Environmentally-responsive poly(amoenoesters): Applications for the delivery of mRNA

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Chem-H Postdoc Retreat
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Some problems require a multi-disciplinary approach

- New cationic pH sensitive materials
- New monomer and polymer synthesis
- Alcohol oxidation catalysis
- Waymouth Lab
- Drug/gene delivery vehicles
- Wender Lab
- In vivo imaging and quantification
- Bioluminescent imaging
- Contag Lab
- Functional biomaterials for gene delivery
Requirements for effective gene transfection

1. Pack and protect mRNA
2. Mediate cell entry
3. Release cargo (escape endosome)
4. Reach ribosome for translation

Cationic Oligomer

mRNA transient expression of “any” gene

<table>
<thead>
<tr>
<th></th>
<th>mRNA</th>
<th>siRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight</td>
<td>ca. 330 kDa</td>
<td>13 kDa</td>
</tr>
<tr>
<td>Length</td>
<td>ca. 1000 nt</td>
<td>2 x 21 nt</td>
</tr>
<tr>
<td>Secondary Structure</td>
<td>Poorly defined single strand</td>
<td>Double stranded double helix</td>
</tr>
<tr>
<td>Size</td>
<td>17-20 nm</td>
<td>2nm x 6 nm</td>
</tr>
<tr>
<td>Effect</td>
<td>Gene induction</td>
<td>Gene silencing</td>
</tr>
<tr>
<td>Limitations</td>
<td>Readily degraded</td>
<td>No protein induction</td>
</tr>
</tbody>
</table>

Release is critical
In vitro gene delivery

siRNA delivery to HaCat cells

This strategy is ineffective for mRNA delivery

Design criteria for siRNA delivery vehicles do not apply to mRNA

• Successful expression of mRNA requires delivery and release

New developments provide another approach

**Catalytic oxidation:** monomer synthesis

**Catalytic oxidation:** monomer synthesis

**Organocatalytic polymerization:** designer materials

**Organocatalytic polymerization:** designer materials

**Functional, biocompatible “smart” materials**

**Functional, biocompatible “smart” materials**

**mRNA delivery vehicles “CARTs”**

**mRNA delivery vehicles “CARTs”**
**Responsive poly(α-aminoesters)**

- **Deprotection**
- **pH 7.4**

\[
y = -0.0386x - 0.0676 \\
R^2 = 0.99513
\]

**First-order Plot**

<table>
<thead>
<tr>
<th>pH</th>
<th>T_{1/2}</th>
<th>Time (min)</th>
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</thead>
<tbody>
<tr>
<td>7.4</td>
<td></td>
<td>&lt;5</td>
</tr>
<tr>
<td>6.4</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>5.4</td>
<td></td>
<td>18</td>
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</table>

*Fast and selective

Cationic polymer → neutral small molecule

*Wilson Ho
*Andrey Rudenko
Proposed charge-canceling mechanism

Native chemical ligation

Fast and Selective
mRNA delivery: cell culture

Using new amphipathic materials we can transfct mRNA and elicit the expression of reporter genes in multiple cell lines.

Flow cytometry: GFP expression

Non-toxic

Higher efficiency than commercial agent lipofectamine

MTT cell viability assay

Structure-activity relationships

This polymerization strategy allows for rapid screening of new oligomers

Block length matters
Non-degrading oligomers don’t work
Lipid is required

McKinley, Blake, et al. PNAS, 2017, E448-E456
To express mRNA, mRNA must escape endosome

Confocal Microscopy
- Allows for independent imaging of transporter and cargo on a cell-by-cell basis

Fluorophores
- Dansyl: attached to transporter
- GFP: indicates expression has occurred
- Cy5: attached to mRNA

Conditions: HeLa cells, 10:1 +/- charge ratio, 4 hours following treatment
mRNA expression via multiple routes of administration in vivo

Intramuscular

Intravenous (tail vein)

Luciferase mRNA administered at t=0
Luciferine administered at time of measurement

Localizes in spleen

7.5 ug mRNA
75uL PBS

McKinley, Blake, et al. PNAS, 2017, E448-E456
Conclusions

• Our oligomerization strategy is a great platform for rapid synthesis and screening of new delivery vehicles

• Rapidly degrading biocompatible oligomers allow for mRNA delivery and robust protein expression

• Release of mRNA from the CART polyplex is required for protein expression
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