Electronic Supplementary Information

Self-Assembly of Mitochondria-Specific Peptide Amphiphiles
Amplifying the Lung Cancer Cell Death through Targeting the VDAC1-Hexokinase-II Complex

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SAXS modelling

To account for possible deviations from spherical entities, the scattered intensity $I(q)$ was modelled as coming from a population of ellipsoids of rotation $I(q)$ can be described in a decoupling approximation (no correlation between size/orientation and position of particles) by the following equation:  

$$I(q) = I(0)P(q)S'(q) + B$$  

Where  

$$P(q) = \langle |F(q)|^2 \rangle$$  

$$S'(q) = 1 + \beta(q)\cdot[S(q) - 1]$$  

$$\beta(q) = |\langle F(q) \rangle|^2/\langle |F(q)|^2 \rangle$$

The inner brackets $\langle \rangle$ in equations (2) and (4) represent an average weighted by the distribution of particle sizes and/or orientations, $I(0)$ is the scattering at zero angle (proportional to concentration of particles, contrast, and particle volume), $P(q)$ is the form factor, $F(q)$ is the amplitude of the form factor, $S(q)$ is the structure factor, and $S'(q)$ is the effective structure factor modified by the anisotropy and polydispersity of particles.

In the case of core shell ellipsoid of rotation of semiaxis $a, b, F(q)$ is expressed as

$$F(q) = 3(\rho_{core} - \rho_{solvent})V_{core} \frac{\sin(qR) - qR\cos(qR)}{(qR)^3} +$$

$$3(\rho_{shell} - \rho_{solvent})V_{shell} \frac{\sin(q(R + T) - q(R + T)\cos(R + T)}{(q + (R + T)^3}$$

(5)

Where  

$$r = [b^2\sin^2 \alpha + a^2\cos^2 \beta]^{1/2}$$

and $\alpha$ is the angle between the axis of ellipsoid $a$ and scattering vector $q$, and $T$ is the thickness of shell. A log normal distribution of $a$ was used in analysis.

The excluded volume interaction calculated with the Percus-Yevick approximation for the closure relation. The detailed expression for the function can be found in.  

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Table S1 Model of ellipsoids of revolution (a, b, b) with excluded volume interaction, polydispersity sigma of an axis as log normal distribution and $<a>$ - mean value of orbital axis.

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Concentration [mg mL$^{-1}$]</th>
<th>$&lt;a&gt;$ [Å]</th>
<th>Sigma [Å]</th>
<th>b [Å]</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHK-pKV</td>
<td>1</td>
<td>48±2.0</td>
<td>1.13±0.20</td>
<td>20.1±0.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>41±2.0</td>
<td>1.03±0.08</td>
<td>20.1±0.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>37±2.0</td>
<td>0.70±0.04</td>
<td>20.1±0.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>36±0.9</td>
<td>0.65±0.02</td>
<td>20.2±0.1</td>
</tr>
<tr>
<td>Pal-pHK-pKV</td>
<td>1</td>
<td>103±3.0</td>
<td>1.4 fixed</td>
<td>42.0±0.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>29±1.0</td>
<td>0.77±0.03</td>
<td>42.3±0.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>31±1.0</td>
<td>0.59±0.02</td>
<td>43.0±0.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32±1.0</td>
<td>0.44±0.02</td>
<td>43 fixed</td>
</tr>
</tbody>
</table>
Secondary structures

The secondary structural data of the CD spectra were analyzed using a CDNN deconvolution program, which is a method based on a neural network theory to deconvolute the CD spectra into five different secondary structures (α-helix, β-sheets, β-turn, parallel, antiparallel and random coil). The obtained results are presented in the following Table S2.

**Table S2** Quantitative content of peptides’ conformation distributions measured by CDNN.

<table>
<thead>
<tr>
<th>Secondary structure</th>
<th>pHK</th>
<th>pHK-pKV</th>
<th>Pal-pHK-pKV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-helix (%)</td>
<td>12.8</td>
<td>20.1</td>
<td>53.9</td>
</tr>
<tr>
<td>Antiparallel β-sheet (%)</td>
<td>20.3</td>
<td>14.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Parallel β-sheet (%)</td>
<td>19.1</td>
<td>13.7</td>
<td>5.2</td>
</tr>
<tr>
<td>β-turn (%)</td>
<td>22.3</td>
<td>19.8</td>
<td>13.5</td>
</tr>
<tr>
<td>Random Coil (%)</td>
<td>43.4</td>
<td>33.7</td>
<td>23.3</td>
</tr>
<tr>
<td>Total Sum (%)</td>
<td>117.9</td>
<td>101.4</td>
<td>100.2</td>
</tr>
</tbody>
</table>
Fig. S1 SAXS patterns of aqueous solutions of Pal-pHK-pKV at high concentrations.

(1.4 mM, 2.8 mM, 5.5 mM, 11 mM, 22 mM).
**MTT assay**

MTT measurements were performed after 24 h, 48 h, and 72 h treatments by peptide formulations. Fig. S2a shows that pHK-pKV exerts effects on the A549 cells viability at essentially higher concentrations (>12.5 μM) as compared to Pal-pHK-pKV. Notably, Pal-pHK-pKV displays a cytotoxic effect already at a very low concentration (3.13 μM Pal-pHK-pKV) (Fig. S2b).

![Graphs showing cell viability](image)

**Fig. S2** Cells treated with increasing concentrations of peptides for various time intervals: a) pHK-pKV and b) Pal-pHK-pKV.
Fig. S3 (a) The purity of peptide pHK analyzed by HPLC. (b) The peptide pHK was identified using mass spectrometry (Mw = 1770.10 g · mol⁻¹).
Fig. S4 (a) The purity of peptide pHK-pKV analyzed by HPLC. (b) The peptide pHK-pKV was identified using mass spectrometry (Mw = 2978.67 g · mol⁻¹).
Fig. S5 (a) The purity of peptide Pal-pHK-pKV analyzed by HPLC. (b) The peptide Pal-pHK-pKV was identified using mass spectrometry (Mw = 3217.08 g · mol⁻¹).
Fig. S6 (a) The purity of peptide pHK-AMC analyzed by HPLC. (b) The peptide pHK-AMC was identified using mass spectrometry (Mw = 1927.27 g · mol⁻¹).
Fig. S7 (a) The purity of peptide pHK-pKV-AMC analyzed by HPLC. (b) The peptide pHK-pKV-AMC was identified using mass spectrometry (Mw = 3135.84 g · mol⁻¹).
Fig. S8 (a) The purity of peptide Pal-pHK-pKV-AMC analyzed by HPLC. (b) The peptide Pal-pHK-pKV-AMC was identified using mass spectrometry (Mw = 3374.26 g · mol⁻¹).

References