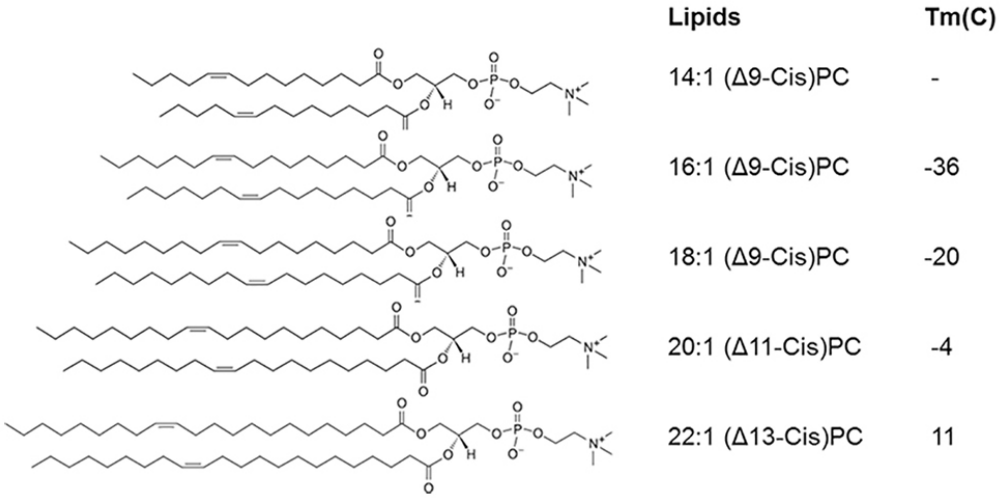
Biophysical Journal, Volume *114*

Supplemental Information

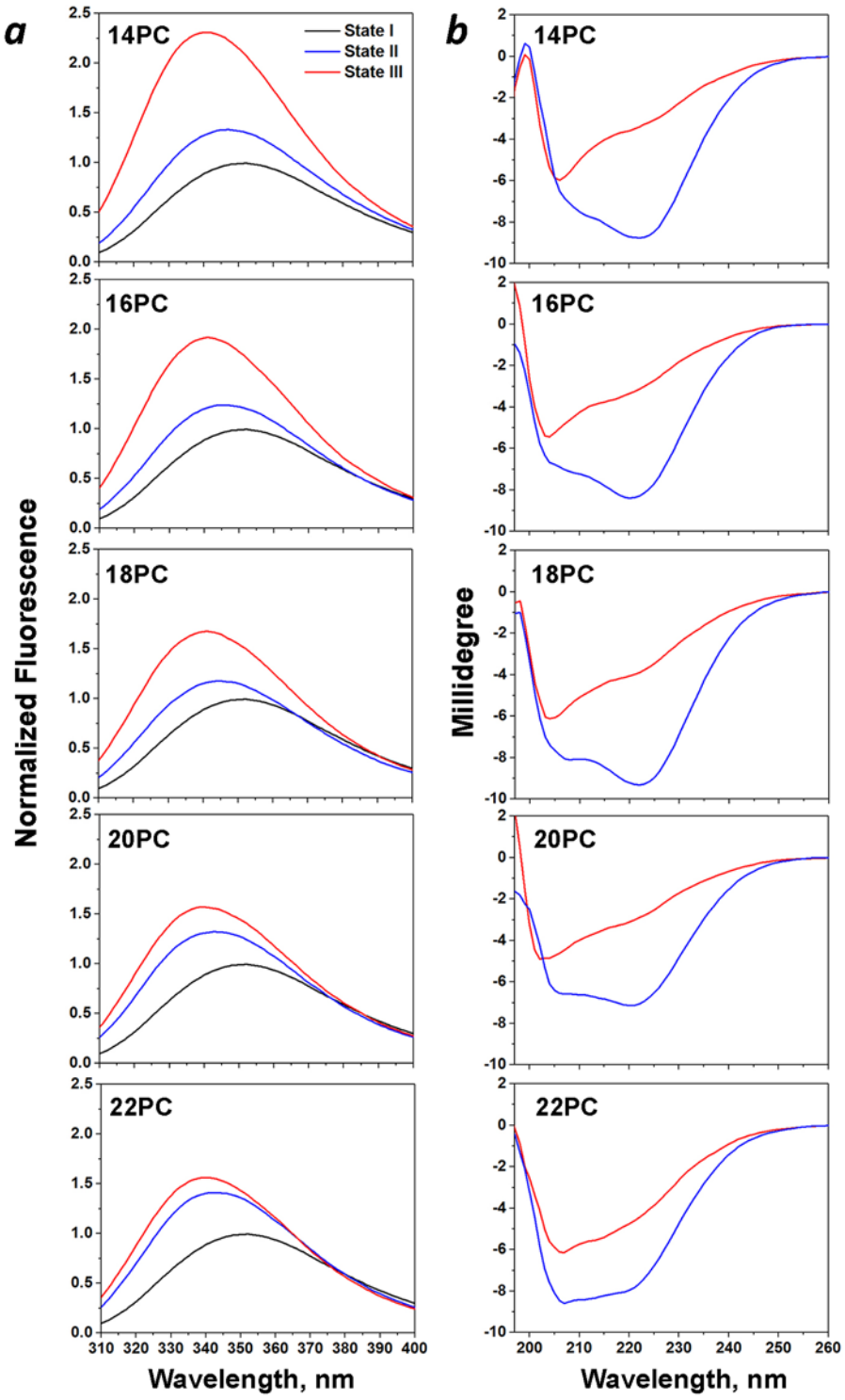
Bilayer Thickness and Curvature Influence Binding and Insertion of a pHLIP Peptide

Alexander G. Karabadzhak, Dhammika Weerakkody, John Deacon, Oleg A. Andreev, Yana K. Reshetnyak, and Donald M. Engelman

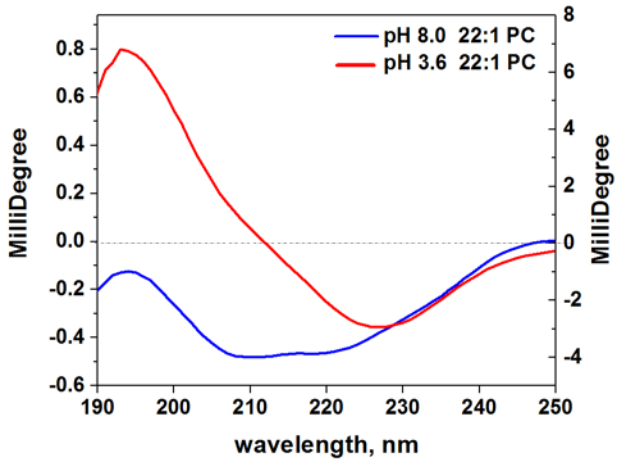
Bilayer thickness and curvature influence binding and insertion of a pHLIP® peptide



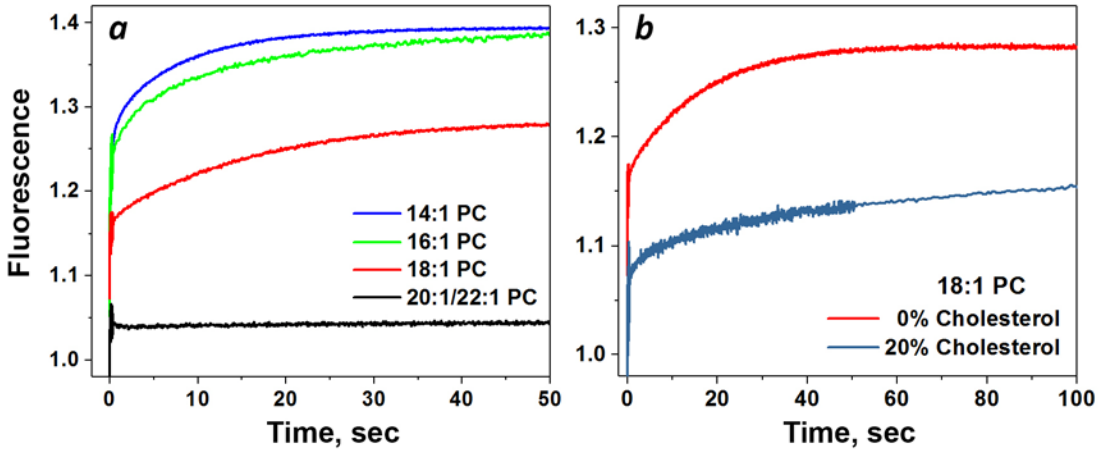
Supplementary Figure S1. Chemical structures and melting temperatures of the monounsaturated lipids used in the study.



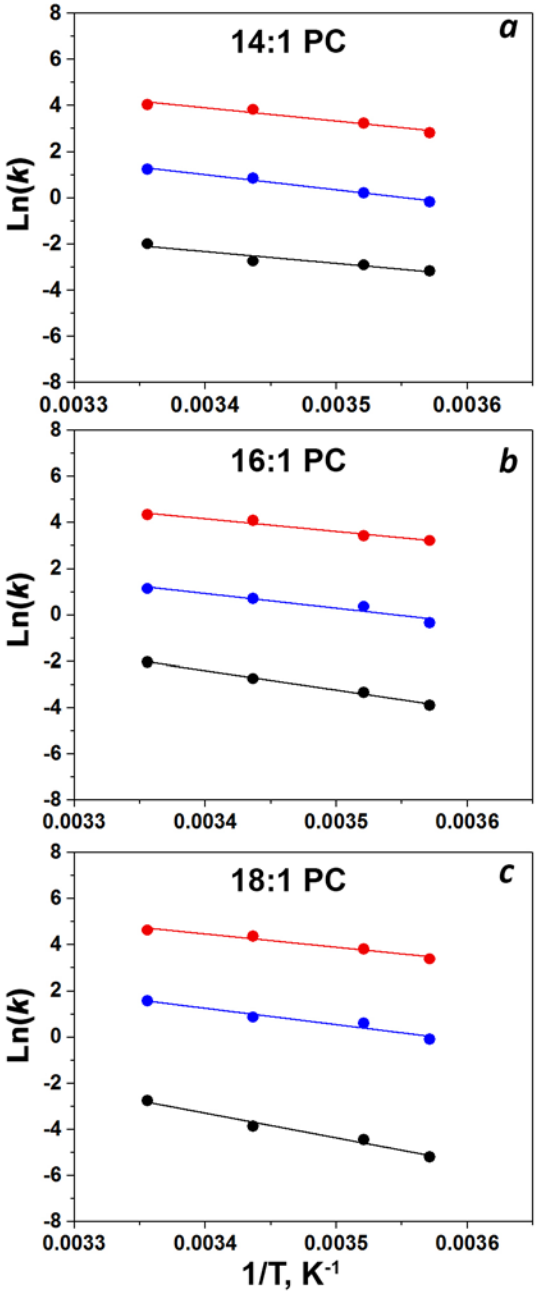
Supplementary Figure S2. Tryptophan fluorescence (*a*) and circular dichroism (*b*) spectra of pHLIP measured in State I at pH 8.0 in the absence of POPC liposomes (black lines), State II at pH 8.0 in the presence of POPC liposomes (blue lines) and State III at pH 3.6 in the presence of POPC liposomes (red lines).



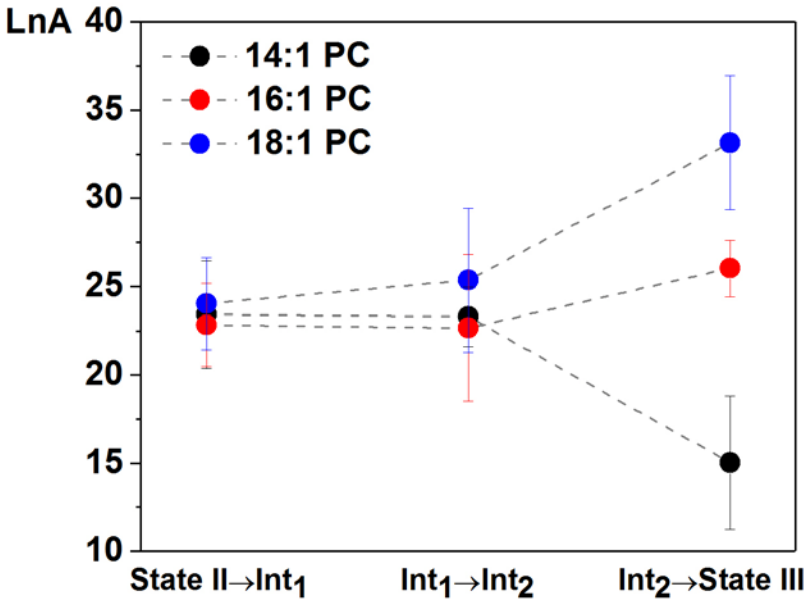
Supplementary Figure S3. Oriented circular dichroism of pHLIP in 22:1 PC membranes at different pHs. The Y-axis on the left shows values for the pH 8.0 signal, The Y-axis on the right shows values for the pH 3.6 signal.



Supplementary Figure S4. Kinetics of pHLIP insertion across the lipid bilayer of 50 nm liposomes of various lipid compositions (***a***) and cholesterol content (***b***) were monitored by changes of tryptophan fluorescence of pHLIP as a result of a pH jump from 8.0 to 3.6. The kinetic parameters are given in Table 1.



Supplement Figure S5. Arrhenius plots for pHLIP insertion across the *di*$C_{14:1}$PC (***a***), *di*$C_{16:1}$PC (***b***), and *di*$C_{18:1}$PC (***c***) lipid bilayers are shown. Rates were obtained by fitting the experimental kinetics data shown on Figure 4a-c using four-state kinetic models, assuming a sequential pathway for the processes of peptide insertion with 3 transitions State I→Int_1; Int_1→Int_2 and Int_2→ State III described by 3 rates, k_i (k_1 is shown in red, k_2 - in blue and k_3 - in black). The Arrhenius plots were obtained by linear fitting and activation energy values were calculated (values are given in Table 2).



Supplement Figure S6. The changes of the Arrhenius frequency factor, *A*, and Standard Errors were calculated for the transitions between four states along the pHLIP insertion pathway by fitting the rates (Supplementary Figures S5) with the Arrhenius equation.