Site-specific drug delivery using Virus-Like Particles
Meet the Wageningen UR team!
Adverse effects conventional drug delivery
Using Synthetic Biology to create site-specific drug carriers

Aim
Improving current medicine by making them site specific.
Safety in the lab and in the application
  – No DNA or RNA present in the final product

Why?
Better for patient’s health
More efficient use of drugs

How?.......

Wild type Virus-Like Particle
Modified VLP
Obtaining the coat protein genes

<table>
<thead>
<tr>
<th>Virus Like Particle</th>
<th>Provided by</th>
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<tbody>
<tr>
<td>Cowpea Chlorotic Mottle Virus (CCMV)</td>
<td>Wageningen UR.</td>
</tr>
<tr>
<td></td>
<td>Dr. Kormelink</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>John Innes Centre, Norwich.</td>
</tr>
<tr>
<td></td>
<td>Prof. Lomonossoff</td>
</tr>
<tr>
<td>Potato Leaf Roll Virus (PoLeRo)</td>
<td>Nature</td>
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</table>
Obtaining PoLeRo

Isolation of RNA from infected potato plant leaves.

Using RT PCR, we made the following natural BioBricks: BBa_K883402 BBa_K883403 BBa_K883404
Overview VLP production and assembly

- Selection of transformants (1 day)
- Production of VLPs in bacteria (1 day)
- Ultracentrifugation (4 hours)
- Dialysis steps (4 days)
- Cell lysis at 15000 psi (1 hour)
VLP Detection

Electron Microscopy
Used to visualize the VLPs

Dynamic Light Scattering
Used to detect and characterize the VLPs
(1) $C = C_{\text{total}} - C_{\text{critical}}$
(2) $\frac{dC}{dt} = \frac{1}{2} k_{v,\text{critical}} C^2 - k_v C C_2$
(3) $\frac{dA}{dt} = \frac{1}{2} k_A C^2$
(4) $\frac{dC}{dt} = k_{v,\text{critical}} C^2 - k_v C \sum_{i=2}^{s-1} C_i - k_A C^2$
(5) $\frac{dC_i}{dt} = k_v C (C_{i-1} - C_i)$; $i = 3, 4, \ldots, s-1$
(6) $\frac{dV}{dt} = k_v C C_{s-1}$
Drug packaging
Drug packaging

Douglas et. al, 2002
Drug packaging

Overhang extension PCR

step I
- first extension

step II
- second extension

step III
- st. 10 prefix

CCMV Δ25 mutant coat protein

st. 10 suffix

result
Drug packaging

Overview VLP production and assembly

- Selection transformants (1 day)
- Ultracentrifugation (4 hours)
- Dialysis steps (4 days)
- Production VLPs in Bacteria (1 day)
- Cell lysis at 15000 psi (1 hour)
The Plug and Apply (PnA) System
VLP standardisation
VLP standardisation
VLP standardisation

VLP with K-coil: M KIAALKEKIAALKEKIAALKEKIAALGDDGGSSGGGSAA
Sequence result: ATG.............................GCTGTGTGTTCAACCTGTATTGTAGAA...
Translation: M KIAALKEKIAALKEKIAALKEKIAALGDDGGSSGGGSAA A VW S N L L L *

No BioBrick...
Modified Virus-Like Particle
Modified GFP

• As part of the PnA system, we fused The E-coil to GFP.

Minten et al, 2009

BBa_K883700
<table>
<thead>
<tr>
<th>Part</th>
<th>Designers</th>
<th>Group</th>
<th>Date</th>
<th>Categories</th>
<th>DNA Planning</th>
<th>Experience</th>
<th>Actions</th>
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<td>BBa_J62003</td>
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<td>2008-07-26</td>
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<td>Protein Domain V5 tag</td>
<td>DNA Planning</td>
<td>Experience: None</td>
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More than half of the BioBricks can still be improved

Need an application to circumvent the quality issue
The Constructor

Optimizing cloning strategies since 2011
Further simplifying this procedure since 2012

E-mail (optional)
E-mail:

Transcription Units (TUs)
Caution: check RPC compatibility!
TU 1:
TU 2:

Publication (letter to the editor):
Journal of Biological Engineering

The Constructor: a web application optimizing cloning strategies based on modules from the registry of standard biological parts (highly accessed)
To investigate the need and perception of our standardised delivery system and synthetic biology
Our Sponsors:

Middelhoven Fund

Mark van Passel

Floor Hugenholtz
Achievements

• We produced, assembled, modified, detected and visualised VLPs.
• Validated DLS as detection method for VLPs.
• We learned.
• We presented at two scientific conferences.
• We published a peer reviewed paper.
• We turned Human Practices in a national effort.
• We designed a medical delivery tool by using the PnA System.
• A safe to use and produce medicine.
Site-specific drug delivery using Virus-Like Particles