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

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Dedicated to Prof. Chad Mirkin in celebration of 20 years of influential research at Northwestern University

Control of function in self-assembled molecular structures is a major challenge in supramolecular chemistry.[1–4] Ion channels, for instance, are pore-forming nanostructures typically comprising multiple molecular subunits assembled via noncovalent interactions.[5] These pores function by facilitating a flux of ions across the hydrophobic barrier of a lipid bilayer. For over a decade, many groups have attempted to mimic the functional properties of ion channels by engineering molecular assemblies that are capable of inducing transmembrane ion flux across lipid bilayers.[6–30] A major goal for engineered ion channels is to control such transmembrane ion fluctuations dynamically.[13,22,27–29,31–35] In nature, ion channels typically alter their ion conducting functional properties in response to external stimuli with the aid of specific chemical groups precisely located within the assembled structure.[5] Recently, we have reported the capability to control the single channel conductance (γ, **Figure 1**B) of ion channels derived from gramicidin A (gA, a natural ion-channel-forming peptide[5,36,37]) in artificial bilayers by manipulating the electrical charge of functional groups attached near the opening of the pore (i.e., on the C-terminus of gA).[16,17,38–42] Here, we demonstrate that C-terminal charged groups on gA can also affect the average channel lifetime (τ, Figure 1C) of conducting gA dimers. We show that this effect is fundamentally interesting and useful for sensing applications (here, for sensing specific wavelengths of light). Channel lifetime represents a second important parameter, in addition to conductance, which dictates the overall functional properties of these self-assembled pores. We present a method to control the lifetime of a gA channel dynamically and reversibly by introducing a light-sensitive molecular switch at the opening of the pore. This switch functions by photochemically controlling the charge presented on the C-terminus of gA.

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In order to explore the effect of charged groups attached to gA on the duration of open channel events, we compared the average open channel lifetimes of permanently charged derivatives of gA as a function of ionic strength in planar lipid bilayers composed of zwitterionic 1,2-diphytanoyl-*sn*-glycero-3-phosphatidylcholine (DiPhyPC) lipids. **Figure 2**B,D shows the fraction of open single channel events ($N(t)/N(0)$) as a function of their duration (t) in the open state in recording buffers containing either 0.1 (Figure 2B) or 2.0 (Figure 2D) M CsCl. $N(0)$ refers to the total number of open channel events and $N(t)$ is the number of channel events with an open channel duration longer than time t. Fitting the data in Figure 2B,D with Equation 1 made it possible to obtain the average lifetime of open channels (τ) in these two electrolytes:[43–46]

$$\frac{N(t)}{N(0)} = e^{\frac{-t}{\tau}} \tag{1}$$

Figure 2B shows that the lifetimes of gA channels with a neutral (**1**), positive (**2**), or negative (**3**) charge at the C-terminus exhibit relatively small differences in τ in a recording buffer with low ionic strength (0.1 M CsCl). In an electrolyte with high ionic strength (2.0 M CsCl), however, the difference in lifetimes of these three channels was more pronounced (Figure 2D,F).[47] Interestingly, this effect of ionic strength on the lifetimes of **1**, **2**, and **3** is opposite in trend to the effect of ionic strength on conductance through these neutral and charged derivatives of gA.[11,38] The difference in conductance values between **1**, **2**, and **3** was most pronounced at low ionic strength and diminished at high ionic strength in the recording electrolyte (Figure 2C,E). Additionally, both neutral (**1**) and, in particular, negatively charged (**3**) channels exhibited increased lifetimes in 2.0 M CsCl compared to those in 0.1 M CsCl, while the lifetime of the positively charged (**2**) channels was not significantly affected by the salt concentration.

Although several groups have reported the absence of any obvious trend in the change in lifetimes of gA-based ion channels as a function of various structural modifications,[48–53] we attempted to ascertain the possible cause for the observed increase in lifetime of pores of **3** compared to pores derived from native gA (**1**) in electrolytes with high ionic strength. We considered two factors that have been proposed[54] to influence the lifetime of gramicidin-based pores:
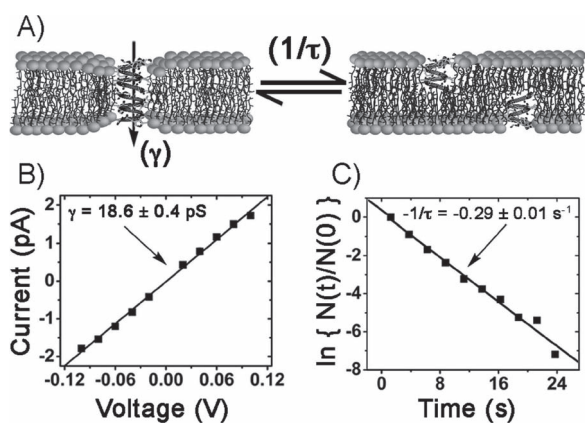
Figure 1. Determination of the two orthogonal functional parameters (γ and τ) of gA pores. A) Cartoon illustrating the reversible dimerization of gA in a phospholipid bilayer and the two associated measurable channel characteristics, conductance (γ) and lifetime (τ). B) Current–voltage (I–V) plot for pores of native gA. C) Plot of the natural log values of the fraction of open single channel events ($N(t)/N(0)$) as a function of their duration (t) in the open state. Fitting this data to the equation: $\ln(N(t)/N(0)) = -t/\tau$ yielded the average single channel lifetime (τ) of native gA. Channel events were recorded in an electrolyte containing 0.1 M CsCl, 1 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), pH 7 in a planar lipid bilayer of DiPhyPC lipids.
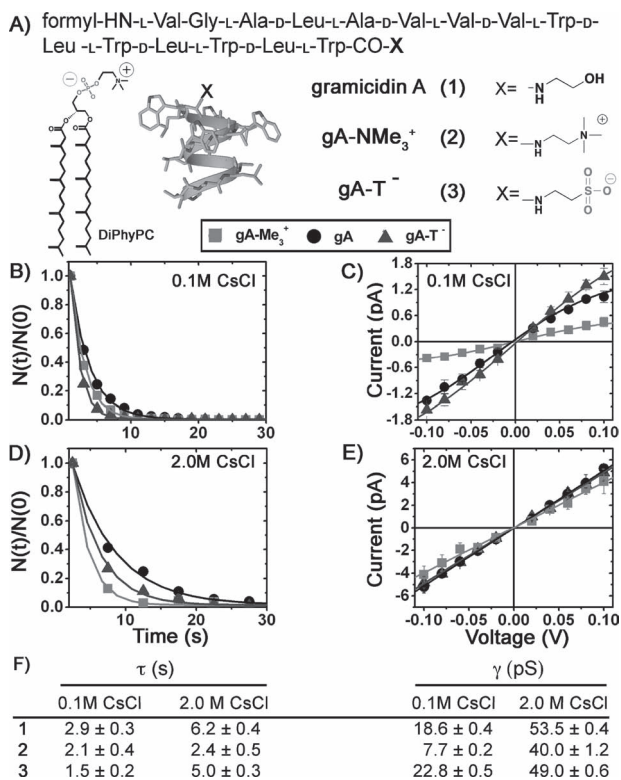

Figure 2. Effects of charge on gA channel lifetime and conductance as a function of ionic strength. A) Amino acid sequence of gramicidin A, structure of gA adapted from 1GRM, structure of a DiPhyPC lipid, and structure of the C-terminus of native gA (**1**), positively charged gA (**2**), and negatively charged gA (**3**). B) Normalized survivor histograms for homodimeric pores of **1**, **2**, and **3**; and C) I–V curves at low ionic strength (0.1 M CsCl, 1 mM HEPES). D) Normalized survivor histograms for pores of **1**, **2**, and **3**; and E) I–V curves at high ionic strength (2.0 M CsCl, 20 mM HEPES). F) Table of the lifetime and conductance values for **1-3** as shown graphically in B–E. All recordings were conducted at pH 7 using DiPhyPC lipids.

1) the hydrophobic mismatch between the thickness of the bilayer and the length of the conducting gA dimer,[36,43–46,55,56] or 2) the π-cation interactions between the lipid headgroups and the tryptophan sidechains in gramicidin peptides.[57–62]

Since a deformation of the lipid bilayers adjacent to the pore occurs upon formation of a channel, the associated energetic cost of this deformation can be experimentally observed through its effect on channel lifetimes.[36,43–46] In the studies reported here, however, differences in hydrophobic mismatch may not fully account for the observed differences in channel lifetime between **1**, **2**, and **3**, since any differences in the degree of hydrophobic mismatch is expected to be small between each of the gA derivatives at a given electrolyte concentration (since the only difference between **1**, **2**, and **3** is the functional group presented on the non-transmembrane C-terminal portion of the gA molecule).[63,64]

Intermolecular π-cation interactions between the positively charged trimethylammonium group of the choline lipid head group in the DiPhyPC lipids and the tryptophan residues near the C-terminus of gA (see Figure S1 in the Supporting Information) have been proposed to stabilize the conducting dimeric form of the channel, and, thus, to increase its lifetime.[57,58] Bezrukov and co-workers proposed that increasing the ionic strength of the electrolyte reduces the hydration shell around the choline lipid head group, which increases the strength of these π-cation interactions and favors longer channel lifetimes.[58] To experimentally test whether these proposed intermolecular π-cation interactions could explain the influence of charged groups in **2** and **3** on the lifetimes of gramicidin channels, we prepared vesicles from DiPhyPC containing gA (2% mol/mol) and measured their UV resonance Raman (UVRR) spectra as a function of CsCl concentration. UVRR spectroscopy has been recently developed by Kim and co-workers as a method to directly observe π-cation interactions between the tryptophan indole ring and inorganic and organic cations.[65] In comparing spectra from a suspension of the gA-incorporated vesicles in 0.0, 0.1, and 2.0 M CsCl, we did not observe any changes in the frequency or intensity of Raman bands (see Figure S2 in the Supporting Information) that could be attributed to intermolecular π-cation interactions between the DiPhyPC lipid headgroups and tryptophan residues on gA. These results suggest that any intermolecular π-cation interaction between the tryptophans in gA and the lipid headgroups in DiPhyPC lipids do not modulate the vibrational structure of the tryptophan sidechains. This result is in contrast to a recent report of the strong change in the vibrational spectrum of indoles (i.e., the sidechain group on tryptophans) induced by cation–π interactions.[65] It is possible that lipid–gA cation–π interactions are simply too weak to create a significant population of spectrally perturbed trytophan residues under these experimental conditions. This finding suggests that charge-induced alterations in cation–π interactions do not adequately account for the observed differences in channel lifetimes between **1**, **2**, and **3** at high ionic strength. We, therefore, conclude that the observed differences in channel lifetimes between **1-3** as a function of ionic strength are not likely a reflection of a single, previously proposed dominant factor,[36,43–46,49,52,53,57,58,66–71] but rather arise from a complex combination of several small

factors that depend on both the sign (since the effect of a positively charged functional group attached to gA on lifetime is different from a negatively charged group) and the magnitude of the charge (which can be tuned by changing the ionic strength of the recording buffer) presented on the C-terminus of gA.

Although the biophysical mechanism for the observed difference in lifetime between **1–3** remains unclear (as it is for other gA-based channels[48–53]), we designed an additional gramicidin derivative to evaluate whether the effects of C-terminal charged groups on gA lifetimes could be predictably extended to other gramicidin-based systems. We, therefore, synthesized a gramicidin derivative carrying a photoswitchable spiropyran moiety attached at the C-terminus (gA-SP, **Figure 3**A) and measured its open channel lifetime and conductance in recording buffers of both high and low ionic strength. We designed this nanopore to demonstrate the capability to dynamically control the functional properties of single channels of a gramicidin-based pore. Upon exposure to UV light ($\lambda = 366$ nm),[72,73] the uncharged spiropyran moiety interconverts reversibly[74] to a positively charged merocyanine form (gA-MC, Figure 3A) under acidic conditions.[75] Based on the results from measurement of τ for gA derivatives **1**, **2**, and **3** at different ionic strengths (Figure 2), we predicted only a small difference in τ between the neutral gA-SP (**4**) and the cationic gA-MC (**5**) at low ionic strength. At high ionic strength, however, we expected a significant difference in τ between **4** and **5**. In agreement with these expectations,

we found that interconversion of **4** to **5** under UV irradiation (or the reverse reaction under irradiation with visible light) resulted in a 20% difference in conductance ($\Delta\gamma_{4-5}$) in 0.1 M CsCl solution, and no significant difference in lifetime ($\Delta\tau_{4-5}$) at these low salt concentrations. In contrast, in 1.0 M CsCl, we observed a 24% difference in τ and a 9% difference in γ between **4** and **5**. The difference in τ between **4** and **5** followed the same trend as the observed difference in τ between **1** and **2**: increasing salt concentrations resulted in larger differences in channel lifetimes and reduced differences in conductance between uncharged and positively charged gA channels. We attribute the reduction in γ upon irradiation of **4** with UV light to a reduction in the local concentration of cesium ions near the mouth of the pore due to the presence of the positive charge in **5**, in agreement with previous reports.[5,11,16,17,38,40]

In conclusion, incorporation of a single electrical charge on gramicidin molecules can have a significant effect on the stability (i.e., lifetime) and rate of transmembrane ion flux (i.e., conductance) of pores formed from these self-assembled nanostructures. We found that regardless of the ionic strength of the recording electrolyte, the lifetimes of all charged derivatives of gA (**2**, **3**, or **5**) were shorter than those of the comparable neutral gA derivatives (**1** or **4**). In addition, the lifetimes of all gA derivatives increased with increasing ionic strength. This increase was strongest for the neutral gA derivatives (**1** and **4**) and, in particular, the negatively charged gA derivative (**3**). By incorporating a photoactive spiropyran functionality at the entrance of a gramicidin channel, we demonstrate the capability to dynamically control the average duration of the channel lifetime and the conductance of ions through the pore by using light to manipulate the charge presented at the C-terminus of gA. The differences in lifetimes of these charged gA derivatives appears to be a result of a combination of several factors that depend on both the sign and magnitude of the charge.

Monitoring the change in conductance upon changing the charge of functional groups presented near the entrance of gramicidin derivatives has been previously used for the detection of chemical and biochemical analytes in situ.[16,17,38,39,42,76] The results presented here show that lifetime measurements may help overcome previous limitations with such a charge-based sensing platform. Since differences in *conductance* between charged and uncharged gA derivatives are often indistinguishable in solutions of high ionic strength,[16,17,38,39,76] the significant and measurable differences in *lifetimes* between charged and uncharged gA derivatives in high ionic strength solutions (Figure 2) may be useful for monitoring chemical reactions under conditions where conductance measurements are not ideal for sensing. The methods reported here for using charge to influence the function of gramicidin channels in membranes represents a step towards achieving the level of control between structure and function of self-assembled structures that is typically observed only in nature.

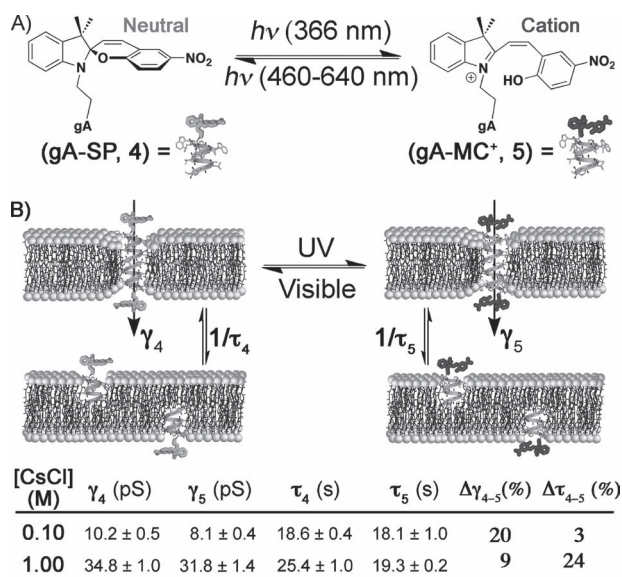| [CsCl] (M)	γ_4 (pS)	γ_5 (pS)	τ_4 (s)	τ_5 (s)	$\Delta\gamma_{4-5}$ (%)	$\Delta\tau_{4-5}$ (%)
0.10	10.2 ± 0.5	8.1 ± 0.4	18.6 ± 0.4	18.1 ± 1.0	20	3
1.00	34.8 ± 1.0	31.8 ± 1.4	25.4 ± 1.0	19.3 ± 0.2	9	24

Figure 3. Conductance and lifetime measurements of a photoactive derivative of gA upon exposure to different wavelengths of light in electrolytes with low and high ionic strengths. A) Chemical structure of a spiropyran moiety covalently linked to the C-terminus of gramicidin (gA-SP, **4**) and the product of its photoconversion induced by irradiation with UV light (gA-MC, **5**). B) Conductance (γ) and lifetime (τ) measurements of **4** (gray) and **5** (black) in electrolyte containing 0.1 or 1.0 M CsCl. All recordings were performed in electrolytes containing 10 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer with a pH of 5.5. Percent differences in γ and τ are reported with respect to the values for gA-SP, **4**.

Experimental Section

Formation of Planar Lipid Bilayers: We formed planar lipid bilayers by the "folding technique" over a hole with a diameter

~150 µm in a Teflon film as described previously.[17] The recording electrolyte was 100–2000 mᴍ CsCl buffered with 1–20 mᴍ HEPES at pH 7.0. Briefly, we spread a solution containing 25 mg mL^{-1} 1,2-diphytanoyl-*sn*-glycero-3-phosphatidylcholine (DiPhyPC) lipids in pentane at the air–water interface of the electrolyte solution in both compartments of the bilayer setup. We aspirated 3 mL of the total volume of 4 mL of electrolyte solution in each bilayer compartment into a syringe, followed by dispensing the electrolyte solution back into each compartment. We repeated this cycle of raising and lowering the liquid levels until we obtained a bilayer that had a minimum capacitance of 70 pF and that was stable (i.e., no significant current fluctuations above the baseline noise level) at 100 mV of applied potential for at least 2 min. After verifying that the membranes were stable, we added gA (**1**, 5–10 µL from 1 ng mL^{-1} in ethanol), gA-T (**3**, 1–10 µL from 100 ng mL^{-1} in ethanol) or gA-NMe$_3$ (**2**, 1–10 µL from 100 ng mL^{-1} in ethanol) directly to the bilayer chambers.

Ion Channel Measurements: We performed single channel recordings in "voltage clamp mode" using Ag/AgCl pellet electrodes (Warner Instruments) in both compartments of the bilayer setup. Data acquisition and storage was carried out using custom software in combination with a BC-535 patch clamp amplifier from Warner Instruments (set at a gain of 10 mV pA^{-1} and a filter cutoff frequency of 3 kHz). The data acquisition board (National Instruments, Austin, TX) that was connected to the amplifier was set to a sampling frequency of 15 kHz.

We performed the analysis of the single channel current traces by computing histograms of the currents from the original current versus time traces with ClampFit 9.2 software from Axon Instruments. From these histograms we extracted the main current values by fitting a Gaussian function to the peaks in the histograms. All conductance values were obtained from the slopes of *I–V* curves.[17] Channel lifetimes were calculated from a table of single channel durations. A minimum of 500 individual channel lifetimes was used for each measurement. In the event that two channel events occurred simultaneously, a random number generator was used to assign a channel opening with a channel closing.[67] Survivor histograms were generated from the tabulated channel durations. Equation 1 was fit to this curve to calculate τ, the average channel duration.[43,58,67,77,78]

Recordings of **4** were conducted under illumination of a halogen light bulb at room temperature. Photoconversion to **5** was accomplished by simultaneous blocking of visible light and irradiation with a 366 nm hand-held UV lamp clamped approximately 15 cm from the recording chamber.

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Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

[1] J. W. Steed, J. L. Atwood, *Supramolecular Chemistry*, John Wiley & Sons Inc, Chichester, UK **2009**.
[2] R. Breslow, *Acc. Chem. Res.* **1995**, *28*, 146.
[3] J. M. Lehn, *J. Chem. Sci.* **1994**, *106*, 915.
[4] J. D. Badjić, A. Nelson, S. J. Cantrill, W. B. Turnbull, J. F. Stoddart, *Acc. Chem. Res.* **2005**, *38*, 723.
[5] B. Hille, *Ionic Channels of Excitable Membranes*, 2nd ed., Sinauer Associates, Sunderland, **1992**.
[6] H. Bayley, P. S. Cremer, *Nature* **2001**, *413*, 226.
[7] N. Sakai, J. Mareda, S. Matile, *Mol. BioSyst.* **2007**, *3*, 658.
[8] A. L. Sisson, M. R. Shah, S. Bhosale, S. Matile, *Chem. Soc. Rev.* **2006**, *35*, 1269.
[9] N. Madhavan, E. C. Robert, M. S. Gin, *Angew. Chem., Int. Ed.* **2005**, *44*, 7584.
[10] H. J. Apell, E. Bamberg, H. Alpes, *J. Membr. Biol.* **1979**, *50*, 271.
[11] H. J. Apell, E. Bamberg, H. Alpes, P. Läuger, *J. Membr. Biol.* **1977**, *31*, 171.
[12] H. J. Apell, E. Bamberg, P. Läuger, *(BBA)-Biomembranes* **1979**, *552*, 369.
[13] H. Bayley, *Mol. BioSyst.* **2007**, *3*, 645.
[14] H. Bayley, L. Jayasinghe, *Mol. Membr. Biol.* **2004**, *21*, 209.
[15] H. Bayley, *Nature* **2009**, *459*, 651.
[16] S. Blake, T. Mayer, M. Mayer, J. Yang, *ChemBioChem* **2006**, *7*, 433.
[17] R. Capone, S. Blake, M. R. Restrepo, J. Yang, M. Mayer, *J. Am. Chem. Soc.* **2007**, *129*, 9737.
[18] B. A. Cornell, V. L. B. Braach-Maksvytis, L. G. King, P. D. J. Osman, B. Raguse, L. Wieczorek, R. J. Pace, *Nature* **1997**, *387*, 580.
[19] S. Futaki, Y. J. Zhang, T. Kiwada, I. Nakase, T. Yagami, S. Oiki, Y. Sugiura, *Bioorg. Med. Chem.* **2004**, *12*, 1343.
[20] S. Matile, N. Sakai, J. Mareda, J. Kumaki, E. Yashima, *J. Recept. Signal Transduct. Res.* **2006**, *26*, 461.
[21] N. Ashkenasy, J. Sanchez-Quesada, M. R. Ghadiri, H. Bayley, *Angew. Chem., Int. Ed.* **2005**, *44*, 1401.
[22] M. R. Banghart, M. Volgraf, D. Trauner, *Biochemistry* **2006**, *45*, 15129.
[23] G. W. Gokel, P. H. Schlesinger, N. K. Djedovic, R. Ferdani, E. C. Harder, J. X. Hu, W. M. Leevy, J. Pajewska, R. Pajewski, M. E. Weber, *Bioorg. Med. Chem.* **2004**, *12*, 1291.
[24] A. Hirano, M. Wakabayashi, Y. Matsuno, M. Sugawara, *Biosens. Bioelectron.* **2003**, *18*, 973.
[25] P. Schoen, T. H. Degefa, S. Asaftei, W. Meyer, L. Walder, *J. Am. Chem. Soc.* **2005**, *127*, 11486.
[26] S. Terrettaz, M. Mayer, H. Vogel, *Langmuir* **2003**, *19*, 5567.
[27] V. Borisenko, D. C. Burns, Z. Zhang, G. A. Woolley, *J. Am. Chem. Soc.* **2000**, *122*, 6364.
[28] M. Banghart, K. Borges, E. Isacoff, D. Trauner, R. H. Kramer, *Nat. Neurosci.* **2004**, *7*, 1381.
[29] J. J. Chambers, M. R. Banghart, D. Trauner, R. H. Kramer, *J. Neurophysiol.* **2006**, *96*, 2792.
[30] D. Trauner, *Angew. Chem., Int. Ed.* **2003**, *42*, 5671.
[31] S. Szobota, P. Gorostiza, F. Del Bene, C. Wyart, D. L. Fortin, K. D. Kolstad, O. Tulyathan, M. Volgraf, R. Numano, H. L. Aaron, *Neuron* **2007**, *54*, 535.
[32] A. Kocer, M. Walko, W. Meijberg, B. L. Feringa, *Science* **2005**, *309*, 755.
[33] P. V. Jog, M. S. Gin, *Org. Lett.* **2008**, *10*, 3693.
[34] D. L. Fortin, M. R. Banghart, T. W. Dunn, K. Borges, D. A. Wagenaar, Q. Gaudry, M. H. Karakossian, T. S. Otis, W. B. Kristan, D. Trauner, *Nat. Methods* **2008**, *5*, 331.
[35] T. Lougheed, V. Borisenko, T. Hennig, K. Rück-Braun, G. A. Woolley, *Org. Biomol. Chem.* **2004**, *2*, 2798.
[36] O. S. Andersen, R. E. Koeppe, B. Roux, *2 Gramicidin Channels: Versatile Tools*, Springer, New York **2007**.
[37] B. Roux, *Acc. Chem. Res.* **2002**, *35*, 366.

[38] M. X. Macrae, S. Blake, M. Mayer, J. Yang, *J. Am. Chem. Soc.* **2010**, *132*, 1766.

[39] S. Blake, R. Capone, M. Mayer, J. Yang, *Bioconjugate Chem.* **2008**, *19*, 1614.

[40] S. Majd, E. C. Yusko, A. D. MacBriar, J. Yang, M. Mayer, *J. Am. Chem. Soc.* **2009**, *131*, 16119.

[41] S. Majd, E. C. Yusko, Y. N. Billeh, M. X. Macrae, J. Yang, M. Mayer, *Curr. Opin. Biotech.* **2010**, *4*, 439.

[42] T. I. Rokitskaya, M. X. Macrae, S. Blake, N. S. Egorova, E. A. Kotova, J. Yang, Y. N. Antonenko, *J. Phys.: Condens. Matter* **2010**, *22*, 454118.

[43] J. A. Lundbæk, A. M. Maer, O. S. Andersen, *Biochemistry* **1997**, *36*, 5695.

[44] J. A. Lundbæk, *J. Phys.: Condens. Matter* **2006**, *18*, S1305.

[45] O. S. Andersen, R. E. Koeppe, *Annu. Rev. Biophys. Biomol. Struct.* **2007**, *36*, 107.

[46] J. A. Lundbaek, R. E. Koeppe, O. S.Andersen, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 15427.

[47] The lifetimes for channels formed from homodimers of **2** or **3** were shorter than for native gA (**1**) at both low and high ionic strength. One possible reason to consider for this observation is the existence of repulsive Coulombic effects between two positive charges in homodimers of **2**-**2** or between two negative charges in homodimers of **3**-**3** across the membrane, which could contribute slightly towards destabilization of the pores. The insulating effects of the lipid bilayer membrane,[5] however, make such repulsive Columbic interactions across the membrane unlikely (personal communication with Sergey M. Bezrukov). The measured lifetime of a heterodimeric channel comprising **2** and **3** in DiPhyPC lipids in 0.1 M CsCl was indistinguishable (within error) from the lifetime of a homodimeric channel of native gA (data not shown), further supporting that Coulombic effects between charged groups on gA across the membrane likely do not significantly affect the lifetime of the channel.

[48] M. D. Becker, D. V. Greathouse, R. E. Koeppe, O. S. Andersen, *Biochemistry* **1991**, *30*, 8830.

[49] O. S. Andersen, D. V. Greathouse, L. L. Providence, M. D. Becker, R. E. Koeppe, *J. Am. Chem. Soc.* **1998**, *120*, 5142.

[50] V. Fonseca, P. Daumas, L. Ranjalahy-Rasoloarijao, F. Heitz, R. Lazaro, Y. Trudelle, O. S. Andersen, *Biochemistry* **1992**, *31*, 5340.

[51] J. Girshman, D. V. Greathouse, *Biophys. J.* **1997**, *73*, 1310.

[52] H. Sun, D. V. Greathouse, O. S. Andersen, R. E. Koeppe, *J. Biol. Chem.* **2008**, *283*, 22233.

[53] A. E. Daily, J. H. Kim, D. V. Greathouse, O. S. Andersen, R. E. Koeppe, *Biochemistry* **2010**, *49*, 6856.

[54] Several additional factors have been shown to influence channel lifetimes, including modification to the N-terminus Hydrogen bonding network,[53,67,68] substitution or chemical alteration of the tryptophan sidechains.[49,52,66]

[55] M. Goulian, O. N. Mesquita, D. K. Fygenson, C. Nielsen, O. S. Andersen, A. Libchaber, *Biophys. J.* **1998**, *74*, 328.

[56] S. M. Bezrukov, *Curr. Opin. Colloid In. Sci.* **2000**, *5*, 237.

[57] F. N. R. Petersen, M. Jensen, C. H. Nielsen, *Biophys. J.* **2005**, *89*, 3985.

[58] T. K. Rostovtseva, H. I. Petrache, N. Kazemi, E. Hassanzadeh, S. M. Bezrukov, *Biophys. J.* **2008**, *94*, L23.

[59] J. P. Gallivan, D. A. Dougherty, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9459.

[60] M. P. Aliste, J. L. MacCallum, D. P. Tieleman, *Biochemistry* **2003**, *42*, 8976.

[61] H. C. Gaede, W. M. Yau, K. Gawrisch, *J. Phys. Chem. B* **2005**, *109*, 13014.

[62] E. K. Esbjörner, C. E. B. Caesar, B. Albinsson, P. Lincoln, B. Nordén, *Biochem. Biophys. Res. Comm.* **2007**, *361*, 645.

[63] R. R. Ketchem, W. Hu, T. A. Cross, *Science* **1993**, *261*, 1457.

[64] J. A. Lundbaek, O. S. Andersen, *J. Gen. Physiol.* **1994**, *104*, 645.

[65] D. E. Schlamadinger, M. M. Daschbach, G. W. Gokel, J. E. Kim, *J. Raman Spectrosc.* **2011**, *42*, 633.

[66] D. A. Kelkar, A. Chattopadhyay, *BBA−Biomembranes* **2007**, *1768*, 2011.

[67] J. T. Durkin, R. E. Koeppe, O. S. Andersen, *J. Mol. Biol.* **1990**, *211*, 221.

[68] C. J. Stankovic, S. H. Heinemann, S. L. Schreiber, *(BBA)-Biomembranes* **1991**, *1061*, 163.

[69] A. Chattopadhyay, S. S. Rawat, D. V. Greathouse, D. A. Kelkar, II Koeppe, E. Roger, *Biophys. J.* **2008**, *95*, 166.

[70] S. Mukherjee, A. Chattopadhyay, *Biochemistry* **1994**, *33*, 5089.

[71] W. Hu, T. A. Cross, *Biochemistry* **1995**, *34*, 14147.

[72] F. M. Raymo, S. Giordani, *J. Am. Chem. Soc.* **2001**, *123*, 4651.

[73] J. T. C. Wojtyk, A. Wasey, N.-N. Xiao, P. M. Kazmaier, S. Hoz, C. Yu, R. P. Lemieux, E. Buncel, *J. Phys. Chem. A* **2007**, *111*, 2511.

[74] J. C. Crano, R. J. Guglielmetti, *Physicochemical Studies, Biological Applications, and Thermochromism*, Organic Photochromic and Thermochromic Compounds, Vol.2, Plenum Publishing Corporation, New York **1999**.

[75] Figure S5 in the Supporting Information includes data for the difference in conductance between neutral gA-SP (**4**) and cationic gA-MC (**5**) as a function of pH and as a function of ionic strength. Based on our empirical observations, we selected pH of 5.5 to highlight the possibility to dynamically switch the conductance and lifetime of the photosensitive gA derivative.

[76] M. X. Macrae, S. Blake, X. Jiang, R. Capone, D. J. Estes, M. Mayer, J. Yang, *ACS Nano* **2009**, *3*, 3567.

[77] D. R. Cox, P. A. Lewis, *The Statistical Analysis of Series of Events*, John Wiley and Sons, Inc., New York, **1966**.

[78] J. A. Lundbæk, S. A. Collingwood, H. I. Ingólfsson, R. Kapoor, O. S. Andersen, *Proc. R. Soc. Interface.* **2009**, *44*, 373.