Construction of a Cell Penetrating Virus-Based Particle

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Short and Long-term Goals

• Construct a virus-based particle capable of self-assembling into a cell-penetrating device with therapeutic value (cargo loading).

• Rigorously characterize the particle so you can incorporate principles of rational peptide/protein design for improved function (assembly)

• Get the particle to penetrate certain cell types specifically (targeting).
<table>
<thead>
<tr>
<th>Well #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{+2}) (mM)</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
<td>0.2</td>
<td>0.1</td>
<td>0</td>
</tr>
</tbody>
</table>

proheads | pentamers

February 9, 2010
Pentamer is soluble protein with significant \( \alpha \)-helical content, which remains prominent after conversion to prohead.

- Quantitative estimate of 22% helix using PCDDDB/DichroWeb.

Pentamer is shifted to higher mass 0.6 - 1.3 MDa by size exclusion when incubated with 5 nm Au nanoparticles.

- Consistent with 1:1 to 3:1 pentamer:gold.

- Analytical ultracentrifuge studies to follow.

[Jack Correia; Univ. of Mississippi]
<table>
<thead>
<tr>
<th>protein</th>
<th>Dmax (angstrom)</th>
</tr>
</thead>
<tbody>
<tr>
<td>orf5</td>
<td>150</td>
</tr>
<tr>
<td>prohead</td>
<td>300</td>
</tr>
</tbody>
</table>

**SAXS**: Maximum size of pentamer
- new information

**SAXS**: Information on size of prohead in solution; spherical
- consistent with TEM and STEM at 27-33 nm
Gold particles 5 nm - 10 nm diameter (up to 15 nm)

dsDNA 21 bp are protected

What we always see: 1-2 particles per head

We can load up to 50% of heads by changing Au/protein

What we never see:

Pentamer edge on

out
in

yes
no
Orf5 + Ca^{2+} + trypsin

Monitor completeness of digestion by SDS-PAGE

LC MS/MS

Orf5 + Au + Ca^{2+} + trypsin

Monitor completeness of digestion by SDS-PAGE

100,000g

Aqueous layer
Glycerol layer

February 9, 2010
Self Assembled Particles

Ca^{2+}
Adaptosome

Prohead attachment | Cell penetration
S – S

Prohead attachment | Cell attachment
S – S

M13 library

Sequence DNA (Peptide)

Prohead attachment | Cell attachment or penetration
S – S

attach

wash away non-binders

elute

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Phage particles (successive 5-fold dilution)

- Phage 1 (YHTAWWP) + Orf 5
- Phage 1 (YHTAWWP) - Orf 5
- Phage 2 (WHDAWWP) + Orf 5
- Phage 2 (WHDAWWP) - Orf 5

GTA-1  WHDAWWP
Pep-1  KETWWETWWTEWSQPKKKRKV

|  S  |  S  |

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Control: Cells alone – bright field

Orf5 pentamers FITC-labeled (no Pep-1) - fluorescence

Orf5 proheads FITC-labeled (no Pep-1) - fluorescence

Control: Cells alone – fluorescence

Orf5 pentamers FITC-labeled with Pep-1, 1:130 - fluorescence

Orf5 proheads FITC-labeled with Pep-1, 1:130 - fluorescence
Outcome(s) and Future Plans

We have made inroads into the physical nature of the GTA prohead and can load it with cargo.

We plan to continue this effort, using X ray structural analysis, analytical biochemical and molecular biological methods

We have begun to develop the means to introduce the particle into cells

Find a robust method for cell penetration using a combination of our phage display head binders with available cell penetrating motifs
Collaborators and Co-workers

• Brian Jones – Bruker, Inc - Small Angle X-ray Structure
  Michal Sabat (UVA, Chemistry)

• Robert Kretsinger (UVA, Biology) – X-Ray Analysis

• Furqan Sami - Crystal structure
  *Hussein Al-Mohammed - Crystal structure

• Tony Spano (UVA, Biology)
  Nikolai Lebedev (NRL)
  *Jennifer Kefauver (Physic/Biology) – gold encapsulation
  *Hillary Sloane (Chemistry) – size exclusion
  * precocious hardworking undergraduates

2008 Seed Project Results  February 9, 2010