Supporting Information for:

Polyarginine Interacts more Strongly and Cooperatively than Polylysine With Phospholipid Bilayers

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Separation of rhodamine-B isomers. Mixed isomers of rhodamine B POPE were separated into the pH-sensitive, *ortho*-, and insensitive, *para*-isomers via thin layer chromatography (TLC). To do this, the mixed isomers were dissolved in chloroform and spotted onto a TLC plate (EMD, 5715-7, silica gel 60 F254) and developed with a mixture of ammonium hydroxide solution and *n*-propanol in a volume ratio of 35:65.

Peptide synthesis. The amino acids, Fmoc-Lys(Boc)-OH and Fmoc-Arg(Pbf)-OH, were used to synthesize H_2N-KKKKKKKKK-NH_2 (Lys_9) and H_2N-RRRRRRRRR-NH_2 (Arg_9) by traditional Fmoc solid phase synthesis methods. Rink amide MBHA resin was used to obtain a C-terminal amide. Amino acid coupling reactions were performed in glass synthesis vessels with N_2 bubbling for agitation. Deprotection of the resin by removal of the Fmoc group was performed by addition of 20% piperidine in DMF. This reaction was performed a first time for 5 min, followed by a DMF wash, then a second time for 15 min, also followed by a DMF wash. Subsequent additions of amino acids were carried out in sequential coupling reactions. Each coupling reaction consisted of the Fmoc-amino acid, HBTU, and DIEA (4, 3.9, and 10-fold molar equivalent to resin amino groups, respectively) in DMF for 4 hrs. After each coupling reaction, the resin was washed with DMF, followed by Fmoc deprotection (same as above) for the next coupling reaction. After completion of the peptide sequences, the *N*-terminal Fmoc group was deprotected and the peptide was cleaved from the resin. The peptide cleavage reaction was performed with TFA containing 2.5% H_2O and 1% triisopropylsilane for 2 hr in order to remove all side chain protecting groups and to cleave the peptide from the resin. Crude peptide in the resulting TFA solution was then washed with cold anhydrous ethyl ether to induce peptide precipitation. After three washes of ethyl ether, the ether was poured off and the crude peptides were dissolved in acetonitrile and lyophilized. Lys_9 and Arg_9 were purified by reversed phase C18 HPLC and their masses were confirmed by MALDI-TOF. The Lys_9 expected mass was 1170.58 amu and its observed mass was 1170.96 amu, while Arg_9 has an expected mass of 1422.70 amu and the observed mass was 1422.74 amu.

pH Titration Curves. Supported lipid bilayers containing 0.5 mol% *ortho*-rhodamine B and varying amounts of POPC and POPG were formed within flow cell devices. Excess vesicles were then flushed away with 10 mM PBS with 150 mM NaCl at different pH values. The fluorescence signal from the bilayers was monitored and measured after stabilization. A sample fluorescence micrograph and its corresponding linescan are shown in Figure S1. These measurements were used to form titration curves of the pH sensitive dye as a function of the POPG concentration in the bilayer. The data points were

normalized to the highest intensity value (lowest pH) and fit with a sigmoidal curve, from which apparent pKa values were extracted (Figure S2). As expected, the apparent pKa of the *ortho*-rhodamine B dye shifted to higher value as the percentage of POPG in the bilayer was increased (Table 1 in main text). This indicated that an increasingly basic solution was required to deprotonate the dye when higher concentrations of negatively charged lipids were embedded in the membranes. Moreover, there is a specific pH range for each bilayer where the change in fluorescence intensity varies roughly linearly with the pH value (Figure S2). It is this range that is exploited below for studying peptide-membrane interactions.

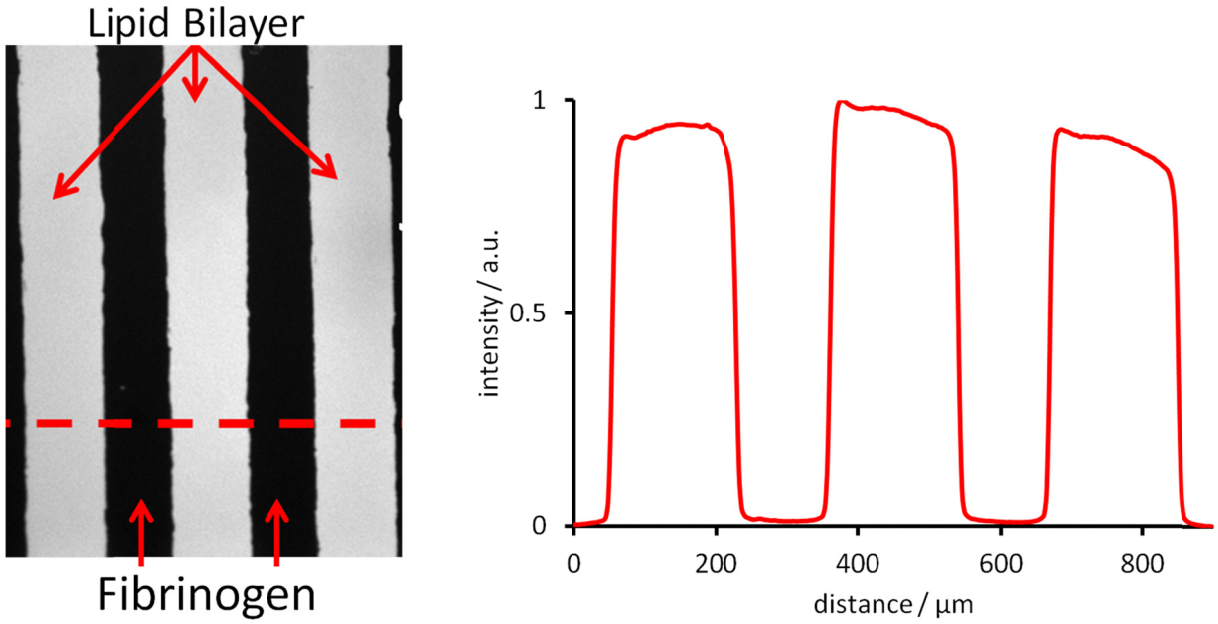

Figure S1. (Left) A fluorescence micrograph of supported lipid bilayers containing 0.5 mol% *ortho*-rhodamine B POPE, 20 mol% POPG and 79.5 mol% POPC patterned on a planar glass support between fibrinogen-passivated regions. Solutions containing different peptide concentrations were flowed over the bilayers, and fluorescence intensity measurements were made accordingly. The line scan on the right shows the fluorescence intensity corresponding to the region marked by the dashed red line in the micrograph.

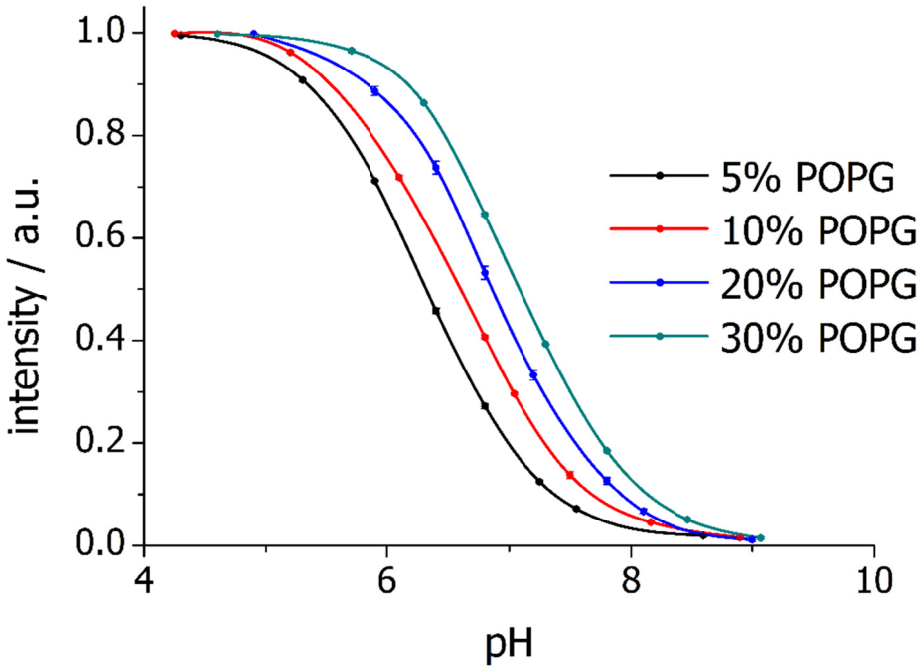

Figure S2. Titration curves for 5, 10, 20, and 30 mol% POPG bilayers with 0.5 mol% *ortho*-rhodamine B POPE in POPC. All titration curves were taken with 10 mM PBS containing 150 mM NaCl.

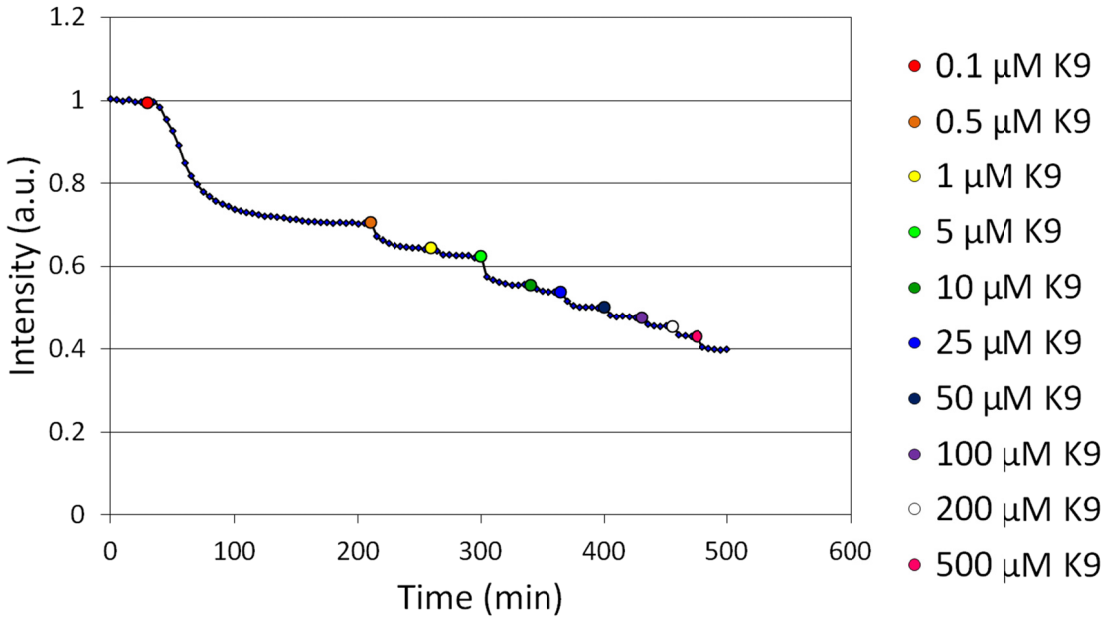

Figure S3. Normalized fluorescence intensity measurements for bilayers composed of 0.5 mol% *ortho*-rhodamine B POPE, 30 mol% POPG and 69.5 mol% POPC as increasing concentrations of Lys_9 peptide are introduced. The buffer used was 10 mM PBS with 150 mM NaCl at pH 6.8.

Effect of *ortho*-rhodamine B on peptide binding. The effect of *ortho*-rhodamine B dye in the bilayer on the dissociation constant of Arg_9 from POPC bilayers was investigated with increasing dye concentrations from 0.05 mol% to 1.0 mol% in POPC. Figure S4a shows the results of these assays. Abstracting the dissociation constants from the curves indicates that increasing the concentration of the dye in the bilayer tightens the binding of Arg_9 (S4b). It would appear that the negative charge on the dye tightens binding in much the same fashion as POPG (i.e. on electrostatic grounds). A hyperbolic function is fit to the data points in S4b and the fit is extrapolated to 0 mol% dye, which yields K_D = 60 µM.

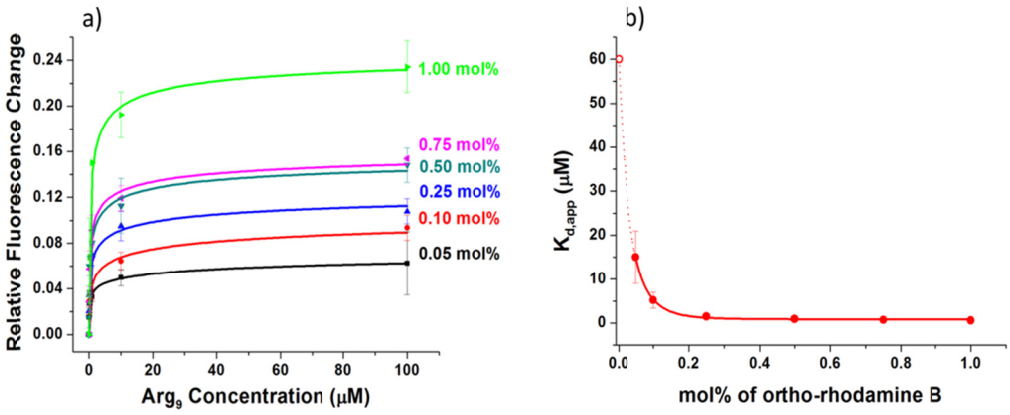

Figure S4. a) *Hill plots for Arg_9 peptide binding to POPC bilayers with different amounts of ortho-rhodamine B dye present in the bilayer. The assays were performed in 10 mM PBS buffer with 150 mM NaCl at pH 6.4. b) Plot of the extracted K_D values from (a) vs. mol% of ortho-rhodamine B. The red curve is the fit of a hyperbolic curve to the data.*

Total internal reflection fluorescence microscopy. 100% POPC bilayers were first formed in the microfluidic channels. No TIRF signal was observed with these bilayers, which indicated that there was no residual fluorophore in either the device or the lipid samples. Solutions of TAMRA-labeled peptides at varying concentrations (from 10 μM to 150 μM) were introduced into each channel and flowed continuously until the overall TIRF signal remained unchanged. Both Lys_9 and Arg_9 experiments were conducted under identical conditions. The measured TIRF signal arose from surface bound peptides as well as peptides in the near surface bulk solution. Peptide molecules in the near surface bulk just gave rise to a linear change in fluorescence intensity with increasing peptide concentration. To subtract off the linear contribution to the signal, experiments were run using a spin-coated PDMS layer in place of the POPC lipid bilayers. The hydrophobic nature of PDMS and similar dielectric constant with glass make PDMS one of the best inert substrate for correcting the bulk effect from unbound dye labeled peptides.[1,2] Figure S5a plots the data for TAMRA-labeled Arg_9 peptides binding to POPC bilayers after the subtraction of the linear contribution. The K_D value abstracted from this data was 70 ± 19 μM. Performing the same experiments with TAMRA-labeled Lys_9 led to no binding behavior, but instead the fluorescence response just changed linearly with peptide concentration (Figure S5b).

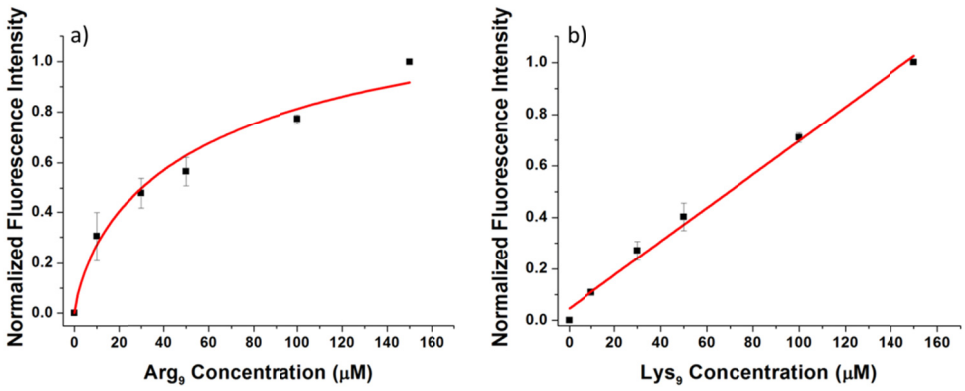

Figure S5. *a) Hill plot fitting with n = 0.75 for TIRF signal change of TAMRA-labeled Arg_9 binding to POPC bilayers. The assay was performed in 10 mM PBS buffer with 150 mM NaCl at pH 6.4, and an apparent K_D of 70 ± 19 μM was extracted; b) Linear fitting for TIRF signal change of TAMRA-labeled Lys_9 on pure POPC bilayers.*

(1) Mark, J. E. *Polymer data handbook*; 2nd ed.; Oxford University Press: Oxford ; New York, 2009.

(2) Tan, C. A., J. *J. Non-cryst. Solids.* **1994**, *169*, 143.