Bacterial-Toxin Inhibition using Multivalent Scaffolds

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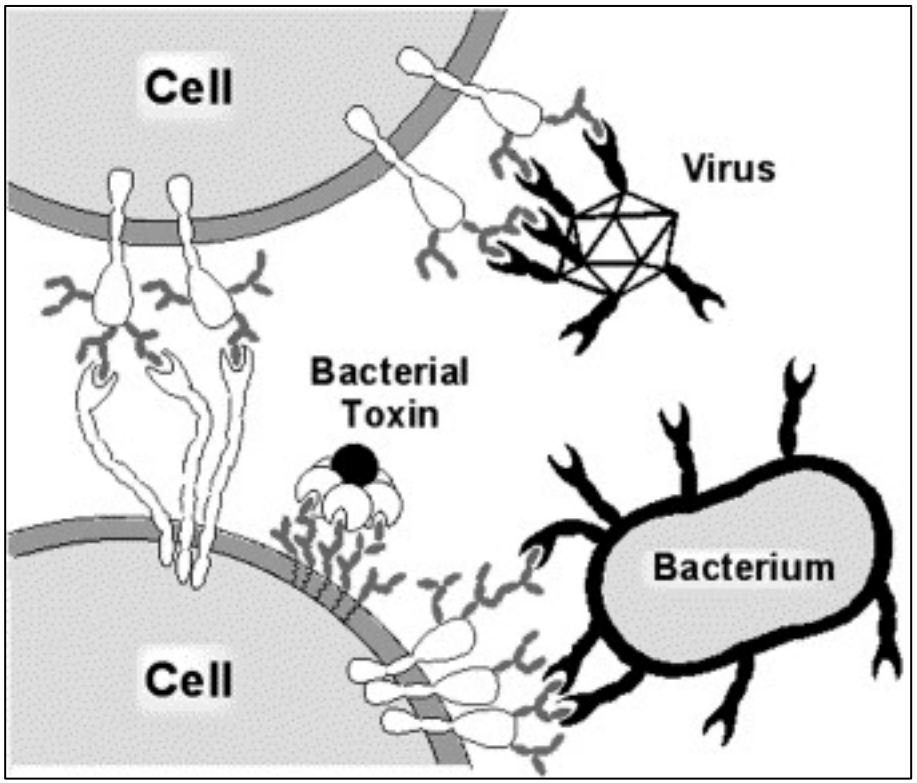
Protein-Carbohydrate Interactions

Cell signalling

Fertilisation

Imflammation

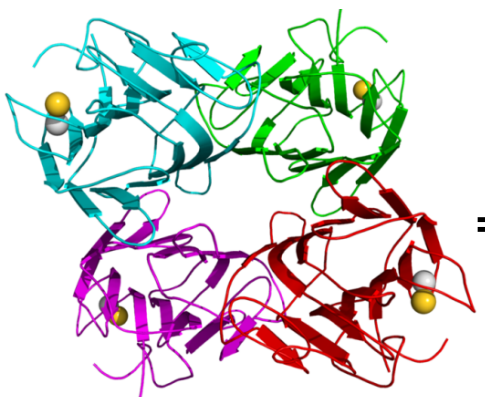


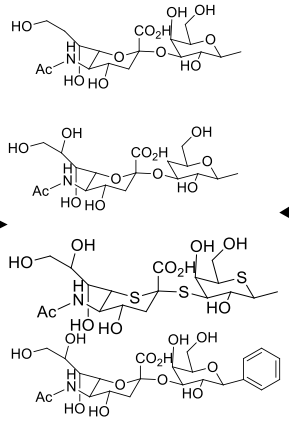
Cellular adhesion of

- Viruses
- Bacterium
- Bacterial toxins

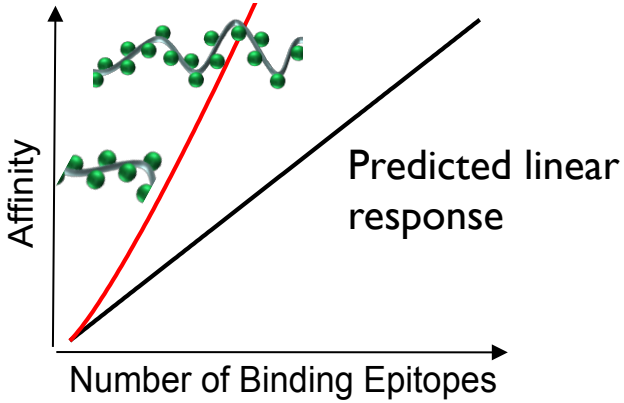
You Houng, *Nat. Mater.*, **2010** 9 485
Miura, *JPOLA,* **2007**, 45, 5031
Spain, *Polym. Chem.*, **2011**, 2, 60
Gamblin, *Chem. Rev*, **2009**, 109, 131
Imberty, *Chem. Eur. J.*, **2008**, 14, 7490
Turnbull, B., *Rev. Mol. Biotech.*, ***2002***, *90, 231*

Why Materials?





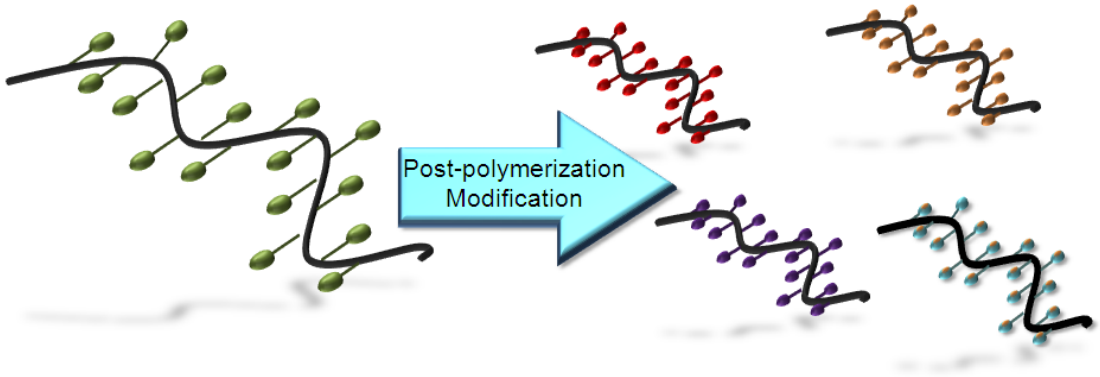
Structural Biology – Organic Synthesis

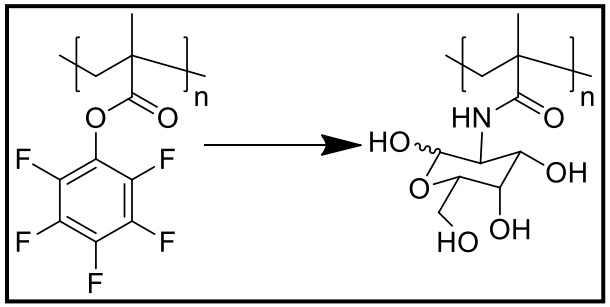


Cell Surface Glycans – Materials Science/multivalency

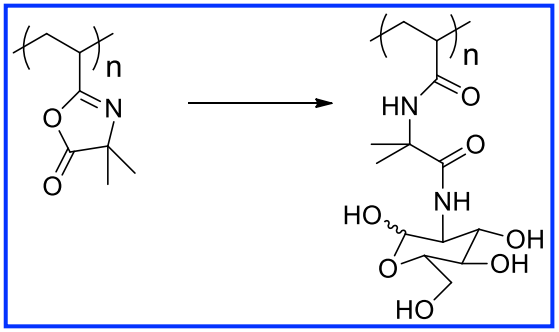
Kiessling, *Angew, Chem.*, **2006**, 45, 22348 Alexander *et al*, *JACS*, **2007**, 129, 11014
Mammam, *J. Med. Chem.*, **1995**, 38, 4179
Spain *et al. Polym. Chem.*, **2011**, 2, 60

Glycopolymers by Post-Polymerisation Modification

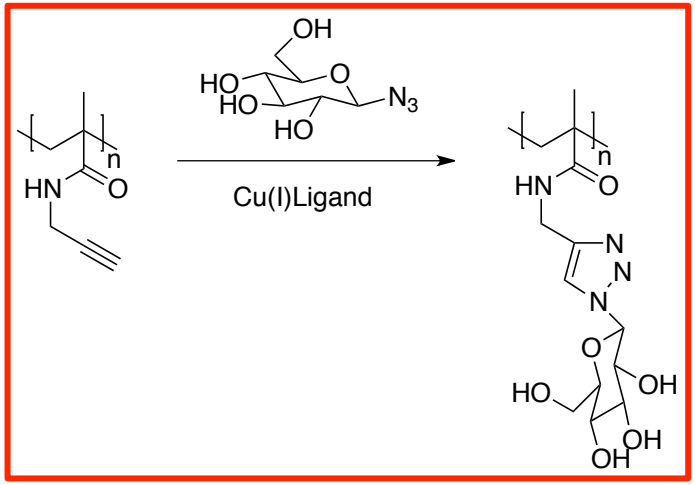




Gibson, M. I. *et al., J. Pol. Sci. A.*, **2009**, 47, 4332



Jones, M.W.; *Polym. Chem.*, **2013**, *In press*

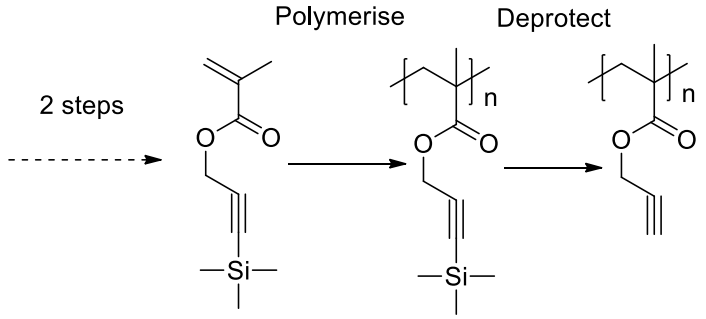


Haddleton, D. M. *et al.*, *JACS*, **2006**, 128, 4823

Spain *et al., J. Pol. Sci. A.*, **2007**, 45, 2059
Gauthier *et al., Angew. Chem.* **2009**, 48, 48

Practicalities

Scaffold synthesis can be inefficient

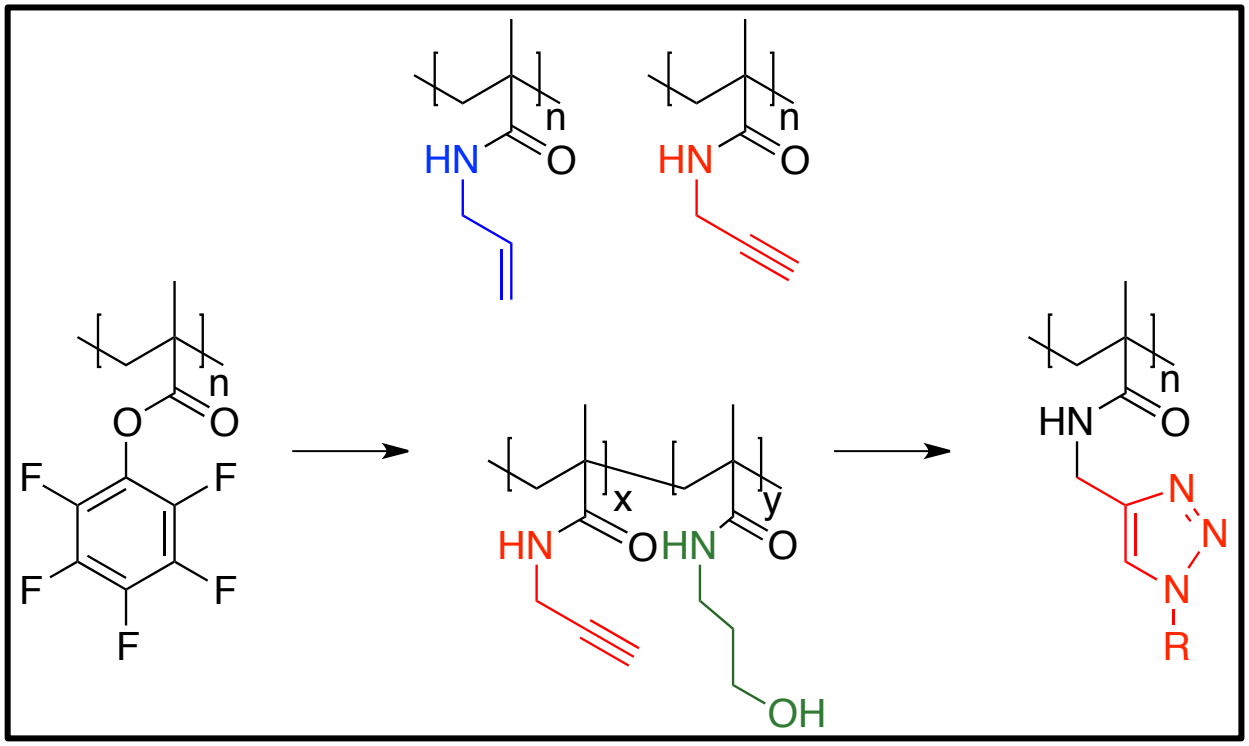


- Monomer synthesis is not always straightforward
- Atom efficiency is poor
- Copolymers require knowledge of reactivity ratios

Variables:
Polymer Length ☑
Carbohydrate ☑
Linker Length X
Co-monomers X
Linking to non-azides X

Our Solution:

"Tandem post-polymerisation modification"



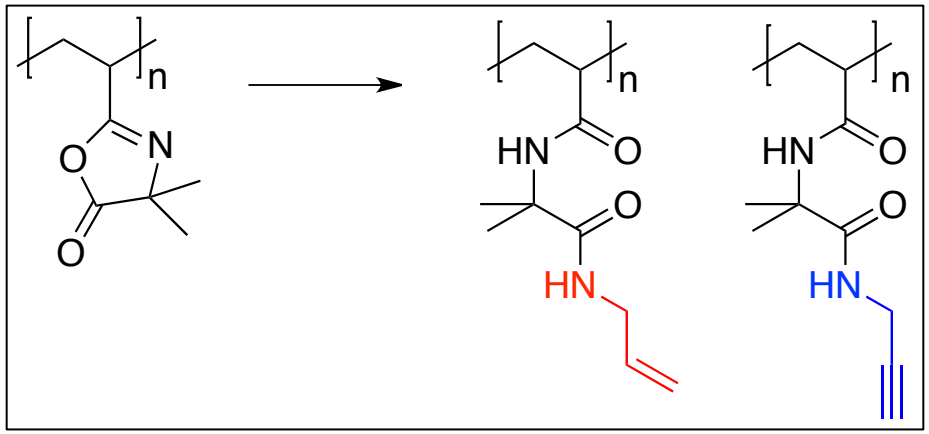
- Easy to make 50 gram scale
- 1 column/distillation
- Compatible with RAFT/ ATRP

- Quantitative functionalisation with non-hindered amines
- Density control

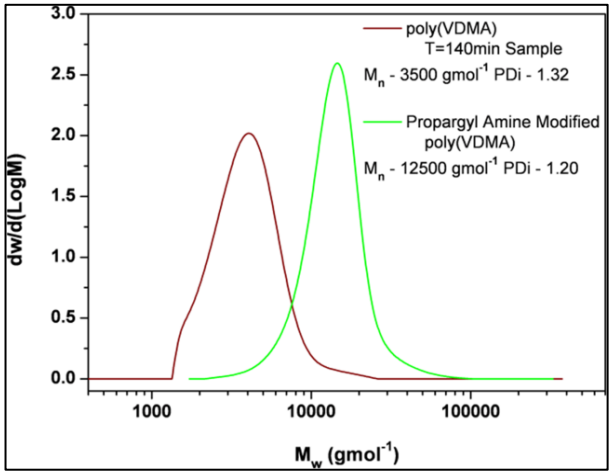
- Sequentially modified polymer libraries

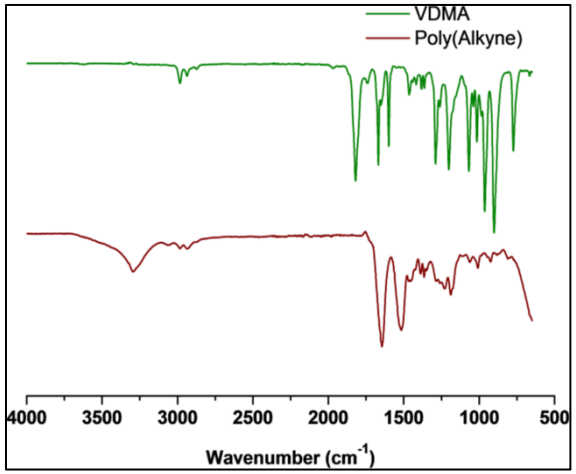
Theato, P.; *J. Pol. Sci. A.*, **2008**, 46, 6677

Improved Synthesis with Poly(azlactones)



- 100 % Atom efficient
- Quantitative conversion with unhindered amines
- Scalable synthesis of monomer
- One-pot, two step synthesis/post-polymerisation modification possible

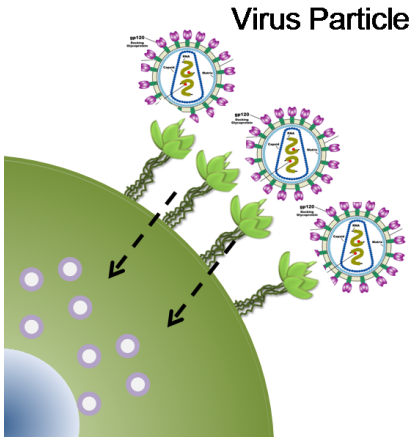


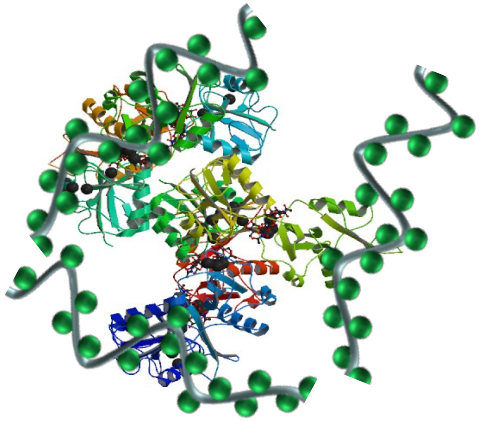


Jones, M.W., Richards, S-J., Haddelton, D. M., Gibson, M. I.; *Polym. Chem.*, **2013**, *In press*

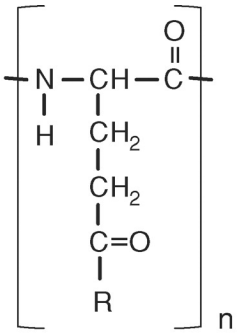
M.E Buck & D. M Lynn, *Polym. Chem.*, **2012**, 3, 66

Applications: Anti-adhesion Therapy

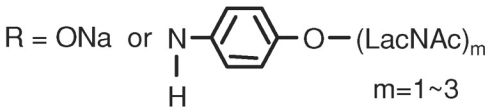


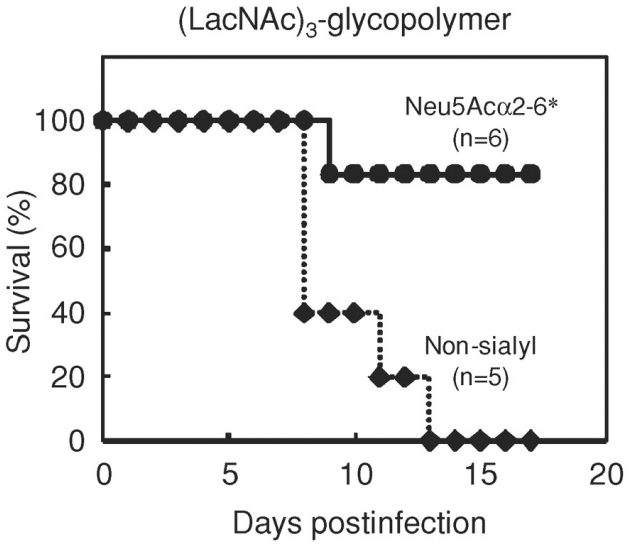


Interactions can be inhibited at ***nM*** *of glycopolymers*



α-polyglutamic acid
(α-PGA)

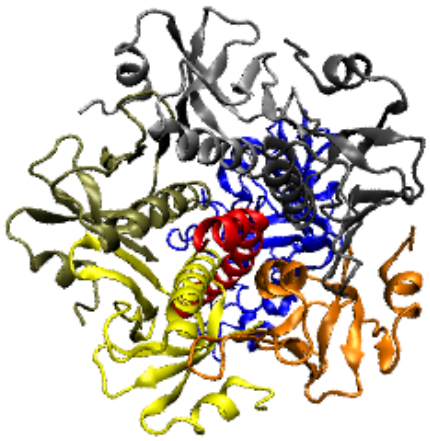




Influenza inhibition in mice

Becer *et al. JACS*, **2010**, 132, 15130
Hidari et al. Glycobiology **2008,** 18, 779

Selective Binding of Cholera-Toxin



Enzymatic domain → Induces toxic effect

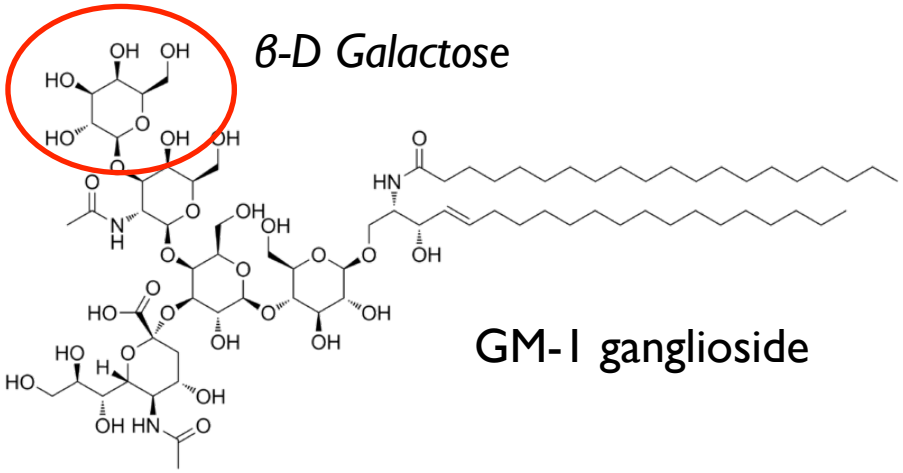
Carbohydrate binding domain → Binds to epithelial cells to promote cell uptake

Anti-adhesion therapy does not target bacteria, so less evolutionary stress

Galectins – at least 13

Sigma-Aldrich – 8 Galactose-'specific' lectins

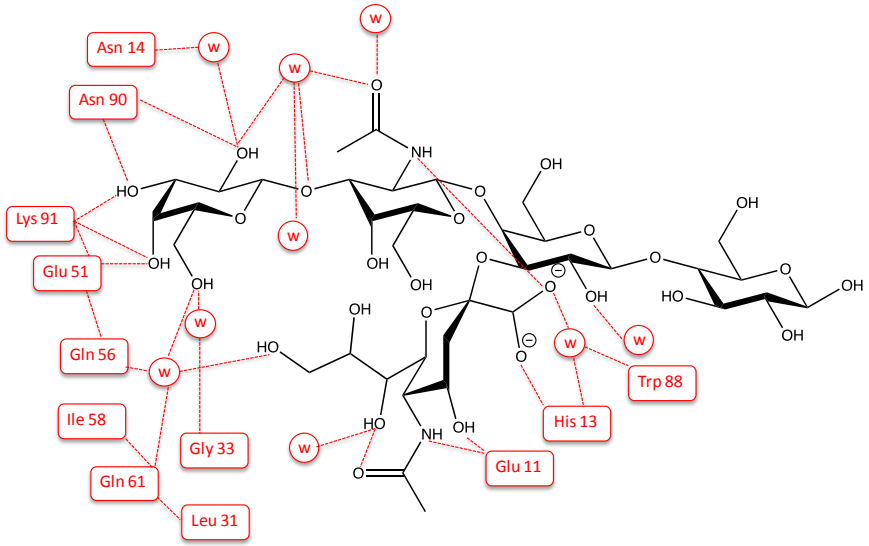
How do we engineer a high-affinity binder for cholera toxin, without total synthesis of complex carbohydrates?



β-D Galactose

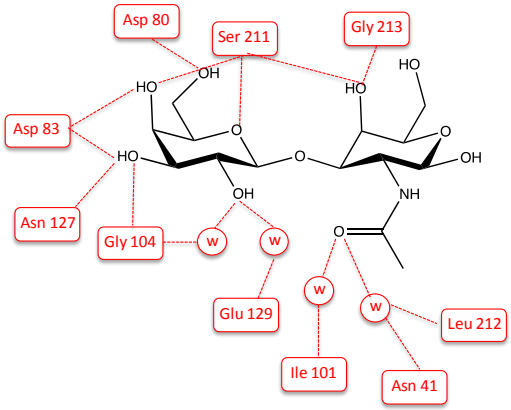
GM-1 ganglioside

Cholera Toxin



16 Å

Peanut Agglutinin

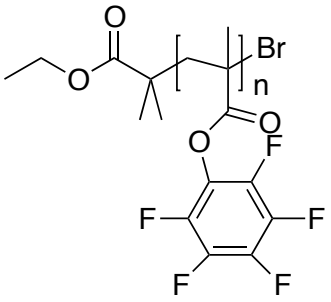


6.3 Å

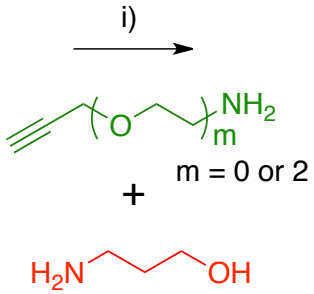
Can glycan accessibility be used as a tool for lectin discrimination?

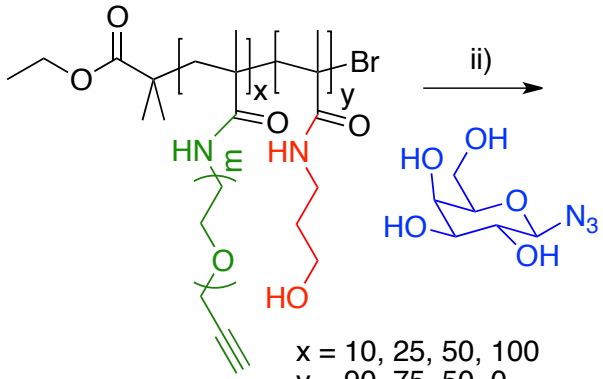
Kiick *et al.*; *Macromol.* **2007**, 40, 7103
Kiick *et al*; *Biomac.*, **2006**, 7 483

Glycopolymer Library

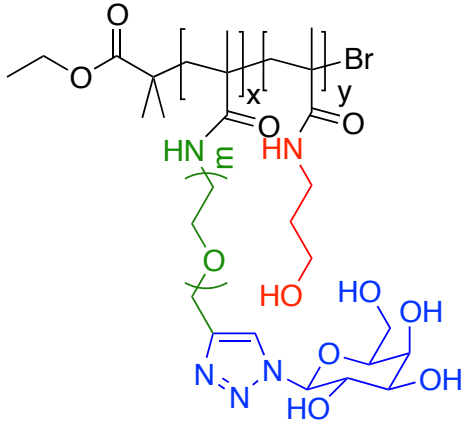


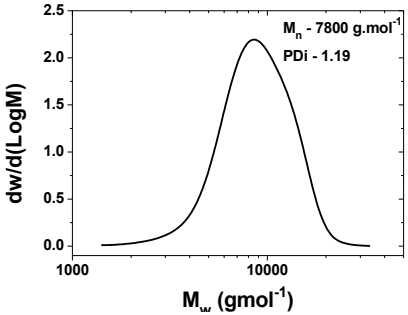
n = 25, 50, or 100

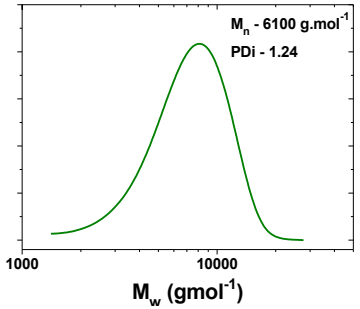


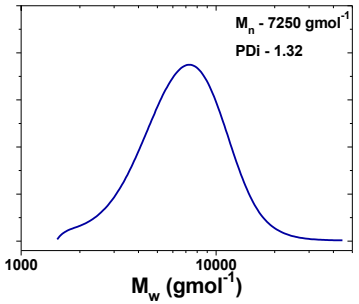


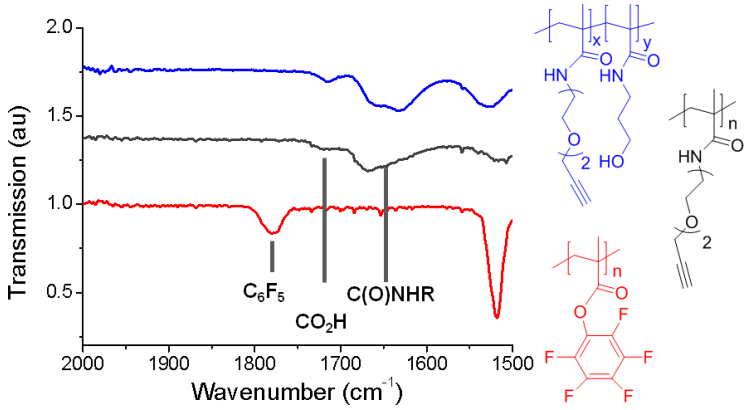
x = 10, 25, 50, 100
y = 90, 75, 50, 0





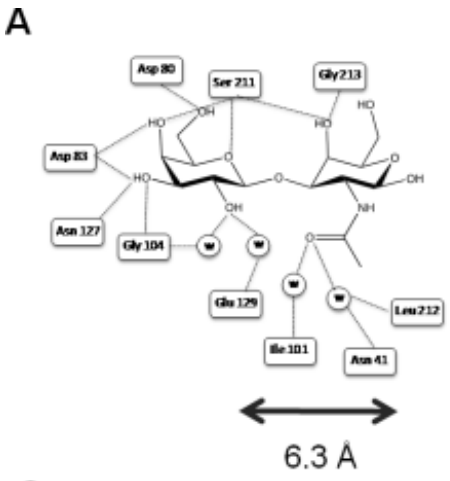


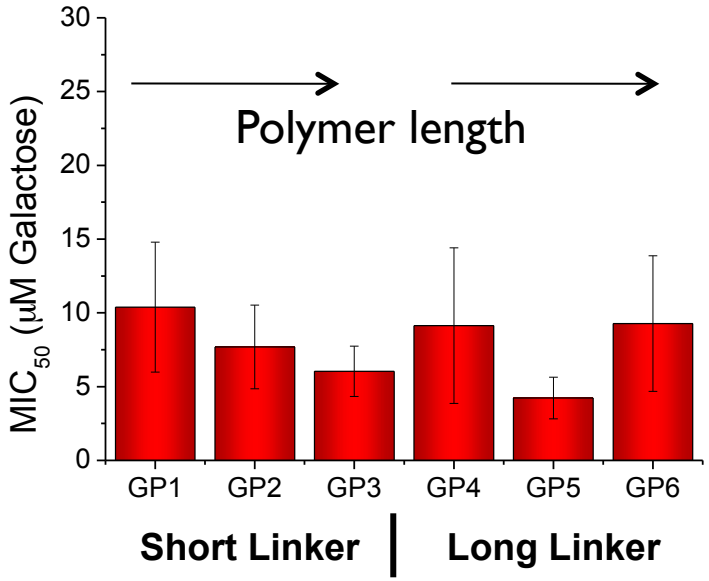




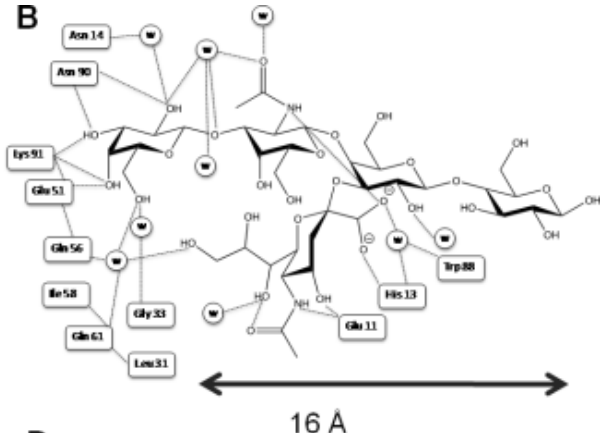
Polymer	DP[a]	Linker[b]	Density[c]	M_w/M_n[d]
GP1	18	Short	100	1.29
GP2	33	Short	100	1.27
GP3	70	Short	100	1.26
GP4	18	Long	100	1.32
GP5	33	Long	100	1.28
GP6	70	Long	100	1.27
GP7	33	Long	50	1.23
GP8	33	Long	25	1.21
GP9	33	Long	10	1.20

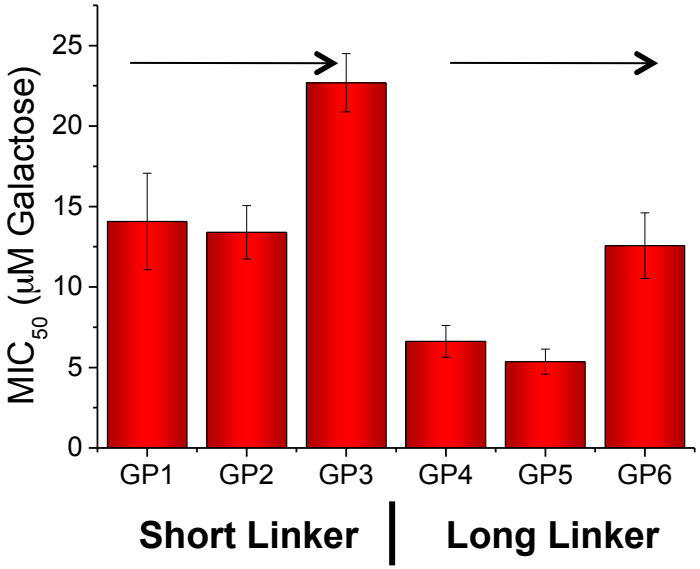
Peanut Agglutinin

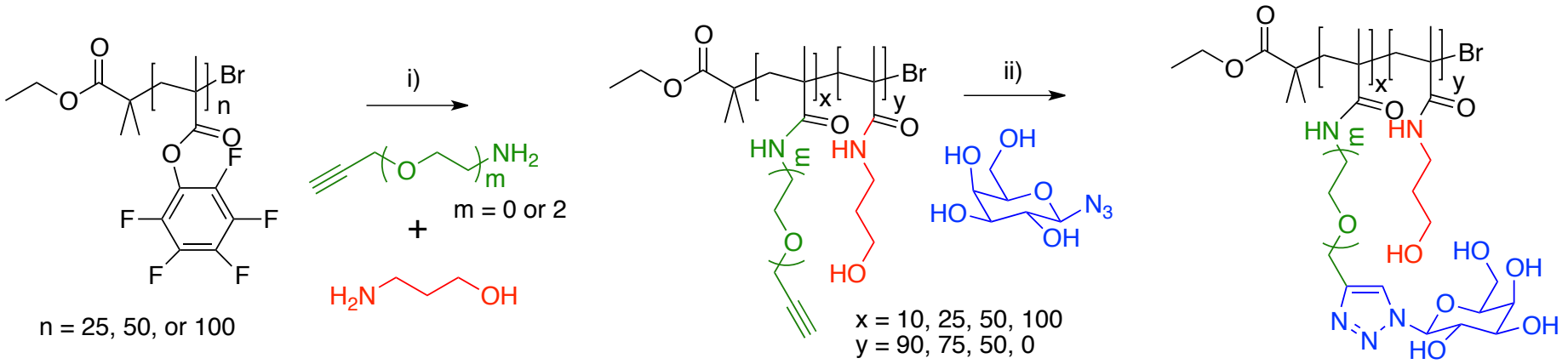




Cholera Toxin



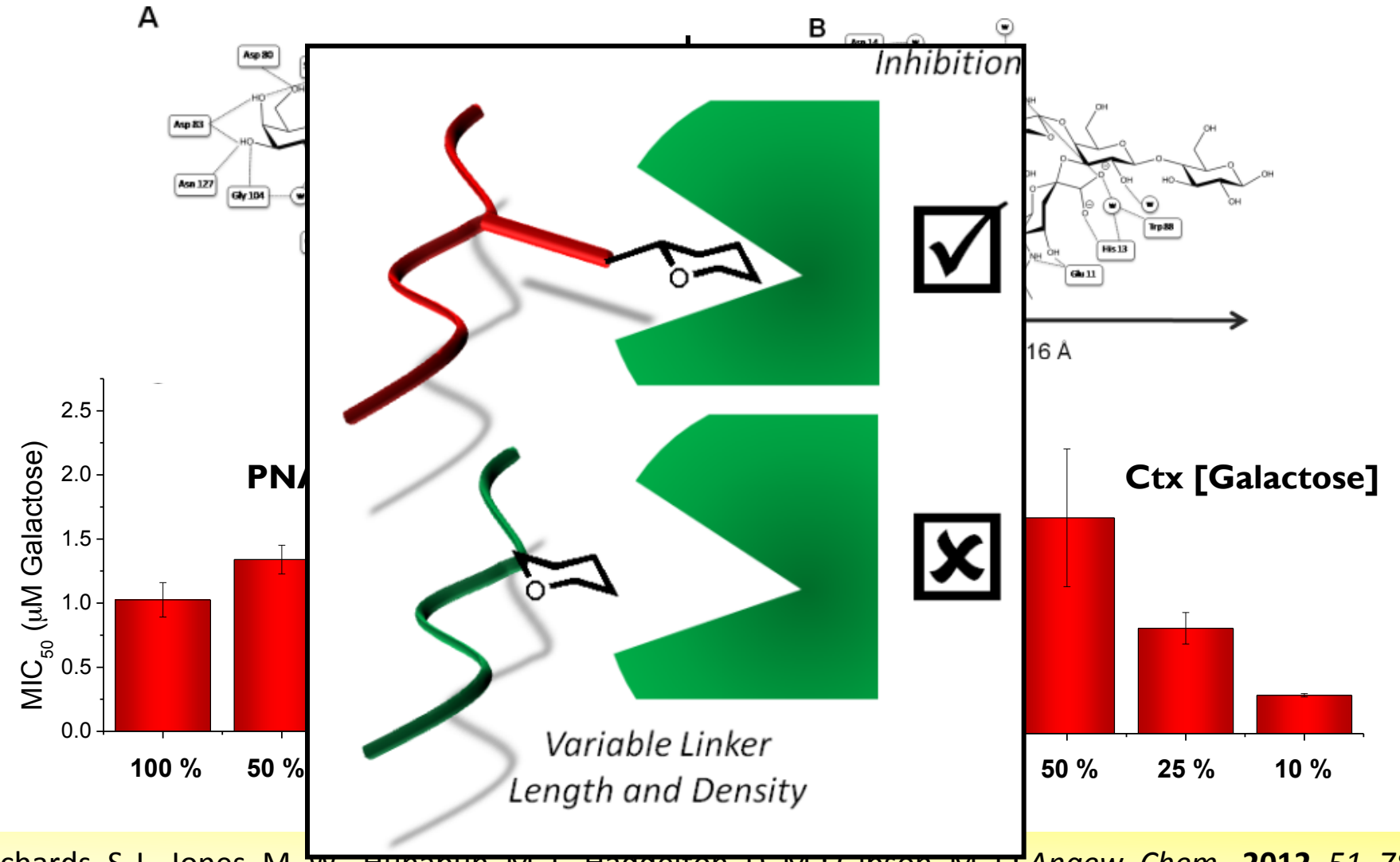




- Degree of polymerisation
- Linker length
- *Carbohydrate density*

Peanut Agglutinin

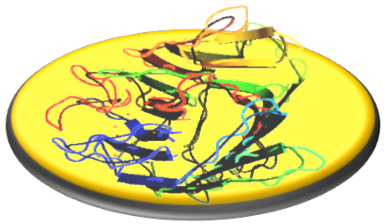
Cholera Toxin



Richards, S-J., Jones, M. W., Hunaban, M. I., Haddelton, D. M.; Gibson, M. I.; *Angew. Chem.*, **2012**, *51, 7812*

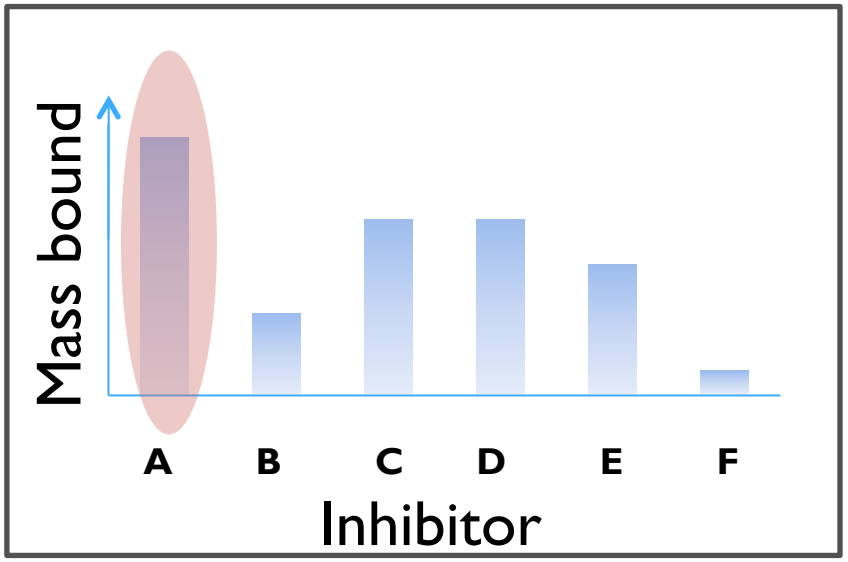
What is the 'best' polymer for lectin binding?

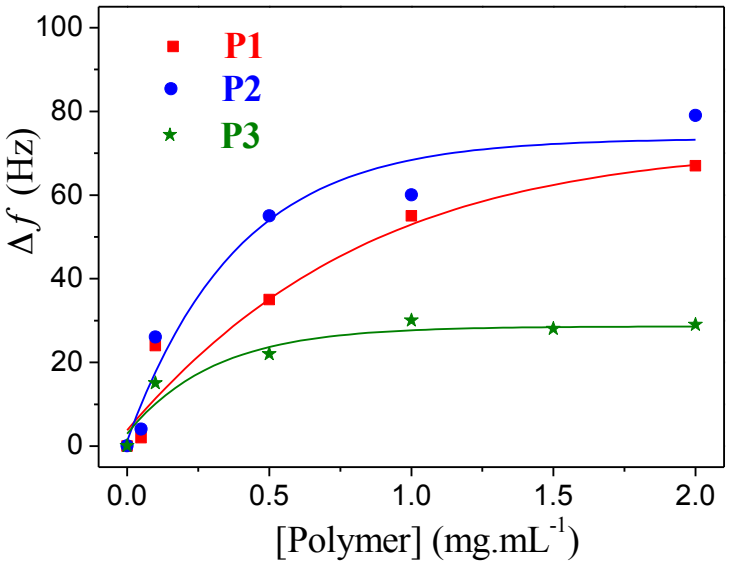
How do you determine what is the best polymer?



Absorption to protein functionalised surface

- Surface Plasmon Resonance (SPR)
- Quartz Crystal Microbalance (QCM)
- Enzyme-linked assays (ELISA)

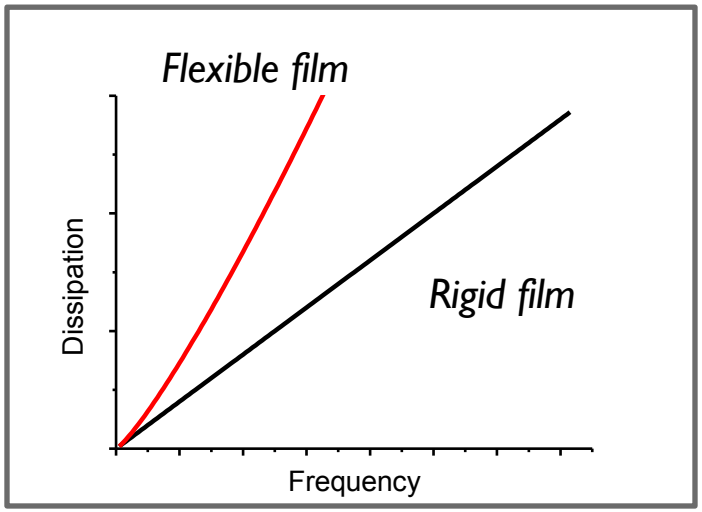


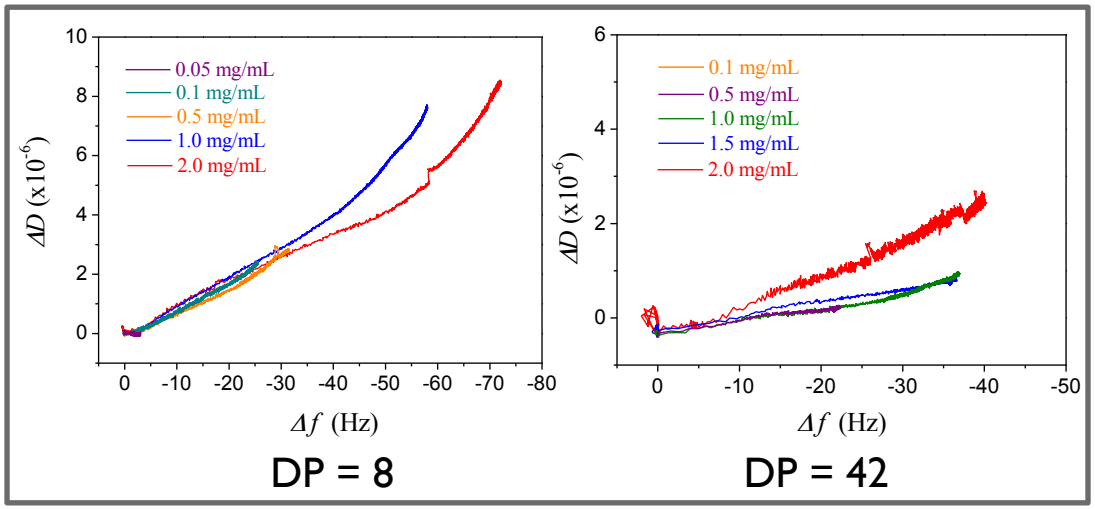


Molecular weight P3 > P2 > P1

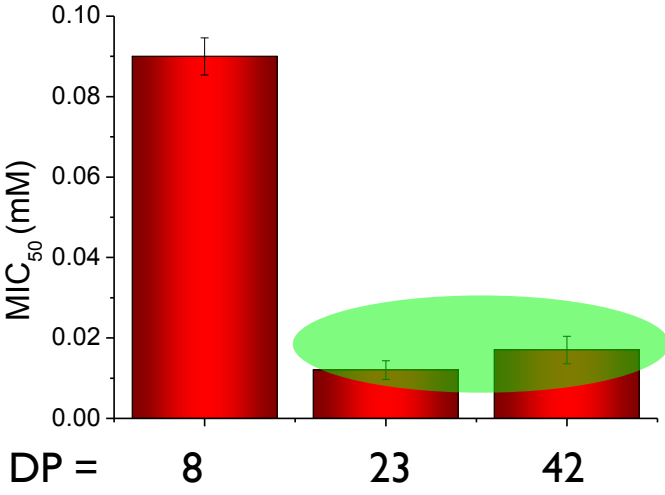
- *Largest* polymer shows *smallest* shifts
- Does this imply weakest binding?
- What is effect of polymer chain length?

QCM-d allows film properties to be probed

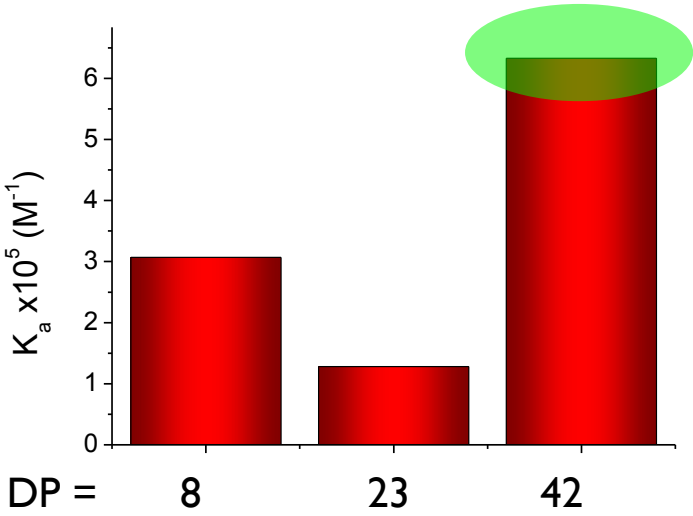


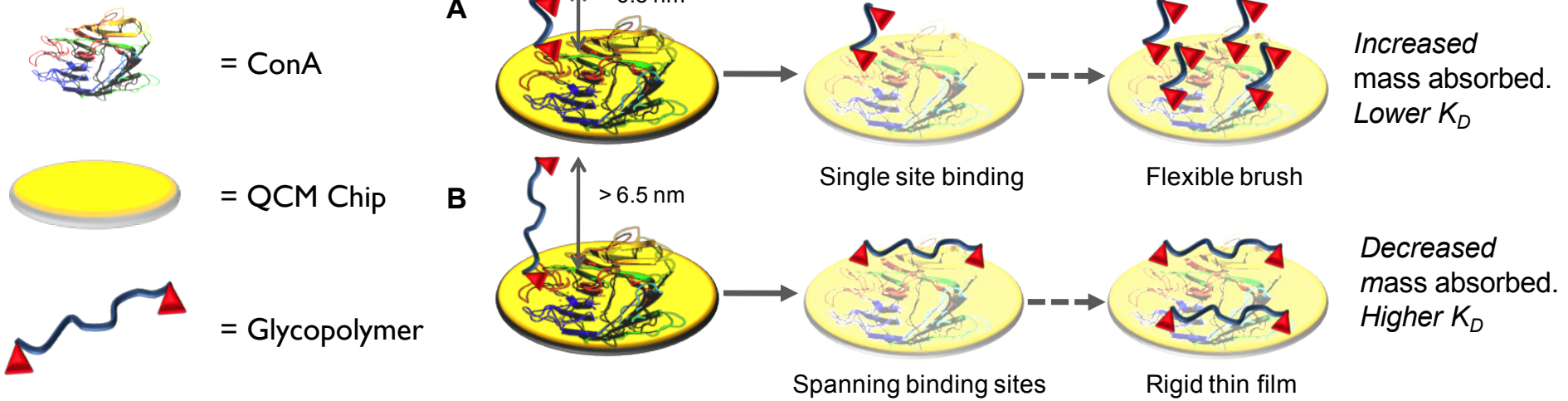


Solution phase inhibition



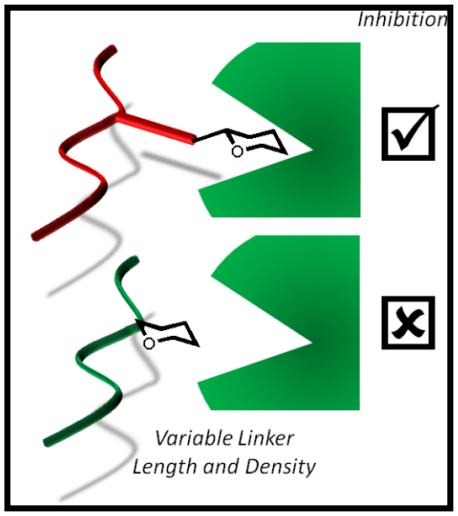
Surface Binding Affinity



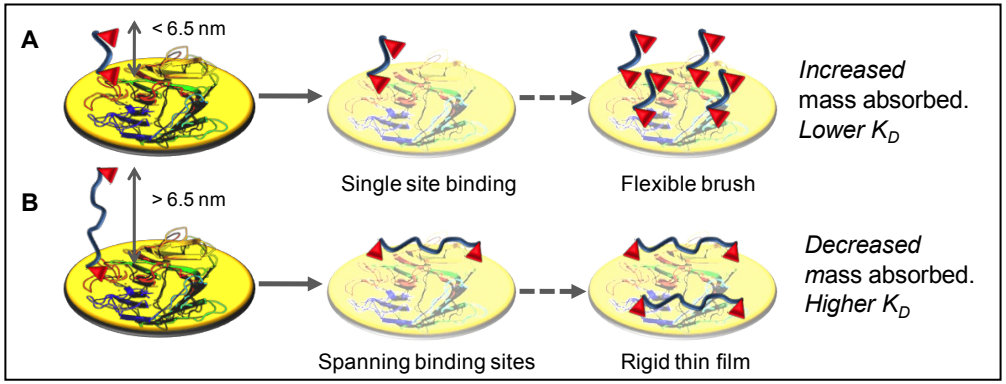


Gou. Y., Richards, S-J., Haddleton D. M., Gibson, M. I.; *Polymer Chemistry,* **2012,** 3, 1634

Summary



- Tandem Post-Polymerisation Modification
- Multivalent inhibitors that have good affinity AND specificity



- A number of techniques are required to determine the 'best' polymer.

Acknowledgements

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MIG Group Current

- *Robert Deller*
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- *Rebecca Williams*

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Bacterial-Toxin Inhibition using Multivalent Scaffolds

Sarah-Jane Richards & Matthew I. Gibson

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7th RSC Biomaterial Chemistry Meeting, Sheffield. 8th and 9th January 2013

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