Glycosylated nanomaterials: Detection and neutralisation of pathogenic bacteria and toxins

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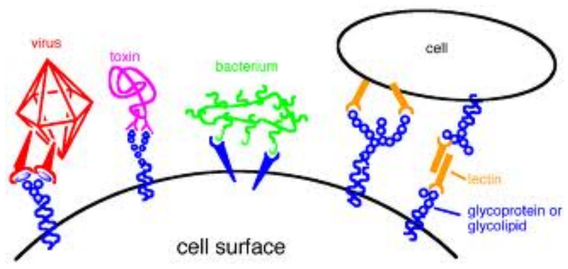
Background

Protein-carbohydrate interactions mediate a multitude of critical biological recognition processes.[1] The proteins responsible for deciphering this information are termed lectins.[2]



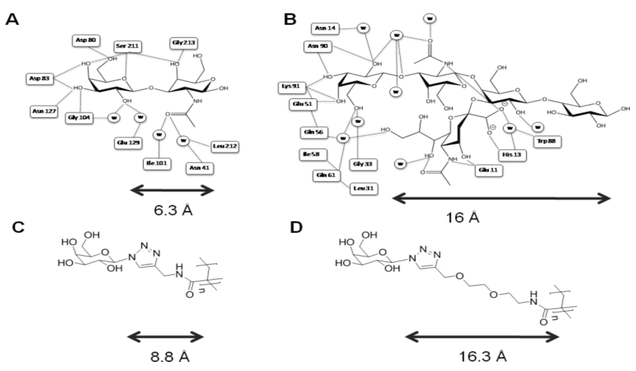
- Polymers with pendent carbohydrate moieties (glycopolymers) interact with lectins and have demonstrated binding affinities several orders of magnitude greater than a single carbohydrate.[3]
- The identification and treatment of bacterial infections remains a major healthcare challenge, especially to ensure appropriate application of a limited spectrum of antibiotics.

Inhibition of Bacterial Toxins

Bacterial Toxin Binding

The cholera toxin (CTx) secreted by *Vibrio cholerae* binds glycosides expressed on the cell surface. Materials with high-affinity and selectivity for these lectins could find applications as anti-adhesive agents.

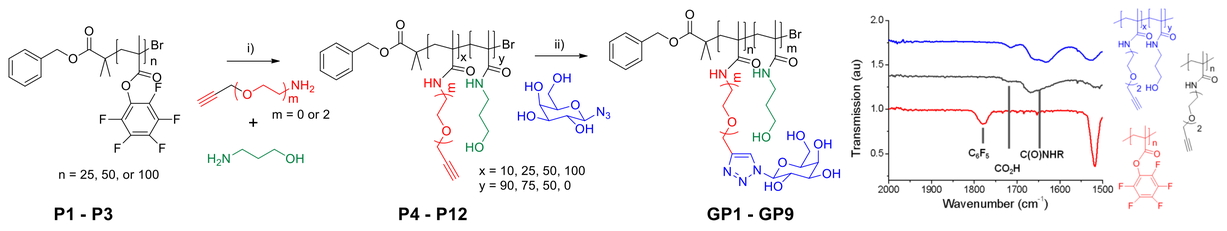
PNA binding site *CTx binding site*



- Probe influence of chain length, carbohydrate density and linker length on binding inhibition.
- Glycopolymer library produced by tandem post - polymerisation modifications.
- Structural biology indicates CTx has deeper binding site than other galactose binding lectins such as Peanut agglutinin (PNA).

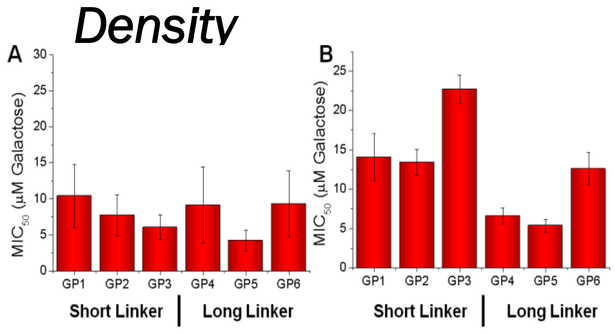
Tandem Post-Polymerisation Modification

'Clickable' units are not compatible with controlled radical polymerisation. Instead, tandem-post polymerisation modification are performed.



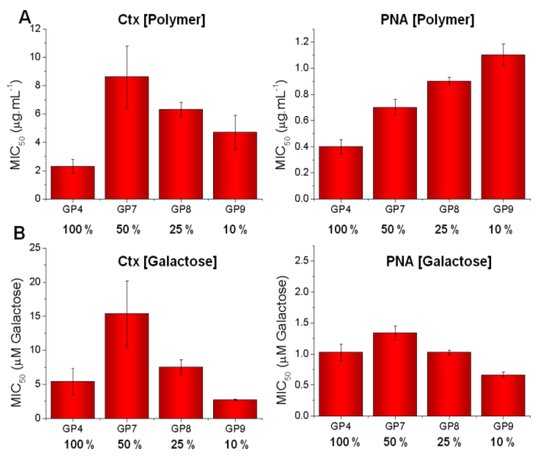
- Poly(pentafluorophenyl methacrylate) for easy modification.
- β-D-galactose was 'clicked-on' to pendent alkyne moieties.
- Results in biocompatible methacrylamide based (co)polymers.

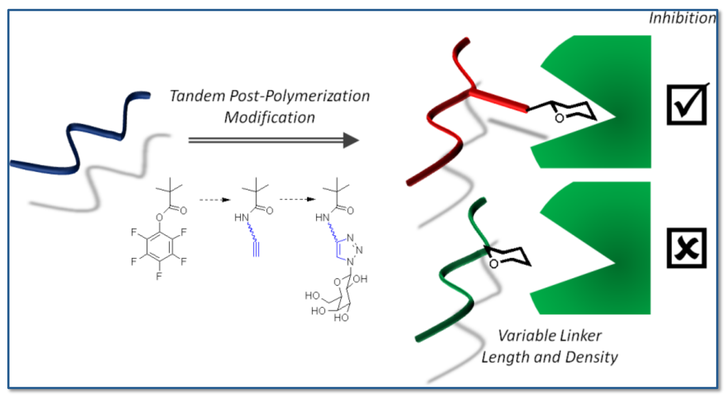
Role of Linker Length and Carbohydrate Density



- Polymers synthesised with ~ 6 Å (short) and 16 Å (long) linkers.
- Linker length has no effect on PNA inhibition (A).
- Longer linker has 2 – 3 fold lower MIC_{50} compared to shorter linker on inhibiting CTx (B).
- 100 X more active than free galactose.

- Polymers synthesised with 10, 25 and 50 % galactose.
- By mass (A), low galactose densities lead to a relative decrease in binding affinity/inhibitory activity.
- By mole of galactose (B), 10 % and 100 % functionalised polymers are the most active suggesting several features (*e.g.* sterics and site spanning) contribute to inhibitory activity.





Summary

- Tandem post-polymerisation modification allows synthesis of polymers from same chain length distribution.
- Longer linker has better binding site accessibility.
- Inhibitors have to be developed for the binding site.

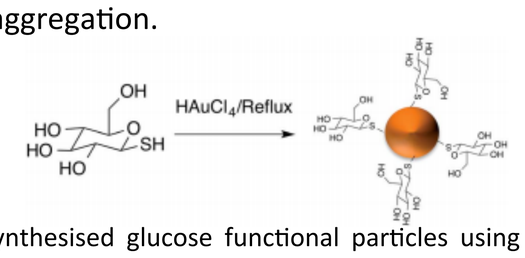
Richards, S-J.; Jones, M. W.; Hunaban, M. I.; Haddleton, D. M.; Gibson M. I. *Angew. Chem Int. Ed.*, **2012**

Jones, M.W. *et. al.*, *Polym. Chem.*, **2013**
Jones, M. W. *et. al.*, *Chem. Sci.*, ***2014***

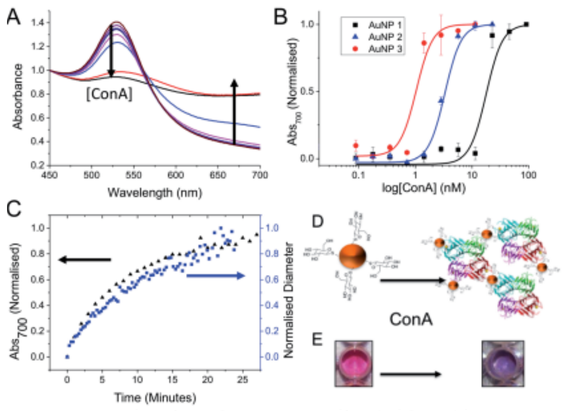
Detection of Lectins and Bacteria

Glycogoldnanoparticles

Goldnanoparticles have interesting optical properties. Red in solution → blue upon aggregation.

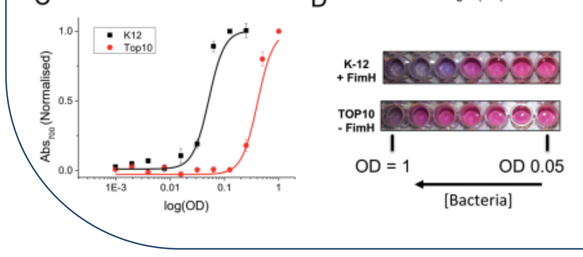


Synthesised glucose functional particles using a one-pot method.



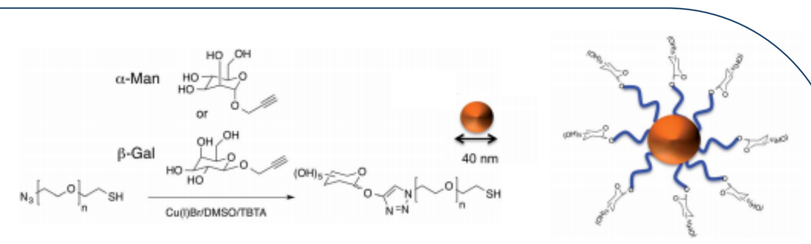
- Concept proved using Concanavalin A (ConA).
- Size dependent aggregation (B).
- Visible colour change (E)

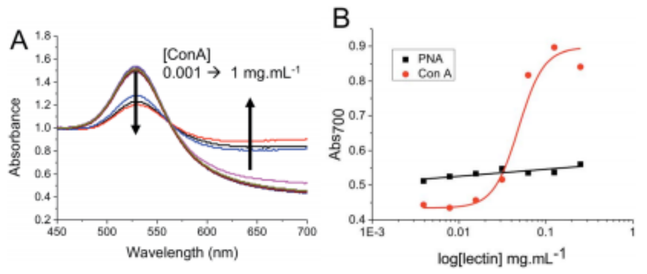
- Tested for aggregation in response to FimH adhesin.
- Colour change noted with K12 (FimH +ve) and not with TOP10 (FimH –ve) until very high bacteria concentration.



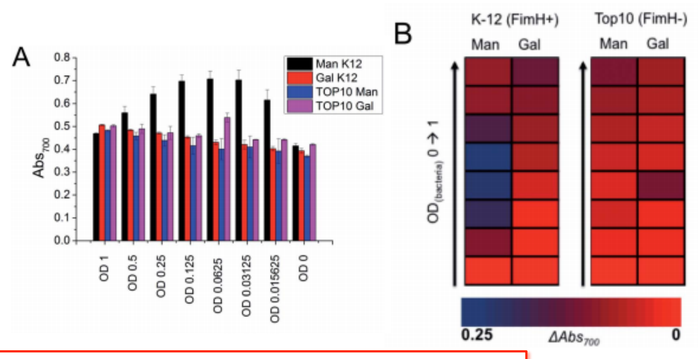
Colour change ☑ Specific ☑ Saline Stable ☒

Increased saline stability by using a Poly(ethylene glycol) linker.
Carbohydrate introduced using a alkyne azide 'click' reaction.





- Tested for response to Type I fimbriated *E. coli*.
- No colour change but increased aggregation noted by Absorbance with Mannose particles with K12 (FimH +ve).



- Stable up to at least 1 M NaCl
- Lectin Specificity – aggregation with ConA (mannose specific) but not with PNA (galactose specific)

Colour change ☒ Specific ☑ Saline Stable ☑

Summary

- Developed a sensitive, rapid colourimetric bioassay for the detection of lectins and Type I fimbriated bacteria.
- PEG layer increases saline stability (important for point of care diagnostics) but visual output is dramatically reduced.

Richards, S-J.; Fullam, L. C.; Besra, G. S.; Gibson M. I., *J. Mater. Chem.*, **2014**

References

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2. M. Ambrosi, N. R. Cameron, B. G. Davis, *Org. Biomol. Chem.*, **2005**, 3, 1593.
3. S. G. Spain, M. I. Gibson, N. R. Cameron, *J. Polym. Sci., Part A: Polym. Chem.*, **2007**, 45, 5031

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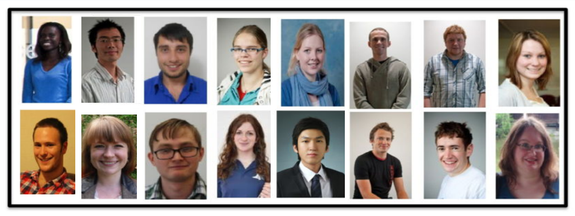

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