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Supporting Information

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Using Charge to Control the Functional Properties of Self-Assembled Nanopores in Membranes

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Using Charge to Control the Functional Properties of Self-Assembled Nanopores in Membranes

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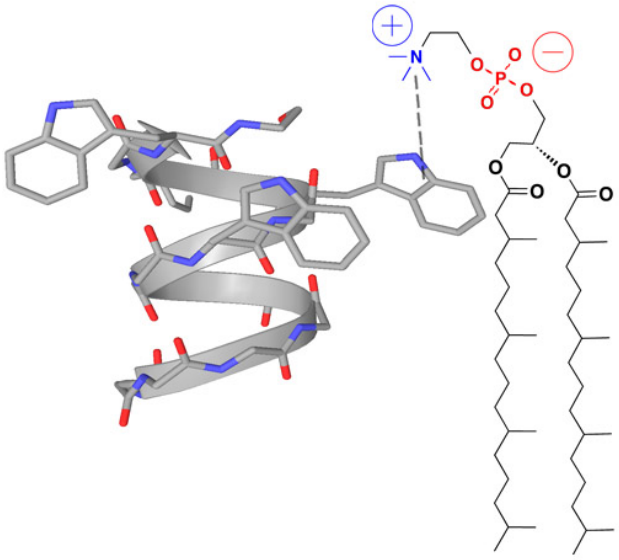


Figure S1. A Cartoon depicting theoretical π-cation interactions between a tryptophan side chain on native gramicidin and the trimethylammonium cation located on the lipid head group of a DiPhyPC lipid.

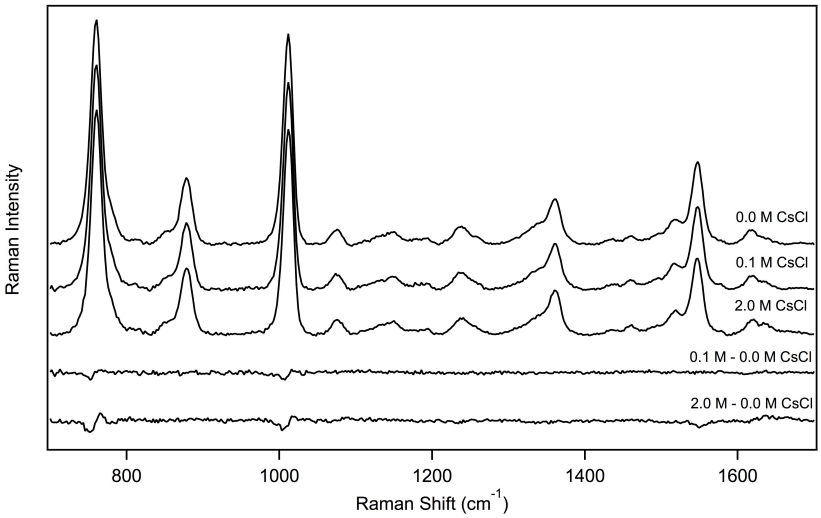


Figure S2. Ultraviolet Resonance Raman spectra (UVRR) for native gramicidin in DiPhyPC lipid vesicles (2% mol/mol) suspended in a 20mM phosphate buffer at pH 7 with various concentrations of CsCl added. The top three data traces are UVRR spectra recorded in aqueous solutions containing 0.0 M, 0.1 M and 2.0 M CsCl added to the buffer. Spectra were normalized for the band at ~ 760 cm^{-1}. The difference between the spectrum in 0.0 M CsCl and the spectrum in 0.1 M CsCl solution (0.1 M - 0.0 M CsCl) and the spectrum in 0.0 M CsCl and the spectrum in 2.0 M CsCl solution (2.0 M – 0.0 M CsCl), respectively, are plotted as the bottom two traces. In the UVRR spectra of gramicidin, 0 – 2 cm^{-1} shifts are observed for all bands when comparing the 0.0 M CsCl spectrum to that of either the 0.1 M or 2.0 M CsCl spectrum. The small, but systematic derivations near ~760 and ~1010 cm^{-1} are not consistent in magnitude or shape with cation-π interaction.[1]

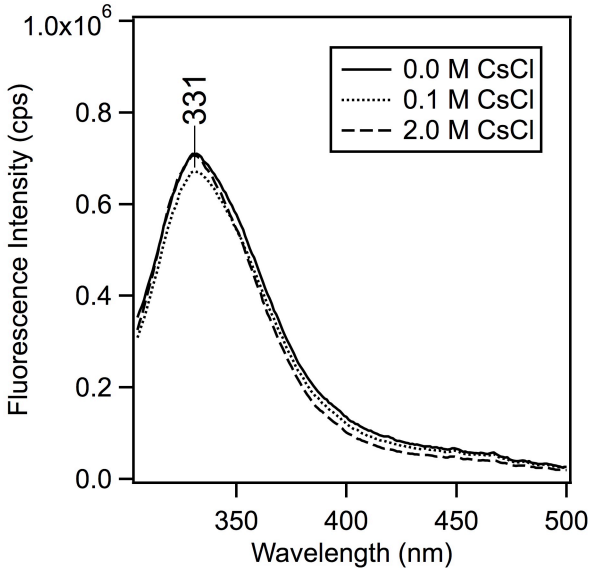


Figure S3. Tryptophan fluorescence spectra of native gramicidin in DiPhyPC lipid vesicles (2% mol/mol) in a 20 mM potassium phosphate buffer pH7 solution. Scattering signal from vesicles was subtracted from each spectrum. Three data traces are shown for fluorescence measurements recorded with 0.0 M, 0.1 M and 2.0 M CsCl added to the buffer. The tryptophan fluorescence peak position is in agreement with previously reported fluorescence spectra for gramicidin tryptophan residues located at the interfacial region of the bilayer, and, in part, confirms the incorporation of gA channels in the lipid vesicles.[2] With the exception of decrease in Rayleigh scattering, the fluorescence spectra do not change with increasing CsCl concentration. This observation indicates that the general environment of the tryptophan is not significantly altered by the high ionic strength.

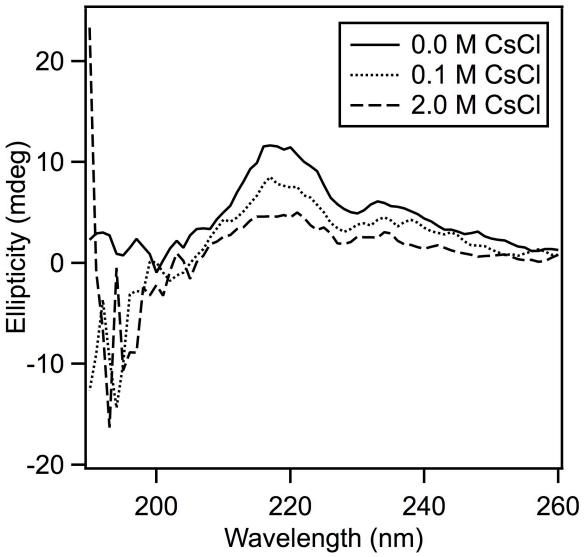
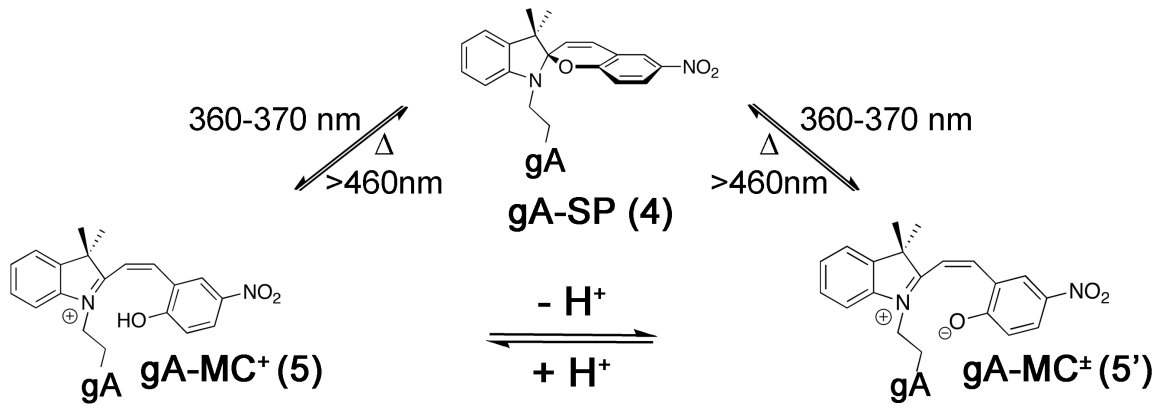
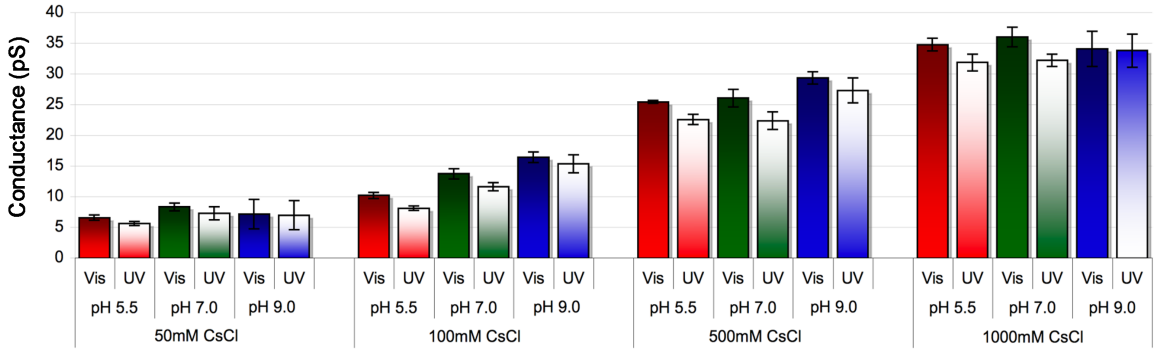


Figure S4. Circular Dichroism (CD) spectra of native gramicidin in DiPhyPC lipid vesicles (2% mol/mol) in a 20 mM potassium phosphate buffer pH 7 solution. Three data traces are shown for CD measurements recorded with 0.0 M, 0.1 M and 2.0 M CsCl added to the buffer. The two peaks located at 215nm and 235nm are consistent with the $\beta^{6.3}$ structure of gramicidin in a lipid bilayer environment.[2-5] The difference in the CD intensities for the three samples is attributed to variations in peptide concentration and low S/N on account of scattering from the vesicles and absorption by CsCl at $\lambda < 215$ nm.

A)



B)



C)

[CsCl] (M)	pH 5.5		pH 7.0		pH 9.0	
	γ_4	γ_5	γ_4	γ_5	γ_4	γ_5
0.05	6.6 ± 0.4	5.6 ± 0.3	8.3 ± 0.6	7.2 ± 1.1	7.1 ± 2.4	6.9 ± 2.4
0.10	10.2 ± 0.5	8.1 ± 0.4	13.7 ± 0.8	11.6 ± 0.7	16.4 ± 0.9	15.3 ± 1.4
0.50	25.4 ± 0.3	22.6 ± 0.9	26.0 ± 1.4	22.4 ± 1.4	29.3 ± 1.0	27.3 ± 2.0
1.00	34.8 ± 1.0	31.8 ± 1.4	36.0 ± 1.6	32.2 ± 1.0	34.1 ± 2.9	33.8 ± 2.7

Figure S5. The conductance of the photoisomers gA-SP **4** and gA-MC **5** as a function of ionic strength and pH. Recordings were performed in DiPhyPC lipids and 0.05 M – 1.00 M CsCl with a 0.05 – 10mM HEPES buffer. A) Schematic of the pH-dependent photoisomeric states of gA-SP (**4**) and gA-MC (**5**). When gA-SP (**4**) is exposed to UV light under acidic conditions it is converted to gA-MC (**5**). Under basic conditions, however, a zwitterionic species is formed as a result of the deprotonation of the acidic phenolic hydrogen to give gA-MC$^{\pm}$ (**5'**). B) A bar graph showing the

general trend for an increase in overall conductance as ionic strength is increased. Three different pH values were used for each of the four CsCl concentrations to discern if uncharged gA-SP (**4**), cationic gA-MC (**5**) and zwitterionic gA-MC$^{\pm}$ (**5'**) displayed different conductance behaviors. The bars shown in red correspond to pH 5.5, bars in green to pH 7.0 and bars in blue to a pH of 9.0. At each of these three pH values, recordings under visible light (darker bar) and recordings under UV light (lighter bar) were conducted. It is expected that for all salt concentrations and all pH values that gA-SP (**4**) is present in the absence of applied UV light. For recordings during the application of UV light, the cationic gA-MC (**5**) is expected at all pH 5.5 values, zwitterionic gA-MC$^{\pm}$ (**5'**) is expected at all pH 9.0 values and some equilibrium between (**5**) and (**5'**) is expected at pH 7.0. C) Table of conductance values for the various photoisomeric states depicted in part B. Based on this study of the interconversion of **4** and **5** on the *conductance* of this switchable ion channel as a function of pH and ionic strength, a pH of 5.5 was selected to investigate the effect of charge on the *lifetime* of channels formed from **4/5** as a function of ionic strength.

METHODS

We purchased all reagents and chemicals from Sigma-Aldrich unless otherwise stated. Gramicidin A (gA) was purchased as gramicidin D from Sigma Aldrich and purified by silica chromatography using a literature procedure[6] (to afford a final purity of 97% of gA). We purchased 1,2-diphytanoyl-*sn*-glycero-3-phosphocholine (DiPhyPC) lipids from Avanti Polar Lipids, Inc. All analyses by HPLC were performed on an Agilent Zorbax C-18 column (4.6 μm × 25 cm) using a gradient of 60 to 100% MeOH in H_2O and a flow rate of 1 mL min^{-1} over 52 min unless otherwise stated.

Synthesis of taurinyl gramicidin (gA-T). Details of the synthesis of taurinyl gramicidin has been reported previously.[7]

Synthesis of trimethyl-gramicidamine (gA-NMe_3). Details of the synthesis of trimethyl-gramicidamine have been reported previously. [8]

Synthesis of spiropyran-gramicidin (gA-SP). Spiropyran-ethylamine was prepared as described previously.[9, 10] We dissolved 4 mg (2.2 μmol) of desethanolamine gramicidin[7] in 0.3 mL of anhydrous tetrahydrofuran. We added 34 μL (238 μmol) of Et_3N and flushed the flask with N_2 gas. The reaction vessel was cooled to 0 °C and 1.2 μL of ethyl chloroformate was added. The solution was stirred at 0 °C for 3.5 h, and then a solution of 1.5 mg of spiropyran-ethylamine (dissolved in 30 μL of anhydrous THF) was added to the solution containing desethanolamine gramicidin. The reaction was stirred for 30 min at 0 °C, warmed to 23 °C, and stirred an additional 12 h. The solution was concentrated *in* v*acuo* and purified by HPLC to give a yield of

64%. The retention time by HPLC was 48.7 minutes. ESI-MS revealed a major peak at *m/z* = 2172.4 corresponding to the expected $[M + H]^+$ of the product.

Formation of Lipid Vesicles.

Lipid vesicles were prepared as reported previously by Rawat, et al.[2] Briefly, stock solutions of native gramicidin in methanol and DiPhyPC in chloroform were combined and dried with a stream of nitrogen. The resulting film was resuspended with 2 mL 0.0 M CsCl, 0.1 M CsCl, or 2.0 M CsCl in 20mM phosphate buffer pH 7 and sonicated for 30 minutes (50% duty cycle) using an ultrasonicator tip. Samples were centrifuged to remove particulates and incubated in a shaker overnight (37° C) for spectral analysis the next day. Resulting samples were ~ 25 μM gramicidin A and 1 mg/mL lipids (2% mol/mol). Insertion of gramicidin into vesicles was confirmed by circular dichroism and fluorescence spectroscopy.[2-5]

Ultraviolet Resonance Raman (UVRR) Measurements.

A detailed description of the UVRR setup has been reported previously.[11] Vibrational spectra of the gA tryptophan residues in lipid vesicles were obtained by setting the fundamental laser wavelength to 912 nm to generate a 228 nm excitation beam. A fresh sample volume of ~ 2 mL was flowed through a vertically mounted fused silica capillary at a rate 0.16 mL/min. The UV power was ~ 3 – 4 mW at the sample. Ten spectra from collection over one-minute intervals were summed for all samples. For each experimental condition (i.e. gA vesicles in a 0.1 M CsCl 20mM phosphate pH 7 buffer), spectra of blank solutions (i.e. no gA present) were also collected and subtracted from all raw spectra.

Fluorescence Measurements.

Tryptophan fluorescence spectra of the gA in vesicles were acquired on a Jobin Yvon Horiba Fluorolog-3 spectrofluorometer. The excitation wavelength was 290 nm, and the entrance and exit bandpass was 3 nm. For each experimental condition (i.e. gA vesicles in a 0.1 M CsCl 20mM phosphate pH 7 buffer), spectra of blank solutions (i.e. no gA present) were also collected and subtracted from all raw spectra.

Circular Dichroism Measurements.

Circular dichroism (CD) spectra of gA in vesicles were acquired on an Aviv202 spectropolarimeter and were obtained by sampling every 1 nm for 5 seconds using a quartz cuvette with a 0.2 cm path length. For each experimental condition (i.e., gA in vesicles in a 0.1 M CsCl 20mM phosphate pH 7 buffer), spectra of blank solutions (i.e., no gA present) were also collected and subtracted from all raw spectra.

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