Understanding the Membrane-Induced Folding Mechanism of an Antimicrobial Peptide: The Effect of Nucleation

Jia Tang[#], Rachel S. Signarvic[&], William F. DeGrado[#,&], and Feng Gai[#,*]

[#]Department of Chemistry and [&]Department of Biochemistry & Biophysics, University of Pennsylvania, Philadelphia, PA 19104

The data presented here were collected at 20 °C.

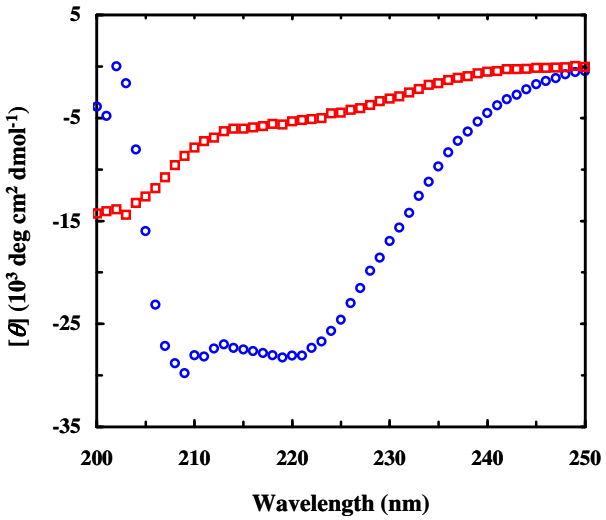

Figure S1. CD spectra of MPx-P15 (10 μM) in 10 mM Tris buffer at pH 7.0 (red) and in 1 mM POPC vesicle solution at pH 7.0 (blue).

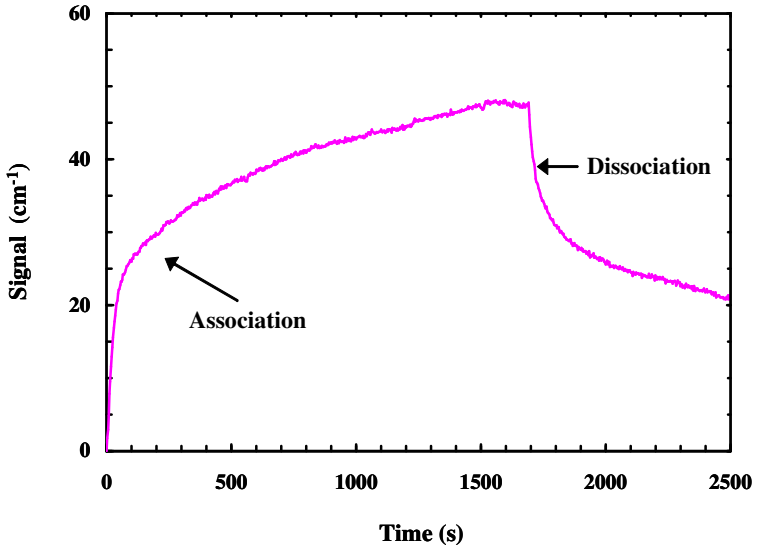

Figure S2. A SPR sensorgram showing the association/dissociation of MPx-P15 to/from a POPC hybrid bilayer membrane (HBM), which was prepared according to published protocols (1, 2). Briefly, the SPR chip was first immersed in a Piranha solution (H_2SO_4/H_2O_2, 7/3, vol/vol) for 15 minutes, and then rinsed thoroughly with Millipore water and ethanol, respectively. The cleaned chip was then immersed into a 0.2 mM octadecanethiol (ODT) ethanol solution for 24 hours to allow the formation of an ODT monolayer on the gold surface. The substrate was then rinsed with copious quantities of ethanol, followed by rapid drying under a steady flow of N_2.

The hydrophobic, ODT modified SPR chip was subsequently installed onto the SPR sample cell. Formation of the HBM was achieved by pumping a 0.5 mM POPC small unilamellar vesicle (SUV) solution through the cell at a flow rate of 0.01 mL/min for 12 hours. After that, any multilamellar lipid structures were removed by a 5-minute pulse of 10 mM NaOH at a flow rate of 2.4 mL/min. The above sensorgram was obtained by first flowing a peptide solution (300 nM in 25 mM HEPES buffer, pH 7.0) and then a HEPES buffer through the sample cell at a flow rate of 1.2 mL/min.

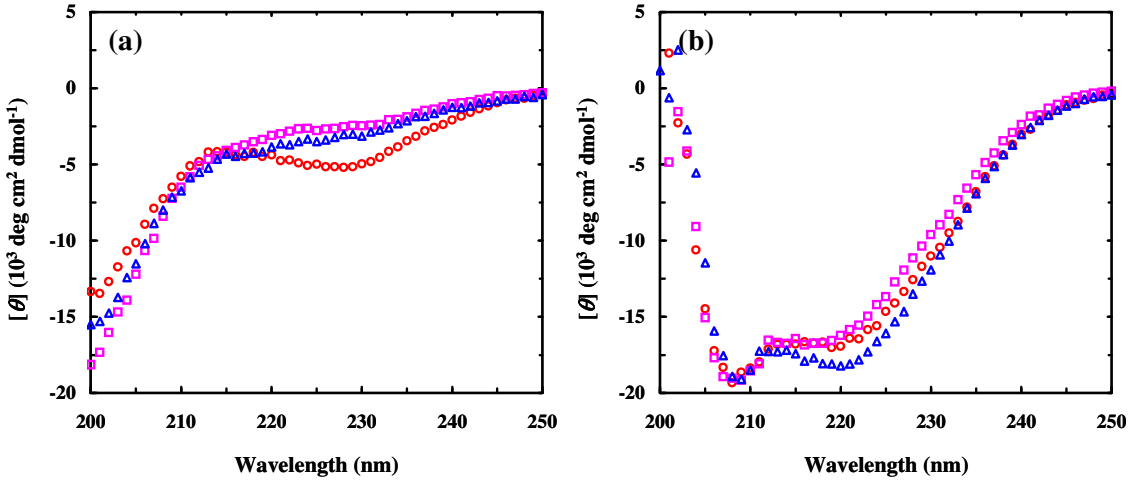

Figure S3. CD spectra of MxHH (pink), Zn^{2+}-bound MxHH (blue), and Ni^{2+}-bound MxHH (red) in (a) Tris buffer (10 mM, pH 7.0) and (b) 1 mM POPC vesicle solution (pH 7.0). The peptide and metal ion concentrations are ca. 10 and 300 μM, respectively. Compared to that of MPx-P15 (Figure S1), the mean residue ellipticity at 222 nm of MxHH in POPC solution is smaller, presumably due to the differences in peptide chain length and sequence, as well as, the relatively large uncertainties in the peptide concentrations determined by Trp absorbance.

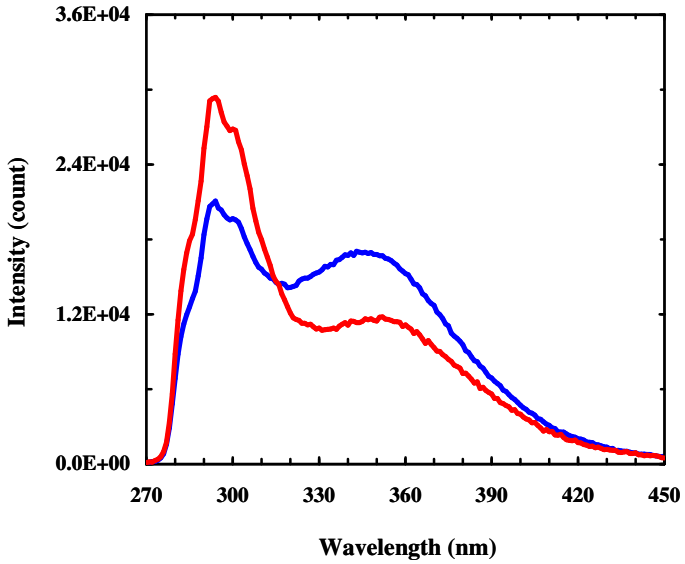

Figure S4. Fluorescence spectra of MPx-P15 in 25 mM HEPES buffer (pH 7.0) (red) and 20% TFE (blue) solution. λ_{ex} = 240 nm.

1. Lingler, S., Rubinstein, I., Knoll, W., and Offenhausser, A. (1997) Fusion of small unilamellar lipid vesicles to alkanethiol and thiolipid self-assembled monolayers on gold, *Langmuir*, *13*, 7085-7091.

2. Mozsolits H., Wirth H. J., Werkmeister J., Aguilar M. I. (2001) Analysis of antimicrobial peptide interactions with hybrid bilayer membrane systems using surface plasmon resonance, *Biochim. Biophys. Acta, 1512*, 64-76.