Supporting Information

Dual-targeted poly(amino acid) nanoparticles on-site deliver drug combinations: an intracellular synergistic strategy to eliminate intracellular bacteria

Dongdong Zhao*, Wenli Fenga, Xiaoxu Kanga, Haofei Lia, Fang Liub, Weitao Zhengc, Guofeng Lia*, Xing Wanga,*

a State Key Laboratory of Organic-Inorganic Composites; Beijing Laboratory of Biomedical Materials, Beijing University of Chemical Technology, Beijing 100029, P. R. China
b Department of Oncology of Integrative Chinese and Western Medicine, China-Japan Friendship Hospital, Beijing, 100029, China
c Hubei Provincial Key Laboratory of Industrial Microbiology, Sino-German Biomedical Center, National “111” Center for Cellular Regulation and Molecular Pharmaceutics, Hubei University of Technology, Wuhan, 430068, Hubei Province, China

* Corresponding author
E-mail address: ligf@mail.buct.edu.cn (G. Li); wangxing@mail.buct.edu.cn (X. Wang)
Scheme S1. Synthetic routes of the F, A\textsubscript{Boc}, M\textsubscript{OAc}, PF, and F(AM).
Figure S1. $^1$H NMR spectra of (a) F, (b) $A_{\text{Boc}}$, and (c) $M_{\text{OAc}}$ (400 MHz, DMSO-$d_6$).
Figure S2. $^1$H NMR spectrum of the PF (400 MHz, DMSO-$d_6$).

Figure S3. GPC curves of PF and F(AM).

Table S1. GPC analysis of PF and F(AM).

<table>
<thead>
<tr>
<th></th>
<th>Mn</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF</td>
<td>7069</td>
<td>1.34</td>
</tr>
<tr>
<td>F(AM)</td>
<td>11451</td>
<td>1.89</td>
</tr>
</tbody>
</table>
Figure S4. $^1$H NMR spectrum of F(A<sub>Boc</sub>M<sub>OAc</sub>) (400 MHz, DMSO-$d_6$).

Figure S5. $^1$H NMR spectrum of F(AM) (400 MHz, DMSO-$d_6$).
Figure S6. Co-localization fluorescence intensity distribution between NR@F(AM) NPs and L929 fibroblasts, which was analyzed using Image J software.

Figure S7. MIC assays of (a) Van and (b) Cur against MRSA.

Figure S8. HPLC standard curve of Van. Van was monitored at a wavelength of 230 nm. The mobile phase composed of 0.01 mol/L potassium phosphate monobasic
monopotassium phosphate solution (pH 3.2) and methanol (spectroscopic grade) (80:20, v/v) at a flow rate of 1.0 mL/min.

**Figure S9.** Absorption spectra of different concentrations of Cur in MeOH solution (left) and its standard curve at 425 nm wavelength (right).

**Table S2.** DLC and DLE of the (Van$_{1.5}$+CUR$_{3.0}$)@F(AM) NPs.

<table>
<thead>
<tr>
<th></th>
<th>DLC(%)</th>
<th>DLE(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Van$<em>{1.5}$+CUR$</em>{3.0}$)@F(AM) NPs</td>
<td>(Van)13.5%</td>
<td>(Van)62.4%</td>
</tr>
<tr>
<td></td>
<td>(Cur)23.2%</td>
<td>(Cur)54.9%</td>
</tr>
</tbody>
</table>

**Figure S10.** Release profiles of Van and Cur from (Van$_{1.5}$+Cur$_{3.0}$)@F(AM) NPs.
**Figure S11.** The variations of size and PDI of (Van$_{1.5}$+Cur$_{3.0}$)@F(AM) NPs under different conditions (H$_2$O and 10% FBS).

**Figure S12.** Zeta potential of F(AM) NPs and (Van$_{1.5}$+Cur$_{3.0}$)@F(AM) NPs.
Figure S13. TEM image of (a) Van@F(AM) NPs and (b) Cur@F(AM) NPs. (c) Size distribution of Van@F(AM) NPs and Cur@F(AM) NPs, testing by DLS. (d) The zeta potential of Van@F(AM) NPs and Cur@F(AM) NPs.

Figure S14. Intracellular antibacterial evaluation. (a) CFU count of intracellular MRSA after different treatments for 24 h. (b) CFU photographs of intracellular MRSA. ns $p > 0.05$, n = 3.
**Figure S15.** CFU of intracellular MRSA after treatment with different concentrations of Van (μg/mL).

**Figure S16.** (a) CLSM observation of FITC@F(AM) NPs (green) in RAW264.7 macrophages and (b) analysis of fluorescence intensity using Image J software. (**** p < 0.0001, n = 6).
Figure S17. (a) CLSM observation of co-localization between FITC-labeled NPs (green) and LysoTracker (red) in RAW264.7 macrophages. (b) Evolution with the time of the Pearson’s correlation coefficients between the signals from the FITC-labeled NPs and LysoTracker.

Figure S18. (a) The cell cytotoxicity of (Van+Cur)@F(AM) NPs against RAW264.7 macrophage. (b) The hemolysis rate of (Van+Cur)@F(AM) NPs with different concentrations.
Figure S19. (a) The cell cytotoxicity of (Van+Cur)@F(AM) NPs against L929 fibroblasts. (b) The cell cytotoxicity of F(AM) NPs against L929 fibroblasts.

Figure S20. The in vivo antibacterial assays of Van (10 mg/kg), Cur (20 mg/kg) and F(AM) NPs (20 mg/kg). (a) CFUs in total, intracellular and extracellular fractions. (b) CFU photographs.
Figure S21. Representative H&E staining images of various organs from mice treated with healthy control, PBS, Van+Cur, and (Van+Cur)@F(AM) NPs (10 mg/kg Van, 20 mg/kg Cur). Scale bars: 200 µm.