Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Targeting Intracellular Bacteria with an Extended Cationic Amphiphilic Polyproline Helix

Manish Nepal,[a] Shankar Thangamani,[b] Mohamed N. Seleem[b] and Jean Chmielewski[a]

[a] *Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907-2027 (USA)*
E-mail: chml@purdue.edu
[b] *Department of Comparative Pathobiology, Purdue University, 627 Harrison Street, West Lafayette, IN 47907-2027 (USA)*
E-mail: mseleem@purdue.edu

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Peptide Characterization

The purity of the peptides were determined using analytical HPLC. Peptides were injected in C18 analytical column at 25-65% acetonitrile/H_20 (with 0.1 % TFA) and allowed to elute for 30 min.

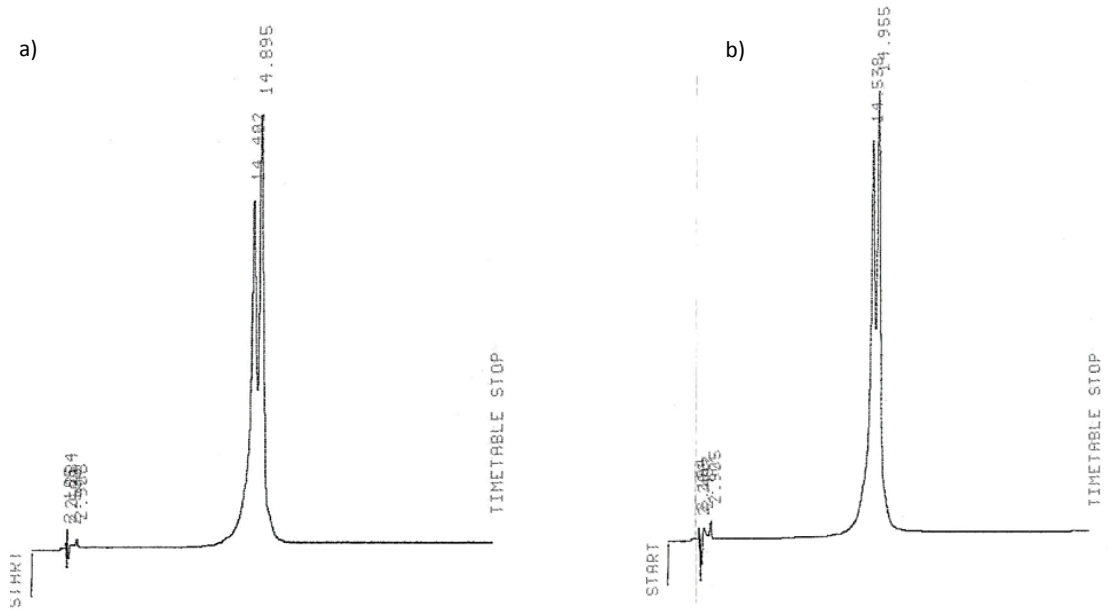

Fig S1. Analytical HPLC spectra of a) **Fl-$P_RP_RP_L$-4** and b) **Fl-$P_RP_RP_L$-5**. Note the 2 peaks in each spectra are due to separation of the different isomers of the attached fluorescein.

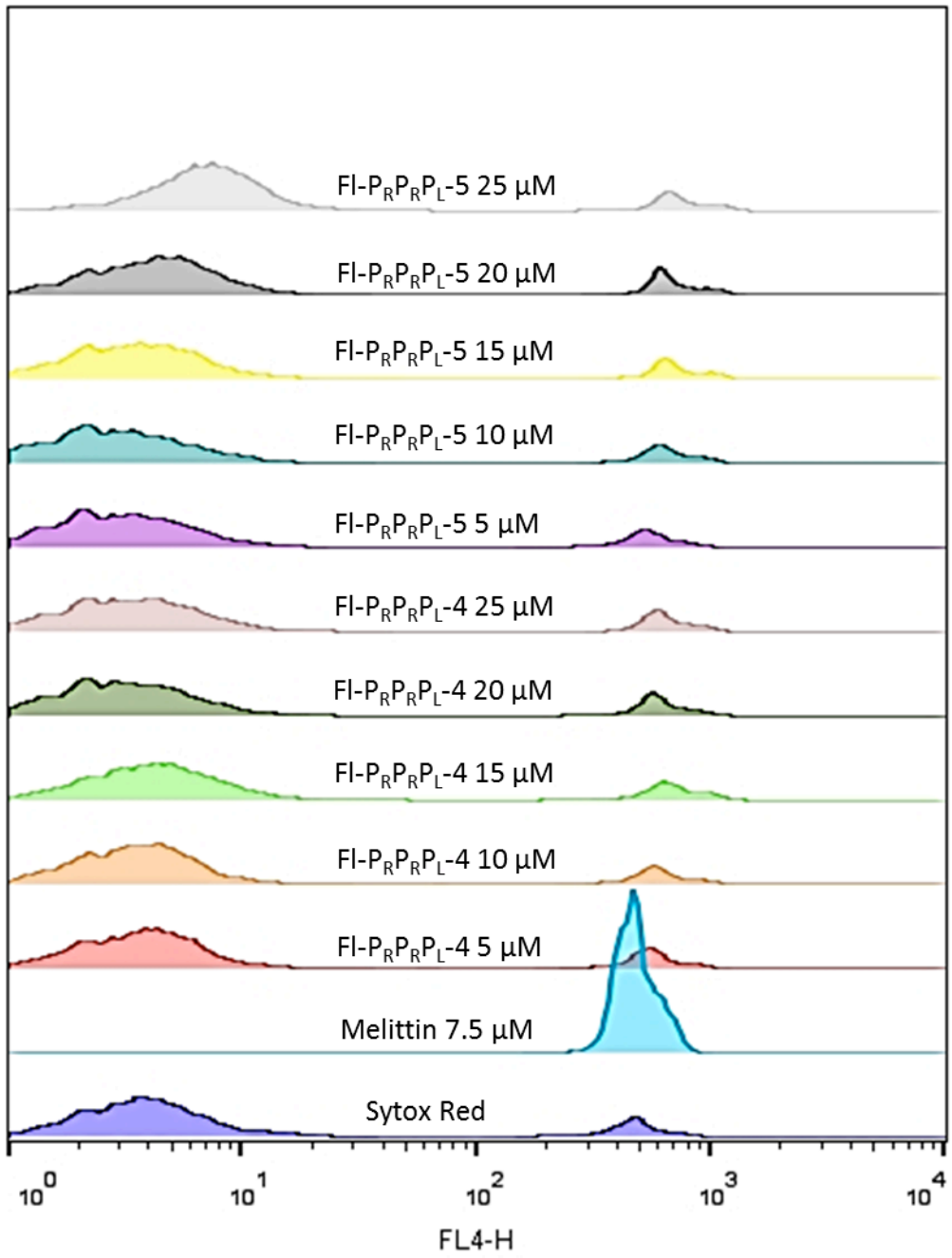

Fig S2: The distribution of cells with compromised and uncompromised membrane as determined by Sytox Red Dead Cell Stain (Life Technologies S34859). The population of uncompromised cells for all concentrations of peptides tested were comparable to the control (Sytox Red dye only) at around 84%. Melittin, an antibacterial peptide known for permeabilizing mammalian cell membranes, was used as a positive control at 7.5 µM. The data was processed using FlowJo software.

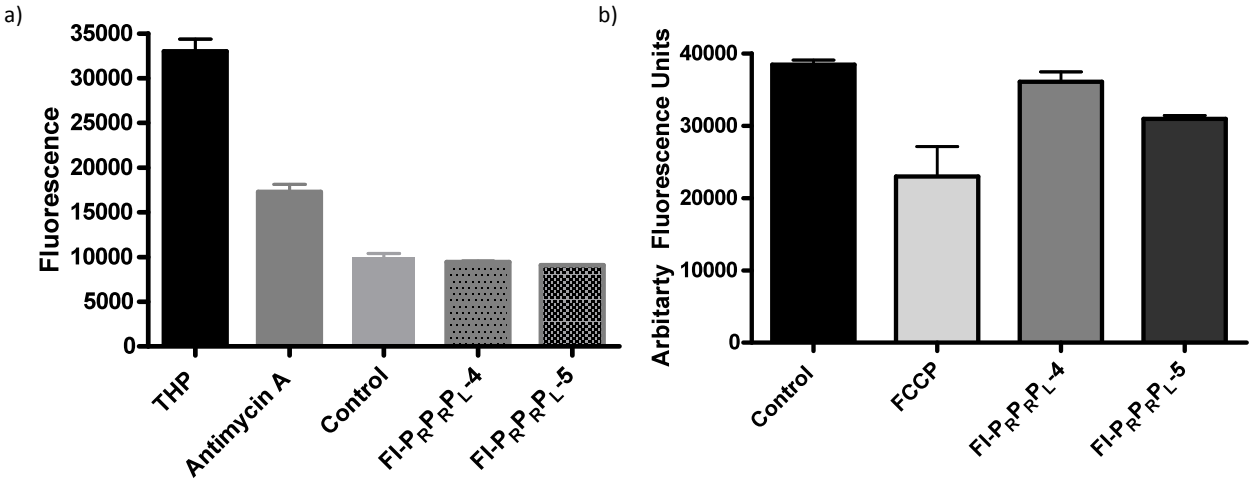

Fig S3: a) Production of *ROS* determined by monitoring oxidation of 50 µM DCFDA after treating cells with tert-Butyl hydroperoxide solution (THP, 100 µM), Antimycin A (100 µM), DCFDA only (control), **Fl-$P_RP_RP_L$-4** (15 µM) and **Fl-$P_RP_RP_L$-5** (15 µM).THP and Antimycin A, a drug that stops production of ATP in mitochondria and subsequently causes formation of large quantities of toxic free radical superoxide, was used as positive control. b) Analysis of mitochondrial membrane depolarization using tetramethylrhodamine ethyl ester (TMRE). J774A.1 cells were either treated with **Fl-$P_RP_RP_L$-4** (15 µM), **Fl-$P_RP_RP_L$-5** (15 µM), carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) (50 µM) or media only (control) followed by TMRE. The cellular fluorescence was measured by flow cytometry.

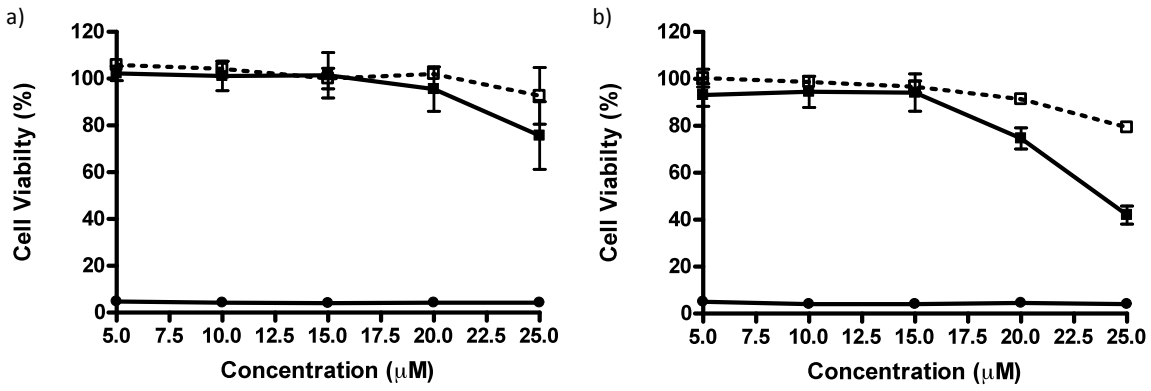

Fig. S4 Cell viability of J774A.1 cells after treating with **Fl-$P_RP_RP_L$-4** (5-25 µM) (hollow square) and **Fl-$P_RP_RP_L$-5** (5-25 µM) (solid square) for a) 4 h and b) 9h. Melittin (solid circle) was used as a positive control.