Label-Free Microarrays to Probe Host-Pathogen Interactions

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Introduction and Background:

Carbohydrates play an important role in mediating several processes in biology including the host-pathogen response[1] making them an important area for research. A class of carbohydrate binding proteins known as lectins mediate these interactions.

Current methods for researching these interactions involve either using labelled proteins which can impact on binding efficiency of the lectin[2] or antibodies both of which are very expensive and not suitable for testing in third world countries where equipment like plate readers is less readily available. Gold nanoparticles (AuNPs) have previously been used in the study of lectins[3] and represent an important label-free method for detection of lectin binding (figure 1).

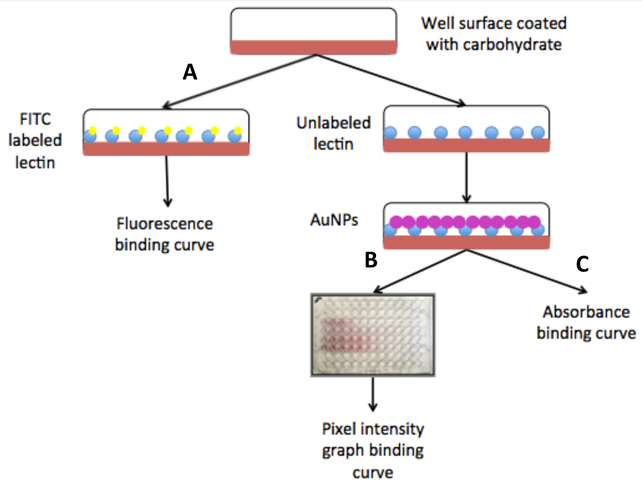

Figure 1: Method A shows the addition of FITC labelled lectin to a carbohydrate coated surface, measurement of fluorescence is then used to produce a binding curve. Method B shows addition of unlabelled lectin to a carbohydrate-coated surface followed by the addition of gold nanoparticles (AuNPs). A picture is then taken and after processing in ImageJ a binding curve is produced using pixel intensity data. Method C is similar to method B but after addition of the gold nanoparticles absorbance is measured.

1. Proof of Principle:

Addition of gold nanoparticles (AuNPs) to a plate functionalised with either bovine serum albumin or G_{M1} showed that the gold nanoparticles will bind selectively to protein and so can be used to visualise protein on a carbohydrate surface. Binding produced a peak in absorbance at around 530 nm indicating the presence of gold nanoparticles (figure 2).

- AuNPs of the ratio 3.5:1 were found to be the best at detecting changes in protein (figure 2B)
- 0.03 mg/ml was the lowest detectable amount of protein.
- This techniques was shown to be reproducible.

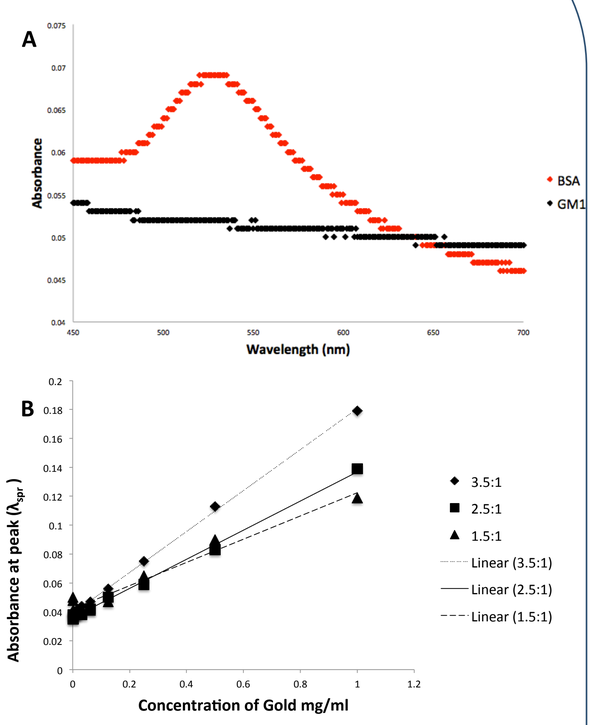

Figure 2: Binding of gold nanoparticles to BSA produced a peak absorbance at 530 nm which was not seen when AuNPs were added to G_{M1} (A). Various different nanoparticle sizes were then compared in their ability to detect protein levels. 3.5:1 was found to be the best (B)

2. Validation of AuNPs vs. fluorescence:

This technique was then applied to 3 model lectins (concanavalin A, cholera toxin subunit B and peanut agglutinin) and results were compared to those obtained when fluorescently labelled lectin is used.

- Detection with AuNPs was as good as detection with fluorescently labelled proteins.
- Detection of fluorescently labelled protein with AuNPs was performed to validate the technique.
- Use of AuNPs to visualise protein binding is faster and significantly cheaper than FITC labelling.

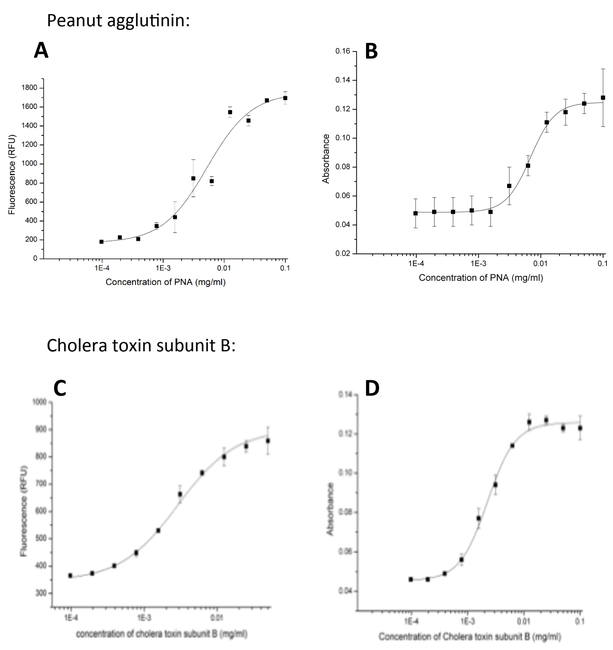

Figure 3: Serial dilutions of various model lectins were added to high binding plates functionalised with their substrate carbohydrates. Data obtained using FITC labelled lectins (A and C) were compared to those obtained using AuNPs (B and D).

3. Evaluation of lectin inhibitors:

- Lectins are involved in pathogenic interactions in the body including cholera infection.
- Inhibitors are needed to prevent the binding of the lectins to their substrates to prevent infection and AuNPs can be used to screen for these.
- Various glycopolymers were tested for their inhibitory effect on lectin binding.
- Polymer 1 was the best polymer and this has a short chain and a short linker[4].
- There was found to be no difference in adding a methyl to mannose when compared to ethyl mannose

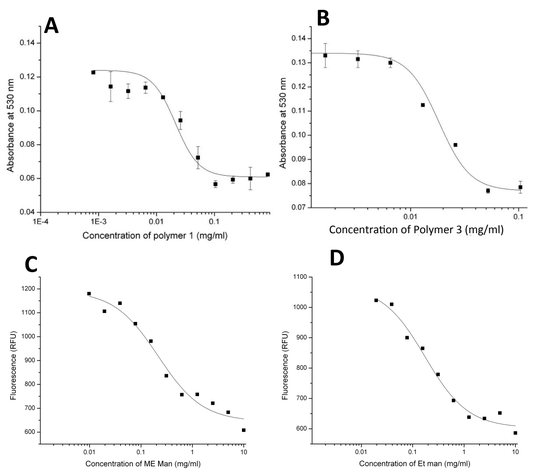

Figure 4: Several inhibitors were tested using both fluorescence and gold nanoparticles. Graphs A and B show polymer 1 and 3 respectively visualised using gold nanoparticles and graphs C and D show two mannose polymers (Methylmannose and ethlymannose respectively).

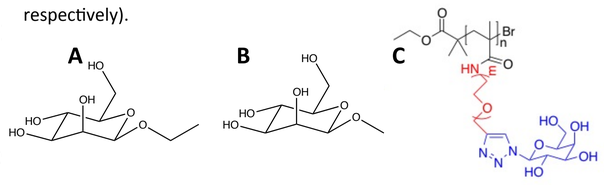

Figure 5: structures of the various inhibitors tested. A is ethylmannose, B is methylmannose and C is the structure of the various other polymers tested.

4. Low cost protein detection:

- Detection of UV-vis to determine gold binding is very effective for detecting protein binding but it requires expensive equipment.
- The binding of gold can be seen visibly as a pink colour.
- A photograph of a plate treated with AuNPs can be used in image analysis to determine protein binding.
- The image analysis technique is simple, cheap and could possibly be implemented on a mobile phone in the future.

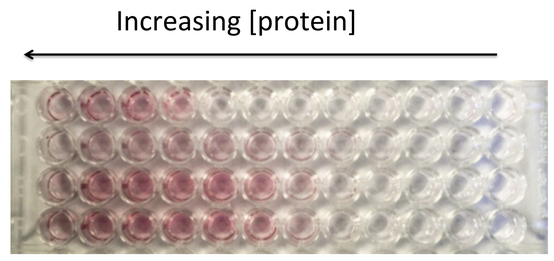

Figure 6: Image of a plate taken after treatment with gold nanoparticles.

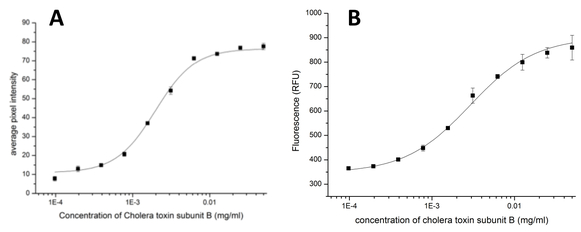

Figure 7: A picture was taken of a plate treated with AuNPs and after processing in ImageJ a binding curve was produced (A). This binding curve was then compared to that achieved using FITC labeled cholera toxin subunit B (B) and the correlation between the two indicating that the two binding curves are equivalent (R^2=0.99).

5. Conclusions:

- Gold nanoparticles can be used to easily detect lectin binding to a carbohydrate surface.
- The data produced strongly agrees with that seen using fluorescently labelled proteins.
- When AuNPs bind there is a clearly visible change in the well and this can be utilised to produce a low cost detection assay using image analysis.

References

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