Synthetic Polymer Nanoparticles: Abiotic Receptors for Peptides, Proteins and Carbohydrates

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Outline

• Introduction to research goals
  • Lessons from biology
  • Research strategy
  • "Plastic" antibodies
• ELISA mimic-expansion of monomer pool
• Catch and release of proteins
  • Protein A mimic
Goal: Develop a general strategy for preparing synthetic polymers with antibody-like affinity and selectivity for peptides, proteins and carbohydrates.

<table>
<thead>
<tr>
<th>Polymer-Ligand Conjugate</th>
<th>Polymer-Antibody Conjugate</th>
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<tbody>
<tr>
<td><img src="image1.png" alt="Diagram of conjugates" /></td>
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Target Protein or Peptide

Lessons to Learn from Protein-Protein Interactions

SARS Protein Attaching to a Host Cell Receptor

![Image of SARS protein attaching to host cell receptor](image2.png)

Recognition results from the cumulative effect of many individually weak interactions over a surface area of 300 - >1000 Å²


Protein-Protein Interactions

![Diagram of protein-protein interactions](image3.png)

Protein-Polymer Interactions?

![Diagram of protein-polymer interactions](image4.png)
Target Peptide = Imprinting Peptide

- Melittin: cytotoxic 26 amino acid peptide, from honey bee venom
- Amphiphilic
- Pore forming toxin, creates unregulated pores in the membrane of targeted cells

Video removed

Nanoparticle Library
Precipitation Polymerization

Color coded distribution of monomers used for polymer synthesis

Interaction Between Co-polymers and Target Peptide Melittin

Hemolysis Neutralization Assay

Both charged (-) and hydrophobic groups are necessary
Nanoparticle Functional Monomer Composition vs RBC Neutralization Constant

Final Step - Melittin Imprinting

Inhibition of Hemolytic Activity by MIP NPs
Capacity of Melittin Neutralization

Video removed.

Are Melittin Specific Imprinted NPs Effective in a Biological Milieu?

In Vitro Neutralization of Melittin Activity

(Inhibition of melittin induced cytotoxicity in cultured cells by MIP/NIP)

In Vivo Studies

(Neutralization of Melittin Toxicity in Mice)
Inhibition of cytotoxicity of cultured HT-1080 human fibrosarcoma cells by NPs is dose dependent.

Therapeutic Polymer Nanoparticles? Can We Use Polymer NPs as a Toxin Antidote?

Determination of survival rate of mice after intravenous injection of melittin w/o polymer NPs

Survival of Melittin-Challenged Mice Followed by Injection of MIP NPs.

Survival curves of mice over 24 hrs after injection (IV) of 5.0 mg kg⁻¹ melittin with 9.4 mg kg⁻¹ (blue), 30 mg kg⁻¹ (red), and without MIPNPs (green).

Surviving rate of mice 24 h after melittin injection with/without (blue triangles/green circles) 9.4 mg kg⁻¹ MIPNPs

This shift is close to what was expected by the neutralization capacity experiment of hemolytic activity.
In vivo imaging with fluorescent labeled melittin and molecularly imprinted nanoparticles (MIPNPs) establishes the MIPNPs accelerate clearance of the toxic peptide (melittin) from blood. The MIPNP peptide complex accumulates in the liver.

NPs and melittin were labeled with different dyes to monitor localization in liver.

Fluorescence microscopy of liver tissue
Affinity Sorting of Plastic Antibodies

Synthetic polymer NPs are not homogeneous materials. They are crude polyclonals with a distribution of sizes and affinities. However, since they are nano size, they can be separated on the basis of affinity just as antibodies and other large proteins.

Temperature Effect on Nanoparticle-Melittin Binding (LCST)

Temperature Effect on Nanoparticle-Melittin Binding

25°C 12°C

Melittin release at low temperature.

Affinity Selection of NPs
Thermal Catch and Release
**Affinity Selection of NPs**

Thermal Catch and Release

- Peptide Immobilized
- Agarose Beads
- Random Copolymer Nanoparticles (NPs)

**Polymer-NP Binding—What’s Important?**

- Hydrogen bonding
- Electrostatic interactions
- Hydrophobic interactions

- Synthetic Polymer NP-Polysaccharide Interactions: A Systematic Study by ITC

- A Systematic Study of Polymer NP-Polysaccharide Interactions

- Binding Forces involved in the NP-Polysaccharide Interactions

- Factors (pH, ionic strength, temp) that affect the NP-polysaccharide interactions

- Potential roles of NPs in inhibition of polysaccharide-protein interactions
**Nanoparticle Synthesis**

a. Polymerization

b. Text

Heparin

a polyanionic carbohydrate

**Thermodynamic Study: Heparin-NPs**

<table>
<thead>
<tr>
<th>Entry</th>
<th>%</th>
<th>$K_a$ (10^6 M⁻¹)</th>
<th>ΔH (kcal/mol)</th>
<th>ΔS (cal/mol K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90% AA</td>
<td>0.40 ± 0.14</td>
<td>0.8 ± 0.3</td>
<td>-51.1 ± 19.8</td>
</tr>
<tr>
<td>2</td>
<td>5% AA</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>3</td>
<td>5% ATC</td>
<td>8.63 ± 0.12</td>
<td>2.10 ± 0.25</td>
<td>-52.7 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>5% AAC</td>
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<tr>
<td>5</td>
<td>10% ATC</td>
<td>16.2 ± 0.28</td>
<td>21.2 ± 0.77</td>
<td>-37.0 ± 1.1</td>
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<tr>
<td>6</td>
<td>2% APM</td>
<td>2.35 ± 0.03</td>
<td>1.52 ± 0.05</td>
<td>-75.4 ± 1.1</td>
</tr>
<tr>
<td>7</td>
<td>5% IM</td>
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<td>8</td>
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**Thermodynamic Study: Heparin-Positive Charged NPs**

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Flexibility of Polymer Chain:
Crosslinking Degree of NPs (2%ATC)

Polymer Main Component: AAm vs. NIPAm

Inhibition of Heparin-Protein Interactions by NPs
Anticoagulant Assay
Conclusions

- Hydrogen bonding, electrostatic interactions and dehydration from polar patches are involved in the NP-polysaccharide interactions. Hydrophobic interactions are less important.

- Charge density, nature of charged groups, cross-linking and the capacity for hydrogen bonding are critical for high NP affinity. Select NPs can inhibit the heparin-protein interaction.

**ELISA-Mimic Screen for Synthetic Polymer Nanoparticles with High Affinity to Target Proteins**

**Functional Monomers for NP Synthesis**

Which functional groups are best for a specific protein?
ELISA-Mimic Screen for Synthetic Polymer NPs

Solution Phase Separation Efficiency

Temperature Effect on Nanoparticle Structure (LCST)
Depletion of Lysozyme from Solutions at RT

Temperature-responsive "catch-and-release" of lysozyme (5 mgmL⁻¹) by NP2 (2.0 mg). Experiments were carried out in PBS (sodium phosphate buffer (35 mm) containing NaCl (150 mm), pH 7.3).
**Protein Composition of Egg White**

Egg White contains approximately 40 different proteins

- 54% Ovalbumin - Nourishment; blocks digestive enzyme
- 12% Ovotransferrin - Binds iron
- 11% Ovomucoid - Blocks digestive enzymes
- 4% Ovoglobulin G2
- 4% Ovoglobulin G3
- 3.5% Ovomucin
- 3.4% Lysozyme
- 1.5% Ovoinhibitor
- 1% Ovoglycoprotein
- 0.8% Flavoprotein
- 0.5% Ovomacroglobulin
- 0.05% Avidin
- 0.05% Cystatin

(Total listed 95.8%)
Can a synthetic polymer NP be engineered to have an intrinsic high affinity to a specific domain of a large biomacromolecule?

Development of a Plastic Protein A

Target Protein IgG

Protein A

Proteins A and G are used to purify IgG on a commercial scale

More than 10,000 kg of IgG are purified a year.

Can we prepare synthetic NPs to capture the Fc fragment of IgG?

Plastic Protein A?

The interactions between NP7 and IgG, BSA, TNFα, and hemoglobin in each of which were immobilized on a QCM surface in PBS (20 mM, pH 7.3 with 150 mM NaCl)
The interaction between NP7 and the Fc fragment in water. Red and Black are NP7 from different batches. The squares plot interactions between NP7 and Fc fragment and the circles plot the interaction between NP7 and the QCM gold surface under the same conditions except the Fc fragment was omitted. It demonstrated that implied that the interaction is not due to batch specific reproducibility.

**pH Dependence of NP-IgG Affinity Competition Studies with Protein A**

Protein A binding site shown on IgG1–Fc. Binding site atoms are colored cyan.

Computationally selected NP binding sites at (a) neutral and (b) low pH. Computational results indicate NP7 is more likely to bind the protein A binding site at lower pH in agreement with experimental observations. Residue side-chains are colored green if the combined score of its surface region is greater than 0.0, and brighter shades of green indicate higher scores.
Water (pH 5.5) Standard deviations were calculated from three independent measurements.

**Binding isotherm of Fc domain to NP7 immobilized on QCM surface**

Conclusions

NPs that bind to Fc fragments of IgG were identified from a library of multifunctional polymer NPs.

NP binding domain(s) on the Fc fragment were identified from the Fc-protein A crystal structure and competition binding experiments.

Affinity could be controlled by adjusting pH of the solution.

NP binding constant (at pH 5.5) was comparable to protein A (at pH 7.2), but the binding capacity was 5 times smaller than protein A.

These results suggest that synthetic polymer NPs can be engineered to have an intrinsic affinity to a specific domain of a large biomacromolecule.

Acknowledgements

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