Electronic Supplementary Information

Complexation and conjugation approaches to evaluate siRNA delivery using cationic, hydrophobic and amphiphilic peptides

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Fig. S1 CD and $T_m$ data. A) CD spectra; **PEP-C-J**, **PEP-H-J**, and **PEP-A-J** exhibit A-form RNA. B) UV absorbances of **PEP-C-J**, **PEP-H-J**, and **PEP-A-J** plotted with respect to temperature; values of $T_m$: natural siRNA, 67.4 °C; **PEP-C-J**, 73.5 °C; **PEP-H-J**, 69.5 °C; **PEP-A-J**, 68.6 °C.
Table S1 ζ-potential of peptide–siRNA complexes

<table>
<thead>
<tr>
<th>molar ratio&lt;sup&gt;a&lt;/sup&gt;</th>
<th>peptide–siRNA complex ζ-potential (+)/mV&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PEP-C-X</td>
</tr>
<tr>
<td>16</td>
<td>-5.34 (±0.417)</td>
</tr>
<tr>
<td>32</td>
<td>-9.92 (±0.193)</td>
</tr>
<tr>
<td>64</td>
<td>-1.73 (±0.209)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number of charges of the peptide divided by the number of charges of the siRNA.  
<sup>b</sup> Mean values from three experiments; standard deviations are given in parentheses. siRNA concentration: 1.00 μM.
NMR spectra

6,6’-Disulfanediylhexan-1-ol (1)

$^1$H-NMR

$^{13}$C-NMR
6-[(4-Bis(4-methoxyphenyl)phenylmethoxy)hexyl]disulfanyl)hexan-1-ol (2)

$^1$H-NMR

$^{13}$C-NMR
6-((6-[Bis(4-methoxyphenyl)phenylmethoxy]hexyl)disulfanyl)-1-[bis(1-methylethyl)phosphoramidyl]cyanocethyhexane (3)

$^1$H-NMR

$^{13}$C-NMR
$^{31}$P-NMR
Table S1 MALDI-TOF mass data and HPLC profiles of peptides

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sequence</th>
<th>Mass Calculated</th>
<th>Mass Found</th>
<th>$t_R$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEP-C</td>
<td>C(PyS) RRRKK</td>
<td>954.2</td>
<td>954.6</td>
<td>9.14</td>
</tr>
<tr>
<td>PEP-H</td>
<td>C(PyS)PFVYLI</td>
<td>961.5</td>
<td>962.7</td>
<td>16.0</td>
</tr>
<tr>
<td>PEP-A</td>
<td>C(PyS)RRRKKPFVYLI</td>
<td>1685.9</td>
<td>1685.9</td>
<td>12.9</td>
</tr>
</tbody>
</table>

HPLC eluents: a gradient from 5% MeCN/0.1 M TEAA buffer (pH 7.0) to 50% MeCN/0.1 M TEAA buffer was run over 30 min at 2.50 mL min$^{-1}$; detection at 254 nm using a semi-preparative VydaC C-18 column (Cat. 218TP510); $t_R$ is the retention time of compound peak in HPLC.

PEP-C

![Graph of PEP-C](image1)

PEP-H

![Graph of PEP-H](image2)

PEP-A

![Graph of PEP-A](image3)
Table S2 MALDI-TOF data and HPLC profiles of oligonucleotides

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mass Calculated</th>
<th>Mass Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>sense strand of VEGF-N</td>
<td>6709.7</td>
<td>6708.2</td>
</tr>
<tr>
<td>antisense strand of VEGF-N</td>
<td>6603.8</td>
<td>6603.9</td>
</tr>
<tr>
<td>5’-thiol modified sense strand of VEGF siRNA</td>
<td>6904.1</td>
<td>6903.5</td>
</tr>
<tr>
<td>5’-Fluorescein labeled antisense strand of VEGF siRNA</td>
<td>7141.4</td>
<td>7141.7</td>
</tr>
</tbody>
</table>

sense strand of VEGF-N

antisense strand of VEGF-N
5’-fluorescence-labeled antisense strand of VEGF-siRNA

The gradient of the HPLC (Agilent Ecilpse XDB-C8 5 μm, 4.6 x 150 mm) mobile phase was then increased linearly over 8 min from 5% MeCN /0.1 M triethylammonium acetate (TEAA, pH 7.2) buffer to 40% MeCN /0.1 M TEAA (pH 7.2) buffer at a flow rate of 1.50 mL/min

5’-Thiol-modified sense strand of VEGF-siRNA
Table S3 MALDI-TOF data and HPLC profiles of peptide–siRNA conjugates

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mass Calculated</th>
<th>Mass Found</th>
<th>(t_R) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEP-C-J</td>
<td>7747.2</td>
<td>7747.8</td>
<td>5.07</td>
</tr>
<tr>
<td>PEP-H-J</td>
<td>7755.2</td>
<td>7755.9</td>
<td>14.0</td>
</tr>
<tr>
<td>PEP-A-J</td>
<td>8480.1</td>
<td>8480.3</td>
<td>11.4</td>
</tr>
</tbody>
</table>

HPLC eluents: buffer A: 0.1 M TEAA buffer solution (pH 7.0); buffer B, MeCN. Elution was a gradient from 5% (0 min) to 50% (25 min) to 5% (30 min) buffer B at a flow rate of 2.5 mL min\(^{-1}\); oligonucleotides were detected at 254 nm; \(t_R\) is the retention time of compound peak in HPLC.

**PEP-C-J**

HPLC chromatogram and MALDI-TOF mass spectrum of **PEP-C-J**. MALDI-TOF MS: \(m/z\) 7747.8 (calcd. 7747.2).

![MALDI-TOF MS: m/z 7747.8 (calcd. 7747.2)](image)
PEP-H-J

HPLC chromatogram and MALDI-TOF mass spectrum of **PEP-H-J**. MALDI-TOF MS: $m/z$ 7755.9 (cald. 7755.2).
PEP-A-J

HPLC chromatogram and MALDI-TOF mass spectrum of **PEP-A-J**. MALDI-TOF MS: \( m/z \ 8480.3 \) (calcd. 8480.1).

\[
\text{Ile-Leu-Tyr-Val-Phe-Pro-Lys-Lys-Arg-Arg-Arg-Cys-NH}_2
\]

\[
\text{O-P-O-GGA GUA CCC UGA UGA GAU CudT-3'}
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