En route to supramolecular functional plasticity: artificial β-barrels, the barrel-stave motif, and related approaches

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This review emphasizes recent or pertinent efforts to create functional plasticity with artificial barrel-stave supramolecules and related motifs. On the structural level, a summary of engineered, *de novo* designed, and artificial β-barrels beyond peptide chemistry is followed by representative α-helix bundles and barrel-stave supramolecules comprising oligonucleotides, inorganic architecture, and organic barrel-stave ion channel models. On the functional level, selected examples are given to highlight divers aspects of molecular recognition, translocation, and transformation that are currently accessible with artificial barrel-stave supramolecules.

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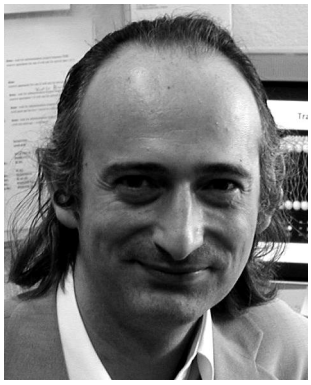

1 Introduction

More than a thousand β-barrel proteins are deposited on the Brookhaven Protein Data Bank.[1] These ubiquitous bio(supra)-macromolecules act as binding proteins, ion channels, or enzymes covering all primary enzyme classification numbers. This overwhelming functional plasticity originates to a good part from the use of β-strands as 'staves', because the opposite orientation of adjacent amino acid side chains permits precise tuning of the chemical nature of outer surface, inner surface, and terminal loops of the stable barrel scaffold (Fig. 1A). Similarly versatile usefulness of artificial β-barrels and related synthetic 'barrel-stave' supramolecules for applications beyond biological function can now be foreseen in view of current progress in the field. The specific aim of this essay is to draw the attention of the reader to novel man-made 'barrel-stave' supramolecules with divers activities by pointing out either very recent pertinent studies or, particularly in the case of the extensively explored α-helix bundles,[2] some perhaps less recognized aspects of functional plasticity.

1.1 What is a barrel?

According to the Oxford Dictionary, a barrel is 'a cylindrical container bulging out in the middle, traditionally made of wooden staves with metal hoops round them' (first option). From these characteristics, only the presence of staves (made from β-strands or other natural and synthetic oligomers of defined length instead of wood) accounts for molecular barrels as well (Fig. 1A). As for their wooden counterparts, however, the most attractive part of molecular barrels is their content or, more precisely, the possibility to fill, to store, perhaps to 'ferment', and to release a product as tasty as a good red wine

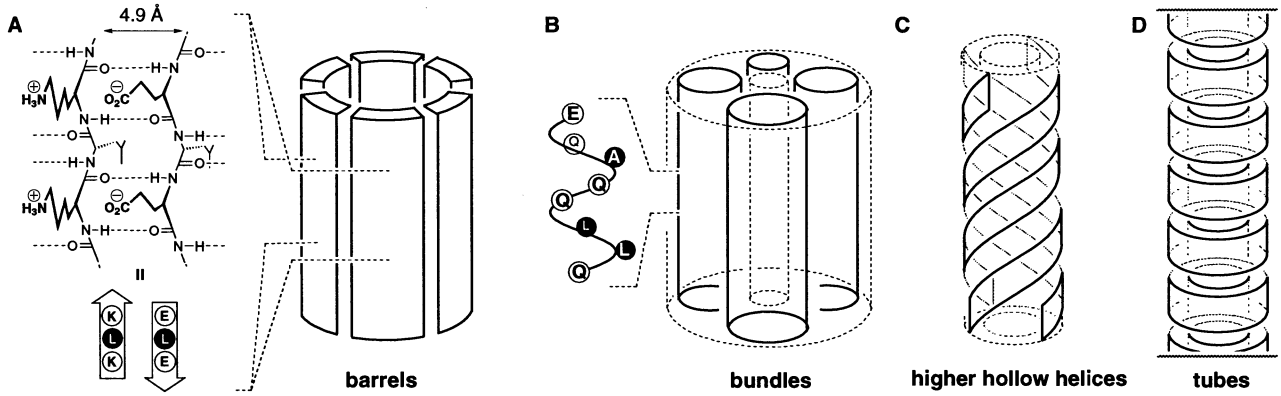

Fig. 1 Barrel-stave architecture (A) in comparison with bundles comprising voluminous staves (B), higher hollow helices comprising helical 'staves' (C), and nanotubes comprising hoops instead of staves (D) (dotted lines ≈ contours). In peptide chemistry, β-barrels (tertiary structure) are made from β-sheets (secondary structure), bundles from α-helices, (higher) hollow helices from β-helical D,L-peptides, and nanotubes from β-helix / β-sheet hybrids of D,L-cyclopeptides. *General abbreviations are defined in insets:* β-Strands are depicted as arrows pointing to the C-terminus, α-helices (and loops) as bold lines, amino acid residues (one letter abbreviation, DX for corresponding D-amino acids) pointing to the exterior of the tertiary structure are depicted black on white, internal residues are white on black. Mutations are given as X*n*X'; X = old, X' = new amino acid, *n* = position in peptide sequence (*e.g.*, Fig. 2).

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by the end of the day. 'Functional plasticity' is suggested as a practical term to indicate such *quality* (of any given motif) *of being adaptable to different activities without global structural changes*.

The term 'bundle' is used instead of (or in addition to) 'barrel' if relatively voluminous staves are present (Fig. 1B). Most popular are α-helix bundles: they represent the second central protein tertiary structure besides β-barrels and exhibit comparable functional plasticity. In sharp contrast to the situation with β-barrels, reliable routes to synthetic α-helical bundles are firmly established.[2] The creation of substantial internal space and precise tuning of its chemical nature is, however, per definition more troublesome with bundles (Fig. 1B) than with 'pure' barrels (Fig. 1A).

All biological β-barrels have a slight helical twist.[1] Increasing helicity transforms barrels into higher hollow helices (Fig. 1C). The formation of higher hollow helices with β-sheet peptides composed of amino acids with ('natural') L-configuration is unfavorable because of the opposite orientation of adjacent amino acid residues and because β-sheets are flat, rigid, and not curved. However, biological and synthetic single- and double-stranded hollow β-helices formed with D,L-peptides (*i.e.*, composed of amino acids with alternating L- and D-configuration) are known. Further 'overtwisting' of helical D,L-peptides ultimately leads to 'barrels' that are composed of hoops instead of staves (Fig. 1D). The term 'nanotube' is used for these supramolecules to emphasize their 'infinite' length.[3] Whereas self-assembly of nanotubes from all-L-cyclopeptides seems as unlikely as that of higher hollow helices, synthetic nanotubes from D,L-cyclopeptides (and many other macrocycles) have attracted much well-deserved scientific attention during the last decade(s).[3] Hollow helices and nanotubes from D,L-peptides are, however, incompatible with 'barrel-type' functional plasticity, because their internal space can, per definition, not be functionalized. (Note that this is not necessarily the case with other synthetic hollow (higher) helices or 'hoops' such as *m*-phenylacetylenes.)[4] Because of their relatively poor comparability with the classical 'barrel-stave' motif, and because superb reviews on this topic are steadily emerging,[3] hollow (higher) helices and stacked macrocycles are not covered in this essay. Also excluded are less related supramolecular motifs[5] such as rosettes (*i.e.*, '2D-barrels'), stacked rosettes (*i.e.*, 'sector nanotubes'),[3] cones (*i.e.*, 'asymmetrically contracted barrels'), spheres (*i.e.*, 'capped truncated barrels'), tapes (*i.e.*, 'unrolled barrels'), as well as porous solids.

2 Artificial β-barrels and functional plasticity

In this chapter, illustrative approaches to artificial β-barrels are summarized with emphasis on the creation of functional plasticity. Current design strategies focusing on directed evolution of biological β-barrels, *de novo* β-barrels, and β-barrel supramolecules exceeding peptide chemistry are covered in this order.

2.1 'New wine from old barrels'

John A. Gerlt has put it so nicely: The biotechnologists' strategy to use biological β-barrels for the creation of functional plasticity can be best imagined as making 'new wine from old barrels'.[6] Over the past few years, this strategy has provided the most impressive illustrations for functional plasticity of the barrel-stave motif with regard to molecular recognition, translocation and transformation.

2.1.1 Molecular recognition. Lipocalins are a fascinating family of eight-stranded β-barrels that serve to bind, stabilize, and transport a broad variety of hydrophobic natural products such as retinoids, carotenoids, and lipids. Bilin binding protein (BBP **1**) is a lipocalin that selectively binds tetrapyrrole metabolites such as biliverdin **2** (Fig. 2). This β-barrel **1** was selected to prepare 'new' lipocalins that are capable of binding, in principle, any organic molecule of choice.[7] To highlight the similarity of this approach with the immunological evolution of antibodies, artificial lipocalins with new binding properties were named anticalins.

This concept was first tested with anticalins that bind fluorescein with a maximal dissociation constant of K_D = 152 nM. More recently, targeted random mutagenesis of four loops of BBP **1** yielded anticalin β-barrel **3** that recognizes cardioactive steroids such as digitoxigenin **4**.[7] Guest binding was assessed by fluorescence titration using six nearby tryptophan residues. Superb results (*i.e.*, K_D = 2.0 nM) were reported for digitoxigenin **4**. Nanomolar K_D's were further found for the corresponding C-3 glucoside digitoxin (K_D = 3.2 nM), digoxigenin with an additional hydroxy group in C-12 (K_D = 30.2 nM) and its C-3 glucoside digoxin (K_D = 31.1 nM). Evidence for molecular recognition was secured from negligible binding of ouabain, testosterone or 4-aminofluorescein. These pioneering studies thus clearly demonstrate functional plasticity of biological barrels with respect to molecular recognition.

2.1.2 Molecular translocation. Lipocalin β-barrels are characterized by a hydrophilic outer and a hydrophobic inner surface. Reversed amphiphilicity, that is hydrophobic exterior and hydrophilic interior, leads to β-barrels that form ion channels in bilayer membranes. Their transmembrane interior is of central interest because the spatial compartmentalization by the surrounding bilayer membrane provides vectorial accessi-

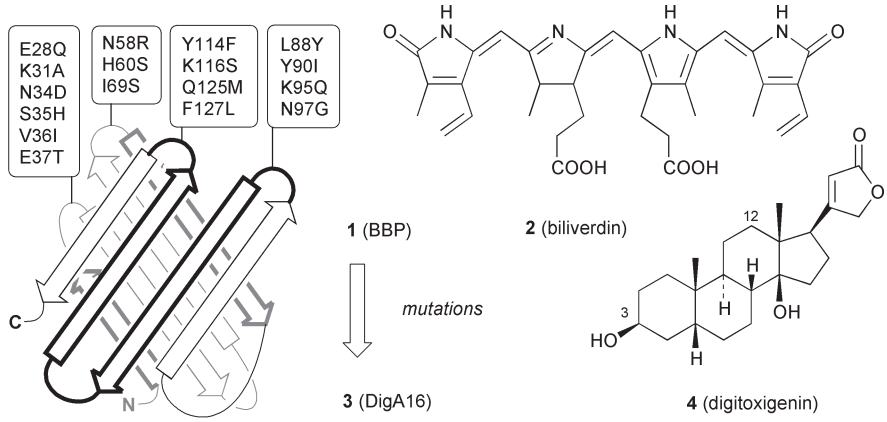

Fig. 2 Transformation of lipocalin **1** capable of binding biliverdin **2** into anticalin **3** able to recognize cardioactive steroids.

bility and allows rapid stochastic detection of intratoroidal processes at the single molecule level.

This unique feature has stimulated inspired expansions of intratoroidal chemistry beyond biological function.[8] Today's β-barrel ion channel of choice for these purposes is a bacterial exotoxin named α-hemolysin (αHL **5**, Fig. 3). The ion channel formed by αHL is composed of seven monomers that self-assemble into a 14-stranded, transmembrane β-barrel with an internal diameter of about 2 nm. The successful creation of 'new' αHL-barrels has been crucial to develop remarkable functional plasticity. In earlier work, one histidine-tag was engineered into one αHL-barrel for sensitive and selective stochastic cation sensing (*e.g.*, Zn^{2+}: K_D = 110 nM). Access to classical organic host–guest chemistry on the stochastic single-molecule level was secured more recently with αHL-barrel **6**. Replacement of a hydrophobic internal residue by a hydrophilic hydrogen-bond-donor/acceptor improved the weak binding of β-cyclodextrin **7** by native αHL-barrel **5** (K_D = 2.1 μM) up to 10^4-times for the new αHL-barrel **6**. With this finding, breathtaking single-molecule third-sphere host–guest chemistry (with β-cyclodextrin as guest of β-barrel **6** as guest of bilayer membranes) has become possible. Stochastic kinetic measurements with representative cyclodextrin guests such as charged adamantanes, antidepressant imipramine and antihistaminic promethazine were performed to illustrate this innovative aspect of functional plasticity combining molecular translocation with molecular recognition.

2.1.3 Molecular transformation. The conversion of β-barrel-like folds within large binding proteins into enzymes has been extensively (and very successfully!) exemplified with catalytic antibodies.[9] More recently, the conversion of cyclophilin **8** into peptidase **9** implied similar functional plasticity for nearly 'pure', low molecular weight β-barrels (Fig. 4).[10] The

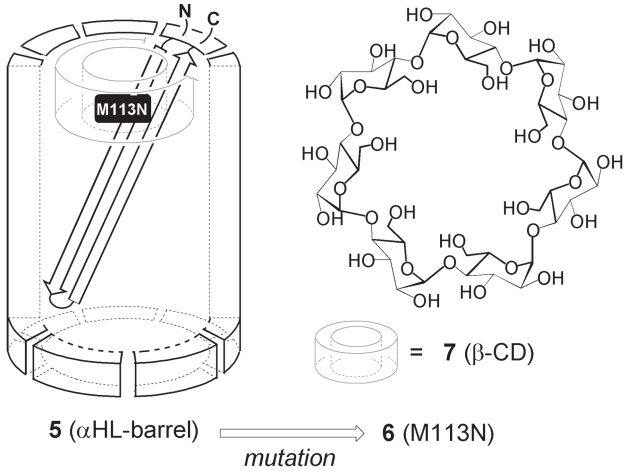

Fig. 3 Transformation of ion channel **5** into single molecule sensor **6** capable of hosting β-cyclodextrin **7** and its guests.

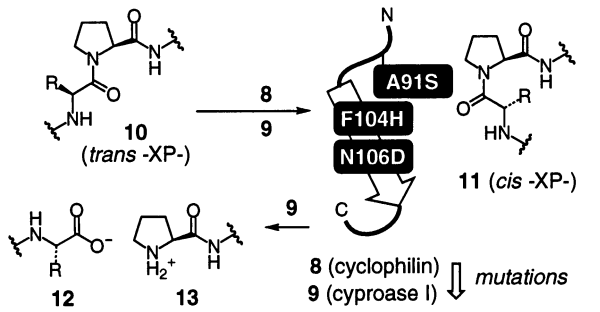

Fig. 4 Conversion of β-barrel **8** (only a schematic view of the active site is depicted) capable of isomerizing *trans*-XP-peptides into peptidase **9** able to hydrolyze *trans*-XP-peptides.

biological function of cyclophilin is to bind *trans*-XP-peptides **10** in *cis*-XP-conformation **11**. Guided by the crystal structure of host–guest complex **8•11**, a catalytic triad was placed near the *cis*-XP-carbonyl electrophile in **9•11**. As a result, peptidase **9** cleaved *trans*-XP-peptides **10** to give fragments **12** and **13** with a surprisingly high k_{cat}/k_{uncat} = 8.3 × 10^8. Removal of proton acceptor (D106, k_{cat}/k_{uncat} = 3.2 x 10^7) or proton acceptor and relay (D106/H104, k_{cat}/k_{uncat} = 0.9 × 10^7) reduced the activity of **9**, although less than expected. Support for a functional catalytic triad include loss in activity upon movement of nucleophile S91 to nearby residues and treatment with 4-(2-aminoethyl)benzenesulfonyl fluoride. Selectivity was evinced by selective hydrolysis of the four XP-amide bonds in a 61-residue snake toxin.

The functional plasticity of so-called α/β-barrel enzymes, comprising a central eight-stranded β-barrel surrounded by eight α-helices, is marvelously displayed in the natural abundance of this motif.[1,11–13] Biomimetic use of this versatile scaffold for rational design and directed 'test-tube' evolution of new catalytic function has recently been demonstrated using the α/β-barrel enzyme **14** (IGPS, Fig. 5).[11] This α/β-barrel **14** catalyzes ring-closure of benzoate **15** to yield indole **16**. Repeated cycles of directed evolution transformed α/β-barrel **14** into the 'new' α/β-barrel **17** that catalyzes the Amadori-rearrangement of anthranilate **18** into benzoate **15**. The origin of the observed 8-fold increased (!) k_{cat}/K_M for **17** compared to the biological α/β-barrel catalyzing the same rearrangement was attributed to contributions from K_M. The new enzyme **17** completely lost the capacity to catalyze the synthesis of indole **16**. Directed evolution of another α/β-barrel catalyzing the same Amadori-rearrangement for different substrates[12] further strengthened implications that the functional plasticity of biological barrels originates from a common, dimeric (or perhaps oligomeric) ancestor.[13] Indeed, *in vitro* dissection of a fluorescent β-barrel monomer into a heterodimer with intact function has been elegantly demonstrated last year.[14]

As with catalytic antibodies,[9] the conversion of biological β-barrel binding proteins into 'semi-synthetic' β-barrel enzymes using synthetic organic chemistry instead of (or in addition to) biotechnology has been reported as well. For instance, the introduction of catalytic phenanthroline groups within the 10-stranded β-barrel of lipid binding proteins allows for internal Cu^{2+} binding and enantioselective esterolysis.[15]

2.2 *De novo* designed β-barrels

In view of the attractive functional plasticity of natural β-barrels, it may surprise that *de novo* design strategies are poorly developed, particularly when compared to α-helix bundles.[2] Most comments on apparent difficulties with β-barrels made 'from scratch'—poor stability, problematic characterization, and frequent precipitation—emphasize the importance of 'negative design'.[2,16] This means that besides design for the desired tertiary structure, it is equally important to design against competing alternative structures to prevent irreversible precipitations.[16]

2.2.1 Molecular recognition. Because most attention focused on the development of reliable design strategies for *de novo* β-barrels, their functional plasticity is nearly unexplored. Two *de novo* metallobarrels have been reported. Whereas the pioneering metallo-'minibody' is a close antibody mimic,[17] the more recent metallo-β-sandwich named betabellin 15D **19** is designed from first principles (Fig. 6).[18] It comprises two four-stranded antiparallel β-sheets with roughly alternating hydrophobic and hydrophilic residues to produce β-sheet amphiphilicity. The central cysteine residue serves to oxidatively crosslink the hydrophobic faces of 'betabellin 15S' dimers in water. In the resulting β-sandwich **19**, two peripheral cation

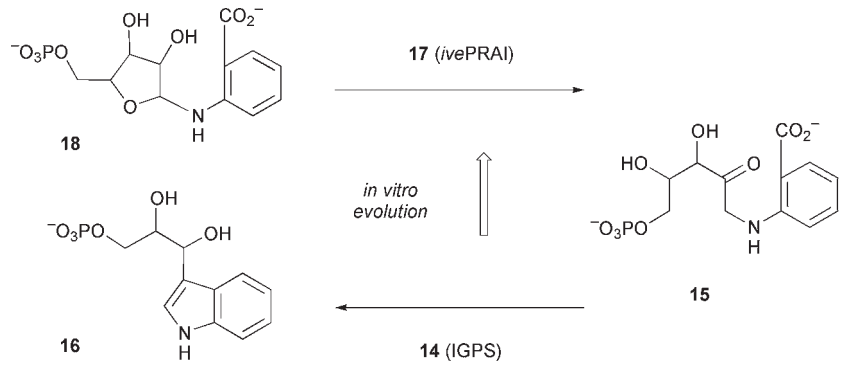

Fig. 5 Conversion of β-barrel **14** capable of catalyzing indole ring closure into β-barrel **17** capable of catalyzing an Amadori-rearrangement.

binding sites are formed by three neighboring histidines each. Cu^{2+} acts as a template for β-sheet formation. Complex stoichiometry (M^{2+} : **19** = 2 : 1) and selectivity (Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+}) were confirmed by elegant electrospray mass spectrometry experiments.

2.3 Rigid-rod β-barrels

In view of the functional plasticity of natural and engineered β-barrels as well as apparent difficulties in constructing *de novo* β-barrels, the development of synthetic β-barrels that bypass folding problems with preorganizing 'non-peptide' staves but maintain the functional plasticity provided by β-strands appears important. Recent results suggest that this may be possible with rigid-rod β-barrels such as **20 – 22** (Fig. 7). Their design is simple: short peptide strands are attached to each arene of a *p*-octaphenyl rod. Self-assembly (**21**, **22**) or programmed assembly of complementary rods (**20**) is driven by stave rigidity and formation of consecutive, interdigitating, intermolecular, antiparallel β-sheets between adjacent rods (*i.e.*, minimized entropy losses and maximized enthalpy gains). The curvature needed to obtain barrels instead of precipitating amphiphilic tapes is initiated by arene–arene torsion angles ≠ 180° in the *p*-octaphenyl scaffold and propagated by peripheral crowding with bulky N- and C-terminal amino acid residues. Supramolecular plasticity of *p*-octaphenyl β-barrels with 'ideal' stability (*i.e.*, ΔG^{H_2O} = −5.2 kcal mol^{-1} from denaturation with guanidinium chloride) was demonstrated with regard to barrel truncation, elongation, contraction and expansion in water and bilayer membranes.

2.3.1 Molecular recognition. Rigid-rod β-barrel **20** is characterized by a hydrophilic outer surface composed of EK-ion pairs to secure solubility in water and a hydrophobic interior composed of leucine arrays to host hydrophobically matching guests such as β-carotene **23**.[19] The most interesting characteristic of the resulting host–guest complex is a red-shifted polyene absorption compared to that in hexane (1616 cm^{-1}). This is consistent with β-carotene planarization by the surrounding tetramer **20**, suggesting biological relevance of rigid-rod β-barrels with regard to similar planarization of vitamin A by biological β-barrels in milk and carotenolipocalins involved in biopigmentation. Molecular recognition was weakly indicated by the apparent incapacity of rigid-rod β-barrel **20** to host mismatched carotenoids.

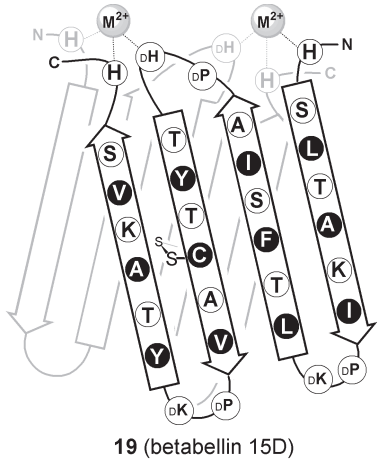

Fig. 6 β-Sandwich **19** with two cation binding sites (depicted as 2 M^{2+}-complex).

2.3.2 Molecular translocation. In contrast to rigid-rod carotenolipocalin **20**, the 'reversed' rigid-rod β-barrel **21** is characterized by a hydrophobic outer surface composed of leucine arrays for solubility in bilayer membranes and a hydrophilic cationic interior with multiple lysines to provide and maintain a large internal space by electrostatic repulsion.[20] Hexameric β-barrel **21** was shown to form giant ion channels in bilayer membranes with a stable, large, functionalized, and transmembrane internal space. The usefulness of this membrane spanning space was exemplified by combining molecular translocation with molecular recognition, that is by the binding of a topologically matching DNA-duplex **24** in 'Watson-Crick'-conformation.[21] The resulting second-sphere host–guest complex unifying central biological metabolites in refined supramolecular architecture (*i.e.*, transmembrane B-DNA **24** within rigid-rod β-barrel **21** within lipid bilayers) was characterized by circular dichroism, fluorescence depth quenching, dye leakage, and membrane conductance experiments (K_D = 177 nM). This result further illustrates functional plasticity of the barrel-stave motif that seems inaccessible with engineered αHL-barrels, or, in other words, demonstrates that synthetic supramolecular chemistry can do more than (often poorly) mimic engineered biomolecules.

2.3.3 Molecular transformation. Studies on the functional plasticity of rigid-rod β-barrels with regard to molecular transformation are in progress. Preliminary results with rigid-rod β-barrel **22** comprising external leucine-arrays as for **21** but internal histidines indicate pH-dependent pore formation in bilayer membranes as well as catalysis of ester hydrolysis. Ongoing characterization of this first synthetic 'catalytic ion channel' focuses on the influence of the spatial compartmentalization on enzyme kinetics (B. Baumeister and S. Matile, unpublished results).

3 Other barrel-stave supramolecules: from terpenoid synthases to inorganic cage architecture

In this chapter, illustrative approaches to hollow 'barrel-stave' supramolecules other than β-barrels are summarized. A discus-

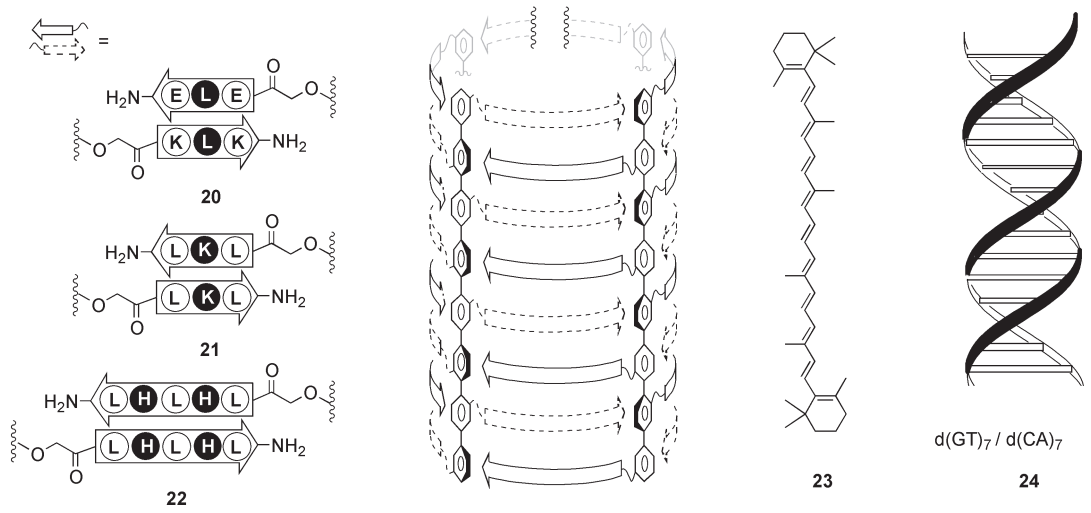

Fig. 7 Functional plasticity of rigid-rod β-barrels with respect to molecular recognition, translocation, and transformation: Tetramer **20** capable of hosting β-carotene **23** in water, hexamer **21** capable of forming ion channels and hosting B-DNA **24** in bilayer membranes, pentapeptide barrel **22** able to permeabilize biomembranes and to catalyze ester hydrolysis.

sion of representative examples on the functional plasticity of the second central protein tertiary structure, *i.e.*, α-helix bundles, is followed by an overview of recent approaches to 'barrel-stave' supramolecules comprising oligonucleotides, inorganic architecture, and synthetic organic amphiphiles instead of peptides.

3.1 α-Helix bundles and functional plasticity

As mentioned in the introduction, the term 'bundle' is used instead of (or in addition to) 'barrel' if relatively voluminous staves are present (Fig. 1). In contrast to the situation with β-barrels, reliable routes to synthetic α-helix bundles are firmly established.[2] Since a comprehensive review of this mature field is far beyond the scope of this essay, selected aspects of functional plasticity are highlighted using representative examples.

3.1.1 Molecular recognition. The solubilization of intact membrane proteins in detergent-free water is of central importance for structural and functional studies. Peptitergents are synthetic α-helix bundles designed to host the hydrophobic part of membrane proteins in their interior, yielding an overall hydrophilic host–guest complex.[22] The sequence of peptitergent **25** was selected to fold into α-helices with a 'flat' hydrophobic face and a large, hydrophilic face (Fig. 8). Terminal charges were neutralized and oppositely charged residues plus ion pairs were placed near each terminus to stabilize the α-helices. In water, facial amphiphilies **25** self-assembled into antiparallel 4-helix bundles **26** with peripheral hydrophilic and central hydrophobic faces. Presumably intact proteins with transmembrane bundles (*e.g.*, rhodopsin **27**) but not β-barrels (*e.g.*, porins) could be dissolved with peptitergent **25** in detergent-free water. More recent results suggest that facial amphiphile **25** solubilizes neither intact Na^+/K^+-ATPases[23] nor GTPases.[24] Peptitergent **25** was further engineered into microsomal cytochrome P450 to replace the native hydrophobic membrane anchor, expressed in *E. coli*, isolated, and purified.[25] The 'new' pepti-P450 mutant was soluble in detergent-free water as oligomer with intact steroid hydroxylase activity.

3.1.2 Molecular translocation. Considering their structural characteristics (Fig. 1), it is not surprising that transmembrane helix bundles generally form ion channels with smaller internal diameter than β-barrels. This makes bundles less attractive for stochastic sensing of single internal guest molecules but

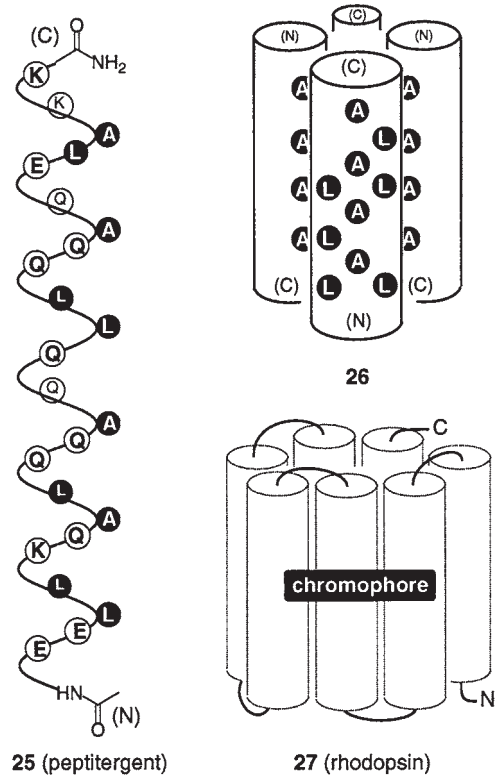

Fig. 8 Peptitergent **25** capable of forming tetrameric bundles **26** and of hosting membrane protein **27** in water.

interesting for, *e.g.*, cell membrane recognition and as oligonucleotide vectors,[26] important aspects of functional plasticity with regard to antibiotic resistance and gene therapy, respectively. KALA **28**, representative of many recent α-helical amphiphiles with similar functional plasticity, was designed for the latter purpose (Fig. 9).[26]

In contrast to peptitergent **25** (Fig. 8), α-helical KALA **28** comprises a major hydrophobic and a minor hydrophilic, cationic face. In biomembranes, this amphiphilicity caused the formation of (not precisely characterized) helix bundles **29** with peripheral hydrophobic and central hydrophilic faces. As with rigid-rod β-barrel **21**, charge repulsion can be expected to create a large bundle interior. The capacity of KALA **28** to mediate dye leakage from spherical biomembranes confirmed the presence of transmembrane space beyond 1 nm. Host–guest complexes with single-stranded antisense phosphodiester/phosphorothionate 27-mer **30** and other oligonucleotides did,

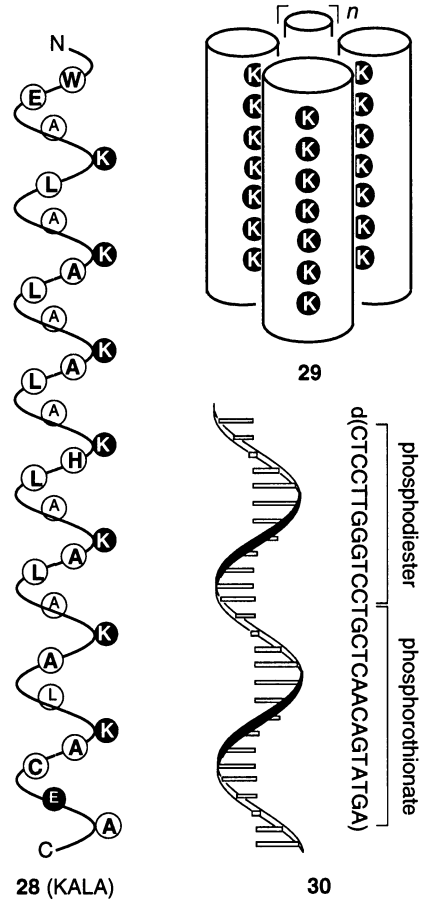

Fig. 9 KALA **28**, a peptide capable of forming membrane-permeabilizing bundles **29**, hosting and translocating single-stranded oligonucleotides in a pH-dependent manner (schematic structures **29** and **30** are mostly hypothetical).

however, not form pores in biomembranes. This functional plasticity of KALA was further pH-dependent, presumably due to the protonation of histidines and glutamates at low pH. This innovative design aspect is significant for controlled oligonucleotide release from acidic endosomes to prevent degradation in the lysosome. Indeed, it was found that KALA can act as nonviral single-component gene delivery system for a variety of cell lines. When used as an endosomolytic component in more complex polymer formulations, the transfection efficiency of commercial agents can be reached.[27]

3.1.3 Molecular transformation. One of the so far unfulfilled promises of synthetic 'barrel-stave' supramolecules is to catalyze templated oligomerizations. Indications that this hope is not that unrealistic come from recent demonstrations that engineered terpenoid synthases can do so.[28,29] The key to insights on this central biooligomerization, leading to the more than 23,000 terpenoids and steroids known today, was the resolution of the first terpenoid synthase structure, *i.e.*, avian FPP synthase **31** (Fig. 10). The dimeric protein comprises an unprecedented, highly expanded bundle formed by ten antiparallel α-helices. Binding sites for the two 'isoprene'-diphosphates **32** and **33** are provided by Mg^{2+}-complexed DDxxD-arrays at the top of the α-barrel, the bottom is hydrophobic. Native barrel **31** catalyzes the synthesis of geranyl diphosphate **34**, as well as elongation of GPP **34** by one more IPP **33** to give the C_{15}-homolog FPP. Elongation of FPP is not possible because of poor substrate binding (K_M(FPP) = 9 μM, K_M(IPP) = 20 μM compared to K_M(GPP) = 0.3 μM, K_M(IPP) = 0.6 μM). Because it was suspected that such chain elongation into the hydrophobic barrel bottom is obstructed by the two bulky F112 and F113 (Fig. 10, inset), they were replaced by smaller residues A and S. [28] The 'new' α-barrel **35** was shown to catalyze templated oligomerization to afford the C_{35}-diphosphate **36** as major oligomer with a length that roughly matches that of the hydrophobic barrel interior (*e.g.*, K_M(FPP) = 0.04 μM, K_M(IPP) = 0.08 μM). Consistent results from many elegant, coinciding and subsequent studies on the expanded bundles used by several terpenoid synthases *and* cyclases have been summarized very recently.[29]

Molecular transformation by *de novo* designed bundles have been reported for self-replicating peptide-coupling, amidation, transamination, hydrolysis, transesterification, and decarboxylation.[30] The supramolecule with highest functional plasticity today is, presumably, bundle **37** comprising two helix-loop-helix peptides **38** with six catalytic histidines each (Fig. 11). The hydrolysis of esters such as **39** by cooperative nucleophilic and acid catalysis in mildly acidic water has been investigated with regard to structural variations of acids **40**, the *p*-nitrophenyl leaving group, and the catalyst itself. Most importantly for the topic of this overview, it was possible to secure qualitative insights on the importance of a hydrophobic bundle interior.[31] Namely, a linear increase of k_{cat}/k_{uncat} was observed for increasing length of the hydrophobic alkyl residues in **39** culminating in a k_{cat}/k_{MeIm} above 700 for *p*-nitrophenyl valerate. Removal of the three N-terminal histidines did not influence this trend. No rate enhancement with increasing ester hydrophobicity was, however, observed when the three C-terminal histidines were removed. Results from further mutations and pertinent NOESY crosspeaks indicate that H34 may be the most potent nucleophile near the hydrophobic core of bundle **37**.

3.2 Higher oligonucleotide helices ('DNA barrels')

Barrel-stave supramolecules can, in principle, be constructed from 'Watson–Crick' duplexes along two distinct routes: Either duplexes are 'compressed' (*e.g.*, A-DNA) or expanded until internal space emerges,[32] or they serve as dimeric staves

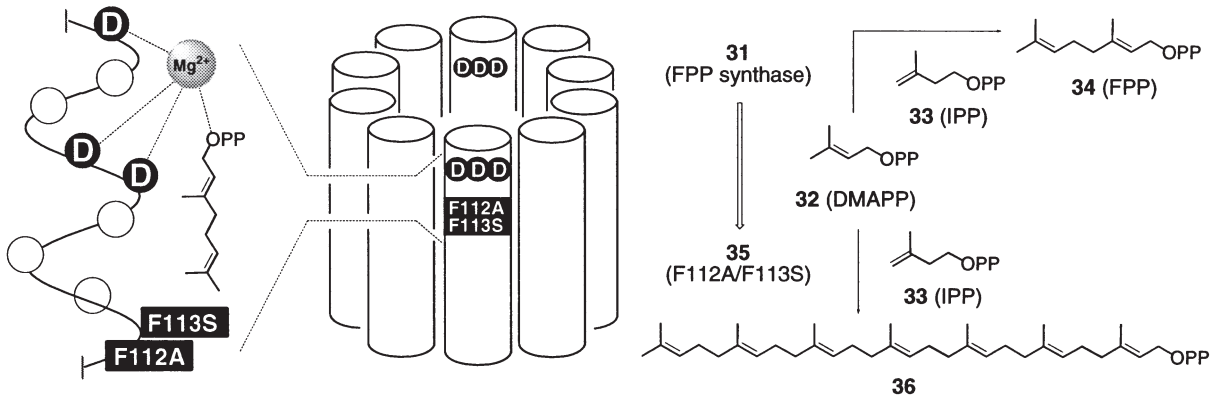

Fig. 10 Conversion of 'barrel' **31** capable of synthesizing C_{10}-terpenoid **34** into 'barrel' **35** able to synthesize C_{35}-terpenoid **36**. The inset shows the enzyme–substrate complex with C_{10}-terpenoid GPP **34**; binding of the corresponding C_{15}-substrate FPP is obstructed in **31** but not in **35**.

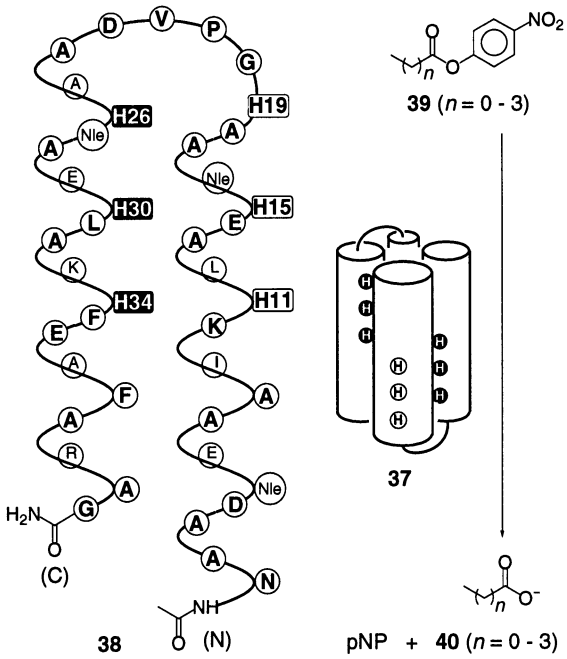

Fig. 11 Peptide **38** capable of forming dimeric bundles **37** and accelerating aryl ester hydrolysis.

themselves.[33] Continuous expansion of duplexes over tri- and quadruplexes has, very recently, culminated in the first example of pentaplexes with internal caesium cations.[32] Barrel-stave supramolecule **41** was constructed based on previous insights on the nature of the G-quartet motif **42** (Fig. 12). Namely, (oligo)guanosines self-assemble in the presence of metal ions to give hydrogen-bonded 'G-quartets' stacked on top of each other with stabilizing cations in between the G-quartet planes. It is assumed that the formation of quartets is preferred because of the 90° angle between the hydrogen-bonding faces. In isoguanosines (isoGs), this sector angle is reduced to 72°, implying that (oligo)isoguanosines should form pentaplex **41**. Elegant gel shift and thymine-photocrosslinking experiments demonstrated that this is the case for oligonucleotides of different length with four central isoGs in the presence of caesium but not potassium cations. The proposed structure of barrel-stave supramolecule **41** has more recently received compelling support from a crystal structure of decameric 5′-*tert*-butyldimethylsilyl-2′,3′-*O*-isopropylidene-isoG with a sandwiched central caesium cation.[34]

Although the potential of G-quartets and thus isoG-quintets with hydrophobic (instead of hydrophilic) staves to form ion channels has been noted, future functional plasticity of this barrel-stave motif seems limited. However, an inspired demonstration that the central cation in G-quartets is not as essential as assumed previously has been reported recently.[35] Together with extensive insights on cyclic hexameric arrays comprising various heterocyclic 60° sectors, this supports the potential of the 'DNA-approach' for the construction of future barrels with appreciable interior and, perhaps, functional plasticity.

3.3 Supramolecular cage architecture ('inorganic barrels')

The usefulness of the precise geometry of metal coordination for molecular architecture is documented in a large number of distinct supramolecules such as helicates, racks, grids, and spheres prepared along this route.[5] Three more recent reports may serve to illustrate the construction of 'inorganic barrels' (Fig. 13). The longest reported 'barrel' **43** comprises three rigid-rod staves with terminal bipyridines.[36] These terminal ligands are complexed *via* Cu^+ to a circular hexadentate ligand with peripheral crowding by phenyl groups to prevent polymerization. Spectroscopic evidence for 'barrel' **43** was consistent with free diffusion of solvent between adjacent staves. Extensive stave variation between the terminal bipyridines demonstrated that stave rigidity and matching length are crucial for barrel formation, and that sub-compartments can be readily installed using central bipyridines or pyrimidines. Crystal structures of truncated analogs of **43** demonstrated that the slight helical stave twist induced by Cu^+ can be avoided by using Ag^+ instead, and that smaller interiors are filled with solvent and/or counterions in solid and, perhaps, also in liquid and gas phase.

'Barrel-stave' triple helicate **44** is representative for a similar approach that focuses on staves of variable rigidity and length with terminal catechol ligands surrounding Ti^{4+} (or other cations).[37] The strained 'barrel-stave-like' supramolecule **44** was chosen as an example from a rapidly growing family because internal guest binding was considered. However, it was found that the presence of potential guests, tetramethylammonium cations, transforms 'barrel' **44** into a tetrahedron.

3.3.1 Molecular recognition. The inorganic architecture with closest similarity to a barrel, however, is tetrameric quinque(3,5-pyridine) **45**.[38] The pyridine staves are planar due to dipole repulsion and joined together in a 90° angle by Pd^{2+}. This intriguing barrel **45** formed *only* in the presence of an internal, topologically matching biphenyl carboxylate (or biphenyl but not *p*-terphenyl or adamantane carboxylate). Host–guest complex **45** was visualized by ESI-MS and upfield shifted guest hydrogen resonances in the ^{1}H-NMR spectrum.

3.4 Synthetic supramolecular 'barrel-stave' ion channels ('organic barrels')

The early appearance of the barrel-stave motif in discussions on possible active structures of ion channels formed by natural

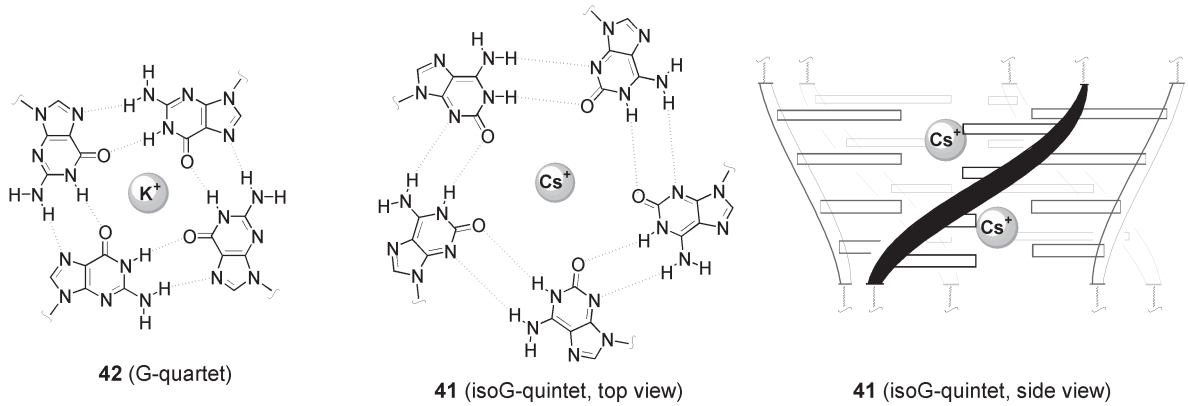

Fig. 12 Barrel-stave supramolecules **41** formed by oligoisoguanosines X-$(isoG)_4$-Y and Cs^+ in comparison with contracted G-quartets **42** (X / Y = T, T_4, or T_8).

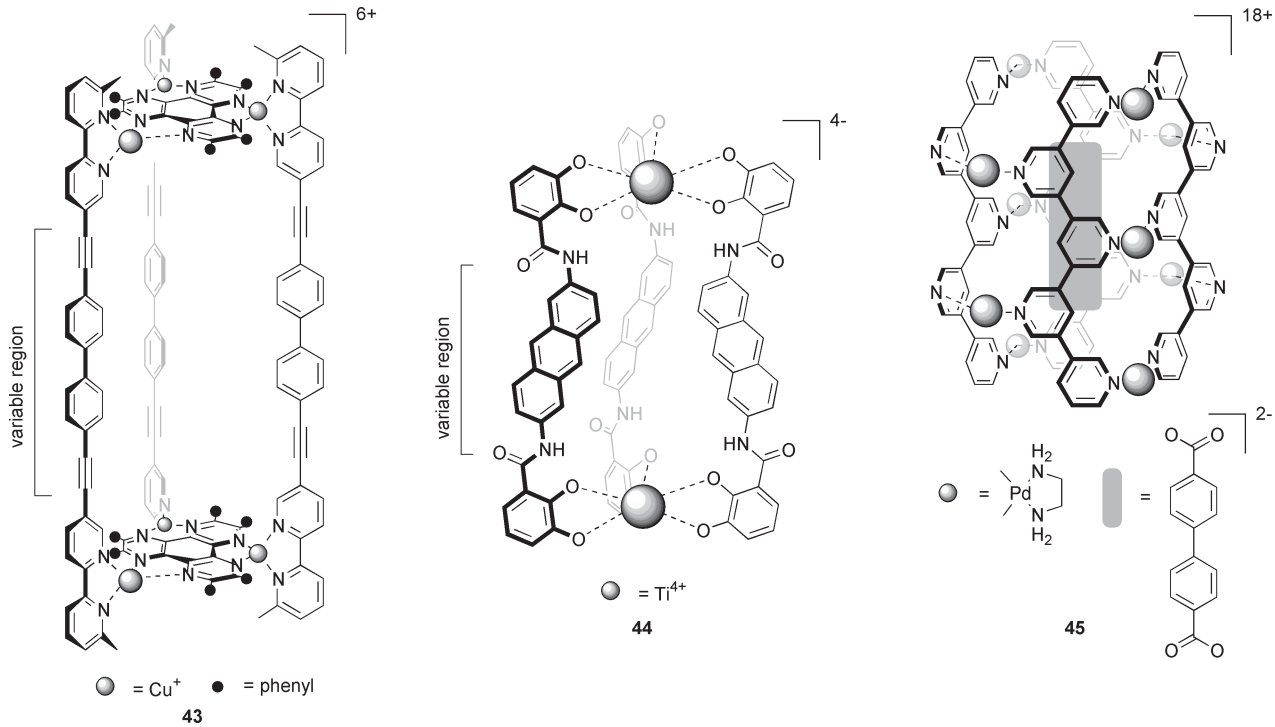

Fig. 13 *Representative* recent barrel-stave and barrel-stave-like supramolecules with (**45**) and without internal guests (**43** and **44**) constructed using coordination chemistry.

products such as amphotericin B is not surprising because the central characteristics of a barrel, its interior (Fig. 1A), is best appreciated as a transmembrane pore. Ironically, it is much more challenging to secure experimental insights on the structure of the barrel-stave supramolecule that actually forms this clearly visible internal space. As a result, most synthetic supramolecular 'barrel-stave' ion channels and ion channel models known today are claimed based on direct or indirect evidence for transmembrane internal space rather than precise information on the suprastructure of the barrel. (Note that this situation is opposite to that with barrels in the liquid phase, where experimental support for the presence and nature of the barrel interior is the most difficult piece of evidence to obtain.) The most recent supramolecular 'barrel-stave' ion channel (models) are summarized in Fig. 14.

3.4.1 Molecular translocation. Relatively rigid, planar steroid scaffolds have been used frequently as 'ministaves'. The cholate derivative **46** is thought to self-assemble into transmembrane 'barrels' because all hydrophobic methyls point to one side and all hydrophilic methoxys to the other to interact with surrounding lipid tails and internal ions, respectively.[39] The terminal ammonium cation may serve to anchor the 'barrel' at the membrane–water interface. Since the cholate scaffold is too short to span a bilayer membrane, 'barrel' dimerization seems necessary to form a transmembrane pore. The formation of such cholate barrels of relatively high stability and small hydrophilic interior was deduced from single channel currents.

The design of cholesterol polyether **47** includes similar considerations.[40] Transport kinetics suggested formation of tetrameric 'barrels'. Attachment of a second sterol nucleus at the amine terminus of **47** affords the corresponding dimers in 'thin' membranes (C14:1, C16:1), while a puzzling transformation into tetrameric 'barrels' occurs in thicker bilayers (C18:1, C20:1). Replacement of the polyether by polyamine tails converts the cation selectivity of **47** into anion selectivity.

Bismacrocycle **48** represents a more flexible, amphiphilic 'stave' that is long enough to span a bilayer membrane.[41] The most interesting asymmetric charge distribution in **48** is thought to account for the sensitivity of active supramolecular 'barrels' to externally applied voltage.

An intriguingly simple 'stave' is provided by biological poly-*R*-3-hydroxybutyrate **49** (PHB).[42] PHBs as well as oligo-*R*-3-hydroxybutyrates (OHBs) of precise length form non-selective, perhaps microcrystalline ion channels in bilayer membranes. PHBs and OHBs are also capable of binding polyphosphates, and the resulting polymer complex forms calcium-selective, voltage-dependent ion channels that can be blocked with transition metals. The latter second-sphere channels have attracted much attention because of their possible role in evolution, as components of biological ion-channel proteins, and as bacterial Ca^{2+}-pumps. A proposed active structure comprises 2_1-helical PHBs (OHBs) **49** as staves forming a barrel filled with calcium polyphosphate (Fig. 14). The presence of an alternative plausible structure with single-stranded PHB helices (Fig. 1C) instead PHB staves (Fig. 1A) illustrates the problematic structure determination in bilayer membrane beyond characterization of a transmembrane interior.

4 Conclusion

The bottom line is that successful extension of the functional plasticity of biological β-barrels to artificial barrel-stave (supra)molecules is increasingly possible. Many routes toward this objective are currently emerging. The construction of either recombinant, *de novo* designed, or synthetic β-barrels beyond peptide chemistry use, in one way or the other, the structural principles that account for functional plasticity of biological β-barrels as well. A rapidly growing number of innovative routes to barrel-stave supramolecules or related motifs with diverse function(s), however, suggests that 'barrel-type' functional plasticity is not necessarily restricted to the presence of β-strands. Ultimately, one would like to enjoy synthetic barrel-stave supramolecules that can be transformed readily to perform desired functions in a predictable manner or, in Gerlt's words, to harvest (*any*) new wine from *new* barrels. The most appealing perspective, however, is to extend the functional plasticity of the barrel-stave motif beyond current understanding, that is to invent new function. Many of the studies presented here were selected to stimulate further progress in this direction.

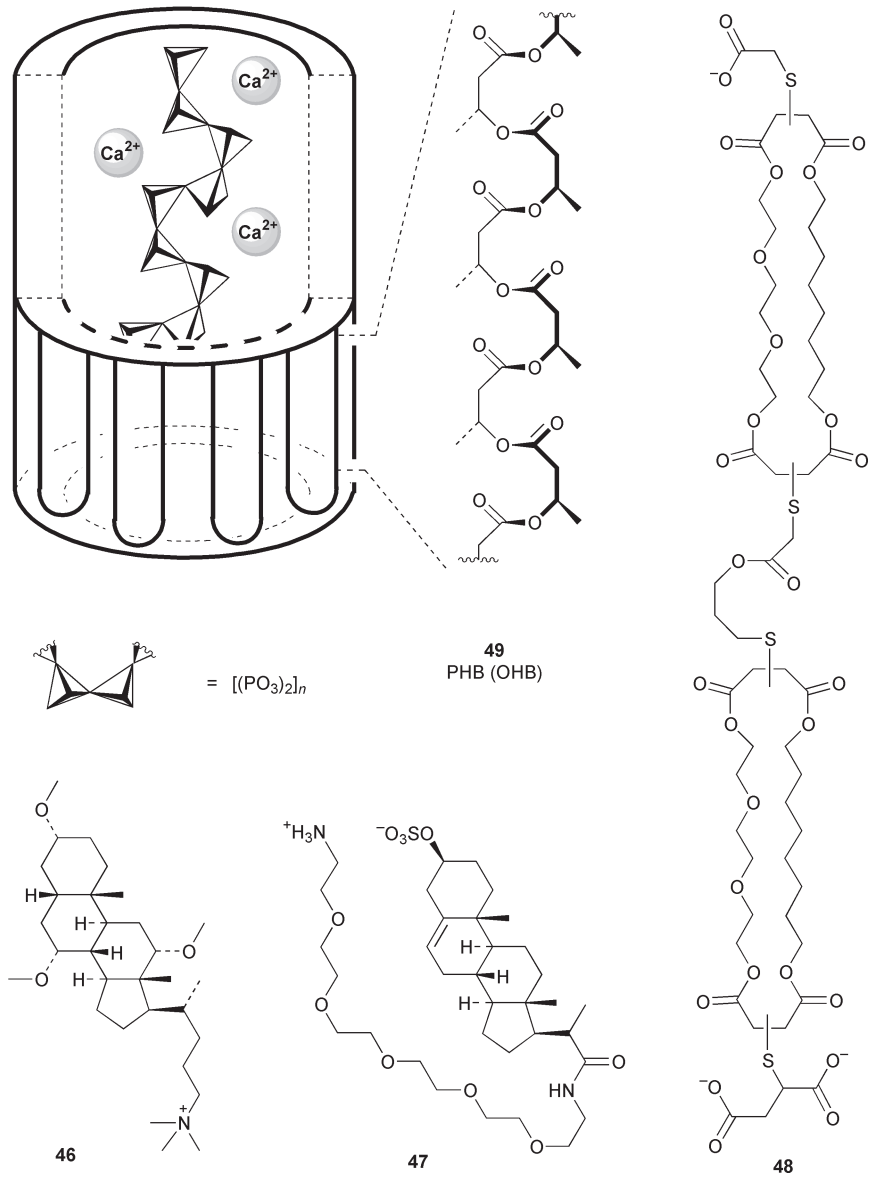

Fig. 14 Most recent 'organic staves' used for the construction of transmembrane barrel-stave supramolecules.

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