Supplementary Material (SM)

## Cholesterol and Phosphatidylethanolamine Lipids Exert Opposite Effects on Membrane Modulations Caused by the M2 Amphipathic Helix

Jianjun Pan,<sup>a\*</sup> Annalisa Dalzini,<sup>b</sup> Likai Song <sup>b\*</sup>

<sup>a</sup>Department of Physics, University of South Florida, Tampa, Florida 33620, United States <sup>b</sup>National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida 32310, United States



**Figure S1** AFM height images of a POPC bilayer before (A) and after (B) treatment with 4  $\mu$ M M2AH. Height scale is indicated by the color bar on the right. Scale bars are 100 nm. (C) Height profiles along the green lines shown in A and B. (D) Height probability distributions of the AFM images in A and B. Gaussian curve fitting resulted in a FWHM of 0.25 and 0.27 nm for POPC and POPC + 4  $\mu$ M M2AH, respectively.



**Figure S2** AFM height images of a POPC+30%Chol bilayer before (A) and after (B) treatment with 4  $\mu$ M M2AH. Scale bars are 200 nm. (C) Height profiles along the green lines shown in A and B. (D) Height probability distributions of the images in A and B. Gaussian curve fitting resulted in a FWHM of 0.26 and 0.48 nm for POPC+30%Chol and (POPC+30%Chol) + 4  $\mu$ M M2AH, respectively.



**Figure S3** AFM height images of a POPC+20%POPG bilayer before (A) and after (B) treatment with 4  $\mu$ M M2AH. Scale bars are 100 nm. (C) Height profiles along the green lines shown in A and B. (D) Height probability distributions of the images in A and B. Gaussian curve fitting resulted in a FWHM of 0.31 and 0.37 nm for POPC+20%POPG and (POPC+20%POPG) + 4  $\mu$ M M2AH, respectively.



**Figure S4** AFM height images of a POPC+20%POPE bilayer treated with 0, 4, and 10  $\mu$ M M2AH. Scale bars are 100 nm. (B) Height profiles along the green lines shown in A. (C) Height probability distributions of AFM images in A. Gaussian curve fitting resulted in a FWHM of 0.31, 0.31, and 0.34 nm at 0, 4, and 10  $\mu$ M M2AH, respectively.



**Figure S5** AFM height images of a POPC+40%POPE bilayer treated with 0, 4, and 10  $\mu$ M M2AH. Scale bars are 100 nm. (B) Height profiles along the green lines shown in A. (C) Height probability distributions of AFM images in A. Gaussian curve fitting resulted in a FWHM of 0.31, 0.31, and 0.32 nm at 0, 4, and 10  $\mu$ M M2AH, respectively.



**Figure S6** EPR spectra of liposomes labeled with 5-SASL for (A) POPC, (B) POPC+30%Chol, (C) POPC+40%POPE, and (D) POPC+20%POPG. The spectra were recorded at 295 K at P/L =1/50. EPR spectra of bare liposomes (black) are overlaid with the spectra in the presence of M2AH (red).



**Figure S7** AFM height images of a POPC+17%Chol bilayer treated with 0, 4, and 10  $\mu$ M M2AH. Scale bars are 100 nm. (B) Height profiles along the green lines shown in A. (C) Height probability distributions of the AFM images in A. Gaussian curve fitting resulted in a FWHM of 0.29, 0.31, and 0.42 nm at 0, 4, and 10  $\mu$ M M2AH, respectively.



**Figure S8** Height probability distributions of AFM images for various lipid bilayers before (A) and after (B) exposure to 4  $\mu$ M M2AH.