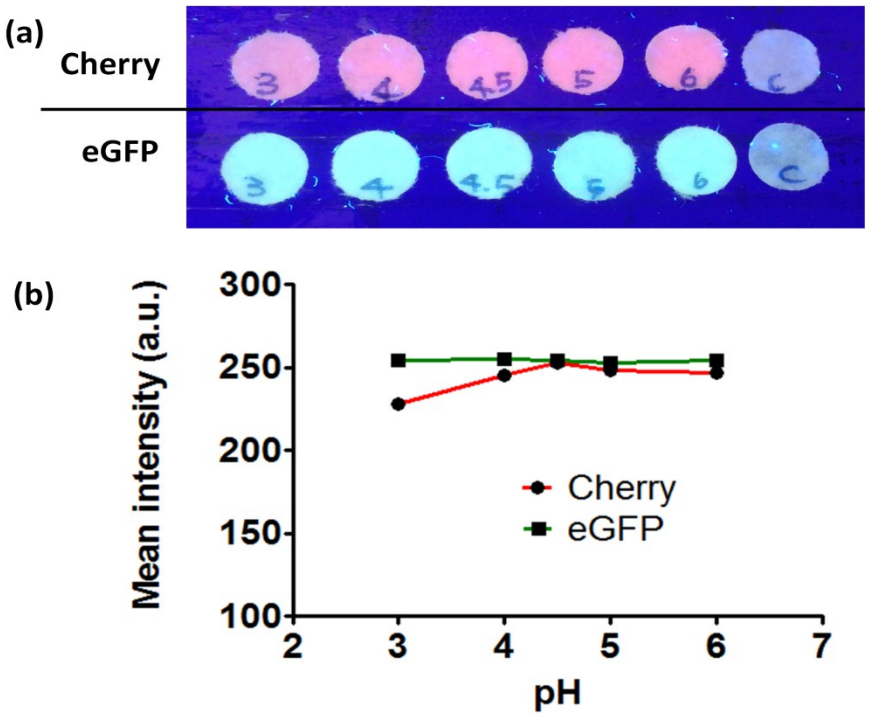


Figure S-4. Investigation of buffer pH on protein immobilization on cellulose paper. (a) Documentation of cherry and eGFP protein immobilized on cellulose papers with different buffer pH under UV_{302nm}. (b) Quantification of fluorescent intensity of fluorescent protein coated cellulose paper disks with different buffer pH using Adobe Photoshop CS2 software.

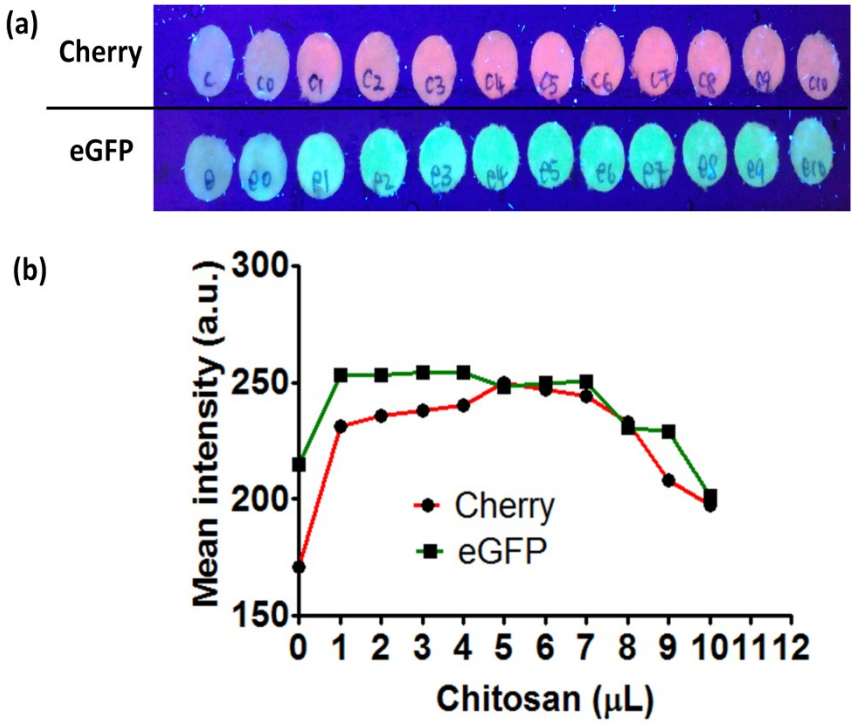


Figure S-5. Investigation of chitosan-glutaraldehyde molar ratio on protein immobilization. (a) Documentation of fluorescent protein immobilized on paper disks with different molar ratio of chitosan and glutaraldehyde under UV_{302nm}. (b) Line graph shows the mean intensity of fluorescent protein with different molar ratio of chitosan and glutaraldehyde.

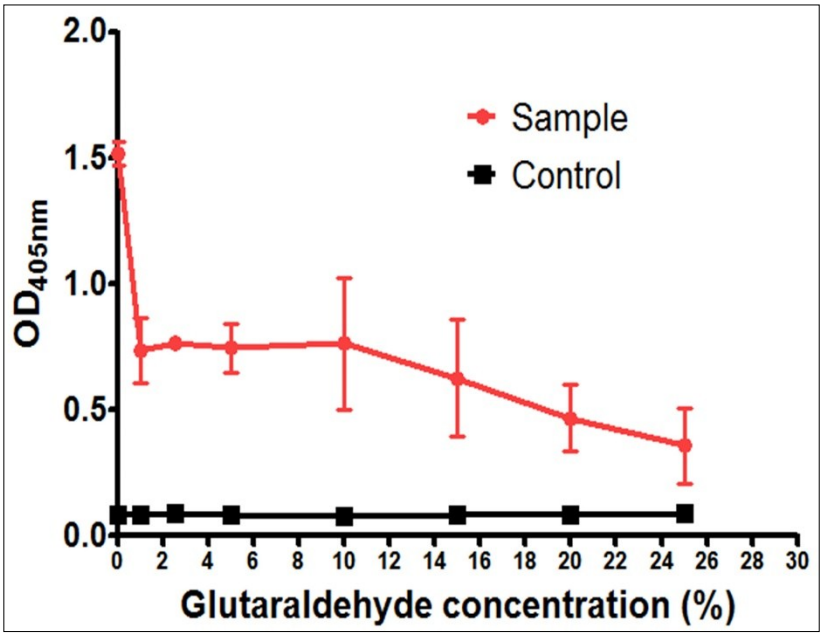


Fig. S-6. Investigation of glutaraldehyde toxicity during protein crosslinking. Each line represents an average from three repeated experiments and the error bars show one standard deviation.

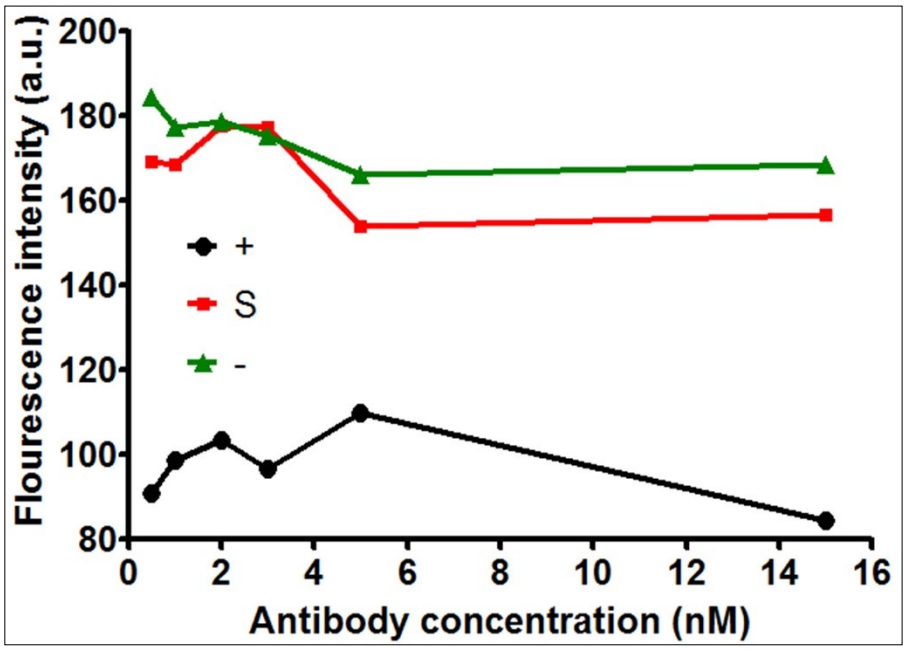


Fig. S-7. Investigation of chitosan-glutaraldehyde modification on protein immobilization. Line graph showing the fluorescence intensity of paper disks incubated with a range of antibody concentrations. (+) paper disk: positive control paper disks coated with antibody; (S) paper disk: unmodified paper disk; (-) paper disk: negative control paper disks modified with chitosan-glutaraldehyde and coated with 1%