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Artificial β-Barrels

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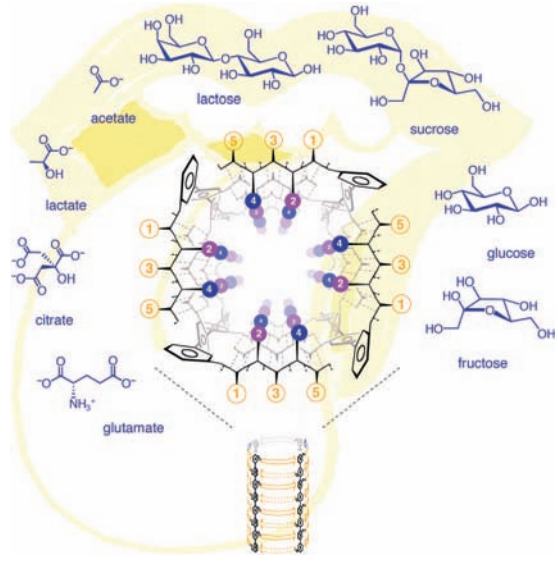
CONSPECTUS

In biology, β-barrels, cylindrically rolled-up forms of β-sheets, are ubiquitous structural motifs within various binding proteins, pores, and enzymes. This biological multifunctionality suggested that synthetic artificial β-barrels would provide access to many different functions beyond the limitations of peptide chemistry. Unlike the relative ease of formation of synthetic (de novo) α-helix bundles, the synthesis of artificial β-barrels remains a challenge. To bypass the folding problems involved, we have employed "unfoldable" rigid-rod scaffolds as privileged staves (staves are the wood strips that form the sides of macroscopic barrels); the resulting barrel-stave supramolecules exhibit their expected multifunctionality. Several "rigid rod" β-barrels that act as receptors, ion channels, pores, catalysts, and sensors have been prepared and studied. The most recent topic of interest concerns the use of artificial β-barrels as multicomponent sensors ("artificial tongues") in complex analyte matrices. For multicomponent sensing, we have designed artificial β-barrels to form pores that can open and close in response to chemical stimulation within lipid bilayers. With use of fluorogenic vesicles, changes in pore activity are readily detectable with either the naked eye or multiwell screening formats. The varying responsiveness to substrates and products makes synthetic pores versatile detectors of chemical reactions, of the activity of the enzymes that catalyze these reactions, and of their inhibitors. In sensing applications, the "perfect" selectivity of enzymes is exploited to generate analyte-specific signals. Reactive signal amplifiers are then covalently linked to the products of enzymatic signal generation to enhance their pore blockage potency. With the help of signal generators and amplifiers, we have employed artificial β-barrel pores to sense sweet (sucrose, lactose), sour (acetate, lactate, citrate), and umami ("deliciousness", glutamate) components in various food samples. This breakthrough naturally led us to design and synthesize refined pores for advanced sensing applications. We have developed methods to build guest-binding sites not only at internal and external barrel surfaces but also near the core or near the periphery of the pore. Further refinements include the introduction of asymmetric staves for voltage gating and anchoring of the pore at the membrane−water interface.



Introduction

Rigid-rod molecules are exceptionally simple, linear, unbendable, and uncompressible compounds.[1−3] Much appreciated in the materials sciences,[1,3] these sturdy rods do not exist in biology. This situation seems regrettable because, after all, rigid-rod molecules can be considered as the ultimate answer to all folding problems. They simply do not fold. No wonder, then, that the introduction of rigid-rod molecules as privileged scaffolds in multifunctional nanoarchitectures was particularly fruitful when applied to one of the more persistent folding problems in peptide and protein chemistry, that is, the synthesis of artificial β-barrels from scratch.[2,3]

β-Barrels are one of the two pure protein tertiary structures. They are ubiquitous in biology, acting as binding proteins, enzymes, and pores in

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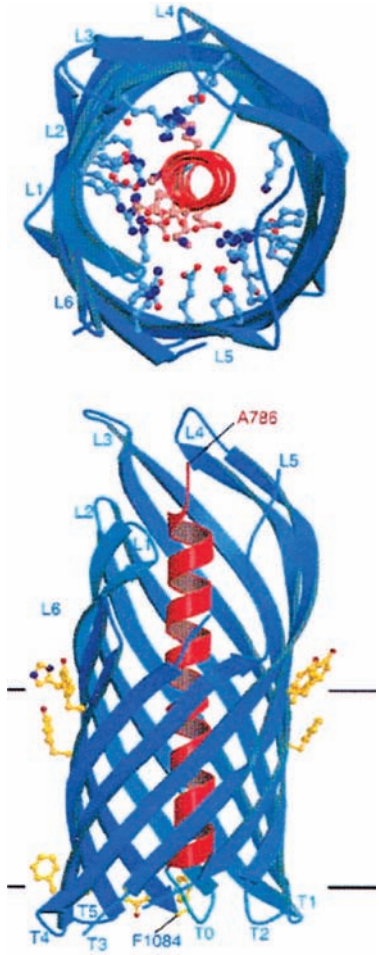

FIGURE 1. The crystal structure of the translocator domain of the bacterial autotransporter NalP from *Neisseria meningitidis* shows a 12-stranded β-barrel pore that is filled with an α-helix blocker. Reprinted by permission from Macmillan Publishers Ltd.: *EMBO J.* (ref 4), Copyright 2004.

many variations. Examples include streptavidin; β-crustacyanin and other lipocalins; the green fluorescent protein (GFP) from jellyfish; superoxide dismutase (SOD), phospholipases, the triose phosphate isomerase (TIM) barrel, enolases, aldolases, and many other enzymes; the porins, α-hemolysin, and autotransporters (Figure 1).[4] This intrinsic and unique functional plasticity of the β-barrel scaffold suggests that synthetic access to artificial β-barrels could lead to new functions beyond the limitations of peptide chemistry. The synthesis of the complementary α-helix bundles is easily achieved,[5] and important progress has been made with the synthesis of β-sheets.[6–8] However, synthetic access to artificial β-barrels remains a challenge. The main problem is the ease with which β-structures misfold into insoluble supramolecular polymers, rather than to assemble into soluble, well-defined supramolecular oligomers such as β-barrels.[9] Best known as a cause of neurodegenerative diseases such as Alzheimer's, the formation of heterogeneous mixtures of supramolecular polymers such as β-fibrils, ribbons, tubes, and so on is by now accepted as the universal expression of misfolding. Variations of this theme include numerous tubular polymer fibrils from modules as small as dipeptides,[10–12] cyclic peptides,[13–15] and oligoureas.[15]

To promote the cylindrical self-assembly of facially amphiphilic β-sheets into supramolecular oligomers of homogeneous molecular weight and shape and exactly defined length, that is, artificial β-barrels, we considered nonplanar rigid-rod scaffolds as shape-persistent turns (Figure 2).[2] This enforced deviation from the β-sheet plane could at the same time help to suppress the competing linear self-assembly into intractable, amyloid-like supramolecular polymers. Rigid *p*-oligophenyl rods were selected for this purpose. The biphenyl torsions along the rigid-rod scaffold introduce the desired nonplanarity. Moreover, the phenyl repeat distance of the scaffold perfectly matches the β-sheet repeat distance of ~5 Å. The dynamic axial chirality as well as intense blue fluorescence makes *p*-oligophenyls additionally valuable as intrinsic probes for structural studies by circular dichroism and fluorescence spectroscopy, respectively.

To synthesize β-barrels of the general structure **1**, short peptide strands were attached to each phenyl ring of the *p*-oligophenyl stave. The synthesized monomers had the general structure **2**. Self-assembly of these monomers was expected to occur by interdigitation of peptides from adjacent staves. This process should afford antiparallel β-sheets. Assisted by facial amphiphilicity of the β-sheets, the rigid-rod turns then should roll the planar β-sheets into the cylindrical β-barrels. In the final barrel, the side chains of N- and C-terminal amino acid residues should be located at the outer barrel surface to avoid internal overcrowding next to the rigid-rod turns. The opposing orientations of adjacent amino acid residues in β-sheets should then place the following amino acid side chains at the inner surface, the next one outside, etc., exactly as in biological β-barrels. This binary β-sheet repeat offered a very simple approach to precisely choose, control, and vary the chemical and physical properties at both inner and outer barrel surfaces. The same β-sheet topology translated into three dimensions contributes to the functional adaptability of biological β-barrels. All peptide sequences that have been attached to date to various rigid *p*-oligophenyl scaffolds to create rigid-rod β-barrels for various purposes are listed in Table 1.

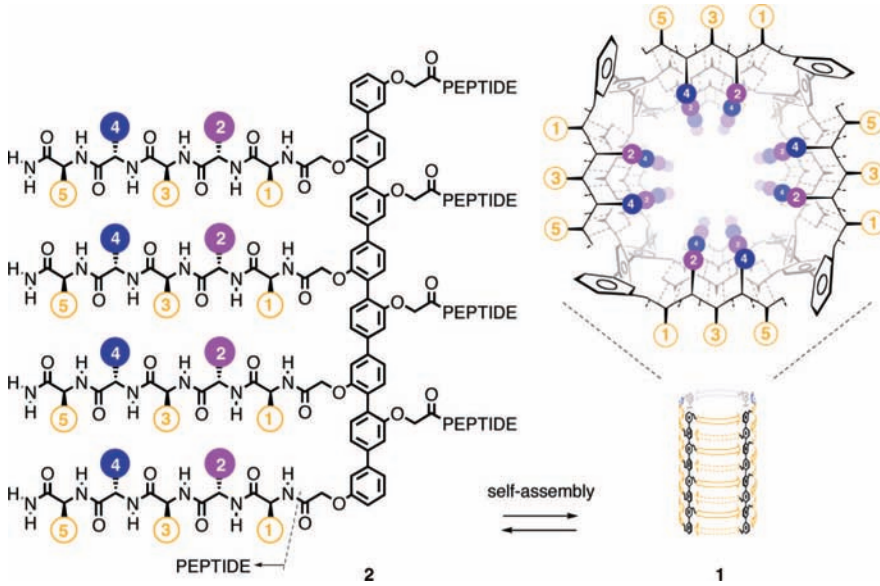

FIGURE 2. Self-assembly of artificial β-barrels **1** from *p*-octiphenyl rods **2**. Peptide backbones in eight-stranded β-sheets of tetrameric rigid-rod β-barrels **1** shown in axial (top) and side view (bottom) are given as arrows (N→C) or as solid lines, hydrogen bonds are given as dotted lines, external amino acid residues are dark on white, and internal ones are white on dark. For specific sequences, see Table 1.

TABLE 1. The Peptide Sequences of Rigid-Rod β-Barrels

entry	sequence[a]	barrel[b]	refs
1	L		
2	KLK	**3**−**7**	16, 17
3	KWK		
4	KHK		
5	ELE	**3**−**7**	16, 17
6	LKL	**23**	36
7	LHL	**20**	34
8	LRL		
9	LEL		
10	LHLHL		35
11	LKLHL	**9**	20, 21, 26, 27, 33
12	LRLHL	**22**	22, 23, 30
13	LRWHV		25
14	Lπ_ALKL	**16**	27, 33
15	LHRHL		18
16	LRLHL ‖ LNLNL	**21**	22
17	LRLHL ⊥ KKKK	**24**	30
18	LDLDL		25

[a] Single-letter abbreviations; π_A = artificial amino acid with π-acidic naphthalenediimide as π-acceptor, see Figures 7 and 8; ‖ = different peptide strands in same β-sheet, see Figure 9, ⊥ = peptide strands not included in the β-sheet, see Figure 9. All C-termini are modified into primary amides, see Figure 2. [b] See Figures 3–9, note that in several cases, the *p*-oligophenyl differs slightly from the one shown in Figure 2.

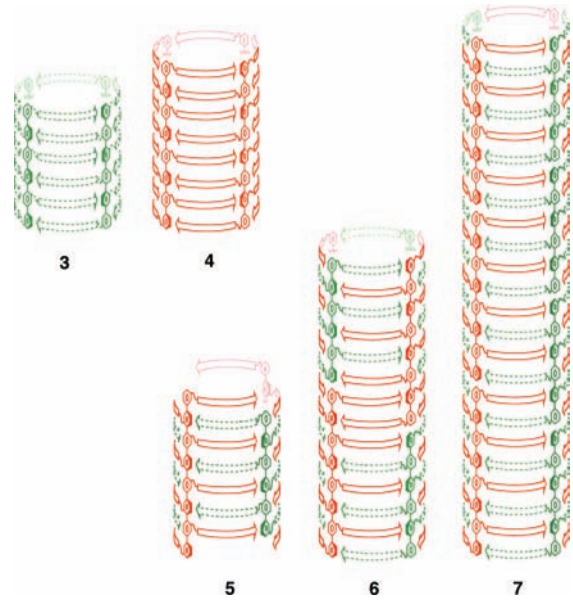

FIGURE 3. Selective assembly of rigid-rod β-barrel **6**. Mixing of anionic *p*-sexiphenyls (green, sequence ELE, Table 1, entry 5) with cationic *p*-octiphenyls (red, sequence KLK, Table 1, entry 2) affords quantitatively octamer **6** as one out of at least five possible architectures **3**−**7**.

Structural Plasticity

The scope and limitations of the rigid-rod β-barrel motif with regard to structural plasticity were explored early on.[16] Rigid-rod β-barrels with short biphenyl and *p*-quaterphenyl staves did not form at all. *p*-Sexiphenyls **3** and longer rods gave barrels of rapidly increasing stability. An intriguing question concerned the programmed assembly of barrels from rods of different length. For example, long *p*-octiphenyls carrying cationic tripeptides along their scaffolds (KLK) and shorter *p*-sexiphenyls containing anionic tripeptides (ELE) mixed together could give at least five higher-order structures. Namely, they could stay apart from each other and form pure anionic *p*-sexiphenyl β-barrels **3** and pure cationic *p*-octiphenyl β-barrels **4**. Alternatively, they could combine as tetramer **5** with a few missing peptide strands and mismatched staves, as octamer **6** with matched staves but mismatched charges, or as 14-mer **7** with matched staves and charges (Figure 3).

The result of the mixing experiment was remarkable: only one product was formed quantitatively, the octamer **6**.[16] This amazingly strong preference for matched rod length over matched charges revealed topological matching as an important driving force in nanoarchitectures with interdigitating rigid-rod scaffolds of different lengths. This finding turned out to be exceptionally useful for the creation of advanced nanoarchitectures with high precision in lipid bilayer membranes[2,3] and, more recently, also on conducting surfaces.

Other structural aspects explored so far include the expansion of the preferred tetramers **1** into hexamers by internal crowding and charge repulsion (Table 1, entries 3 and 4). The complementary contraction of hexamers into tetramers was achieved by internal templation.[17] However, the introduction of rigid-rod β-barrels was particularly important because it allowed us to support the ongoing shift of attention from structure to function, that is, to expand the marvelous functional plasticity of the β-barrel scaffold beyond the limitations of biological amino acids. Highlights from functional studies with artificial β-barrels will be briefly recalled in the following.

Focus on Function

In rigid-rod β-barrels, the side chain of the N-terminal amino acid residue *i* is expected to locate at the outer barrel surface to avoid internal overcrowding near the rigid-rod turns (Figure 2). The simple dyad repeat of β-sheets should impose the same structural constraint for $i + 2$ and $i + 4$ residues. With pentapeptide strands, the amino acid residues $i + 1$ and $i + 3$ are expected to define the physical and chemical properties at the inner barrel surface. The introduction of hydrophilic amino acid residues at the outer surface and hydrophobic residues at the inner surface afforded artificial β-barrels that are soluble in water and have a hydrophobic interior (Table 1, entries 2−5). A hydrophobic exterior and a hydrophilic interior produced β-barrels that are soluble in organic solvents and form pores in lipid bilayer membranes (Table 1, entries 1 and 6−18).

Molecular recognition by artificial β-barrels has been explored in many variations. Artificial β-barrels that can host carotenoids in water can be considered as lipocalin mimics.[17] Astaxanthin was a particularly attractive guest because its decomplexation from the biological β-barrel host β-crustacyanin caused by thermal denaturation is responsible for the color change during the cooking of lobsters and shrimps.

Molecular recognition by artificial β-barrels that form pores in lipid bilayers has been studied intensely, and it is the basis of all practical applications of artificial β-barrel pores. External and internal barrel design was introduced to create pores that open (ligand gating) and close (blockage) in response to chemical stimulation.[18] Internal pore design, that is, the introduction of guest binding sites at the inner β-barrel surface, was particularly attractive to couple molecular recognition within the pore with molecular translocation through the pore and across the membrane. Realized examples reach from magnesium cations or phosphate anions to molecules such as nucleotides, carbohydrates, inositol phosphates, pyrenes, calixarenes, or fullerenes and to macromolecules such as oligosaccharides, RNA, DNA, peptides, or synthetic polymers.[2,3] α-Helix recognition within artificial β-barrel pores was of interest to study the importance of topological matching in molecular recognition.[2,18,19] Rigid-rod α-helix mimics were introduced to determine the role of α-helix folding in α-helix recognition.[20] Later on, the envisioned supramolecular structure, that is, an α-helix bound inside of the β-barrel pore was found to occur also in a biological system (Figure 1).[4,18] Our attempt to gain structural insight on α-helix recognition within artificial β-barrel pores by atomic force microscopy (AFM) failed because polyglutamate α-helices produced large spherical particles on mica.[21] More shape-persistent polymers **8** were thus needed to observe, according to statistical analysis of the AFM images, polymer blockers bound in the artificial β-barrel pores **9** (Figure 4).[21] In the resulting polymer−pore complex **10**, a giant pseudorotaxane, the polymer exhibited an interesting template effect on the barrel. Namely, AFM images and molecular dynamics simulations revealed that the artificial β-barrel contracts forming a diamond-shaped conformation in order to fully bite into the polymer thread.

Circular dichroism spectroscopy of the same pore−polymer complex in lipid bilayers readily revealed the opposite template effect, that is, the induction of polymer chirality by the barrel, and supported the existence of the structure found in AFM images. The change from pseudorotaxanes to inclusion complexes when moving from polymer to small molecule blockers will be discussed later on.[22]

Attractive applications of molecular recognition by artificial β-barrels include catalysis and sensing. The conversion of substrates while passing through a pore across a lipid bilayer remains an amusing, challenging, and essentially unexplored topic of interest.[23] The prospect of detecting single reactive intermediates is particularly appealing.[24] The other main application of molecular recognition by artificial β-barrels is sensing,[25−27] a topic that is attracting increasing attention. The emerging, very general sensor systems promise practical usefulness in applications reaching from enzyme assays to drug discovery and diagnostics. In the current system, artificial β-barrel pores serve as general optical transducers of reac-

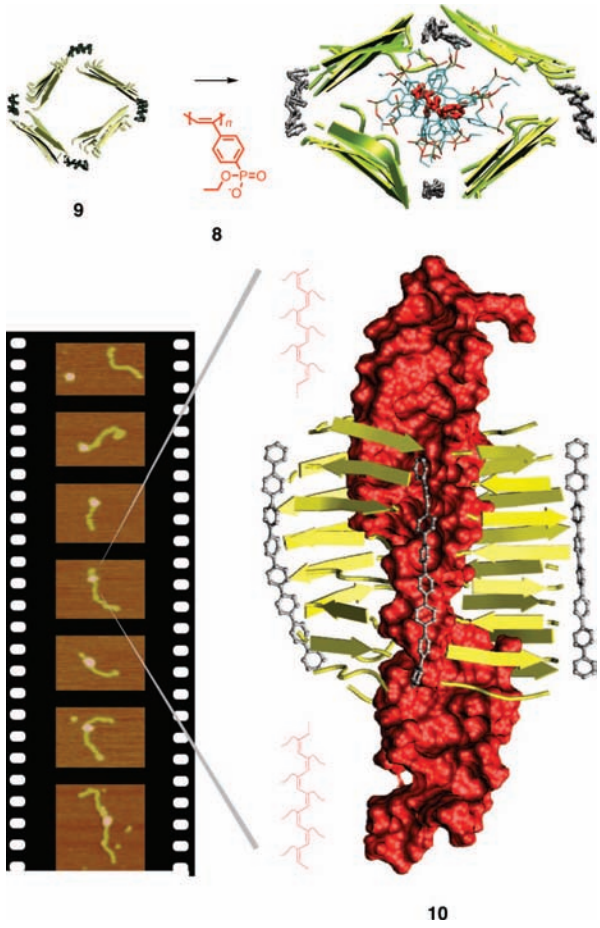

FIGURE 4. AFM snapshots and molecular dynamics simulations of a single poly(ethyl(4-ethynylphenyl)phosphonate) blocker **8** (red) moving through a single artificial β-barrel pore **9** (yellow). The molecular model of the pseudorotaxane **10** seen in the AFM images (bottom left) is shown in axial view (top right) and in side view (bottom right). Adapted from ref 21, Copyright 2005 Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

tions (Figure 5). This method is based on the fluorometric detection of changes in pore activity in response to chemical stimulation. In practice, this is quite easy. Vesicles are loaded with 5(6)-carboxyfluorescein (CF) or other fluorescence probes at concentrations high enough for self-quenching to occur. CF efflux through an added pore turns on CF emission due to local dilution, which reduces self-quenching.

To detect the closing (or opening) of artificial β-barrel pores in response to chemical stimulation, the change in pore activity is measured as a function of blocker (or ligand) concentration. A reaction is detectable if the IC_{50} (the concentration needed to cause 50% pore blockage) of substrate and product differ by more than 3-fold (a discrimination factor $D > 3$). To follow a reaction, aliquots are taken at intervals in a similar manner to chromatographic methods such as TLC or HPLC, and the changing ability of the reaction mixture to block the pore is recorded as changes in CF emission.

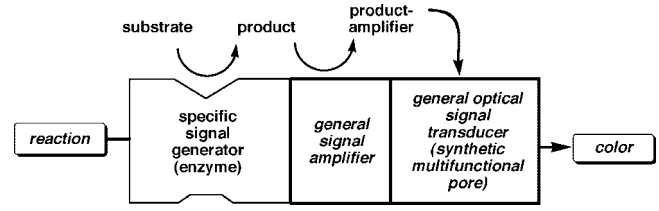

FIGURE 5. Artificial β-barrel pores as sensors. The sensing system reports the consumption of analyte during a reaction as a change in color or fluorescence. Enzymes are used to generate selectively the signal for a specific substrate analyte in complex matrices. If needed, signal amplifiers are then covalently linked to the product to enhance its pore-blocking efficiency. Synthetic pores, finally, are used as signal transducers that respond with high sensitivity to the binding of the analyte−amplifier conjugates (see Figures 6−8 for examples). Enzymes detected with artificial β-barrel pores include acetate kinase, aldolase (RAMA), apyrase, citrate lyase, DNA exonuclease, DNA polymerase, elastase, esterase, ficin, galactosyltransferase, galactosidase, heparinase, hexokinase, hyaluronidase, invertase, lactate oxidase, papain, phosphatase, phosphofructokinase, pronase, RNase, subtilisin, transaminase, and triose phosphate isomerase (TIM).[25−27]

With sensitivity toward changes in charge or size, optical transduction with synthetic pores is compatible with a broad range of reactions. To detect otherwise elusive reactions due to poor blockage ability (sensitivity) or discrimination between substrate and product blockers (selectivity), we recently introduced the concept of reactive signal amplification (Figure 5).[27] Reactive signal amplifiers are defined as pore blockers that react spontaneously and selectively with the functional group produced or consumed during an otherwise undetectable reaction. If the product−amplifier conjugate blocks the pore better than the amplifier itself, the reaction is detectable as pore closing. Optical transduction with artificial β-barrel pores with or without reactive signal amplification was particularly attractive in combination with enzymes as specific signal generators. The broad adaptability achieved with reactive signal amplification assured detectability of many different enzymatic reactions with the same pore transducer. Pertinent realized examples are listed in Figure 5.

The sensor system composed of enzymes as signal generators, reactive blockers as signal amplifiers, and synthetic pores as signal transducers is applicable to a wide variety of topics. Highlights include examples for drug discovery (inhibitor screening assays for otherwise problematic enzymes of medicinal interest)[28] and diagnostics (multicomponent sensing in complex matrices).[26,27]

To provide experimental evidence for multicomponent sensing in complex matrices with artificial β-barrel pores, the creation of artificial tongues was an irresistible topic because on our own tongues the sensation of taste is mediated by

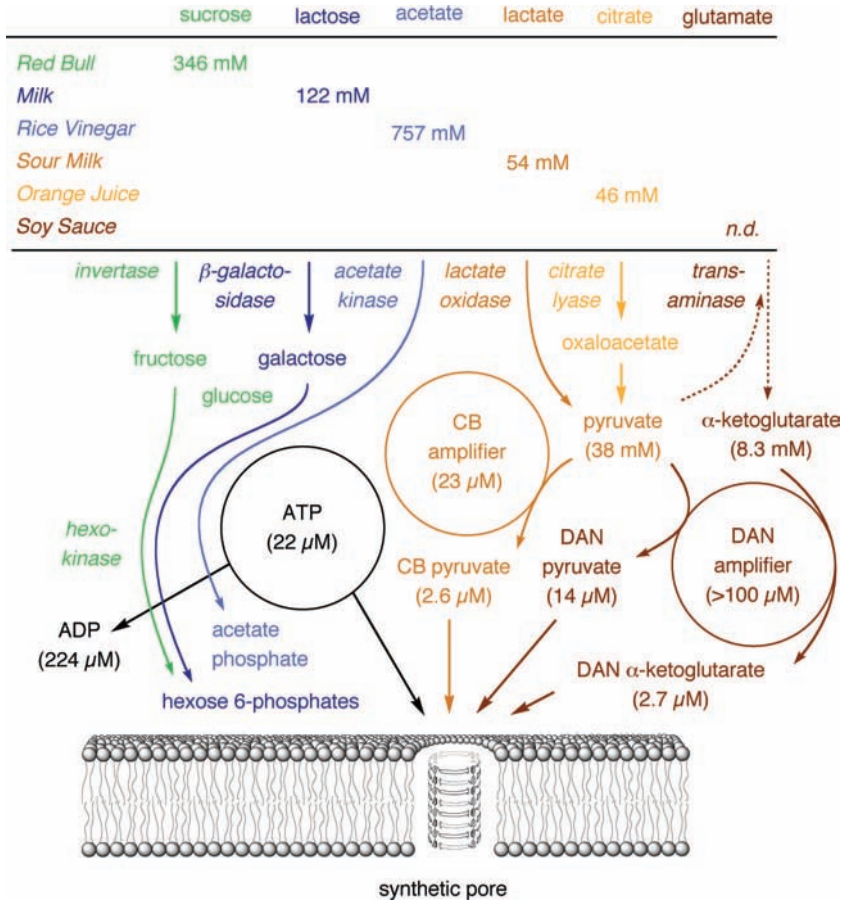

FIGURE 6. An artificial tongue, made to demonstrate multicomponent sensing in complex matrices with synthetic pores and reactive signal amplifiers. The representative samples from the supermarket were first separated from intractable material and then incubated with enzymes and cosubstrates to selectively produce or consume detectable pore blockers (e.g., ATP). The pore blockage efficiency of poorly recognized analytes was amplified after enzymatic signal generation with reactive CB or DAN amplifiers for detection as amplifier−analyte conjugates (compare Figure 7). Concentrations in parenthesis indicate IC_{50} values for blockage of pore **9** or **16** (DAN data only).

transmembrane pores that open and close in response to chemical stimulation. To begin with sweet sensations, the sensing of sugar in soft drinks such as Coca Cola or Red Bull was possible without reactive signal amplification (Figure 6). This opportunity occurred because the conversion of cosubstrate ATP into ADP during signal generation with invertase and hexokinase was detectable with pore **9** with high sensitivity and selectivity.[26] Essentially the same strategy with different enzymatic signal generators was applicable to lactose sensing in milk and acetate sensing in vinegar (Figure 6).[27] In clear contrast, lactate sensing in sour milk with artificial β-barrel pores was not possible without reactive signal amplification. Lactate oxidase was available as a signal generator. However, synthetic pores that respond sensitively to either lactate or pyruvate are not available. This sensitivity problem with lactate sensing was readily solved by covalent capture of the pyruvate product with CB (Cascade Blue) hydrazide **11** (Figure 7). The resulting CB hydrazone **12** blocked the artificial β-barrel pore **9** with high sensitivity and without interference from the smaller and less anionic amplifier **11**. Reactive signal amplification increased the sensitivity of the lactate sensor by more than 4 orders of magnitude.

Replacement of the selective signal generator from lactate oxidase to citrate lyase readily transformed the artificial β-barrel pore **9** from a lactate sensor into a citrate sensor (Figures 6 and 7).[27] In a typical procedure, orange juice was incubated first with a hydrazine-rich resin. The removal of all aldehydes and ketones as hydrazones was important to minimize interference. The orange juice was then exposed to citrate lyase to convert citrate into oxaloacetate. This specific signal generation was followed by signal amplification with hydrazide **11** and signal transduction with pore **9**. The question whether or not oxaloacetate spontaneously decarboxylates to pyruvate before or during signal amplification is irrelevant for citrate sensing with amplifier **11** and pore **9**.

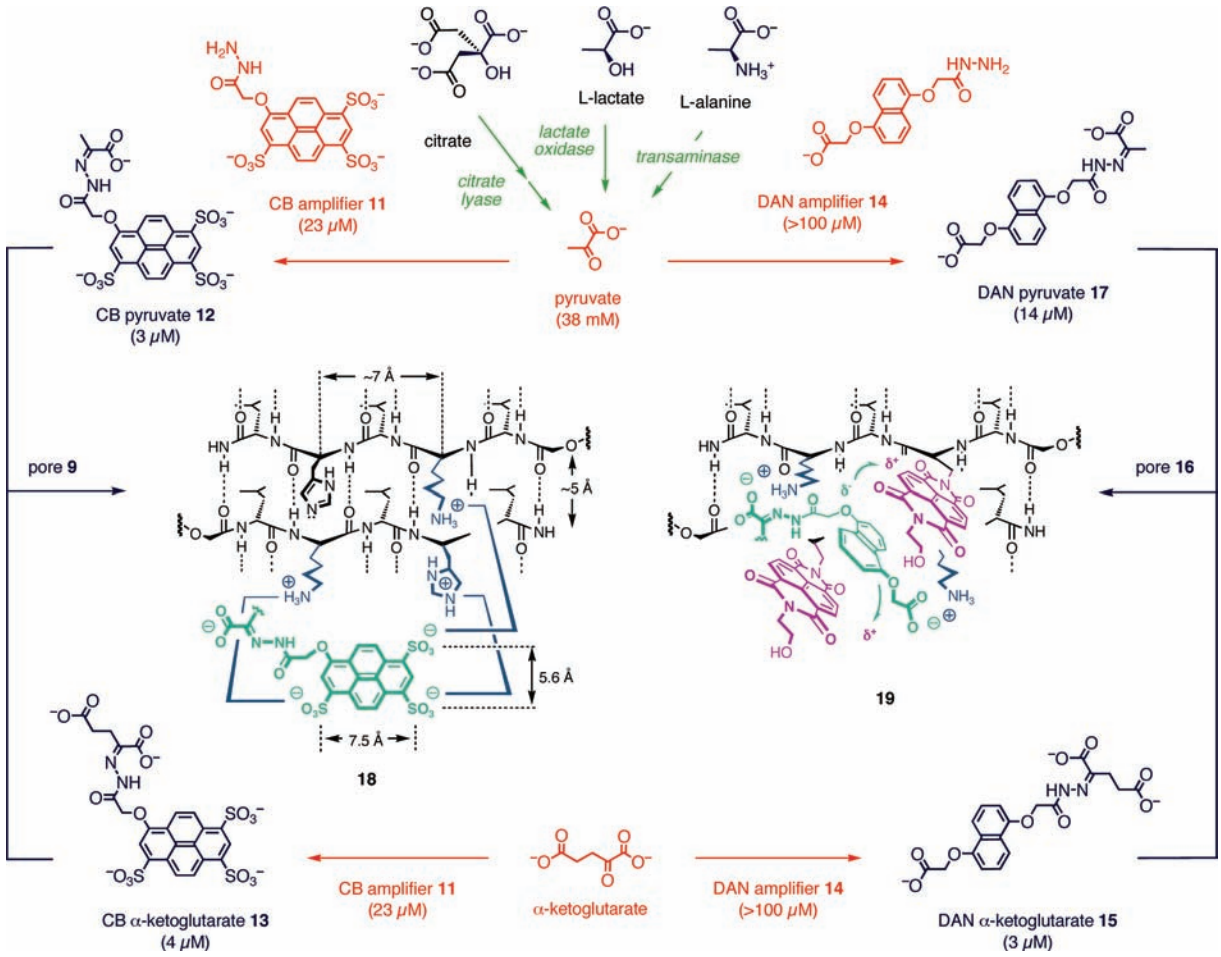

FIGURE 7. Adaptability of synthetic pore sensors illustrated with signal generation for citrate, lactate, and alanine (green), followed by amplification of the product pyruvate with CB hydrazide **11** and DAN hydrazide **14** (red) for recognition by multipoint ion pairing within pore **9** and adhesive π-clamping within pore **16** (Figure 8), respectively. IC_{50} values for pore blockage are given in parentheses.

Moving on from sweet and sour components in milk, vinegar, orange juice, or coke, umami sensing was envisioned to further refine the artificial tongue.[27] Umami, a Japanese word meaning "savory" or "deliciousness", is one of the five basic tastes. Glutamate, present in meat, cheese, tomato, etc., or added as taste enhancer MSG (monosodium glutamate), is chiefly responsible for the umami sensation, although other contributors are known. Glutamate sensing with aminotransferases as signal generators was particularly attractive from an academic point of view. The challenge was how to make the product α-ketoglutarate send a signal selectively in the presence of the cosubstrate pyruvate. Signal amplification with CB hydrazide **11**, for example, was not sufficiently selective for this purpose. Artificial β-barrel pore **9**, perfectly capable of discriminating between ATP and ADP, failed to discriminate between hydrazones **12** and **13**. A quite extensive screening identified dialkoxynaphthalene (DAN) hydrazide **14** as ideal amplifier. Covalently captured α-ketoglutarate, that is, DAN hydrazone **15**, blocked pore **16** with high sensitivity and without interference from DAN pyruvate **17**. Selective signal amplification was not applied to soy sauce because glutamate sensing with glutamate oxidase and conventional signal amplification is obviously more straightforward in practice.

Advanced Architectures

Internal Barrel Design. The introduction of active sites at the inner surface of artificial β-barrel pores has been explored in many variations (Table 1, entries 6−18). Multipoint ion pairing between pore and blocker was particularly effective. The three anions of the planar 1,3,6-pyrenetrisulfonate scaffold, for example, are precisely positioned to block artificial β-barrel pores. Namely, ion pairing with three charged residues in three corners of the 5 Å × 7 Å rectangle on one face of a β-sheet provides complex **18** within pores such as **9** (Figure 7). This simple recognition motif has been exploited extensively to create artificial β-barrel pores with catalytic activity.[23] General applicability of the recognition motif has been demonstrated recently with macrocyclic peptide catalysts.[29] However,

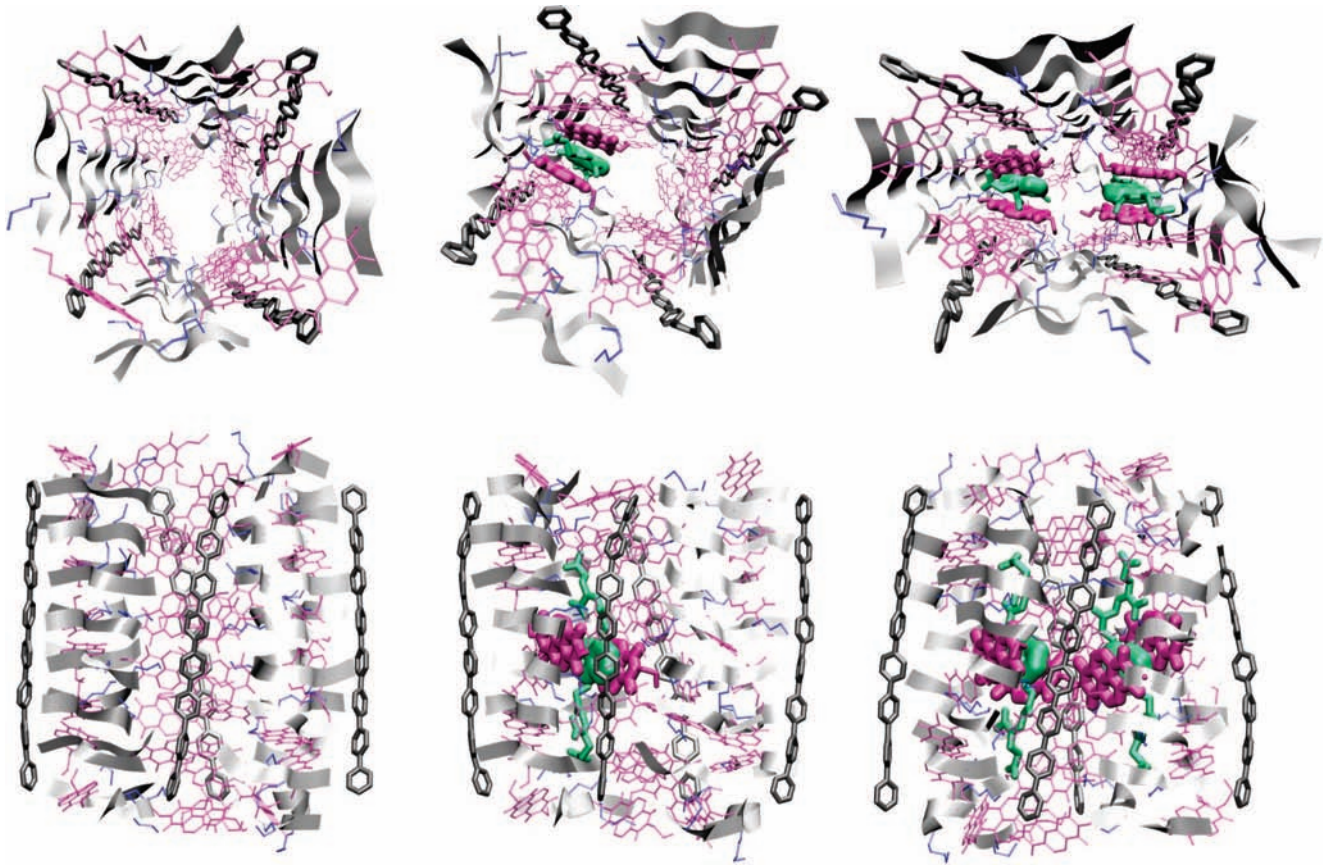

FIGURE 8. Molecular dynamics simulations of synthetic β-barrel pore **16** (right) in the process of catching one (middle) and two (right) α-ketoglutarate-amplifier conjugates similar to **15** (green) with internal π-acidic NDI clamps (purple) to form the complex **19**, see Figure 7. Adapted from ref 33, reproduced by permission of The Royal Society of Chemistry.

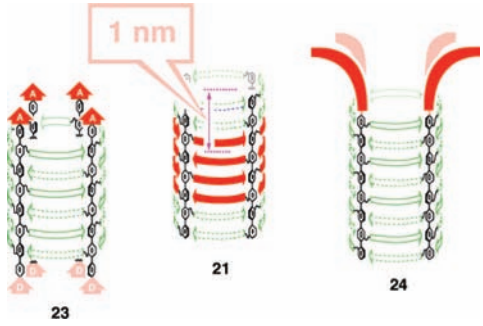

FIGURE 9. Advanced architectures realized for artificial β-barrel pores with refined function includes push−pull staves for voltage-gated formation of pore **23** (donor D = methoxy, acceptor A = methylsulfone), active-site contraction toward the middle of pore **21** for voltage-sensitive molecular recognition, and "hydrophilic anchoring" of pore **24** for hypersensitive sensors. For peptide sequences, please see Table 1.

so far there is no structural evidence available for host−guest complexes of pyrene-3,6,8-trisulfonates and cationic β-sheet as depicted in **18**.

The same multipoint ion pairing with pyrenetrisulfonates has been used to introduce the concept of reactive signal amplification for multicomponent sensing in complex matrices with artificial β-barrel pores (Figures 7 and 8).[27] For *selective* signal amplification, however, this versatile motif in CB amplifiers **11** was too potent. Stoichiometric binding obscured all selectivity.[30] To accomplish selective signal amplification, for example, for glutamate sensing with transaminases as signal generators for α-ketoglutarate without interference from pyruvate, advanced internal pore design for refined amplifier recognition was unavoidable. In artificial β-barrel pore **16**, the artificial amino acid π_A with a naphthalenediimide (NDI) side chain was placed together with lysine residues at the inner pore surface (Figures 7 and 8; Table 1, entry 14). With a positive quadrupole moment much larger than that of hexafluorobenzene, NDIs are extremely π-acidic.[3] For this reason, they are capable of conducting electrons and of binding anions. Moreover, the introduction of π-donating core substituents produces attractive push−pull fluorophores with easily varied frontier orbital energy levels.[31] Their π-acidity and planarity further facilitates face-to-face π-stacking. Face-to-face π-stacking with π-basic aromatics such as DANs produces charge-transfer complexes.[31−33] Placed diagonally on one face of a β-sheet, two NDIs were thus thought to act like sticky

π-clamps or tweezers that can catch π-basic aromatics by aromatic electron donor−acceptor (AEDA)[32] interactions. As with WW-clamps in nucleotide binding proteins and artificial β-sheets,[7] lysines were added to complete the 5 Å × 7 Å rectangle on one face of a β-sheet to assist π-clamping with ion pairing.

Introduced within artificial β-barrel pores, π-acidic NDI π-clamps thus expanded molecular recognition from multipoint ion pairing to orthogonal AEDA interactions.[33] Formal double mutant cycles confirmed that AEDA interactions account for the excellent recognition of the DAN amplifier **14** within NDI pore **16**, that is, the occurrence of adhesive π-clamping in inclusion complexes such as **19** (Figure 7).[33] Molecular dynamics simulations of this complex synthetic functional system were in agreement with the experimental results (Figure 8).

Core Engineering and the Depth of Molecular Recognition. Giant pseudorotaxanes are formed when polymers enter and move through artificial β-barrels (Figure 4).[21] With small molecules instead of polymers as blockers within artificial β-barrels, we obtain inclusion complexes instead of pseudorotaxanes as a central suprastructural motif (Figure 8). A general key question with inclusion complexes concerns the depth of inclusion and its possible importance for molecular recognition.[22] Although quite difficult to assess in solution, the depth of molecular recognition is readily measured within pores because it directly relates to function. Namely, the voltage sensitivity of pore blockage increases with the distance that a blocker travels from the pore entrance to the binding site.

Early on it became clear that molecular recognition by artificial β-barrel pores **20** composed of tripeptide β-sheets is very weak and insensitive (Table 1, entry 7).[34] The disappointing lack of responsiveness of these tetrameric pores has been attributed to an interior that is too small for guest inclusion. Physical barrel expansion by replacing the tripeptide β-sheets with pentapeptide β-sheets improved the molecular recognition by the corresponding pores by more than 3 orders of magnitude (Table 1, entries 10−13).[35] The increase in recognition can be considered as demonstration of the power of guest inclusion, although contributions from other parameters may be important as well. The voltage sensitivity of molecular recognition did, however, not increase correspondingly. This poor voltage sensitivity was in good agreement with rate-limiting guest binding to the first available active sites near the barrel entrance. To achieve voltage-sensitive molecular recognition by "in-depth" inclusion, the synthesis of artificial {242}-β-barrel **21** appeared unavoidable (Figure 9). This refined artificial β-barrel contains central and peripheral domains of different composition and dimension.

The synthetic challenge of this approach was to attach peptides with different sequences to the same rigid-rod scaffold. For this purpose, *p*-octiphenyls with orthogonally protected acids along the rigid-rod scaffold were synthesized. Reductive cleavage of benzyl esters without affecting *tert*-butyl esters liberated the peripheral group of carboxylic acids for coupling with the N-termini of the first pentapeptide; subsequent acidic hydrolysis of the central *t*-butyl esters produced the second group of carboxylic acids for coupling with the second pentapeptide.

In {242}-β-barrel **21**, the four central β-sheets were selected to have RH dyads positioned at the inner barrel surface to bind anionic guest such as nucleotides by multipoint ion pairing (Figure 9 and Table 1, entry 16). The two peripheral β-sheets were designed to contribute "inactive" NN dyads to the inner barrel surface. Hence, this refined architecture is denoted with the {242} prefix. Compared with classical artificial β-barrels **22** (Table 1, entry 12), this formal active-site contraction toward the middle of artificial β-barrel pore **21** still caused a 2-fold increase in molecular recognition. This outcome validated previous suspicions that it is the location of the active site rather than the number of charges, that is, the depth of guest inclusion, that determines molecular recognition by artificial β-barrel pores. In-depth molecular recognition by the {242}-β-barrel **21** was as voltage sensitive as expected for an active-site contraction by 1 nm toward the middle of the pore.

Rigid-Rod Push−Pull β-Barrels. Whereas "in depth" voltage-sensitive molecular recognition by synthetic pores has been realized only recently with pore **21**,[22] the already challenging voltage-gated formation of synthetic pores had been explored early on with pore **23** (Figure 9).[36] Biological β-barrel pores have poor voltage sensitivity because β-sheets have no macrodipole. Closing rather than opening can occur with biological β-barrel pores at high voltage. This effect has been attributed to β-sheet destruction by enforced alignment of the peptide carbonyls. Voltage-gated ion channel opening is much easier with parallel α-helix bundles because of their strong, amplified macrodipole. Attempts to introduce macrodipoles in β-structures with β-peptides have so far not been verified experimentally.[13]

Considering this situation in biology and chemistry, the creation of an artificial β-barrel pore that opens at high voltage was an attractive objective.[36] In rigid-rod push−pull β-barrel pore **23**, the rigid-rod scaffold was used to create the macrodipole needed for voltage gating. A π-donor D such as a methoxy group was attached at one terminus and a π-accep-

tor A such as a methylsulfone at the other. The resulting macrodipole along the rigid-rod scaffold has several advantages compared with the α-helical dipole. For instance, it cannot disappear with conformational changes. It can, however, be turned off permanently without global structural changes. The reduction of a sulfone acceptor to a sulfide donor, which is the formal removal of two oxygens, is, in principle, already possible in situ. Furthermore, the fluorescence of rigid push−pull rods can serve as intrinsic probe for structural studies on dipole−potential interactions.

In solution, dipole−dipole attraction was expected to direct the antiparallel self-assembly into rigid-rod β-barrels without macrodipole. In polarized lipid bilayer membranes, however, dipole−potential interactions were likely to overcompensate dipole−dipole repulsion and enforce the parallel self-assembly into push−pull β-barrels with an amplified macrodipole. The found Hill coefficient $n = 4$ and gating charge $z_g = 1$ demonstrated the voltage-gated formation of a tetrameric ion channel **23**. Indications for biphasic self-assembly were secured, with the formation of single β-sheets being possibly detectable in single-channel current experiments. Once formed at high voltage, parallel push−pull β-barrels **23** remained oriented in depolarized membranes and were neatly destroyed by inversion of the applied voltage.

Interface Engineering with Artificial β-Barrel Pores. End-group engineering as in push−pull barrel **23** was recently expanded to address the possible interactions of transmembrane synthetic β-barrel pores at the membrane−water interface.[30] In the advanced artificial β-barrel **24**, the classical artificial β-barrel pore **22** is elongated with tetralysine peptides (Figure 9, Table 1, entry 17 vs entry 12). Rather than participating in the antiparallel β-sheets formed by interdigitating LRLHL pentapeptides, these tetralysines were designed to extend from the transmembrane β-barrel into the membrane−water interface.

This "hydrophilic anchoring" of artificial β-barrel pore **24** was encouraged by increasing evidence that the apparent activities found in lipid bilayer membranes are often determined by solubility in water. Indeed, hydrophilic domains attached to one end of functional transmembrane architectures may be beneficial for many reasons. For instance, it may ensure delivery to the vesicle as well as intervesicular transfer by preventing competing precipitation from water. Moreover, hydrophilic anchors were expected to enforce vectorial partitioning, prevent disappearance in the hydrophobic membrane core, and promote transmembrane orientation and parallel self-assembly of the pore.

High expectations for hydrophilic anchoring were supported by many examples from biological pores and promising breakthroughs with pioneering synthetic approaches. With regard to the activity of artificial β-barrel pore **24**, the key impact of hydrophilic anchoring was a dramatic increase in cooperativity. This change confirmed successful suppression of the competing self-assembly and precipitation of prepores from the aqueous phase. The resulting, excellent characteristics reduced the effective concentration of pore **24**. This increase of pore activity was the key to maximize sensitivity and selectivity in sensing applications. Current efforts with interface engineering focus on topics such as targeted pore formation and immunosensing.

Conclusion

With the introduction of rigid-rod molecules as shape-persistent staves, the difficulties in synthesizing artificial β-barrels were overcome.[2,3] Over the years, a rich collection of rigid-rod β-barrels have been made and studied. The early elucidation of fundamental aspects such as the variation of the length of both rigid-rod staves and β-sheet hoops revealed *p*-octiphenyls and pentapeptides as the best combination, particularly when embedded in a lipid bilayer membrane. The synthesis of more refined *p*-octiphenyl staves provided access to more sophisticated β-barrel architectures. They include rigid-rod β-barrels with more than one peptide sequence to create domains of different dimension, location, and function or to expand structural plasticity beyond the β-barrel scaffold.[22,30] Rigid-rod push−pull β-barrels with permanent axial macrodipoles are of interest in the context of voltage-gated ion channels and natural antibiotics.[36] The found preference for equal rod length identifies the programmed assembly of rods of different length as a simple approach for the creation of sophisticated architectures with high precision.[16]

Variation of the peptide sequence is of interest to vary and fine-tune the function of artificial β-barrels without global structural changes. The realized functional plasticity reaches from lipocalin models that can bind carotenoids in water to multifunctional pores that open or close in response to physical and chemical stimulation. This includes changes in pH, ionic strength, voltage, molecular recognition, and catalysis. Recent breakthroughs include the creation of artificial β-barrel pores that can act as multicomponent sensors in complex matrices.[27] In this application, the β-barrel pores function as general optical transducers together with reactive amplifiers for the covalent capture of elusive analytes after enzymatic signal generation. The use of artificial amino acids to install

sticky π-clamps within artificial β-barrel pore sensors has been particularly fruitful in this context.[33]

The success of advanced architectures to make artificial tongues provides strong support for future efforts toward the creation of more complex rigid-rod β-barrels, also from a practical point of view. However, plans to further expand the barrel-stave motif beyond *p*-oligophenyls as staves and β-sheets as hoops are also enormously appealing. Recent efforts in this direction introduce rigid oligonaphthalenediimide[3] and oligoperylenediimide rods as photoactive anion$-\pi$ slides. Alternatively, the replacement of β-sheet hoops with naphthalenediimide π-stacks provides access to advanced photoactive architectures for applications in photosynthesis and photovoltaics.[31] Another appealing perspective of research on artificial β-barrels is to apply the lessons learned to topics of current concern in chemical biology. Recent highlights concerning the functional relevance of dipole−potential interactions for natural antibiotics or the dominant role of counteranions for the function of cell-penetrating peptides[37] and voltage-gated biological potassium channels also justify the highest expectations in this direction.

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Stefan Matile received his education from the University of Zurich (Diploma, 1989; Ph.D., 1994) and Columbia University (postdoctoral work, 1994−1996). He is interested in the synthesis and study of multifunctional architectures such as artificial supramolecular leaves and tongues (Georgetown University 1996−1999, then Geneva; www.unige.ch/sciences/chiorg/matile/).

FOOTNOTES

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