VOLUME 38 NUMBER 2
ACCOUNTS of CHEMICAL RESEARCH®
FEBRUARY 2005

Rigid-Rod Molecules in Biomembrane Models: From Hydrogen-Bonded Chains to Synthetic Multifunctional Pores

NAOMI SAKAI, JIRI MAREDA, AND STEFAN MATILE*
Department of Organic Chemistry, University of Geneva, Geneva, Switzerland

Received October 19, 2004

ABSTRACT
Synthetic ion channels and pores formed by rigid-rod molecules are summarized. This includes work on hydrogen-bonded chains installed along membrane-spanning rigid-rod scaffolds to transport protons. As a second topic, programmed assembly of *p*-septiphenyls with terminal iminodiacetate-copper complexes for potassium transport by cation−π interactions is described. The third topic concerns rigid push−pull rods as fluorescent α-helix mimics to probe the importance of dipole−potential interactions for voltage gating, both on the functional and the structural level. Topic number four deals with *p*-octiphenyl staves as key scaffolds for the synthesis of rigid-rod β-barrel pores. The description of internal and external design strategies for these rigid-rod β-barrels covers a rich collection of pH-, pM-, voltage-, ligand-, and enzyme-gated synthetic multifunctional pores that can act as hosts, sensors, and catalysts. As far as practical applications are concerned, the possibility to detect chemical reactions with synthetic multifunctional pores appears most attractive. Recent molecular mechanics simulations are presented as a valuable approach to insights on the elusive suprastructures of multifunctional pores made from rigid rods.

Introduction

Rigid-rod molecules are rod-shaped molecules that cannot be bent and compressed.[1] Somehow the antithesis of foldamers,[2,3] these sturdy rods have attracted much interest in the materials sciences.[1,4] They remain, however, largely ignored in the life sciences. We therefore wondered whether rigid-rod molecules could serve in the life science as well as they do in the materials sciences to address challenging topics of current concern.

Still far from being fully explored, the chemical diversity of rigid-rod molecules is remarkable.[1] To initiate the bioorganic chemistry of rigid-rod molecules, we selected *p*-oligophenyls as model rods because they are nonplanar and fluorescent. The former characteristic is of use to preorganize supramolecular architecture, whereas the latter provides intrinsic fluorescent probes. Moreover, the synthesis of *p*-oligophenyls is comparably straightforward. Even relatively complex rods such as the *p*-octiphenyl-peptide conjugate **1**, designed to form voltage-gated anion channels (see below), can be prepared from commercial biphenyl **2** in overall 15 steps (Figure 1).[5] Other functional *p*-oligophenyl rods mentioned in this Account were prepared similarly.

This Account focuses on rigid-rod molecules that function as synthetic ion channels and pores in lipid bilayer membranes. Hydrophobic matching of rod length and bilayer thickness was found to be the key for the construction of functional transmembrane architecture from membrane-spanning *p*-septiphenyl (30 Å) and *p*-octiphenyl scaffolds (34 Å).[6] After a primer on synthetic ion channels and pores, we will summarize how trans-

Naomi Sakai received her B.S. from Keio University (1987) and her Ph.D. from Tokushima Bunri University (1994) for work on natural products. After a postdocoral stay in the group of Professor Koji Nakanishi at Columbia University (1994−1996), she has been working on the bioorganic chemistry of rigid-rod molecules, first at Georgetown University (1996−1999) and then at the University of Geneva (1999-present).

Jiri Mareda studied chemistry at the University of Geneva where he obtained the Diploma (1975) and his Ph.D. (1980) under the supervision of Professors C. W. Jefford and U. Burger for studies and computer modeling of hypervalent cationic species. Subsequently, he was a postdoctoral research associate in Professor Ken Houk's group (1980−1983) where he conducted theoretical studies of factors controlling the regioselectivity and stereoselectivity in cycloadditions. He then joined the Department of Organic Chemistry at the University of Geneva, where his current research interests are in the field of computational investigations of mechanisms of organic reactions and modeling of reactive intermediates and rigid-rod molecules.

Stefan Matile received both Diploma (1989) and Ph.D. from the University of Zurich (1994) for contributions to the bioorganic chemistry of porphyrins studied in the group of Wolf Woggon. After postdoctoral work on porphyrin circular dichroism in the group of Koji Nakanishi at Columbia University (1994−1996), he focused on the bioorganic chemistry of rigid-rod molecules, first at Georgetown University (1996−1999) and then at the University of Geneva.

10.1021/ar0400802 CCC: $30.25
Published on Web 01/25/2005

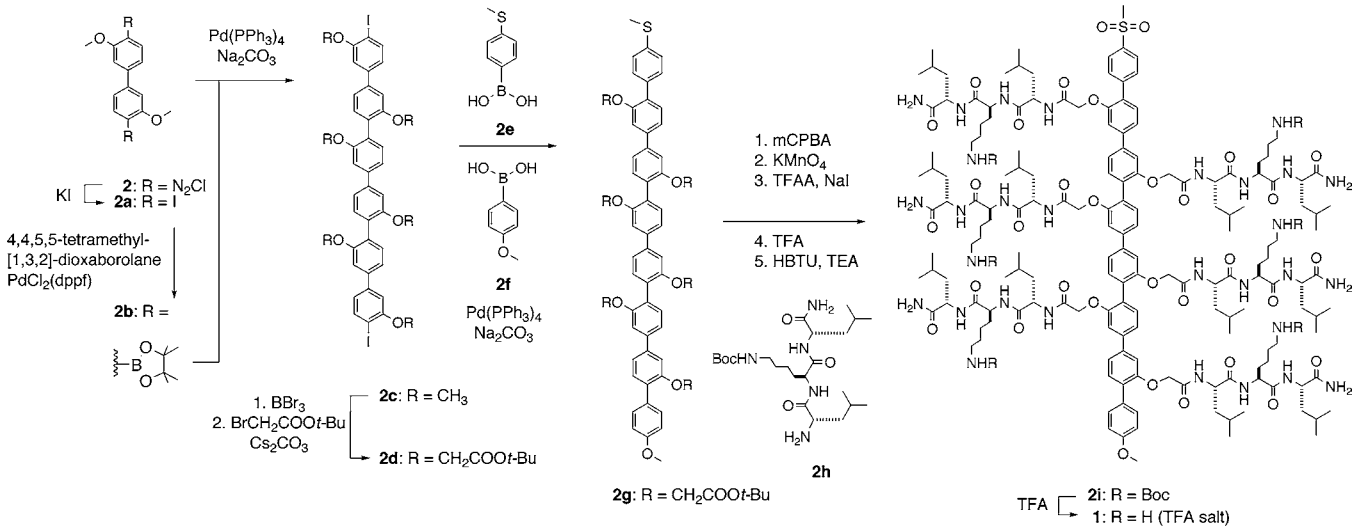


FIGURE 1. Representative multistep synthesis of a functional rigid-rod molecule designed to form voltage-gated anion channels in biomembrane models (see Figure 5F).

membrane rigid-rod scaffolds can be used to address current topics in this field.

Synthetic Ion Channels and Pores

The emerging field of synthetic ion channels and pores builds on the ability to make molecules, that is, synthetic organic chemistry, to probe pertinent themes in biomembrane research, that is, biochemistry, biophysics, molecular biology, and so on. This subject is reviewed regularly.[7] Without going into details, some key terms and topics are briefly reiterated in a highly simplified manner.

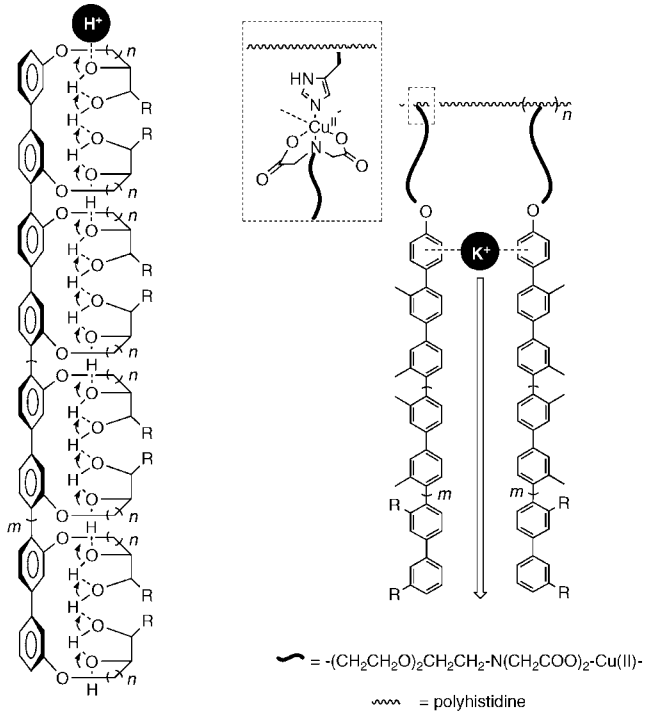
Herein, the terms ion channel and pore are used for transmembrane transport of inorganics and organics, respectively. The term "synthetic ion channels and pores" is applied for compounds that have abiotic scaffolds (i.e., scaffolds that are not found in biological ion channels and pores). Different from synthetic carriers, they do not shuttle across the lipid bilayer membrane during action and may, therefore, be inactive in phase-transfer experiments in bulk liquid membranes. Different from detergents (including "micellar pores," "toroidal pores" and "carpets"), they do not modify the suprastructure of bilayer membranes, a drastic change that can be detected by several methods. In conductance measurements using planar bilayers, these three, eventually overlapping[7] mechanisms can be differentiated in general. The stochastic appearance and disappearance of currents in planar bilayers is often considered as the key characteristic of ion channels and pores. The conductance represents the rate of ion transport; thus it can be indicative of the minimal inner diameter of synthetic ion channels ($g \approx 1-100$ pS) and pores ($g \approx 0.1-5$ nS). Similarly dependent on experimental conditions, the lifetime of single ion channels and pores can reveal poor ($\tau < 1$ ms), intermediate ($\tau \approx 10-100$ ms), or high kinetic stability ($\tau > 1$ s).

Significant characteristics of synthetic ion channels and pores accessible in planar and[8] spherical bilayers (i.e., vesicles or liposomes) using electrochemical and spectroscopic methods, respectively, include inner diameter, thermodynamic stability, voltage dependence, pH dependence, ion selectivity, ligand gating, and blockage. Specifically, inner diameters can be estimated by size-exclusion experiments. The thermodynamic stability of active supramolecules is indicated by Hill coefficients $n > 1$ (i.e., dependence on the nth power of monomers) for endergonic self-assembly and $n \leq 1$ for exergonic self-assembly.[9] The Hill coefficient can be indicative of the number of monomers per active pore. The terms "ohmic" and "nonohmic" describe ion channels and pores that obey and violate Ohm's law, that is, their transport abilities exhibit linear and exponential voltage dependence, respectively.[8] Weak ($z_g \approx 0-0.5$) and strong ($z_g > 0.5$) nonohmic behavior is characterized by the gating charge, z_g [with single channels and pores, nonohmic behavior can be expressed as nonohmic open probabilities, lifetimes, or currents (rectification)].

Usually, synthetic ion channels and pores with anionic interior select cations, and vice versa. The recognition of specific cations and anions is reflected in selectivity sequences such as the Eisenman topologies.[10] The subtle interconnection of selectivity and blockage on one hand and ligand-gated opening of synthetic ion channels on the other can be considered as molecular recognition events and described by dissociation constants (K_D), Hill coefficients (n), and, for blockage in polarized membranes, Woodhull distances (δ) from entrance to active site. The designation of apparent dissociation constants K_D from Hill analysis as effective concentrations (EC_{50}) for 50% pore opening by ligands and inhibitory concentrations (IC_{50}) for 50% pore closing may describe the experimental situation more accurately.[11]

Ion Selectivity

Polyols **3**−**5** were designed to give a hydrogen-bonded chain along their rigid-rod scaffold for transmembrane transport of protons by a two-step hop-and-turn mechanism that attracts much attention in bioenergetics (Figure 2).[12−14] Proton selectivity was measured in vesicles



	R	*n*	*m*	*activity*
3	-H	1	1	++
4	-H	3	1	+++
5	-*n*Pr	1	1	+++
6	-H	1	0	+

	R	*m*	*activity*
7	-OEt	1	+
8	-Me	1	-
9	-OAd	1	-
10	-Me	2	-

	A	D	*recognition*
11	-SO_2Me	-SMe	+
12	-SMe	-SMe	-
13	-$SO_2CH_2CH_2NH_3^+$	-SMe	+
14	-SO_2Me	-$SCH_2CH_2NH_3^+$	-
15	-SMe	-$SCH_2CH_2NH_3^+$	-

FIGURE 2. Rigid-rod molecules with hydrogen-bonded chains for selective proton transport (**3**−**6**), as ligand-gated π-slides for potassium transport (**7**), and as rigid push−pull rods with axial dipoles for the recognition of polarized membranes (**11**, **13**, and **14**). OAd = 2(1-adamantyl)ethyleneoxy.

with internal pH-sensitive probes using proton and potassium carriers to specifically accelerate one of the H^+/M^+ antiport processes, which was disfavored by rods **3**−**5**. In the presence of potassium carriers, polyols **3**−**5** mediated rapid proton gradient collapse. The rate increase obtained with the potassium carrier valinomycin suggested that the proton selectivity of rod **3** was $H^+/K^+ > 16$. Enantiomeric rods **3**, either all-*S* or all-*R*, had identical activity and proton selectivity. Compared to rod **3**, the increased hydrophobicity of rods **4** and **5** resulted in increased activity without losses in proton selectivity. Rods **3**−**5** did not mediate the transmembrane transport of large anions such as CF. Structural studies by fluorescence depth quenching (FDQ) confirmed transmembrane orientation of hydrophobically matching *p*-octiphenyl fluorophores **3**−**5**, whereas the shorter *p*-sexiphenyl **6** with poor activity accumulated near the membrane−water interface.

Rigid-rod π-slide **7** was studied at a time when cation−π interactions were under discussion as a possible molecular origin of selectivity of biological potassium channels.[10,15] The prediction that potassium recognition by arene arrays with fixed arene−arene distances would produce the biologically less relevant Eisenman VI ($K^+ > Na^+ \gg Rb^+$) selectivity sequence has been supported experimentally with resorcin[4]arene-based synthetic channels. Rigid-rod π-slide **7** was expected to probe the predicted access to the biologically relevant Eisenman IV ($K^+ > Rb^+ \gg Na^+$) using flexible arene arrays. Complex **7** is composed of *p*-septiphenyls with steric bulk R near one terminus to prevent spontaneous self-assembly. The other terminus contains an iminodiacetate group. Mediated by Cu(II), multivalent polyhistidine ligands were expected to assemble the *p*-septiphenyls into flexible arene arrays. The final complex **7** was expected to comprise seven noncovalent calix[*n*]arene-like macrocycles on top of each other to mediate selective potassium hopping across a bilayer membrane.

Cation selectivity of complex **7** was determined in vesicles with internal pH-sensitive probes by measuring the rate of pH-gradient collapse as a function of H^+/M^+ antiport. A biomimetic Eisenman IV topology ($K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$) and biomimetic blockage with tetraethylammonium cations were found. Without polyhistidine, the cation selectivity changed to a less pronounced Eisenman III topology. Polyhistidine-induced *p*-septiphenyl assembly of complex **7** was confirmed by CD spectroscopy. Replacement of the ethoxy groups R by smaller (**8**) and larger groups (**9**) resulted in inactivity

because of tightly packed, cation-impermeable *p*-septiphenyl assemblies and poor affinity for bilayer membranes, respectively. Poor membrane affinity of asymmetric (**9**) and mismatched rods (**10**) was confirmed by FDQ. Amusingly, it turned out that topologically mismatched rods such as **9** and **10** did not interact well with phospholipid vesicles because they preferred to self-assemble into rigid-rod vesicles by themselves.[16]

The crystal structure of a biological potassium channel has ruled out cation−π interactions as the origin of cation selectivity but confirmed cation−π interactions as the origin of blockage by tetraethylammonium cations.[17] The biomimetic selectivity topologies obtained with complex **7** may, therefore, be considered as experimental support for the validity of a chemically attractive concept that ultimately turned out not to apply in nature.

Voltage Gating

Rigid push−pull rods[18] are α-helix mimics[19] that may help to clarify contributions of axial rod dipoles to the voltage gating of ion channels and pores.[20] Push−pull rods are at least partially conjugated rigid rods with an electron-donating π donor D at one terminus and a π acceptor A at the other terminus. In rigid push−pull rod **11**, a sulfide is used as π donor and a sulfone as π acceptor.[21] Different from the macrodipole of α-helices, the axial dipole of rigid push−pull rods does not depend on experimental conditions. For example, the α-helical dipole of polyglutamate disappears with the α-helix itself at high pH, whereas the axial dipole of rigid push−pull rod **11** does not depend on pH. The axial dipole of rigid push−pull rods can, however, be permanently removed without global structural changes, whereas dipole-free α-helical rods do not exist. For example, reduction of the sulfone acceptor in push−pull rod **11** yields the symmetric rigid rod **12** of nearly identical global structure but without axial dipole. Finally, and most importantly for structural studies by FDQ, push−pull *p*-oligophenyls are fluorescent, whereas α-helices as such are not.

Push−pull rods **11**, **13**, and **14** and the "push−push" controls **12** and **15** were equipped with ion-conducting azacrown modules. Moreover, terminal ammonium cations were introduced to dissect the importance of dipole−potential interactions and phase transfer of fixed charges for voltage gating.[21] Voltage-gated ion channel formation was determined in planar bilayers and elaborated in doubly labeled neutral (EYPC) and anionic (egg yolk phosphatidylglycerol) spherical bilayers (SUVs) with inside negative membrane potentials, an internal pH-sensitive dye and an external potential-sensitive dye.

In unpolarized, neutral, and anionic SUVs, cationic ionophores **13**−**15** were more active than neutral rods **11** and **12**. These differences were attributed to an improved water solubility of cationic rods instead of specific bilayer membrane recognition. Support for the formation of unimolecular ion channels was obtained from linear dependence of activity on monomer concentration, inability to mediate transport of large organic molecules, and small single-channel conductances.

Increased activity of neutral push−pull rod **11** compared to the dipole-free analogue **12** in polarized vesicles confirmed model studies on the importance of dipole−potential interactions for the recognition of polarized membranes obtained with *p*-octiphenyl rods carrying cyano acceptors and methoxy donors.[18] Conductance experiments in planar bilayers indicated that single-channel rectification contributes to the high activity of push−pull rods in polarized membranes (Supporting Information of ref 21).

The situation becomes more interesting with push−pull rod **13** with an ammonium cation near the negative end of the axial dipole and isomer **14** with an ammonium cation near the positive end of the axial dipole. With inside-negative polarization, push−pull rod **13** can adopt transmembrane orientation with constructive dipole−potential interactions without the need to phase transfer ammonium cations across the membrane. With push−pull rod **14**, the same favorable transmembrane orientation is accessible only if the ammonium cation is transferred across the bilayer. Increased activity of the push−pull rod **13** compared to isomer **14** in vesicles with inside-negative membrane potential suggested that hindered phase transfer of ammonium cations across the bilayer is more important for voltage gating than overall rod asymmetry. Taking advantage of the *p*-octiphenyl as an intrinsic fluorescent probe, it was possible to confirm this interpretation on the structural level by FDQ.

Since push−pull rod **13** simulates the dipole−charge distribution of the bee toxin melittin and isomer **14** that of natural antibiotics, such as magainin, these studies helped to clarify the question of why the latter avoid mammalian membranes. Namely, voltage-gated pore formation in lipid bilayers with moderate polarization is hindered by unfavorable phase transfer of fixed charges across the bilayer. The recent crystal structure of biological potassium channels has attracted renewed attention to voltage gating by phase transfer of multiple fixed guanidinium cations.[22] Different from voltage gating with antibacterial pores and rigid push−pull rods **13** and **14**, however, phase transfer of oligoarginines seems to occur via the reversible exchange of scavenged counteranions.[23]

Artificial β-Barrels

The introduction of rigid-rod molecules provided synthetic access to a ubiquitous protein tertiary structure with unique structural as well as functional plasticity, that is, β-barrels.[24−28] Rigid-rod β-barrels are the only artificial β-barrels known today. To synthesize rigid-rod β-barrels, the N-termini of short peptide strands **16** were coupled with the carboxylic acids along the scaffold of *p*-octiphenyl **17** (Figure 3). Cylindrical self-assembly (or programmed assembly) of the resulting *p*-octiphenyl−peptide conjugates **18** yielded artificial β-barrels such as **19**−**24** with rigid-rod "staves" that are held together by β-sheet "hoops." The biphenyl torsions, that is, the nonplanarity

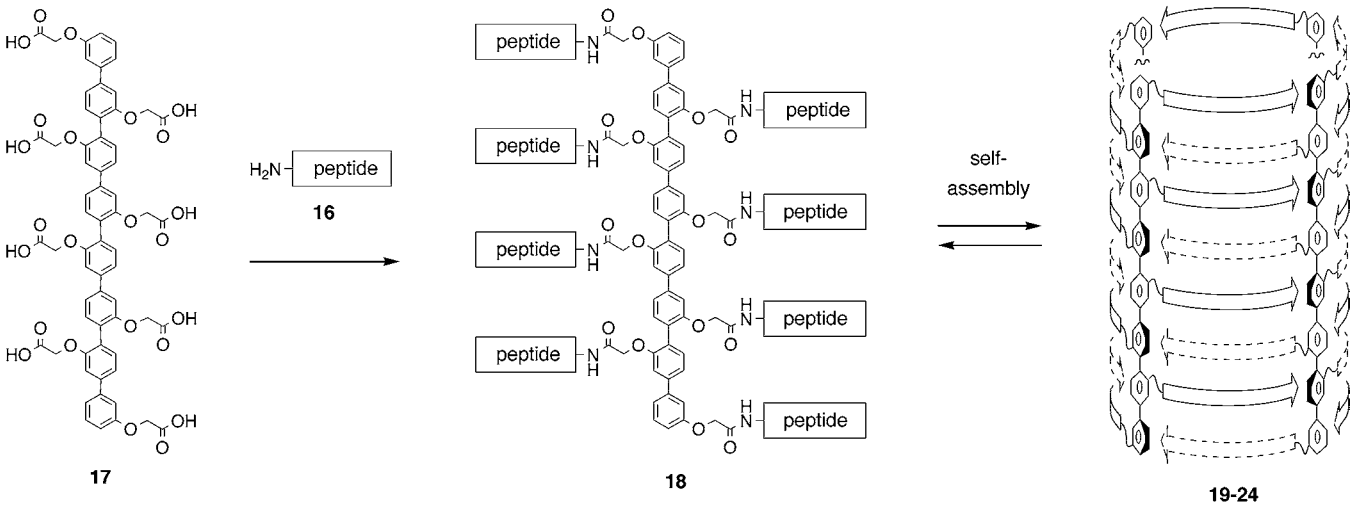
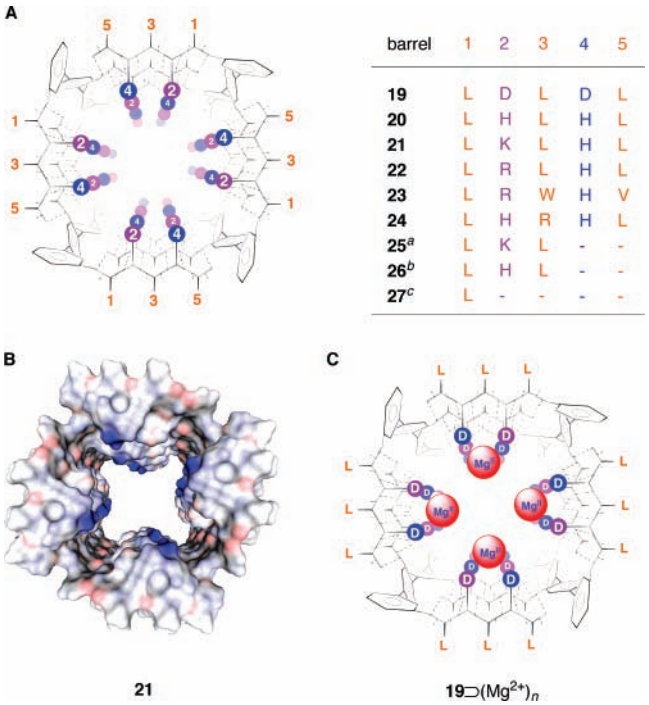


FIGURE 3. Synthesis of rigid-rod β-barrels **19**−**24** from *p*-octiphenyl **17** (arrows between *p*-octiphenyl staves indicate 8-stranded β-sheets (N → C). For axial views of rigid-rod β-barrels and peptide sequences, see Figure 4).

of rigid *p*-octiphenyl rods, are thought to be crucial to hinder the linear self-assembly of the planar β-sheets into precipitating supramolecular polymers (i.e., β-fibrils) and to yield the cylindrical oligomeric self-assembly (i.e., β-barrels).

Rigid-rod β-barrels appear as discrete objects in atomic force microscopy (AFM) images.[25] They are clearly distinct from supramolecular "nanotubes", for example, macrocycles that self-assemble into supramolecular polymers of "infinite" length. The number of rods per barrel was estimated by methods such as Hill analysis of concentration dependence[9,28] or size-exclusion chromatography,[26,27] inner barrel diameters by Hille analysis of single-pore conductances,[9,28] and size-exclusion experiments.[27,28] The stability of these barrel-stave supramolecules depends on many parameters such as *p*-octiphenyl length,[26] peptide length, and peptide sequence.[9] Because of the alternating orientation of neighboring amino acid residues of peptides in β-sheet conformation, both inner and outer surface of rigid-rod β-barrels can be functionalized in a flexible, precise, and straightforward manner (Figure 4A). Positioning of hydrophilic amino acid residues at the outer and hydrophobic amino acid residues at the inner barrel surface gives water-soluble barrels that can host—like biological β-barrels of the lipocalin family—hydrophobically matching guests such as carotenoids.[27] Reversal of cylindrical amphiphilicity, that is, external hydrophobicity and internal hydrophilicity, gives barrels that can form multifunctional pores in bilayer membranes, that is, **19**−**27** (Figure 4A). Highlights on this topic are discussed in the following; a more detailed discussion can be found in a recent comprehensive review.[28]



barrel	1	2	3	4	5
19	L	D	L	D	L
20	L	H	L	H	L
21	L	K	L	H	L
22	L	R	L	H	L
23	L	R	W	H	V
24	L	H	R	H	L
25[a]	L	K	L	-	-
26[b]	L	H	L	-	-
27[c]	L	-	-	-	-

FIGURE 4. (A) Synthetic multifunctional pores formed by rigid-rod β-barrels **19**−**27** with (B) molecular model of **21** (MMFF94, lysine, 50% protonation; histidine, 0% protonation) and (C) magnesium complex of **19**: (a) rigid-rod β_3-barrel **25** is a hexamer; (b) rigid-rod β_3-barrel **26** is a tetramer with a structure analogous to that of β_5-barrels **19**−**24**; (c) rigid-rod β_1-barrel **27** is a dimer. Peptide backbones in eight-stranded β-sheets of tetrameric rigid-rod β_5-barrels **19**−**24** are given as solid lines; hydrogen bonds are given as dotted lines; external amino acid residues are dark on white; internal ones white on dark (single-letter abbreviations); compare with side view in Figure 3. We caution that all shown suprastructures are in part speculative simplifications that are, however, consistent with experimental data and molecular models. Panel B was reproduced with permission from ref 9. Copyright 2004 American Chemical Society.

Synthetic Multifunctional Pores

Internal pore design was of interest to introduce functional groups that can interact with ions and molecules that move across the internal space of rigid-rod β-barrels from one side of a bilayer membrane to the other. The axial view on the molecular model of pore **21** visualizes how the lysine−histidine (KH) dyads functionalize the internal "nanospace" of rigid-rod β-barrels ($d \approx 16$ Å, Figure 4B).[9]

The interaction of synthetic multifunctional pores with inorganic ions provided access to counterion-mediated

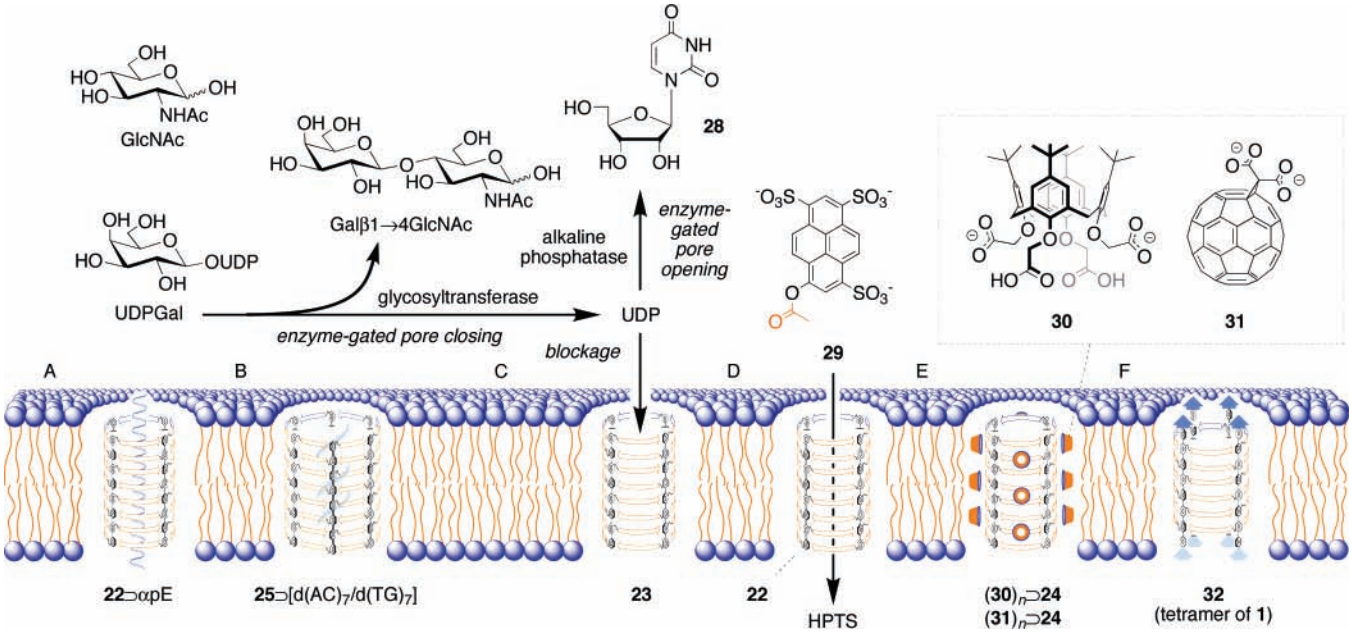


FIGURE 5. Examples of synthetic multifunctional pores as hosts (A, B), sensors (C), and catalysts (D), as well as for ligand (E) and voltage gating (F). Panel A shows recognition of α-helices (poly(L-glutamic acid)) by pore host **22**. Panel B shows recognition of DNA duplexes $[d(AC)_7/d(TG)_7]$ by pore host **25**. In panel C, production of UDP blockers allows observation of reactions catalyzed by glycosyltransferases as enzyme-gated closing of pore **23**, whereas conversion of UDP blockers by phosphatases can be "seen" as the complementary enzyme-gated pore opening. In panel D, synthetic catalytic pore **22** hydrolyzes ester **29** during its translocation across the membrane. In panel E, pore **24** with external and internal active sites opens in response to chemical stimulation by ligands such as calixarene **30** and fullerene **31** (compare Figure 6). Panel F shows parallel self-assembly of rigid-rod push−pull β-barrel pore **32** in polarized membranes. See Figures 1, 3, and 4 for pore structures.

function. For example, internal complexation of Mg^{2+} deleted the cation selectivity of polyanionic pores **19** (EC_{50} = 5.3 mM). It produced metallopores with internal "biomimetic"[29] aspartate−Mg^{2+} complexes that were permeable for organic anions as big as CF (Figure 4C). The pH-gated cation selectivity of polycationic pores **22** and **23** with internal arginines (R) was shown to originate from internal charge inversion by scavenged inorganic phosphates. The implications of the concept of counteranion-mediated function for research on arginine-rich cell-penetrating peptides (CPPs), voltage-gating paddles of ion channels,[22] and organic synthesis are being currently investigated.[23]

Most rigid-rod β-barrel pores with internal acids or bases exhibit substantial pH gating. The bell-shaped, often sharp pH profiles with maxima at pH ≥ 7 (**25**), pH = 6 (**19**), pH = 5 (**21**), and pH ≤ 5 (**20**, **22**, and **23**) have been summarized in the internal charge repulsion (ICR) model: intermediate internal charge repulsion best stabilizes internal space. In other words, partial protonation/deprotonation of internal bases/acids gives maximal activity.[30] Pore stability depends strongly on the peptide sequence. Pores **20**−**23**, for example, cover all variations of thermodynamic and kinetic stabilities: barrel **20** is stable and labile, **22** is stable and inert, **23** is unstable and labile, and most attractive for practical applications, **21** is unstable and inert.[9] All pores analyzed by FDQ were, however, confirmed to be true barrel-stave pores with membrane-spanning rigid-rod scaffolds. This was even true for the fragile pore **23**, demonstrating that preference for hydrophobic matching of rigid rods and membrane overruled the interfacial preference of tryptophan.

Because of the interaction of organic molecules with internal and external active sites, synthetic multifunctional pores can serve as supramolecular hosts, sensors, and catalysts. Highlights of a rich collection of guests, analytes, and substrates are brought together in Figure 5.

Molecular Recognition. Molecular recognition by synthetic multifunctional pores with internal active sites partially or fully hinders molecular translocation of non-recognized molecules across the same pore. Symmetric pore hosts that act by guest inclusion mediate, therefore, the selective translocation of their own guest. This concept of guest selectivity represents a translation of the mechanism of selectivity of biological potassium channels[17] to organic chemistry within synthetic multifunctional pores. It has been verified experimentally for pore **22** as host selective for HPTS as guest.[31]

Molecular recognition by pore hosts **19**−**25** has been studied in many variations.[28] The recognition of α-helical poly(L-glutamate) (αpE) by β_5-pore **22** in polarized membranes was attractive because it provided an example for α-helix recognition affected by remote control, that is, dipole−potential interactions. The apparent dissociation constant of the inclusion complex **22**⊃αpE was K_D = 150 nM in unpolarized and K_D = 13 nM in polarized membranes (Figure 5A).[28] Comparison with biological pores revealed remarkable molecular recognition by rigid-rod β-barrel pore hosts. Indeed, more than 10 000-times higher poly(L-glutamate) concentrations were needed to

block the pores formed by the bee toxin melittin, an α-helical peptide with monomer charge and tetrameric active structure comparable to that of pore **22**.[32]

The ion selectivity of synthetic multifunctional pores naturally influences molecular recognition. For instance, cation-selective pore **19** with internal aspartate arrays did not mediate CF efflux and could be blocked by polylysine or polyarginine, whereas metallopore **19**⊃$(Mg^{2+})_n$ with internal aspartate−Mg^{2+} complexes mediated CF efflux and could be blocked by many organic anions, such as thiamine pyrophosphate or ATP. Efficient blockage of pore host **23** by polyasparagine exemplified recognition of neutral guests with matching topology (IC_{50} = 37 nM).[33]

The internal space of the hexameric β_3-pore host **25** was sufficiently large to accommodate oligonucleotide duplexes in B-DNA conformation (Figure 5B). The internal space of the contracted, tetrameric β_3-pore **26** was, by contrast, too small for substantial guest inclusion. The dimeric mini-β_1-barrel **27** recognized and transported inorganic cations only.

Sensing Applications. Elaborating on synthetic hosts that sense the age of Scotch, Wiskur and Anslyn observed that "the inherent low selectivities of some synthetic systems can actually be an advantage for certain applications and that such applications can be attractive targets for supramolecular and analytical chemists to contemplate."[34] Modest but not negligible molecular recognition is indeed the key prerequisite to use synthetic multifunctional pores to detect chemical reactions (or to sense the corresponding substrates, products, and catalysts) in a general manner as widely applicable as chromatographic systems such as TLC, HPLC, or GC.[32,33,35]

This practical application of molecular recognition by synthetic multifunctional pores is exemplified here with glycosyltransferases (Figure 5C).[35] The development of assays for these enzymes and their substrates is a topic of current concern in several domains including organic (polysaccharide) synthesis. Galactosyltransferase converts UDPGal and GlcNAc into disaccharide Galβ1 → 4GlcNAc and UDP. Among the compounds involved in this reaction, pore **23** recognized the product UDP best. With increasing reaction time, an aliquot taken from the reaction mixture exhibited, therefore, increasing ability to block pore **23**. Such decreasing pore activity with increasing reaction time is readily detectable by several methods. Arguably the simplest method of detection follows the increase in emission during release of 5(6)-carboxyfluorescein (CF) from vesicles through open pores. Using pore **23** and CF vesicles, it was possible to "see" the action of galactosyltransferase with the "naked eye". Other enzymes detected with the same synthetic multifunctional pore include aldolases, apyrases, phosphatases, RNases, exonucleases, proteases, kinases, polymerases, heparinases, and hyaluronidases.[32,33,35]

As far as "smart" synthetic ion channels and pores are concerned, the response of synthetic multifunctional pore **23** to the action of galactosyltransferase can be considered as "enzyme-gated pore closing". The complementary "enzyme-gated reopening" of pore **23** was achieved with the conversion of the nucleotide blocker UDP into the nonrecognized nucleoside **28** by alkaline phosphatase.[35] In a very qualitative sense, such "enzyme gating" is reminiscent of the function of enzymes such as acetylcholine esterase to regulate ion channels such as the acetylcholine receptor.

Synthetic Catalytic Pores. The vision of a substrate entering a synthetic multifunctional pore from one side of the bilayer membrane and the product being ejected on the other side is amusing.[36] This combination of molecular recognition, translocation, and transformation was explored with histidine−arginine (HR) barrel **22** as a synthetic catalytic pore and pyrenetrisulfonate ester **29** as a model substrate (Figure 5D). A ground-state stabilization $\Delta G^{\circ}_{ES} = -29.6$ kJ/mol and a transition-state stabilization $\Delta G^{\circ}_{TS} = -52.2$ kJ/mol were indicative of substantial catalytic activity.

Inside-negative polarization of vesicles loaded with substrate **29** increased the catalytic activity of pore **22**, most pronounced for product release ($V_{max}^{polarized}/V_{max}^{unpolarized} = 1.3$). This finding was interpreted as proof-of-principle for remote control of catalysis within pores by electrostatic steering. Namely, the membrane potential possibly guided the intravesicular anionic substrate **29** into the catalytic pore **22** and the anionic product HPTS out into the extravesicular media. Work on substrate diversity (RNA, amides, carbamates), active-site modifications, and the introduction of cofactors is summarized elsewhere.[28]

Ligand Gating. All applications of synthetic multifunctional pores **19**−**23** and **25** as hosts, sensors, and catalysts operate on pore closing in response to molecular recognition. To construct pores that open in response to chemical stimulation, external rather than internal pore design was introduced.[11] A polycationic surface was created with external leucine−arginine−leucine (LRL) triads to weaken the interaction of barrel **24** with the hydrophobic core of lipid bilayers (Figure 4). Amphiphilic oligoargininophiles such as calix[4]arene **30** (EC_{50} = 440 nM) or fullerene **31** (EC_{50} = 10 nM) were then introduced as ligands that promote constructive barrel−membrane interactions to position open supramolecular pores (**30**)$_n$⊃**24** or (**31**)$_n$⊃**24** (Figure 5E). Note that the terms "ligand gating" and "open" refer here to function without structural implications.

Ligand-gated pores such as (**30**)$_n$⊃**24** still contained free internal active sites for noncompetitive blockage with matching guests such as α-helices. The formation of complex (**30**)$_n$⊃**24**⊃αpE was supported on the structural level by fluorescence resonance energy transfer experiments from the rigid-rod staves as intrinsic fluorescent donors to acceptors in the membrane, as well as by molecular mechanics simulations (Figure 6). One control experiment was particularly instructive because it demonstrated how precisely internal and external pore design are reflected in pore function: whereas calixarene **30** acted as ligand to open pore **24** with external arginine arrays, the same oligoargininophile **30** acted as a blocker of pore **22** with internal arginine arrays and did not change the activity of pore **20** without arginine arrays. Practical

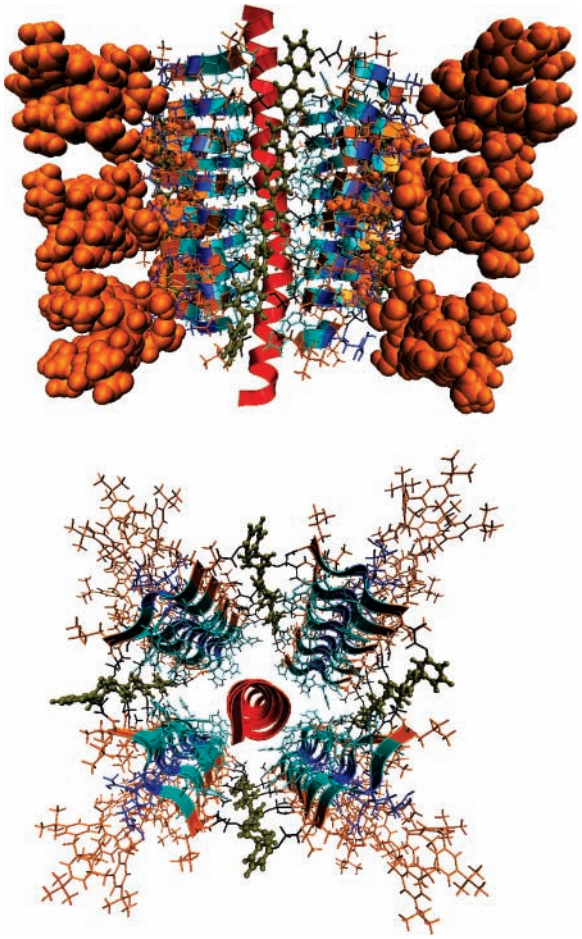


FIGURE 6. Side (top) and axial (bottom) views of the molecular model of complex (**30**)$_n$⊃**24**⊃αpE formed by pore **24** with external calixarene ligands **30** (gold) and internal α-helix blockers (αpE, red). *p*-Octiphenyl staves are highlighted in ball-and-stick; α-helix and β-sheets are depicted as ribbons. Adapted with permission from ref 11. Copyright 2004 American Chemical Society.

usefulness of ligand gating was indicated by experimental evidence for the continuous detection of chemical process with synthetic multifunctional pores **24**, a mode of detection that is problematic with synthetic multifunctional pores that operate by blockage.

Voltage Gating. Pores formed by rigid-rod β-barrels **19**−**23**, **25**, and **26** are ohmic because the amide dipoles are canceled out in the symmetric β-sheet architecture. However, earlier studies on rigid push−pull rods such as **11** as α-helix mimics (Figure 2) suggested that the introduction of push−pull staves may afford voltage-gated rigid-rod β-barrel pores. This concept was verified with *p*-octiphenyl-peptide conjugate **1** (Figure 1).[5] Voltage-gated ($z_g \approx 0.9$) formation of anion-selective push−pull tetramers **32** was confirmed in planar and spherical bilayers (Figure 5F, $P_{Cl^-}/P_{K^+} = 3.7$, $I^- \geq Cl^- \geq OAc^- \geq F^-$).[8] Different from voltage-gated pores formed by α-helix bundles, inert push−pull β-barrels **32**, once formed, exhibited ohmic behavior and closed only in response to sign inversion of the applied voltage. Also in contrast to α-helical pores, the role of the axial dipole for voltage gating could be corroborated by voltage-independent self-assembly of the corresponding push−push pores of identical global structure except for two terminal methoxy donors. As a final difference from α-helical pores, it was possible to determine the structural basis of voltage gating by FDQ studies in polarized vesicles using the *p*-octiphenyl stave as an intrinsic fluorescent probe.

Perspectives

The work described in this Account suggests that rigid-rod molecules can serve as attractive scaffolds to address challenging questions in bioorganic biomembrane chemistry and beyond. The scope of bioorganic chemistry à la baguette appears overwhelming. For instance, rigid-rod molecules beyond *p*-oligophenyls remain nearly untouched. Current efforts to improve on this situation focus on the design and synthesis of functional rigid-rod molecules that offer stronger interactions along their scaffold than the cation−π interactions used for potassium transport by **7** (Figure 2). As far as barrel-stave supramolecules are concerned, a broad variety of "hoops" other than β-sheets is conceivable. Current efforts in this area focus on the replacement of β-sheets in rigid-rod β-barrels by π-stacks to study and exploit electron-transfer processes in biomembrane models.[37] Even the limited suprastructural space of *p*-oligophenyl β-barrels leaves immense room for future excitement. Topics of interest include refined β-barrel architecture with, for example, more than one peptide sequence per stave,[38] the integration of artificial amino acids, and the creation of refined external and internal active sites for ligand gating and blockage.

We are grateful to all co-workers and collaborators named in the references for their contributions. This work was supported by the Swiss NSF (Grant 200020-101486 and National Research Program "Supramolecular Functional Materials" Grant 4047-057496).

Abbreviations

CD	circular dichroism
CF	5(6)-carboxyfluorescein
EC_{50}	effective ligand concentration for 50% pore opening
EYPC	egg yolk phosphatidylcholine
FDQ	fluorescence depth quenching
HPTS	8-hydroxy-1,3,6-pyrenetrisulfonate
IC_{50}	inhibitor concentration for 50% pore blockage
K_D	dissociation constant
LUV	large unilamellar vesicle
pE	poly(L-glutamate)
αpE	α-helical poly(L-glutamate)
SUV	small unilamellar vesicle

References

(1) Schwab, P. F. H.; Levin, M. D.; Michl, J. Molecular rods. 1. Simple axial rods. *Chem. Rev.* **1999**, *99*, 1863−1934.
(2) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. A field guide to foldamers. *Chem. Rev.* **2001**, *101*, 3893−4012.
(3) Gellman, S. H. Foldamers: a manifesto. *Acc. Chem. Res.* **1998**, *31*, 173−180.
(4) Müllen, K., Wegner, G., Eds. *Electronic Materials: The Oligomer Approach*; Wiley-VCH: Weinheim, Germany, 1998.
(5) Sakai, N.; Matile, S. Recognition of polarized bilayer membranes by *p*-oligophenyl ion channels: from push−pull rods to push−pull β-barrels. *J. Am. Chem. Soc.* **2002**, *124*, 1184−1185.
(6) Matile, S. Bioorganic chemistry à la baguette: studies on molecular recognition in biological systems using rigid-rod molecules. *Chem. Rec.* **2001**, *1*, 162−172.

(7) Matile, S.; Som, A.; Sordé, N. Recent synthetic ion channels and pores. *Tetrahedron* **2004**, *60*, 6405−6435.
(8) Sakai, N.; Houdebert, D.; Matile, S. Voltage-dependent formation of anion channels by synthetic rigid-rod push−pull β-barrels. *Chem.—Eur. J.* **2003**, *9*, 223−232.
(9) Litvinchuk, S.; Bollot, G.; Mareda, J.; Som, A.; Ronan, D.; Shah, M. R.; Perrottet, P.; Sakai, N.; Matile, S. Thermodynamic and kinetic stability of synthetic multifunctional rigid-rod β-barrel pores: evidence for supramolecular catalysis. *J. Am. Chem. Soc.* **2004**, *126*, 10067−10075.
(10) 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*, 540−543.
(11) Gorteau, V.; Perret, F.; Bollot, G.; Mareda, J.; Lazar, A. N.; Coleman, A. W.; Tran, D.-H.; Sakai, N.; Matile, S. Synthetic multifunctional pores with external and internal active sites for ligand gating and noncompetitive blockage. *J. Am. Chem. Soc.* **2004**, *126*, 13592−13593.
(12) Sakai, N.; Brennan, K. C.; Weiss, L. A.; Matile, S. Toward biomimetic ion channels formed by rigid-rod molecules: length-dependent ion transport activity of substituted oligo(*p*-phenylene)s. *J. Am. Chem. Soc.* **1997**, *119*, 8726−8727.
(13) Weiss, L. A.; Sakai, N.; Ghebremariam, B.; Ni, C.; Matile, S. Rigid rod-shaped polyols: functional nonpeptide models for transmembrane proton channels. *J. Am. Chem. Soc.* **1997**, *119*, 12142−12149.
(14) Ni, C.; Matile, S. Side-chain hydrophobicity controls the activity of proton channel forming rigid rod-shaped polyols. *Chem. Commun.* **1998**, 755−756.
(15) Kumpf, R. A.; Dougherty, D. A. A mechanism for ion selectivity in potassium channels: computational studies of cation-π interactions. *Science* **1993**, *261*, 1708−1710.
(16) Sidorov, V.; Douglas, T.; Dzenukov, S. M.; Abdallah, D.; Ghebremariam, B.; Roepe, P. D.; Matile, S. Self-assembled single-chain oligo(*p*-phenylene) amphiphiles: reversed micelles, vesicles, and gels. *Chem. Commun.* **1999**, 1429−1430.
(17) Doyle, D. A.; Morais Cabral, J.; 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*, 69−77.
(18) 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*, 7961−7962.
(19) Orner, B. P.; Ernst, J. T.; Hamilton, A. D. Toward proteomimetics: terphenyl derivatives as structural and functional mimics of extended regions of an α-helix. *J. Am. Chem. Soc.* **2001**, *123*, 5382−5383.
(20) Sakai, N.; Matile, S. On the electrostatics of cell membrane recognition: from natural antibiotics to rigid push−pull rods. *Chem.—Eur. J.* **2000**, *6*, 1731−1737.
(21) Sakai, N.; Gerard, D.; Matile, S. Electrostatics of cell membrane recognition: structure and activity of neutral and cationic rigid push−pull rods in isoelectric, anionic, and polarized lipid bilayer membranes. *J. Am. Chem. Soc.* **2001**, *123*, 2517−2524.
(22) Jiang, Y.; Ruta, V.; Chen, J.; Lee, A.; MacKinnon, R. The principle of gating charge movement in a voltage-dependent K^+ channel. *Nature* **2003**, *423*, 42−48.
(23) Sakai, N.; Matile, S. Anion-mediated transfer of polyarginine across liquid and bilayer membranes. *J. Am. Chem. Soc.* **2003**, *125*, 14348−14356.
(24) Sakai, N.; Majumdar, N.; Matile, S. Self-assembled rigid-rod ionophores. *J. Am. Chem. Soc.* **1999**, *121*, 4294−4295.
(25) Das, G.; Ouali, L.; Adrian, M.; Baumeister, B.; Wilkinson, K. J.; Matile, S. β-Fibrillogenesis from rigid-rod β-barrels: hierarchical preorganization beyond microns. *Angew. Chem., Int. Ed.* **2001**, *40*, 4657−4661.
(26) Das, G.; Matile, S. Topological diversity of artificial β-barrels in water. *Chirality* **2001**, *13*, 170−176.
(27) Baumeister, B.; Matile, S. Rigid-rod β-barrels as lipocalin models: probing confined space by carotenoid encapsulation. *Chem.—Eur. J.* **2000**, *6*, 1739−1749.
(28) Sakai, N.; Matile, S. Synthetic multifunctional pores: lessons from rigid-rod β-barrels. *Chem. Commun.* **2003**, 2514−2523.
(29) Tarshis, L. C.; Proteau, P. J.; Kellogg, B. A.; Sacchettini, J. C.; Poulter, C. D. Regulation of product chain length by isoprenyl diphosphate synthases. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 15018−15023.
(30) Baumeister, B.; Som, A.; Das, G.; Sakai, N.; Vilbois, F.; Gerard, D.; Shahi, S. P.; Matile, S. On the importance of intermediate internal charge repulsion for the synthesis of multifunctional pores. *Helv. Chim. Acta* **2002**, *85*, 2740−2753.
(31) Ronan, D.; Sordé, N.; Matile, S. Blocker efflux through blocked pores. *J. Phys. Org. Chem.* **2004**, *17*, 978−982.
(32) Sordé, N.; Matile, S. On sensitivity and selectivity of synthetic multifunctional pores as enzyme sensors: discrimination between ATP and ADP and comparison with biological pores. *Biopolymers* **2004**, *76*, 55−65.
(33) Sordé, N.; Das, G.; Matile, S. Enzyme screening with synthetic multifunctional pores: focus on biopolymers. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11964−11969.
(34) Wiskur, S. L.; Anslyn, E. V. Using a synthetic receptor to create an optical-sensing ensemble for a class of analytes: a colorimetric assay for the aging of Scotch. *J. Am. Chem. Soc.* **2001**, *123*, 10109−10110.
(35) Das, G.; Talukdar, P.; Matile, S. Fluorometric detection of enzyme activity with synthetic supramolecular pores. *Science* **2002**, *298*, 1600−1602.
(36) Sakai, N.; Sordé, N.; Matile, S. Synthetic catalytic pores. *J. Am. Chem. Soc.* **2003**, *125*, 7776−7777.
(37) Gust, D.; Moore, T. A.; Moore, A. L. Mimicking photosynthetic solar energy transduction. *Acc. Chem. Res.* **2001**, *34*, 40−48.
(38) Ronan, D.; Baudry, Y.; Jeannerat, D.; Matile, S. Synthesis of {323}-*p*-octiphenyls: orthogonal functionalization along a rigid-rod scaffold for refined supramolecular architecture. *Org. Lett.* **2004**, *6*, 885−887.

AR0400802