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# Directional Potassium Transport through a Unimolecular Peptide Channel

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***Abstract:*** *Three unimolecular peptide channels have been designed and prepared by using the β-helical conformation of gramicidin A (gA). The new peptides bear one to three $NH_3^+$ groups at the N-end and one to three $CO_2^-$ groups at the C-end. These zwitterionic peptides were inserted into lipid bilayers in an orientation-selective manner. Conductance experiments on planar lipid bilayers showed that this orientation bias could lead to observable directional $K^+$ transport under multi-channel conditions. This directional transport behavior can further cause the generation of a current across a planar bilayer without applying a voltage. More importantly, in vesicles with identical external and internal KCl concentrations, the channels can pump $K^+$ across the lipid bilayer and cause a membrane potential.*

Natural ion channels, such as potassium channels, are able to directionally conduct ions across lipid bilayers, which has been considered as a type of ion rectifying behavior.[1] The main role of this rectifying behavior is to maintain the membrane resting potential and to regulate the action potential in electrically excitable cells.[2] It has been proposed that this behavior of natural channels is related to the selective alignment of their asymmetrical porous structures in the bilayers and the presence of charges at one or two ends of the backbones.[3] The construction of artificial systems that mimic this important biological behavior not only provided simple models for investigating the rectifying mechanism but also may produce energy-related materials.[4,5] Recently, an important advance has been achieved in the design of artificial rectifying nanopores by utilizing the etching and lithography of solid materials.[6] In this context, the rectifying behavior at the multi-pore level, also called macroscopic level, has been utilized to develop new nano-diodes.[7] Examples of molecular channels that exhibit rectifying behavior across lipid bilayers are also available.[8] These channels are supramolecular and operate well at the single-channel level, but cannot be investigated at the macroscopic level, owing to the difficulty in controlling the asymmetrical alignment of multiple channels in the lipid bilayer. Herein, we report that three unimolecular zwitterionic peptide channels can rectify $K^+$ transport at the macroscopic level by controlling their orientation in lipid bilayers. The macroscopic rectifying behavior could even lead to the channel pumping $K^+$ from outside to inside of vesicles containing identical external and internal KCl concentrations.

It has been established that gramicidin A (gA), whose alternately arranged L- and D-amino acid residues induce the backbone to adopt a β-helical conformation,[9] can mediate the transmembrane transport of cations by forming hydrogen-bonded head-to-head dimers in membranes (Figure 1a). We

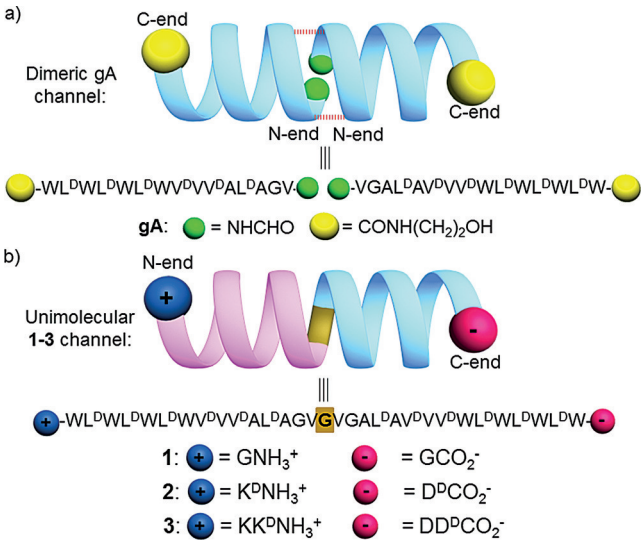


***Figure 1.*** Schematic of the structures of the a) gA channel and b) peptides **1**–**3**.

and other groups demonstrated that unimolecular tubular structures exhibited very high membrane-insertion capability for transporting discrete species.[10,11] We therefore prepared peptide **1**, which contained alternating L- and D-amino acid residues, using solid phase synthesis with 2-chlorotrityl chloride resin and Fmoc-protected amino acids as starting materials. The peptide was purified by preparative reverse-phase HPLC using acetonitrile and water as the eluent. Peptide **1** formally consisted of one gA amino acid sequence and one gA-analogous sequence (Figure 1b), with the N-end of the first segment being linked through an additional glycine (G) residue to the C-end of the second one. The whole backbone was expected to maintain the β-helical conformation of the dimeric gA channel.[12] Because the two ends of **1** bear one $NH_3^+$ and one $CO_2^-$, respectively, the tubular peptide could be structurally regarded as an electronic diode.[13] We envisioned that insertion of molecules of **1** from one side of a bilayer would generate a bias in the alignment of the molecules across the membranes. We further

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prepared peptides **2** and **3** by introducing one or two more ionic lysine (K) and aspartic acid (D) residues at the two ends, respectively, to investigate the influence of the charge number on the rectifying behavior. The structures of the peptides **1–3** had been characterized by $^1$H NMR and mass spectroscopy and the purity was determined to be higher than 95% by HPLC (see Section S2 of the Supporting Information).

Circular dichroism (CD) spectroscopy has been widely used to characterize the β-helical conformation of gA and gA analogues.[14] We therefore investigated the conformation of the new peptides in lipid bilayers composed of egg yolk L-α-phosphatidylcholine (EYPC) by comparing the CD spectra of **1–3** with that of gA (see Section S3 of the Supporting Information). The CD spectrum of gA showed two characteristic peaks of positive ellipticity at 214 and 245 nm and a valley at 228 nm (Supporting Information, Figure S10), which are consistent with the reported CD spectrum of gA.[15] These ellipticities were assigned to the characteristic single-stranded β-helical conformation.[15] Under the same conditions, peptides **1–3** showed the positive ellipticity at around 210 and 240 nm and a valley at 225 nm. These ellipticities are close to that of gA, supporting that, similar to gA channel, peptides **1–3** also adopted a β-helical conformation in the lipid bilayers.

The capability of **1–3** to form transmembrane channels was investigated by performing conductance measurement experiments on a planar lipid bilayer composed of diphytanoylphosphatidylcholine (diPhyPC) at the single-channel level (see Section S4 and Figure S11 of the Supporting Information).[16] At a concentration of $1.0\times10^{-16}$ M and potential of −200 mV, the square-like single-channel conductance signals could be observed (Figure 2), which strongly supported the formation of transmembrane channels in the lipid bilayers. Kinetic experiments for **2** at different concentrations were also conducted. The logarithm of the current value versus the logarithm of the concentration exhibited a linear relationship with a slope approaching one, which indicates that the channels operated through a unimolecular mechanism (Supporting Information, Figure S12).[17] The lifetimes of **1–3** and gA were determined to be 0.05, 0.2, 0.4, and 0.8 s, respectively (see Section S4 of the Supporting Information). In principle, the unimolecular channels **1–3** should live longer than dimeric gA channel. However, their terminal charges might cause the channel molecules to sway in the bilayers. Thus, their lifetimes decreased as a result.

The single-channel currents ($I_s$) of **1–3** at different voltages were measured. All of the peptide channels displayed non-linear current–voltage ($I$–$V$) relationships in the range of −200 to +200 mV (Figure 3a), indicating a weak gating behavior as a result of the voltage-induced conformation variation.[18,19] The current ($I_s^-$) at negative voltage was obviously higher than the current ($I_s^+$) at the corresponding positive potential, which clearly indicated the rectifying behavior of the transport process.[20] To investigate the rectifying mechanism, the selectivity of the three channels for $K^+$ and $Cl^-$, expressed as the permeability ($P$) ratio of the two ions, was assessed by adding 0.3 M KCl to the cis compartment and 1.0 M KCl to the trans compartment (Supporting Information, Figures S13–15). The $P_{K^+}/P_{Cl^-}$ values of **1–3** were determined to be 10.8, 13.3, and 13.8, respectively, by using the Goldman–Hodgkin–Katz equation (Supporting Information, Table S2).[21] These high permeability ratios clearly indicate a high selectivity for $K^+$ over $Cl^-$. Because the cavity of the β-helix of gA can only accommodate dehydrated cations,[22] it is reasonable to propose that $K^+$ transported through the cavity of **1–3** should also be dehydrated. The negative C-end of **1–3** should be more favorable than the positive *N*-end for this process. Thus, the above rectifying behavior may be attributed to the more favorable insertion of $K^+$ into the channels from their C-end (Figure 3b). The directional rectifying behavior for $K^+$ also indicated that the negative C-end of **1–3** all pointed to the cis (outward) compartment. The $I_s^{-200}/I_s^{+200}$ ratios of **1–3** were 1.6, 1.9 and 2.0, respectively, reflecting an increase of their rectifying capability. This increase is consistent with the trend of the $K^+$ selectivity of **1–3** and may be rationalized by

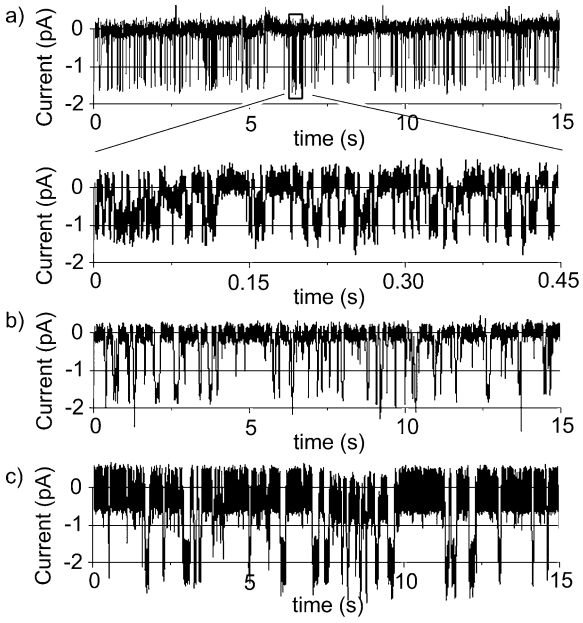


***Figure 2.*** Single-channel current traces of a) **1**, b) **2**, and c) **3** ($1.0\times10^{-16}$ M) in planar lipid bilayers at −200 mV. Both cis and trans compartments were filled with KCl (1.0 M).

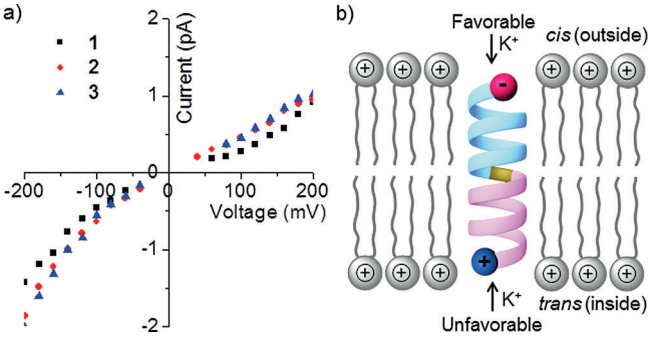


***Figure 3.*** a) $I$–$V$ plots for **1–3** ($1.0\times10^{-16}$ M) at the single-channel level in planar lipid bilayers. b) Schematic of the current rectification of **1–3** at the single-channel level in bilayers.

considering the increase of the negative charges at the C-end, which was favorable for the dehydration of $K^+$.

To investigate the multi-channel (macroscopic) behavior of **1**–**3**, conductance measurements were also performed at the higher concentration of $1.0\times10^{-11}$ M. Upon addition of **1**–**3** to the cis compartment, the channels were rapidly inserted into the bilayers and reached equilibrium within 10 min, as indicated by the formation of a stable current at a constant voltage. Macroscopic currents ($I_m$) were then recorded by applying the ramp potential range from −200 to 200 mV across the bilayer. The corresponding *I*–*V* plots are shown in Figure 4a. Similar to the *I*–*V* plots of the above single-channel conductance (Figure 3a), the plots also showed asymmetrical

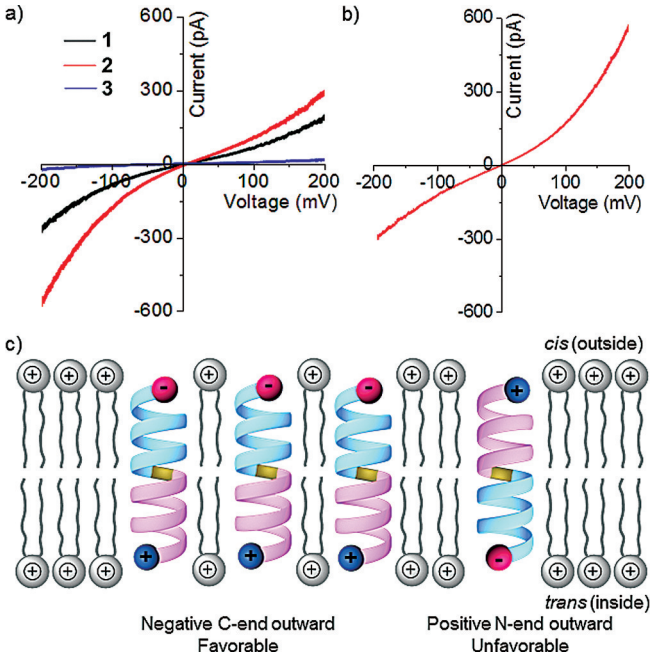


***Figure 4.*** *I*–*V* plots measured under multi-channel conditions in the planar lipid bilayer by adding a) **1**–**3** ($1.0\times10^{-11}$ M) to the cis compartment and b) **2** ($1.0\times10^{-11}$ M) to the trans compartment. c) Schematic of the alignment and current rectification of channels **1**–**3** under multi-channel conditions in the bilayer.

non-linear *I*–*V* relationships, again supporting $K^+$ transport rectification. Under the multi-channel conditions, the flux of $K^+$ from the cis side to trans side was also favorable. The $I_m^{-200}/I_m^{+200}$ ratios of **1**–**3** were calculated to be 1.5, 1.8, and 1.6, respectively. For **1** and **2**, the ratio is very close to that for single-channel conditions, indicating a comparable rectifying ability. However, for **3** under multi-channel conditions, the rectifying ability clearly decreased compared with that under single-channel conditions owing to the decreasing difference in the orientation of the channel alignment (see below). Addition of **2** to the trans compartment led to a higher $I_m^+$ than $I_m^-$ (Figure 4b), indicating that the rectification direction was reversed. This result further confirmed that the rectifying direction was determined by the membrane-insertion direction of the channels.

The number of outward negative C-end ($N_{no}$) and positive N-end ($N_{po}$) channels of **1**–**3** could be calculated from the $I_s^{-200}$and $I_m^{+200}$ and the channel open probability ($P$) (see the Supporting Information), and the results are listed in Table 1.

***Table 1:*** The calculated channel numbers of **1**–**3** in the lipid bilayers under multi-channel conditions.

| Channel | $N_{no}$[a] | $N_{po}$[b] | $N_{no}/N_{po}$ | $N_{no}+N_{po}$ |
|---|---|---|---|---|
| **1** | 334 | 46 | 7.3 | 380 |
| **2** | 793 | 78 | 10.2 | 871 |
| **3** | 47 | 13 | 3.6 | 60 |

[a] $N_{no}$ = negative C-end outward channel number. [b] $N_{po}$ = positive *N*-end outward channel number.

It was found that, for all the three channels, the $N_{no}$ value was much higher than the $N_{po}$ value, with the $N_{no}/N_{po}$ ratios being 7.3, 10.2, and 3.6, respectively, demonstrating that their negative C-end remarkably preferred to be oriented outward, albeit not in an exclusive manner (Figure 4c). This large orientation difference led to the rectifying direction under multi-channel conditions. Peptide **3** showed a notable decrease in the $N_{no}/N_{po}$ value compared with **1** and **2**, revealing the decrease in orientation difference. Since the channels were zwitterions, we tentatively attributed their large orientation bias in the bilayers to the electrostatic attraction between their negative C-end and the positive head group of the lipid molecules on the side of the bilayer in which the channels were added. The difference in the number ($N_{no}+N_{po}$) of the membrane-inserted channels of **1**–**3** reflected their different membrane-insertion ability, which might be caused by the different charge number at the two ends. The multi-channel conductance in KCl solutions at pH 4.0 and 10 was further measured for **2** (Supporting Information, Figure S16), which exhibited the highest membrane-insertion ability. The $I_m^{-200}/I_m^{+200}$ values were 1.2 and 1.4, respectively. Both values were smaller than that (1.8) under neutral conditions, which indicated that the rectification ability decreased as a result of the protonation of the $CO_2^-$ group or the deprotonation of the $NH_3^+$ group.[23]

The selectivity of **1**–**3** followed the sequence $K^+ > Rb^+ > Cs^+ > Na^+ > Cl^-$ (Supporting Information, Figure S13–15 and Table S2), which was different from the gA channel.[24] This unusual selectivity sequence was probably caused by the conformation of the terminal $COO^-$ group, which might be more favorable for the dehydration of $K^+$. To investigate the influence of the ionic selectivity on the rectifying capability, the conductance of **2** in CsCl, RbCl, and NaCl solutions was also monitored (Supporting Information, Figure S17). The $I_m^{-200}/I_m^{+200}$ values were 1.1, 1.1, and 1.0, respectively, indicating a very weak rectifying capability for all the solutions owing to a poor cation/$Cl^-$ selectivity (Supporting Information, Table S2). The result is consistent with the above ion selectivity sequence. This observation also suggests the importance of the high $K^+/Cl^-$ selectivity in KCl solution for **2** to achieve the directional transport for $K^+$. To investigate the influence of anionic lipids on the rectifying capability of **2**, the conductances were measured in the diPhyPC bilayers containing 2% and 5% (molar

ratio) 1,2-diphytanoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (diPhyPG) (Supporting Information, Figure S18). The $I_m^{-200}/I_m^{+200}$ values under these conditions were 1.3 and 1.1, respectively. These values were obviously smaller than that in the absence of diPhyPG (1.8), demonstrating that the anionic diPhyPG decreased the rectifying capability of **2**. The decrease of the rectifying capability should be caused by a decrease of the orientation-selectivity of the channel molecules due to the opposite charges of the head-groups of diPyhPC and diPhyPG.

It has been reported that natural ion channels, such as the outer membrane protein F,[25] can transport $K^+$ cross the lipid bilayers in symmetric KCl solutions without applying a voltage due to their high rectifying abilities.[26] The possibility of **2** for generating such a capability was also investigated by measuring the conductance under multi-channel conditions. For this measurement, the cis and trans compartments were both filled with 0.5 M KCl solution, and the DMSO solution of **2** was added to the cis compartment to reach a final concentration of $1.0\times10^{-9}$ M, and then the current was monitored without applying a voltage across the bilayer. The inserted channels did produce a negative macroscopic current, which increased gradually and reached the maximum of −7.2 pA after 10 min (Figure 5). This observation demonstrated that $K^+$ could be directionally transported through the channels without the driving force of an applied voltage. The generation of a negative current indicated that $K^+$ was transported from the cis side to the trans side, which is consistent with the rectifying direction of the channel. This result further supported that the negative C-end of the molecules of **2** preferred to be oriented outward.

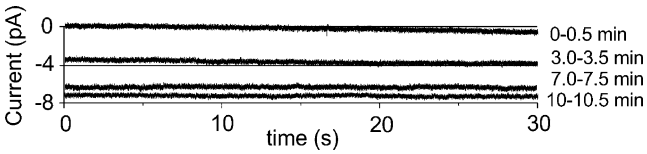


***Figure 5.*** Current traces of **2** ($1.0\times10^{-9}$ M) in the planar lipid bilayers without applying a voltage. Both the cis and trans compartments were filled with KCl (0.5 M).

The above directional potassium transport behavior of **2** in the planar lipid bilayer at 0 mV holds a promise for biasing the identical concentration of $K^+$ in the external and internal solutions of lipid vesicles. We thus explored this possibility by assessing the ability of **2** to polarize the membrane of lipid vesicles that had the identical external and internal KCl concentrations (0.1 M) (see Section S5 of the Supporting Information). Safranin O ($1.8\times10^{-7}$ M), a fluorescent probe for membrane potential,[27] was added to the solution of the EYPC vesicles. Adding **2** (10 μM) to the solution led to an obvious increase of the emission of Safranin O (Figure 6a), suggesting that a negative membrane potential was produced from outside to inside of the vesicles.[28] After six minutes, valinomycin (20 nM), a selective $K^+$ carrier, was added to the vesicle solution. The fluorescence intensity of Safranin O was found to decrease quickly and reached the intensity of the solution in the absence of **2**, indicating that the membrane was

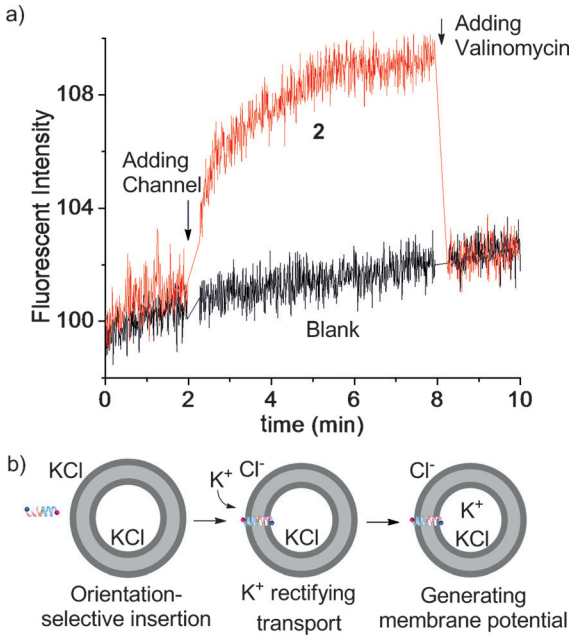


***Figure 6.*** a) Fluorescence versus time plots of Safranin O ($1.8\times10^{-7}$ M, $\lambda_{ex}=522$ nm, $\lambda_{em}=581$ nm) in Mes buffer (pH 6.4) containing EYPC vesicles with and without the addition of **2** (10 μM). $[KCl]_{inside}=[KCl]_{outside}=0.1$ M. b) Schematic of the mechanism of generating membrane potential.

depolarized due to the valinomycin-mediated $K^+$ inside-to-outside transport. This result demonstrated that the external-to-internal membrane potential caused by the directional outside-to-inside transport of **2** for $K^+$, also supporting that the negative C-end of **2** preferred to be oriented to the outside of the vesicles. The molecules of **2** could be inserted into the lipid bilayer with high orientation selectivity ($N_{no}/N_{po}=10.2$, Table 1) as a result of the asymmetric channel structure, in which both ends were oppositely charged. This high orientation-selective alignment caused a flux of $K^+$ from outside to inside of the vesicles, which resulted in a separation of $K^+$ and $Cl^-$ and thus a membrane potential (Figure 6b). It has been established that the membrane potential could be created by the oriented polarized synthetic channel.[29] However, the directional transport of the asymmetric channel demonstrated in this work represents a new strategy for producing a membrane potential. The fact that $K^+$ could be transported from outside to inside of vesicles that had identical external and internal KCl concentration suggests that peptide channel **2** could formally work as an ion pump, although there was no energy input into the channel molecules.

In conclusion, we have achieved directional $K^+$ transport under multi-channel conditions by designing new unimolecular peptide channels that mimic the β-helical structure of the natural dimeric gA channel. The new unimolecular channels, which are zwitterionic, were inserted into a lipid bilayer in an asymmetric manner. That is, the negative C-end was preferentially oriented to the side of the bilayer in which the channel was added. The orientation bias was shown to cause a macroscopic observable directional ionic transport as evidenced by conductance measurements. Moreover, this bias can pump $K^+$ across bilayers without the driving force of

  

a voltage. The peptides could be viewed as a new type of molecular diode, which may find potential application in the construction of novel molecular electronic devices.[30]


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**Keywords:** conductance measurements · directional transport · ion channels · potassium · rectifying behavior

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