Peptide-based Sensing Utilizing Gold Nanoparticles for the Development of a Protease Biosensor

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Outline

• Introduction
• Methodology
• Results
• Conclusion
• Future
Introduction

• Development of a fluorescent peptide-based biosensor

• Detection of medical relevant analytes:
  • Human thrombin/trypsin
Methodology
Gold Nanoparticles

- Au Nanoparticles can be utilized as:
  - Fluorescent enhancers
  - Fluorescent quenchers

- Depends on:
  - Size
  - Shape
  - Distance
  - Orientation of dipoles

2nm-20nm
Plasmon absorption
# Peptide Sequences

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CF6</td>
<td>H$_2$N-Cys-dPhe-Pro-Arg-Gly-Lys(Ahx-Fluorescein)-OH</td>
<td></td>
</tr>
<tr>
<td>51-4</td>
<td>H$_2$N-Cys-Gly-Val-Pro-Gly-Val-Lys(Ahx-Fluorescein)-OH</td>
<td></td>
</tr>
</tbody>
</table>

Peptide was cleaved at enzyme sensitive region

**Proline-Arginine-Glycine**
Surface Immobilization

- Simple covalent binding due to the strong interaction between gold and cysteine on the n-terminal of the peptide
Sensing Mechanism

Gold Nanoparticle

Fluorescein-labeled Peptide

Protease

Cleaved Fluorescein-labeled Peptide

λ₀

λ₁

λ₂
Results
Fluorescence Recovery in Presence of Trypsin

Time Response - Trypsin Cleavage of Gold Nanoparticles/CF6 Peptide over Time

- Initial
- 20 Sec
- 1 Min
- 2 Min
- 3 Min
- 4 Min
- 10 Min

Intensity (cps)
- 1.5x10^5
- 1.0x10^5
- 5.0x10^4
- 0

Wavelength (nm)
- 510
- 520
- 530
- 540
- 550
Percent Signal Change

Change in Intensity over Time

Intensity Change (%) vs Time (Seconds)
Dosage Response - Percent Intensity Change with Different Concentrations of Trypsin at 20 sec
Conclusion

• It is possible to immobilize peptides onto metallic nanoparticles for a very fast and sensitive response to trypsin

• Dosage response indicated limit of detection in the range of 6.1 µg/ml with a near immediate response time
Future Work

• Continue to optimize conjugation protocol to decrease limit of detection

• Amend to detect additional analytes
  – Explosives (TNT, RDX, etc.)
  – Neurotoxins (Botulinum)

• Incorporating design into a lab-on-chip based device
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Questions?