Electronic Supplementary Information

for

Membrane Electrostatics Sensed by Tryptophan Anchors in Hydrophobic Model Peptides Depends on Non-Aromatic Interfacial Amino Acids: Implications in Hydrophobic Mismatch

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S1 Preparation of unilamellar vesicles

Unilamellar vesicles (ULVs) of POPC or POPC/POPG (70/30, mol/mol) containing 2 mol% of the peptide (WALP23 or KWALP23 or GWALP23) were prepared as described earlier,[1] with minor modifications. 550 nmol of total lipid and 11 nmol of each peptide in methanol, along with a few drops of chloroform, were mixed well by vortexing. The resultant solution was dried, while being warmed gently (~35 °C), under a stream of nitrogen gas. This was followed by further drying under high vacuum for at least 3 h. Subsequently, 1 ml of 10 mM sodium phosphate, 150 mM sodium chloride, pH 7.2 buffer was added to the dried lipid film to induce swelling. Each hydrated lipid sample, vortexed intermittently for 3 min to ensure uniformity in lipid dispersion, yielded homogeneous multilamellar vesicles (MLVs). These MLVs were sonicated to clarity (~40 min in short bursts) using a Sonics Vibra-Cell VCX 500 sonifier (Sonics & Materials Inc, Newtown, CT) fitted with a titanium microtip. The lipid suspensions were purged with argon prior to sonication and cooled in an ice/water mixture during each sonication step to ensure negligible lipid damage. Titanium particles shed from the microtip during sonication were removed by centrifuging the sonicated samples in a Heraeus Biofuge centrifuge (DJB Labcare, Buckinghamshire, UK) for 15 min at 15,000 rpm.

S2 Steady state fluorescence measurements

Steady state fluorescence measurements were acquired with a Fluorolog-3 Model FL3-22 spectrofluorometer (Jobin Yvon, Edison, NJ) at room temperature using semi-micro quartz cuvettes. Slit widths of 2 and 4 nm were used for excitation and emission, respectively. Spectra were recorded in the corrected spectrum mode. Contributions from solvent Raman peaks and other scattering artifacts were corrected by subtraction of background intensities (from samples without peptide) from sample spectra. Data shown is representative of at least three independent measurements. Fluorescence emission maxima were assigned based on the centre of mass of each spectrum and in each case, the observed emission maximum was identical to or within ±1 nm of the reported values.

Values of fluorescence anisotropy were calculated using the following equation:[2]

$$r = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad \text{(S1)}$$

where I_{VV} and I_{VH} represent fluorescence intensities (after appropriate background subtraction) measured with a configuration consisting of vertically oriented excitation polarizer and vertically and horizontally oriented emission polarizer, respectively. G denotes the grating correction factor and is the ratio of efficiencies of the system for the detection of vertically and horizontally polarized light (I_{HV}/I_{HH}). Data shown is representative of at least three independent measurements.

S3 Time-resolved fluorescence measurements

Time-resolved fluorescence intensity decays were acquired using a Delta-D TCSPC system (Horiba Jobin Yvon IBH, Glasgow, UK) with EzTime software version 3.2.2.4 (Horiba Scientific, Edison, NJ) in the time-correlated single photon counting (TCSPC) mode, as described earlier.[3] In order to optimize the signal-to-noise ratio, 10,000 photon counts were collected at the peak channel. Data acquisition was carried out with an emission band pass of 8 nm or less and a neutral density filter (ND1) in the excitation path. The sample and scatterer profiles were acquired in 10 alternate cycles of 1,000 photon counts (10% acquisition) at each of the peak channels, to compensate for shape and timing drifts that might occur during data acquisition. This arrangement has the additional advantage of preventing prolonged exposure and subsequent photodamage of the fluorophore.

Data were stored and analyzed using in-built plugins in the EzTime software version 3.2.2.4 (Horiba Scientific, Edison, NJ). Fluorescence intensity decay curves were deconvoluted with the IRF and analyzed as a sum of exponential terms given by the equation:

$$F(t) = \Sigma_i \alpha_i \exp(-t/\tau_i) \quad \text{(S2)}$$

where F(t) is the fluorescence intensity at time t and α_i is a pre-exponential factor denoting the fractional contribution to the time-resolved decay of the i^{th} component with a lifetime of τ_i, such that $\Sigma_i \alpha_i = 1$. The decay parameters were recovered using a nonlinear least squares iterative fitting program based on the Marquadt algorithm.[4] The program also includes subroutine packages associated with statistical analysis and plotting.[5] The goodness of fit of a given data set to a chosen function was evaluated by the χ^2 values, the weighted residuals[6] and the autocorrelation function of the weighted residuals.[7] A fit was considered acceptable when plots of the weighted residuals and their autocorrelation function exhibited random deviation about zero, with a χ^2 value of not more than 1.2. Intensity-averaged mean fluorescence lifetimes ($<\tau>$) for triexponential fluorescence decays were calculated from the decay times and pre-exponential factor using the equation:[2]

$$<\tau> = \frac{\alpha_1\tau_1^2 + \alpha_2\tau_2^2 + \alpha_3\tau_3^2}{\alpha_1\tau_1 + \alpha_2\tau_2 + \alpha_3\tau_3} \qquad \text{(S3)}$$

Fluorescence lifetimes were represented as intensity-averaged mean values since this parameter does not depend on the method of analysis employed and the number of exponential terms used to deconvolute the time-resolved fluorescence decay.

Fig. S1
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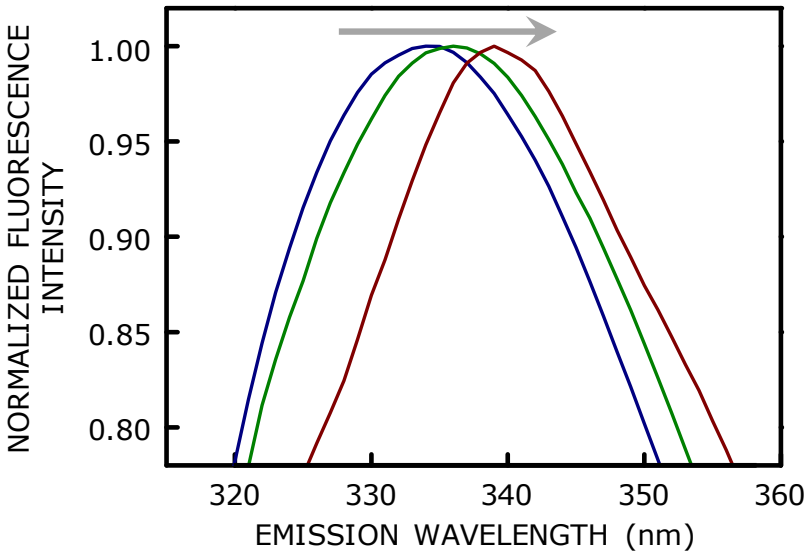

Fig. S1 Representative fluorescence emission spectra of WALP tryptophans in POPC membranes showing the shift in emission maximum upon red edge excitation. Spectra were acquired upon excitation at 280 nm (blue), 305 nm (green) and 310 nm (maroon) in the corrected spectrum mode. The shift in the emission maximum on increasing the excitation wavelength is highlighted by a gray arrow. Each spectrum was intensity-normalized at the corresponding emission maximum and subjected to a moderate degree of smoothening only for the purpose of representation. Smoothening was carried out with the adjacent-averaging program in MicroCal Origin version 8.0 (OriginLab, Northampton, MA), while exercising caution to ensure that the overall spectral shape and emission maxima remain unaltered. The concentration of WALP was 11 μM and the lipid/peptide ratio was 50 (mol/mol). All other conditions are as in Fig. 2a. See Experimental and Section S2 for more details.

Fig. S2
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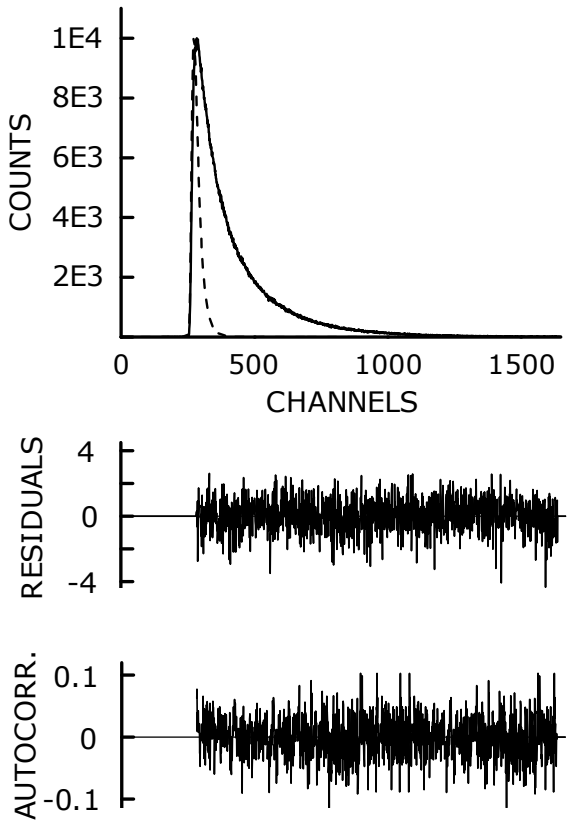

Fig. S2 Representative time-resolved fluorescence intensity decay profile of WALP tryptophans in POPC membranes. Excitation wavelength was 297 nm corresponding to the pulsed light emitting diode (LED) used as the light source and emission was monitored at the emission maximum (334 nm). The sharp peak on the left (shown as a black dotted line) is the instrument response function (IRF) and corresponds to the LED profile. The relatively broad peak on the right (shown as a black solid line) is the decay profile, fitted to a triexponential function. The two lower plots show the weighted residuals and the autocorrelation function of the weighted residuals obtained on fitting to the triexponential function. The concentration of WALP was 11 μM and the lipid/peptide ratio was 50 (mol/mol). All other conditions are as in Fig. 3a. See Experimental and Section S3 for more details.

References

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