

# Glycopolymer–Lectin Interactions and Inhibition of Pathogens using Multivalent Scaffolds

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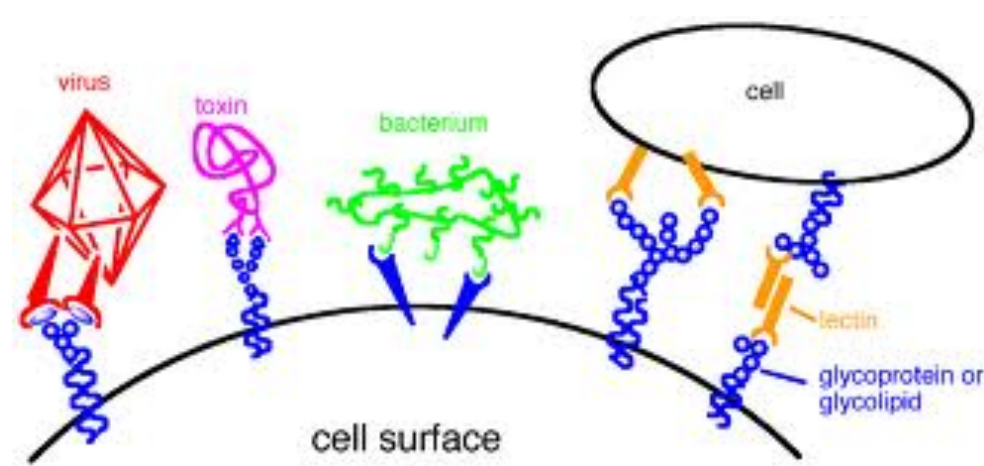
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## Background

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

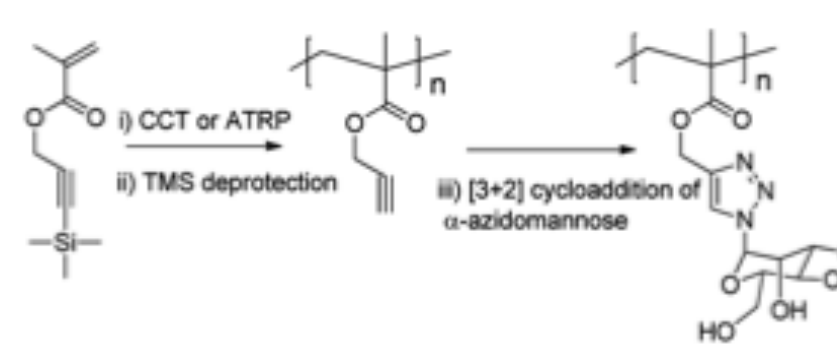


- Polymers with pendent carbohydrate moieties (glycopolymers) interact with lectins and have demonstrated binding affinities several orders of magnitude greater than a single carbohydrate.<sup>3</sup>
- Interference at this early stage is known as anti-adhesion therapy and does not kill the pathogen.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

## 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.<sup>2</sup> In this study, we probed multivalent interactions in the  $\alpha$ -mannose - Concanavalin-A (ConA) pairing:

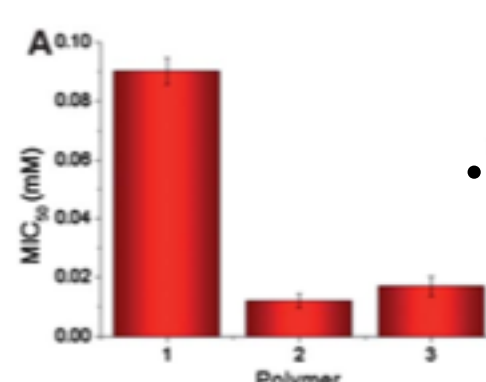
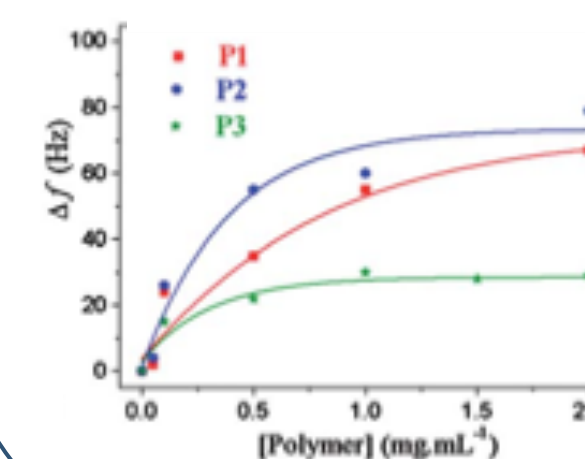


- Post-polymerisation Modification:  $\alpha$ -D-mannose was 'clicked-on' to a poly-(propargyl methacrylate) backbone.

- 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).

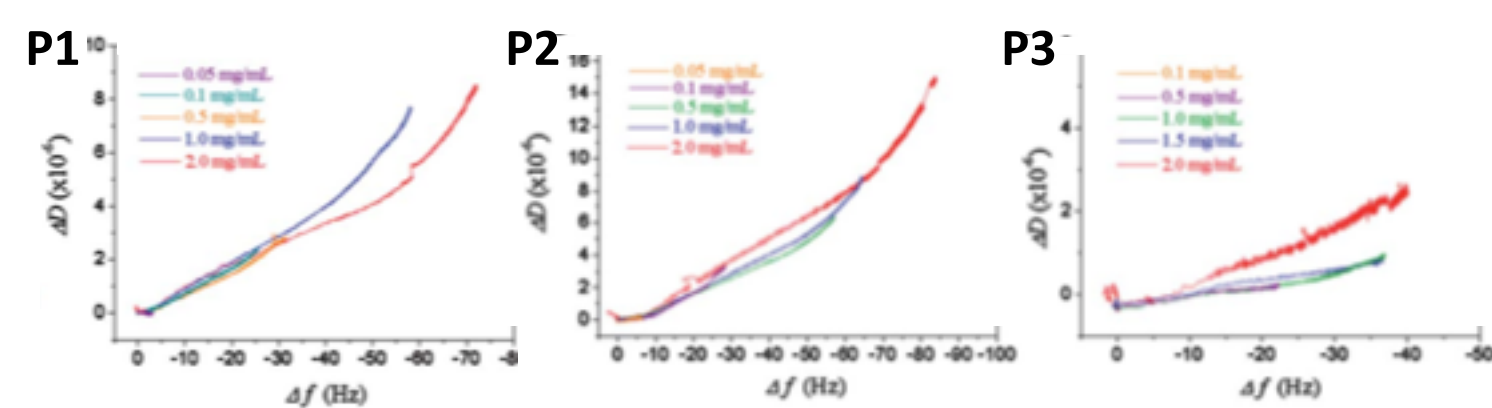
- Higher binding affinity = increased mass of glycoside binding to the lectin surface. This property is used to screen new inhibitors.



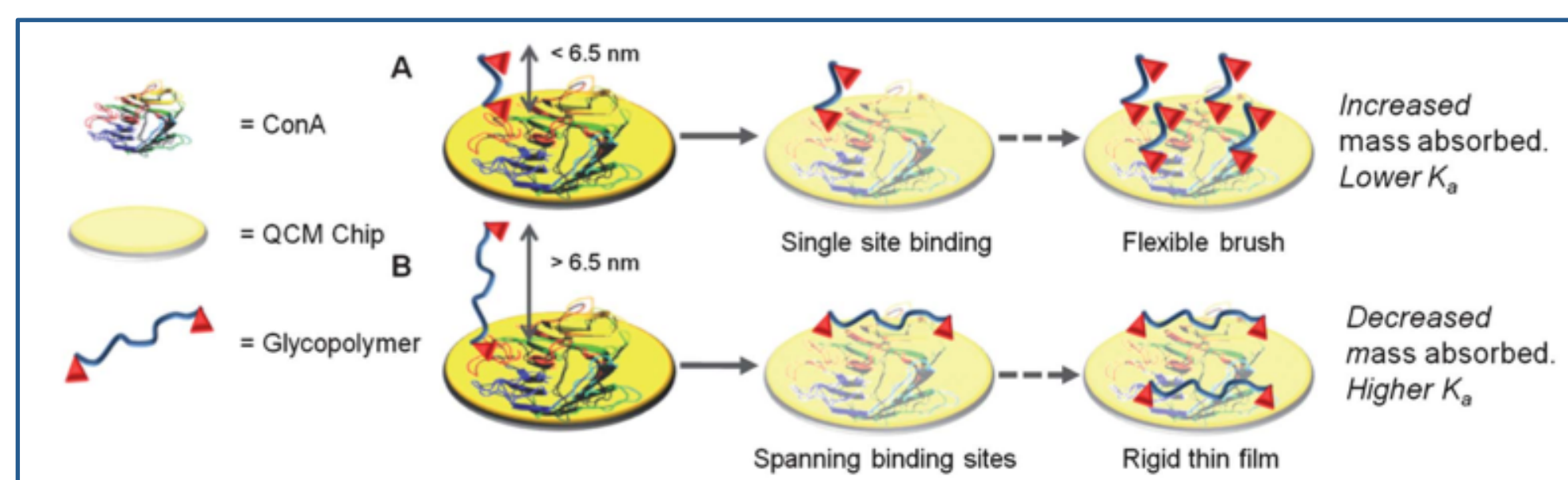
## Binding Modes

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 ( $\Delta D$ ) indicate a flexible film, whilst small  $\Delta D$  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.

Gou, Y.; Richards, S-J.; Haddleton D. M.; Gibson, M. I. *Polymer. Chem.*, 2012

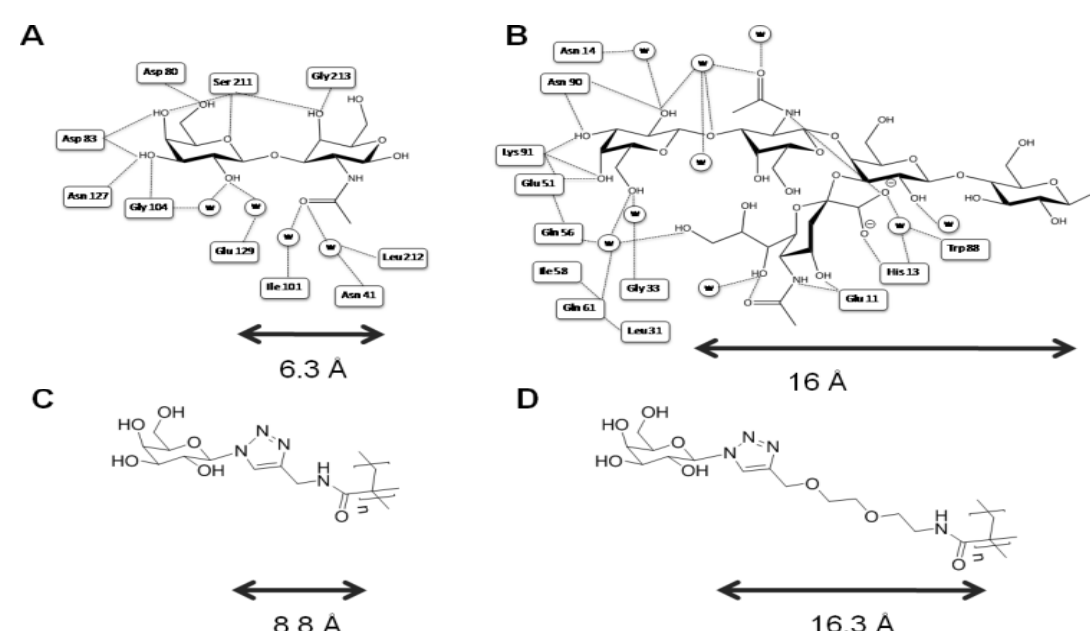
## Inhibition of Bacterial Toxins

### Bacterial Toxin Binding

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

#### PNA binding site

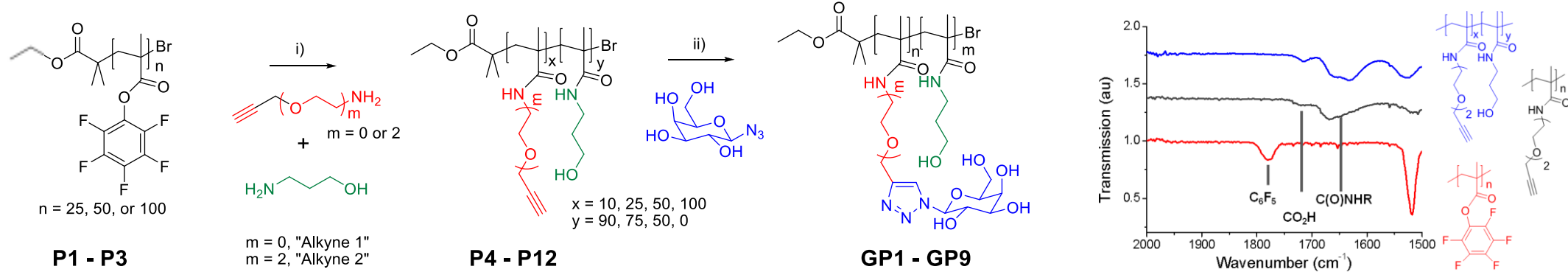
#### CTx binding site



- 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

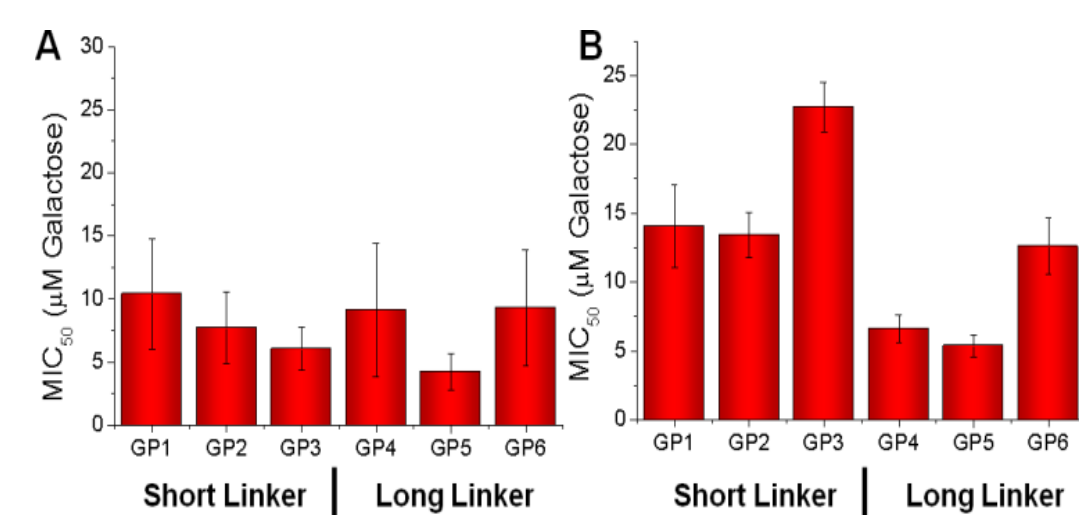
'Clickable' units are not compatible with controlled radical polymerisation. Instead, tandem-post polymerisation modification techniques were used.



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

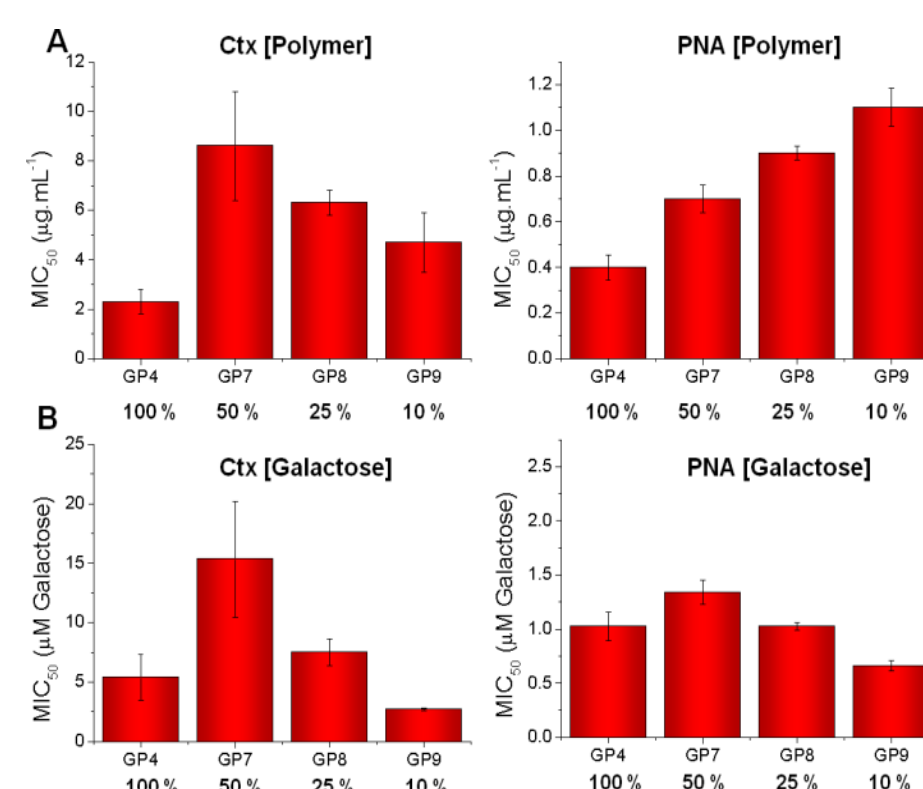
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## Role of Linker Length and Carbohydrate Density



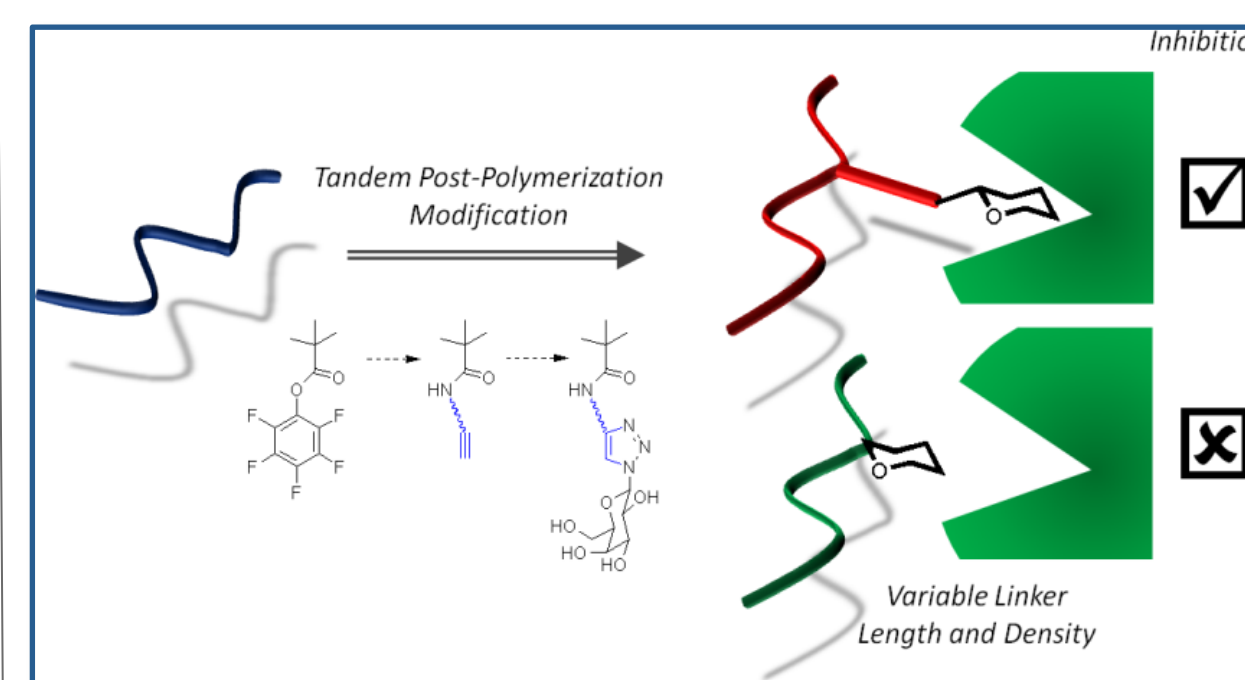
- Polymers synthesised with  $\sim 6 \text{ \AA}$  (short) and  $16 \text{ \AA}$  (long) linkers.
- Linker length has no effect on PNA inhibition (A).
- Longer linker has 2 – 3 fold lower  $MIC_{50}$  compared to shorter linker on inhibiting CTx (B).
- 100 X more active than free galactose.

- 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.



## References

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