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 Houning, *Nat. Mater.*, **2010** 9 485
Miura, *JPOLA*, **2007**, 45, 5031
Why Materials?

Structural Biology – Organic Synthesis

Cell Surface Glycans – Materials Science/multivalency

Glycopolymers by Post-Polymerisation Modification


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


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 ×
Linking to non-azides ×

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

\[
\text{\( \alpha \)-polyglutamic acid (\( \alpha \)-PGA)}
\]

\[
R = \text{ONa or } \text{ON} \quad \text{(LacNAc)}_m \\
m = 1 \sim 3
\]

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 → Binds to epithelial cells to promote cell uptake → Induces toxic effect

Carbohydrate binding domain

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

i) \[ \text{Br} \quad \text{O} \quad \text{O} \quad \text{F} \quad \text{F} \quad \text{F} \quad \text{F} \quad \text{F} \quad \text{n = 25, 50, or 100} \]

\[ \text{H}_2\text{N} \quad \text{OH} \]

\[ m = 0 \text{ or } 2 \]

ii) \[ \text{H}_2\text{N} \quad \text{OH} \quad \text{Br} \quad \text{O} \quad \text{O} \quad \text{F} \quad \text{F} \quad \text{F} \quad \text{F} \quad \text{F} \quad \text{x = 10, 25, 50, 100} \]

\[ y = 90, 75, 50, 0 \]

<table>
<thead>
<tr>
<th>Polymer</th>
<th>DP(^a)</th>
<th>Linker(^b)</th>
<th>Density(^c)</th>
<th>(M_w/M_n)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP1</td>
<td>18</td>
<td>Short</td>
<td>100</td>
<td>1.29</td>
</tr>
<tr>
<td>GP2</td>
<td>33</td>
<td>Short</td>
<td>100</td>
<td>1.27</td>
</tr>
<tr>
<td>GP3</td>
<td>70</td>
<td>Short</td>
<td>100</td>
<td>1.26</td>
</tr>
<tr>
<td>GP4</td>
<td>18</td>
<td>Long</td>
<td>100</td>
<td>1.32</td>
</tr>
<tr>
<td>GP5</td>
<td>33</td>
<td>Long</td>
<td>100</td>
<td>1.28</td>
</tr>
<tr>
<td>GP6</td>
<td>70</td>
<td>Long</td>
<td>100</td>
<td>1.27</td>
</tr>
<tr>
<td>GP7</td>
<td>33</td>
<td>Long</td>
<td>50</td>
<td>1.23</td>
</tr>
<tr>
<td>GP8</td>
<td>33</td>
<td>Long</td>
<td>25</td>
<td>1.21</td>
</tr>
<tr>
<td>GP9</td>
<td>33</td>
<td>Long</td>
<td>10</td>
<td>1.20</td>
</tr>
</tbody>
</table>
Peanut Agglutinin

- Polymer length: Short Linker (6.3 Å) vs. Long Linker
- MIC<sub>50</sub> (μM Galactose): GP1 < GP2 < GP3 < GP4 < GP5 < GP6

Cholera Toxin

- Polymer length: Short Linker (16 Å) vs. Long Linker
- MIC<sub>50</sub> (μM Galactose): GP1 < GP2 < GP3 (25 < GP4 < GP5 < GP6)
- Degree of polymerisation
- Linker length
- Carbohydrate density
Peanut Agglutinin

Cholera Toxin

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 $P_3 > P_2 > P_1$

- 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

![Flexible film graph](image)

Rigid film

![Rigid film graph](image)

DP = 8

DP = 42
**Solution phase inhibition**

- MIC<sub>50</sub> (mM) vs DP
  - DP = 8: 0.08
  - DP = 23: 0.06
  - DP = 42: 0.04

**Surface Binding Affinity**

- $K_a \times 10^5$ (M<sup>-1</sup>) vs DP
  - DP = 8: 3
  - DP = 23: 2
  - DP = 42: 1

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**A**
- Flexible brush: $< 6.5$ nm
- Rigid thin film: $> 6.5$ nm

**B**
- Single site binding
- Spanning binding sites
- Flexible brush
- Rigid thin film

*Increased mass absorbed. Lower $K_D$*

*Decreased mass absorbed. Higher $K_D$*

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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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