A "Holey" Supramolecular Approach to the Detection of Enzyme Activity

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Synthetic transmembrane channels, while possessing much simpler structures than their biological counterparts,[1, 2] have the potential to serve as selective membrane transport conduits. Several different approaches have been taken to the construction of these species by using either single molecules or multicomponent assemblies. In early work, Yoshiaki Kobuke and co-workers found that carboxylate-terminated polyglycol chains surround dioctadecyldimethylammonium cations in lipid bilayer membranes and allow cation transport through the assembly.[3] More recently, Kobuke has reported ion-channel formation and transport of potassium cations by resorcin[4]arenes appended with amphiphilic cholic acid moieties.[4] Tom Fyles and co-workers have constructed transmembrane channels from single molecules containing a central crown ether unit attached to walls consisting of glycol and alkyl chains terminated with hydrophilic sugar head groups.[5] This group has also reported voltage-gated synthetic ion channels based on bis-macrocyclic bola-amphiphiles.[6] Jean-Marie Lehn and co-workers have synthesised "bouquet"-type molecules containing a central crown ether or cyclodextrin core linked to polyglycol chains terminated in carboxylate groups. These species were shown to mediate both sodium and lithium cation transport.[7, 8] Crown ethers have been employed by other groups in different ways. For example, Normand Voyer has constructed polypeptides with appended [21]crown-7 units such that, when the peptide adopts an α-helical conformation, the crown ethers line up and form a channel.[9, 10] Hicham Fenniri and co-workers have taken an alternative approach using crown ether functionalised heteroaromatic bicyclic bases to form stacks of hydrogen-bonded rosettes.[11] In addition to his early work on cation channels,[12] George Gokel and co-workers reported a peptidic synthetic chloride membrane transporter system whose design incorporated structural patterns from naturally occurring chloride transporters.[13]

Yet transmembrane channels can do more than mediate the transport of chemical species. For example, if control of transport across a membrane can be linked to a separate chemical process occurring in the system, then one possible application of the channel would be to function as a component of a sensor. Stefan Matile and co-workers Gopal Das and Pinaki Talukdar at the University of Geneva have recently demonstrated this principle by developing a new high-throughput fluorometric method for monitoring enzyme activity that employs selective blocking and unblocking of synthetic peptidic transmembrane channels.[14]

There are a number of structural options available when designing a peptidic artificial channel to span a membrane (Figure 1). These include the use of assembly of D,L-cyclopeptides into hydrogen-bonded peptide nanotubes (Figure 1A). Reza Ghadiri and co-workers at Scripps have been pioneers in this area, constructing a variety of channels that are capable of transmembrane transport.[15] The transport of chemical species through these channels is dependant on the size of the pore. For example, glucose and glutamic acid are too large to pass through octapeptide nanotubes, although they may pass freely through larger decapeptide nanotubes.[16, 17] In addition, these assemblies have been shown to possess antibiotic properties as they disrupt the structural integrity of the membrane.[18] An alternative approach to channel construction is to employ bundles of α-helical peptides (Figure 1B). These types of channel consist of several α-helical staves with amphiphilic surfaces that assemble in the membrane and so form a channel with a hydrophobic exterior surface and hydrophilic groups lining the channel interior. Such channels have been shown to allow transport of single-stranded DNA through bilayer membranes.[19] Biological helical peptides, such as gramicidin,[20–22] have provided the structural backbone for new photoswitchable ion channels. Andrew Woolley and co-workers prepared a gramicidin analogue containing a photoswitchable azobenzene side chain.[23] Photoisomerism of this group changes its dipole moment, thereby modulating the sodium and caesium cation conductance of the channel. Alternatively, one may construct higher-order helical assemblies from peptide chains (Figure 1C) or construct a β-barrel—a cylindrical structure formed by β-sheets of peptides (Figure 1D). Hagan Bayley and co-workers have explored the use of modified staphylococcal α-haemolysin (αHL) pores (the transmembrane portion of which consists of a β-barrel) to detect a variety of species, including metal ions, organic molecules and proteins, passing across lipid bilayers. In these cases, the detection of transmembrane transport of the analyte was achieved by measuring the current across the lipid bilayer.[24] In landmark work, attachment of an individual oligonucleotide within the lumen of the α-haemolysin pore was also achieved thereby forming a "DNA nanopore". The binding of single-stranded DNA to the tethered DNA modulated the ionic current flowing through the pore allowing identification of individual DNA strands with single base resolution.[25]

Matile has recently developed new synthetic supramolecular β-barrels that are constructed from molecular units containing a rigid octiphenyl stave to which short peptide chains are attached (Figure 2).[26] When chains on adjacent octiphenyl groups interdigitate they form

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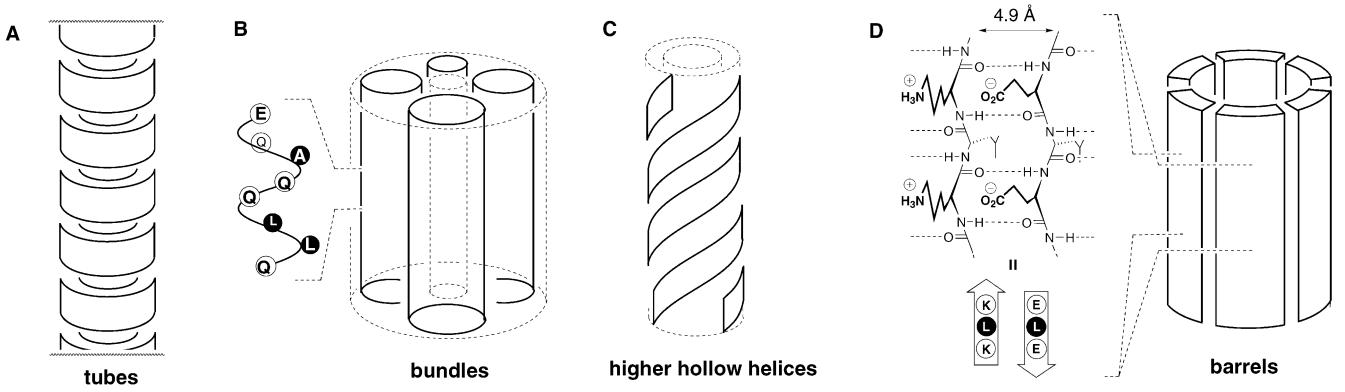


Figure 1. *A) Nanotubes composed of cyclic molecules rather than staves, B) bundles comprising voluminous staves, C) higher hollow helices and D) barrel stave architecture. β-Strands are depicted as arrows pointing to the C terminus, α-helices (and loops) as bold lines, amino acid residues (one letter abbreviation) pointing to the exterior of the tertiary structure are depicted black on white, internal residues are white on black. Adapted from an illustration appearing in ref. [27], Copyright 2001, reproduced by permission of the Royal Society of Chemistry.*

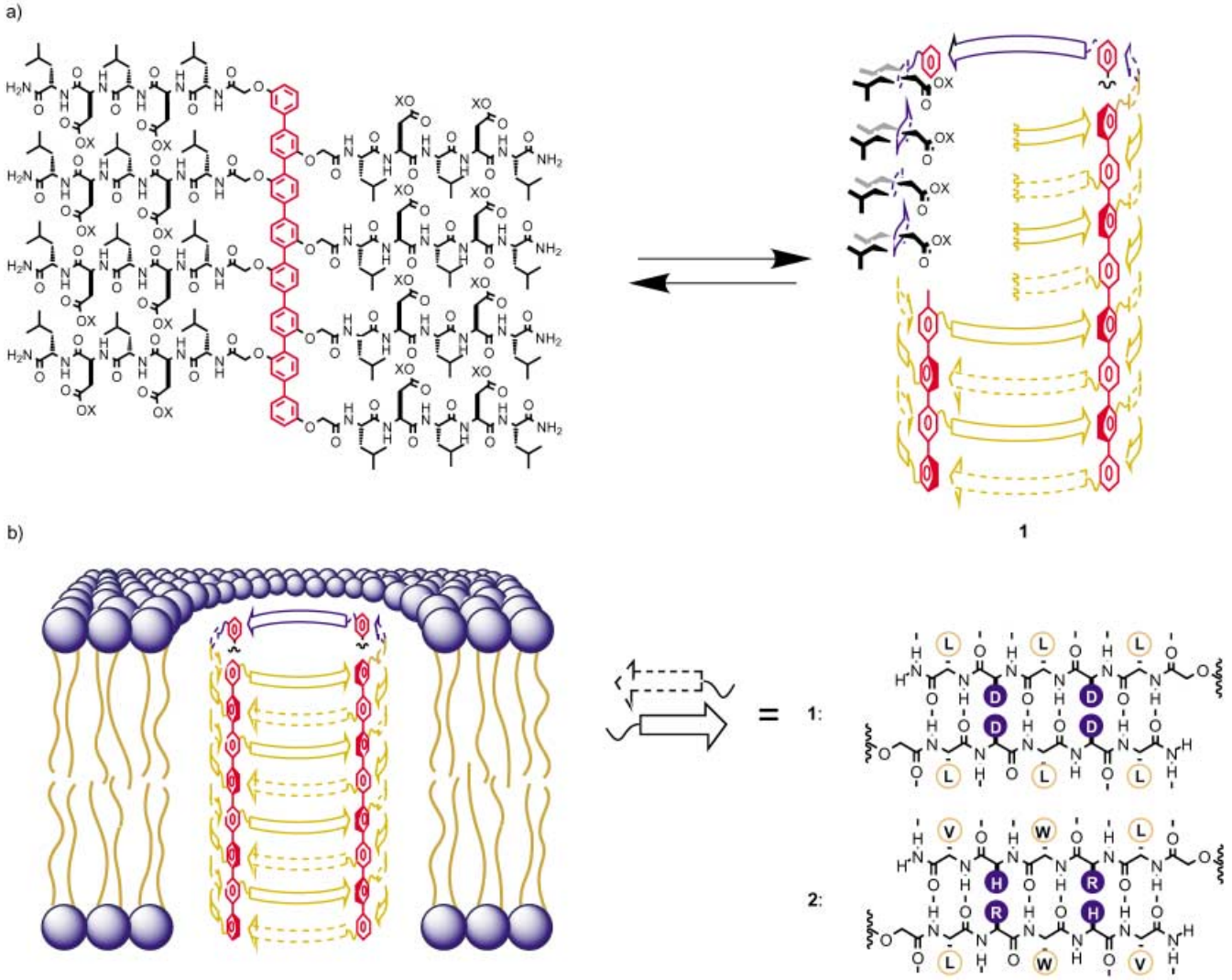
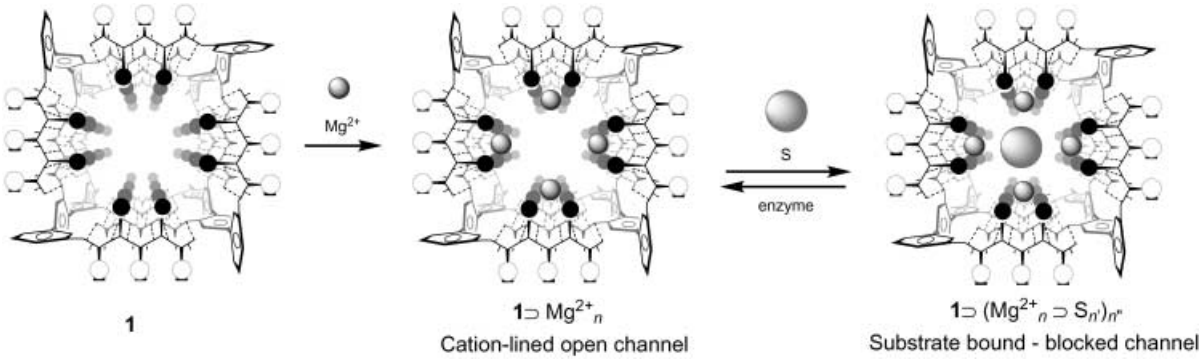


Figure 2. *A) Self-assembly of peptide-functionalised* p*-octiphenyl monomeric rods. The octiphenyl staves are highlighted in red whilst the exterior surface of the barrel is brown and the interior surface blue. X = H, M; M = metal ion: 0.5 Mg^{2+}, 1.0 Na^{+} etc. depending upon conditions. b) The β-barrel in a lipid bilayer. Amino acids located on the outer surface are black on white; internal ones are white on blue. One letter abbreviations used (D, Asp; H, His; L, Leu; R, Arg; V, Val; W, Trp).*



Scheme 1.

antiparallel β-sheets, and, because of twists in the stave due to the arene–arene torsion angles (∓180 °C) and steric crowding of the *N*- and *C*-terminal amino acid residues, the self-assembled structures are curved, so allowing the formation of rigid-rod β-barrel structures. Perhaps the most important feature of this approach to synthetic-channel formation is its versatility. It is possible to choose the identity of the amino acid residues that coat the exterior and interior surfaces of the barrel and hence tune the barrel's solubility (exterior) and transport ability (interior). Matile calls this "functional plasticity"[27] as the barrels are adaptable for use in different applications without making changes to the underlying structure that constitutes the cylinder. For example, barrel **1** shown in Figure 2 has leucine groups on its exterior that confer solubility in a lipophilic environments (such as within a cell membrane) and a polar interior lined with Asp groups.

Matile introduced magnesium ions into barrel **1** that bound to the aspartate groups so transforming the interior surface into a cationic channel **1** ⊃ $Mg^{2+}{}_n$ (Scheme 1). This allowed the barrel to act as a host for a variety of larger anionic species.[28] Matile found that ATP would block the channel but that AMP and P_i were ineffective channel blockers. This finding allowed the channel to be used to monitor the effectiveness of phosphatase enzymes (e.g. potato apyrase, a nonspecific ATPase) that convert ATP into the smaller phosphate-containing species. The channels were incorporated into large unilamellar vesicles (LUVs) containing high (self-quenching) concentrations of a fluorescent dye [5(6)-carboxyfluorescein (CF)]. When channel **1** ⊃ $Mg^{2+}{}_n$ containing ATP was unblocked by the action of the enzyme, the CF dye passed through the channel and escaped from the vesicle. Enzyme activity could then be monitored quickly and easily by following the increase in fluorescence as the concentration of dye within the vesicle fell. Matile demonstrated that weak binding of the magnesium cations inside the channel led to high dissociation constants of the substrates from the channels and hence designed barrel **2** (Figure 2) to overcome this problem. Barrel **2** contains histidine–arginine dyads on its interior walls while valine, tryptophan and leucine groups constitute its exterior surface. The interior arginine groups form strong complexes with phosphates,[29, 30] leading to reduced dissociation constants with the substrates and hence higher sensitivities (> 1000) for activity sensors constructed from barrel **2** as compared to **1** ⊃ $Mg^{2+}{}_n$. The detection system was also applied to other classes of enzymes (and phosphate substrates) that are useful in the development of enzymatic carbohydrate and oligosaccharide synthesis. Bovine milk galactosyltransferase is an enzyme that can have the opposite effect on barrel **2**. The enzyme produces uridine diphosphate (a good channel blocker) from UDPGal and GlcNAc (poor channel blockers) and hence the activity of this enzyme may be monitored by a decrease in the transport ability of the channel. This system also demonstrates that it is possible to switch on *or* switch off the transport of species through barrel **2** solely by using enzymes. One could therefore regard these channels as being "enzyme-gated".

The broad applicability to a wide variety of enzymes and substrates of Matile's approach to sensing activity (and the avoidance of patch-clamp techniques[31, 32] to monitor transport of species through the channels) suggests that this technique will become an important tool for the chemical biologist for future high-throughput screening of enzyme activityl.[24] Meanwhile, applications for Matile's β-barrel channels in other areas such as catalysis are already being discovered.[33]

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