Self-Assembly of Facial Amphiphiles in Water

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1 HEAD/TAIL AMPHIPHILES VERSUS FACIAL AMPHIPHILES

Amphiphiles, from the Greek *amphis* for "both" and *philia* for "friendship," are chemical compounds containing both a hydrophilic and a hydrophobic segment. Owing to their affinity for both hydrophilic and hydrophobic domains this class of molecules often interacts with interfaces between hydrophobic and hydrophilic phases and they are also often referred to as surfactant (from surface active agent). In the absence of free hydrophobic/hydrophilic interfaces, these amphiphiles can interact with each other because of hydrophobic interactions between the hydrophobic segments and assemble in water in a wide variety of architectures. Because of these properties, amphiphiles are widely found in everyday life fulfilling many different functions.

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There are many different amphiphiles with a large variety of chemical structures and sizes, ranging from simple surfactants consisting of one or more hydrophobic alkyl chain connected to a single hydrophilic head group, gemini and bolaform surfactants containing two head groups, up to macromolecular block copolymers containing a hydrophilic and a hydrophobic polymer segment. The most common class of amphiphiles consists of head/tail amphiphiles, where the hydrophilic and the hydrophobic group are distributed along the length axes of the molecule. These amphiphiles adopt a cylindrical or a cone-like structure in water• (Figure 1a). To prevent exposure of the hydrophobic moiety to the aqueous phase, these amphiphiles aggregate by bringing their hydrophobic moieties together. Because the hydrophobic moieties are exposed on a cylindrical surface, self-assembly extends in directions perpendicular to the normal of the surface (Figure 1b). In other words, the hydrophobic interactions of normal head/tail amphiphiles are divergent and their self-assembly results in larger aggregates. The morphology of such aggregates can be predicted by the structure of the amphiphile via the structure-shaped concept.[1] For each head/tail amphiphile, a so-called packing parameter (p) can be calculated as $p = V_H/l_c a_0$ in which V_H is the volume of the hydrophobic segment, l_c is the length of the molecule, and a_0 is the cross-sectional area of the head group. This packing parameter is directly related to the architecture formed. If $p < 1/3$ spherical micelles are expected, for $1/3 < p < 1/2$ cylindrical micelles are expected, for $1/2 < p < 1$ lamellar structures such as bilayers and vesicles are expected, and for $p > 1$ inverted micelles are expected (Figure 2).

Beside the head/tail amphiphiles, there are other classes of amphiphiles with a distinct architecture and different properties. One of these classes of amphiphiles is formed

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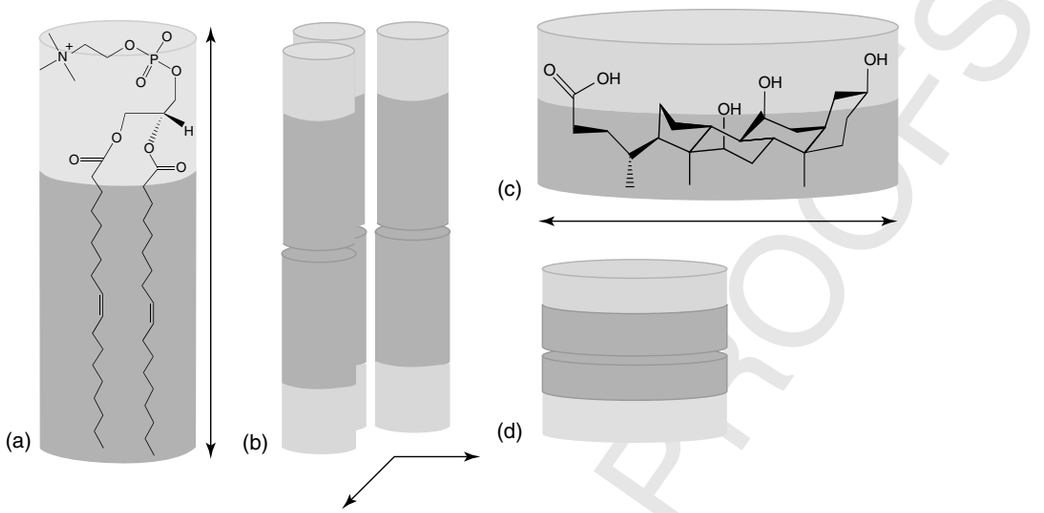

Figure 1 (a) Structure of dioctadecylphosphatidylcholine (DOPC), a classical head/tail amphiphile. (b) Hydrophobic interactions of such amphiphiles are divergent, and therefore their self-assembly can extend in any direction away from the cylindrical surface (black lines). (c) Structure of cholic acid, a typical facial amphiphile, in which the hydrophilic and hydrophobic groups are separated along the long axis of the amphiphile. (d) Self-assembly of facial amphiphiles is self-complementary and convergent. Dark gray areas indicate hydrophobic domains, whereas light gray areas indicate hydrophilic domains.

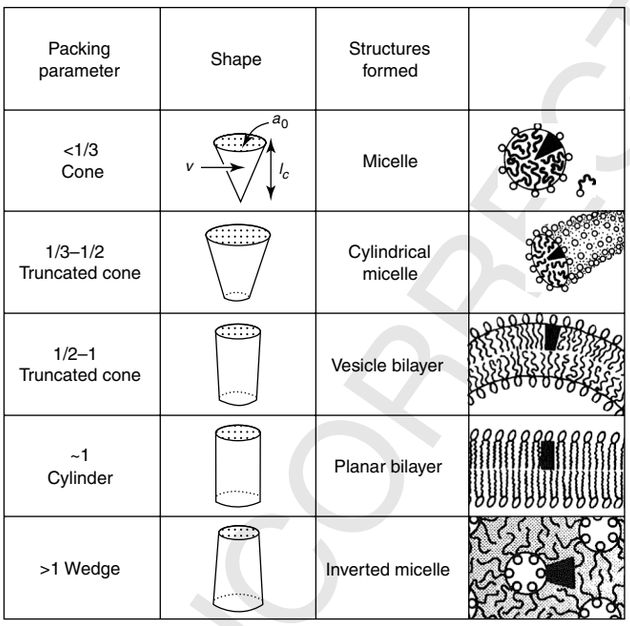

Figure 2 The structure-shaped concept of head/tail amphiphiles. The packing parameter of the amphiphile determines the structure of the aggregate formed on self-assembly. (Reproduced from Ref. 1. © Royal Society of Chemistry, 1976.)

by the so-called facial amphiphiles. Facial amphiphiles are distinguished from head/tail amphiphiles by the distribution of their hydrophobic and hydrophilic groups. In the classic head/tail amphiphiles, the hydrophobic and hydrophilic segments are separated along the short molecular axis, but in facial amphiphiles they are separated along the long molecular axis (Figure 1c).[2] Because of their separation along the longitudinal axis, the hydrophobic and hydrophilic moieties are almost fully exposed on either face of the amphiphile, hence the name facial amphiphiles. For these type of amphiphiles, it can be argued that the packing parameter $p \sim 1$, which according to the structure-shaped concept would lead to the formation of bilayers. However, research in the past decades has shown that facial amphiphiles usually form different kind of assemblies. Because with facial amphiphiles, the hydrophobic moieties are almost fully exposed at one face of the molecule, interaction with one other hydrophobic domain of a similar amphiphile will almost completely covers both hydrophobic domains from the solvent. In other words, self-assembly of these facial amphiphiles is self-complementary and the exposure of their hydrophobic interactions is convergent (Figure 1d). Therefore, the self-assembly behavior of facial amphiphiles is markedly different from the better-known head/tail amphiphiles, but they also show distinct behavior at hydrophobic/hydrophilic interfaces, such as air/water or oil/water interface. Most interestingly, these different properties are associated with a number of interesting and important biological functions and applications which are unique to facial amphiphiles. In this article, we gave an overview of the self-assembly and interfacial behavior of various natural as well as synthetic facial amphiphiles and discuss their most important biological functions and applications.

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2 STRUCTURAL FEATURES OF FACIAL AMPHIPHILES

Just like head/tail amphiphiles, facial amphiphiles are found in a variety of chemical structures and sizes, ranging from small molecules such as bile acids to biopolymers such as amphiphilic alpha helices.

A common class of small molecular facial amphiphiles are the bile acids. Bile acids and their salts are found mainly in the bile of mammals and are made in the liver by oxidation of cholesterol.[3] Cholesterol is a relatively flat-fused hydrocarbon ring system and therefore has two hydrophobic faces. Enzymatic oxidation in the liver leads to the attachment of three hydroxyl groups as well as a carboxylic acid to one face of the fused ring system rendering it hydrophilic, whereas the other unchanged face remains hydrophobic (Figure 1c). Many studies on bile acid derivates showed that these compounds are very effective in stabilizing emulsions but can also serve as antimicrobial agents.[4] Both topics are discussed in following sections.

Facial amphiphilic peptides are another class of facial amphiphiles, which play an important role in many biological processes involving lipid bilayer membranes. Because of the large surface area of the amphiphilic domains, they are prone to interact with the hydrophilic/hydrophobic interface of lipid bilayers, which is necessary to assist in membrane fusion or transmembrane pore formation. In case of the pore-forming antibiotics, the peptides are often relatively small (between 25 and 100 amino acids) and the entire peptide becomes facially amphiphilic on folding into the secondary structure. In case of the membrane fusion or curvature inducing proteins only the peptide fragment, which interacts with the bilayer membrane, is facially amphiphilic.

The largest group of facial amphiphilic peptides consists of the alpha-helical peptides. Facial amphiphilic alpha helices, often referred to as facial amphiphilic alpha helices, are not amphiphilic in their random coil conformation and their amphiphilicity is not directly obvious from their sequence. However, folding of the peptide into its preferred secondary structure, leads to the formation of an alpha helix, of which the hydrophilic amino acids occupy one face and the hydrophobic amino acids are located at the other face.[5] Alpha-helical peptides have a periodicity of 3.6 amino acid residues per turn, and because of this, for two turns, roughly every third and seventh amino acids are on the same face of the alpha helix. In order to make a helix amphiphilic, the sequence of amino acids should alternate between hydrophobic and hydrophilic every three to four residues, which becomes more clear in a helical wheel representation (Figure 3). An example of such a facial amphiphilic alpha helix is magainin 2, a 23 amino acid antibiotic peptide.[6] Studies have shown that magainin

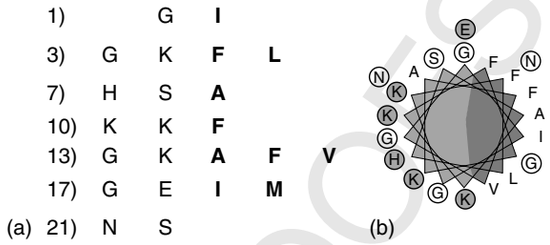

Figure 3 (a) Amino acid sequence of magainin 2 with hydrophobic amino acids highlighted. (b) Helical wheel presentation of magainin 2, an amphiphilic peptide.

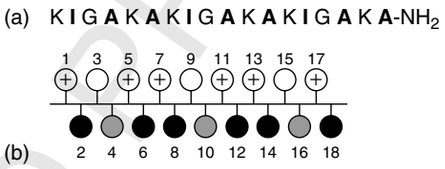

Figure 4 (a) Amino acid sequence of antibiotic peptide described by Prasad Kari and coworkers with hydrophobic amino acids highlighted in bold and (b) its beta-strand diagram. Dark shaded circles are hydrophobic amino acids, whereas white circles are hydrophilic. (Reproduced with permission from Ref. 9. © American Society for Biochemistry and Molecular Biology, 2001.)

2 has a random coil conformation in solution.[7] However, incorporating of magainin 2 into a lipid bilayer induces folding into a facial amphiphilic alpha helix (Figure 3).

Another class of facial amphiphilic protein domains are the amphiphilic beta sheets. Like alpha helices, amphiphilic beta sheets can be a substructure of larger proteins, as in beta-barrel proteins, or these beta sheets can be the entire peptide, as is the case with the smaller antibiotic facial amphiphilic beta sheets. If the sequence of beta-sheet peptides alternates between hydrophobic and hydrophilic residues,[8] one face of the beta sheet is hydrophilic and the other is hydrophobic. An example such a facial amphiphilic beta-sheet peptide strand is the synthetic antibiotic described by Prasad Kari and coworkers.[9] This 18-sequence peptide has alternating hydrophobic and hydrophilic amino acids and it has been shown that it folds into a facial amphiphilic beta sheet in the presence of a bilayer (Figure 4).

3 SELF-ASSEMBLY OF FACIAL AMPHIPHILES IN WATER

In normal head/tail amphiphiles, the hydrophobic interactions are spatially divergent, but because of their specific molecular architecture the hydrophobic interactions of facial amphiphiles are much more convergent. They

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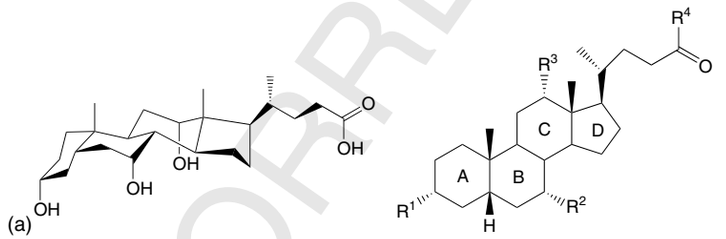
also have relatively large hydrophilic and hydrophobic surfaces, which are roughly equal in size. As a consequence, the properties of facial amphiphiles are clearly different from those of normal head–tail amphiphiles. For instance, assemblies of facial amphiphiles have relatively low aggregation numbers and have excellent solubilizing and emulsifying properties. The latter properties are discussed in more detail in the next section. Facial amphiphiles give rise to not only a wide variety of self-assembled architectures, including spherical-like[10,11] and worm-like micelles[12] and vesicles,[13,14] but also less common morphologies unknown for head/tail amphiphiles, such as molecular clips and two molecule micelles.

One key property of facial amphiphiles is the low aggregation number found with many micellar assemblies of facial amphiphiles. For instance, the aggregation numbers of bile acids **3**, **6**, and **7** are approximately 6, 3, and 5, respectively (Figure 5a and b).[15,16] This is in strong contrast with the much larger aggregation numbers for micelles formed by head-to-tail surfactants, e.g. ∼80 for CTAB[17] or ∼50 for SDS[18] (CTAB, cetyl trimethylammonium bromide, SDS, sodium dodecyl sulphate), making bile acids very efficient in the formation of micelles. It should be noted that the critical micelle concentrations of bile acids are in the same range as for common head/tail surfactants (0.9 mM for CTAB[19] and 8.2 mM for SDS[20]).

This marked difference in aggregation number is a direct result of the facial amphiphilicity. Since the hydrophobic domain is mainly located on one face, self-assembly is complementary and convergent. This is confirmed by the model of bile acid micelles proposed by Small where two or four molecules assemble because of a hydrophobic collapse between the cholesterol domain. The carboxylic acid side groups are on opposite sides of the micelle protecting these sides from water (Figure 5c).

Several studies have been conducted on derivatives of cholic acid, which were easily be obtained by functionalization of the hydroxyl groups. For instance, to further increase the hydrophilicity, these hydroxyl groups have been replaced by quaternary amines resulting in larger spherical micelles with a higher aggregation number in aqueous solution.[25] Other examples involve functionalization with sugars,[2] carboxylates,[26,27] amino acids,[28] and sulfonates.[29] If, however, only the carboxylic acid moiety is functionalized with a long-alkyl chain, the cholic ester stacks to form highly ordered sheets[30] or fibers, depending on the solvent.[31,32] Another example of facial amphiphiles that form aggregates with a low aggregation number is the amphiphilic clips shown in Figure 6(a).[33,34] Depending on the counterions, these facial amphiphiles assemble into dimers or larger-sized aggregates in a wide range of solvents including water.[35,36]

Self-assembly of synthetic facial amphiphile is, however, not restricted to assemblies with low aggregation numbers. For instance, very large and well-defined supramolecular assemblies are formed by the rigid-flexible macrocycle constructed from a rigid aromatic oligomer as the hydrophobic face and a flexible oligo (ethylene glycol) as



	R^1	R^2	R^3	R^4	Cmc (mM)	Agg #
1 NaC	OH	OH	OH	ONa	13.8	
2 NaCDC	OH	OH	H	ONa	7.0	
3 NaDC	OH	H	OH	ONa	5.25	5.82
4 NaGC	OH	OH	OH	$NHCH_2CO_2Na$	12.02	
5 NaGDC	OH	H	OH	$NHCH_2CO_2Na$	4.26	
6 NaTC	OH	OH	OH	$NHCH_2CH_2SO_3Na$	4.33	2.59
7 NaTDC	OH	H	OH	$NHCH_2CH_2SO_3Na$	2.98	5.42

(b)

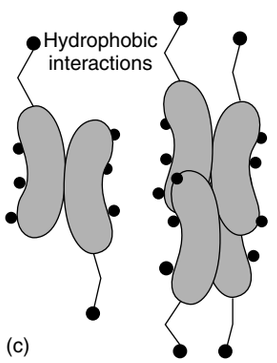


Figure 5 (a and b) Chemical structure of the sodium salt of several bile acids: **1** cholic acid, **2** chenodeoxycholate, **3** deoxycholic acid, **4** glycocholic acid, **5** taurocholic acid, and **6** tauro deoxycholic acid. (c) Structures of micelles from cholic acid derivatives, proposed by Small and coworkers. Two or four molecules assemble because of hydrophobic interactions between the cholesterol groups. The hydroxyl groups (black dots on cholesterol) and the carboxylic acids side group shield the hydrophobic domain from water. (Reproduced with permission from Ref. 21. © Indian Academy of Sciences, 2004.) (Refs. 22–24 for cmcs and Ref. 16 for aggregation numbers.)

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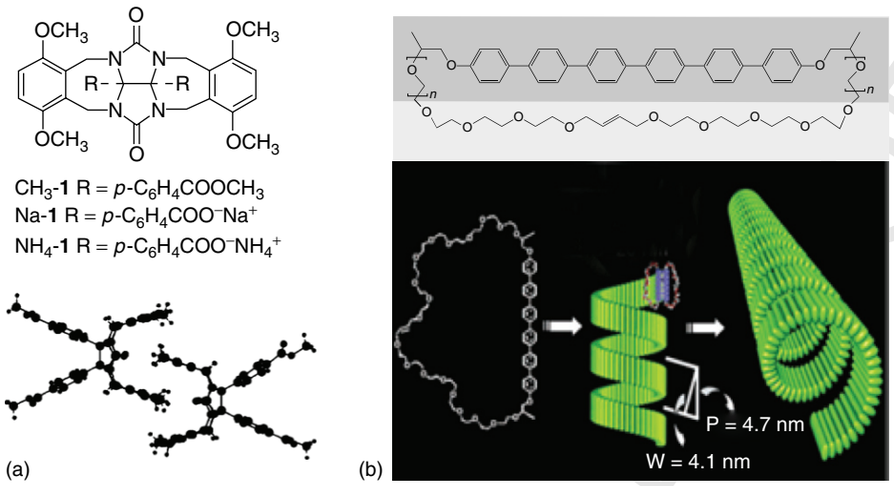

Figure 6 (a) Facial amphiphilic molecular clips forming dimers. (Reproduced with permission from Ref. 33. © Springer, 2001.) (b) Rigid-flexible cyclic facial amphiphile assembling in a bilayer strap which in turn folds into a tubular structure. (Reproduced with permission from Ref. 38. © American Chemical Society, 2006.)

the hydrophilic face (Figure 6b). In the absence of solvent, these macrocycles tend to aggregate into highly ordered bilayers with the rigid hydrophobic aromatic domains in the core due to $\pi-\pi$ stacking.[37] In aqueous solution, these molecules assemble into an infinite bilayer strap, which in turn folds into a helix, giving a helical tubular structure with a hydrophilic interior and exterior.[38]

Not only small molecular facial amphiphiles have been described but also polymeric facial amphiphiles. Amphiphilicity can be induced in polymers by using hydrophilic and hydrophobic blocks. Self-assembly of such head–tail amphiphilic block copolymers has extensively been described and is controlled by phase separation of the hydrophilic and hydrophobic blocks and their volume fractions.[39] Contrary, facial amphiphilic polymers can be obtained from alternating copolymers of hydrophilic and hydrophobic monomers, or by polymerization of facial amphiphilic monomers.

An interesting example of such facial amphiphilic polymers is given by McCullough *et al*.[40] These facial amphiphilic polythiophenes consist of alternating hydrophilic ethylene glycol/hydrophobic alkyl chains substituted thiophenes (Figure 7a). This polymer self-assembles into well-ordered monolayers at an air–water interface because of hydrophobic interactions between the alkyl chains as well as $\pi-\pi$ interactions between the thiophene moieties. This high degree of organization can be related to the large surface area of the hydrophobic and hydrophilic face as well as the rigidity of the polythiophene backbone.

A system analogous to the amphiphilic polythiophenes is based on oligophenylene ethynylene, which is turned into a facial amphiphilic polymer by the attachment of ammonium groups on one face and aliphatic tails on the other (Figure 7b). These facial amphiphiles self-assemble in aqueous solution to form ordered layers and were found to be efficient stabilizers of oil–water emulsions.[41,42] The stabilization of emulsions by facial amphiphiles is discussed in more detail in the next section.

Another class of natural facial amphiphilic polymer able to self-assemble into supramolecular architectures is the so-called leucine zippers.[43] Owing to their large hydrophobic and hydrophilic surfaces, these amphiphiles can form architectures with low aggregation numbers. These facial amphiphilic alpha helices are characterized by seven amino acid repeating unit (a–b–c–d–e–f–g), where a and d are hydrophobic and on folding an amphiphilic alpha helix is created. Two of such helices can aggregate through hydrophobic interactions, as well as hydrogen bonding to form a coiled-coil. Several mutants have been investigated and show that mutations in both the hydrophobic and hydrophilic domain result in the formation of dimers, trimers, or tetramers.[44]

Dendrimers are another type of macromolecules, which are frequently used as building blocks of supramolecular architectures because of well-defined structures. Numerous examples of amphiphilic dendrons and dendrimers exist, giving rise to various architectures on self-assembly. In some cases, dendrimers behave as facial amphiphiles although this is not obvious from the molecular structure (Figure 8a). The aryl ether dendrimers shown in Figure 8 contain both hydrophobic and hydrophilic substituents at each generation, and as a consequence they adopt a facial amphiphilic conformation in aqueous solution (Figure 8b), *which* self-assembles into micellar aggregates of 10–40 nm in diameter.[45]

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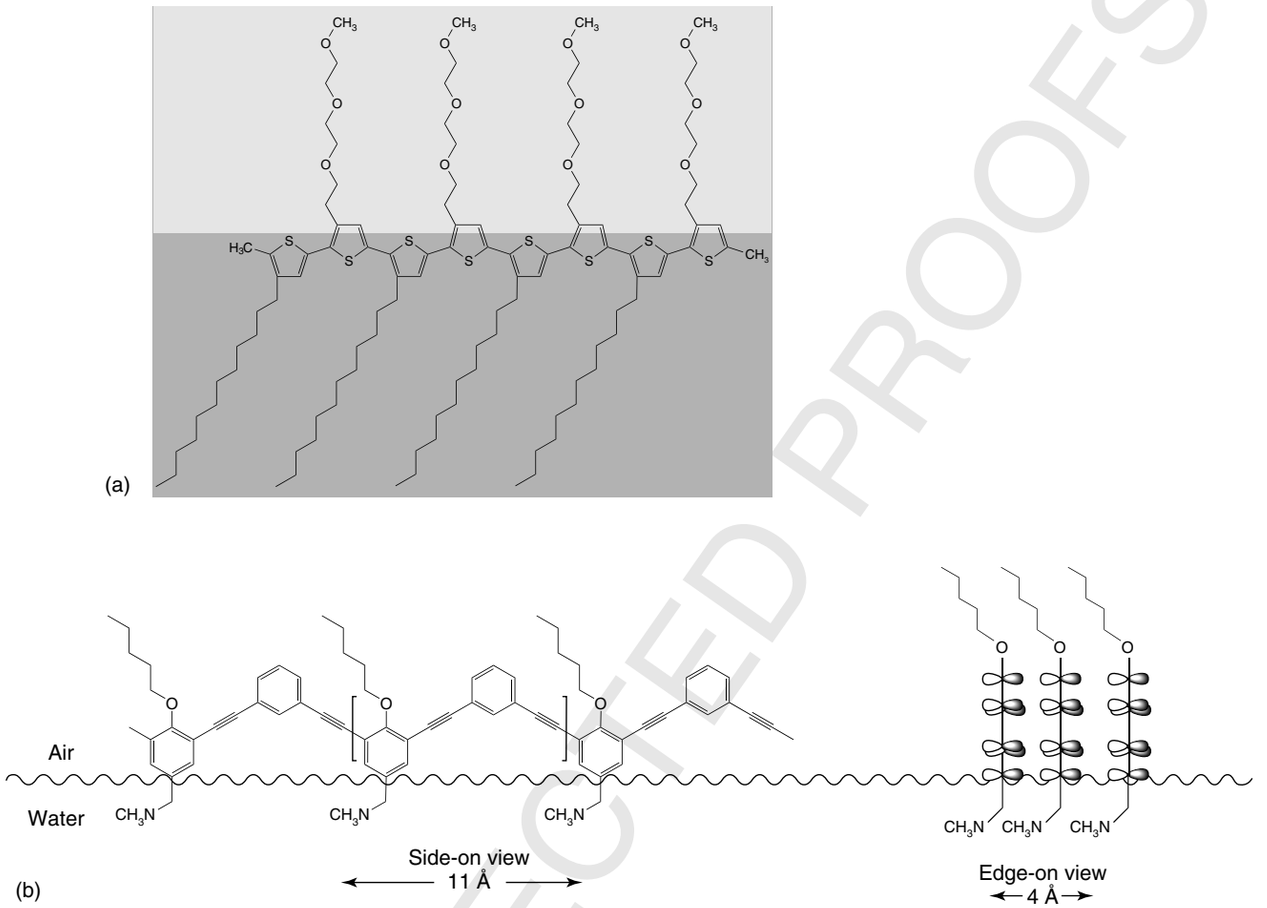

Figure 7 (a) Facial amphiphilic polythiophenes described by McCullough and coworkers and (b) the rigid oligophenylene ethynylenes described by Arnt and coworkers are known to form ordered structures at a water–air interface because of hydrophobic interactions and $\pi-\pi$ stacking. (Reproduced with permission from Ref. 42. © American Chemical Society, 2003.)

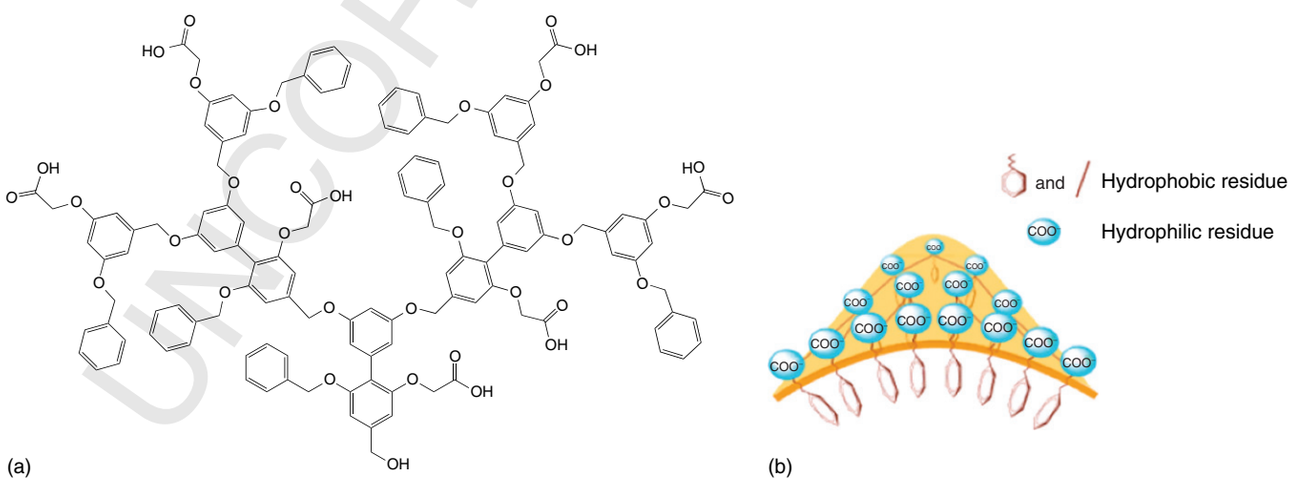

Figure 8 (a) Molecular structure of a dendrimers described by Thayumanavan *et al*. (b) Facial amphiphilicity of the molecule becomes clear in aqueous solution. (Reproduced with permission from Ref. 45. © American Chemical Society, 2006.)

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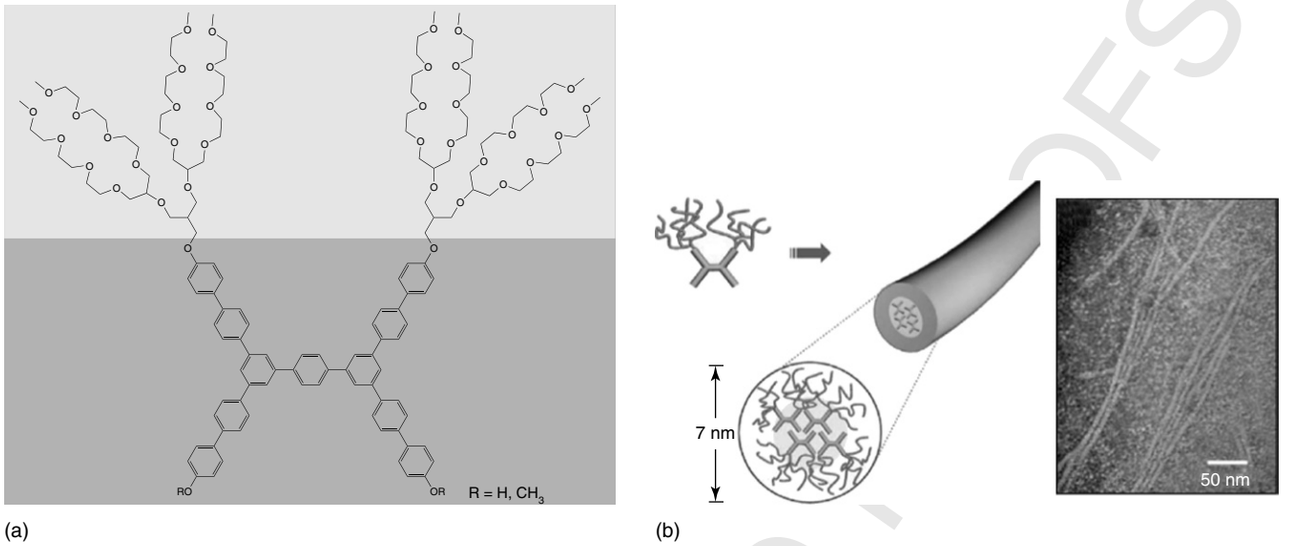

(a) (b)

Figure 9 (a) Molecular structure of the series of facial amphiphilic dendrons. (b) These dendrons aggregate into elongated micelles in water. (Reproduced from Ref. 46. © Royal Society of Chemistry, 2007.)

Another type of facial amphiphilic dendrons based on an aromatic segment and an oligo (ethylene oxide segment) is described by Lee and coworkers (Figure 9a).[46] Self-assembly of these amphiphiles gives rise to elongated micelles with an average width of 7 nm (Figure 9b). As the fully extended molecular length is about 4 nm, the diameter of 7 nm is consistent with a bilayer packing of the aromatic segments.

From these examples, it is clear that self-assembly of facial amphiphiles results in the formation of a variety of common and less common aggregate morphologies. Most notable is the low aggregation number of micellar assemblies and the high degree of order in interfacial assemblies. These properties are related to the large and comparable size of the hydrophobic and hydrophilic surface areas of facial amphiphiles compared to those of classic head/tail amphiphiles.

4 FACIAL AMPHIPHILES AS SOLUBILIZING AGENTS

Another property of facial amphiphiles, which is related to the large hydrophobic and hydrophilic areas, is their effectiveness as solubilizing and emulsifying agents. In fact, one of the main functions of facial amphiphiles in molecular biology is the solubilization of hydrophobic molecules in micellar assemblies. For instance, the already mentioned bile acids are biologically the most important dissolution agents and assist in the transport and digestion of triglycerides[47] and the dissolution of hydrophobic vitamins such as vitamin E.[48] Bile acids are commercially used in detergents, as food additive and in laboratories they are used to dissolve bilayers for protein purification.[49]

Because of the high efficiency of bile acids to form micelles and stabilize emulsions, researchers started to investigate nonsteroidal facial amphiphiles. An example of such a nonnatural facial amphiphilic compound is described by Sorrells and Menger,[50] consisting of a rigid hydrophobic segment functionalized on one face with hydrophilic sulfates (Figure 10). These amphiphiles are effective at stabilizing an oil–water interface. As a consequence, the size distribution in an oil–water emulsion is unaffected for six months in the presence of 0.6 mM of the facial amphiphilic compound. Again the large hydrophobic and hydrophilic surfaces play a key role in this stabilizing behavior.

Not only do facial amphiphiles act at the oil/water interface, natural facial amphiphiles also interact strongly with lipid bilayers such as cell membranes. Depending on the nature of the facial amphiphile, its interaction with biomembranes can lead to membrane bending, to pore formation, or even complete dissolution of the membrane. The dissolution of membranes by facial amphiphiles leads to cell death, and therefore the secretion of bile acids to the intestine of vertebrates is tightly regulated. The "carpet" model describes the mechanism of membrane dissolution by facial amphiphiles. Pore formation and membrane bending by facial amphiphiles are described in the next sections.

The carpet model was first described for the mode of action of the alpha-helical facial amphiphilic peptides dermaseptin S[51] and later for cecropins[52] as well as LL-37.[53] These compounds display antibiotic behavior

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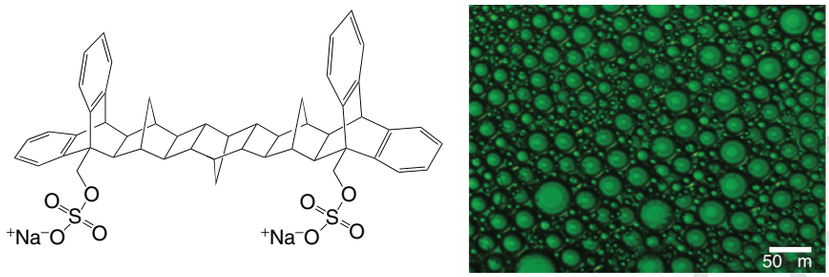

(a) (b)

Figure 10 (a) A nonsteroid facial amphiphilic compound described by Sorrells *et al.* (b) Oil droplets in water stabilized by the nonsteroid facial amphiphile. (Reproduced with permission from Ref. 50. © American Chemical Society, 2006.)

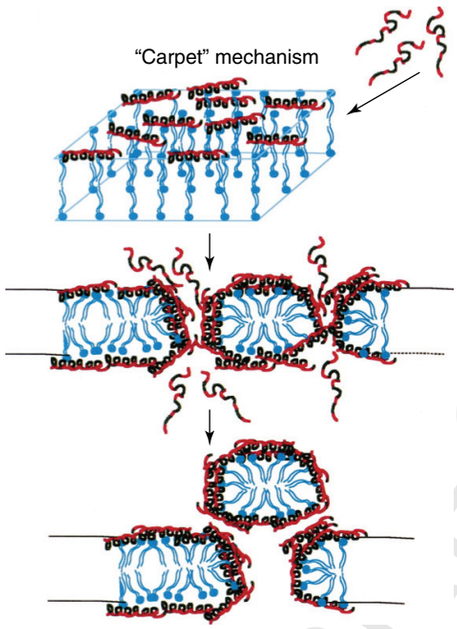

Figure 11 (a) Carpet mechanism of membrane dissolution; facial amphiphilicity is induced by the membrane bilayer. (b) If the concentration amphiphiles reach a threshold concentration they can incorporate into the bilayer. (c) By forming a carpet around a patch of bilayer these amphiphiles can dissolve the bilayer. (Reproduced with permission from Ref. 55. © Elsevier, 1999.)

and are short peptide sequences isolated from frog skin, moths, and humans, respectively. In the carpet model, the peptides interact with the hydrophilic/hydrophobic interface of the membrane, both via electrostatic attraction and hydrophobic interactions. The hydrophilic face of the antibiotic alpha helices is mainly cationic and therefore they have a preference for the negatively charged bacterial phospholipids. At high concentration on the membrane, the alpha helix monomers can form a "carpet" on the bilayer, these high concentrations can either be reached by covering the entire membrane surface or alternatively by locally association of the monomers. The hydrophobic face can then rotate and reorient toward the hydrophobic core of the membrane. If the concentrations of alpha helices on both sides of the membrane are high enough the membrane can be solubilized in a detergent-like manner.[54] As a consequence the bilayer dissolves, causing leakage of the cellular contents leading to cell death (Figure 11). This process of dissolving of the bilayer membrane to induce membrane leakage requires high (local) concentrations of facial amphiphilic peptide. This is demonstrated by the calcein release of vesicle as a measure of activity of dermaseptin.[51] Significant leakage was observed at peptide:lipid ratios of approximately 200 : 1 for soybean vesicles.

5 FACIAL AMPHIPHILES AS TRANSMEMBRANE PORE-FORMING AGENTS

In the previous section, we showed that, because of their large surface areas, facial amphiphiles are active at hydrophobic/hydrophilic interfaces. This is not only the case for oil/water interfaces but also for the hydrophobic/hydrophilic interface of phospholipid bilayers. Because of this property, facial amphiphilic molecules have been found to carry out a variety of biological functions related to their interaction with cell membranes. One of the most prominent functions of facial amphiphiles is the formation of pores in bilayer membranes. In the case of peptide antibiotics and toxins, depending if the peptide is specific for bacterial or mammalian cells, respectively, the formation of pores leads to lysis of the cells and ultimately cell death. Many other facial amphiphiles form pores to regulate transmembrane transport, such as the ion channels for selectively transport ions, or release osmotic pressure.

Pore formation by facial amphiphiles can occur via several mechanisms, mainly depending on the structure of the amphiphile. Extensive research has been done on the mechanism of action for antibiotic alpha-helical facial

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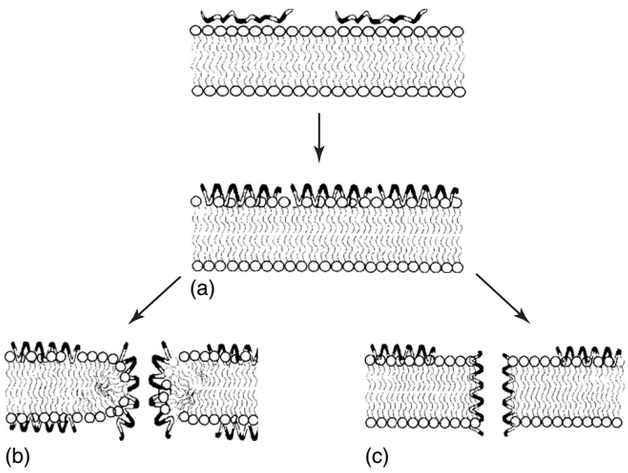

Figure 12 Mechanism of proposed pore formation by amphiphilic alpha helices. (a) Single helices combine with the bilayer. (b) If the concentration monomer is sufficient, the monomers insert in the hydrophobic core and align via the "barrel-stave" model or (c) via the "toroidal" model. (Reproduced from Ref. 57. © John Wiley & Sons, Ltd, 2000.)

amphiphiles such as the magainins[56] and others.[57] Mostly these alpha helices are relatively short (less than 50 amino acid residues) and show activity against various pathogens, including gram-positive and gram-negative bacteria, fungi, and protozoa.

Pore formation by alpha helices has been proposed to proceed via one of two general mechanisms (Figure 12).[55] The first model, referred to as the "barrel-stave" model, describes the formation of membrane pores by bundles of alpha helices.[58] Like the previous discussed carpet model, single helices bind at the surface of the bilayer with the hydrophobic domains inserting in the hydrophobic core of the membrane, and the hydrophilic segments remaining in the aqueous phase (Figure 12a). The perpendicular insertion of a single alpha helix into the bilayer, that is, with the helical axis parallel with the bilayer normal such that the peptide spans the membrane, is highly unfavorable since this would lead to exposure of the hydrophilic peptide domains to the hydrophobic bilayer interior. However, if the concentration of alpha-helical monomers on the bilayer surface is sufficiently high the monomers can insert in the bilayer and align their hydrophilic faces to overcome the unfavorable interaction between the hydrophobic membrane core (Figure 12b).

An example of pore-forming alpha helix acting via the "barrel-stave" models is the toxin pardaxin. This 33-residue peptide is found in the Red Sea Moses and used to repel Sharks.[59] It is found that this peptide binds to bilayer surfaces and forms transmembrane pores consisting of 6 ± 3 peptides. Remarkable is the efficiency of pore formation, studies have shown that calcein leakage takes place at lipid:peptide ratios as low as $2000 : 1$.[60]

A second model, referred to as the "toroidal" model, is fairly similar to the "barrel-stave" model for the first step. The "toroidal" model differs from the barrel-stave model in that the peptides are associated with the lipid head groups and stay bound to these head groups through the entire process, even when they are perpendicularly inserted in the lipid bilayer (Figure 12c). It has been suggested that the previous discussed magainin 2 forms transmembrane pores via the "toroidal" model[61] and only four to seven peptides can form a transmembrane pores at lipid:peptide ratios of $100 : 1$.[62,63]

In both models facial amphiphilicity is the key factor. If the peptides are also long enough, they are able to span the entire membrane. Because of their facial amphiphilicity, self-assembly into pores is self-complementary and as a result pores can form with low aggregation numbers, typically around five monomers. As a result, the diameter of the pores formed by alpha-helical peptides are relatively small, however, sufficiently large to induce leakage of small molecules and ions, and becoming lethal for the cell.

The distribution of charge in pore-forming alpha-helical peptides is shown to be important for its biological function and specificity. Facial amphiphilic peptides with a relative large net positive charge distributed along the axis of the peptide backbone show antimicrobial behavior [e.g., magainin $(+5)$[7], cecropin $(+7)$,[64] and dermaseptin S3 $(+5)$[65]]. In contrast, peptides with a low net positive or negative charge show high lytic behavior toward mammalian cells [e.g., pardaxin $(+1)$[59] and δ-hemolysin (0)].[66] It has been proposed that these different modes of action are governed by electrostatic interactions between the peptide and the phospholipid membranes.[55] The phospholipid membrane of mammalian cells is zwitterionic and the cell membrane interfacial potential is slightly negative.[67] Bacterial membranes, on the other hand, contain acidic phospholipids resulting in much more negative interfacial potential.[68] Therefore, cationic facial amphiphiles prefer to interact with bacterial cells, whereas slightly positive or negative charged or uncharged facial amphiphiles prefer to interact with mammalian cells.

In addition to amphiphilic alpha helices, also peptides with a beta-sheet secondary structure are known, which are capable of forming transmembrane pores on insertion in lipid bilayers. Although the interaction of beta-sheet type of peptides with lipid bilayers operate via many different mechanisms, facial amphiphilicity is one of the key factors for the formation of pores.

One specific case where beta sheets are involved in pore formation is in the case of beta barrel-forming proteins. Beta barrels are transmembrane proteins that contain beta-sheet peptide fragments incorporated in the lipid bilayer.

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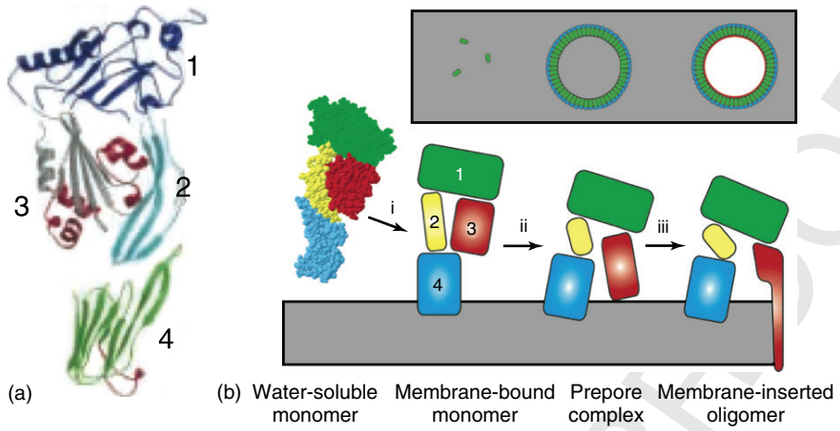

Figure 13 (a) The structure of PFO as determined by X-ray crystallography. The colors refer to different domains of the protein. (Reproduced with permission from Ref. 72. © Elsevier, 1999.) (b) Mechanism of pore formation in bilayer (gray). (Reproduced with permission from Ref. 73. © American Chemical Society, 2001.)

The beta-sheet peptide fragment is facial amphiphilic and decreases the interfacial energy between the hydrophobic core of the bilayer and the aqueous phase to allow the formation of a pore with an aqueous interior. There are many different families of beta barrel-forming proteins with pore diameter ranging from a few nanometers larger than 10 nm, where the pore can be formed beta-sheet peptide fragments located on a single protein, or by oligomerization of beta-sheet peptide fragments located on different proteins.[69] Such pore formation can have several functions, for example, pore formation can lead to leakage of ions leading to cell death by beta-barrel toxins, but other pores can also regulate the uptake and release of ions and small molecules in a controlled way. The mechanism of incorporation and pore formation differs drastically per family and an extensive discussion would be beyond the scope of this article.

A nice example of pore formation by beta-barrel proteins is the Perfringolysin O (PFO) pore-forming protein. PFO is part of the family of cholesterol-dependent cytolysins (CDCs), a class of pore-forming toxins which form pores as large as 30 nm in diameter by oligomerization of 40–50 monomers.[70] These CDCs are secreted by bacteria and act as toxins to cholesterol containing membranes. X-ray crystallographical studies have demonstrated that this protein consists of four domains (Figure 13).[71] On pore formation, domain 4 shallowly inserts into the bilayer membrane after which two small alpha helices in domain 3 rearrange to form two antiparallel facial amphiphilic beta hairpins. These two beta hairpins also incorporate in the membrane and together with beta hairpins from other monomers, form the wall of the beta barrel (Figure 13).[72]

Besides the channel-forming peptides and proteins, there are also small molecule channel-forming agents. A well-studied facial amphiphile is the antibiotic cholesterol derivative squalamine, which first has been isolated from the dogfish shark (Figure 14a).[74] Squalamine is a broad spectrum antibiotic with a minimal inhibitory concentration (MIC) of 1–2 $\mathrm{g\,ml^{-1}}$ against *E. coli*. It has been proposed that the hydrophilic polyamine chain covers the hydrophobic face of the steroid when inserting in a membrane resulting in an amphiphilic compound (Figure 14b).[75] This hypothesis was confirmed by studies with squalamine analogs, in which the position of the polyamine chain has been exchanged with position of the sulfonate group, but nevertheless retained the antimicrobial properties (MIC of 16 $\mathrm{g\,ml^{-1}}$ against *E. coli*). Several models have been suggested for pore formation by squalamine. Regen and coworkers described a model in which squalamine monomers embedded in one leaflet of the bilayer are in equilibrium with bilayer-spanning dimers.[76] These dimers create pores which can transport ions and are therefore lethal to the cell (Figure 14b).

The previous discussed cholic acid can also serve as a squalamine scaffold. If, for instance, cholic acid is functionalized with guanidine moieties on the hydroxyl positions, higher antimicrobial activities as compared to squalamine were found (MIC = 0.31 $\mathrm{g\,ml^{-1}}$ against *E. coli*).[77,78]

Inspired by these natural antibiotics, researchers have also investigated facial amphiphiles as pore-forming agents and antibiotic activity. In 2000, Gellman and coworkers investigated nonnatural amphiphilic helices made from β-amino acids for antibiotic activity.[79,80] The advantage of β-amino acids over natural amino acids is their increased stability to proteases, because they do not occur in nature.[81] Facial amphiphilic helices were designed by taking an hydrophilic residue for every first and third residues, because helices composed of β-amino acids have five

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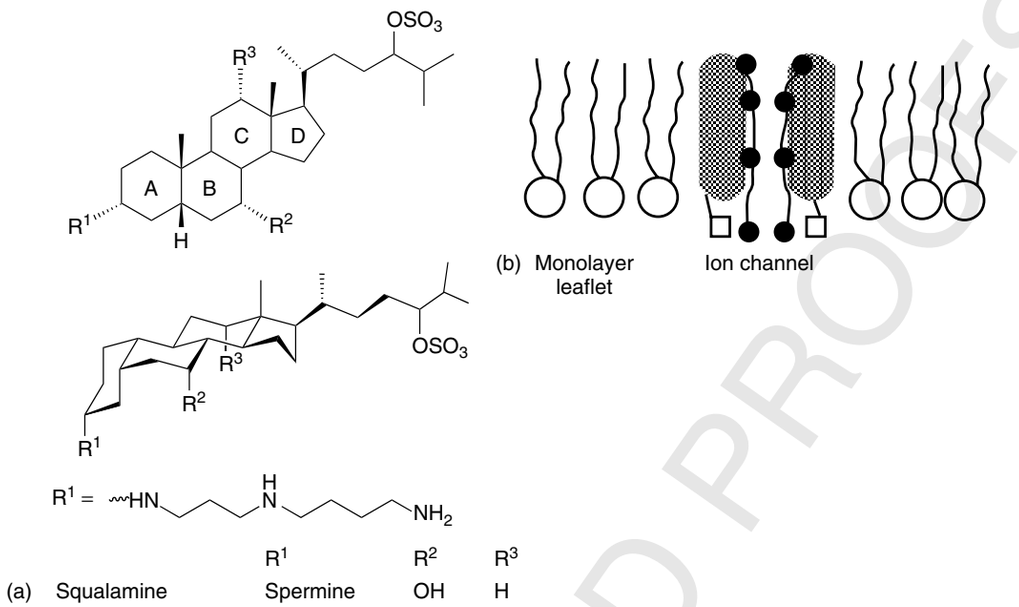

Figure 14 (a) Squalamine, a natural compound and although facial amphiphilicity is not directly obvious it is proposed to be induced by bilayers (b). (Reproduced with permission from Ref. 76. © American Chemical Society, 1998.)

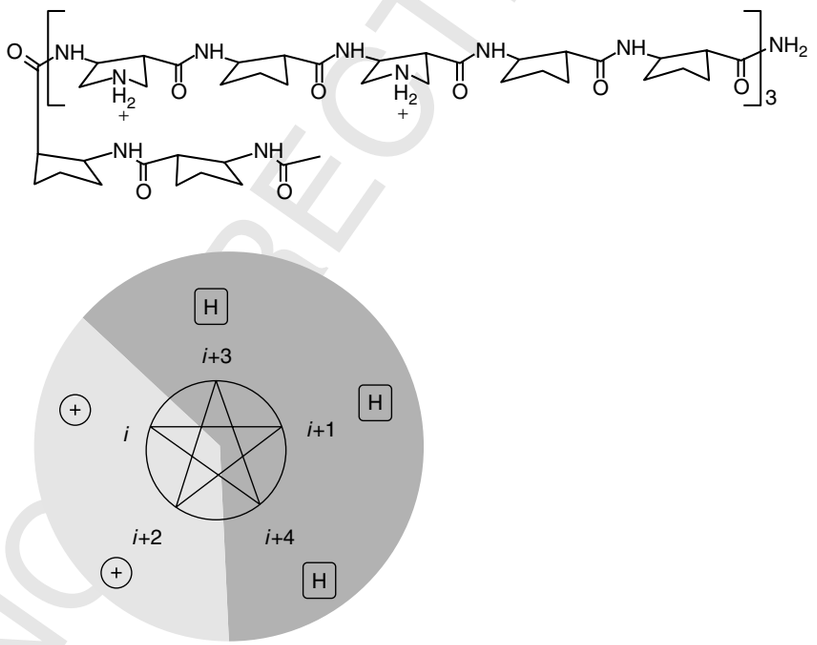

Figure 15 Sequence of β-17 with a hydrophilic cationic residue on position i and $i+2$. On self-assembly, a facial amphiphilic helix is formed (hydrophilic light gray and hydrophobic dark gray). (Reproduced with permission from Ref. 79. © Nature Publishing Group, 2000.)

residues per two turns (Figure 15). The antibiotic activity of this facial amphiphile (β-17) was found to be comparable to synthetic antibiotic magainin derivatives against different bacteria. For instance, against *E. coli* a MIC of 6.3 $\mathrm{g\,ml^{-1}}$ was found.

Another successful example of an antimicrobial β-peptide is described by DeGrado and coworkers.[82] This synthetic peptide consists of a repeating sequence of hydrophobic β-leucine, hydrophobic β-valine, and hydrophilic β-lysine. On folding, with three residues per turn, a

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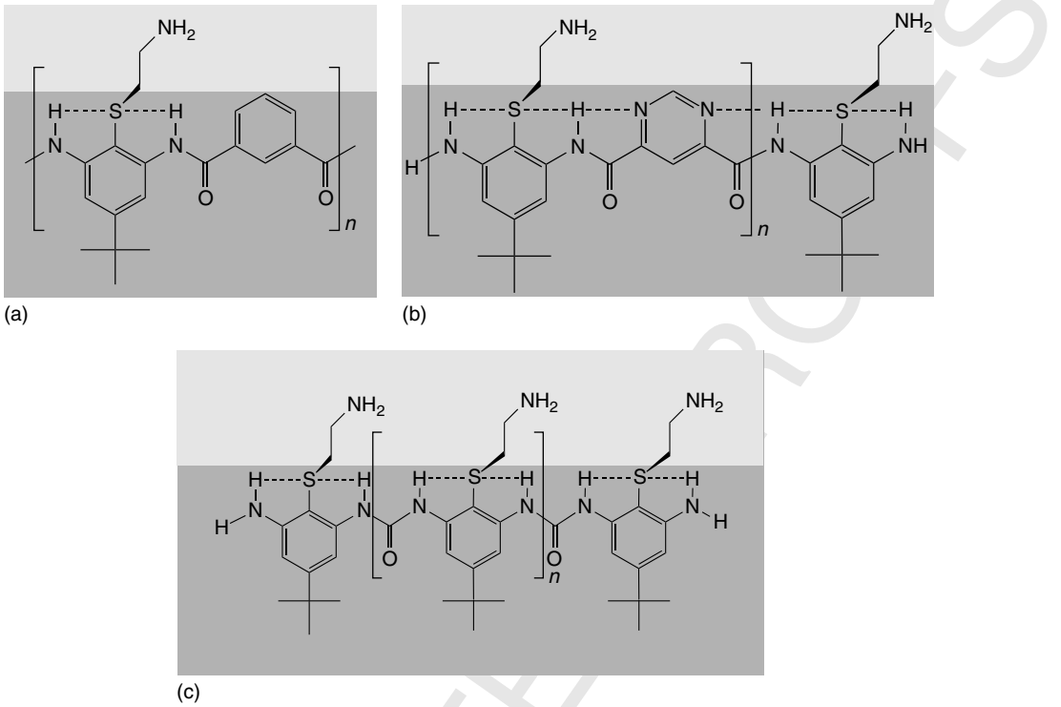

Figure 16 (a) A rigid polyamide with high antimicrobial activity. (b) Rigidity is induced by hydrogen bonding. (c) Additional rigidity is acquired if the benzene ring is replaced with a hydrogen bond accepting pyrimidine ring. A rigid facial amphiphilic polyurea with high antimicrobial activity against *E. coli*.

facially amphiphilic helix is formed. Cell penetrating activity was measured against *E. coli* and human erythrocytes as bacterial and mammalian cell models, respectively. The 12-residue helix showed highest selectivity for the bacterial cell with MIC values of 1.1 g ml^{-1}. These examples illustrate the possibility of mimicking biological antimicrobial alpha helices.

DeGrado and coworkers also described an extensive investigation of the effect of the rigidity of the backbone of the polymer on its facial amphiphilic character. These studies are based on aryl amide oligomers containing hydrophilic amines and hydrophobic *t*-butyl-groups (Figure 16a). Owing to intramolecular hydrogen bonding between the thioether and the amides, the polymer backbone becomes rigid and the hydrophilic side groups remain on one face, resulting in a facial amphiphile.[83] Antimicrobial studies showed that oligomers with $n = 8$ possessed high antimicrobial activity (MIC of 7.5–15 g ml^{-1} against *E. coli*).[84]

In an attempt to make the oligomers more rigid, and thereby increase their facial amphiphilic character, a polyamide was synthesized similar to the previous described compound. In this case, however, the benzene ring was replaced with a pyrimidine, bearing two additional nitrogen atoms and capable of forming hydrogen bonds (Figure 16b).[85] For $n = 1$, the triaryl compound showed highest antimicrobial activity, with a more than 10-fold lower MIC (MIC = 0.8 g ml^{-1} against *E. coli*) than the less rigid triaryl thioether analog described above. Moreover, this monomer showed high selectively toward the *E. coli cells* with respect to human red blood cells.

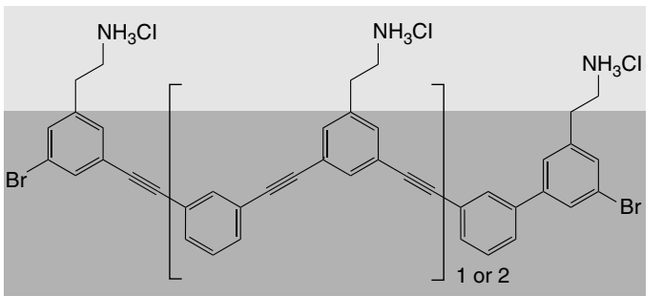

Figure 17 A rigid oligophenylene ethylene showing antibiotic behavior with a MIC of 0.1 g ml^{-1} against *E. coli*.

The high antibiotic activities of these relatively simple facially amphiphilic oligomers prompted researchers to further explore facial amphiphiles with rigidified oligomer backbones as possible antibiotics. For instance, a similar system based on urea-linked oligomers was investigated by Tew *et al.* (Figure 16c).[86] These compounds show comparable antimicrobial activities (MIC = 0.7 g ml^{-1} against *E. coli*).

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Another attempt to increase the rigidity of these compounds by Tew and coworkers is by using oligophenylene ethylene (Figure 17). These oligomers form ordered layers at air–water interfaces and also display antimicrobial behavior.[87] Again, the triaryl compound showed the highest antibiotic behavior against *E. coli* (MIC = 0.1 $g\,ml^{-1}$)[88] and *several other* antibiotic-resistant bacteria such as *Meticilline-resistant Staphylococcus aureus* (MRSA bacteria) and *Enterococcus faecalis.*

This study shows that rigidity of the polymer backbone, analogous to the rigidity induced by hydrogen bonding in alpha helices, is of high importance for antibiotic activity. Next to that, in most examples, the triaryl shows the highest antibiotic activity. This observation suggests that the match between the length of these triaryl molecules and the bilayer thickness allow them to span the bilayer, whereas the longer oligomers might fail to do so because they are too long. It is tempting to speculate that these relatively short and rigid molecules form transmembrane pores similar to other rigid facial amphiphiles, but more insights into the origin of the antibiotic activity are required.

Not all facial amphiphilic amphiphiles need support from a bilayer to induce their folding into a channel. In the case of a polycholate, the apolar environment provided by solvents such as hexane and CCl_4 induces its folding to hollow helices due to intramolecular interactions between the slightly concave hydrophilic faces (Figure 18a). Most likely, also solvophobic effect contributes to the driving force for their folding. These helices have a hydrophilic interior with a pore size of roughly 1 nm and a hydrophobic exterior in apolar solvents (Figure 18b).[89,90]

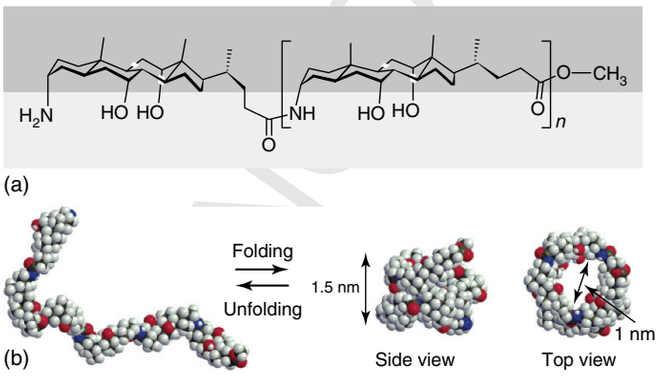

Figure 18 (a) Molecular structure of oligocholate which, on intramolecular self-assembly, forms helices with a hydrophilic interior (b). (Reproduced with permission from Ref. 89. © American Chemical Society, 2005.)

6 MORPHOLOGICAL SENSING AND CONTROL BY FACIAL AMPHIPHILIC STRUCTURES

In addition to the well-known antibiotic and pore-forming properties, facial amphiphilic proteins are also involved in several nonlethal functions in connection to bilayer membranes, especially in sensing and controlling membrane curvature. Membrane curvature plays an important role in essential processes such as cell division, exocytosis and endocytosis, vesicle trafficking, and motility. Unlike in model systems such as vesicles and liposomes in which the bilayer membrane adopts a minimal curvature, the internal membranes of cells bear regions with high (local) curvatures, such as tubules, pores, bends, flagella, and so on. Moreover, natural bilayer membranes are continuously changing shape and curvature, because of the high turnover of the processes they are involved in,[91] and because many of these processes are related to membrane curvature, the ability to sense and regulate membrane curvature is of primary importance for living cells.

Several different proteins for the sensing and regulation of membrane curvature are known, and not all of them involve facial amphiphilic peptides. For instance, membrane curvature can be induced by the pushing or pulling force by the cytoskeletal network or by the insertion of entire proteins which have intrinsic curvature.[92]

A well-studied class of facial amphiphiles involved in curvature sensing are facial amphiphilic alpha-helical peptides, which are part of a variety of proteins involved, for example, protein coat assembly and disassembly.[93] Membrane curvature sensing by facial amphiphilic alpha helices can be explained by the following model. In case of a membrane curvature sensing facial amphiphilic alpha-helical domain, the alpha helix generally bears a small net positive charge, in order to interact with the negatively charged phospholipid bilayer. (Highly) curved membranes are known to come with defects in the phospholipid bilayer resulting in packing stress and curvature strain (Figure 19c).[1] The hydrophobic face of the facial amphiphilic alpha helix is proposed to incorporate shallowly within these defects and thereby alleviating the curvature strain (Figure 19b).[93,94] This hypothesis is further supported by the observation that the ability to sense curvature is affected by the composition of the bilayer membrane. The presence of lyso-lipids, known to incorporate into bilayer defects at convex interfaces, drastically diminishes the curvature sensing ability of membrane curvature sensing proteins.[95]

A second membrane curvature sensing otif is the Bin/Amphiphysin/Rvs (BAR) domains, which is found in, for instance, amphiphysin and involved in endocytosis, and

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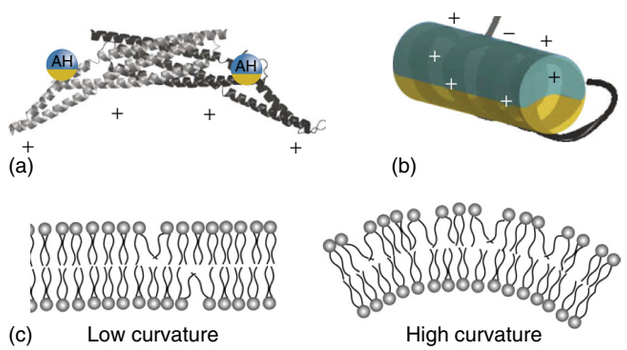

Figure 19 (a) The BAR domain a crescent-shaped protein domain with a cationic concave surface. (b) Facial amphiphilic alpha helices can incorporate in lipid defects alleviating this membrane stress, which is the mechanism for curvature sensing of these facial amphiphiles. (c) Membrane curvature results in defects in the bilayers, resulting in membrane stress. (Reproduced with permission from Ref. 99. © Elsevier, 2010.)

is suggested to be part of a much wider range of proteins including nexins, centaurins, and oligophrenins.[96] These domains are able to sense membrane curvature and preferentially bind to curved membranes, and at higher protein concentration these domains can also deform the membrane and induce curvature,[97] essential, for example, vesicle budding, and tubulation. The exact sensing mechanism of membrane curvature sensing of these BAR domains is part of discussion. The BAR domain is a crescent or banana-shaped quaternary protein structure, having positive charges on the concave surface (Figure 19a). Until recent, membrane sensing has been thought to occur by inserting the cationic charges on the membrane surface and the binding constant depends on the similarity between the membrane curvature and the intrinsic curvature of the protein. However, recent studies have shown that mutations changing either the charge of the concave surface or the curvature of the crescent do not change the membrane sensing properties.[98] Moreover, the preferred membrane curvature sensed by these mutated proteins does not change either.

BAR domains are also often found in combination with N-terminal facial amphiphilic helices (making them N-BARs) and several studies have shown that mutations on these facial amphiphilic helices change the membrane sensing ability drastically. These observations led to the suggestion that membrane sensing by these N-BAR domains actually takes place via a mechanism, which involves facial amphiphilic alpha helices. In fact, it has been suggested that all membrane sensing BAR domains would contain such an adjacent facial amphiphilic alpha helices.[99] After sensing the membrane curvature with the facial amphiphilic alpha helix, the BAR domain can induce curvature by the overall banana-shaped structure (Figure 20).[100]

An example of a membrane curvature inducing protein without a BAR domain is epsin, which is known to assists in membrane deformation during clathrin-mediated endocytosis.[101] Epsin is a well-characterized member of accessory proteins and its primary function is to add bending stress to membranes which induces curvature, necessary for vesicle formation. A specific domain of this protein, the ENTH domain, can bind specifically to phosphatidylinositol-4,5-bisphosphate [PtdIns(4,5)P_2], a membrane phospholipid. On binding, unstructured residues at the N-terminus of the ENTH domain fold to a facial amphiphilic alpha helix. This facial amphiphilic alpha helix inserts into the inner leaflet of the bilayer and acts as a wedge pushing aside the surrounding phospholipids. To overcome the induced local bilayer stress, the total membrane reorients and bends at the insertion site of the protein (Figure 21).[102,103]

In both membrane curvature sensing and inducing by facial amphiphilic helices, the mechanism has to do with incorporation of the helix into the bilayer. In the case of membrane curvature *sensing* incorporation is driven by alleviating the membrane stress, present in curved membranes. In the case of membrane curvature *inducing* proteins a facial amphiphilic alpha helix is *forced* into the bilayer, for example, binding of a protein domain to the phospholipids, which results into bilayer curving.

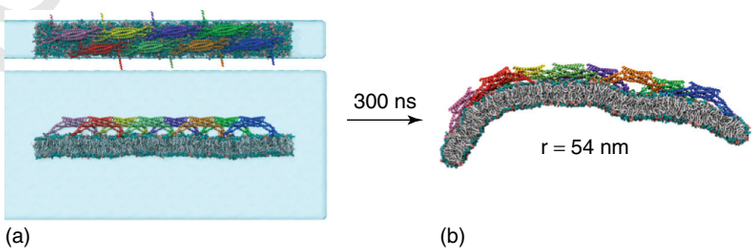

Figure 20 All atom simulations of inducing membrane curvature by N-BAR domain. (a) The N-BAR domain can bind a flat membrane with facial amphiphilic alpha helices after which it can bend the membrane with a radius of 54 nm (b). (Reproduced with permission from Ref. 100. © Elsevier, 2009.)

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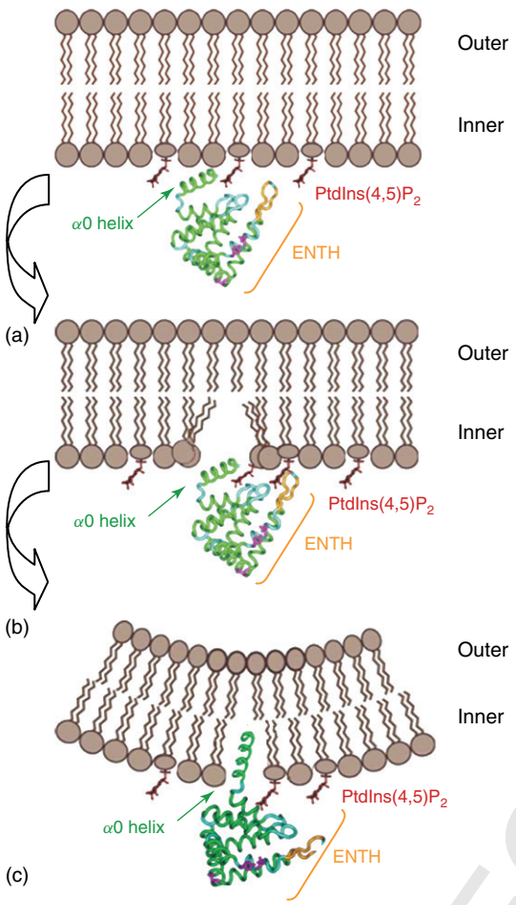

Figure 21 Epsin-mediated membrane curvature inducing. (a) Membrane curvature induced by incorporation of a facial amphiphilic alpha helix. (Reproduced with permission from Ref. 101. © Elsevier, 2007.)

7 CONCLUSIONS

In this article, an overview is given concerning facial amphiphiles in nature, bio-, and supramolecular chemistry. In facial amphiphiles, the hydrophilic and hydrophobic parts are separated by the longitudinal axis of molecule, this in contrast to head–tail amphiphiles in which the hydrophilic and hydrophobic groups each occupy one end of an elongated molecule. This different distribution of hydrophilic and hydrophobic groups with respect to the overall molecular shape leads to a number of properties and functions which are unique for facial amphiphiles. In nature facial amphiphiles occur mainly as low molecular weight compounds such as the bile acids, and as a variety of peptides, alone or as part of larger proteins. Interestingly, these peptides gain their facial amphiphilicity from folding into alpha-helical or beta-sheet secondary structures. In nature these compounds serve a variety of functions, including emulsification and solubilization of hydrophobic compounds, as antibiotics and pore-forming agents in bilayer membranes, and as membrane curvature sensing and regulating agents.

In supramolecular chemistry, facial amphiphiles have been synthesized ranging from low molecular weight to polymers and dendrimers for the purpose of novel building blocks for supramolecular architectures and to mimic the natural functions of facial amphiphiles. This approach has led to novel building blocks for common morphologies such as vesicles and micelles, and also new architectures, such as coils and complex networks. Many of these synthetic facial amphiphiles have similar properties as their natural counterparts with regard to emulsification and solubilization, and their interaction with bilayer membranes. Therefore facial amphiphiles are gaining more and more interest as new antibiotics and toxins, and transport channel agents.

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ABSTRACT: In this article, an overview is given concerning facial amphiphiles in nature, bio-, and supramolecular chemistry. In facial amphiphiles, the hydrophilic and hydrophobic parts are separated by the longitudinal axis of molecule, this in contrast to head-tail amphiphiles in which the hydrophilic and hydrophobic groups each occupy one end of an elongated molecule. This different distribution of hydrophilic and hydrophobic groups with respect to the overall molecular shape leads to a variety of functions which are unique for facial amphiphiles, including emulsification and solubilization of hydrophobic compounds, as antibiotics and pore-forming agents in bilayer membranes, and as membrane curvature sensing and regulating agents. In nature facial amphiphiles occur mainly as low molecular weight compounds such as the bile acids, and as a variety of peptides, alone or as part of larger proteins. Synthetic facial amphiphiles have been synthesized ranging from low molecular weight to polymers and dendrimers for the purpose of novel building blocks for supramolecular architectures and to mimic the natural functions of facial amphiphiles. In this article, we gave an overview of the self-assembly and interfacial behavior of both natural as well as synthetic facial amphiphiles and discuss their most important biological functions and applications.

KEYWORDS: facial amphiphile, self-assembly, antibiotics, pore formation, membrane curvature sensing

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