JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
pubs.acs.org/JACS
Communication

Foldamer-Based Potassium Channels with High Ion Selectivity and Transport Activity

Shuaiwei Qi, Chenyang Zhang, Hao Yu, Jing Zhang, Tengfei Yan, Ze Lin, Bing Yang, and Zeyuan Dong*

Cite This: J. Am. Chem. Soc. 2021, 143, 3284−3288
Read Online
ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Small molecules that independently perform natural channel-like functions show greatly potential in the treatment of human diseases. Taking advantage of aromatic helical scaffolds, we develop a kind of foldamer-based ion channels with lumen size varying from 3.8 to 2.3 Å through a sequence substitution strategy. Our results clearly elucidate the importance of channel size in ion transport selectivity in molecular detail, eventually leading to the discoveries of the best artificial K^+ channel by far and a rare sodium-preferential channel as well. High K^+ selectivity and transport activity together make foldamers promising in therapeutic applications.

Ion transmembrane movement is essential in the central nervous system and even human diseases. Normal physiological processes require a selective permeation pathway for ions to cross cellular membranes.[1−4] Ion channel proteins play a central role in such a selective ion permeation pathway.[5−7] However, ion channel dysfunctions generally lead to ion channelopathies. Recently, several seminal reports have demonstrated that small-molecule-mediated transmembrane translocation is able to compensate for missing protein transport functions, which gives such small molecules great potential in the treatment of human diseases.[8,9] Therefore, small molecules that independently perform natural channel-like functions are eagerly pursued for possible therapeutic use.

Nature encodes a series of elegant and sophisticated signature sequences to govern ionic flow across cellular membranes.[10] For instance, the selectivity filter of potassium channels is a highly conservative amino acid sequence containing four groups of close-spaced K^+ binding sites.[11,12] The characteristic sequences occurring inside potassium channels give rise to extremely high selectivity for transporting K^+ ions but not smaller Na^+ ions. Because K^+ channels are responsible for the transmembrane conduction of K^+ ions, their absence or dysfunctional physiological actions can cause severe diseases, like hyperkalemia.[13] Therefore, the development of K^+ channel mimics provides an alternative pathway to compensate for the functional deficiency. Inspired by the characteristic structure of K^+ channels with a pore aperture of 2.8 Å, chemists have endeavored to build numerous synthetic nanopores for mimicry recently.[14,15] However, the relationships between channel size and ion selectivity are still underexplored, partly because it would not only be hard to engineer protein structures of natural ion channels but also be extremely difficult to construct a pore matching ion size in synthetic molecule-level channel systems.[16−24]

Recently, we reported a class of artificial ion channels based on pore-forming aromatic helical scaffolds with the concept of a pyridine-oxadiazole helical codon, in which helically folded structures are stabilized by electrostatic repulsion of heteroatoms in molecular backbones.[25−27] This type of hollow helical structures is conformationally stable and easily predictable. Importantly, they can be tuned in lumen size via sequence substitution by using various monomers. Preliminary studies have demonstrated that helically folded polymers perform significant transport functions as unimolecular transmembrane ion channels.[26] This exciting result inspired us to design specific channels, to simulate a type of highly selective synthetic K^+ channels (K^+/Na^+ selectivity ratio of 22) based on helical foldamers composed of pyridine-oxadiazole repeating units.[25] Moreover, Zeng et al. very recently reported pyridine-oxadiazole-based helical polymer channels with a high K^+/Na^+ selectivity ratio of up to 16.[28] These significant findings suggested that helical foldamer channels are a promising research direction to manufacture artificial ion channels with potential in treating human diseases. According to the fundamental features of drug discovery, we empirically speculate that small-molecule foldamers that can autonomously fulfill the transport functions of K^+ channels might be better for therapeutic utilization than polymer-based models. Furthermore, improvements in both the ion selectivity and transport activity of foldamer channels are prerequisites for the discovery of potential drug precursors at present. For such a purpose, we precisely govern the lumen size to investigate the transport properties of foldamer channels. By a sequence substitution strategy in which the helical sequence is changed by using different monomers, three pore-containing helical foldamers, **1**−**3**, with slightly varied lumen sizes were obtained, and importantly, all of them present excellent ion-selective transmembrane transport functions (Figure 1b). Very excitingly, tiny but definite variations in lumen size have a remarkable influence on the ion selectivity of foldamer

Received: November 30, 2020
Published: March 1, 2021

https://dx.doi.org/10.1021/jacs.0c12128
J. Am. Chem. Soc. 2021, 143, 3284−3288

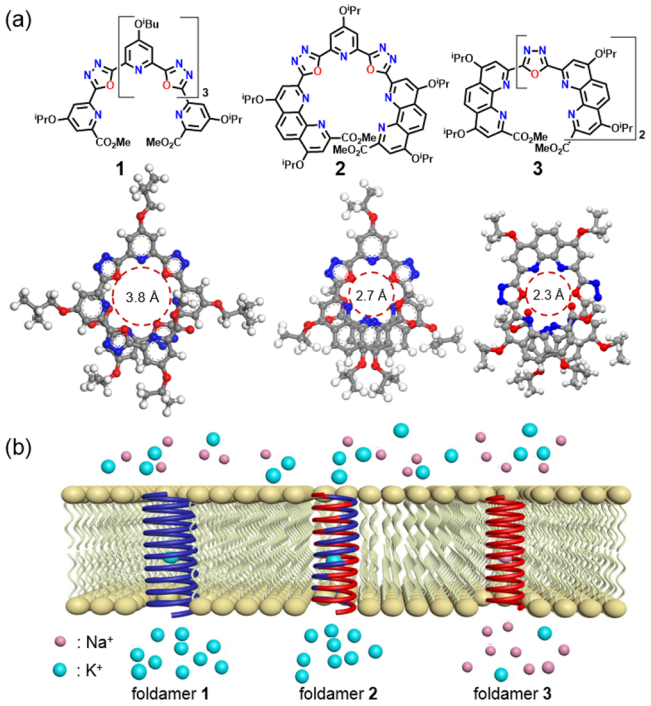

Figure 1. (a) Molecular structures of foldamers **1**−**3** and energy-minimized conformations of **1** and **2** as well as crystal structure of **3** with pore diameters of 3.8, 2.7, and 2.3 Å, respectively. (b) Schematic representation on ion channels formed by self-assembly of foldamers **1**−**3** with high K^+ or Na^+ selectivity.

channels. We eventually find a small-molecule surrogate for K^+ channels, **2**, that shows the highest K^+ selectivity and transport activity among reported channel mimics.[29−32]

Foldamers **1**−**3** were straightforwardly synthesized and fully characterized (see the Supporting Information). We previously reported a type of artificial K^+ channels with O and N atoms fully populated inside the channel surface, such as in foldamer **1**.[25] To improve the properties of artificial channels, we thus retain the landmark characteristic structure in the channel and attempt to adjust the lumen size. When monomer pyridine is substituted by *o*-phenanthroline, foldamer **3** can be obtained. The substitution of helical sequence achieves the channel aperture convergence, leading to a smaller lumen size in foldamer **3** than in **1**. At the same time, foldamer **2** was rationally designed by combining *o*-phenanthroline and pyridine monomers into helical sequences which can regulate the lumen size between those of **1** and **3**. The crystal structure of **3** confirms the helical conformation and gives a lumen size of 2.3 Å (Figure 1a). The simulated structure of **3** is similar to its crystal structure (Figure S21), suggesting that such aromatic foldamers are structurally predictable. Correspondingly, the lumen sizes from the energy-minimized structures (Figure 1a) of foldamers **1** and **2** can reasonably be 3.8 and 2.7 Å, respectively. These small-molecule foldamers **1**−**3** are thus established with gradually constricted electron-rich pores. Moreover, their respective stacking channel structures, formed by self-assembly of foldamers **1**−**3**, were confirmed by atomic force microscopy (Figure S22).[25]

Next, the ion transport properties of foldamers **1**−**3** were investigated via vesicle-based kinetic techniques.[33,34] As shown in Figure 2a−c, a significant increase in normalized fluorescence intensity is observed, indicating that foldamers **1**−**3** can remarkably accelerate transmembrane movement of

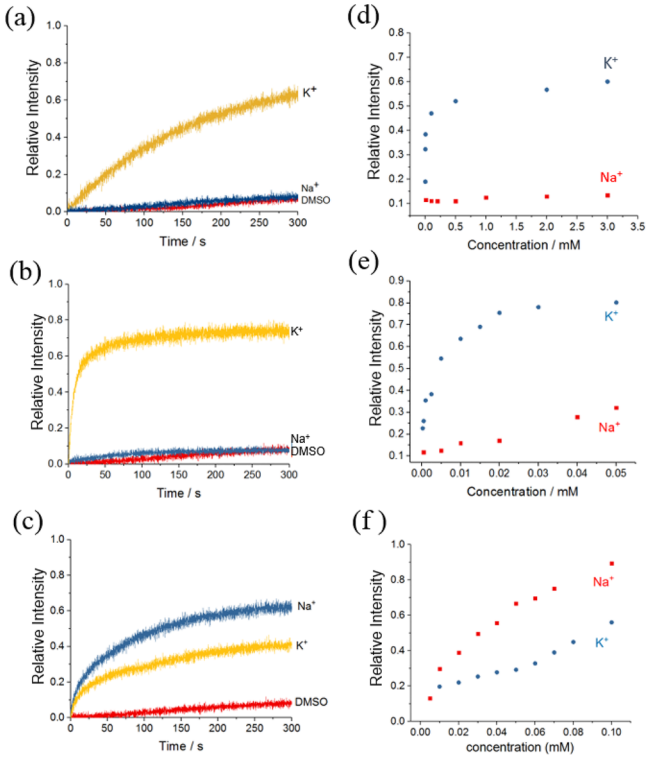

Figure 2. Normalized K^+ and Na^+ transport activities of foldamer **1** at 3 mM (a), foldamer **2** at 0.01 mM (b), and foldamer **3** at 0.05 mM (c). Hill analysis of the dose−response profile of K^+ and Na^+ transport for **1** (d), **2** (e), and **3** (f).

ions. As can be seen in Figure 2d−f, the concentration−activity curves are nonlinearly fitted via the Hill equation, and the half-maximal effective concentration (EC_{50}) can thus be quantitatively evaluated (Figures S1−S3). As listed in Table 1, EC_{50} values of foldamers **1**−**3** are 3.1 μM, 35 nM, and 280 nM, respectively. Notably, the transport activity of **2** is almost 2 orders of magnitude higher than that of **1**. Such a low EC_{50} value of 35 nM gives foldamer **2** great potential for therapeutic utilization.

Table 1. Quantitative Analyses on the Lumen Sizes, Transport Activities, and Ion Selectivity Ratios of Foldamers 1−3

	foldamer		
	1	**2**	**3**
lumen size[a]	3.8 Å	2.7 Å	2.3 Å
EC_{50}	3.1 μM	35 nM	280 nM
$S_{K/Na}$	4.0−22.7	10.2−32.6	−
$S_{Na/K}$	−	−	4.2−5.2

[a]Defined by van der Waals surfaces.

The ion selectivity of foldamers was investigated by vesicle-based kinetic analysis. As can be seen in Figure 2a, foldamer **1** displays high transport selectivity for K^+ over Na^+. Very strikingly, foldamer **2** shows extremely high K^+ transport selectivity. As can be observed in Figure 2a,b, the relative fluorescence intensity of **2** reaches saturation in a shorter time and at a much lower concentration than that of **1**. Based on pseudo-first-order initial transport rates,[35,36] the K^+/Na^+ selectivity ratio ($S_{K/Na}$) of **2** was calculated to be 10.2−32.6

https://dx.doi.org/10.1021/jacs.0c12128
J. Am. Chem. Soc. 2021, 143, 3284−3288

at different concentrations, which is higher than that of **1** ($S_{K/Na}$ = 4.0−22.7). It is reasonable that the $S_{K/Na}$ value will be given as a range with the concentration change of foldamers.[31,35] Notably, the $S_{K/Na}$ value of **2** can reach up to 32. This result demonstrates that **2**, with a smaller cavity (2.7 Å), shows a higher $S_{K/Na}$ than **1** (3.8 Å), indicating that the lumen size does play a crucial role in ion selectivity. This finding inspired us to explore what would happen to **3** with a smaller lumen size (2.3 Å). Unexpectedly, foldamer **3** preferentially transports Na^+ over K^+ (Figure 2c), and its selectivity ratio $S_{Na/K}$ was calculated to be 4.2−5.2 (Figures S10−S14). This result indicates that **3** is a rare Na^+-preferred channel. This observation further supports the importance of lumen size in ion selectivity. When the lumen size becomes smaller, e.g., 2.3 Å in **3**, the selectivity is prone to the smaller Na^+ (2.04 Å) over K^+ (2.76 Å), eventually resulting in an opposite Na^+-preference phenomenon. It should be emphasized that the lumen size of the selectivity filter (4.6 Å) of natural Na^+ channels is bigger than that of the selectivity filter (2.8 Å) of natural K^+ channels.[12,37,38] These results imply that artificial sodium channel **3** performs a dehydrating Na^+ transport, differing from the natural sodium channels, of which the ion transport mechanism involves water molecules. Additionally, the critical transformation lumen size of K^+ and Na^+ selectivity is in the range of 2.3−2.7 Å. We envisage that the ion selectivity of channels originates from their ion affinity. To prove it, K^+ and Na^+ binding behaviors of foldamers **1**−**3** were studied by fluorescence titrations. As observed (Figure S16), the fluorescence intensity of both **1** and **2** largely changes with the addition of K^+ ions but varies slightly for Na^+ ions, suggesting that foldamers **1** and **2** can specifically recognize K^+ over Na^+, and particularly that the K^+ binding capacity of **2** is higher than that of **1** (Figures S16a,b and S17a). Surprisingly, the fluorescence intensity of foldamer **3** changes a little bit upon addition of K^+, but it is significantly decreased in the presence of Na^+ (Figures S16c and S17b). Although attempt failed to measure the association constants of foldamers **2** and **3** due to strong self-assembly features, the results from fluorescence titrations suggest that ion binding capacities of foldamers **1**−**3** are well consistent with their ion transport selectivity. These observations demonstrate that ion selectivity can be essentially controlled by tuning the lumen size.

Single-channel electrophysiological experiments were carried out to further prove the ion transport selectivity of foldamers **1**−**3**. As shown in Figure 3a, all foldamers **1**−**3** exhibit clear square signals, demonstrating the features of ion channels rather than transporters in lipid membranes (Figures S18 and S19).[39,40] Moreover, asymmetric bilayer lipid membrane (BLM) experiments confirm that permeability ratios for K^+/Na^+ ($P_{K/Na}$) of **2** calculated from the reverse potential (V_{rev} = −75 mV, Figure 3b) by using the Goldman−Hodgkin−Katz equation[40] (Figure S20) are high, up to 18.2. The result from asymmetric BLM experiments is in accord with that of the LUVs assay (Table 1). To our knowledge, foldamer **2** is a K^+ channel with the highest $S_{K/Na}$ so far.[16−19,21,41,42]

To underpin the K^+ selectivity of **2**, membrane polarization experiments were carried out by using safranine O as a membrane-potential-sensitive probe (Figure 3c).[43,44] In the presence of the K^+-selective transporter valinomycin, the fluorescence intensity of safranine O increases and eventually reaches a relatively stable state. With the addition of foldamer **2**, the fluorescence intensity of safranine O can increase to almost as high as that of valinomycin. This result demonstrates

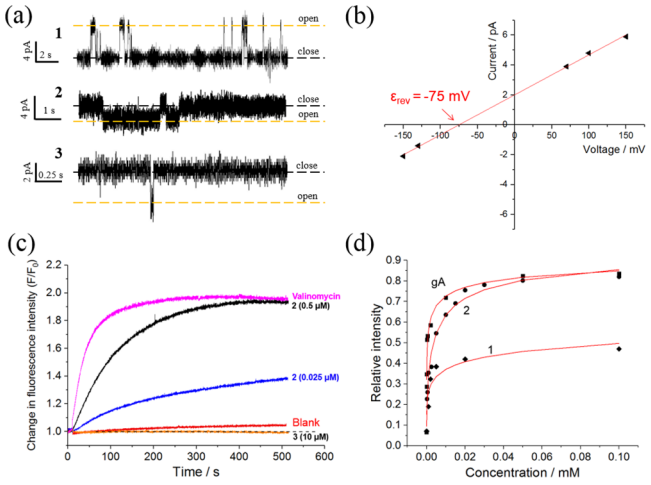

Figure 3. (a) Single-channel current traces recorded for **1**−**3**. (b) Current−voltage relationship from asymmetric BLM experiments of **2**. (c) Time-dependent fluorescence intensities of safranine O in the presence of **2**, **3**, and valinomycin (0.5 nM). (d) Hill analyses on concentration-dependent K^+ transport of **1**, **2**, and gA.

that foldamer **2** and valinomycin have very similar capacities for membrane polarization generated by K^+ efflux. In contrast, sodium-selective channel **3** is unable to polarize the membrane under identical conditions, which allows Na^+ influx and thus prevents safranine O from moving into the hydrophobic area of the membrane.[44] This observation provides unambiguous evidence regarding the high K^+ selectivity of foldamer **2**.

To assess the transport activity of potassium channel **2**, a native channel, gramicidin A (gA), with an approximately 4.0 Å, highly conducting pore, is chosen for comparison. As can be seen in Figure 3d, the transport activity of gA is high (Figure S15), and its EC_{50} can be calculated to be 5.0 nM. Very excitingly, the small-molecule foldamer **2** also exhibits high transport activity (EC_{50} = 35 nM), with an almost 100-fold enhancement in transport activity compared to foldamer **1**. These results indicate that the transport activity of the self-assembling channel from foldamer **2** is close to that of native gA.

In conclusion, we have developed a kind of foldamer-based ion channels with gradually constricted electron-rich pores by means of a sequence substitution strategy. Our results clearly elucidate the importance of channel size in ion transport selectivity, eventually leading to the discoveries of the best artificial K^+ channel by far as well as a rare sodium-preferential channel. High K^+ selectivity and transport activity together make foldamers greatly promising in therapeutic application. Our study not only provides a clear understanding on the relationship between channel size and ion transport selectivity but also shows a successful proof-of-concept demonstration to create synthetic ion channels by a size-guided approach. To facilitate future medical applications of synthetic channels, rational design of foldamers for better ion channels with more specific selectivity is under investigation.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.0c12128.

Materials and methods, synthetic and experimental details of compounds, crystal data of **3**, experimental

https://dx.doi.org/10.1021/jacs.0c12128
J. Am. Chem. Soc. 2021, 143, 3284−3288

details of LUV preparation, cation transport, and planar lipid bilayer experiments, including Figures S1−S69 (PDF)

Accession Codes

CCDC 2046777 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

AUTHOR INFORMATION

Corresponding Author

Zeyuan Dong − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China;* orcid.org/0000-0001-6509-9724; Email: zdong@jlu.edu.cn

Authors

Shuaiwei Qi − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China*

Chenyang Zhang − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China*

Hao Yu − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China*

Jing Zhang − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China*

Tengfei Yan − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China*

Ze Lin − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China*

Bing Yang − *State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China;* orcid.org/0000-0003-4827-0926

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.0c12128

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of China (21722403, 22071078, and 21574054) and the Program for JLU Science and Technology Innovative Research Team (JLUSTIRT) (2019TD-36).

REFERENCES

(1) Hille, B. *Ionic channels of excitable membranes*; Sinauer Assoc.: Sunderland, MA, 1984.

(2) Fraústo da Silva, J. J. R.; Williams, R.J.P. *The biological chemistry of the elements*; Clarendon Press: Oxford, 1991.

(3) Hodgkin, A. L.; Huxley, A. F. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **1952**, *117* (4), 500−544.

(4) Møller, J. *Biomembranes: Structural and functional aspects*, Vol. 2; Shinitzky, M., Ed.; VCH-Verlagsgesellschaft: Weinheim, 1994.

(5) Catterall, W. A. From Ionic Currents to Molecular Mechanisms: The Structure and Function of Voltage-Gated Sodium Channels. *Neuron* **2000**, *26* (1), 13−25.

(6) Emery, E. C.; Luiz, A. P.; Wood, J. N. Nav1.7 and other voltage-gated sodium channels as drug targets for pain relief. *Expert Opin. Ther. Targets* **2016**, *20* (8), 975−983.

(7) MacKinnon, R. Potassium Channels and the Atomic Basis of Selective Ion Condution (Nobel Lecture). *Angew. Chem., Int. Ed.* **2004**, *43* (33), 4265−4277.

(8) Grillo, A. S.; SantaMaria, A. M.; Kafina, M. D.; Cioffi, A. G.; Huston, N. C.; Han, M.; Seo, Y. A.; Yien, Y. Y.; Nardone, C.; Menon, A. V.; Fan, J.; Svoboda, D. C.; Anderson, J. B.; Hong, J. D.; Nicolau, B. G.; Subedi, K.; Gewirth, A. A.; Wessling-Resnick, M.; Kim, J.; Paw, B. H.; Burke, M. D. Restored iron transport by a small molecule promotes absorption and hemoglobinization in animals. *Science* **2017**, *356* (6338), 608−616.

(9) Muraglia, K. A.; Chorghade, R. S.; Kim, B. R.; Tang, X. X.; Shah, V. S.; Grillo, A. S.; Daniels, P. N.; Cioffi, A. G.; Karp, P. H.; Zhu, L.; Welsh, M. J.; Burke, M. D. Small-molecule ion channels increase host defences in cystic fibrosis airway epithelia. *Nature* **2019**, *567* (7748), 405−408.

(10) Zakon, H. H. Adaptive evolution of voltage-gated sodium channels: the first 800 million years. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 10619−10625.

(11) Zhou, Y.; Morais-Cabral, J. H.; Kaufman, A.; MacKinnon, R. Chemistry of ion coordination and hydration revealed by a K^+ channel−Fab complex at 2.0 Å resolution. *Nature* **2001**, *414* (6859), 43−48.

(12) Doyle, D. A.; Cabral, J. M.; Pfuetzner, R. A.; Kuo, A.; Gulbis, J. M.; Cohen, S. L.; Chait, B. T.; MacKinnon, R. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **1998**, *280* (5360), 69−77.

(13) Cummings, C. C.; McIvor, M. E. Fluoride-induced hyperkalemia: The role of Ca^{2+}-dependent K^+ channels. *AM. J. EMERG. MED* **1988**, *6* (1), 1−3.

(14) Howorka, S. Building membrane nanopores. *Nat. Nanotechnol.* **2017**, *12* (7), 619−630.

(15) Zheng, S. P.; Huang, L. B.; Sun, Z. H.; Barboiu, M. Self-Assembled Artificial Ion-Channels Toward Natural Selection of Functions. *Angew. Chem., Int. Ed.* **2021**, *60* (2), 566−597.

(16) Tanaka, Y.; Kobuke, Y.; Sokabe, M. A Non-Peptidic Ion Channel with K^+ Selectivity. *Angew. Chem., Int. Ed. Engl.* **1995**, *34* (6), 693−694.

(17) Sun, Z. H.; Barboiu, M.; Legrand, Y.-M.; Petit, E.; Rotaru, A. Highly Selective Artificial Cholesteryl Crown Ether K^+-Channels. *Angew. Chem., Int. Ed.* **2015**, *54* (48), 14473−14477.

(18) Otis, F.; Auger, M.; Voyer, N. Exploiting Peptide Nanostructures to Construct Functional Artificial Ion Channels. *Acc. Chem. Res.* **2013**, *46* (12), 2934−2943.

(19) Gokel, G. W.; Negin, S. Synthetic Ion Channels: From Pores to Biological Applications. *Acc. Chem. Res.* **2013**, *46* (12), 2824−2833.

(20) Benke, B. P.; Aich, P.; Kim, Y.; Kim, K. L.; Rohman, M. R.; Hong, S.; Hwang, I.-C.; Lee, E. H.; Roh, J. H.; Kim, K. Iodide-Selective Synthetic Ion Channels Based on Shape-Persistent Organic Cages. *J. Am. Chem. Soc.* **2017**, *139* (22), 7432−7435.

(21) Xin, P.; Kong, H.; Sun, Y.; Zhao, L.; Fang, H.; Zhu, H.; Jiang, T.; Guo, J.; Zhang, Q.; Dong, W.; Chen, C.-P. Artificial K^+ Channels Formed by Pillararene-Cyclodextrin Hybrid Molecules: Tuning Cation Selectivity and Generating Membrane Potential. *Angew. Chem., Int. Ed.* **2019**, *58* (9), 2779−2784.

(22) Shen, J.; Fan, J.; Ye, R.; Li, N.; Mu, Y.; Zeng, H. Polypyridine-Based Helical Amide Foldamer Channels: Rapid Transport of Water and Protons with High Ion Rejection. *Angew. Chem., Int. Ed.* **2020**, *59* (32), 13328−13334.

(23) Shen, J.; Ye, R.; Romanies, A.; Roy, A.; Chen, F.; Ren, C.; Liu, Z.; Zeng, H. Aquafoldmer-Based Aquaporin-like Synthetic Water Channel. *J. Am. Chem. Soc.* **2020**, *142* (22), 10050−10058.

(24) Gong, B. Artificial water channels: inspiration, progress, and challenges. *Faraday Discuss.* **2018**, *209* (0), 415−427.

(25) Lang, C.; Deng, X.; Yang, F.; Yang, B.; Wang, W.; Qi, S.; Zhang, X.; Zhang, C.; Dong, Z.; Liu, J. Highly Selective Artificial Potassium Ion Channels Constructed from Pore-Containing Helical Oligomers. *Angew. Chem., Int. Ed.* **2017**, *56* (41), 12668−12671.
(26) Lang, C.; Li, W.; Dong, Z.; Zhang, X.; Yang, F.; Yang, B.; Deng, X.; Zhang, C.; Xu, J.; Liu, J. Biomimetic Transmembrane Channels with High Stability and Transporting Efficiency from Helically Folded Macromolecules. *Angew. Chem., Int. Ed.* **2016**, *55* (33), 9723−9727.
(27) Zhang, C.; Tian, J.; Qi, S.; Yang, B.; Dong, Z. Highly Efficient Exclusion of Alkali Metal Ions via Electrostatic Repulsion Inside Positively Charged Channels. *Nano Lett.* **2020**, *20* (5), 3627−3632.
(28) Chen, F.; Shen, J.; Li, N.; Roy, A.; Ye, R.; Ren, C.; Zeng, H. Pyridine/Oxadiazole-Based Helical Foldamer Ion Channels with Exceptionally High K^+/Na^+ Selectivity. *Angew. Chem., Int. Ed.* **2020**, *59* (4), 1440−1444.
(29) Matile, S.; Vargas Jentzsch, A.; Montenegro, J.; Fin, A. Recent synthetic transport systems. *Chem. Soc. Rev.* **2011**, *40* (5), 2453−2474.
(30) Tedesco, M. M.; Ghebremariam, B.; Sakai, N.; Matile, S. Modeling the Selectivity of Potassium Channels with Synthetic Ligand-Assembled π Slides. *Angew. Chem., Int. Ed.* **1999**, *38* (4), 540−543.
(31) Gilles, A.; Barboiu, M. Highly Selective Artificial K^+ Channels: An Example of Selectivity-Induced Transmembrane Potential. *J. Am. Chem. Soc.* **2016**, *138* (1), 426−432.
(32) Su, G.; Zhang, M.; Si, W.; Li, Z.-T.; Hou, J.-L. Directional Potassium Transport through a Unimolecular Peptide Channel. *Angew. Chem., Int. Ed.* **2016**, *55* (47), 14678−14682.
(33) Kano, K.; Fendler, J. H. Pyranine as a sensitive pH probe for liposome interiors and surfaces. pH gradients across phospholipid vesicles. *Biochim. Biophys. Acta, Biomembr.* **1978**, *509* (2), 289−299.
(34) Sakai, N.; Matile, S. The determination of the ion selectivity of synthetic ion channels and pores in vesicles. *J. Phys. Org. Chem.* **2006**, *19*, 452−460.
(35) Li, Y. H.; Zheng, S.; Legrand, Y. M.; Gilles, A.; Van der Lee, A.; Barboiu, M. Structure-Driven Selection of Adaptive Transmembrane Na^+ Carriers or K^+ Channels. *Angew. Chem., Int. Ed.* **2018**, *57* (33), 10520−10524.
(36) Feng, W.; Sun, Z.; Zhang, Y.; Legrand, Y.; Petit, E.; Su, C.; Barboiu, M. Bis-15-crown-5-ether-pillar [5]arene K^+-Responsive Channels. *Org. Lett.* **2017**, *19* (6), 1438−1441.
(37) Chung, S.-H.; Kuyucak, S. Ion channels: recent progress and prospects. *Eur. Biophys. J.* **2002**, *31* (4), 283−293.
(38) Payandeh, J.; Scheuer, T.; Zheng, N.; Catterall, W. A. The crystal structure of a voltage-gated sodium channel. *Nature* **2011**, *475* (7356), 353−358.
(39) Matile, S.; Sakai, N. *The Characterization of Synthetic Ion Channels and Pores*; John Wiley & Sons, Ltd, 2007.
(40) Fyles, T. M. Synthetic ion channels in bilayer membranes. *Chem. Soc. Rev.* **2007**, *36* (2), 335−347.
(41) Hall, C. D.; Kirkovits, G. J.; Hall, A. C. Towards a redox-active artificial ion channel. *Chem. Commun.* **1999**, *18*, 1897−1898.
(42) Ren, C.; Shen, J.; Zeng, H. Combinatorial Evolution of Fast-Conducting Highly Selective K^+-Channels via Modularly Tunable Directional Assembly of Crown Ethers. *J. Am. Chem. Soc.* **2017**, *139* (36), 12338−12341.
(43) Winum, J.-Y.; Matile, S. Rigid Push−Pull Oligo(p-Phenylene) Rods: Depolarization of Bilayer Membranes with Negative Membrane Potential. *J. Am. Chem. Soc.* **1999**, *121* (34), 7961−7962.
(44) Woolley, G. A.; Kapral, M. K.; Deber, C. M. Potential-sensitive membrane association of a fluorescent dye. *FEBS Lett.* **1987**, *224* (2), 337−342.

https://dx.doi.org/10.1021/jacs.0c12128
J. Am. Chem. Soc. 2021, 143, 3284−3288