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Supplemental information

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Supporting Information

Roles of Key Proline/Arginine Residues and Changes in Lipid Dynamics Reveal Interactions between the pH-Low Insertion Peptide (pHLIP) and Model Bilayers at Intermediate pH Values

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Quantitative Analysis of ^{31}P Relaxation Spectroscopy. Quantitative analysis to the ^{31}P relaxation spectroscopy was conducted to obtain the correlation times of fast- and slow-motion at nanosecond and microsecond timescales, respectively. The analysis algorithm was derived based on previous literature methods[1,2] with approximations made for the current sample.

To start, the ^{31}P resonance peaks in T_1 or T_2 spectra were integrated over a 0.5 ppm range (approximately the full width at half maximum of the peak). The normalized peak volumes were plotted as a function of delay times and fitted to exponential functions for T_1 or T_2 relaxation to obtain the decay time constants τ_1 or τ_2, respectively:

$$I(t) = I_0 \exp(-t/\tau_2) \text{ for } T_2 \quad (1)$$

$$I(t) = I_0 - 2I_0 \exp(-t/\tau_1) \text{ for } T_1 \quad (2)$$

The rate constant for the T_1 and T_2 decay, denoted as R_1 and R_2 respectively:

$$R_1 = 1/\tau_1 \quad (3)$$

$$R_2 = 1/\tau_2 \quad (4)$$

The fast and slow-motion correlation times, denoted τ_f and τ_s respectively, are related to the relaxation decay rate constants according to the following quadratic equations from literature:

$$R_1 = \frac{2}{15}\omega_{31P}^2\sigma^2\left(1+\frac{\eta^2}{3}\right)\left[\frac{S^2\tau_s}{1+(\omega_{31P}\tau_s)^2}+\frac{(1-S^2)\tau_f}{1+(\omega_{31P}\tau_f)^2}\right] \quad (5)$$

$$R_2 = \frac{1}{15}\omega_{31P}^2\sigma^2\left(1+\frac{\eta^2}{3}\right)\left\{\left[\frac{S^2\tau_s}{1+(\omega_{31P}\tau_s)^2}+\frac{(1-S^2)\tau_f}{1+(\omega_{31P}\tau_f)^2}\right]+\frac{4}{3}\left[s^2\tau_s+(1-S^2)\tau_f\right]\right\} \quad (6)$$

In the present work, approximation was made based on the magnitude of the terms in these equations to simplify the calculation. We consider the orders of magnitude of τ_s (~ 10^{-9}), τ_f (~10^{-6}) and the values of constants in Eqs. 5-6, where ω_{31P}, σ, η were the spectrometer frequency, the CSA and the asymmetric parameter of ^{31}P respectively, and had the values $2\pi \times 242\ MHz$, 160 ppm and 0.57. The order parameters *S* was estimated as 0.2 based on our previous studies using similar lipid bilayer models[3]. The first terms in both R_1 and R_2 are neglectable because they are ~ 10^{-15} while all other terms are between 10^{-8} and 10^{-10}. Furthermore, $\left(\omega_{31P}\tau_f\right)^2$ is ~ 4-10 considering the average value of τ_f for non-pHLIP and pHLIP-containing samples and constant ω_{31P}, and is considerably larger than 1. Therefore, the second terms in both R_1 and R_2 are estimated as $\frac{2}{15}\sigma^2\left(1+\frac{\eta^2}{3}\right)(1-S^2)\tau_f^{-1}$ and $\frac{1}{15}\sigma^2\left(1+\frac{\eta^2}{3}\right)(1-S^2)\tau_f^{-1}$ respectively. Eqs. 5-6 are therefore estimated as:

$$R_1 = \frac{2}{15}\sigma^2\left(1+\frac{\eta^2}{3}\right)(1-S^2)\tau_f^{-1} \quad (7)$$

$$R_2 = \frac{1}{15}\sigma^2\left(1+\frac{\eta^2}{3}\right)(1-S^2)\tau_f^{-1} + \frac{4}{45}\omega_{31P}^2\sigma^2\left(1+\frac{\eta^2}{3}\right)\left[s^2\tau_s + (1-S^2)\tau_f\right] \quad (8)$$

The values of τ_s and τ_f are calculated based on Eqs. 7-8 and plotted in the main text for different pH values.

The uncertainties of τ_s and τ_f are derived from the error propagation of Eqs. 7-8:

$$\sigma_{\tau_f} = \frac{C1}{\omega_{31P}^2}\sigma_{T_1} \quad (9)$$

$$\sigma_{\tau_s} = \sqrt{\left(\frac{-C2}{T_2^2}\right)^2\sigma_{T_2}^2 + \left(\frac{C3}{T_1^2} - C4\right)^2\sigma_{T_1}^2} \quad (10)$$

, where C1-C4 are constants derived from the ^{31}P Larmor frequency, the CSA, the asymmetry parameter and the order parameters, and their values are 8.74×10^9, 4.29×10^{-9}, 2.20×10^{-9} and 9.58×10^{-8}, respectively.

Natural Abundance Correction for ^{13}C-^{15}N REDOR applied to T19-P20. The overall 13C' signal contains three parts: the isotope labeled Proline 13C', the natural abundance contribution from other unlabeled amino acids on pHLIP; the natural abundance contribution from lipids ester groups (2 ester carbonyl carbons on each lipid molecule, POPC). Considerations: (1) the molar ratio between pHLIP and lipids for all samples was set to be 1:75; (2) the polarization transfer efficiency are different for pHLIP and lipid molecules on NMR because they may have different dynamics.

Overall S_0 signal:

$$S_0 = S_{0,lipid-na} + S_{0,pHLIP-na} + S_{0,Pro} \quad (11)$$

, where $S_{0,pHLIP-na} = 36 \times 0.011 S_{0,Pro}$ (Natural abundance of ^{13}C, 0.011; 36 other natural abundant carbons), $S_{0,lipid-na} = 75 \times 2 \times 0.011 \times f S_{0,Pro}$ (Natural abundance of ^{13}C, 0.011; 75-fold lipid molecules with 2 carbonyl carbons each, *f* describes the coefficient due to different polarization transfer between peptides and lipids)

Overall S_1 signal:

$$S_1 = S_{1,lipid-na} + S_{1,pHLIP-na} + S_{1,Pro} \quad (12)$$

, where $S_{1,pHLIP-na} = 35 \times 0.011 S_{0,Pro}$ (Assuming one natural abundance carbonyl carbon was completely suppressed because of the ^{13}C-^{15}N dipolar coupling, which is T19 C'), $S_{1,lipid-na} = S_{0,pHLIP-na}$ (Assuming no lipid carbonyl carbons are close to the T19 NH, likely to be true at least for pH 6.1, 5.8 and 5.3)

Therefore,

$$\left(\frac{\Delta S}{S_0}\right)^{exp} = \frac{1}{(150f+36)\times 0.011+1}\left(\frac{\Delta S}{S_0}\right)^{corr} + \frac{0.011}{(150f+36)\times 0.011+1} \quad (13)$$

$$(\frac{\Delta S}{S_0})^{corr} = [(150f + 36) \times 0.011 + 1](\frac{\Delta S}{S_0})^{exp} - 0.011 \quad (14)$$

Use pH 5.3 experimental data to find out the best-fit *f* (because it is known that Proline adopts "Trans" configuration in the *State III*[4]). It is determined that f = 0.16. Use this best-fit parameter to perform natural abundance corrections for the other pH values. Corrected values are plotted as open symbols in Figure 5 in main text.

Reference:

1. Yang, Y., Yao, H. & Hong, M. Distinguishing bicontinuous lipid cubic phases from isotropic membrane morphologies using (31)P solid-state NMR spectroscopy. *J. Phys. Chem. B* **119**, 4993-5001 (2015).
2. Chang, S.-L. & Tjandra, N. Temperature dependence of protein backbone motion from carbonyl 13C and amide 15N NMR relaxation. *J. Magn. Reson.* **174**, 43-53 (2005).
3. Qiang, W., Doherty, K. E., Klees, L. M. & Tobin-Miyaji, Y. Time-Dependent Lipid Dynamics, Organization and Peptide-Lipid Interaction in Phospholipid Bilayers with Incorporated β-Amyloid Oligomers. *J. Phys. Chem. Lett.* **11**, 8329-8336 (2020).
4. Hanz, S. Z. *et al.* Protonation-Driven Membrane Insertion of a pH-Low Insertion Peptide. *Angew. Chemie. Int. Ed.* **55**, 12564-12569 (2016).