Electronic Supporting Information for

“Uncorking” of Liposomes by Matrix Metalloproteinase-9

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**Synthesis of the peptide P1 and the lipo-peptide LP1:**

**Synthesis of peptide P1:** The peptide was synthesized on a Rainin Symphony Quartet automatic peptide synthesizer, using CLEAR resin as the support and HBTU-HOBt as the coupling reagents. Each coupling step was for three hours and repeated twice with five-fold excess of reagents. Cleavage was performed for 3 h using a cocktail of CF$_3$CO$_2$H-anisole and water (95%-2.5%-2.5%). The crude peptide P1 was purified by RP-HPLC using a linear gradient of 0 – 70% acetonitrile in water over 40 minutes. Each solvent contained 0.1% trifluoroacetic acid. For P1, MH$^+$ (MALDI-TOF) calcd. for C$_{88}$H$_{137}$N$_{28}$O$_2$: 2066.00. Found: 2066.12.

Analytical HPLC conditions: Vydac C18 analytical HPLC column (238TP5415); eluant: linear gradient of 0 – 70% acetonitrile in water over 40 minutes; both solvents contained 0.1% CF$_3$CO$_2$H; flow rate: 1.5 mL/min.

Semi-preparatory HPLC conditions: Vydac C18 HPLC column (238TP152022); eluant: linear gradient of 0 – 70% acetonitrile in water over 40 minutes; both solvents contained 0.1% CF$_3$CO$_2$H; flow rate: 5 mL/min.

The analytical HPLC chromatogram of the purified P1 is shown below.

![Analytical HPLC Chromatogram](image)

**Figure S1.** The analytical HPLC elution profile of purified P1 is shown.
**Synthesis of lipopeptide LP1**: Conjugation with stearic acid was performed using the same procedure as the amino acid coupling with 5 fold excess of reagents. A shaker was used for better mixing of reagents. Cleavage conditions were the same as that for P1. Crude LP1 was purified by RP-HPLC, employing a Vydac semipreparatory diphenyl column (RP 219TP510). For LP1, MH⁺ (MALDI-TOF) calcd. for C₁₀₆H₁₇₂N₂₈O₃₁: 2333.27. Found: 2333.32.

Analytical HPLC conditions: Vydac analytical HPLC column (219TP5415); eluant: linear gradient of 0 – 70% acetonitrile in water over 40 minutes; both solvents contained 0.1% CF₃CO₂H; flow rate: 1.5 mL/min.

Semi-preparatory HPLC conditions: Vydac HPLC column (219TP510); eluant: linear gradient of 0 – 70% acetonitrile in water over 40 minutes; both solvents contained 0.1% CF₃CO₂H; flow rate: 5 mL/min.

The analytical HPLC chromatogram of the purified LP1 is shown below.

![Analytical HPLC chromatogram](image)

**Figure S2.** The analytical HPLC elution profile of purified LP1 is shown.
Circular Dichroism Spectroscopy:

CD spectra were recorded on Applied Photophysics PiSTAR instrument using a cell of 0.2 mm pathlength. The concentration of P1 or LP1 was 1 mg/mL in 10 mM phosphate buffer, pH = 4.0. The solutions were stored for 12 hours at 4 °C before recording the spectra. For the temperature dependent CD spectra, the sample was equilibrated for 20 minutes at each temperature before recording the spectra.

Figure S3. Temperature dependent CD Spectra of P1 and LP1 is shown. [P1 or LP1] = 1 mg/mL in 10 mM phosphate buffer, pH 4.0. The peptide solution was stored at 4 °C for 12 h before recording the spectra.
Cloning, expression and purification of MMP-9:

The catalytic and fibronectin domains (truncating the hemopexin domains from the full length enzymes) of human MMP-9 were cloned in pET20b vector (Novagen), and over-expressed the enzymes in BL21(DE3) Escherichia coli cells. The expressed proteins were primarily recovered from the inclusion bodies. The purified MMP-9 showed single band on SDS gel electrophoresis. The yield from 1 liter of bacterial culture was in the range of 20-30 mg. Details of this will be published elsewhere.

Cleavage studies with P1 and LP1 with MMP-9 and trypsin:

For the cleavage studies, the conditions are: \([\text{P1}] = 1 \text{ mg/mL}\) in 25 mM HEPES buffer, pH = 8.0 containing 10 mM CaCl\(_2\); [enzyme] = 5 nM; the reaction was stopped by adding 1 µL of CF\(_3\)CO₂H. The products were analyzed by RP-HPLC and the elution conditions are the same as reported for the analytical HPLC analysis of P1 and LP1. The HPLC chromatograms for the cleavage studies with P1 and MMP-9 are shown below.

![Cleavage of P1 with MMP-9](image)

Liposome formation and leakage studies:

Liposome formation: 10% MMPP_4HFA and 90% DSPC (by mole, total lipid concentration of 1 mg/mL) were dissolved in CHCl\(_3\). A thin film was prepared by evaporating the solvent using a rotary evaporator. The film was placed under high vacuum for 12 h. The film was then hydrated with 150 mM 5-carboxyfluorescein solution (prepared in in 25 mM in HEPES buffer, 10 mM CaCl\(_2\) at pH = 8.0) for an hour at 60 °C followed by sonication for another hour at 60 °C. Non encapsulated dye was separated from liposomes through gel filtration chromatography. Before passing through column the osmolarity of the elution buffer (with same composition) was adjusted with liposome solution. This liposome solution was diluted 10 times for the leakage assays.
Leakage assays: MMP-9 (10µL of 200 nM solution) was added to a 2 mL of liposome solution in 25 mM HEPES buffer, pH = 8.0, containing 10 mM CaCl₂. The emission spectra of the control and liposome + MMP-9 solution were measured. The emission intensity at 520 nm (excitation: 480 nm) was followed as a function of time for 5 h. The conditions for the leakage studies with trypsin were the same as those for MMP-9.