Bacterial-Toxin Inhibition using Multivalent Scaffolds

Sarah-Jane Richards & Matthew I. Gibson
Department of Chemistry, University of Warwick, UK

S-J.Richards@warwick.ac.uk      www.warwick.ac.uk/go/gibsongroup

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Protein-Carbohydrate Interactions

Cell signalling

Fertilisation

Inflammation

Cellular adhesion of
- Viruses
- Bacterium
- Bacterial toxins

Miura, *JPOLA*, 2007, 45, 5031
Gamblin, *Chem. Rev.*, 2009, 109, 131
Affinity

Number of Binding Epitopes

Why Materials?

Predicted linear response

Cell Surface Glycans – Materials
Science/multivalency

Glycopolymers by Post-Polymerisation Modification


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

Jones, M. W.; Polym. Chem., 2013,

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
- Co-monomers

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


Applications: Anti-adhesion Therapy

Interactions can be inhibited at \( nM \) of glycopolymers

\[
\begin{align*}
\text{\( \alpha \)-polyglutamic acid (\( \alpha \)-PGA)} & \quad \text{(LacNAc)}_3\text{-glycopolymer} \\
& \quad R = \text{ONa or } \text{N}\text{H} \quad \text{Neu5Ac}\alpha\text{-2-6*} \\
& \quad m = 1\sim 3 \quad \text{Neu5Ac}\alpha\text{-2-6*} \quad (n=6) \\
& \quad \text{Non-sialyl} \quad (n=5)
\end{align*}
\]

Survival (%) over Days postinfection

Influenza inhibition in mice

Becer et al. \textit{JACS, 2010}, 132, 15130
Hidari et al. \textit{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?

GM-1 ganglioside

β-D Galactose
Can glycan accessibility be used as a tool for lectin discrimination?
Glycopolymer Library

Polymer | DP<sup>a</sup> | Linker<sup>b</sup> | Density<sup>c</sup> | M<sub>w</sub>/M<sub>n</sub><sup>[d]</sup>  
--- | --- | --- | --- | ---  
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   

<sup>[a]</sup> Polymer DP range

<sup>[b]</sup> Linker type: Short or Long

<sup>[c]</sup> Density range: 10, 25, 50, 100

<sup>[d]</sup> M<sub>w</sub>/M<sub>n</sub> range: 1.20 to 1.32
Peanut Agglutinin

A

Cholera Toxin

B

Polymer length

MIC\textsubscript{50} (\mu M Galactose)

Short Linker | Long Linker

MIC\textsubscript{50} (\mu M Galactose)

Short Linker | Long Linker
- Degree of polymerisation
- Linker length
- *Carbohydrate density*
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

Flexible film

Rigid film

Dissipation vs. Frequency

DP = 8

DP = 42
Solution phase inhibition

![Bar chart showing MIC₅₀ values for different DP values (8, 23, 42).]

Surface Binding Affinity

![Bar chart showing Kₐ values for different DP values (8, 23, 42).]

- ConA
- QCM Chip
- Glycopolymers

A = ConA
B = QCM Chip

- < 6.5 nm: Single site binding
- > 6.5 nm: Spanning binding sites

Increased mass absorbed. Lower Kₐ
Decreased mass absorbed. Higher Kₐ

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.
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