Glycopolymer–Lectin Interactions and Inhibition of Pathogens using Multivalent Scaffolds

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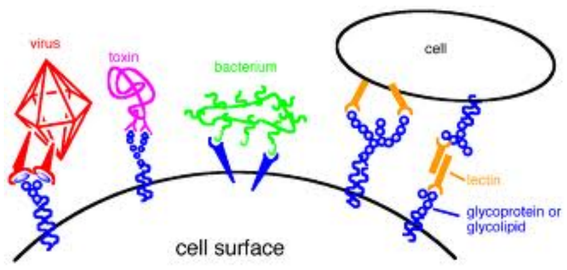
Background

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

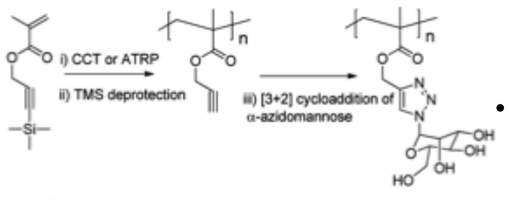


- Polymers with pendent carbohydrate moieties (glycopolymers) interact with lectins and have demonstrated binding affinities several orders of magnitude greater than a single carbohydrate.[3]
- Interference at this early stage is known as anti-adhesion therapy and does not kill the pathogen. Importantly, it prevents binding and hence internalisation which markedly reduces the chance of becoming resistant.[4]

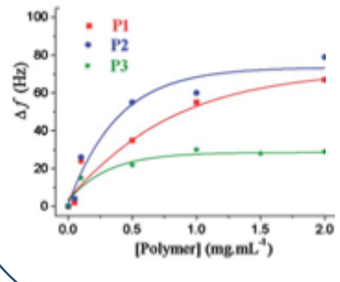
Comparison of Surface Binding with Inhibitory Activity

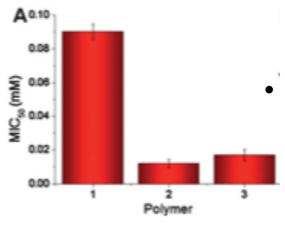
Glycopolymer–Lectin Interactions

The nature of the interactions between glycopolymers and lectins, and the structural features necessary to obtain high-affinity materials are not fully understood.[2] In this study, we probed multivalent interactions in the α-mannose - Concanavalin-A (ConA) pairing:



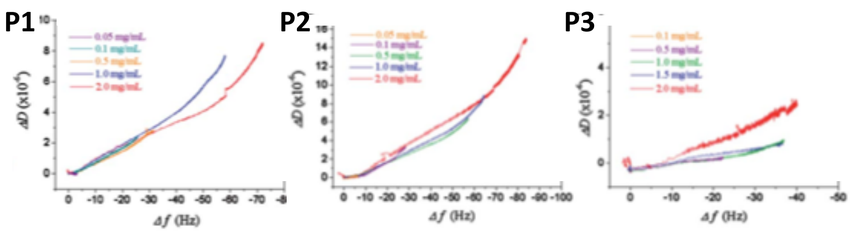
- Post-polymerisation Modification: α-D-mannose was 'clicked-on' to a poly-(propargyl methacrylate) backbone.
- Polymer Chain Lengths: 2 (**P1**), 6 (**P2**) and 11 nm (**P3**).
- Binding inhibition was assessed using Quartz-crystal microbalance with dissipation monitoring (QCM-d) and Fluorescence-linked sorbent assay (FLSA).
- Higher binding affinity = increased mass of glycoside binding to the lectin surface. This property is used to screen new inhibitors.



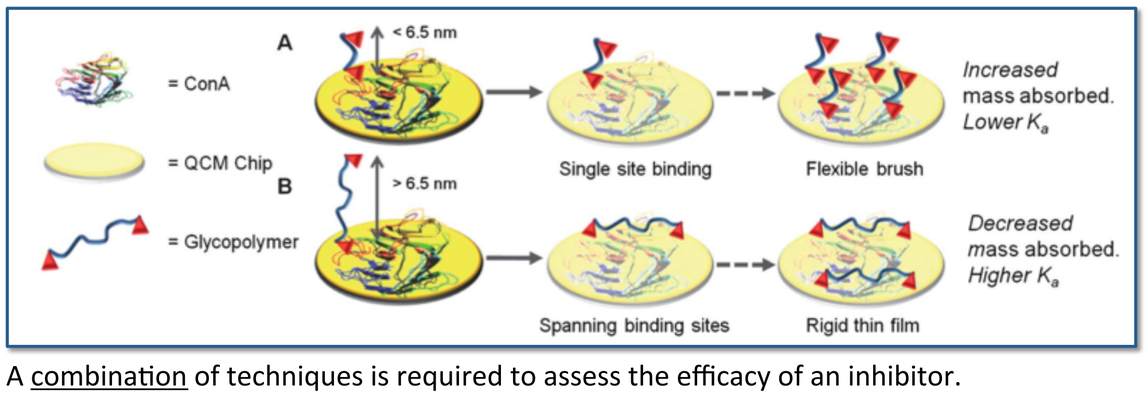


Binding Modes

P2 and **P3** inhibited ConA 10x more effectively than **P1**. However, this is in contrast to the QCM studies:



- QCM-d also probes the viscoelasticity of films formed on a surface.
- Large changes in dissipation (ΔD) indicate a flexible film, whilst small ΔD values suggest a rigid, non-flexible coating: The longer chain polymer (**P3**) spans the binding site of ConA whereas the shorter polymers (**P1/P2**) can only bind one site.



- A combination of techniques is required to assess the efficacy of an inhibitor.

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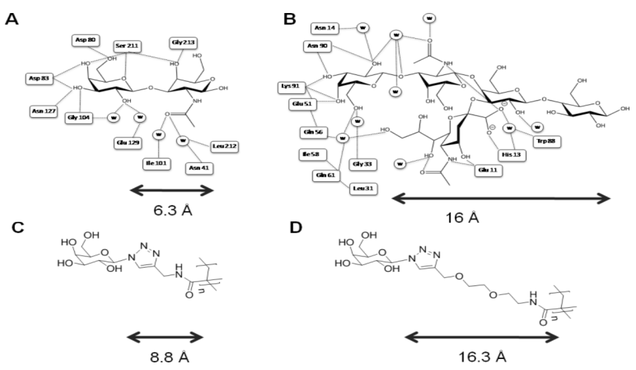
Inhibition of Bacterial Toxins

Bacterial Toxin Binding

The cholera toxin (CTx) secreted by *Vibrio cholerae* binds glycosides expressed on the cell surface.[5] Materials with high-affinity and selectivity for these lectins could find applications as anti-adhesive agents.[4]

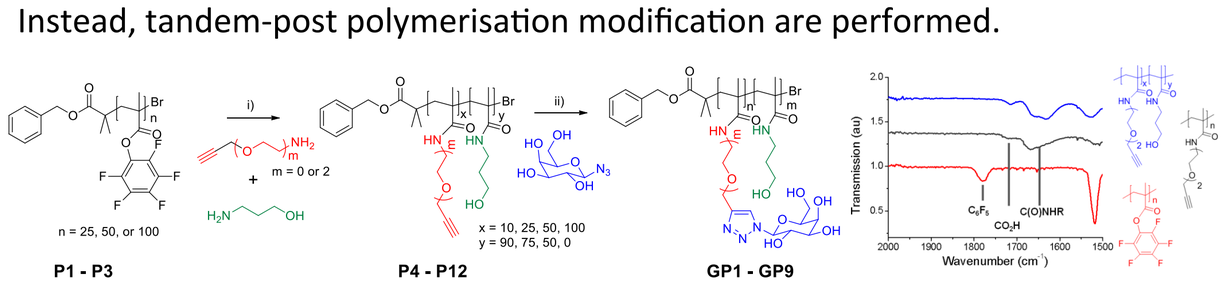
PNA binding site *CTx binding site*



- 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).

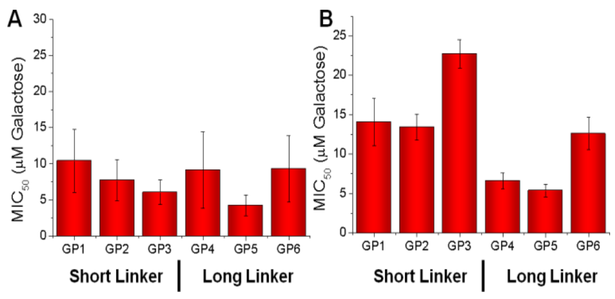
Tandem Post-Polymerisation Modification

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



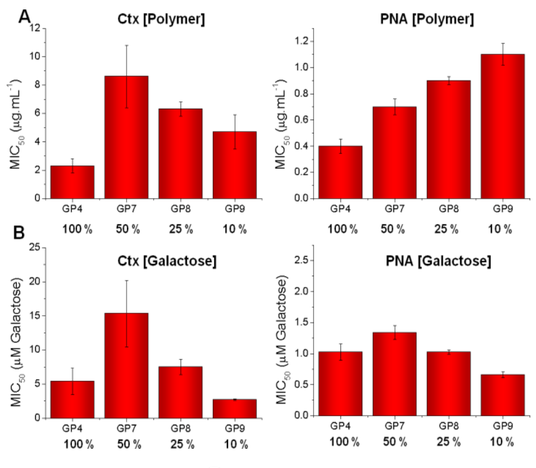
- Poly(pentafluorophenyl methacrylate) for easy modification.
- β-D-galactose was 'clicked-on' to pendent alkyne moieties.
- Results in biocompatible methacrylamide based (co)polymers.

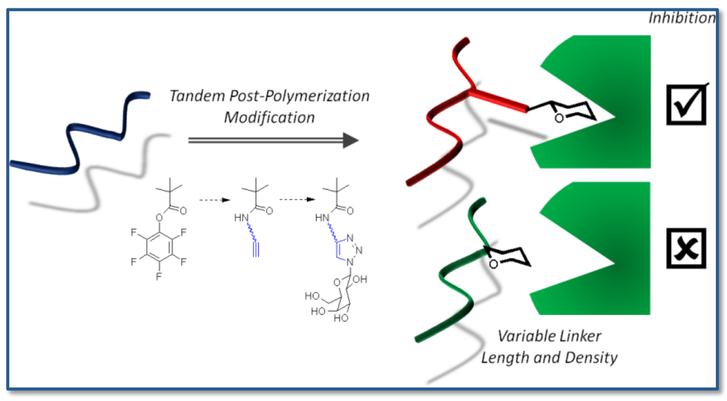
Role of Linker Length and Carbohydrate Density



- 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.
- Carbohydrate density has an effect.
- 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**

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Acknowledgements

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