Studying the Effects of Concave and Confined Surfaces on Immobilized Proteins

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Outline

• Motivation
  ○ Surface curvature and nano-bio interactions

• Methodology
  ○ Preparation of nanomaterials
  ○ Probe the nano-bio interactions
    □ Protein uptake
    □ Immobilized protein activity

• Conclusion
Motivation: Nano-bio Interactions

Controllable by people

- Our task to understand and utilize

Nano Structure

- Morphology
  - Curvature
  - Shape
- Atomic structure
  - Surface Energy
- Chemistry
  - Substrate Materials
  - Surface Modification

Nano-bio Interaction

- Fundamental exploration

Topographical Chemical Effects

Protein:
- NANOMETER scale (usually <50 nm)
- Structure
- Stability
- Function
Nano-bio interactions

- Morphology effect: Surface curvature


SBP on CNT ( ● )/graphite ( ▲ )
Nano-bio interactions

**Negative surface curvature**

- Negative surface curvature

Gold Nanocage (AuNG)

- Bio-compatible
- Electromagnetic properties
Au-Ag nanocage: a platform


$\text{HAuCl}_4 + \text{Ag} \rightarrow \text{Au} + 3\text{Ag}^{+} + 4\text{Cl}^{-}$
Nanocages (AuNG)
Nanomaterials Preparation

**Surface ligand**

- Poly(vinylpyrrolidone), PVP
  - Binding to cage via carbonyl group
  - Poor protein affinity

[11-Mercaptoundecanoic acid], MUA
- Binding to cage via sulfide bond
- pKa=5.7

X-ray Photoelecton Spectroscopy (XPS)

<table>
<thead>
<tr>
<th>XPS</th>
<th>Before</th>
<th>MUA 1 Day</th>
<th>MUA 2 Day</th>
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<tbody>
<tr>
<td>Au</td>
<td>120</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>N</td>
<td>80</td>
<td>40</td>
<td>40</td>
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<tr>
<td>S</td>
<td>200</td>
<td>275</td>
<td>220</td>
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</tbody>
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Lysozyme Uptake of AuNC and AuNG

**AuNG-Lyz**

- 100% exterior coverage
- $R^2=94\%$

**AuNC-Lyz**

- $R^2=84\%$
- 100% exterior coverage
Lysozyme Uptake of AuNG and AuNC: Summary

Protein uptake isotherm

- AuNG and AuNC behave similarly at low free protein concentrations
- AuNC saturates at high protein concentration, where AuNG doesn’t
- AuNG’s internal surfaces are available for protein uptake, and high protein concentration increased their accessibility
Enzymatic Activity Assays

- Cell wall assay (*Micrococcus lysodeikticus*)

- Fluorescence assay \( 4\text{-Methylumbelliferyl } \beta\text{-d-N,N2,N22-\text{triaxety}} \) get into nanocages cleaved by lysozyme and has fluorescent effect \( \text{ex@355 nm, em@460 nm} \)
Activity Assays

![Graph showing relative activity of AuNG-Lyz and AuNC-Lyz in fluorescence and M. Lysodeikticus assays.](image)

- **Relative Activity (%)**
  - X-axis: Fluorescence Assay, M. Lysodeikticus Assay
  - Y-axis: Relative Activity

- **Graph Legend**
  - Blue: AuNG-Lyz
  - Red: AuNC-Lyz
The Two Substrates and Their Accessibilities
Investigating the Two Assays

Lyz sees AuNG & AuNC’s external surface similarly. For AuNG:
\[ N(\text{int}) = 85\% \, N(\text{ext}) \]

For the fluorescence assay:
\[ A_{\text{ext}} N_{\text{ext}} + A_{\text{int}} N_{\text{int}} = A_{\text{AuNG}} N_{\text{AuNG}} \]

A: Specific activity
N: Protein amount
The Activity of Lyz Inside AuNG

$$A_{ext}N_{ext} + A_{int}N_{int} = A_{AuNG}N_{AuNG}$$

![Graph showing relative activity of AuNG-Ext, AuNG-Int, and AuNC.](image-url)
Conclusions & Future work

• A significant amount of proteins can be internalized into AuNG
• And more importantly, remain substantial functional
• More precise characterizations of the immobilized proteins are being developed
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