Supporting Information

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Text

pHLIP Peptide. The pHLIP sequence: AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGT was prepared by solid-phase peptide synthesis using standard Fmoc (9-fluorenylmethyloxycarbonyl) chemistry and purified by reverse phase chromatography at the W. M. Keck Foundation Biotechnology Resource Laboratory at Yale University. For use of the peptide, the lyophilized powder is dissolved in a solution containing 3 M urea and transferred to working buffer by using a G-10 size-exclusion spin column. The concentration of the peptide was determined by absorbance (ε_{280} = 13,940 $M^{-1}cm^{-1}$).

Vesicle Preparations. Large unilamellar vesicles (LUVs) were prepared by extrusion. POPC (1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine; Avanti Polar Lipids) in chloroform was desolvated on a rotary evaporator and dried under vacuum for several hours. The phospholipid film was rehydrated in 10 mM phosphate buffer, pH 8.0, vortexed for 2 h, and passed through the extruder (100-nm membrane) 15 times. Fluorescent vesicles were prepared by mixing 1 mol % of Rhodamine-PE (1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine-*N*-(Lissamine Rhodamine B Sulfonyl; Avanti Polar Lipids) with PC lipids. The concentration of the fluorescent lipids was determined by using the absorbance of the fluorescent dye. The concentration of the nonfluorescent lipids was calculated from scattered light (PC1 spectrofluorometer; ISS), calibrated with the signal of a known concentration of vesicles containing 1% phospholipids with fluorescently labeled headgroups prepared by exactly the same method described above.

Fluorescence Measurements. Tryptophan fluorescence measurements were carried out on a PC1 spectrofluorometer (ISS) with regulated temperature-control units. Tryptophan fluorescence spectra were recorded from 310–400 nm with the spectral widths of excitation and emission slits set at 2–4 nm and 2 nm, respectively, using an excitation wavelength of 295 nm. The polarizers in the excitation and emission paths were set at the "magic" angle (54.7° from the vertical orientation) and vertically (0°), respectively, to reduce Wood's anomalies from the reflecting holographic grating. The injection of small aliquots of POPC in 10 mM phosphate buffer, pH 8.0 or 5.0, into a solution of peptide in 10 mM phosphate buffer, pH 8.0 or 5.0, was performed by using an ISS computer-controlled titrator.

Isothermal Titration Calorimetry Measurements. Isothermal titration calorimetry was performed using a VP-ITC ultrasensitive microcalorimeter (MicroCal). Solutions were degassed under vacuum. A water-to-water injection was performed before each run to ensure that the system contained no traces from previous runs. In the first set of experiments, small aliquots of peptide (10 μl of a 7 μM solution of pHLIP, pH 8.0) were injected into a solution of vesicles (800 μM of POPC, pH 8.0 in V_{cell} = 1.4267 ml). In control experiments, the heat released when the peptide solution was injected into the buffer (no lipids) and when buffer solution was injected into the vesicle solution were measured. In a second type of titration experiment, the peptide solution (starting at 3.3 μM) was first loaded in the calorimetric cell (V_{cell} = 1.4267 ml), and then vesicles (4 mM POPC) were injected in a sequence of steps. For controls, the buffer solution was injected into the peptide solution and the vesicles were injected into the buffer solution.

To conduct an isothermal acid-titration calorimetry experiment, pHLIP was preequilibrated with vesicles (7 μM of pHLIP and 1:200 peptide/lipid ratio) at pH 8.0 (V_{cell} = 1.4267 ml), and increments of acid (50 mM HCl) were injected (4 μl) to lower the pH. In separate experiments, acid was injected into the solution of POPC at various temperatures to measure the heat of dilution. Additionally, separate experiments were implemented to measure pH values at each titration step by using a MI-415 pH microelectrode (Microelectrodes).

Data Analysis. The experimental data fitting was performed in Mathematica 5 (Wolfram Research), Statistica (StatSoft, Inc. 2006) and Origin7.0 (Original Lab Corp.).

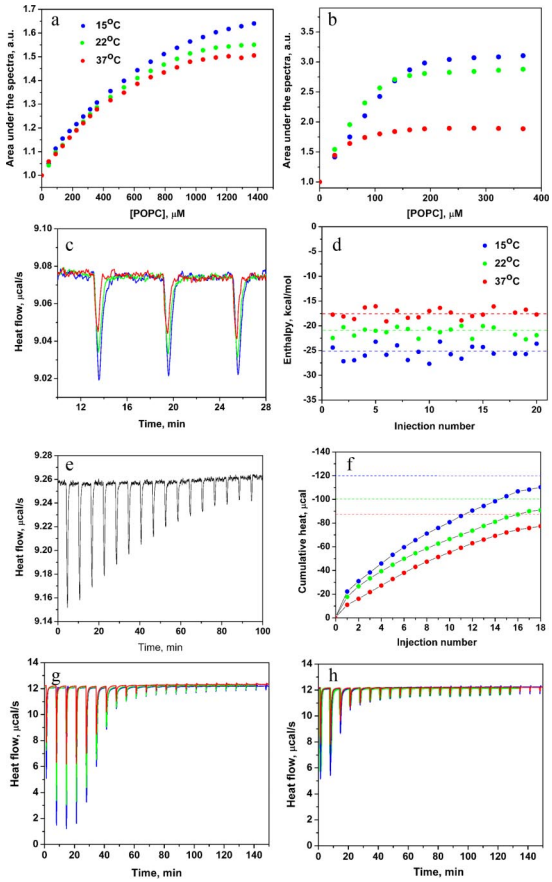

Fig. S1. Titrations of pHLIP with POPC vesicles monitored by changes of tryptophan fluorescence. The titration curves are obtained by calculation of areas under the tryptophan fluorescence spectra of pHLIP measured with progressive increments of POPC vesicles at different temperatures at pH 8.0 (*a*) and pH5.0 (*b*). Areas were calculated over the range of wavelengths from 330–360 nm (short wavelength emission was excluded from consideration to minimize the contribution of scattered light). The excitation wavelength was 295 nm. (*c–f*) Titrations of pHLIP with POPC vesicles at pH 8.0 monitored by changes of heat release measured by ITC. (*c*) Calorimeter tracing: Downward peaks indicate exothermic reactions, produced by injection of small aliquots of peptide into a solution of vesicles. (*d*) The heat per injection is calculated from the areas underneath the peaks in *c*. In control experiments, we measured the heat released when the peptide solution was injected into the buffer (no lipids) and when the buffer solution was injected into the vesicle solution. In both cases, the heat of reaction was significantly lower than the heat registered during the peptide–lipid interaction. (*e*) Calorimeter tracing of exothermic reactions, obtained by titration of the peptide solution with aliquots of POPC vesicles at pH 8.0. Progressive injections produced decreasing exothermic heats of reaction (h_i). For controls, the buffer solution was injected into the peptide solution, or the vesicles were injected into the buffer solution. In both cases, the heat of dilution was significantly lower than the heat released during the titration experiment. (*f*) The cumulative heat of reaction after the *i*th injection (the titration curve) calculated as

$$\delta H_i = \sum_{k=1}^{i} \delta h_k.$$

Complete saturation was not achieved; however, asymptotic values can be found from the averaged heat of reaction (ΔH) obtained in the peptide-into-lipid injection scheme described above:

$$\delta H_{\text{total}} = \frac{\Delta H}{n_p},$$

where n_{p} is the total amount of peptide in the cell (V_{cell} is the volume of cell and P_t is the peptide concentration in a cell at the starting point):

$$n_{\text{p}} = V_{\text{cell}} \cdot P_{\text{t}}.$$

The calculated asymptotic values for different temperatures: 15°C, 22°C, and 37°C were −120, −100, and −83 μcal, respectively. These values are presented as dotted lines. (*g–h*) Acid titrations of pHLIP preequilibrated with POPC vesicles at pH 8.0 monitored by changes of heat release measured by ITC are presented. (*g*) Calorimeter tracing obtained by titration of pHLIP preequilibrated with vesicles with increments of acid. (*h*) Calorimeter tracing obtained by titration of the vesicles with acid in the absence of peptide.

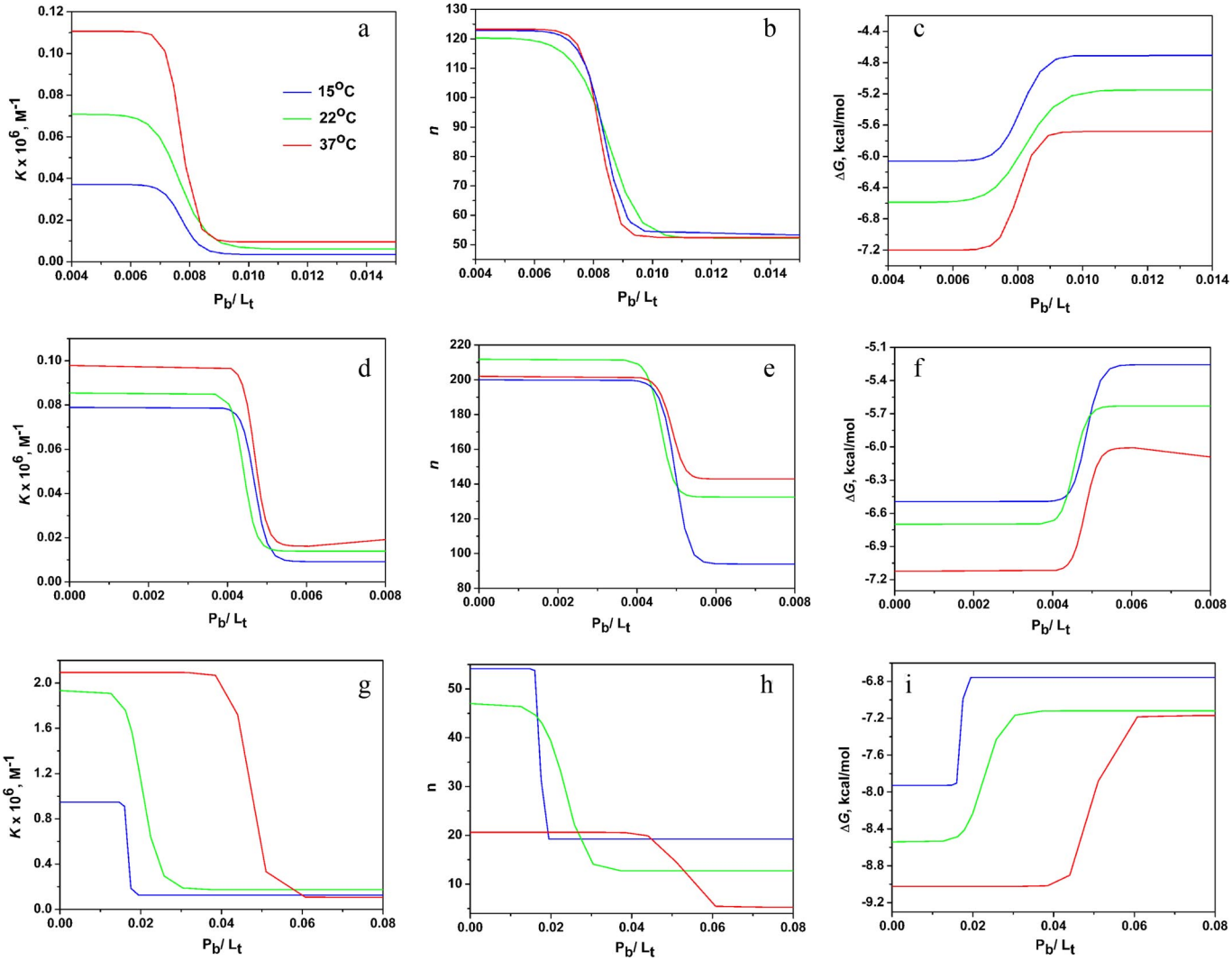

Fig. S2. The changes of adsorption constant (K) coordination number (n), and Gibbs free energy ΔG with increasing lipid/peptide ratios at different temperatures are presented (all numbers can be found in Tables S1 and S2). (*a–c*) The parameters calculated based on the analysis of fluorescence titrations data obtained at pH 8.0. (*d–f*) The parameters calculated based on the analysis of ITC titration data obtained at pH 8.0. (*g–i*) The parameters calculated based on the analysis of fluorescence titration data obtained at pH 5.0. The variation of the adsorption constant $K(x)$ and coordination number $n(x)$ during the transition are calculated based on Eq. **6** as:

$$K(x) = K_1 - K_1 n_1 x + \left(\frac{K_1 n_1 \exp\left(\frac{x_t}{x_w}\right) + K_2 n_2 \exp\left(\frac{x}{x_w}\right)}{\exp\left(\frac{x}{x_w}\right) + \exp\left(\frac{x_t}{x_w}\right)} \right) x + (K_2 n_2 - K_1 n_1) x_t + (K_1 n_1 - K_2 n_2) x_w \ln\left[\exp\left(\frac{x}{x_w}\right) + \exp\left(\frac{x_t}{x_w}\right) \right]$$

$$n(x) = -\left[K_1 n_1 \exp\left(\frac{x_t}{x_w}\right) + K_2 n_2 \exp\left(\frac{x}{x_w}\right) \right]$$

$$\left\{ -\exp\left(\frac{x_t}{x_w}\right)(K_1 - K_1 n_1 x_t) + K_2 n_2 x_t + \exp\left(\frac{x}{x_w}\right)[-K_2 n_2 (x + x_t) + K_1(-1 + n_1(x + x_t))] - \left[\exp\left(\frac{x}{x_w}\right) + \exp\left(\frac{x_t}{x_w}\right) \right](K_1 n_1 - K_2 n_2) x_w \ln\left[\exp\left(\frac{x}{x_w}\right) + \exp\left(\frac{x_t}{x_w}\right) \right] \right\}^{-1}$$

Table S1. Thermodynamic parameters of pHLIP adsorption at the surface of a lipid bilayer at pH 8.0

	15°C	22°C	37°C
Fitting of fluorescence titration data, pH 8.0			
K_1 (M^{-1})	3.3×10^3	6.2×10^3	9.3×10^3
n_1	56	52	57
span style='font-family:Symbol'DG_1 (kcal mol^{-1})	−4.7	−5.2	−5.7
K_2 (M^{-1})	3.7×10^4	7.1×10^4	11.1×10^4
n_2	123	125	124
ΔG_2 (kcal mol^{-1})	−6.1	−6.6	−7.2
x_t	7.7×10^{-3}	7.6×10^{-3}	7.7×10^{-3}
x_w	3.1×10^{-4}	4.5×10^{-4}	2.5×10^{-4}
Fitting of ITC titration data, pH 8.0			
K_1 (M^{-1})	9.2×10^3	13.9×10^3	16.0×10^3
n_1	94	132	144
ΔG_1 (kcal mol^{-1})	−5.3	−5.6	−6.0
K_2 (M^{-1})	7.9×10^4	8.5×10^4	9.8×10^4
n_2	200	212	202
ΔG_2 (kcal mol^{-1})	−6.5	−6.7	−7.1
ΔH_2 (kcal mol^{-1})	−25.5	−21.2	−17.6
ΔS_2 (kcal $mol^{-1}K^{-1}$)	−0.066	−0.049	−0.034
x_t	4.7×10^{-3}	4.4×10^{-3}	4.7×10^{-3}
x_w	1.9×10^{-4}	1.5×10^{-4}	1.5×10^{-4}

K is the adsorption constant, n is the coordination number, x_t and x_w are the transition point and width of transition, respectively ($x = P_b/L_t$), ΔG, ΔH, and ΔS are changes of free energy, enthalpy, and entropy of peptide–membrane interaction. The subscripts 1 and 2 refer to the two regions seen in the Scatchard plots.

Table S2. Thermodynamic parameters of pHLIP insertion/folding in a lipid bilayer

	15°C	22°C	37°C
Fitting of fluorescence titration data, pH 5.0			
K_1 (M^{-1})	1.3×10^5	1.7×10^5	1.1×10^5
n_1	19	13	6
ΔG_1 (kcal mol^{-1})	−6.8	−7.1	−7.2
K_2 (M^{-1})	9.6×10^5	19.3×10^5	20.9×10^5
n_2	54	47	21
ΔG_2 (kcal mol^{-1})	−7.9	−8.5	−9.0
x_t	1.7×10^{-2}	2.0×10^{-2}	4.7×10^{-2}
x_w	2.8×10^{-4}	2.0×10^{-3}	2.0×10^{-3}
Fitting of ITC titration data			
ΔH (kcal mol^{-1})	−8.04	−6.68	−3.33
No. of H^+	1.5	1.6	1.8

K is the adsorption constant, n is the coordination number, x_t and x_w are the transition point and width of transition, respectively ($x = P_b/L_t$), ΔG, ΔH, and ΔS are changes of free energy, enthalpy, and entropy of peptide–membrane interaction. The subscripts 1 and 2 refer to the two regions seen in the Scatchard plots.